Florida Department of Health
Bureau of Onsite Sewage Programs
Research Review and Advisory Committee Meeting

DATE AND TIME: **May 29, 2008 at 9:30 am**

PLACE: **Orange County Health Department**
Administrative Services
6101 Lake Ellenor Dr.
Orlando, FL 32809
(407) 858-1400

This meeting is open to the public

AGENDA: FINAL 5/27/2008 Elke Ursin

1. Introductions
    a. New RRAC Members / Alternates
2. Review Minutes of Meeting 1/23/2008
3. Discussion on Legislative Session
4. Brief discussion on TMDL for Wekiva
5. Brief updates on Ongoing Projects
    a. UCF Research Station
    b. Passive Nitrogen Removal Assessment
    c. Optical Wastewater Tracers Study (old Remote Sensing project)
    d. Manatee Springs, Performance of Onsite Systems Phase II Karst Study
    e. Taylor County Source Tracking Study
    f. Keys Performance Based Treatment System Performance Assessment
6. Brief updates on Future Projects
    a. RRAC Priorities from last meeting
    b. 319 Project on Performance and Management of Advanced Onsite Systems
    c. Town of Suwannee Study
7. Other Business
8. Public Comment
9. Closing Comments, Next Meeting, and Adjournment

Research Review and Advisory Committee for the Bureau of Onsite Sewage Programs

Approved Minutes of the Meeting held at the Orange County Health Department, Orlando, FL
May 29, 2008
Approved by RRAC July 30, 2008

In attendance:

- **Committee Membership and Alternates**: David C. Carter (Chairman, member, Home Building Industry); Paul Davis (member, DOH-Environmental Health); Anthony Gaudio (member, Septic Tank Industry); Marc Hawes (alternate, Home Building Industry); Bill Melton (member, Consumer); Eanix Poole (alternate, Consumer); Jim Rashley (alternate, DOH-Environmental Health); Patti Sanzone (alternate, Environmental Interest Group); Clay Tappan (member, Professional Engineer); Pam Tucker (member, Real Estate Profession); and Ellen Vause (alternate, Septic Tank Industry)
- **Not represented**: Restaurant Industry, State University System
- **Visitors**: Damann Anderson (Hazen & Sawyer); Rick Baird (Orange County Environmental Protection Division); Quentin Beitel (Markham Woods Association); Alice Berkley (State Representative Bryan Nelson's Office); Dominic Buhot (Greens Environmental Services); John Byrd (Aide to Orange County Commissioner Brummer); Blaine Carter (Florida Home Builders Association); Ron Davenport (Infiltrator Systems); Lindsay Faloh (E-2 Tanks); Francisco Gonzalez (Seminole County Environmental Health); Roxanne Groover (Florida Onsite Wastewater Association); Karl Henry (Seminole County Environmental Health); John Higgins (Markham Woods Association); Mary Howard (Orange County Environmental Health); Dan Meeroff (Florida Atlantic University); Harley Pattee (E-2 Tanks); Daniel Smith (Applied Environmental Technology); Gary Smith (Orange County Environmental Health)
- **Department of Health (DOH), Bureau of Onsite Sewage Programs**: Paul Booher; Sonia Cruz; Kim Duffek; Eberhard Roeder; and Elke Ursin

1. **Introductions:** Seven out of nine groups were present, representing a quorum. Chairman David Carter calls the meeting to order at 9:40 am. James H. Peters is the new alternate for the Professional Engineer's and was added to the committee as of the last meeting. Elke Ursin asked a procedural question on whether the RRAC members would be interested in having a CD-ROM mailed to them with the meeting material, as opposed to hard paper copies, in order to save resources, time, and money. The RRAC indicated that they prefer to have the paper copies so that they do not have to print out the materials themselves.

2. **Review of Previous Meeting Minutes**: Motion by Clay Tappan, seconded by Paul Davis:
   The minutes were approved as submitted.
   The members voted and all were in favor with none opposed, the motion passed. Later in the meeting John Byrd mentioned that the location information on the first page was incorrect, and this change will be made to reflect the correct information.

3. **Discussion on Legislative Session**:

   a. **Senate Bill 1318:** Appoints a local government representative to RRAC and TRAP and eliminates certification requirement for individuals working under an engineer. Those working under an engineer would still need to go through the soil certification requirements with the Department of Health. Bill Melton asks what the intent was to eliminate the certification requirements. Clay Tappan stated that the Florida

Engineering Society took exception to the requirement for those individuals working under the direct responsible charge of an engineer to be a Certified Environmental Health Professional. This was seen as an encroachment into the engineer's profession. The local government representative would be knowledgeable about domestic wastewater treatment and is to be recommended by the Florida Association of Counties and the Florida League of Cities.

b. **Special Appropriation 1682:** $1,000,000 study on passive nitrogen, $150,000 inventory of onsite systems, and a report by October 1, 2008 identifying costs for 5-year inspection program. This item was introduced to the RRAC for consideration and will be discussed at future meetings if/when the appropriation has been signed into law. The million-dollars would come from DEP, and the $150,000 would come from the research trust fund. Patti Sanzone states that she has concerns that the amount of money specified in this appropriation is not very much. David Carter stated that he understands this inventory to be more of a records review type project. Elke Ursin states that some of the initial concepts on how to approach this are similar to how the Wekiva numbers were generated: get a list of developed properties from the county property appraisers and subtracting those that have a sewer bill. Patti Sanzone states that this inventory will help with the inspection program mentioned in the last part of the appropriation. Paul Davis states that it will be easier to do this inventory with the large utilities, but much harder for the small ones. Anthony Gaudio mentioned that an inventory was previously done in Leon County and that there may be good information available to help develop this project. There was a question from the audience whether RRAC is in favor of this and the general consensus is that RRAC carries forward with whatever charge is given to them. This appropriation is much broader than just the Wekiva issue, the focus is more statewide.

c. Both are waiting to be signed into law.

4. **Wekiva Update:** There is no new information on rulemaking; options are still being discussed with the governor's office. At the last RRAC meeting Dr. Eberhard Roeder presented revised the input and loading estimates. The RRAC was given an opportunity to provide comments, the report has been finalized, and is available on the DOH website:

   http://www.doh.state.fl.us/environment/ostds/wekiva/WekivaEstimateFinal.pdf

   There was a discussion on how this report could be final without RRAC having approved the report. Any report generated by or for the department is presented to the RRAC for review and comment, per the statute requirement. The RRAC was allowed to comment at the last meeting. The RRAC does not generally vote on a report. The RRAC is an evolving study group where if new information comes to light it can be considered at that time.

   A brief overview of the Total Maximum Daily Loads (TMDL) process was given by Dr. Eberhard Roeder. Elke Ursin provided a brief update of the TMDL for the Wekiwa Spring, Rock Spring, Wekiva River, Rock Springs Run, Little Wekiva Canal & River, and seven lakes. The reports were released in April. DEP did not receive objections and is going forward to final adoption. Surface loading and springs contribute the majority of nutrient load into Wekiva River/Rock Springs Run. Septic tanks are one source of nutrient loading in surface runoff and springs. Implementation of TMDLs will require a close look at septic tank loadings along with all other watershed sources. TMDL implementation plan (Basin Management Action Plan, or BMAP) development will begin this summer. The reports are available here:

http://www.dep.state.fl.us/water/tmdl/final_tmdl.htm

An update was presented on the FDEP Phase II study looking at what impacts fertilizer use has on water quality in residential areas in the Wekiva Basin. DEP has contracted with SJRWMD and there is an anticipated start date for sampling sometime in June.

5. Brief updates on other projects

a. Ongoing projects

i. **University of Central Florida Research Facility** – UCF has a grant with FDEP to look at nutrient reducing onsite systems and to develop a research facility with test beds. Dr. Ni-Bin Chang presented to the RRAC the current status of the project.

ii. **Passive Nitrogen Removal Assessment** – Elke Ursin presented a quick overview of the progress since the last RRAC meeting in January. The final laboratory experiment report has been received. The draft economic analysis of passive systems report and the recommendations for the passive nitrogen system report have both been received. Dr. Smith presented a paper on the preliminary results of the laboratory experiments at the National Onsite Wastewater Recycling Association (NOWRA) Nitrogen Symposium in April. The laboratory experiment sampling has been extended for five additional sampling events over a five month period due to the interesting preliminary results. At the end of the sampling, the station will be disassembled and the treatment media will be evaluated. The draft final project report was received two days before the meeting, the full report, including the appendices, was emailed to the RRAC, and printed copies of the report, excluding the appendices, were passed out with some extras for the audience.

Dr. Smith presented on the final report. He worked on this with collaboration from Dr. Dick Otis with Otis Environmental Consultants, LLC and Mark Flint with Watermark Engineering Group, Inc. Dr. Otis was much help on the literature review and the recommendations portions of the project, and Mr. Flint was central to the economic analysis and other components of the overall project. He acknowledged FDOH staff, the RRAC, Damann Anderson, and Hillsborough County for allowing use of the site. The objectives of the study were to evaluate enhanced nitrogen removal from on-site wastewater using passive systems. The influent for the laboratory experiment came from the effluent of a septic tank. The overall goal is to reduce total nitrogen. The presentation will cover the literature review and database portion of the contract, the experimental evaluation (which is now completed), the economic analysis, and the recommendations.

The definition of passive was defined as "A type of onsite sewage treatment and disposal system that excludes the use of aerator pumps and includes no more than one effluent dosing pump in mechanical and moving parts and uses a reactive media to assist in nitrogen removal." These are the restraints that form the basis of the project. The literature review portion of the project looked at several references and what has been done in the field, to come up with some constraints to formulate an approach for the laboratory experiments. Some of the technology constraints are that there are no aerators, which would require an unsaturated filter for Stage 1. Stage 1 is to accomplish the first stages of nitrogen removal which is ammonification and nitrification. There was a constraint of having only one pump, and the question was where to put the pump. A reactive media was required, which would require an electron donor for denitrification, to provide alkalinity, and to possibly provide ion exchange in the whole process.

The two stage filter concept was developed in response to the constraints. There are two filters in series: Stage 1 is unsaturated and aerobic to get organic nitrogen and ammonia removal and to produce a low amount of TKN in the effluent, Stage 2 is a saturated anoxic filter to convert to oxidized nitrogen, provide an electron donor for denitrification and produce a low nitrogen oxide effluent. The electron donor is provided by passive aeration through the media. The next step is to decide where the pump should go. It was decided to put it prior to the Stage 1 filter as nitrification/ammonia removal is one of the most sensitive processes. This allows time dosing to the media, the potential for recycling of the effluent, allowing for flow equalization to the entire treatment train, and gravity flow after the pressure dosing of the effluent through the rest of the system. Septic tank effluent was pumped to the top of the unsaturated media in Stage 1 by a peristaltic pump. This runs through trickle flow into the Stage 2 horizontal saturated anoxic filter with reactive media. One could think that this is a good small scale representation of what would happen in a full size system. Dr. Smith sees these as different modules that could handle different flow rates and influent concentrations that could be plugged into different systems. He sees these working for onsite and as well as for management of sanitary water onsite in an urban environment. Anthony Gaudio asks what a peristaltic pump is and Dr. Smith states that it is a pump that provides positive displacement and does well with low flow rates. The systems were dosed with wastewater once every 30-minutes. Dr. Roeder asked how the air gets into the top of the unsaturated filter and whether it comes into the bottom as well and Dr. Smith stated that there are air holes in the top and no air holes at the bottom. The water is dosed to the top and trickles down. During normal operation all the effluent is put back into the septic tank, and when a sample is taken it was collected as it came out of the Stage 1 or 2 filters. He developed a method to measure the effluent volume as it comes out. Two of the filters (clinoptilolite and the expanded clay) had a constant flow rate but the tire crumb filter varied. The dissolved oxygen was high as it came out of Stage 1, which shows that the media has a high aeration capacity, better than sand.

An important factor for the media is particle size, specific surface area, external porosity, air filled porosity, water retention capacity, and cation exchange capacity (CEC). These were all researched in the literature review portion of the project and the information gathered was used to select the media. There are three two stage systems in parallel. The first used Zeo-Pure clinoptilolite which is an ion exchange zeolite, a naturally mined mineral from the Death

Valley area. Zeolites are used in some industrial water treatment processes to take different ions out, were once used in water softeners, found in aquarium filters, and are used in cat litter boxes to keep the ammonia down. It was also used at one secondary wastewater treatment facility for ammonia removal. Paul Davis stated that a byproduct of the mining is silicate which can be harmful if inhaled and Dr. Smith stated that when he received the material it had a little dust and that when placing the material precautions should be taken. The second is an expanded clay which comes from Georgia. You take clay and you put it in a cement kiln, it opens up and is called a lightweight aggregate. It's lighter than concrete and is great for biofilm reactors like this one. The expanded clay is sold for construction purposes, and is used for drinking water treatment filtration processes. When Dr. Smith spoke with the manufacturers they stated that if the demand was there someone would set up a distribution center. Paul Booher asked whether this is the same type of material used in the Keys Demonstration Project and Damann Anderson said it was a different brand but basically the same process. The LECA (Lightweight Expanded Clay Aggregate) comes from Norway and is now called Filtralite P for phosphorus removal that includes calcium carbonate to aid in phosphorus sorption. The third media used was tire-crumb which is a recycled product available from many places here in Florida. It is used as an aggregate substitution in drainfields. The first two media have a high capillarity; they are able to retain a lot of water vs. the tire crumb. The three media each have different characteristics. Hauling the media for one system would be more costly, but for more than one it becomes more cost effective. This will be discussed later in the cost analysis section.

Paul Booher stated that this process could be seen as the Culligan Man system where there is ion exchange. You come in and backflush and there was no residue to dispose of later. Dr. Smith said that one way of looking at this is that the material is being regenerated onsite through biological regeneration. Mr. Booher said there can be a factory that makes modules of these systems, which is closer to the Culligan Man system. Dr. Smith said that this is similar to how cars are designed and manufactured. Every time someone wants to design a new car, mass production is always the end goal. These are mass produced depending on what model you want and they come with a users manual that says that you change the oil every 5,000 miles, and at 10,000 miles you have to look at and replace certain components. He sees this as a modular thing that can be built, where you know that it can perform, and then you can plug it in to different designs. He stated that this study is creating a space for innovation and where we go with it is up to us.

The Stage 2 media was selected based on it being reactive, having a specific electron donor release rate (not too fast (used up too quickly) and not too slow (greater potential for a breakthrough of nitrogen)), a media with longevity, having a supply of alkalinity, and a high anion exchange capacity (a media that would also remove phosphorus). One of the selected media is expanded shale, which comes from Utah and is also called utilite. The expanded shale is similar to the LECA but came out on top for phosphorus sorption in one specific study, and would probably be cheaper as it comes from Utah as opposed to

Norway. Elemental sulfur is another media which provides an electron donor. Oyster shell is the third media and it provides alkalinity. The Stage 2 media is a mixed media. The process for nitrogen reduction is totally autotrophic. There is nitrification in the first stage and autotrophic denitrification in the second stage.

There were three two-stage systems shown in the diagram below. The first stage is unsaturated aerobic that had three columns (one with each media type: zeolite, expanded clay, and tire crumb) that have a three-inch diameter and 24-inch depth. Each were stratified in similar ways with larger particle sizes on top going to smaller sizes and ending with some larger particles so that the smaller particles do not get flushed out. The reason for this was because septic tank effluent may not be completely filtered, and to handle some of the larger solids that may come through. There is a possibility that the 24-inch depth may not be needed, but this will need to be evaluated after future study. The second stage is saturated anoxic that had three columns (each with different combinations of non-stratified sulfur, oyster shell, and expanded shale) that have a 1.5-inch diameter and 24-inch depth. There is an interpretation problem with each Stage 1 filter feeding into another State 2 filter and that was understood.

<table>
<tr><th>Stage</th><th>Filter</th><th>Column ID, inch.</th><th>Media depth, inch</th><th>Media placement</th><th>Media</th></tr>
<tr><td rowspan="3">Stage 1 unsaturated aerobic</td><td>1A</td><td rowspan="3">3.0</td><td rowspan="3">24.0</td><td rowspan="3">Stratified</td><td>Clinoptilolite
depth (in.) | diameter (mm) | top
8 | 3 - 5
8 | 0.8-2.3
6 | 0.5-1.1
1 | 0.8-2.3
1 | 3 - 5 | bottom</td></tr>
<tr><td>1B</td><td>Expanded Clay
depth (in.) | diameter (mm) | top
8 | 3 - 5
8 | 0.8-2.3
6 | 0.5-1.1
1 | 0.8-2.3
1 | 3 - 5 | bottom</td></tr>
<tr><td>1C</td><td>Tire Crumb
depth (in.) | diameter (mm) | top
8 | 3 - 5
8 | 0.8-2.3
6 | 0.5-1.1
1 | 0.8-2.3
1 | 3 - 5 | bottom</td></tr>
<tr><td rowspan="3">Stage 2 saturated anoxic</td><td>2A</td><td rowspan="3">1.5</td><td rowspan="3">24.0</td><td rowspan="3">Nonstratified</td><td>75% elemental sulfur
25% oyster shell</td></tr>
<tr><td>2B</td><td>60% elemental sulfur
20% oyster shell
20% expanded shale</td></tr>
<tr><td>2C</td><td>45% elemental sulfur
15% oyster shell
40% expanded shale</td></tr>
</table>

Pam Tucker asked where the septic tank effluent came from and Dr. Smith stated that it came from Flatwoods Park in Hillsborough County. The wastewater was mixed from a single family ranger residence with four people and a day-use bathroom. There was one power failure which was after the initial sampling events.

This system operated as a one pass design, which means there was no recirculation. There were 48 doses per day, and a chart was presented outlining several other features. The average residence time was 9 hours in Stage 1 and 12 hours in Stage 2. This experiment was run for 60 days. The first 21 days were start-up to allow the system to stabilize. The nitrogen loading (how much nitrogen per area of filter per time) was reasonably consistent for purposes of the study and was around 7 and 11 grams/square meters/day. The total nitrogen from the septic tank was an average of 77.4 mg/L, which was mostly ammonia. The total nitrogen coming out of the final stage was around 2 mg/L for the clinoptilolite and expanded clay; and the tire crumb went through a progression of decrease through the study ending around 15 mg/L but averaging around 44 mg/L. The effluent was dominated by organic nitrogen, which shows that nitrification has occurred. The tire crumb Stage 1 system did not do a good job at getting rid of the ammonia, so the total nitrogen in the final effluent after Stage 2 is much higher than the others. Ammonia in an anoxic filter is going to behave conservatively, and not be removed. David Carter pointed out that column 1A had an average total nitrogen much lower than column 1B, but the average total nitrogen coming out of 2A and 2B are about the same. This would seem to show that column 2B was more efficient than 2A. Damann Anderson stated that the clinoptilolite is a good ion-exchanger with ammonia, so the ammonia is being held there and is perhaps held long enough to nitrify and then denitrify in that first stage. Dr. Smith stated that he expects that the expanded clay would accept less loading than the clinoptilolite. David Carter asked whether Dr. Smith expects the tire crumb to stay around 15 mg/L and if so taking an average would not be a good representation of this media. The tire crumb could have had a longer stabilizing time than the others. Damann Anderson stated that this is why the experiment is going to run longer. Dr. Smith stated that these systems do have start-up phase and it appears the tire crumb did take longer to acclimate. Paul Booher made a point that the loading at 3 pounds per square foot per day could be a biological overloading and not a nutrient overloading. Damann Anderson did not agree completely and thinks the loading could be higher and still work because of the coarser material on top. Dr. Roeder stated that column 2C (receiving the effluent from the tire crumb column) did not seem to remove the nitrate even though it gets very little and Dr. Smith stated that this was an interesting result. Pam Tucker asked whether the tire crumbs would be more repellant than the other two media and Dr. Smith agreed that there is less residence time with the tire crumb.

The removal efficiency average after Stage 1 showed ammonia nitrogen being removed at 99.9% for the clinoptilolite and the expanded clay; and the tire crumb was 60.5%. The removal efficiency average for total nitrogen after

Stage 2 for the clinoptilolite and the expanded clay was around 97%; and the tire crumb was 33%. This could be considered start-up data.

The results of the field parameters were discussed next. The influent dissolved oxygen (DO) was low, as expected. The Stage 1 (aerobic) results were much higher showing that aeration occurred. Stage 2 was low. The pH was maintained at neutral. Alkalinity is very important for nitrification/denitrification and is an important parameter to measure. Alkalinity was high in the septic tank effluent and very low as it left the Stage 1 filter and went up in Stage 2 due to the oyster shells.

The theoretical longevity of the sulfur is an important question. This can be calculated and was presented on a graph showing the results for different levels of pre-denitrification. The range for some of the scenarios he presented was between 30 – 50 years. This needs to be tested.

Some of the conclusions from the laboratory experiment were:

- Proof of the two stage filter concept for passive nitrogen removal, comparable to wastewater treatment plants
- Single pass loading at 3 gallons per square feet per day
- Total nitrogen removal of 97%
- Total nitrogen reduced from 77 to 2 mg/L
- Longer term operation needed

Anthony Gaudio asked whether the high 77 mg/L influent had any impact on what the results are and Dr. Smith stated that even at such a high level the system brought it down to very low levels. He stated that in the future with more water conservation, that number can go up. In doing these types of studies you are looking to find out the resilience and get a design envelope for how much you can push the system. John Byrd asked at the end when there is a total cost, would this be marketable at this point in time, is there anyone out there that can do this. Dr. Smith stated that Damann Anderson could do it. Damann Anderson said that this is why we need studies, to develop this into a marketable product. Paul Davis stated that none of this is patented at this point. Dr. Smith stated that this is public domain information.

The next part of the project was to provide an economic analysis and develop some examples of how to configure this on site. Dr. Smith showed a diagram with a septic tank (primary treatment) with a pump going to the unsaturated Stage 1 and saturated Stage 2 filters and then to soil dispersal. His objective is to do a life cycle cost analysis and to provide an equitable comparison of alternatives. The life cycle costs look at installation costs, what needs to be done every year (maintenance), etc. All of the costs are split up by unit costs (making things comparable to 2008 dollars), uniform annual cost (take the life cycle cost and break it into a uniform cost over the years), and present worth. He acknowledged Mark Flint for his work on this task. This estimate includes costs for equipment, materials, installation, energy, maintenance, monitoring, media replacement, and residuals. The base case used for these calculations is a 300 gallon per day system. He used existing tanks and materials to come up with these estimates, but if this is to be mass produced specific designed modules could be created that optimize some of the design factors.

There were multiple scenarios considered varying the Stage 1 filter plan area (varying loading rate), the media, and the Stage 2 filter volume/media replacement intervals. A total of 12 different systems were analyzed by varying the different scenarios.

A table was shown with the operation and maintenance costs. David Carter asked what monitoring will be done annually for $100. Dr. Smith states that nitrogen, that is TKN and NOx, are what should be measured, perhaps CBOD too. Samples would be pulled from the final effluent from the Stage 2 filter. He stated that you could take septic tank influent samples too, but if you are designing a resilient system the end of the treatment train is what you need to know. You would probably monitor more things initially and then as the system stabilizes only monitor for specific parameters. David Carter asked whether there are any other systems in the state that have regular chemical monitoring. Dr. Roeder stated that it depends, but that in most cases there is no sampling. Dr. Roeder noticed that the operating permit is not on the list and that generally takes the place of sampling. David Carter stated that he wants to compare apples to apples and while it is a good thing to do, if we are not sampling anything now maybe that number should not be part of a direct comparison. Damann Anderson stated that the operating permit is missing so it is a wash. Ellen Vause stated that because there is a media that needs to be replaced, you have to do some sort of monitoring of the constituents coming out of the filter media to know when to replace it. Dr. Smith stated that there are three media replacement options: a large system with infrequent replacement, an intermediate, and a small system with frequent replacement (Culligan Man model). David Carter stated that at the end of all this, these numbers need to compare with other types of systems. He wants to find what things are in common with other systems and which are additional things special for this system. David Carter asked whether there were any other studies that Dr. Smith looked at that went over this type of cost estimate. Damann Anderson stated that this was done for the Keys Study. The uniform annual cost for all the alternatives range from around $2,100 to about $2,500 per year. The present worth, including the primary treatment and drainfield, ranges around $36,000 - $44,000. The nitrogen-removing (nitrification-denitrification) processes take approximately 80% of the total cost. O & M overall is about 35% of the total. The cost per gallon treated is around $0.21 and the cost per pound of nitrogen removed is between $22 to $29.

A breakdown of the cost distribution for one of the 12 scenarios was presented as an example, was the focus of much of the discussion, and is presented here:

	2008$	% of Total
Primary Treatment / Pumping		
Installation	**2,800**	**7.0**
O&M	**1,284**	**3.2**
Total	**4,084**	**10.2**
PNRS Stage 1		
Installation	**3,770**	**9.4**
O&M	**6,801**	**16.9**
Total	**10,571**	**26.3**
PNRS Stage 2		
Installation	**3,417**	**8.5**
O&M	**6,801**	**16.9**
Media replacement	**10,844**	**27.0**
Total	**21,062**	**52.4**
Total PNRS Component	**31,633**	**78.7**
Drainfield	**4,500**	**11.2**
Total Installation	**14,487**	**36.0**
Total O&M	**14,886**	**37.0**
Total System Life Cycle Cost	**40,217**	**100.0**

The total system life cycle cost consists of the total O & M (37%) + the total installation (36%) + the media replacement for Stage 2 (27%) = 100%.

There are many unknowns in this cost estimate. One of the questions would be the cost for the media replacement in Stage 2. John Higgins stated that he understood that the time dosing was important. Would this mean having to install a larger septic tank to handle the peak flows? The answer was no. Damann Anderson stated that this is similar to a mound system which is dosed. There are overrides within these systems to handle times when there are parties with lots of flow. John Higgins asked whether the system would be degraded if it is being overused and Damann Anderson said for the long term probably not, but for a day or two it may be. Dr. Smith said that instead of getting 99% removal, a couple of times a year you may get less than that, but over the year it may not be important.

Dr. Roeder stated that the costs presented here are much lower per pound of nitrogen removed than when he tried to do the analysis for Wekiva. Part of the reason for this may be because this system is more effective (2 mg/L from 77 mg/L) than what Dr. Roeder was looking at (10 mg/L from 40mg/L).

Paul Davis stated that the electrical costs for a system recently installed in his county was over $1,000/year which does not compare to the number presented here ($124). Dr. Smith stated that this system only has one pump running, and no aerators. There is also the option of powering this off the grid with solar panels if needed.

Anthony Gaudio asked how the installation costs were calculated. Dr. Smith stated that the primary treatment costs were estimates from Mark Flint who has designed and installed systems. The costs for Stage 1 include the media costs gathered from various vendors and shipping. If there are enough of these systems there needs to be a distribution center that receives the media and prepares it. Anthony Gaudio stated that he is excluding the costs for Stage 1 and 2 but the installation and drainfield costs are a knowable amount and the cost seems high. Ellen Vause stated that $6,500 for a standard septic tank and drainfield is a little high. Damann Anderson stated that it depends on what part of the state you are in.

David Carter asks for clarification on the media replacement and whether the entire media is used up at the end. Dr. Smith stated that the Stage 1 media would not need to be replaced but Stage 2 would. Most of the sulfur would still be there but it might have some sort of coating on it, or flow is not moving through as it should, performance is deteriorating and the media needs to be replaced. Dr. Smith clarified that the media replacement cost is a conservative estimate to replace all of the media. The questions are: how to design the system (either large with infrequent replacement, or small with frequent replacement) and how do you manage the media (possible reuse)? David Carter pointed out that the replacement frequency costs were not very different between the different scenarios and suggested to design a system that makes the most sense from a consumer standpoint. Clay Tappan asked whether Dr. Smith sees the media becoming dirty as the bacteria grow, die, and slough off. Dr. Smith stated that so far this has not been seen in the laboratory experiments and there is no evidence of hydraulic overload. Ellen Vause asked what the disposal method would be for the sulfur removed from the system and Dr. Smith stated that there are several options. One possibility could be to regenerate and reuse it in the process; another could be land application as a crop soil amendment; as a last resort it could be put in the landfill. David Carter stated that much of what has been considered lately is how much does it cost to buy and operate, and Dr. Smith stated that that would be around $14,500. If the media was not going to be replaced, the total installation and O & M would be around $29,200. David Carter asked whether the O & M cost would be common among other performance based treatment systems and Dr. Smith said that it would be. John Higgins asked whether the installation costs include the first order of media and Dr. Smith said that it does include the media. David Carter stated that the media replacement cost is an annualized value over the life of the system. Pam Tucker asked whether the costs were only for a 5-year period, and David Carter clarified that if you wanted to have a system for the next 30-years you would need to write a check for $40,217. David Carter asked whether the sampling results would dictate when the media should be replaced, and Dr. Smith said that the annual monitoring would be a way to trigger the need for media replacement. There is uncertainty as to how long the media would last. Dr. Smith stated that if you design a system that you know is going to last a certain number of years, then you would not need to test it and instead replace it at 70% of the lifetime. David Carter related this to pumping out a tank, some can go longer before a pump out and some will go less. Dr. Smith stated if you have a conservative estimate on the expected life of the system, perhaps a simple checkup prior to this date would be sufficient.

John Higgins asked that the tables and the report make it clearer, add a footnote, that there is a cost escalation factor of 3.75% per year.

Anthony Gaudio stated that there is current technology available that can get the effluent to a 70% reduction, as opposed to the 97% with this media, but there is a drip irrigation drainfield added on to the end of the system which would give an even greater reduction. He asked whether development costs are included in the cost estimates. He also brought up Dr. Roeder's Wekiva cost estimate for $140 per pound of nitrogen removed, and how he thought that number was too high. He stated that to think that a system could get 97% reduction based on the results of a small scale controlled study might not be realistic. Dr. Smith stated that this is a start, and he firmly believes that these systems adequately represent the performance factors that would occur in a larger system. This would need to be tested in a full-scale setting, attached to a single family home. Anthony Gaudio stated that it would be good to have a discussion with Mark Flint to sharpen up these numbers to make it more realistic. Damann Anderson stated that these answers are unknown until this has been scaled up and studied, which would be the next phase. Dr. Smith stated that this is all experimental, and the next step is to see how this would operate under real life conditions.

David Carter asked where Dr. Smith expects this system to break down; where will it fail first? Dr. Smith stated that the mechanical items would be the first to break: the pump, electronic control system, and electrical supply. One of the advantages of passive systems is that the bugs grow on the surface and don't do much. If it is designed right so that it does not clog up, and you maintain the water moving through, it will work fine as long as the mechanical parts are working. Dr. Smith is fairly confident that Stage 1 will work fine; Stage 2 is more unknown and would need to be tested.

Paul Booher compared the system with a sewer system. He said his sewer bill runs around $35 per month and asked whether it would be a fair statement to say that this system would be about twice as high a sewer system. David Carter stated that the $35 does not include the impact fees and installation costs so it may not be comparable. Paul Booher stated that the life cycle cost of a conventional system would be around $8,600 and the nutrient reducing system would be $40,217. Anthony Gaudio stated that the $8,600 might not be accurate as the drainfield on the nutrient reducing system would last much longer than one on a conventional system due to the change in the quality of the influent, and it may be more around $20,000 for a conventional system. Dr. Smith stated that an estimate of the costs for sewer as compared to upgrading the existing systems was done for Marco Island and the costs were comparable. Dr. Smith stated that it is important to compare life-cycle costs and that for centralized sewer that would include construction, O&M, and energy costs, and also include any federal grant funding received.

Jim Rashley asked what code changes would need to be made: do we need a 2-year permit, or can we go to a 5-year permit, do we need to do a dig-out, what would the water separation requirement need to be, etc. He is very interested in this study and where it could go. Dr. Smith thinks the loading could be higher too and maybe there does not need to be as much drainfield.

Anthony Gaudio stated that the cost analysis part of this study would be great to look at again after the larger scale study, and that it is important to understand that costs go down with demand.

Dr. Smith showed a cost comparison between the passive system and a recirculating sand filter. Sand filters are not used much in Florida, but are common nationally. The numbers he presented for the passive nitrogen system are not too far off from currently available numbers for other similar systems.

He went over some of the design recommendations. He recommended having a tank with a built in pump chamber, having screened septic tank effluent, measure the daily flow, pressure dosing to Stage 1, and install sampling ports. Some of the installation recommendations are to develop a complete hydraulic profile, pay attention to the media preparation and placement, and perform watertightness testing. Some of the permitting recommendations are to perform some full scale evaluations which could lead to NSF certification. There is also the additives rule that would need to be considered to make sure what is leaving the system will not harm the environment. Dr. Roeder stated that one of the byproducts might be sulfuric acid. Some of the maintenance and monitoring recommendations are to check the counters and meters every 6-months, and once a year inspect all electrical/mechanical parts, flush, and test the flow distribution to the system. Monitoring would be done once a year for Stage 1 and Stage 2 effluent for TKN, NOx, CBOD5, and DO.

Dr. Smith went over some of the recommended next steps. One would be to continue the operation of the small scale columns to verify continued performance, which is currently being done. Another would be to do a full scale demonstration which would test various process configurations and media configurations. Some other next steps would be to look at the longevity of the denitrification process, refine the design and the cost analysis, and examine other water quality parameters such as phosphorus, pathogens, and emerging contaminants.

Paul Davis stated that the budget for the Phase II of the Passive Nitrogen Removal Study is currently $200,000 coming from the research funds, and the research is important and exciting. John Higgins applauded Dr. Smith for using a present value approach and he urged RRAC to set a standard to require all vendors to submit costs using this approach. David Carter stated that this is a good point. Most people look at what it costs to buy the system, not what it costs to maintain and operate. There seemed to be consensus that the work Dr. Smith has done was very impressive and he was thanked for doing a thorough job.

iii. **Optical Wastewater Tracers Study (old Remote Sensing of Optical Brighteners Study)** – Contract executed between DOH and DEP in May. Grant extension received from EPA to extend to end December. The purpose of this study is to test the feasibility of detecting wastewater inputs to Florida surface waters using optical characteristics such as optical brighteners from laundry detergents as tracers. Project budget: $66,680.

iv. **Manatee Springs, Performance of Onsite Systems Phase II Karst Study** – The immediate task is to complete the upgrades of the systems to include nitrogen reduction. A new research collecting permit has been received from DEP. The tanks were pumped and certified and site evaluations were performed. DOH is currently working on repair permit applications. The budget for upgrades and some sampling is $34,000. A new agreement will need to be prepared to perform the sampling once the systems have been installed. FSU expressed interest in being the provider with an anticipated budget of $24,000.

v. **Taylor County Source Tracking Study** – Draft report on Phase I submitted for comments from RRAC. Final report from Florida Atlantic University submitted and distributed to RRAC members. Grant ends 6/30/2008. The tasks that are remaining are developing a tri-fold brochure and write the final grant report. FAMU MPH intern currently working on designing tri-fold, creating a mailing list, and developing any policy change recommendations. Some of the main points in the tri-fold:

- Beaches were all about the same regardless of development
- Going upstream (creeks and canals) generally worse water quality than beaches
- Environmental factors like rainfall and temperature effect the numbers
- No big difference between OSTDS and sewer, use whatever is appropriate for the desired density and existing OSTDS conditions

vi. **Monroe County Performance Based Treatment System Performance Assessment** – The revised sampling protocol is to be reviewed by the RRAC for the remaining work.

b. Projects coming up

i. **2008 Research Priorities** - Top 5 ranked projects presented to TRAP. All were approved by TRAP at 1/24/2008 meeting.

1. **Restoration of the University of South Florida (USF) Lysimeter Station** - $20,000 - $50,000 approximate cost. If restored, several projects that RRAC wanted to pursue could potentially be conducted at the station. USF faculty has expressed interest in partnering on this, could be linked with Kiran C. Patel Center for Global Solutions which has ranked potable water and sanitation as a top priority in their research agenda. The restoration is dependant on updating the memorandum of understanding between USF and FDOH which is currently being routed internally.
2. **Phase II of the Florida Passive Nitrogen Removal Project** - $200,000 approximate cost. Build on the results of the Phase I study to go from a lab scale project to a prototype scale project. Could be accomplished under special appropriation 1682
3. **Wekiva Onsite Sewage Treatment and Disposal System (OSTDS) Seasonal Variability Assessment** - $200,000 approximate cost. Investigate if there is a seasonal variability of nitrogen concentrations from OSTDS in the Wekiva Study Area of Central Florida. Could be accomplished under special appropriation 1682.
4. **Alternative Drainfield Product Assessment** - $200,000 - $300,000 approximate cost. Compare the functioning of alternative drainfield materials to standard aggregate. This project may need to wait until the next budget cycle.
5. **Long-term deformation of tanks of different materials** - $20,000 approximate cost. Draft request for proposal submitted for RRAC review. Current draft proposal emphasizes less on other tank materials, but the intent is to include these. Proposing two phases:
    a. Phase I: literature review on plastic tanks with assessment protocol to include different tank materials (fiberglass, concrete)
    b. Phase II: field sampling numerous tanks of different materials based on the Phase I protocol

ii. **319 Project on Performance and Management of Advanced Onsite Systems** – Grant is still in review process with DEP. Task 1 is the Monroe County project discussed previously. Task 2 is the statewide database of advanced systems based on permit records. Draft Request for Proposal submitted to RRAC for review for a vendor to provide an electronic database of all advanced OSTDS in Florida with permit information. Cost $6,000.

iii. **Coastal Management Program Grant Funding Opportunity** – Grant to resample the Town of Suwannee to see what effects sewering has had on water quality. DEP has included the project with the state's application to NOAA in the amount of $68,000. DEP is currently working on the grant agreement. NOAA will decide around July 1, 2008, if they approve we will begin solicitation for a contract provider. Draft Invitation to Negotiate scope of work submitted for RRAC's consideration.

6. **Budget Discussion** – A draft budget for the 2008 – 2009 fiscal year has been suggested:

Operating Budget	$90,000
Phase II Manatee Springs	$34,000
Columbia County River Front Survey	$5,000
Lysimeter Station Restoration	$50,000
Passive Nitrogen Phase II	$100,000
Deformation of Tanks Study	$20,000
Wekiva Seasonal Variability Study	$100,000
Alternative Drainfield Product Assessment	$100,000
Other	$30,000
Total	**$529,000**

FY 07-08 Budget was $242,475

7. **Other Business** – There was no other business discussed.
8. **Public Comment -** The public was allowed to comment throughout the meeting.
9. **Closing Comments, Next Meeting, and Adjournment**
10. **Next Meeting**: No date was set for the next meeting. Next meeting anticipated to be some time in July 2008 at a location to be determined with a preference for the Gainesville area. Some of the items for the next meeting are to select a provider for Suwannee Study, and to respond to Special Appropriation 1682 if/when signed by the Governor. Motion by Clay Tappan, seconded by Anthony Gaudio to adjourn. The meeting adjourned at 3:55 pm.

List of Packet Contents

1. Agenda
2. Map of Meeting Location
3. RRAC Member List
4. 2008 RRAC Research Priorities
5. Draft Meeting Minutes January 23, 2008 RRAC Meeting
6. 2008 Legislative Session SB 1318 and SA 1682
7. Parts of FDEP's TMDL for Wekiva
8. Wekiva Nitrogen Reduction Options
9. Wekiva Paper for NOWRA's Nitrogen Symposium
10. Wekiva Revised Estimate Draft
11. Florida Passive Nitrogen Removal Study Final Task 2 Report
12. Florida Passive Nitrogen Removal Study Draft Task 3 Report
13. Florida Passive Nitrogen Removal Study Draft Task 4 Report
14. Florida Passive Nitrogen Removal Paper for NOWRA's Nitrogen Symposium
15. Attachment I of Optical Wastewater Tracer's Study
16. Draft Attachment I Phase II Manatee Springs Karst Study
17. Taylor County Coastal Coliform Study Draft Report on Phase I
18. Taylor County Coastal Coliform Study Parts of Final Report on Phase II (FAU)
19. Keys PBTS Study Draft Wastewater Sampling Protocol
20. Draft MOA between DOH and USF for Lysimeter Station
21. Draft RFP for Tank Deformation Project
22. Draft ITN for Town of Suwannee Effects of Sewering Study
23. Draft Solicitation Document for Statewide Inventory of Advanced Systems (part of 319 grant)

Florida Department of Health
Bureau of Onsite Sewage Programs
Research Review and Advisory Committee Meeting

DATE AND TIME: **May 29, 2008 at 9:30 am**

PLACE: **Orange County Health Department**
Administrative Services
6101 Lake Ellenor Dr.
Orlando, FL 32809
(407) 858-1400

This meeting is open to the public

AGENDA: FINAL 5/27/2008 Elke Ursin

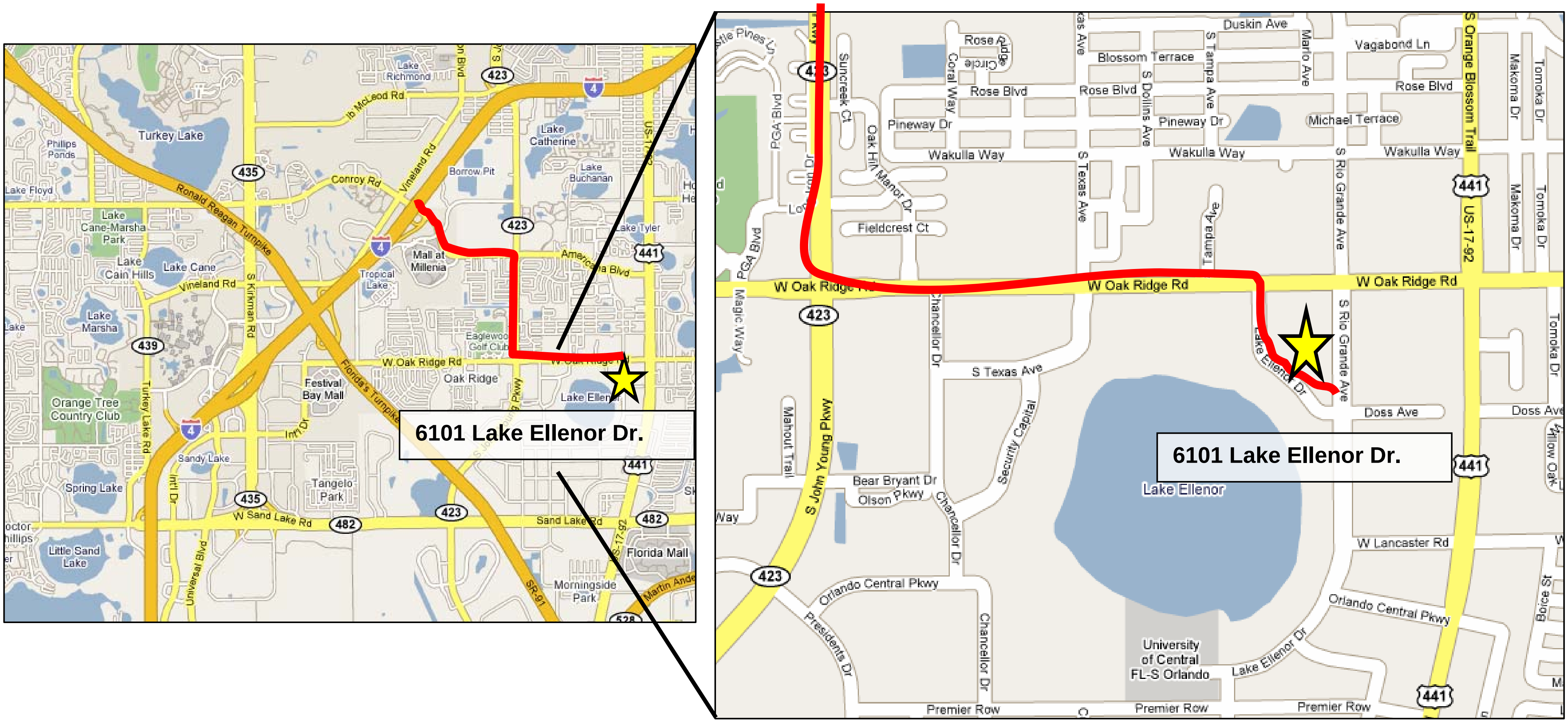
1. Introductions
    a. New RRAC Members / Alternates
2. Review Minutes of Meeting 1/23/2008
3. Discussion on Legislative Session
4. Brief discussion on TMDL for Wekiva
5. Brief updates on Ongoing Projects
    a. UCF Research Station
    b. Passive Nitrogen Removal Assessment
    c. Optical Wastewater Tracers Study (old Remote Sensing project)
    d. Manatee Springs, Performance of Onsite Systems Phase II Karst Study
    e. Taylor County Source Tracking Study
    f. Keys Performance Based Treatment System Performance Assessment
6. Brief updates on Future Projects
    a. RRAC Priorities from last meeting
    b. 319 Project on Performance and Management of Advanced Onsite Systems
    c. Town of Suwannee Study
7. Other Business
8. Public Comment
9. Closing Comments, Next Meeting, and Adjournment

Directions to Orange County Administrative Services Building
Research Review and Advisory Committee (RRAC) Meeting
May 29, 2008 9:30 a.m.

From I-4 E or W:

Take exit 78 for Conroy Rd	0.7 mi
Go East on Conroy Rd	1.1 mi
Turn Right/South at S John Young Pkwy/SR-423 S	1.0 mi
Turn Left/East at W Oak Ridge Rd	0.9 mi
Turn Right/South at Lake Ellenor Dr	0.3 mi
Building is NW corner of Lake Ellenor and S. Rio Grande	

Orange County Health Department
Administrative Services Building
6101 Lake Ellenor Dr.
Orlando FL, 32809
407-858-1400



Florida Department of Health
Bureau of Onsite Sewage Programs

Research Review & Advisory Committee Members

Updated on March 8, 2013

The list has the same order as 381.0065(4)(o) Florida Statutes
Format is: Represented group (nominating organization if applicable), term expiration date, member, member address, alternate, alternate address

Bureau of Environmental Health, Department of Health

(term expires 01/2015)

Member:	Alternate:
• **Paul Davis** Citrus County Health Department 3650 W. Sovereign Path, Suite 2 Lecanto, FL 34461-8071	• **Tom Higginbotham** Highlands County Health Dept. 7205 S. George Boulevard Sebring, FL 33875 • **Taylor Brown** Lee County Health Dept. 2295 Victoria Avenue, Room 206 Fort Meyers, FL 33901

Septic Tank Industry (Florida Onsite Wastewater Association)

(term expires 01/2015)

Member:	Alternate:
• **Wayne B. Crotty** 3806 181st Road Live Oak, FL 32060	• **Bob Himschoot** P.O Box 27 Fort Myers, FL 33902-0027

Home Building Industry (Florida Home Builder's Association)

(term expires 01/2016)

Member (Vice-Chair):	Alternate:
• **Carl Ludecke** 18650 Hwy 441 Mt. Dora, FL 32757	• **Ed Dion** 1105 Lothian Drive Tallahassee, FL 32312

Environmental Interest Group (Florida Chapter, Sierra Club)

(term expires 01/2015)

Member:	Alternate:
• **Craig Diamond** 405 Inglewood Drive Tallahassee, FL 32301	• **Vacant**

Consumer

(term expires 01/2014)

Member:	Alternate:
• **Bill Melton** 4619 Louvinia Dr. Tallahassee, FL 32311	• **Eanix Poole** 6310 Birchwood Rd. Marianna, FL 32448-5202

State University System:
(term expires 01/2014)

Member:	Alternate:
John Schert The Bill Hinkley Center for Solid and Hazardous Waste Management The University of Florida, College of Engineering PO Box 116016 Gainesville, FL 32611	**John Dryden** Construction Management Department University of North Florida 1 UNF Drive Jacksonville FL 32224-2645

Professional Engineer (Florida Engineering Society)
(term expires 01/2016)

Member (Chair):	Alternate:
Clay Tappan, P.E. Camp Dresser, McKee, Inc. 1715 N. West Shore Blvd. Suite 875 Tampa, FL 33607	**James H. Peters, P.E.** Senior Consultant Brown and Caldwell 850 Trafalgar Ct #300 Maitland, FL 32751

Real Estate Profession (Florida Association of Realtors)
(term expires 01/2016)

Member:	Alternate:
Quentin (Bob) Beitel 4 Quail Run Longwood, FL 32779	**Tony Macaluso** Portside Properties, Inc. 9492 Bloomfield Drive Palm Beach Gardens, FL 33410

Restaurant Industry (Florida Restaurant Association)
(term expired 01/2015)

Member:	Alternate:
Geoff Luebkemann Florida Restaurant and Lodging Association 230 S. Adams Tallahassee, FL 32301	**Susan McKinley** Florida Restaurant and Lodging Association 230 S. Adams Tallahassee, FL 32301

Local Government (Florida Association of Counties and Florida League of Cities)
(term expires 01/2014)

Member:	Alternate:
David Richardson Gainesville Regional Utilities 301 S.E. 4th Avenue Gainesville, Florida 32601	**Ms. Nancy Gallinaro** Palm Beach County Water Utilities 8100 Forest Hill Boulevard West Palm Beach, Florida 33413

Florida Department of Health (FDOH)
Research Review and Advisory Committee (RRAC)
2008 Research Priorities

“Research projects shall be initially approved by the technical review and advisory panel and shall be applicable to and reflect the soil conditions specific to Florida.” – Chapter 381.0065(3)(j) F.S.

1. **Restoration of the University of South Florida (USF) Lysimeter Station**
    a. $20,000 - $50,000 approximate cost
    b. Dependant on updating the memorandum of understanding between USF and FDOH
    c. If restored, several projects that RRAC wanted to pursue could potentially be conducted at the station

2. **Phase II of the Florida Passive Nitrogen Removal Project**
    a. $200,000 approximate cost
    b. Build on the results of the Phase I study to go from a lab scale project to a prototype scale project

3. **Wekiva Onsite Sewage Treatment and Disposal System (OSTDS) Seasonal Variability Assessment**
    a. $200,000 approximate cost
    b. Investigate if there is a seasonal variability of nitrogen concentrations from OSTDS in the Wekiva Study Area of Central Florida

4. **Alternative Drainfield Product Assessment**
    a. $200,000 - $300,000 approximate cost
    b. Compare the functioning of alternative drainfield materials to standard aggregate

5. **Long-term deformation of tanks of different materials**
    a. $20,000 approximate cost
    b. Project is in response to problems observed in the field

Research Review and Advisory Committee for the Bureau of Onsite Sewage Programs

Draft Minutes of the Meeting held at Sylvan Lake Park, Sanford, FL
January 23, 2008
Draft by Elke Ursin 5/12/2008

In attendance:

- **Committee Membership and Alternates**: Sam Averett (alternate, Septic Tank Industry); David C. Carter (Chairman, member, Home Building Industry); Paul Davis (member, DOH-Environmental Health); Anthony Gaudio (member, Septic Tank Industry); John Glenn (member, Environmental Interest Group); Marc Hawes (alternate, Home Building Industry); Bill Melton (member, Consumer); Eanix Poole (alternate, Consumer); Patti Sanzone (alternate, Environmental Interest Group); Pam Tucker (member, Real Estate Profession); and Ellen Vause (alternate, Septic Tank Industry)
- **Not represented**: Professional Engineers, Restaurant Industry, State University System
- **Visitors**: Damann Anderson (Hazen & Sawyer); Rick Baird (Orange County Environmental Protection Division); John Byrd (Aide to Orange County Commissioner Brummer); Ron Davenport (Infiltrator Systems); Doug Everson (Plastic Tubing Inc.); Christopher Finkbeiner (Aide to Representative Bryan Nelson); Robert Harper (Harper Realty and Development); Ken Jones (Markham Woods Association); Steve Meints (Averett Septic); Cory Mong (Economy Septic); Len Moore (Eco-Pure, Inc.); Daniel Smith (AET); Ron Suchecki (Hoot Systems)
- **Department of Health (DOH), Bureau of Onsite Sewage Programs**: Paul Booher; Dr. Eberhard Roeder; and Elke Ursin

1. **Introductions:** Six out of nine groups were present, representing a quorum. Chairman David Carter calls the meeting to order at 9:35 am. Several new members and alternates have been added to the committee as of the last meeting: Eanix Poole (alternate for Consumer, replaced John Heber), Geoff Luebkemann (member for Restaurant Industry, replaced Adam Parmer), Susan McKinley (alternate for Restaurant Industry, replaced Richard Turner), and Anthony Gaudio (member for Septic Industry, replaced Scott Womble). David Carter pointed out that the agenda items on the budget and the prioritization for research directions were put off from the last meeting, and is something that could take half a day.

2. **Election of Chair and Vice-Chair**: This is something that should have been done last year, but with the Wekiva project it was delayed until now. David Carter is the current chair. Patti Sanzone was the vice-chair previously and it has been several years since there was a vote on this. The duties of the chair are to run the meeting and to review the agenda prior to the meeting. The vice-chair will take over the meeting if the chair is out. Both the chair and vice-chair need to be committed to attending the meetings. David Carter stated that he has been chair for several years and has been thinking of stepping down. He stated that he could stay on for the next two meetings. The floor was opened for nominations. Bill Melton read the list of current members and alternates. There was a discussion on whether the chair has to be a member, or whether they can be an alternate. Elke Ursin stated that Roberts Rules did not prohibit anyone from being the chair, but that the voting privileges remain with the members. Anthony Gaudio made a motion which was seconded by Bill Melton:

   > **David Carter to remain chair for the next two meetings and then this issue will be revisited.**

   The members voted and all were in favor with none opposed, the motion passed.

DRAFT

3. **Discussion on Travel Reimbursement**: Elke Ursin briefly went over the travel reimbursement requirements for RRAC members/alternates.

4. **Review of Previous Meeting Minutes**: Motion by Bill Melton, seconded by Paul Davis:
   The minutes were approved as submitted.
   The members voted and all were in favor with none opposed, the motion passed.

5. **Wekiva Update:** There is no new information on rulemaking; options are still being discussed with the governor's office. Dr. Eberhard Roeder has revised the input and loading estimates and has written a draft report that is available on the FDOH website:

http://www.doh.state.fl.us/environment/ostds/research/01-23-08Materials/Revised_Nitrogen_Estimates.pdf

Any comments or questions can be sent directly to Eberhard_Roeder@doh.state.fl.us within the next two weeks from any interested party. The presentation does not address the load to surface water discharge as that was not part of the legislative mandate. The following describes what numbers were used for the inputs:

- OSTDS based on Wekiva Study
- Wastewater Treatment Plants based on available discharge records, prorated by capacity (MACTEC report)
- Fertilizer based on sales, attributed to land uses based on recommended application rates
- Livestock based on livestock density (MACTEC report)
- Atmospheric Deposition based on UCF records

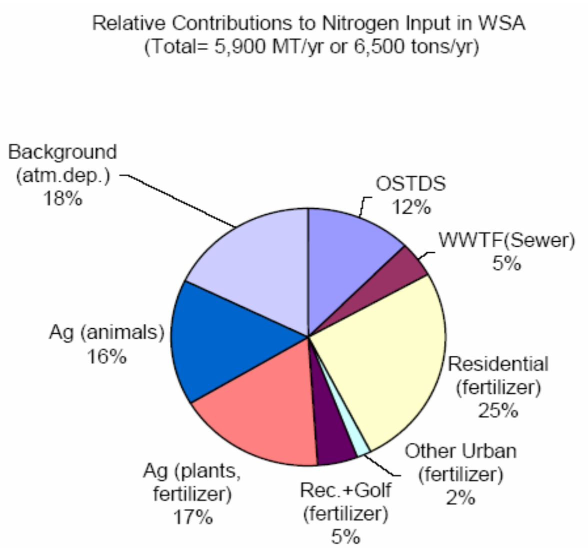
The nitrogen inputs in the Wekiva Study Area were shown to be increasing. The fertilizer sales for farm fertilizer were shown to be relatively steady over time, with non-farm fertilizer sales increasing.

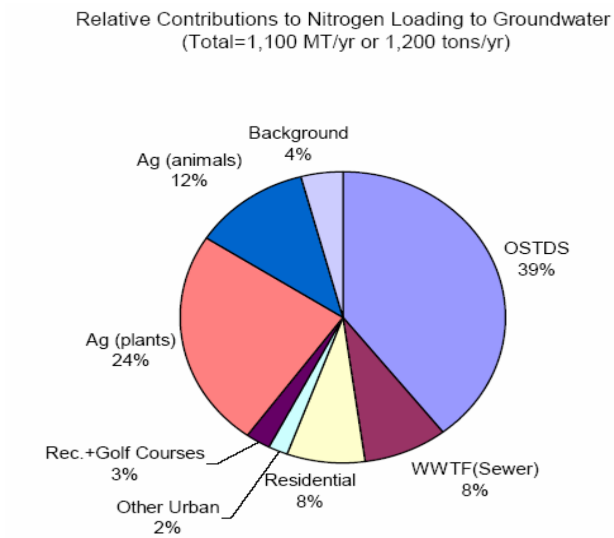
The revised loading estimates were also discussed. The following describes what numbers were used for the loadings:

- OSTDS based on Wekiva Study
- Centralized Wastewater reduction from inputs based on EPA guidance
- Land uses based on groundwater concentration times recharge
  - Residential and urban land use concentrations based on Wekiva Study
  - Agricultural tree crops concentration and recharge based on Best management Plan (BMP) study
  - Background based on TN=0.2 mg/L

DRAFT

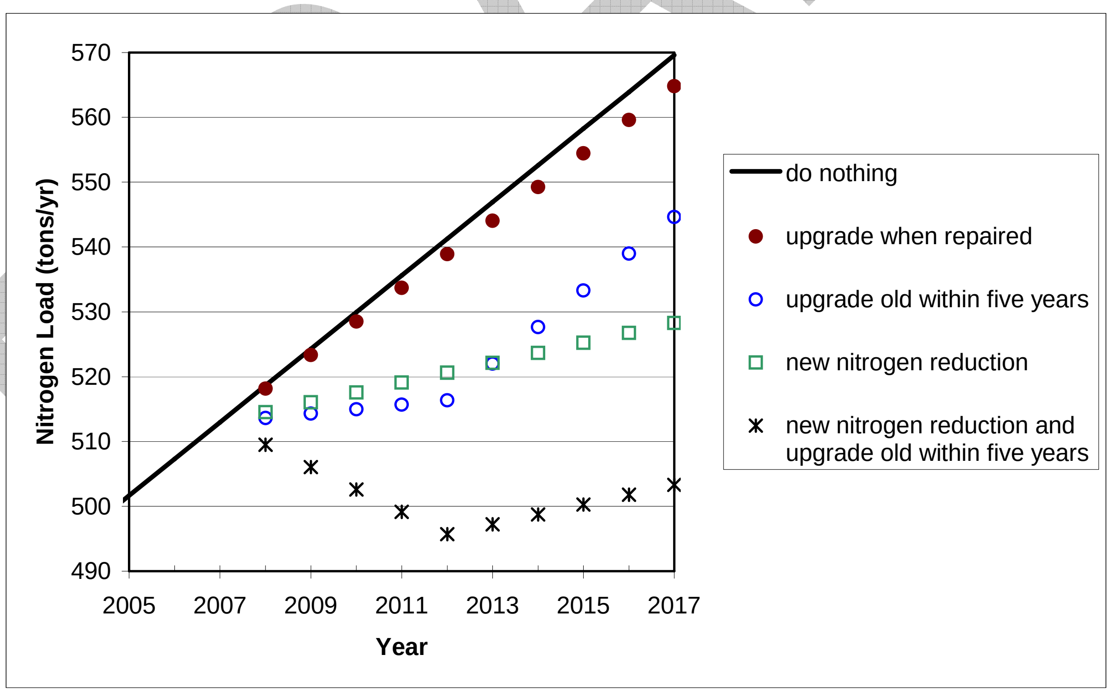
The draft revised input and loading pie charts were presented and discussed:





Input to the Environment **Loading to the Groundwater**

The projections of onsite nitrogen-load based on various management options were discussed:



There was a discussion on why this revised estimate for input and loading was being brought up. Dr. Roeder explained that at the June 2007 meeting, RRAC provided two options with regard to further revisions of input and loading estimates: to wait for FDEP to revise their numbers or staff to bring new information back to the RRAC as it became available. This is in response to reviewing and refining new information.

An update was presented on the FDEP Phase II study scope that was released in November 2007. There were questions why this study only focused on fertilizers and no additional work was to be done with onsite systems. Patti Sanzone stated that FDOH is dealing with the onsite systems and that FDEP does not have regulatory authority over onsite systems.

Pam Tucker made a motion which was seconded by John Glenn:

> **RRAC recommends a request to FDEP expand the design of the Phase II study to include groundwater testing of onsite systems in the wet and dry seasons to obtain accurate numbers for onsite systems in the Wekiva Study Area.**

There was a discussion on this motion. Sam Averett stated that RRAC should not tell FDEP to study onsite systems. Ellen Vause suggested withdrawing the motion, that FDEP's study will confirm or debate Dr. Roeder's revised calculations. She stated that significance can still not be determined for fertilizer inputs and loadings until FDEP completes their study and RRAC makes a decision. The RRAC members voted with four members against (Anthony Gaudio, Bill Melton, Paul Davis, and David Carter) and two members for (Pam Tucker and John Glenn). **The motion did not pass.**

Bill Melton made a motion which was seconded by Paul Davis:

> **To ask FDEP to get their information in a format that is as comparable as possible with the FDOH numbers and to provide Total Nitrogen numbers and to use the atmospheric deposition numbers used in the FDOH report.**

The members voted and all were in favor with none opposed, the motion passed.

New labeling and content regulations are required for lawn fertilizers. This is effective December 30, 2007. The Urban Turf rule reduces phosphorus content for maintenance, limits the how much nitrogen fertilizer can be applied to the lawn per application and also how much can be applied in total, and has expected reductions of 20-25% of TN and 50-70% of Phosphorus. The Consumer Fertilizer Task Force was described, and some costs and effects were discussed.

6. Brief updates on other projects

a. Ongoing projects

i. **Passive Nitrogen Removal Assessment** – The final literature review report and database has been received. The Quality Assurance Project Plan (QAPP) has been finalized. The media evaluation experiments are currently in progress. Dr. Smith, the contract provider, expressed that this study is very short. The contract is expected to complete before the end of this fiscal year (June 30, 2008).

ii. **Remote Sensing of Optical Brighteners Study** – Development of revised contract with FDEP is in progress. Several phone conferences were had between FDOH and FDEP to go over the content of the new contract.

iii. **Manatee Springs, Performance of Onsite Systems Phase II Karst Study** – Manuscript from Florida State University was accepted by Water Research. Due to contractual and timing issues, this contract has expired and must be re-contracted. Grant end date was extended by EPA.

iv. **Taylor County Source Tracking Study** – The draft final report was submitted for comment. Some of the conclusions from the study were discussed:
   - No significant differences in ammonia trends between sewer & OSTDS
   - Nitrate levels low for all sampling events
   - Caffeine and optical brighteners ineffective tracers due to dilution, low development density, etc.
   - Good correlation between Enterococcus and E. coli and the change from seasonal low water table (SLWT) and seasonal high water table (SHWT)
   - E. coli violations were nearly 4 times more frequent at sewered sites as compared to OSTDS sites, and the number of violations was higher in 2007 than in 2006 (thought that because sewer was only recently installed previous contamination may still be reflected) (any thoughts???)
   - High TN with high Enterococcus indicates greater contribution of nutrients from septic systems as opposed to runoff contributions
   - Nitrogen isotope analysis seems to implicate fertilizers at beach communities
   - Background sites had a low Enterococcus/E. coli ratio, and beach sites had high ratios showing human-derived sources of pollution
   - Sewered areas do not show improved water quality in comparison to areas that remain on OSTDS

   Elke Ursin requested comments be sent to her by January 27'th so that she can compile and send to the provider to develop the final report. Grant end date was extended by EPA. The final task is for FDOH to develop a tri-fold brochure on the results of the study, and to write a final report for EPA.

- **Monroe County Performance Based Treatment System Performance Assessment** – Dr. Eberhard Roeder stated that Monroe County appears to have remained below budget for the study so far and would like direction on what RRAC would like for them to study with the remainder of the allocated money. Ellen Vause stated that resampling the locations previously sampled would be a good option and there was general agreement on this.

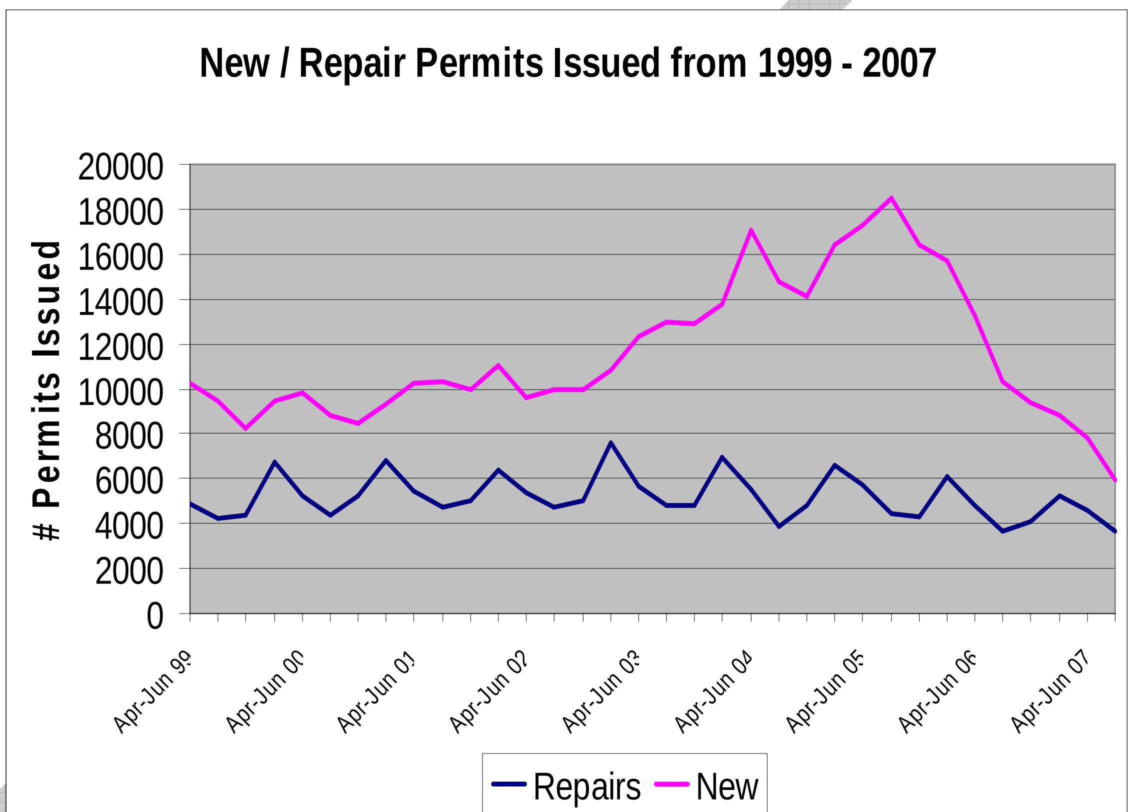
b. Projects coming up

i. **319 Project on Performance and Management of Advanced Onsite Systems** – A TRAP teleconference was held on November 20, 2007 to discuss approval of this project. TRAP recommended approval of the initial process of the 319 monitoring study to secure the funding with the condition that the project be brought before RRAC for discussion on the protocol and sampling details and then be presented back to TRAP. This agreement is going through the routing process with FDEP and EPA. The first task is to develop a database of all advanced systems in the state and Elke Ursin asked the RRAC to start thinking about what fields to incorporate. She will send a reminder out later with a list of the potential data fields.

ii. **Coastal Management Program Grant Funding Opportunity** – FDOH submitted the grant proposal on November 14, 2007 to resample the Town of Suwannee. A final decision should be made prior to the next RRAC meeting.

iii. **University of Central Florida Research Facility** – UCF has a grant with FDEP to look at nutrient reducing onsite systems and to develop a research facility with test beds. UCF provided some slides that were provided to the RRAC members for review. FDOH and UCF have developed a memorandum of understanding (MOU) to allow this facility to be built, and is subject to various conditions.

7. **Budget Discussion** – The total revenue from the $5 surcharge on new permits for 2006-2007 fiscal year (FY) was $181,747 and the expenditures were $342,895 leaving an ending cash balance of $882,955. A discussion was had on the limitations for spending the cash, and how it is subject to budget authority which is beyond the Bureau's control. A graph was presented showing the decline in permitting, which results in a reduced research budget. Damann Anderson stated that the research fee was set in 1985 and that it is time for this fee to be increased.



8. **Prioritization of Future Projects** – The RRAC had a discussion on priorities for future projects, and each member was given an opportunity to add additional projects to the prepared list. After all the projects were compiled, each member listed their top three research priorities. From that selection, five projects had more than 1 vote. Editorial note: Approximate costs below were staff estimates after the RRAC-meeting for purposes of TRAP-discussion.

   1. **Restoration of the University of South Florida (USF) Lysimeter Station**
      a) $20,000 - $50,000 approximate cost
      b) Dependant on updating the memorandum of understanding between USF and FDOH
      c) Several projects, that RRAC wanted to pursue, could potentially be conducted at the station if restored
   2. **Phase II of the Florida Passive Nitrogen Removal Project**
      a) $200,000 approximate cost

b) Build on the results of the Phase I study to go from a lab scale project to a prototype scale project

3. **Wekiva Onsite Sewage Treatment and Disposal System (OSTDS) Seasonal Variability Assessment**
   a) $200,000 approximate cost
   b) Investigate if there is a seasonal variability of nitrogen concentrations from OSTDS in the Wekiva Study Area of Central Florida
4. **Alternative Drainfield Product Assessment**
   a) $300,000 approximate cost
   b) TRAP approved this project 2-years ago
   c) Contract in place and then canceled due to industry concerns
   d) Compare the functioning of alternative drainfield materials to standard aggregate
5. **Long-term deformation of tanks of different materials**
   a) $20,000 approximate cost
   b) Project is in response to problems observed in the field

This list is to be presented to the TRAP for their approval at the TRAP meeting the following day (January 24, 2008).

9. **Public Comment -** The public was allowed to comment throughout the meeting.

10. **Closing Comments, Next Meeting, and Adjournment**

    a. No date was set for the next meeting. Next meeting anticipated to be some time in April or May 2008 at a location to be determined. The meeting adjourned at 3:04 pm.

DRAFT

An act relating to onsite sewage treatment and disposal systems; amending ss. 381.0065 and 381.0068, F.S.; providing that a member of local government who is knowledgeable about domestic wastewater treatment be added to the research review and advisory committee and the technical review and advisory panel established by the Department of Health for purposes of onsite sewage treatment and disposal system regulation; amending s. 318.0101, F.S.; exempting certain persons who are performing site evaluations relating to wastewater treatment and disposal systems from having to be certified as an environmental health professional by the Department of Health; providing that such persons must have completed a soils morphology course approved by the department and be working under the direct responsible charge of a licensed engineer; providing an effective date.

Be It Enacted by the Legislature of the State of Florida:

Section 1. Paragraph (o) of subsection (4) of section 381.0065, Florida Statutes, is amended to read:

381.0065 Onsite sewage treatment and disposal systems; regulation.--

(4) PERMITS; INSTALLATION; AND CONDITIONS.--A person may not construct, repair, modify, abandon, or operate an onsite sewage treatment and disposal system without first obtaining a permit approved by the department. The department may issue permits to carry out this section, but shall not make the

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issuance of such permits contingent upon prior approval by the Department of Environmental Protection, except that the issuance of a permit for work seaward of the coastal construction control line established under s. 161.053 shall be contingent upon receipt of any required coastal construction control line permit from the Department of Environmental Protection. A construction permit is valid for 18 months from the issuance date and may be extended by the department for one 90-day period under rules adopted by the department. A repair permit is valid for 90 days from the date of issuance. An operating permit must be obtained prior to the use of any aerobic treatment unit or if the establishment generates commercial waste. Buildings or establishments that use an aerobic treatment unit or generate commercial waste shall be inspected by the department at least annually to assure compliance with the terms of the operating permit. The operating permit for a commercial wastewater system is valid for 1 year from the date of issuance and must be renewed annually. The operating permit for an aerobic treatment unit is valid for 2 years from the date of issuance and must be renewed every 2 years. If all information pertaining to the siting, location, and installation conditions or repair of an onsite sewage treatment and disposal system remains the same, a construction or repair permit for the onsite sewage treatment and disposal system may be transferred to another person, if the transferee files, within 60 days after the transfer of ownership, an amended application providing all corrected information and proof of ownership of the property. There is no fee associated with the processing of this supplemental information. A person may not contract to construct, modify, alter, repair, service, abandon, or maintain any portion of an onsite sewage treatment and disposal system without being registered under part III of chapter 489. A property owner who personally performs construction, maintenance, or repairs to a system serving his or her own owner-occupied single-family residence is exempt from registration requirements for performing such construction, maintenance, or repairs on that residence, but is subject to all permitting requirements. A municipality or political subdivision of the state may not issue a building or plumbing permit for any building that requires the use of an onsite sewage treatment and disposal system unless the owner or builder has received a construction permit for such system from the department. A building or structure may not be occupied and a municipality, political subdivision, or any state or federal agency may not authorize occupancy until the department approves the final installation of the onsite sewage treatment and disposal system. A municipality or political subdivision of the state may not approve any change in occupancy or tenancy of a building that uses an onsite sewage treatment and disposal system until the department has reviewed the use of the system with the proposed change, approved the change, and amended the operating permit.

(o) The department shall appoint a research review and advisory committee, which shall meet at least semiannually. The committee shall advise the department on directions for new research, review and rank proposals for research contracts, and review draft research reports and make comments. The committee is comprised of:

1. A representative of the Division of Environmental Health of the Department of Health.

2. A representative from the septic tank industry.

3. A representative from the home building industry.

4. A representative from an environmental interest group.

5. A representative from the State University System, from a department knowledgeable about onsite sewage treatment and disposal systems.

6. A professional engineer registered in this state who has work experience in onsite sewage treatment and disposal systems.

7. A representative from local government who is knowledgeable about domestic wastewater treatment.

8.~~7.~~ A representative from the real estate profession.

9.~~8.~~ A representative from the restaurant industry.

10.~~9.~~ A consumer.

Members shall be appointed for a term of 3 years, with the appointments being staggered so that the terms of no more than four members expire in any one year. Members shall serve without remuneration, but are entitled to reimbursement for per diem and travel expenses as provided in s. 112.061.

Section 2. Subsection (2) of section 381.0068, Florida Statutes, is amended to read:

381.0068 Technical review and advisory panel.--

(2) The primary purpose of the panel is to assist the department in rulemaking and decisionmaking by drawing on the expertise of representatives from several groups that are affected by onsite sewage treatment and disposal systems. The panel may also review and comment on any legislation or any existing or proposed state policy or issue related to onsite sewage treatment and disposal systems. If requested by the panel,

the chair will advise any affected person or member of the Legislature of the panel's position on the legislation or any existing or proposed state policy or issue. The chair may also take such other action as is appropriate to allow the panel to function. At a minimum, the panel shall consist of a soil scientist; a professional engineer registered in this state who is recommended by the Florida Engineering Society and who has work experience in onsite sewage treatment and disposal systems; two representatives from the home-building industry recommended by the Florida Home Builders Association, including one who is a developer in this state who develops lots using onsite sewage treatment and disposal systems; a representative from the county health departments who has experience permitting and inspecting the installation of onsite sewage treatment and disposal systems in this state; a representative from the real estate industry who is recommended by the Florida Association of Realtors; a consumer representative with a science background; two representatives of the septic tank industry recommended by the Florida Onsite Wastewater Association, including one who is a manufacturer of onsite sewage treatment and disposal systems; <u>a representative from local government who is knowledgeable about domestic wastewater treatment and who is recommended by the Florida Association of Counties and the Florida League of Cities;</u> and a representative from the environmental health profession who is recommended by the Florida Environmental Health Association and who is not employed by a county health department. Members are to be appointed for a term of 2 years. The panel may also, as needed, be expanded to include ad hoc, nonvoting representatives who have topic-specific expertise. All rules proposed by the

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department which relate to onsite sewage treatment and disposal systems must be presented to the panel for review and comment prior to adoption. The panel's position on proposed rules shall be made a part of the rulemaking record that is maintained by the agency. The panel shall select a chair, who shall serve for a period of 1 year and who shall direct, coordinate, and execute the duties of the panel. The panel shall also solicit input from the department's variance review and advisory committee before submitting any comments to the department concerning proposed rules. The panel's comments must include any dissenting points of view concerning proposed rules. The panel shall hold meetings as it determines necessary to conduct its business, except that the chair, a quorum of the voting members of the panel, or the department may call meetings. The department shall keep minutes of all meetings of the panel. Panel members shall serve without remuneration, but, if requested, shall be reimbursed for per diem and travel expenses as provided in s. 112.061.

Section 3. Subsection (3) of section 381.0101, Florida Statutes, is amended to read:

381.0101 Environmental health professionals.--

(3) CERTIFICATION REQUIRED.--No person shall perform environmental health or sanitary evaluations in any primary program area of environmental health without being certified by the department as competent to perform such evaluations. ~~The requirements of~~ This section does not apply to: ~~shall not be mandatory for~~

(a) Persons performing inspections of public food service establishments licensed under chapter 509; or~~.~~

(b) Persons performing site evaluations in order to

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<u>determine proper placement and installation of onsite wastewater treatment and disposal systems who have successfully completed a department-approved soils morphology course and who are working under the direct responsible charge of an engineer licensed under chapter 471.</u>

Section 4. This act shall take effect July 1, 2008.

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1682 SPECIAL CATEGORIES CONTRACTED SERVICES FROM GENERAL REVENUE FUND . . . . 8,225 FROM ECOSYSTEM MANAGEMENT AND RESTORATION TRUST FUND . . . . 6,750 FROM FEDERAL GRANTS TRUST FUND . . . . . . 30 FROM LAND ACQUISITION TRUST FUND . . . . . 1,100 FROM PERMIT FEE TRUST FUND . . . . 5,370 FROM WATER PROTECTION AND SUSTAINABILITY PROGRAM TRUST FUND . . . . 1,000,000 From the funds in Specific Appropriation 1682 , $1 million from the Water Protection and Sustainability Program Trust Fund shall be transferred to the Department of Health to further develop cost-effective nitrogen reduction strategies. The Department of Health shall contract, by request for proposal, for Phase I of an anticipated 3-year project to develop passive strategies for nitrogen reduction that complement use of conventional onsite wastewater treatment systems. The project shall be controlled by the Department of Health's research review and advisory committee and shall include the following components: 1) comprehensive review of existing or ongoing studies on passive technologies; 2) field-testing of nitrogen reducing technologies at actual home sites for comparison of conventional, passive technologies and performance-based treatment systems to determine nitrogen reduction performance; 3) documentation of all capital, energy and life-cycle costs of various technologies for nitrogen reduction; 4) evaluation of nitrogen reduction provided by soils and the shallow groundwater below and down gradient of various systems; and 5) development of a simple model for predicting nitrogen fate and transport from onsite wastewater systems. A progress report shall be presented to the Executive Office of the Governor, the President of the Senate and the Speaker of the House of Representatives on February 1, 2009, including recommendations for funding additional phases of the study.

The Department of Health shall also submit a report to the Executive Office of the Governor, the President of the Senate and the Speaker of the House of Representatives by no later than October 1, 2008, which identifies the range of costs to implement a mandatory statewide 5-year septic tank inspection program to be phased in over 10 years pursuant to the Department of Health's procedure for voluntary inspection, including use of fees to offset costs.

From the research fees collected pursuant to section 381.0066, Florida Statutes, $150,000 shall be used by the Department of Health to provide a statewide inventory of onsite treatment and disposal systems.

Version 1.2
Comparison of Nitrogen Reduction Options in the Wekiva Study Area

Draft E. Roeder 12/14/2007

Summary:

The following is an estimate of the change in nitrogen load to groundwater from onsite wastewater treatment systems for different management strategies.

The strategies are from most effective to least long-term effective in regards to nitrogen loading to groundwater in the Wekiva Study Area:

1) require all new developments to utilize nitrogen reducing onsite systems, and upgrade all old systems to current new construction standards within five years by requiring a five-year inspection. After ten years this strategy is projected to reduce nitrogen load by 9 tons/year from 2007 levels.
2) require all new developments to utilize nitrogen reducing onsite systems. After ten years this strategy is projected to increase nitrogen load by 15 tons/year
3) upgrade all old systems to current new construction standards within five years by requiring a five-year inspection. After five years this management strategy loses effectiveness because all old systems will have been upgraded. The projected increase in the nitrogen load is 32 tons/yr.
4) upgrade all old systems to current new construction standards whenever they are evaluated under current requirements (repair or modification). The projected increase in nitrogen load after ten years is 52 tons/yr.
5) do nothing. This is projected to increase nitrogen loading after ten years by 56 tons/yr.

Assumptions:

<u>Nitrogen Inputs and Loads per System:</u>
Based on the department's study the input per system for a typical treatment system serving 2.6 people is 29 lbs/year. Data supporting this revision from the 20 lbs/year were discussed in the task 4 report.
Load per system: The field work included two systems in Tavares with deep water table and passage through clay zones. For these the estimated removal was between a 25 – 50 %, this was higher than the 10% estimated in task 2, possibly due to clay. In the Myakka site the estimated removal was a 33 %, this was lower than the 50% TKN or >90% (NO3) estimated in task 2. The average removal observed at the three sites was 40%, leading to a load estimate of 17 lbs N/year.
As noted input and load are variable by specific site conditions.

Lbs of nitrogen/ year	Existing System in Groundwater	Standard System compliant with 24 inch water table separation	70% nitrogen reduction in pretreatment	Sewer (90%)
Input	29	29	8.7	2.9
Load	29	17	5.2	1.7

Management Options:

0: Current Rule Remains in Place. No reduction in nitrogen loading by existing systems, full addition input and load from new systems

1: Upgrade of old systems to current new system standards in regard to water table separation, surface water setbacks, and tank standards: a) whenever a system needs a repair, modification or reapproval b) within five years (five year inspection program)

2: Require nitrogen reduction technology: a) new systems only b) new and systems needing repair, modification or reapproval c) all systems within five years

3: Sewer: providing service and enforce connection requirement to 80% of their service areas (90% of OSTDS) within ten years.

Number of system per years impacted under the management options:

New systems per year: On average 700 new standard systems were installed annually between 2000-2005. These systems add additional input and load.

Systems in the water table: Approximately 50% of existing systems (28,000) were built before the 24 inch water table separation requirement was in effect (ecfrpc-census data). Current repair standards allow these old systems to have a minimum separation of 6 inches from the water table. Current repair standards allow a minimum 12 inch separation. Under these conditions no reduction would be expected between input and load. Site investigations during the Wekiva field study suggested that even some systems installed after 1982 may not meet code standards, perhaps due to changes in the water table subsequent to development. Based on current permitting data as well as data from the department study, it is estimated that 7.5% of existing systems or 4200 systems require additional separation from the water table.

Repairs: 901 repairs were annually permitted on average 2000-2005. An additional 160 systems per year were evaluated for modification or existing system evaluations It is assumed that approximately 7.5% of repairs or 831 systems will require an upgrade to lift out of the water table. During an inspection program, all systems would be looked at over a period of five years (11,000 systems per year). Those system not in conformance with new code standards would have to be upgraded.

Sewering: Most (90%) of the onsite systems within the WSA are in sewer service area. One option therefore, is to extend sewer lines and hookup onsite systems over, e.g. a ten-year period. This would assume about 4000 hookups of existing systems per year.

Cost estimates:

A new standard system is assumed to cost $4,400, and a new nitrogen reducing system is assumed to cost $12,400. Wakulla County's experience suggests that prices may drop noticeably for such systems once a larger volume can be distributed into an area.

A repair that does not elevate the drainfield to 24" seasonal high water table separation is assumed to cost $5,500 (drainfield repair of mound to repair code), while a repair to reach 24" separation is assumed to cost $6,300 (total system repair of mound system). Repairs of gravity systems without water table separation issues are estimated to cost $3,750.

For upgrades to nitrogen reducing systems in repair situations the estimated cost is $11,800 a weighted average (92.5%/7.5%) of the price survey results for subsurface systems ($11,700) and systems too close to the water table ($13,600). These costs includes drainfield replacement. For upgrades to nitrogen reducing systems, while reusing the existing tanks and drainfield, the price is assumed to be $7,900.

For sewering, a $10,000 cost per unit for connecting to sewer and abandoning the old sewer are assumed.

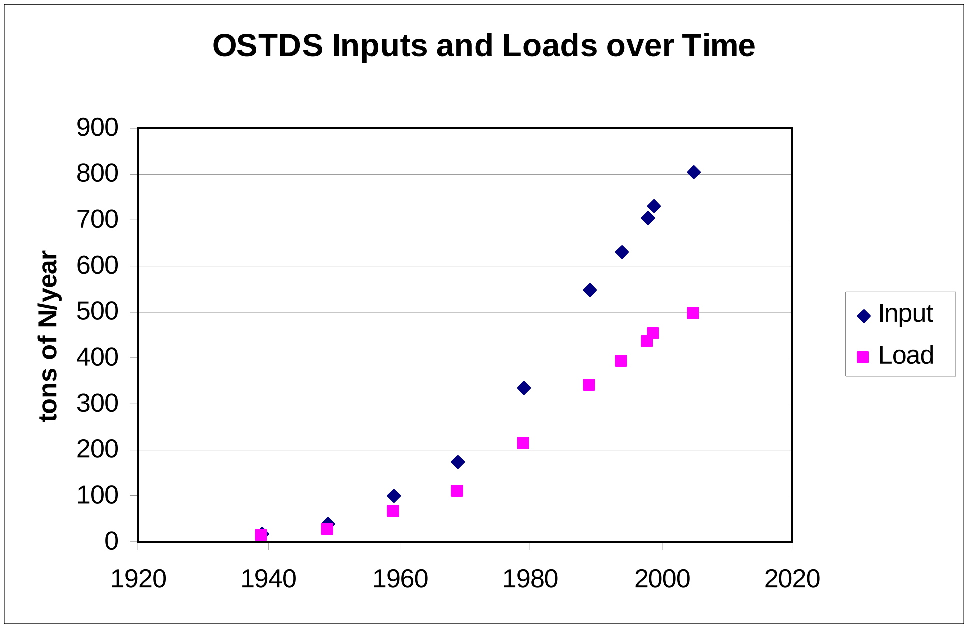
Installation costs are annualized assuming a 20 year useful life and a 6% interest rate.

Annual operating and maintenance costs are assumed to be $1000. For sewer operating costs $500/year or a $40 monthly utility bill are assumed.

The uniform annual costs are calculated by adding the annualized installation costs to the annual operating and maintenance costs. The resulting uniform annual costs for a mound system are $640/year.

Results:

The annuallized cost effectiveness for nitrogen removal is about $5/lbs for increasing the water table separation. For performance based treatment systems it ranges from about $100 to $150/lb N removed. Under the given assumptions ($10,000 connection cost), sewer is more cost effective than site-specific nitrogen reducing treatment systems. At $16,000 connection cost the cost effectiveness reaches $100/lb N, and at $25,000; $150/lb N. This allows an assessment in which areas sewer is going to be more cost effective than individual onsite systems.


OSTDS Inputs and Loads over Time
tons of N/year
900
800
700
600
500
400
300
200
100
0
1920
1940
1960
1980
2000
2020
Input
Load

Inputs and Loads in tons of N/year	Option 0	Option 1 Repair to new standards		Option 2 70% Nitrogen reduction			Option 3 Sewer extension and hookup
	No Change	a) When Repair becomes necessary	b) Within five years (inspection requirement)	a) new systems only	b) new and repair	c) all systems within five years	Extend lines and enforce hookup for 80% of service areas within ten years
# of new systems/year	700			700	700	700	
Input Addition from new Systems (tons N/yr)	10.15			-6.41	-6.41	-6.41	
Load Addition from new Systems (tons N/yr)	5.95			-4.13	-4.13	-4.13	
# of repairs/upgraeds/year	1060	80	831		901	11083	3990
Input Reduction from Repairs (tons N/yr)	0	0	0		-8.24	-101.41	-48.08
Load Reduction from Repairs (tons N/yr)	0	-0.41	-4.99		-5.32	-65.39	-30.52
For each Option							
Marginal Input Change after a year (tons N/yr)	10.2	0.0	0.0	-6.4	-14.6	-107.8	-240.4
Marginal Load Change after a year (tons N/yr)	6.0	-0.4	-5.0	-4.1	-9.4	-69.5	-152.6
In Combination with Option 0							
Net change in input/year after a year (tons N/yr)	**10.2**	**10.2**	**10.2**	**3.7**	**-4.5**	**-97.7**	**-38.9**
Net change in load/year after a year (tons N/yr)	**6.0**	**5.5**	**1.0**	**1.8**	**-3.5**	**-63.6**	**-25.1**

Marginal Costs of Options ($)and Cost Effectiveness per system	Option 1 Repair to new standards		Option 2 70% Nitrogen reduction			Option 3 Sewer extension and hookup
	a) When Repair becomes necessary	b) Within five years (inspection requirement)	a) new systems only	b) new and repair	c) all systems within five years	Extend lines and enforce hookup for 80% of service areas within ten years
New System Installation						
N-load difference (lbs N/year)			11.8	11.8	11.8	15.3
Cost difference new ($)			$8,000	$8,000	$8,000	$5,600
Annualized installation cost difference ($/yr)			$697	$697	$697	$488
Operation Cost Difference Per New System ($/yr)			$996	$996	$996	$496
Uniform annual cost ($/yr)			$1,693	$1,693	$1,693	$984
($/lb)			$143	$143	$143	$64
Existing System Upgrades/Repairs						
N-load difference (lbs N/year)	12.0	12.0		11.8	11.8	15.3
cost difference upgrade/repair ($)	$800	$800		$7,919	$4,419	$6,119
Annualized installation cost difference ($/yr)	$70	$70		$690	$385	$533
Operation Cost Difference Per Upgrade/Repair ($/yr)	$0	$0		$993	$910	$493
Uniform annual cost ($/yr)	$70	$70		$1,684	$1,295	$1,027
($/lb)	**$5.8**	**$5.8**		**$143**	**$110**	**$67**

AN ASSESSMENT OF NITROGEN CONTRIBUTION FROM ONSITE WASTEWATER TREATMENT SYSTEMS (OWTS) IN THE WEKIVA STUDY AREA OF CENTRAL FLORIDA

Elke L. Ursin[1] and Eberhard Roeder[2]

ABSTRACT

Nitrogen from wastewater can be one contributor to water quality problems. The onsite sewage, or septic tank discharge, part of this source has been hard to assess due to institutional, technological, and economic constraints. The water for the Wekiva River in Central Florida comes from rainfall runoff as well as several aquifer fed springs, for which nitrogen pollution of ground water is important. This paper will discuss the Florida Department of Health's Wekiva Study that was completed in the first half of 2007 in response to a legislative mandate to address nitrogen contamination of ground water by onsite wastewater treatment systems (OWTS). The study included tasks ranging from field sampling around drainfields to locate and measure the nitrogen plume, estimating how much nitrogen is released into the environment and how much reaches the ground water, to assessments of nitrogen releases from all sources, and management options. The field work results showed that effluent plumes can be identified in the ground water and that approximately one-half to one-third of the nitrogen released from the septic tank reaches the ground water. While soil types were a good indicator of how nitrogen behaves once it leaves the OWTS, variability and limited extent of nitrogen removal indicated that soils cannot be relied on solely to nitrify/denitrify the effluent. To determine the relative significance of OWTS nitrogen contributions, the amount of nitrogen from all sources was evaluated. Approximately 6% to 12% of the nitrogen from all sources came from onsite systems. To reach springs protection levels all sources of nitrogen must be addressed. To reduce the amount of nitrogen in the study area from OWTS, the Department proposed that existing systems should be upgraded to current setback requirements to seasonal high water table and surface water and that new systems should meet a 70% reduction in nitrogen pretreatment performance standard. A strong maintenance and management program is key to ensuring performance results are sustained.

INTRODUCTION

Protection of public health and the environment is a priority for the Florida Department of Health (FDOH). Onsite Wastewater Treatment Systems (OWTS) are a permanent solution to

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wastewater treatment in many locations throughout the State of Florida. Approximately one-third of the population of Florida utilizes an OWTS for wastewater treatment, creating one of the largest artificial ground water recharge sources in the state. Ninety percent of the water used for drinking comes from the ground water (Florida Department of Environmental Protection, 2006). It is necessary to take care of this resource to protect public health and the environment.

Excessive nitrogen can have negative effects on public health and the environment. There are various sources of nitrogen pollution: fertilizer from both agricultural and residential land uses; atmospheric deposition; agricultural sources such as livestock, feedlots, and manure; wastewater treatment plants; drainage wells; OWTS; and other sources such as sinking streams. The combined affect from all these sources add up to the total nitrogen load that travels to ground and surface waters.

A focus of recent research on OWTS in the State of Florida has been on the contribution of nitrogen to ground water and surface water bodies. Research has shown that properly installed and maintained OWTS effectively reduce the concentration of pathogens found in normal wastewater, but that nitrogen is reduced to a lesser extent (Ayres Associates, 1993; Sherblom 1998). A recently completed FDOH study looked at how quickly and far effluent and nitrogen from conventional OWTS moves in a karst environment (Florida Department of Health, 2004; Roeder et al, 2005). Karst features are found throughout the state of Florida and are characterized by conduits in the underlying limestone. The study found that effluent tracers moved very quickly and nitrogen concentrations remained high in wells in the effluent plume, illustrating the conduits between the ground surface and the surficial aquifer in karst environments that make them more sensitive to nitrogen pollution.

As the population of Florida increases, development in the state increases. This increase in development has led to an increase in the number of new OWTS and a corresponding increase in the amount of nitrogen contributed by OWTS to the ground water. The largely spring-fed Wekiva River in Central Florida (Figure 1) is an area that is currently evaluating nitrogen impacts from all sources of nitrogen, including OWTS. The Wekiva River Basin ecosystem has been recognized as a valuable natural resource with nineteen springs fed by the Floridan Aquifer. This is a karst environment where the ground surface and the underlying aquifer are interconnected and sensitive to nitrogen pollution (Cichon et al. 2005).

In response to proposed increases in development, the Wekiva River Basin Coordinating Committee (WRBCC) was established in 2003 by then-Governor Jeb Bush. This committee, which included various stakeholders in the area, was tasked to develop recommendations on how to protect the Wekiva River through sound development decisions.

Figure 1. Wekiwa Springs State Park, Source Water for the Wekiva River

The local water management district, St. Johns River Water Management District (SJRWMD) found that the nitrogen levels in the Wekiva River were elevated levels of nitrogen as compared to spring-fed streams without development and to be ecologically imbalanced (Mattson et al., 2006).

By setting total maximum daily loads (TMDL's) EPA has set goals of up to 95% reduction in nitrogen output for springs contributing to the Wekiva River (US EPA 2005). These reductions would allow the nitrogen levels in the river to return to ecologically healthy levels. The SJRWMD report set a pollution load reduction goal to reach up to an 85-percent reduction in the nitrate levels for the springs that contribute the most flow to the river (Mattson et al., 2006).

The WRBCC delineated the Wekiva Study Area (WSA) in August of 2003 (Figure 2) as a planning and legislative approximation of the land area that contributes surface and ground water to the Wekiva River system. The Wekiva Study Area consists of 475 square miles and includes parts of Orange, Seminole, and Lake Counties located in Central Florida. According to information developed by counties and the Florida Department of Environmental Protection, the study area contains over 55,000 OWTS.

The 2006 legislature tasked FDOH and its onsite sewage research review and advisory committee (RRAC) with assessing whether OWTS in the WSA are a significant contributor of nitrogen to ground water as compared to other sources, and if so to recommend cost-effective strategies to reduce this impact. The project was split into four tasks: field work, literature review of expected nitrogen reduction in the drainfield, estimation of nitrogen inputs from all sources in the Wekiva Study Area, and recommendations of a range of cost-effective solutions to reduce the nitrogen impact. The first three tasks were accomplished in cooperation with outside contractors.

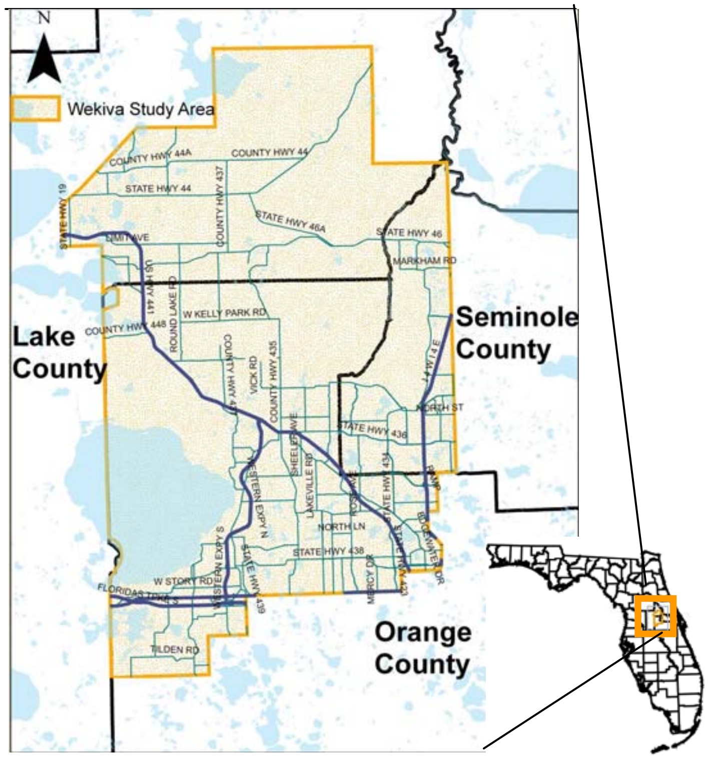

Figure 2. Wekiva Study Area of Central Florida

METHODS

Task 1: Field work

This task was to determine what amount of nitrogen one OWTS contributes to the ground water through performing detailed field sampling. Three systems in the WSA were sampled to determine how much nitrogen comes out of the septic tank, and how much makes it to the ground water. Samples were analyzed in the septic tank and under the drainfield at the top of the ground water table. The effluent plume was also identified as it moved away from the source.

Each of the selected sites met several requirements that included: having one site in each of the three effected counties, a water table that was reachable with the equipment used to sample the ground water, a variety of different water table conditions, minimum and maximum age criteria for the system, the lot size had to be large enough to contain the nitrogen plume, the household fertilizer usage had to be minimal, no availability of reclaimed water for irrigation, and the property was a single-family residence. A Florida licensed master septic tank contractor and county health department staff performed site evaluations for each of the sites to determine the size and location of the existing system. A diagram outlining the field work approach can be seen in Figure 3.

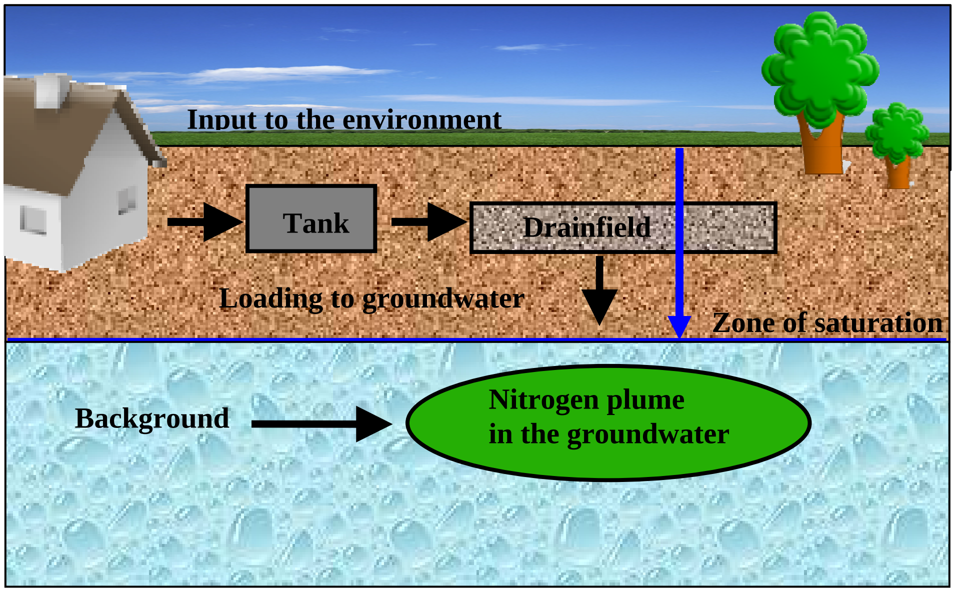

Figure 3. Wekiva Study Field Work Approach

In determining how much nitrogen is released into the drainfield from the septic tank, effluent samples were taken within the solids deflection device at the outlet of the tank. These effluent grab samples were taken at three separate times during the field sampling period and were evaluated for total Kjeldahl nitrogen (TKN), total nitrogen (TN), $CBOD_5$, total suspended solids (TSS), total phosphorus (TP), and several other field parameters. The effluent strength was multiplied with measured flow rates for each house to determine the amount of nitrogen that was discharged to the drainfield.

The ground water flow direction and ground water elevation were estimated utilizing piezometers. Samples of the ground water were taken by direct push technology in strategic locations to best capture the horizontal and vertical extent of the effluent plume in the shallow aquifer and record background concentrations. The field parameters measured were electrical conductivity, dissolved oxygen (DO), pH, temperature, turbidity, oxidation reduction potential (ORP), chloride, total iron, and ferrous iron. The ground water samples were analyzed by a laboratory for total Kjeldahl nitrogen (TKN), nitrate-nitrogen (NO_3-N), and total nitrogen (TN). A subset of the samples were analyzed for $CBOD_5$, total suspended solids (TSS), total phosphorus (TP), fecal coliform bacteria, and for nitrogen isotopes to help determine human or other animal versus inorganic sources such as fertilizers. The soil grain size distribution and soil organic content (SOC) was also measured at each of the three sites.

Task 2: Literature review of expected nitrogen reduction in the drainfield

This task was to determine what performance boundaries and categories should be considered to determine what amount of nitrogen one system could contribute to the environment. The denitrification process was broken down to the basic requirements to determine what categories would be important to complete the conversion. Two performance boundaries were considered: what leaves the last treatment component prior to the drainfield and what enters the ground water after treatment in the drainfield and the soil.

Task 3: Estimation of nitrogen input and load in the Wekiva Study Area

This third task was to determine an estimate of the nitrogen input and load in the Wekiva Study Area from OWTS. An input is defined as the amount of nitrogen that is released into the environment. An example would be the amount of nitrogen from a bag of fertilizer applied to the ground surface. A load is the amount of nitrogen that reaches the ground water. An example would be the remaining nitrogen from a bag of fertilizer that reaches the ground water after the plants and the soil have utilized (denitrified) portions of the nitrogen that was originally considered an input. In order to determine the relative significance of the input and load contributions from OWTS in the WSA, the contributions from other nitrogen sources were required. Slightly before FDOH's effort, a contractor for FDEP also performed a literature review study on the nitrogen inputs to the soil from various sources, and this information was used. That study (Tucker and Bidlin, 2007) looked at various sources of nitrate in the Wekiva River Basin and Springshed. This area was slightly larger than the WSA which FDOH addressed. FDEP used total nitrogen (TN) data when nitrate data was not available or reported, and assumed it to be a surrogate for nitrate (Table 1).

Table 1. Nitrogen sources, nitrogen type, and method of calculation considered in FDEP and FDOH studies

Nitrogen Source	FDEP Study	FDOH Study
Industrial, Commercial, and Domestic wastewater from Centralized wastewater facility effluents	• Used nitrate • Reclaimed water used for irrigation, assumed to replace fertilizer use	• Used total nitrogen • Review of FDEP system permit records in WSA, including nitrogen in reuse water, using the actual discharge by the concentration
OWTS effluents	• Used total nitrogen • Obtained estimates from previous FDOH studies	• Used total nitrogen • Used estimates based on the results of this FDOH study
Fertilizer: agricultural (row crop, citrus, nurseries, pasture), residential, golf course, and 'other' (ball fields, roadside, etc.)	• Used total nitrogen • Recommended application rates on pervious land area	• Used total nitrogen • Recommended application rates on pervious land area
Livestock	• Used total nitrogen • Literature values for feedlots and pasture land	• Used total nitrogen • Literature values for feedlots and pasture land
Atmospheric deposition	• Used nitrate • Utilized US EPA's Clean Air Status and Trends Network rural wet nitrate deposition data for the Indian River Lagoon	• Used total nitrogen • Urban literature values for Orlando area for wet deposition, and 30% of total for dry deposition

A second estimate of nitrogen input contributions was obtained by modifying two aspects of the estimate. First, the actual sales of fertilizers in the area and their distribution between farm and non-farm uses were considered. These data are collected by the Florida Department of Agriculture and Consumer Services by county and showed that, given the area of the Wekiva Study Area and the three counties, only about half as much nitrogen was sold as first estimated based on recommended application rates. Secondly, the inputs of nitrogen by OWTS measured in the Wekiva Study Area during Task 1 were included, which were larger than initially assumed.

For FDOH's assessment, inputs and loads were scaled down from the Wekiva River Basin to the Wekiva Study Area utilizing much of the same methodology as FDEP. Total nitrogen values were used for all sources (Table 1). The results of Task 2 were used to obtain a more refined estimate for nitrogen input and loading from onsite systems.

There are over 55,000 onsite systems in the Wekiva Study Area (Figure 4). Both FDEP's study and this study assumed a contribution of 30 lbs/nitrogen leaving the septic tank annually for a typical system. Utilizing GIS, the number of septic systems located in each soil map unit was counted. The estimated nitrogen removal potential from Task 2 was applied to each point to determine a total nitrogen loading estimate for the Wekiva Study Area.

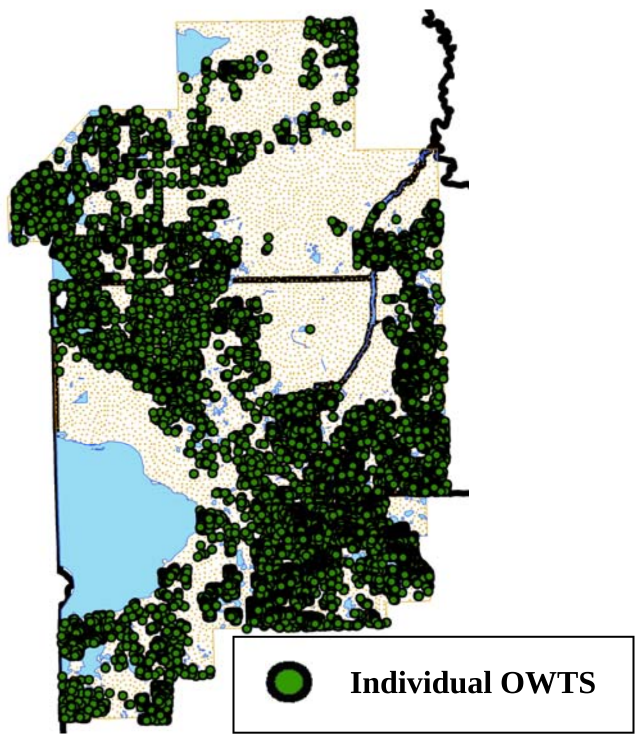

Figure 4. Distribution of Onsite Wastewater Treatment Systems in the Wekiva Study Area

Task 4: Cost-effective solutions to reduce the nitrogen impact

This task was to find cost-effective solutions to reduce any impacts from nitrogen in the WSA. Cost information on construction, operation, and maintenance was gathered from each county in the WSA. The framework built on EPA's voluntary onsite management guidelines (US EPA 2003). Some of the strategies that were researched were providing funding mechanisms for cost-effective projects, to keep the nitrogen loadings the same or lower, evaluating watershed impacts, establishing routine maintenance and inspection programs, and keeping an inventory of location and condition of all systems.

RESULTS

Task 1: Field work

The sample results helped to describe how ground water with an effluent influence behaves as it moves both horizontally and vertically. The wastewater effluent plume in two of the three sites was found not to move in the apparent direction of ground water flow. One possible reason for this could be the underlying discontinuous layers of clay in the soil.

The estimated nitrogen input per capita leaving the septic tank varied between 7.3 and 14.7 pounds per person per year. This can be compared to previous literature ranges from between 7.3 to 17.5 pounds per person per year (Crites et al., 1998) and 4.8 to 13.7 pounds per person per year (US EPA, 2002). The average household size of 2.6 in the Wekiva Study Area leads to an estimated input of a typical septic system of 29 lbs/year.

The nitrogen plumes were identified at all three sites, and electrical conductivity was found to be the best tracer to determine the extent and direction of movement of the effluent plume. All three sites found nitrogen concentrations exceeding 10 mg/L to depths of approximately ten to fifteen feet below the ground water table. Plumes traveled horizontally. Reduction in concentrations as a result of either denitrification or dilution was observed directly below the drainfield and diminished at further distances. Anderson (2006) summarized earlier literature and estimated that between 50 to 90 percent of nitrogen from onsite systems is loaded to the ground water. The field study found, based on the concentration reduction between septic tank effluent and shallowest ground water samples, that approximately one-half to three-quarters of the nitrogen input from the septic tank was loaded to the shallow ground water (Figure 5).

On a per capita basis, the mass loading of nitrogen to the ground water ranged from 3.95 to 9.65 pounds per person per year. This resulted in a mid-range of about 17 lbs nitrogen per year per system.

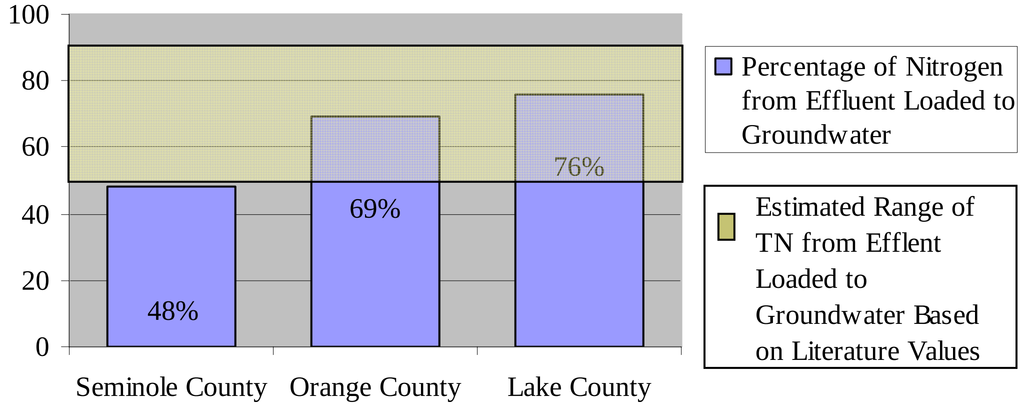

Figure 5. Percentage of Nitrogen from Effluent Loaded to the Ground Water in the Wekiva Study Area from each of the Field Sites

Task 2: Literature review of expected nitrogen reduction in the drainfield

The results of the literature review indicated that two of the main factors influencing nitrogen loading to ground water are the level of pre-treatment prior to release to the drainfield, and the soils underneath the drainfield.

If the amount of nitrogen is reduced in pre-treatment, then the corresponding nitrogen load to the ground water is also reduced. There are two ways to reduce the nitrogen in pre-treatment: by reducing the amount of nitrogen in the raw wastewater coming in, and by adding additional treatment processes prior to release in the drainfield. Nitrogen levels can be reduced up to 75-percent in pre-treatment with commonly available technologies.

Soil factors that appeared important for nitrification/denitrification were depth to the zone of saturation, organic content in the soil, and drainage class. In Florida, some of the requirements for a new drainfield include that it cannot be installed lower than 30-inches below the ground surface, a 24-inch separation is required from the bottom of the drainfield to the estimated wet season zone of saturation, and the organic horizon of the soil is required to be removed. The literature survey indicated that the depth to the zone of saturation under the drainfield can influence the amount of nitrogen that is loaded to the ground water. A traditional drainfield could have a nitrogen removal range between 0 – 35-percent. Having a mounded drainfield increases the nitrogen removal potential, and having a low-pressure dosed system or a sand filter increases this potential further.

The amount of organic content in the soil can aid in denitrification by feeding heterotrophic bacteria which convert nitrogen in nitrified wastewater to nitrogen gas. The drainage class describes how quickly water moves in the soil. For excessively drained well aerated soils, in which a majority of the OWTS in the WSA are installed, plenty of air is available to nitrify the effluent but these types of soil do not provide much organic matter to denitrify the nitrified effluent, and also have a very short retention time. For poorly drained soils that are oxygen deficient there is plenty of organic matter to denitrify the effluent, but if the effluent is not nitrified first it cannot be denitrified. Therefore, it is important to assure conversion of nitrogen to the nitrate form to allow denitrification, such as by maintaining at least a two foot separation from the bottom of the drainfield to the estimated seasonal high zone of saturation.

The literature survey showed that soils cannot be completely relied on to nitrify and denitrify. The best conditions for denitrification are that the zone of saturation is no deeper than 3.5-feet below grade and that there is a good chance of finding organic content in the soil. The estimated nitrogen removal potential in soils found in the Wekiva Study Area based on this classification ranged between 0 – 100-percent with an average of 33-percent.

Task 3: Estimation of nitrogen inputs in the Wekiva Study Area and determination of significance

An estimate was calculated for each individual nitrogen source, and is shown in Figure 6. The estimates were based on the calculation methods outlined in Table 1 for FDOH. In this scenario 71% of inputs are estimated to come from fertilizer sources, and 6% of inputs are estimated to come from onsite systems.

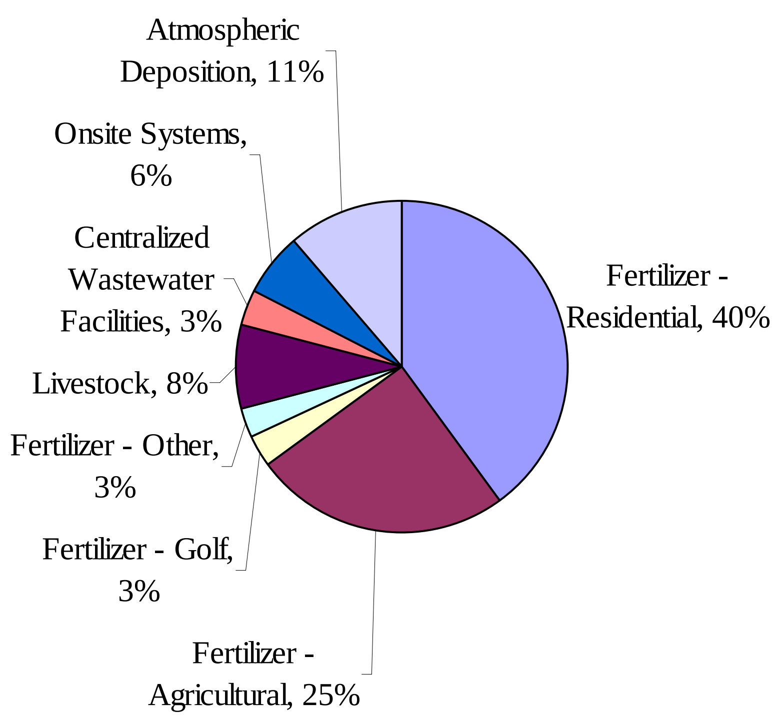

Figure 6. Nitrogen Inputs to the Wekiva Study Area by Source from FDOH Report Utilizing Recommended Application Rates for Fertilizers, Total is 18-Million Pounds per Year

The revised estimates, including fertilizer sales information (about half) and the results of the field study on inputs from onsite systems (29 vs. 20 lbs/year), is shown in Figure 7. 54% of nitrogen inputs are estimated from fertilizer and 12% from onsite systems.

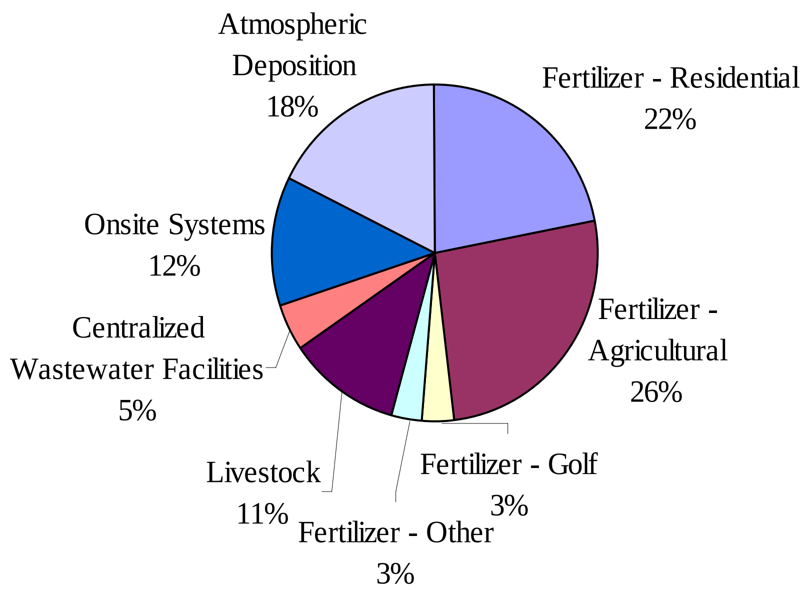

Figure 7. Revised Nitrogen Inputs to the Wekiva Study Area by Source. Changes are Due to Consideration of Fertilizer Sales and OWTS Field Measurements (29-Pounds per Year per System), Total is 13-Million Pounds per Year

The loading to ground water from OWTS was calculated based on the information gathered in Task 2 and overlaying it with soil GIS information specific for the WSA. A comparison between the calculated inputs and the medium soil-specific loading estimates indicated an 18% reduction in the nitrogen between the septic tank and the ground water table. The resulting average load for systems in the Wekiva Study Area (16 lbs/yr) is similar to the average load estimated from the observed OWTS nitrogen inputs and somewhat larger observed nitrogen losses (40% on average) during the field work (17 lbs/yr). Within the time frame of the study methodological issues about how to estimate ground water contributions by other sources were not resolved.

In order to determine whether OWTS are a significant contributor of nitrogen to the ground water the criteria used to determine *significance* need to be defined. Two approaches were considered: determining if the contribution is significant as compared to other sources, or determining if the contribution is significant to reach springs protection levels. The first approach was precluded because no consensus on the nitrogen contribution to loads from other sources was reached. Therefore, the approach was taken to look at springs protection levels. That nitrogen pollution of the ground water and surface water in the Wekiva Study Area occurs has been demonstrated by work leading to pollution load reduction goals by St. Johns River Water Management District (SJRWMD) and total maximum daily loads by the Florida Department of Environmental Protection (FDEP). There is no one entity that is the main contributor of nitrogen in the WSA. Instead, many people, though classified into broad categories, contribute small amounts of nitrogen individually that cumulatively result in the estimated inputs and loadings. Nitrogen impacts overall are significant so all contributing sources will need to do something to meet these goals.

Task 4: Cost-effective solutions to reduce the nitrogen impact

In order to determine the most cost-effective solution to reduce the impact of nitrogen, given that it is a cumulative impact, there has to be a mechanism to prioritize and categorize. One method of categorization would be to look at nitrogen that is entering the environment. This would result in fertilizers being the main contributor, next would come atmospheric deposition, and then wastewater. The focus could also be on the different land uses from which the nitrogen originates. The largest human influenced land use in the WSA is residential followed by agricultural, so addressing nitrogen from residential and agricultural land uses would be most important.

An important component to any program is developing a funding mechanism. Two funding mechanisms were proposed: establishment of a grant program to solicit cost-effective nitrogen reduction projects from any nitrogen source funded by all source contributors, and establishment of wastewater management entities that are funded by onsite system owners to reduce nitrogen load. The first mechanism will require a great deal of cooperation between all nitrogen source contributors and may lead to the most cost-effective way to reduce nitrogen impacts as a whole. The latter of the two can be an existing utility, new management entity, or a county health department that provides grants or loans to upgrade systems. This mechanism can provide the most cost-effective wastewater solutions to reducing nitrogen, or if used in conjunction with the first mechanism, can provide the best option to resolving the nitrogen issue in the WSA.

The costs to system owners will vary on which strategies are implemented. If everyone on an OWTS in the WSA contributes $60 per year per system, this could fund an area-wide grant program for nitrogen reduction, such as upgrades to wastewater treatment plants. If this cost is increased to $200 per year per system a program to upgrade failing systems to provide nitrogen reduction by pretreatment could be implemented. Alternatively, each system owner will be individually responsible for nitrogen reduction.

Another important component for a comprehensive program to reduce nitrogen impacts is to have an accurate inventory of all OWTS. This helps to identify sensitive or densely populated areas that may be better suited for connection to centralized sewer. A large number of OWTS in the WSA were installed prior to the current rule requirements, which in some soil types makes it very likely that they were not installed with a proper separation to the ground water table, increasing the nitrogen load.

A further consideration is the rate at which upgrades to existing OWTS are implemented. One option, which is currently followed in Florida, is that upgrades are only required whenever the system needs a new, repair, or modification permit after failure or changes to the original permit conditions. Another option could be a requirement to upgrade every system within a certain number of years.

Reducing nitrogen loads from OWTS will grow in importance because the Wekiva Study Area is growing in population leading to additional OWTS. These new OWTS add to the existing load. New systems can be installed with reductions in the amount of nitrogen they discharge. Compared to an aerobic treatment unit (ATU)-requirement without specifying nitrogen reduction levels, a more cost-effective level of nitrogen reduction for OWTS is 70%. Existing systems that are in need of repair or modification can be upgraded to meet current setback standards. This results in some reduction of nitrogen loads from systems that are currently so close to the ground water that no reduction occurs. Based on soil types and ages of buildings, about 5-10% of systems in the WSA could be in this category, in which no reduction of input occurs instead of approximately 40% found during the field work.

Figure 8 illustrates the impact of different management strategies on nitrogen loads from OWTS in the future based on permitting trends in 2000-2005.

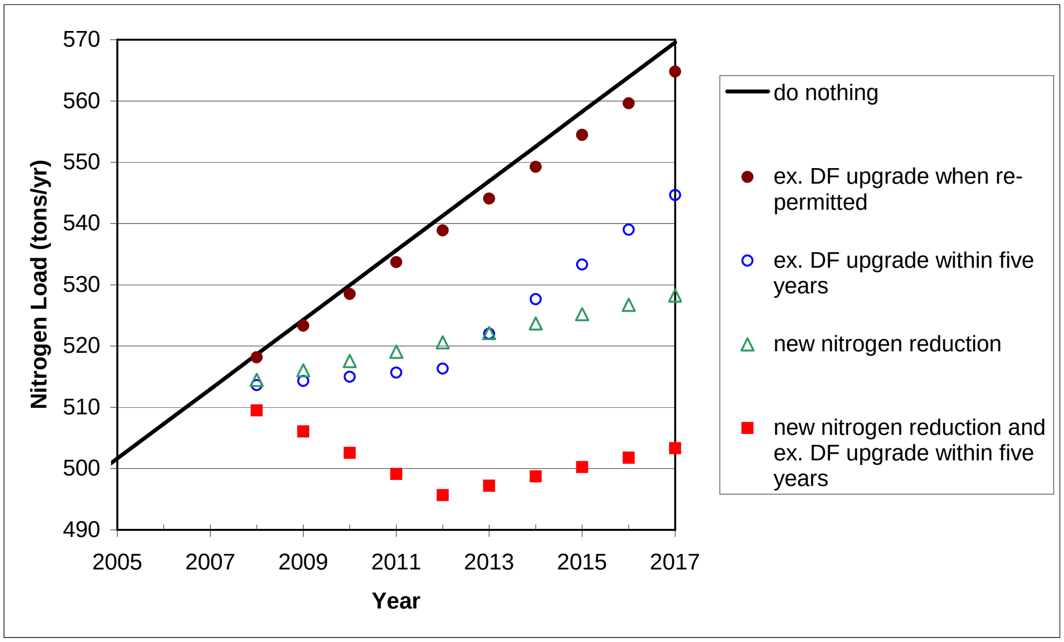

Figure 8. Nitrogen Load Projections for the Wekiva Study Area from Onsite Wastewater Treatment Systems

DISCUSSION

The amount of nitrogen pollution in the WSA was assessed in this study. In forested areas, where the major source of nitrogen is atmospheric deposition, the background levels were generally 0.1 mg/L (Tucker and Diblin, 2007). Field work performed in this study showed background nitrogen levels in residential areas to have an average concentration of 1-2 mg/L. Approximately ten times this level was found in the well defined effluent plume underneath OWTS.

By necessity, an estimate of inputs and loads based on average behavior allows for some variation in the determined input and load contribution from onsite systems. This assessment does not include processes once it is in the ground water prior to discharge at the spring. The FDEP estimate of loads was based on an average of literature values and came to 14 pounds per year per system. The Task 1 field work estimate was based on an average of three sampled sites and came to 17 pounds per year per system. The Task 2 and Task 3 estimates were based on soils and system construction and came to 16.3 pounds per year per system.

Medium density residential, with 2-5 dwelling units per acre, is where most of the OWTS are located in the Wekiva Study Area. This type of land use is increasing, whereas agricultural types are deceasing. This study finds that the contribution of nitrogen from OWTS in medium density residential areas to the underlying aquifer is significant. The relative impact of OWTS as compared to other sources has not been determined, but when comparing to springs protection goals efforts should be made to reduce nitrogen from OWTS as well as other sources of nitrogen. All nitrogen contributors must work together to reduce impacts. The combined effect of fertilizers reveals that they are a major source of nitrogen pollution in the WSA. Onsite systems are not the major source of nitrogen input, but are similar to livestock and centralized wastewater. The solution to reducing nitrogen impacts to the ground water and the springs is to address all sources, with fertilizers being one of the most critical to tackle. FDEP is planning to conduct a field assessment to determine impacts from the residential use of fertilizer in more detail in the next year.

The soil cannot be relied on by itself to significantly reduce nitrogen levels. Adding a mechanism to reduce nitrogen prior to discharge to the soil can be an effective method of achieving nitrogen reduction goals. A previous FDOH study in the Florida Keys (Anderson et al., 1998) demonstrated that nitrogen can be reduced significantly through implementation of performance based standards. Proper operation and maintenance is critical to ensure that these systems continue to perform at the intended levels.

Several recommendations have been made as a result of the study. One is to set a nitrogen discharge fee for all sources to fund cost-effective projects. This will allow for a cost-effective method to target key issues. The establishment of a maintenance program is another recommendation. This program can either be similar to the US EPA Model 4 program (US EPA 2003) where a utility collects fees to provide maintenance, repairs, upgrades, and sewer connections; or to require that all systems have an operating permit, and be inspected and pumped a minimum of once every 5-years. A portion of any fees can be used to fund a grant program for low-income home-owners. Another recommendation is to eliminate grandfathering provisions for older systems as it relates to minimum lot sizes and surface water setbacks. All existing systems needing repair or modification should be upgraded to new system requirements for separation to water table and surface water setbacks. New systems add nitrogen and nitrogen reducing systems will help reduce this. All new systems should be performance based with nitrogen reduction to a level of 10 mg/L or a 70% reduction of nitrogen from raw wastewater as compared to treated wastewater. All onsite systems should be inventoried to help locate areas with older systems closer to the water table and assess the overall impact. The prohibition of land spreading of septage is another recommendation. Finally, the economic feasibility of sewering high density areas should be considered. Implementation of these recommendations will reduce OWTS impacts in the Wekiva Study Area.

This study combined information gathered through reviewing existing literature and field work to evaluate how nitrogen behaves in OWTS, the soil, and the ground water; and what the overall contributions of nitrogen by OWTS to groundwater are. As a result of this study, rule language has been proposed to achieve the recommended changes.

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Revised Estimates of Nitrogen Inputs and Nitrogen Loads in the Wekiva Study Area

Draft 1.6
February 22, 2008

Prepared
by
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Bureau of Onsite Sewage Programs
Florida Department of Health

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Executive Summary

This report presents estimates of relative contributions of nitrogen to groundwater in the Wekiva Study Area. It is a follow-up to the report submitted by the Florida Department of Health in June of 2007 to the Governor. A goal of that study was to determine if OSTDS were a "significant source of nitrogen to the underlying groundwater relative to other sources".
The methodology and terminology of this report follows closely the previous Wekiva nitrogen assessments (MACTEC, 2007; Young, 2007). In particular, input is the amount of nitrogen that is released to or near the surface of the environment, while load is the amount of nitrogen that enters the ground or surface water. Figure 0-1 illustrates this distinction.

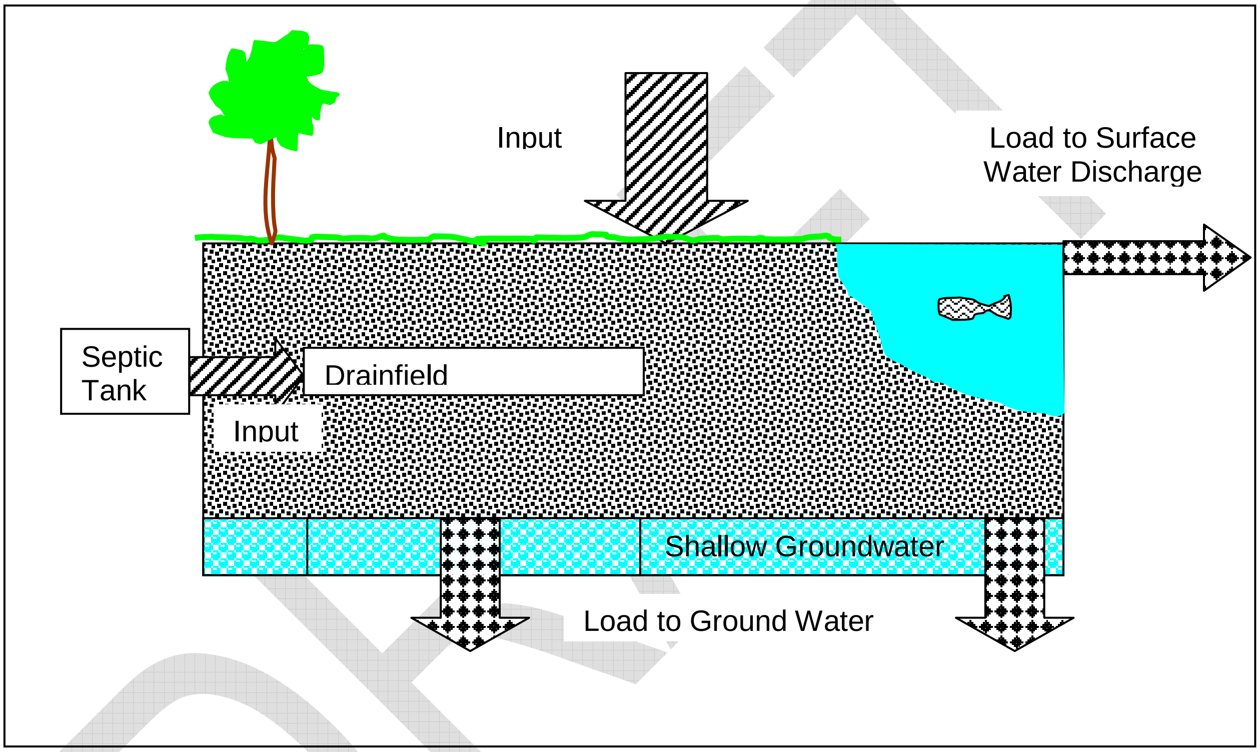

Figure 0-1. Conceptual sketch of distinction between inputs and loads.

Two issues raised in the 2007 report are addressed in this revised input estimate: First, the field work during the Department's study indicated a larger nitrogen contribution for an OSTDS than considered in the assessment (29 lbs/yr instead of 20 lbs/yr). Second, the estimated amount of fertilizer used in the Wekiva Study Area was twice the amount one would estimate based on pro-rating by area the total fertilizer sales registered by the Department of Agriculture and Consumer Services in Lake, Orange and Seminole Counties.

Inputs were determined by estimating atmospheric deposition, fertilizer use, livestock waste, and wastewater effluent discharged into the Wekiva Study Area. The revised relative contributions to nitrogen inputs to the Wekiva Study Area are shown in figure 0-2. The total input was estimated at 6,500 tons/yr or 5,900 metric tons (MT)/yr. Inputs are grouped together by land use category, except for wastewater and atmospheric deposition, which was uniform throughout the area. The figure illustrates that many sources, covered by a variety of jurisdictions, contribute to the nitrogen problem. The contribution by wastewater treatment facilities (WWTF) accounts already for nitrogen reduction accomplished there. Without restrictive nitrogen treatment standards for these facilities, the inputs could be about 1,800 MT/yr higher.

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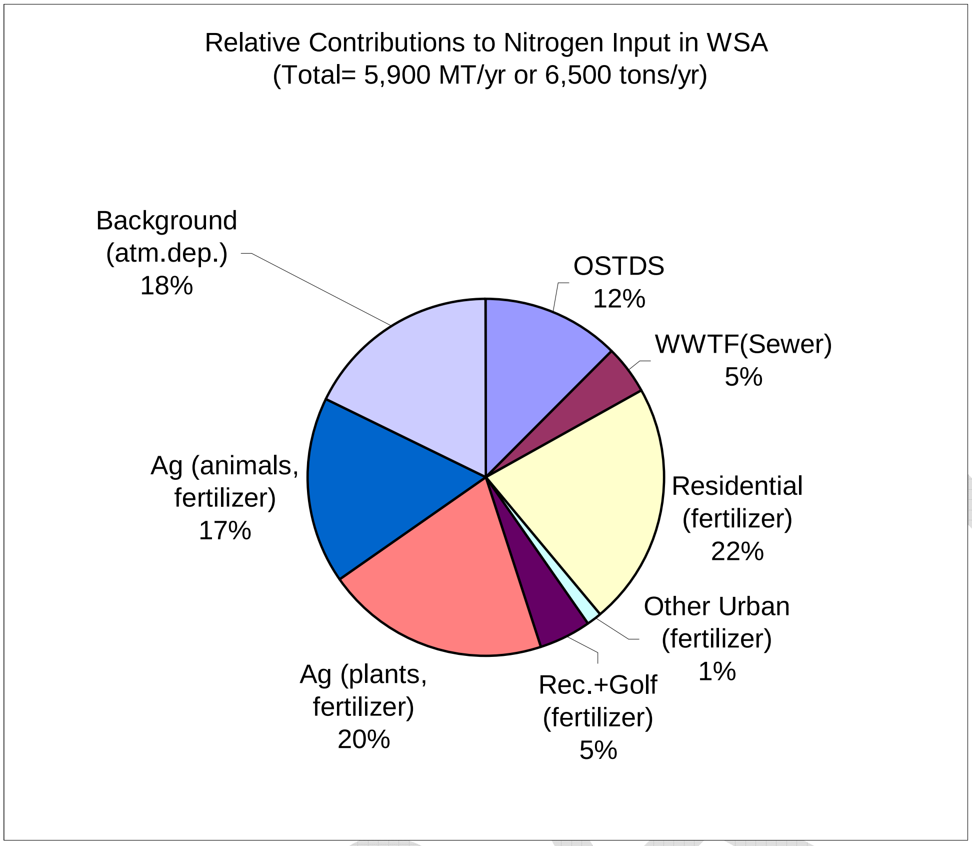

Figure 0-2. Relative contributions to nitrogen input by land use, wastewater and background.

Loads were generally determined by multiplying concentrations with flow rates. For land use classifications the concentrations were shallow groundwater concentrations and the flow was the groundwater recharge rate, which was with one exception obtained from the Groundwater flow model of the St. Johns River Water Management District. The exception was the agricultural tree crops land use classification, for which best management practices irrigation resulted in a much larger flow and therefore loading rate. Loads for each land use were adjusted for a hypothetical background load determined by multiplying a background concentration of 0.2 mg/L total nitrogen with the groundwater recharge rate. Wastewater loads were determined by considering the concentration reduction observed under the discharge areas relative to concentrations and flows that determined the input. The concentration reduction (40%) for OSTDS was based on the results of the 2007 Wekiva Study field work.

Figure 0-3 presents the estimate for relative contributions to groundwater loading in the Wekiva Study Area. The shift in relative contributions is a result of the apparent treatment effectiveness of soil. For low nitrogen and water application rates, such as for atmospheric deposition, soil removed about 95% of the nitrogen, while for high nitrogen and water application rates, such as for rapid infiltration basins, OSTDS and tree crops, soil removed half or less of the nitrogen. This indicated that the amount of irrigation is an important loading factor that should be addressed in future studies.

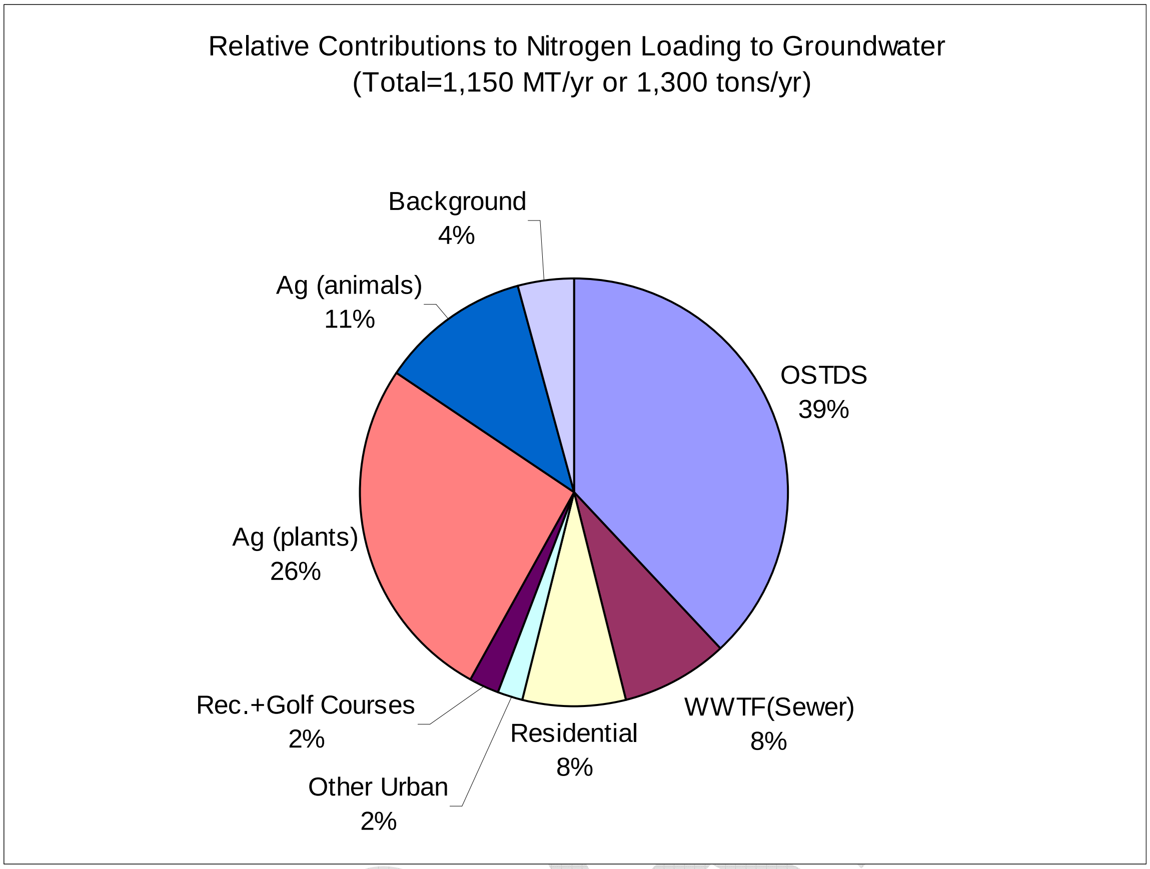

Figure 0-3. Relative contributions to total nitrogen loads to groundwater.

In addition, an estimated 600 tons/yr or 550 MT/yr of nitrogen were discharged via stormwater runoff. Overall, these two estimates indicated that about 70% of the nitrogen input to the Wekiva Study Area is not transferred to ground or surface water.

In order to reduce nitrogen loads to groundwater and surface water in the Wekiva Study Area, better management practices for sources are needed and future population growth must be addressed. This includes OSTDS, for which the Department has proposed nitrogen reduction strategies both for existing and new systems.

1 Introduction

The objective of this report is to present revised estimates of relative contributions of total nitrogen to waters in the Wekiva Study Area. The 2007 Wekiva Study by the Florida Department of Health assessed nitrogen contributions by onsite sewage treatment and disposal systems (OSTDS) to the Wekiva Study Area. The assessment focused on total nitrogen. A goal of the study was to determine if OSTDS were a "significant source of nitrogen to the underlying groundwater relative to other sources". This included an assessment of the relative contribution of nitrogen inputs by onsite systems compared to other sources (Young, 2007). As the summary report (Briggs et al., 2007) pointed out, two pieces of information were not considered in that assessment: First, the field work during the Department's study indicated a larger nitrogen contribution for a typical OSTDS than considered in the assessment; Second, the estimated amount of fertilizer used in the Wekiva Study Area, which comprises only parts of Lake, Orange and Seminole Counties, appeared unlikely high relative to the total fertilizer sales registered by the Department of Agriculture and Consumer Services in these three counties.

The methodology and terminology of this report follows closely the previous Wekiva nitrogen assessments (MACTEC, 2007; Young, 2007). In particular, input is the amount of nitrogen that is released to or near the surface of the environment, while load is the amount of nitrogen that enters the ground or surface water. Either inputs or loads quantify the variety of sources of nitrogen to the underlying groundwater. For most sources, the difference between inputs and loads reflects largely treatment processes in the soil. Because of this, loads characterize the impact on groundwater better than inputs. Figure 1-1 illustrates this distinction.

The Wekiva Study Area encompasses 305,000 acres in Lake, Orange and Seminole Counties in central Florida. While boundaries are not hydrological they encompass most of the springsheds and surface watersheds that contribute water to the Wekiva River before it merges with the St. Johns River. Figure 1- 2 shows the location of the Wekiva Study Area in relation to surface drainage basins and springs recharge areas.

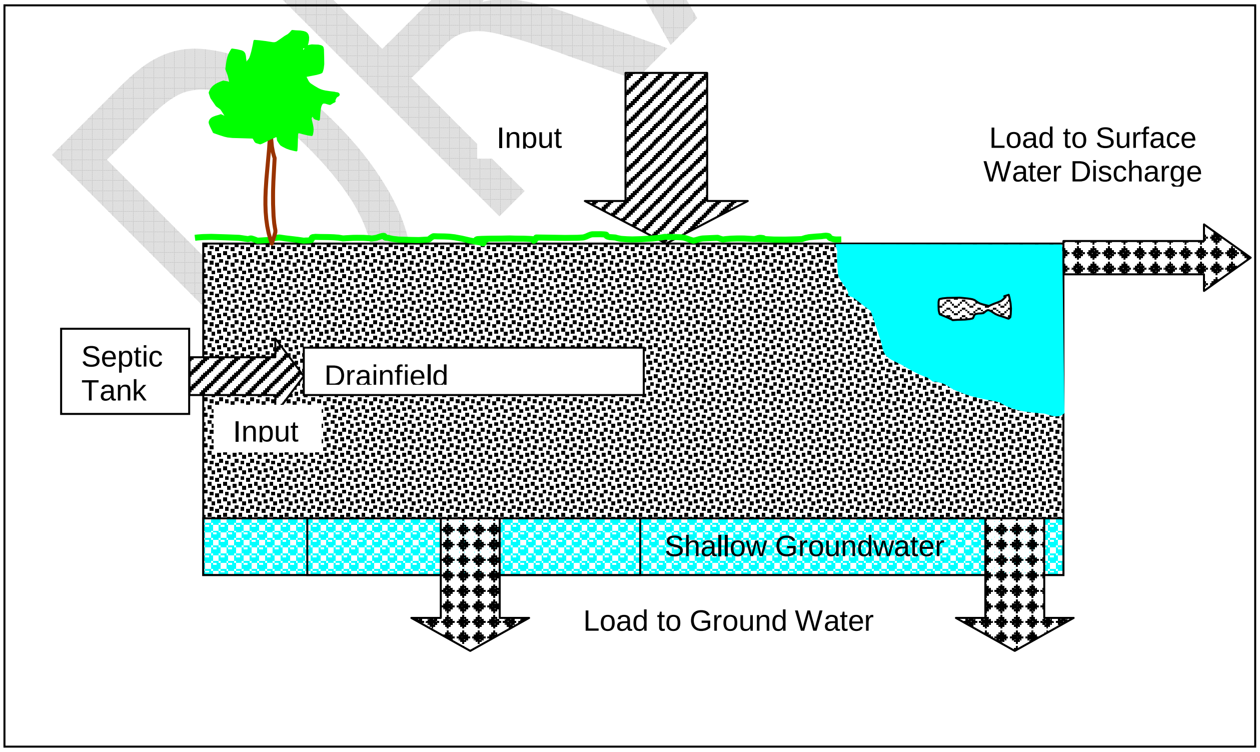

Figure 1-1. Conceptual sketch of distinction between inputs and loads.

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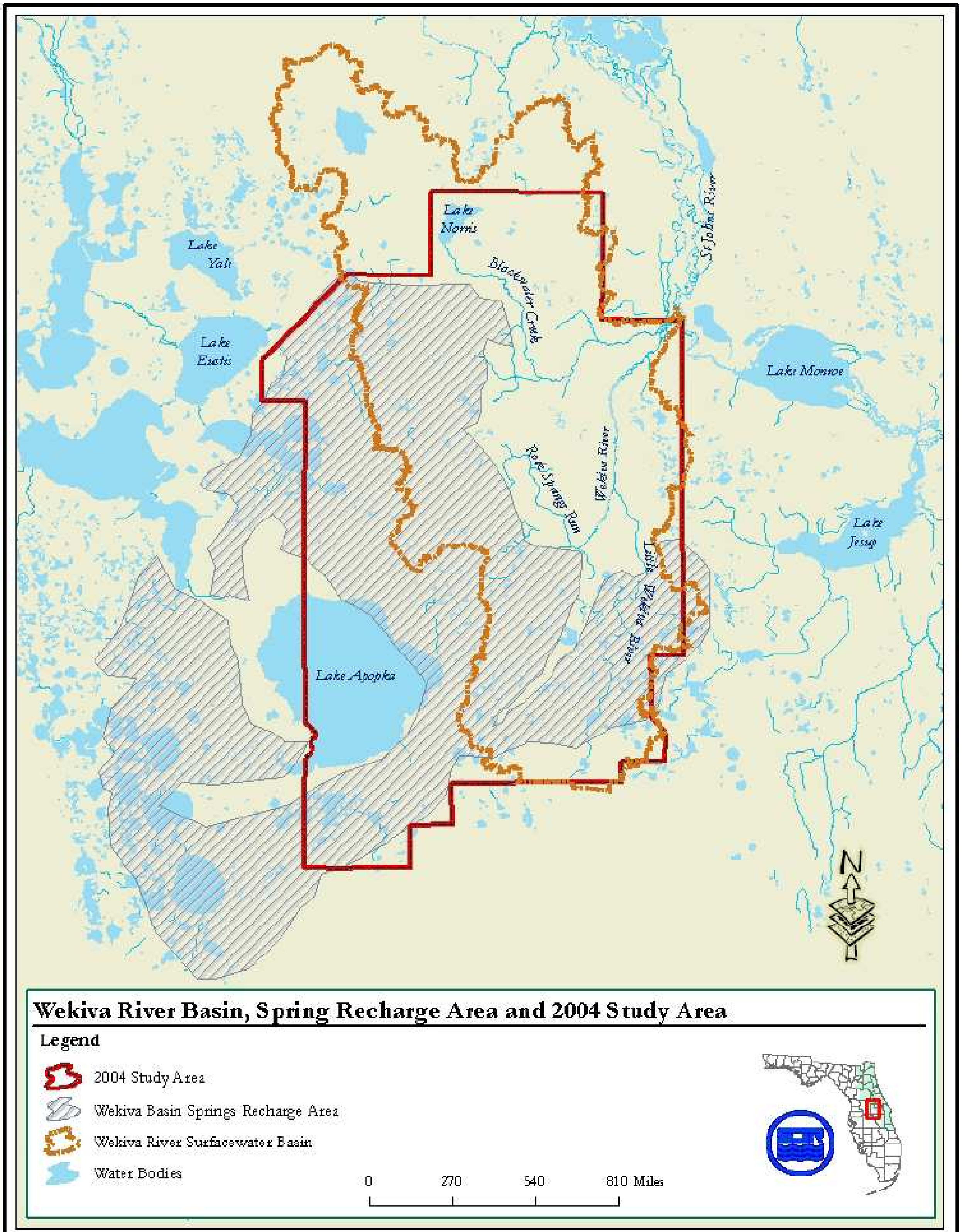

Figure 1-2. Location of Wekiva Study Area relative to springs recharge area and surface drainage basins (from Mattson et al., 2006).

2 Input Assumptions

2.1 Input by OSTDS

The input per system for a typical onsite sewage treatment and disposal system serving 2.6 people, the average household size, was taken as 29 lbs/year. This was based on the mid-range per-capita nitrogen release from the septic tanks observed in the DOH Wekiva Study field work. Such an input was consistent with other recent literature surveys of nitrogen discharged by septic systems. Data supporting this revision were discussed in the task 4 report of the Department's 2007 Wekiva Study (Roeder, 2007). For 55,417 OSDTS in the Wekiva Study Area at the end of 2005 this results in an estimated input of 730 MT/yr or 804 tons/year.

An estimate of how nitrogen inputs by OSTDS have developed over time was obtained by combining census data on house ages in the Wekiva Study Area with onsite permit information and is shown in figure 2- 1. The number of onsite systems estimated for 2005 were prorated by the age of the structures in the WSA given in census files, under the assumption that 91% of all systems were present by the end of 1998.

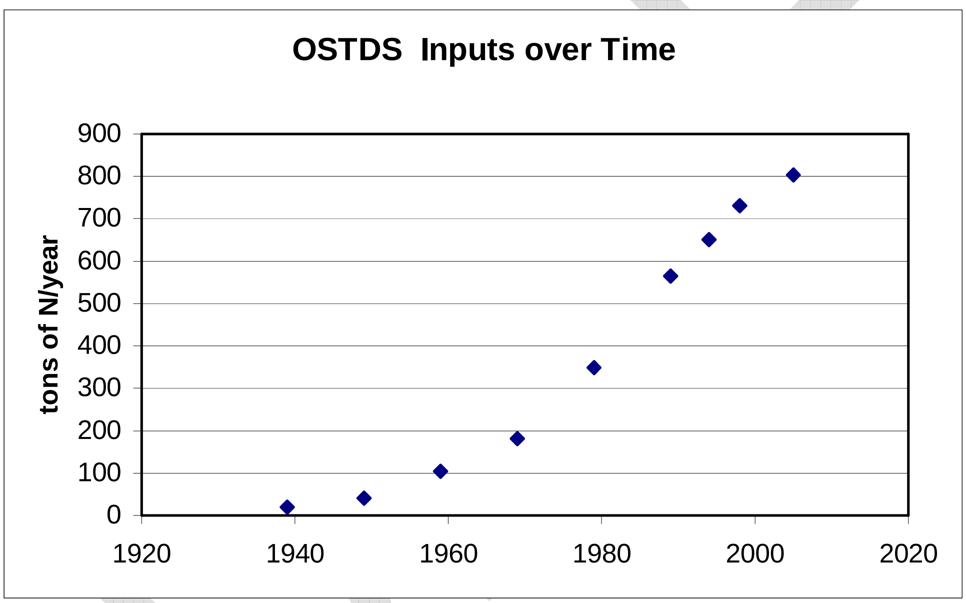

Figure 2-1. Estimated nitrogen inputs from OSTDS in the Wekiva Study Area.

2.2 Input by Sewer (Centralized Wastewater Treatment Facilities):

The estimates for inputs by centralized wastewater treatment facilities are: 28.8 MT/yr that are discharged to surface water; 72.6 MT/yr discharged to groundwater, and 164.7 MT/yr reused, for a total of 266 MT/yr or 293 tons/year. During the previous Wekiva Study Area assessment (Young, 2007), discharge flows and concentrations of wastewater treatment facilities in the Wekiva Study Area were reviewed. Information was available for approximately 80% of permitted capacity. The estimate prorated inputs based on permitted capacity for treatment systems with missing information. It also assumed that 10% of discharge by the Conserv II facility, a large regional facility for the distribution of treated sewage, occurs in the Wekiva Study Area.

A consistency check is achieved by comparison between this estimate and a coarse estimate of treated sewage generated. The number of households on not on onsite systems (157,000) multiplied by an annual input of 29 lbs/household and an average treatment effectiveness of 87% would result in about the same input. The average total nitrogen discharge concentration for wastewater treatment facilities with data was 6.1 mg/L. The sewer input calculation did not consider losses due to exfiltration or import or export of nitrogen from or to areas outside of the WSA.

A similar estimate allowed an assessment of how large nitrogen inputs from wastewater would be if not for centralized wastewater treatment facilities. Without this treatment 2,100 MT/yr of nitrogen instead of 266 MT/yr would be discharged from sewers in addition to the nitrogen from onsite systems.

2.3 Input by Atmospheric Deposition:

The estimated nitrogen input to WSA from atmospheric deposition was 1,050 MT/year or 1,150 tons/yr. Compared to the MACTEC (2007) report, the estimate of nitrogen input from atmospheric deposition was changed in two ways: Data from a station in the Orlando area were used to estimate wet deposition of nitrate and ammonia rather than only nitrate. Nickerson and Madsen (2005) provided trend functions for wet ammonia and nitrate deposition recorded in Orlando from 1978 to 1997. Ammonia did not show a linear increase over time, with 1.02 meq/m^2 month or 1.7 kg/ha.yr as the constant value. Nitrate showed a positive trend for the monthly wet deposition: q= 1.33+0.044*(year-1978) meq/m^2month, which results in a yearly wet deposition of 4.2 kg/ha yr for the end of 2004. The estimated wet total nitrogen deposition is then 5.94 kg/ha year. Dry deposition was assumed to be 30% of the total deposition, the average of the 15% recorded by the CASTNET Indian River Lagoon monitor and the 44% reported by Poor, et al. (2001) for Tampa Bay, or 2.55 kg/ha year. This fraction is similar to 37% dry deposition cited by Dixon (1994) for the Gainesville area in a review of nitrogen deposition. Figure 2-2 shows the regressions of wet deposition with seasonal variability and the estimated total deposition over the period 1978-2004.

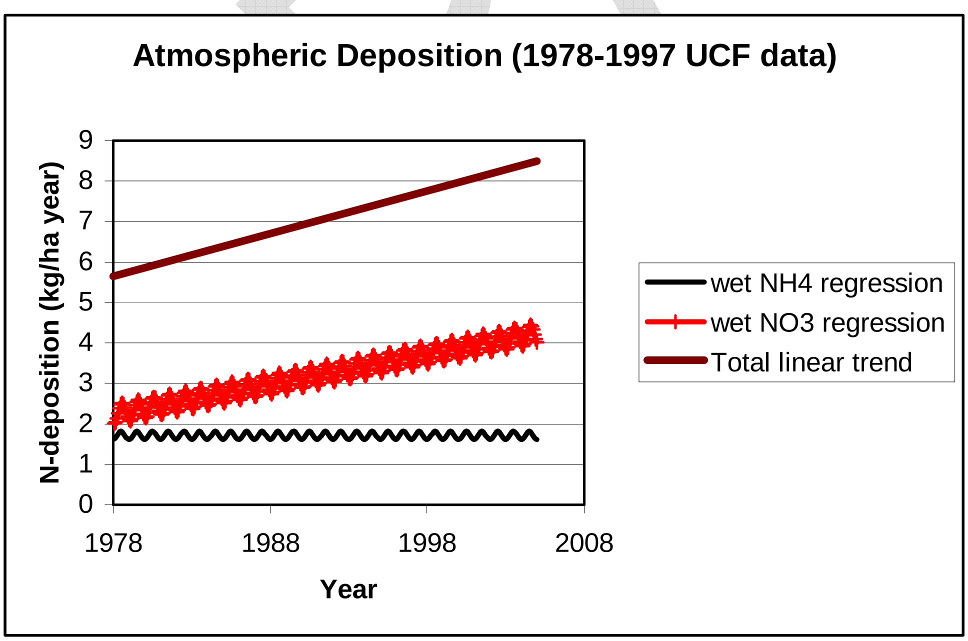

Figure 2-2. Estimated development of yearly wet and total nitrogen deposition based on 20-year observations at University of Central Florida. Regressions reported by Nickerson and Madsen (2005)

Thus, the total nitrogen from atmospheric deposition was estimated to be 8.5 kg/ha year. This value was higher but within the error bounds reported by Poor et al. (2001) for the Tampa Bay, and somewhat higher than the value of 7.6 kg/ha yr given as an estimate for urban bulk loading by Dixon (1994). It is somewhat lower than the 11.4 kg/ha yr obtained by Heyl (1992) for Sarasota Bay.

The input from atmospheric deposition was calculated by multiplying the deposition rate by the area for each land use/land cover classification. .

2.4 Inputs by Fertilizers

2.4.1 Fertilizer Sales

The nitrogen fertilizer sale estimates for the WSA are 1,470 tons/year (1,300 MT/yr) for farm use and 1,980 tons/year (1,800 MT/yr) for non-farm use, for a total of 3,450 tons/year (3,100 MT/yr). This estimate was developed from fertilizer sales data, published by the Department of Agriculture and Consumer Services (http://www.flaes.org/). These data included nitrogen sold and a split between farm and non-farm use of fertilizer for each of the three Wekiva counties for the time period 1998-2007. Non-farm total N sales increased steadily over this period by about 520 tons/year. The average non-farm fraction over the ten-year period was 47%. This is illustrated in Figure 2-3.

In order to estimate how much fertilizer was used in the Wekiva Study Area the following approach was used:
Farm fertilizer nitrogen, estimated as the county farm-use fraction of fertilizer multiplied by county nitrogen sales, was prorated by the county's total area in the Wekiva Study Area. Non-farm fertilizer nitrogen, estimated as the county non-farm use fraction of fertilizer multiplied by county nitrogen sales, was prorated by the county's population in the Wekiva Study Area. Because population is relatively concentrated in the Wekiva Study Area, this approach leads to somewhat higher fertilizer use estimates than an approach that only considers total area as suggested by Anderson (2006). The consistency of the tons/person of non-farm fertilizer sales between the three counties supports the assumption that non-farm uses, such as residential fertilization, are more dependent on the number of people than on the area. Table 1 shows the resulting fertilizer sales for the Wekiva Study Area.

A further consistency check was possible by comparing the census estimate for the population increase in the three counties between 2000 and 2006 (U.S. Census Bureau, 2007) with the increase in non-farm fertilizer use. The population increased by about 44,800 person per year between 2000 and 2006. Multiplying the number of people by the estimate for per capita non-farm nitrogen use of 0.0117 tons/capita year resulted in an estimated increase of 520 tons/year in non-farm use, which matched the observed increase in non-farm nitrogen sales.

The resulting nitrogen fertilizer use estimates for the WSA were 1,470 tons/year (1,300 MT/yr) for farm use and 1,980 tons/year (1,800 MT/yr) for non-farm use, for a total of 3,450 tons/year (3,100 MT/yr). This was noticeably higher than prorating a gross average area sales rate to the Wekiva Study Area, (2,700 tons/year), or even a county area-weighted average (3,000 tons/year) for the Wekiva Study Area. Still, a comparison with the estimates for fertilizer inputs based on application rates as given in the previous assessment suggests that the application rates based approach results in estimates higher by a factor of close to two (6,300 tons/ year for WSA). This discrepancy occurred similarly in the MACTEC study area where simple area-prorating of fertilizer sales lead to an estimated 3,700 tons/year sold and the application rate-based estimate resulted in an estimate of 8,400 tons/year nitrogen applied.

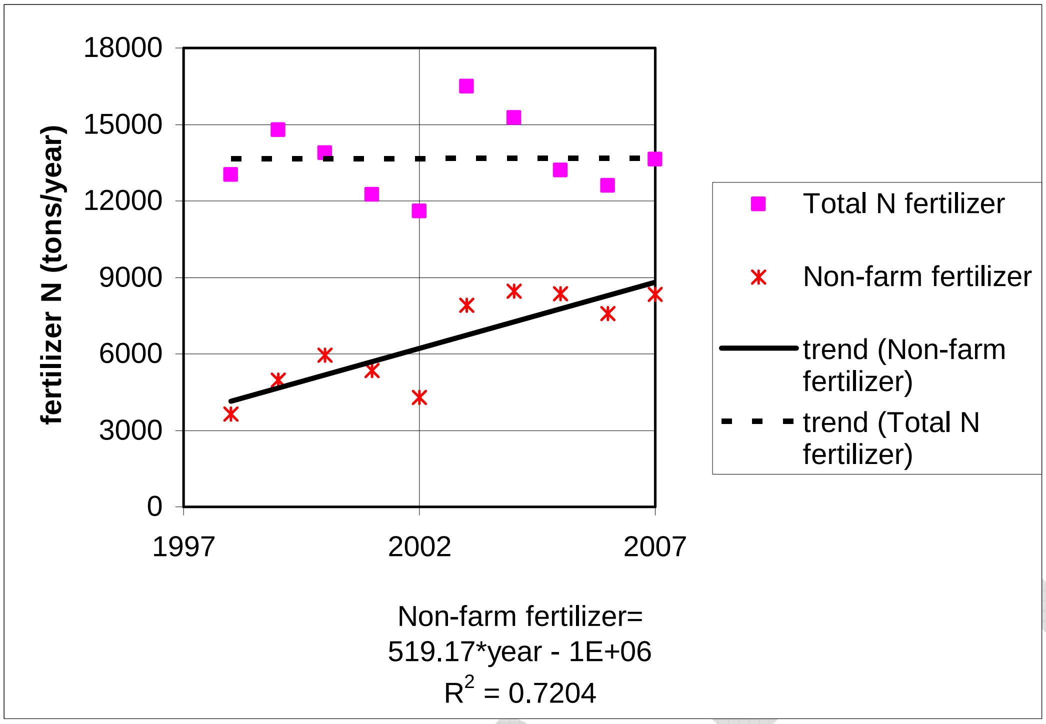

Figure 2-3. Farm and non-farm sales of total nitrogen fertilizer in the three counties, showing an increase by 520 tons/year for non-farm fertilizer between 1998 and 2007.

Table 1. Estimates of fertilizer use in the Wekiva Study Area, based on 1998-2007 average fertilizer sales, areas and 2000 populations.

Area	County Area from GIS (acres)	Area in WSA (acres)	Fraction of County in WSA	County Farm Fertilizer (tons/year)	WSA Farm Fertilizer (tons/year)	tons/ acre year
Lake	743,040	101,395	0.14	2,262	309	0.0030
Orange	645,120	163,731	0.25	3,712	942	0.0058
Seminole	221,440	39,655	0.18	1,214	217	0.0055
Total	1,609,600	304,780		7,188	1,468	0.0048
Population	County Population 2000 from census	Population in WSA 2000	Fraction of County in WSA	County Non-Farm Fertilizer (tons/year)	WSA Non-Farm Fertilizer (tons/year)	tons/ person year
Lake	210,528	98,644	0.47	1,204	564	0.0122
Orange	896,344	259,774	0.29	3,424	992	0.0132
Seminole	365,196	127,054	0.35	1,221	425	0.0096
Total	1472068	485,472		5,849	1,981	0.0117

2.4.2 Effective Fertilizer Application Rates

To determine fertilizer inputs by land use, application rates can be multiplied by the fertilized area. In order to address the lower overall fertilizer numbers a modification in the approach was necessary. The previous model assumed that a fertilizer application rate derived from literature values applied to all area of a land use not classified as impervious (covered by hard surfaces). As no new literature was identified that would shed more light on application rates, the question was rephrased to assess if the fraction of a land use classification to which the application rate applies could be less than previously assumed.

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The split between farm and non-farm use does not strictly align with land uses. In particular, “nurseries” is given as an example for fertilizer classified as non-farm use, even though the land use is agricultural (http://www.flaes.org/). DACS-staff provided guidance on the fertilizer category likely applied to the various land uses (William Cox, written communication). Fertilizer classified as farm and as non-farm use may be used in nurseries, tree nurseries, ornamentals, and floriculture. Because nurseries could use a sizable proportion of non-farm fertilizer sales, this issue introduces some uncertainty about the relative contribution of agriculture and non-agriculture fertilizer inputs. The Department of Agriculture and Consumer Services (DACS) is developing improved methods of capturing information to provide more insight into fertilizer use by user group in the future. For the purposes of this revised estimate, fertilizer use on these land use classifications were split evenly between farm and non-farm use.

The fraction of the area fertilized depended on two factors, how much area was impervious and how much of the pervious area was actually fertilized.

The first factor concerned perviousness as an indicator of usable area for plants that might need fertilizer. The stormwater model WMM, which was applied by CDM (2005) in the Wekiva area, utilizes directly connected impervious area (DCIA), the fraction of the land surface area that is directly connected to the storm water drainage system. The total impervious area can be larger by a factor of about 2 (Rouge River National Wet Weather Demonstration Project, 1998, p.18). Lee and Heaney (2002) reported both DCIA and total impervious areas from four sites in south Florida, which also showed larger impervious fractions than directly connected impervious area. Values for impervious fractions for residential land uses were based on Lee and Heaney’s values. For other land uses the maximum of the DCIA-value given in the CDM (2005) stormwater report and the impervious fraction given in the MACTEC (2007) report were utilized.

The second factor indicated how much of the remaining pervious area was fertilized. The previous assessments (MACTEC, 2007; Young, 2007) assumed that all pervious areas in non-agricultural land uses would be fertilized at the rate for turf grass. This approach was followed here for golf-courses. For other land uses it was assumed that only a fraction of the remaining area was fertilized. For example, tree groups may be fertilized less and canopy cover in Broward county has been estimated between 11 and 45 % (Morrow et al. 2001).

The fraction of fertilized pervious area was adjusted separately for agriculatural and non-agricultural land use classifications in 5% increments until the farm and non-farm fertilizer use estimates were within 4% of the sales estimate. This resulted in an estimate that 60% of non-agricultural pervious area and 85% of agricultural pervious land area could be fertilized at the assumed application rates. Pervious fraction multiplied by turf grass fertilization fraction yields an overall estimate of what fraction of each non-agricultural land use could be covered by turf grass. While the fertilizer application rate was based on turf grass, it should be noted that other landscaping and ornamental plants were included in this use and not captured separately.

As a consistency check, these residential fertilizer estimates were compared to more direct estimates of the lawn area.
Hodges et al (1994, p.79) estimated 1.1 acre of lawn per single family household in Florida. If one applied this estimate to the 120,000 detached single unit structures present in the Wekiva Study Area in 2000 according to census data, 132,000 acres would be covered by lawn. This is about twice the total land use area for low and medium density residential land uses combined. Obviously, the average lawn must be smaller in the Wekiva Study Area. The MACTEC impervious assumption estimates resulted in an average lawn size of 0.4 acres per detached structure. The pervious area and fertilized fraction estimates given for this revision resulted in an average fertilized area for low and medium density residential areas of about 0.17 acre. This is similar to a national average lawn size of 0.2 estimated by Vinlove and Torla (1995). Such an average could be comprised of smaller lawns in medium density residential land uses (2-5

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units/acre) and larger lawns in low density residential land uses. Phelps (2004) cited results of an evaluation of aerial photographs in Marion County by Jones et al (1996) that indicated that 34% of high density residential land use area was covered by turf, 66% of medium density, and 17% of low density. These ratios would result an average lawn size of 0.28 acre for low and medium density residential land uses, about half way between the MACTEC and this revised estimate. If the fertilized area is indeed larger then estimated here, then the application rate would have to be smaller for fertilizer use to remain within the fertilizer sales statistics.

2.4.3 Estimated Fertilizer Nitrogen Input

After the revisions discussed above, the total estimated fertilizer input was 3,200 MT/year or 3,500 tons/year. This was close to the 3,130 MT/yr or 3,450 tons/year estimated as the prorated county nitrogen sales data. Figure 2-4 shows the distribution of fertilizer by land use. For this graphic, low, medium and high density land uses were aggregated into a residential land use category.

The estimate suggested that around 2002 agricultural fertilizer use was the largest source of nitrogen fertilizer applied in the Wekiva Study Area, followed by residential fertilizer. The fertilizer sales over the ten-year period indicated a marked increase in the non-farm fraction while sales overall remained constant. This indicated that increasing urbanization is decreasing agricultural fertilizer inputs but does not decrease fertilizer inputs overall.

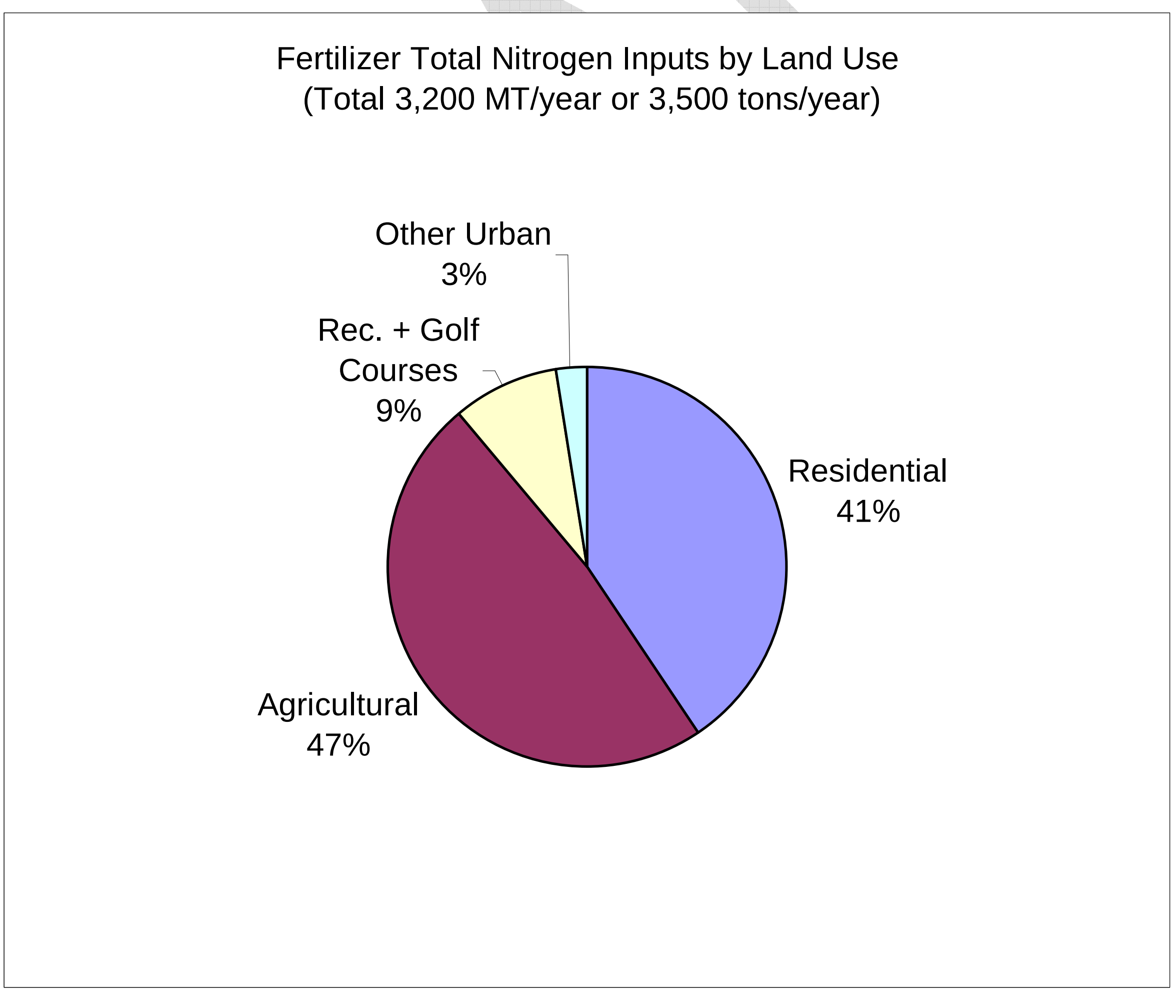

Figure 2-4. Distribution of estimated fertilizer nitrogen input between land uses.

2.5 Animal Waste:

The input assumptions of the previous assessments (MACTEC, 2007; Young, 2007) remained the same. The resulting estimate for the animal waste contribution to the Wekiva Study Area was 650 MT/yr or 720 tons/year nitrogen. This estimate only considered livestock but not wildlife or pet contributions, for which no literature sources were found.

3 Relative Contributions to Inputs

3.1 Inputs without consideration of centralized wastewater treatment

A first approach to input assessment was an estimate of nitrogen that enters the land surface before consideration of the effectiveness of centralized wastewater treatment facilities. This includes fertilizer sales, all wastewater before treatment, atmospheric deposition, and live stock waste. The contributions of these inputs are shown in figure 3-1 and table 2. Fertilizer is the largest input, followed by human wastewater.

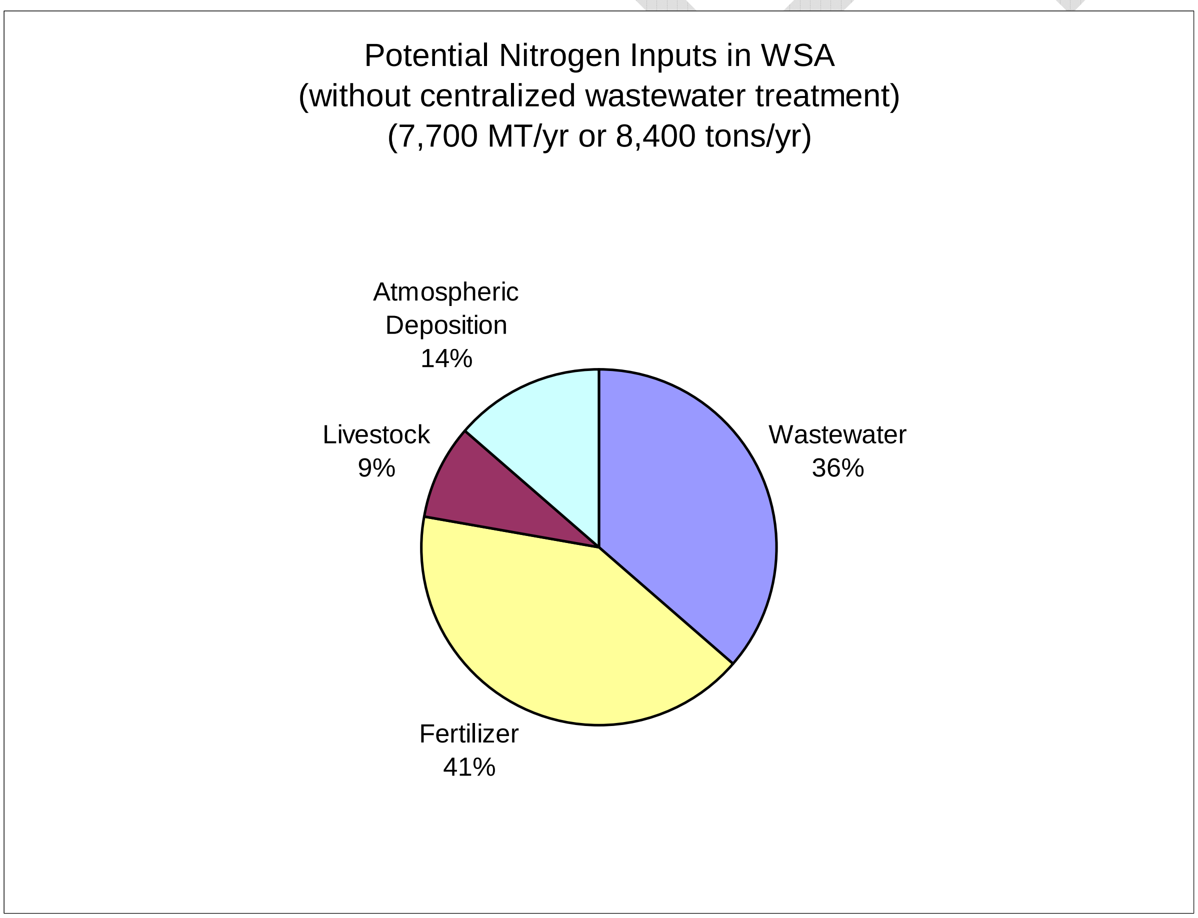

Figure 3-1. Relative contributions to overall nitrogen inputs in the Wekiva Study Area, without consideration of centralized wastewater treatment effectiveness.

Table 2. Nitrogen inputs in the WSA without consideration of centralized wastewater treatment.

	Input (MT/yr)	Input (tons/yr)
Wastewater	2,797	3,080
Fertilizer	3,171	3,492
Livestock	653	719
Atmospheric Deposition	1,048	1,154
Sum	7,669	8,446

3.2 Inputs including wastewater treatment facilities

Upon consideration that centralized wastewater treatment facilities control already part of the potentially available nitrogen, the picture shifted. The difference between figure 3-1 and 3-2 represents the effectiveness of centralized wastewater treatment, which effect a reduction of over 25% of nitrogen input between these estimates.

The estimated input of 5,900 MT/yr nitrogen was about 27% less than the 8,100 MT/yr estimated during task 3 of the DOH Wekiva Study (Young, 2007). The reduction was largely due to the consideration of fertilizer sales in estimating this input, which resulted in a 45% reduction of this input. OSTDS input increased by 45% with the inclusion of results from the 2007 DOH Wekiva Study. Of the inputs, OSTDS, atmospheric deposition, and non-farm fertilizer use had increasing tendencies. Fertilizer sales overall appeared to remain at a constant level. For livestock and sewer no historic data were researched.

Figures 3-2 and 3-3 and tables 3 and 4 present the estimated inputs released to the waters and soils of the Wekiva Study Area. The difference between the two presentations is in the role of land use. Looking ahead to the loading estimate, all inputs on a land use (except wastewater and a natural background) will result in a common loading to water. To make inputs and loads comparable and to provide somewhat more detail for management discussions it was considered helpful to aggregate by land use. The following categories were used: residential (low, medium, high), background (atmospheric deposition and inputs from extensively managed land uses, such as open range, upland forest), other urban (commercial, institutional, transport, utilities, extractive), recreational and golf, plant agriculture (all crops), animal agriculture (all pasture, horse farms, aquaculture, feeding operations).

4 Nitrogen Loading

As the MACTEC report (2007) outlined, three pathways are distinguished in this assessment of loadings. The loading mass rates are estimated as the product of flow and concentration. Stormwater runoff and recharge, or percolation of a part of rainfall to groundwater, are the two pathways that transport diffuse sources as a function of land use. For these diffuse loads, estimated concentrations, which vary by land use, and estimated flows, which vary by land use or location, were multiplied with each other. For more identifiable sources, in particular wastewater, the mass rate of loading was estimated as a fraction of the input, which was equivalent to calculating the discharge flow times a commonly observed reduction in concentration.

Anderson (2007), in commenting on the MACTEC-report suggested that “the relative contributions of each nitrogen source should be based on estimated inputs until such time that field data is available to more accurately calculate loadings from each source in a consistent fashion”. The consistency concern related apparently chiefly to the estimation of flow rates as illustrated by his example in which local groundwater concentrations under a drainfield were

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multiplied by a diffuse recharge rate, thereby ignoring the available information on local wastewater flow out of a drainfield. A drawback of a loading contribution estimate based solely on input information is that it assumes that soil is equally effective in removing inputs from various sources and along various transport pathways. Such a simplifying assumption disregards much information regarding both concentration and flow.

The following presents a loading estimate based on current information. As additional information becomes available, such as results of additional inquiries in residential fertilizer fate and transport, this estimate can be updated. The loading estimate may also point towards areas where additional information can be most useful.

Table 3. Nitrogen inputs in the Wekiva Study Area by source

Source	Input (MT/yr)	Input (tons/yr)
OSTDS	730	804
WWTF(sewer)	266	293
Fertilizer	3,171	3,492
Livestock	653	719
Atmospheric Deposition	1,048	1,154
Sum	5,868	6,462

Table 4. Nitrogen inputs in the Wekiva Study Area by land use, wastewater, and background

Land Use	Input (MT/yr)	Input (tons/yr)
OSTDS	730	804
WWTF(Sewer)	266	293
Residential (fertilizer)	1,290	1,421
Other Urban (fertilizer)	82	90
Rec.+Golf (fertilizer)	274	302
Ag (plants, fertilizer)	1,194	1,315
Ag (animals, fertilizer)	984	1,083
Background (atm.dep.)	1,048	1,154
Sum	5,868	6,462

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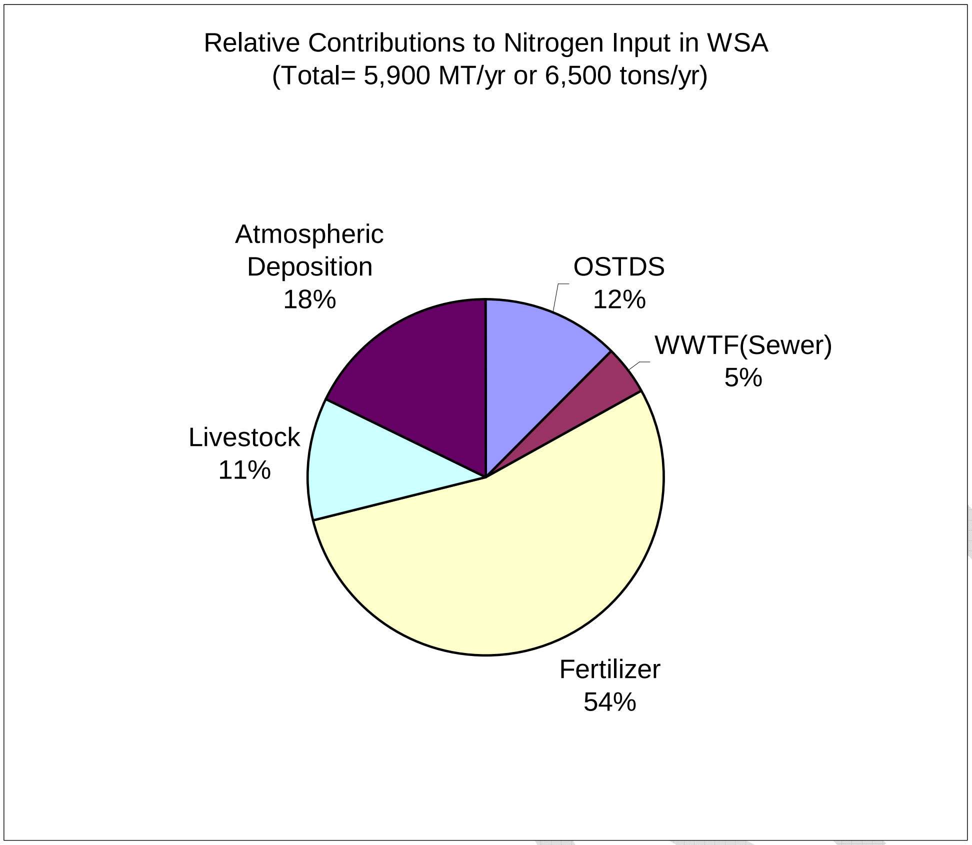

Figure 4-1. Estimated relative contributions to nitrogen input in the Wekiva Study Area.

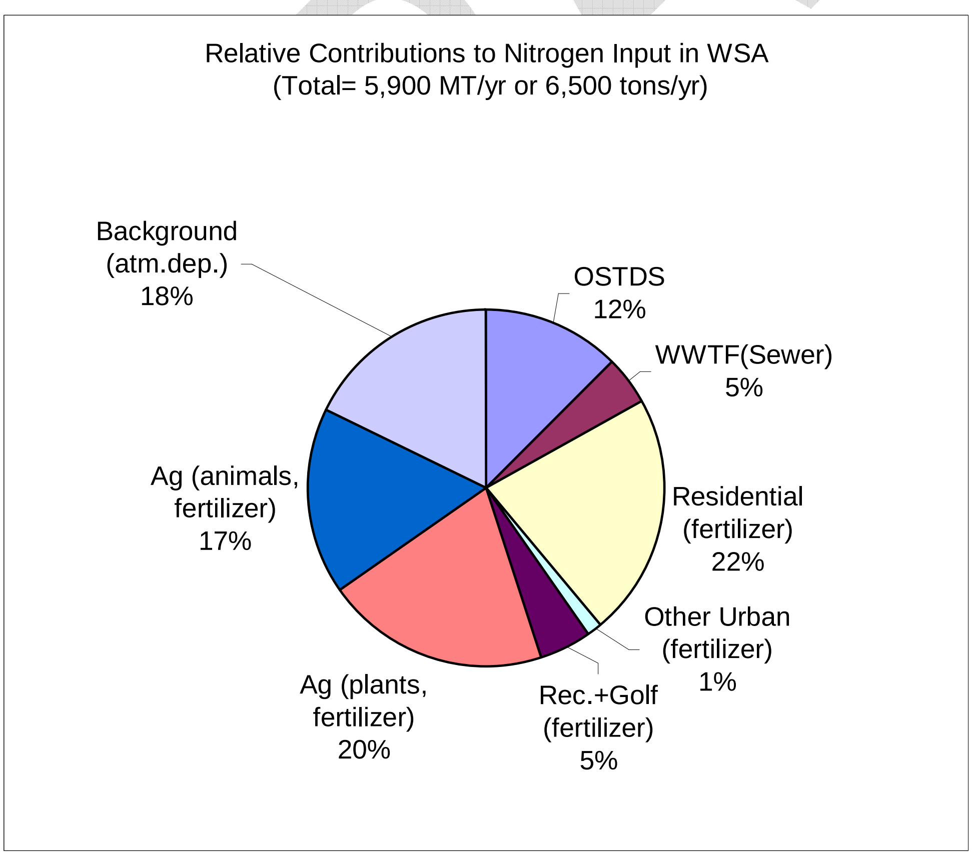

Figure 4-2. Relative contributions to nitrogen input by land use, wastewater and background.

4.1 Water budget for the Wekiva Study Area

The first step in the loading assessment was an estimate of the water flows involved in transport of nitrogen. This was accomplished by an approximate water budget for the Wekiva Study Area. To allow comparisons between areas and account for the fact that the WSA has political and not hydrological boundaries, the amount of water was conveniently expressed as the annual depth of water on top of the area. CDM (2005) gave an average precipitation of approximately 50.3 in/yr. Average groundwater recharge values by land use were obtained by Dr. Young in the course of task 3 of the 2007 Wekiva Study from an overlay of land use and recharge values for the regional groundwater flow model of the St Johns River Water Management District. The area-weighted average recharge was 7.6 in/year. This value was consistent with results by Wanielista et al (2005), who estimated an average spring discharge of at least 7 in/yr in the 450 square miles of springshed.

Estimates for non-spring discharge by rainfall and stormwater runoff or possibly diffuse groundwater discharge were obtained by looking at the gaging station of the Wekiva River at SR 46, where the Wekiva River leaves the Wekiva Study Area, River Basin and MACTEC's area of analysis. Wanielista et al. (2005) estimated that at least 58% of the flow at this point stems from spring discharge. This left about 42% of the discharge that could be attributed to rainfall and stormwater runoff, which was 8.7 in/year. The value was very similar to 9.1 in/yr found by Wanielista et al. (2005) for part of the Little Wekiva River watershed within the Wekiva Study Area.

The Wekiva Study Area extends further west than the surface watershed of the Wekiva River, into an area where recharge is more important than runoff. Mattson et al. (2006) estimated the fraction of springs discharge in the Wekiva River flow higher. Both facts suggested that 8.7 in/year is an upper bound of surface water discharge that is not stemming from springs.
The remainder of the water, 50.3-7.62-8.74 = 33.9 in/yr, was an estimate for the amount of water returned to the air as evapotranspiration. These values for spring discharge and evapotranspiration were similar to those obtained for water balances for springs on the west coast of Florida (Knochenmus and Yobbi, 2001). In that area no surface water discharge was present, and instead a similarly large diffuse groundwater flow provided outflow from the area.

Water supply was excluded from this gross water balance. For the purposes of this assessment the assumption was that human water use is supplied by water from the Wekiva Study Area and returned to the Wekiva Study area in a closed loop. Thus, this closed loop had on the scale of the Wekiva Study Area no net effect on the water balance and only the effect of flushing nitrogen into the groundwater.

For domestic use resulting in wastewater the amount of water could be quantified. The number of people living in the Wekiva Study Area (485,500 in 2000) multiplied by a daily per capita use of 68.6 gallons resulted in a yearly water use estimate of 12.2 billion gallons, or about 1.5 in/year over the Wekiva Study Area.

Water use for agricultural irrigation was only estimated for tree crops (discussed below). The irrigation for this land use was estimated to recycle 0.7 in/year water over the entire Wekiva Study Area. If other land uses also experienced much irrigation the amount of water recycling through the Wekiva Study Area would become more important relative to the amount of water that flows simply from recharge areas to the springs and river. This effect was not assessed here in any more detail, but could be included in further studies.

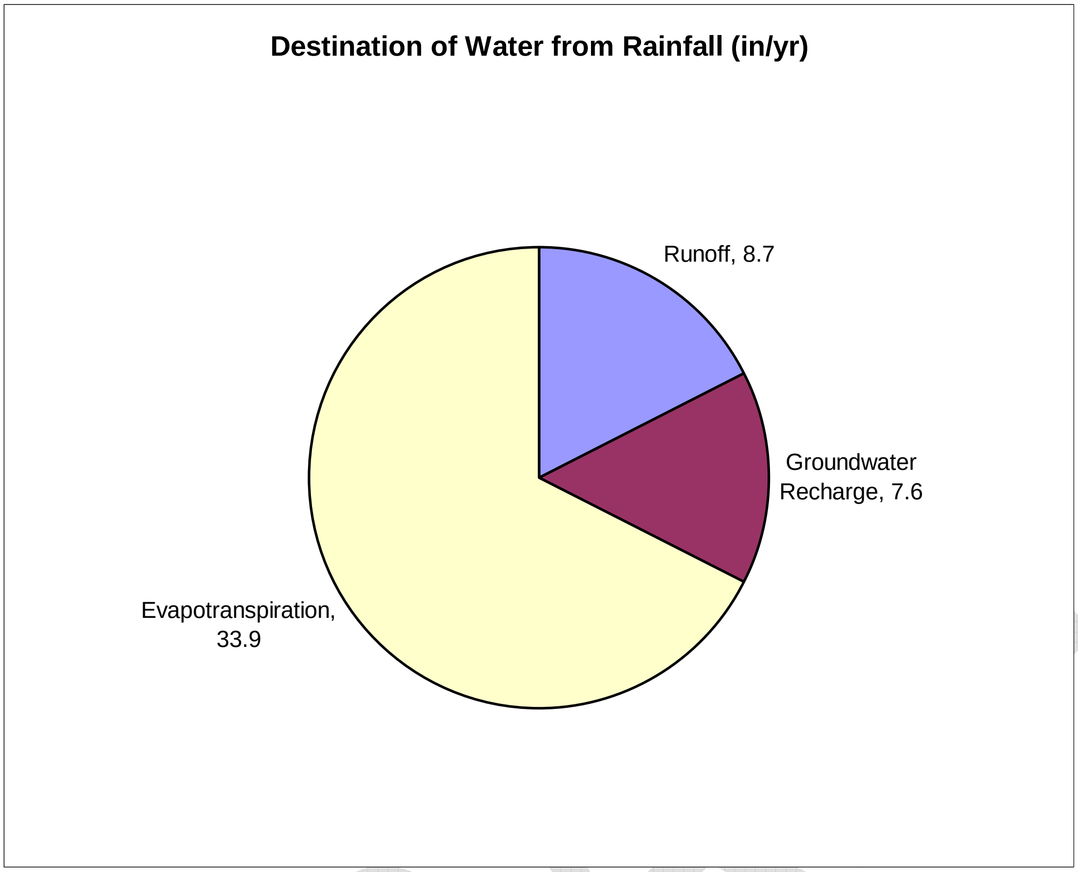

Figure 4-3. Water budget for the Wekiva Study Area based on the Wekiva River gaging station at SR46. For comparison, wastewater generation amounted to approximately 1.5 in/year and was assumed to not cause a net change in the water balance.

4.2 Load to Groundwater

4.2.1 Load from OSTDS

The average removal observed at the three sites of the field work was 40%, leading to a load estimate per system of 17.4 lbs N/year. The field work performed during the Department's Wekiva Study in 3007 included two systems in Tavares soil with low water table and passage through clay zones. For these the estimated removal was between 25% and 50%. This was higher than the 10% estimated by Otis (2007) in a separate task of the study, possibly due to the presence of clay. In the third site, in Myakka soil, the estimated removal was a third, this was lower than the 50% for discharge as TKN or >90% for discharge in nitrate form estimated by Otis (2007).

The average estimated removal fraction based on field work was noticeably higher than estimated in a draft report for task 3 of the Wekiva Study as the weighted average of soil denitrification potential. As Otis (2007) pointed out, nitrogen removal can be very site specific and depends on several factors. The 40% removal estimate is within the range of 10-50% given in Anderson and Otis (2000), but higher than the 30% removal estimated in the MACTEC (2007) report. In this report, 40% removal was assumed, which resulted in an OSTDS loading estimate of 438 MT/year or 482 tons/year.

Drainfields that don't maintain the modern requirements for separation from the water table are likely to experience less nitrogen removal. A coarse estimate based on soil types and system ages suggested that between 5 and 10% of systems may be in such a situation, which would increase the load from OSTDS by about 3%.

4.2.2 Load from wastewater treatment facilities

Loading from wastewater treatment facilities to groundwater varied by discharge mechanism. For groundwater discharge via rapid infiltration systems and similar technologies, 40% removal was assumed. This removal fraction was within the range given by EPA for rapid infiltration systems (EPA, 2003, 2006), and the same removal effectiveness as assumed for OSTDS. It was only somewhat lower than the 50% suggested by FDEP's former reuse coordinator David York in his comments included in MACTEC report. For reuse applications, a similar removal fraction as given by EPA (2002) for slow rate land treatment was assumed (70%). This resulted in a groundwater load of 93 MT/yr or 102 tons/yr of nitrogen from wastewater treatment facilities. This load did not include exfiltration from wastewater transport networks.

4.2.3 Load from diffuse sources

The mass loading rate brought about by water recharging the ground water was determined by estimation of flow and concentration. Concentrations were adjusted for background concentrations to capture the increase in loading due to land uses.

The estimation method considered that the input was applied over large areas with little or no water, and subsequently only the percolating fraction of water facilitated transport. This was the case for transport of fertilizer input and livestock input and atmospheric deposition towards groundwater. To account for such more diffuse sources, MACTEC suggested the approach to utilize shallow groundwater concentrations, as an indicator of the nitrogen that has arrived in the water and multiply them with the recharge rate, which represents the flow of water that had the apparent concentrations. The variation in the amount of water available to transport nitrogen to the groundwater meant that the mass loading was spatially variable.

The shallow groundwater concentrations in the MACTEC report were applied here, with three exceptions:

First, background concentrations were assumed to be 0.2 mg/L total nitrogen, rather than 0.1 mg/L nitrate-nitrogen. This value was consistent with the concentrations observed in the unimpacted Alexander and Juniper Springs (Wetland Solutions, Inc, 2004; Mattson et al., 2006), and observations in wells in forests that appeared unimpacted by fertilization and human disturbances (Phelps, 2004, Toth and Fortich, 2002). Generally, such samples have a high fraction of TKN and a low fraction of nitrate. 0.2 mg/L nitrate-nitrogen has also been used as a cut-off value to distinguish background groundwater values from impacted groundwater (O'Reilly et al., 2007)

Second, for low density residential land uses field work during the 2007 Wekiva Study indicated that total nitrogen concentration under low density residential land uses are usually lower than 3 mg/L given by MACTEC (2007). That value was based on lysimeter studies. During the 2007 field work, background samples in shallow ground water unimpacted by drainfields averaged between 0.5 and 2 mg/L at the three sites. The mid-range of 1.3 mg/L or slightly less than half the previous estimate was the number used in the following for residential and urban land uses. This concentration was applied to all fertilized land uses that previously were assigned a 3 mg/L concentration in recharge water. This number is similar to nitrate-nitrogen well concentrations observed in shallow wells under residential land uses in the Silver Springs Basin by Phelps (2004). Nitrate-nitrogen dominated nitrogen species in that study.

Third, for tree crops among the agricultural land uses, data became available from a BMP verification study (Citrus Research and Education Center, 2007). The total nitrogen concentrations in shallow groundwater varied around 10 mg/L, somewhat lower than the 15 mg/L given by MACTEC (2007). The yearly fertilizer input for the years 2004-2006 for the 8 sites for which the yearly sums are given averaged around the 227 kg/ha yr given in the MACTEC report. The water balances for these 8 sites showed average yearly evapotranspiration of 43.8 in, rainfall of 47.2 in, irrigation of 41.8 in, and drainage to the water table of 45.8 in. Irrigation resulted in a recharge rate of 46 in/yr instead of 11 in/yr estimated from the groundwater recharge model. The resulting estimate for groundwater loading was 112 kg/ha yr, or half of the fertilizer input. These monitoring data pointed to the importance of irrigation for the mobilization of nutrient, which the MACTEC (2007) discussed in the context of turf grass. The estimated nitrogen transfer to groundwater was larger by a factor of two than what was observed in lysimeters during leaching events over the same time frame. These lysimeters measured an average load of 42 kg/ha yr, or only 20% of the input. Both the relative magnitude of evapotranspiration and recharge, and the groundwater concentrations around 10 mg/L are in agreement with earlier modeling predictions by Harrison et al. (1999) for BMP practices. For consistency, the product of recharge rate and shallow groundwater concentration was used in the following.

The question arose if the areas of land uses should be adjusted to account for impervious surfaces and non-fertilized areas. This adjustment appeared unnecessary for the following reason: the recharge rates were obtained by a regional groundwater model that did not distinguish between pervious and impervious surfaces for the recharge rate and therefore the average recharge rate accounted for variations in the local recharge between pervious and impervious surfaces. The non-fertilized areas accounted for the yearly nitrogen fertilizer application rates, while the shallow groundwater concentrations were not finely resolved enough to distinguish between fertilized and not-fertilized areas. Fertilized area was only considered in the agricultural tree crops land use, for which the recharge rate was not determined from the groundwater flow model but from measurements within the citrus grove. Therefore the load from this land use was estimated by multiplying the groundwater concentration times the recharge due to irrigation times the estimated effective fertilized area fraction of 0.85.

The results indicated that 620 MT/yr or 680 tons/year of nitrogen enters the groundwater as part of the diffuse recharge to ground water. Agriculture is the largest source, in turn dominated by tree crops. Tree crops, as a result of the consideration of irrigation, contributed slightly more than half of the agricultural nitrogen on a sixth of the agricultural area. The difference between the estimate using the assumptions of the MACTEC (2007) report and the BMP-based estimate was about 100 MT/yr for the agricultural tree crop land use. If other crops or urban landscapes are irrigated to a similar extent, the estimate of 620 MT/yr would need to be increased.

A comparison of inputs and loads provided an estimate of the apparent nitrogen losses occurring between the surface and the shallow groundwater. Background groundwater concentrations indicated about 95% removal relative to atmospheric deposition. Other land uses saw on average about a 85% apparent loss. The heavily irrigated tree crops show a 50% reduction of fertilizer input, similar to the estimated removals for onsite systems and rapid infiltration wastewater disposal facilities.

4.2.4 Total load to groundwater

The approximate overall nitrogen load to groundwater was estimated as 1,150 MT/yr or 1,300 tons/yr nitrogen by adding onsite systems and land applications of wastewater to the diffuse loads discussed in the previous section. This groundwater load was effective on water that can eventually discharge from springs. Table 5 shows the contribution of groundwater loadings by sources.

The average concentration from this load was 4 mg/L, determined by dividing the load of 1,150 MT/yr by 7.6 in/yr recharge over 305,000 acres of area. This was by a factor of about two higher than the total nitrogen concentrations estimated for Wekiva (2.1 mg/L) and Rock Springs (1.6 mg/L) from the sum of nitrate and organic nitrogen (Wetland Solutions, Inc., 2004 table 2-7). The loading assumptions appear unlikely be too high by this factor of two. A plausible explanation is that some nitrogen removal occurs during transport from shallow groundwater to the springs. The extent of this removal is likely to depend on aquifer vulnerability and travel time between shallow groundwater and springs. If these factors are correlated with land use, relative contributions could shift, for example, the more common occurrence of OSTDS in more vulnerable areas could increase their contributions to loads relative to background contributions from less vulnerable areas. Such shifts are expected to be limited. A future more detailed study, such as a ground water quality model that incorporates conduit flow could quantify the impact of such attenuation factors.

Table 5. Estimated nitrogen loads to groundwater by source

Loading Ground Water	Load (MT/yr	Load (tons/yr)
OSTDS	438	482
WWTF(Sewer)	93	102
Residential	88	97
Other Urban	22	24
Rec.+Golf Courses	28	31
Ag (plants)	303	334
Ag (animals)	130	143
Background	47	52
Sum	1,150	1,266

4.2.5 Relative contributions to nitrogen loading to groundwater

Figure 4-2 presents the estimated relative contributions of nitrogen loading to groundwater. Among the sources of nitrogen considered, OSTDS is prominent with about 40%. Its share of wastewater loads has increased relative to inputs because of the higher apparent nitrogen removal rate of slow rate applications and the diversion to surface discharge of some treated wastewater. OSTDS contribution relative to fertilizer has increased because fertilizer loads are more reduced relative to inputs, except in heavily irrigated situations. Still, fertilizer contributions to the load overall are similar to OSTDS. Background load contributions have much decreased relative to inputs, reflecting the low concentrations found in unimpacted springs.

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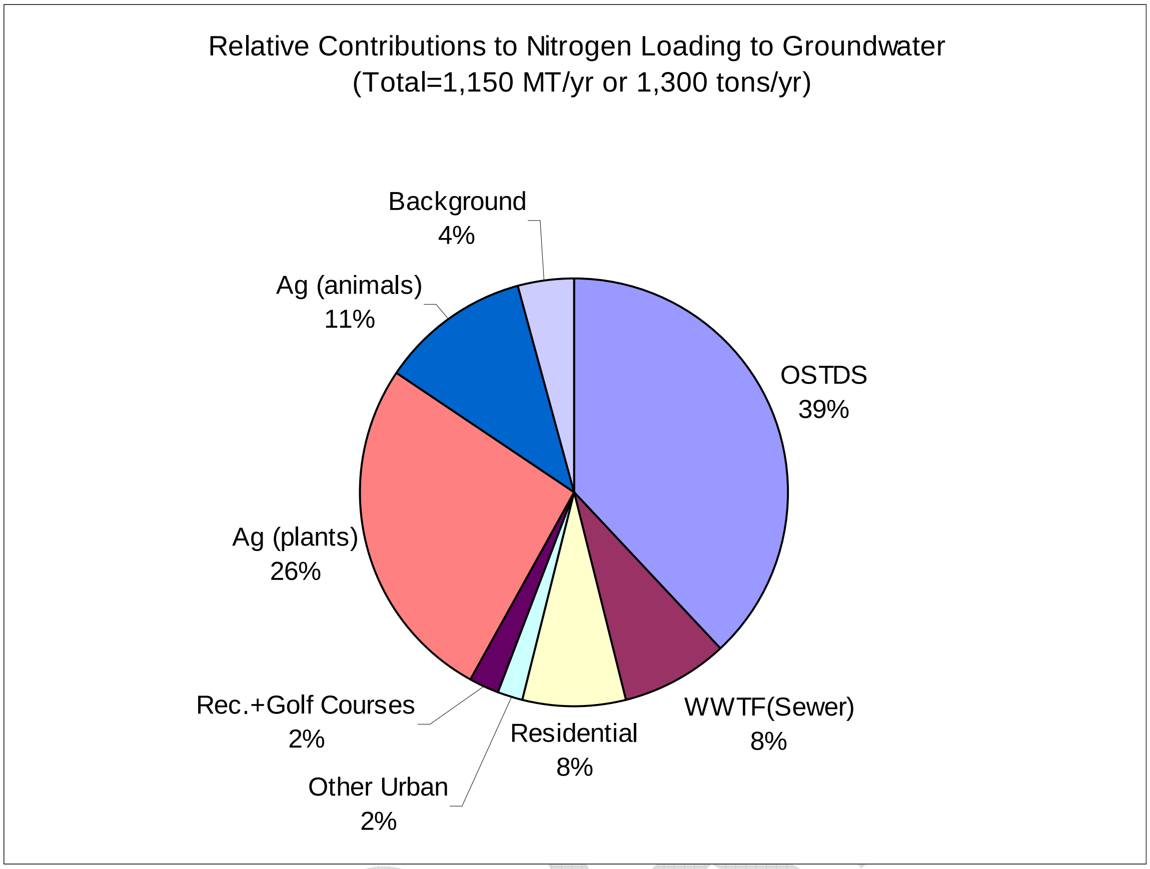

Figure 4-4. Relative contributions to total nitrogen load to groundwater.

4.3 Non-spring surface water discharge loading

The second transport mechanism of nitrogen from the land surface to water is storm water or runoff as surface water. The MACTEC report utilized event mean concentrations only for nitrate-nitrogen, not for total nitrogen, and did not provide loading rates for all land uses. Therefore, this revised estimate utilized values for event mean concentrations and directly connected impervious area fractions provided in the Wekiva Stormwater Model (CDM, 2005).

4.3.1 Rainfall-runoff coefficients

The coefficients suggested by CDM (2005) for predicting runoff assume that 20% of rainfall runs off as surface runoff even for pervious surfaces. This is higher than the conceptual model presented by Wanielista et al. (2005), and the assessment by Gao (2007) for the Wekiva River. Such an estimate would result in an average runoff of 19 in/year, which is more than twice than what the water balance for the Wekiva River indicates as an upper limit for surface water discharge and what gaging stations in the area suggests for river flow as analyzed by Wanielista et al. (2005). A lower effective value for runoff coefficients for watershed-scale models was also observed by Hendrickson and Hart (2007) in the lower St Johns River.

Various runoff coefficients could be chosen to meet the constraint that the overall runoff estimate can not exceed 9 in/yr in accordance with the water balance. A secondary constraint used here was that the pervious runoff coefficient should be at least five times smaller than the impervious runoff coefficient to agree with the relative importance assigned to the two by CDM (2005). Loading estimates overall were not very sensitive to changes in the parameters, given that the total runoff was fixed and event mean concentrations vary only within a factor of two except for agricultural feeding operations. A runoff coefficient of 0.06 for pervious surfaces results in a runoff of about 3.3 in/year. This value is close to those obtained for USGS gaging stations in high recharge areas in the Clermont area and thus appeared consistent for runoff from areas with much groundwater recharge and little impervious area (Wanielista et al., 2005). To meet the overall runoff limit, a 0.54 runoff coefficient for impervious surfaces was chosen.

4.3.2 Nitrogen concentrations

Event mean concentrations for total nitrogen by land use were also taken from the CDM (2005) report. Some information was available on treatment effectiveness with regard to downstream water bodies (CDM, 2005; Harper, 2007). Hendrickson and Hart (2007) cautioned that they found on larger scales event mean concentrations for nitrogen that were only 2/3 of other literature values. Little information was available on the treatment effectiveness with regard to groundwater recharge from retention facilities. On the scale of the Wekiva Study Area no explicit treatment effectiveness by stormwater management measures was considered in this report. Some effectiveness is implied by the lower runoff coefficient for impervious surfaces used here (0.54), which is about a third lower than proposed by CDM (2005).

Surface water contamination by onsite systems was not considered separately, but assumed to be addressed by the event mean concentrations for residential land uses. Stormwater loading models such as the one used here provide options to increase loads due to large numbers of systems that fail and discharge to the land surface instead of to ground water. Generally, this contribution is minor (Rouge River National Wet Weather Demonstration Project, 1998). Gao (2007) provides an estimate of 206 for the number of onsite system that are located within 200 m of river segments in the Wekiva River Basin. Yearly repair rates for the three counties having part of the Wekiva Study Area are on the order of 1.5% to 2% (Roeder, 2007). Both numbers suggest that a surface water contribution rate of 10% of onsite systems as suggested by Rouge River National Wet Weather Demonstration Project (1998) is much too high for consistent discharge to surface water in the whole Wekiva Study Area. Furthermore, the 10% estimate is based in part on the number of systems for which the drainfield is below the ground water table, which for the purposes of this report should be part of groundwater loading. There may be localized areas of higher failure rates or higher numbers of systems that don't meet modern construction standards where higher contribution rates could be justified in a more detailed assessment.

4.3.3 Rainfall-runoff or stormwater loading, including background load

Loads were estimated as the product of runoff, area and event mean concentration. Background contributions were estimated as those stemming from undeveloped land (DCIA fraction =0.005) with the event mean concentration for undeveloped land. The loading contribution from each land use was determined as the difference between background load and the load estimated for that land use with updated DCIA and event mean concentration. The fraction of load stemming from background concentrations varied between half for an impervious to pervious runoff coefficient ratio of four to somewhat more than a third for a ratio of 15. An intermediate estimate with a 0.06 runoff coefficient for pervious surfaces and a 0.54 runoff coefficient for impervious surfaces resulted in an estimate of 520 MT/yr or 570 tons/yr, of which background contributions were 42%.

4.3.4 Total surface water discharge load

To obtain the overall estimated surface water loading, surface water discharges by wastewater treatment facilities had to be added. These consisted of 29 MT/yr or 32 tons/yr. No additional in-stream reduction was considered. Although Gao (2007) and Wetland Solutions, Inc (2005) provided evidence that removal of nitrogen, in particular nitrate, occurs within the water body, the objective of this report was to provide a loading estimate to surface water rather than a river water quality model.

Table 7 provides the estimated nitrogen loading contributions from different land uses and wastewater. The overall load estimate was 550 MT/yr or 600 tons/yr. Division of this load by the 8.7 in/yr estimated runoff resulted in an average concentration of 2 mg/L. This appeared to be within a factor of two compared to the measured concentrations around 1 mg/L in the Wekiva River and Little Wekiva River (Wetland Solutions, Inc., 2004). Mattson et al., (2006) provide TN concentrations of 1.25 at the Wekiva River at SR 46 and 1.68 mg/L in the Rock Spring Run. They also discuss an apparent reduction in nitrogen concentrations with distance downstream from the springs. In addition to in-stream removal processes, such a reduction could be caused by dilution of more contaminated spring water with cleaner wetland and lake surface water discharge.

Figure 4-3 illustrates the nitrogen loading to surface water due to rainfall runoff or stormwater, and direct sewer discharge. Residential land uses represented a third of the stormwater nitrogen load, and a sixth of the stormwater nitrogen load came from the “other urban” category. Overall, about half of estimated surface water loading was associated with residential and urban land uses. Even without an increase in impervious surfaces and event mean concentrations due to urbanization, about 40% of the load would remain. More than half of this background load was provided by the flow out of wetlands and lakes. Agriculture, recreation and golf contributed only minor amounts to the estimated stormwater load, because very little runoff is attributed to them. About half of the estimated agricultural surface water load was from animal feeding operations.

Table 6. Estimated nitrogen loads to surface water other than springs discharge

Loading Surface Water Discharge	MT/yr	Tons/yr
OSTDS	0	0
WWTF(Sewer)	29	32
Residential	192	212
Other Urban	87	96
Rec.+Golf	4	5
Ag (plants)	5	6
Ag (animals)	14	15
Background	217	239
Sum	549	604

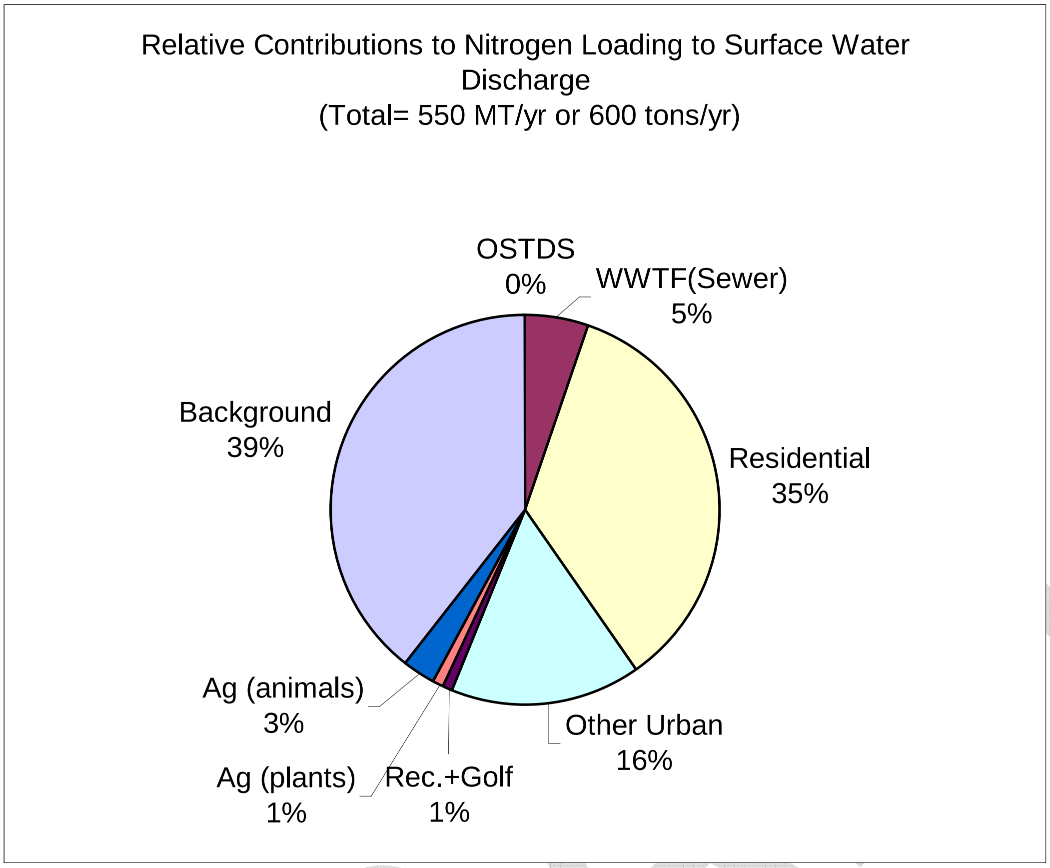

Figure 4-5. Relative contributions to total nitrogen load to surface water discharge.

4.4 Total Load to Waters in the Wekiva Study Area

Addition of the loads via different pathways and from different sources and land uses resulted in a load estimate to waters of the Wekiva Study Area shown in table 9 and figure 4-4. This aggregation is most appropriate for nitrogen loads to the Wekiva River at SR46, where both surface and ground water contribute to the nitrogen load. By averaging surface water and groundwater load contributions, which had very different patterns, the aggregated pie chart provides fewer insights into transport mechanisms and possible management approaches. Overall, these two estimates indicated that about 70% of the nitrogen input to the Wekiva Study Area is not transferred to water but removed before entering groundwater or a river.

OSTDS were a prominent contributor with 26% of the estimated load, all of which as load to ground water. OSTDS increased contribution to load relative to centralized wastewater treatment facilities was due to the higher removal effectiveness assumed for reuse slow-rate applications. OSTDS contributions are expected to increase with continued population growth unless this source is addressed.

Agricultural land uses together provided a contribution of 25% of the total nitrogen load, most of that as ground water load. This contribution is expected to decrease over time as agriculture is replaced by residential and urban land uses.

Table 7. Estimated total load of nitrogen to waters of the Wekiva Study Area

Loading to Water	Load (MT/yr)	Load (tons/yr)
OSTDS	438	482
WWTF(Sewer)	122	134
Residential (fertilizer)	281	309
Other Urban (fertilizer)	109	120
Rec.+Golf Courses	32	35
Ag (plants)	309	340
Ag (animals)	144	159
Background	265	291
Sum	1,698	1,871

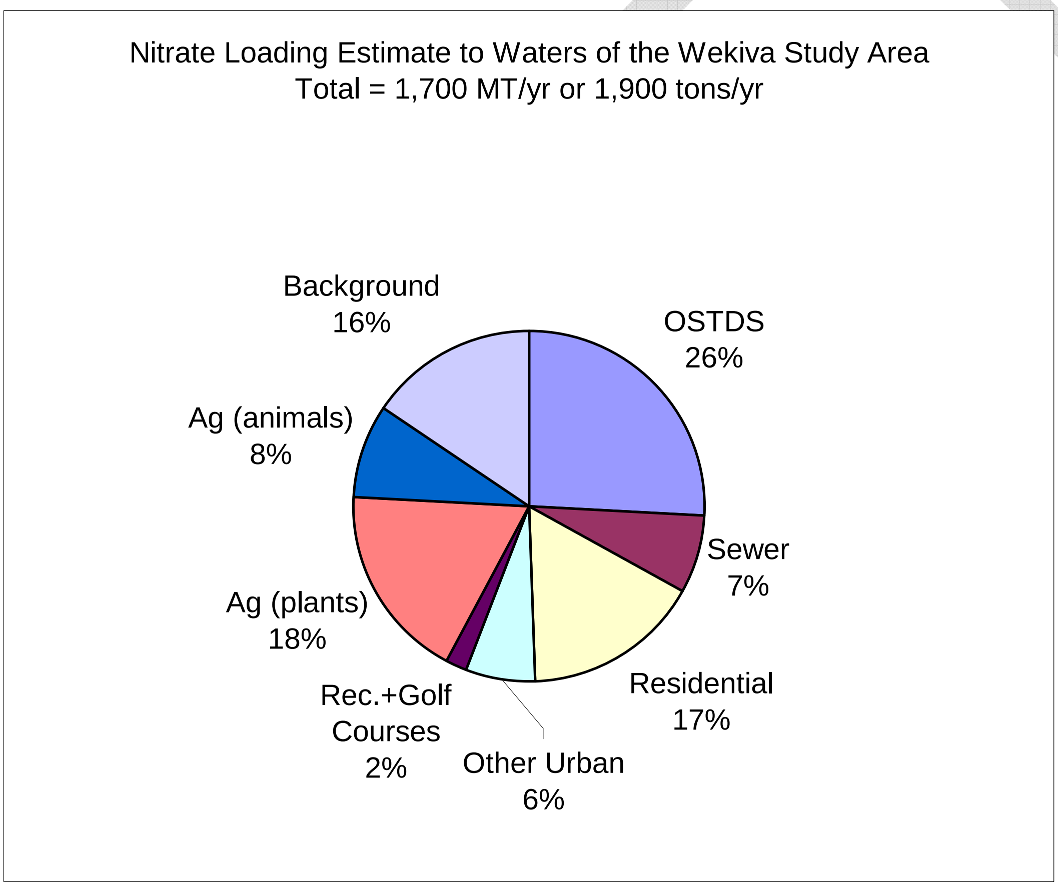

Figure 4-6. Relative contributions to total nitrogen load to all waters in the Wekiva Study Area

Residential and urban land uses together contributed a similar 23%. About three quarters of this contribution occurred in the form of stormwater. Non-farm fertilizer use was the largest considered input for these land uses. This contribution is expected to increase with new development and the increased fraction of non-farm fertilizer sold.

Background contributions were estimated at 16%. This contribution was determined by runoff from a hypothetically undeveloped Wekiva Study Area and recharge as if shallow groundwater concentrations were unimpacted. This contribution is unlikely to change unless increased atmospheric deposition eventually affects it.

Sewer contributions were estimated at 7%. They are expected to decrease in the short term as the Wekiva-specific rules promulgated by FDEP come into effect. In the long run, increases in population may lead again to an increase.

4.5 Equitable and Cost-Effective Solutions

In order to achieve the nitrogen pollution reductions goals for the springs and river (35% to 85%), all controllable sources must be reduced to a large extent. One way to approximate an equitable distribution of reductions would be to ensure that the costs paid per pound of nitrogen removed or the fees paid per pound of nitrogen discharged are similar across sources. This was the motivation for the proposal of a nitrogen discharge fee in the Department's 2007 Wekiva Study report. Such a fee could fund cost effective nitrogen reduction measures in the Wekiva Study Area.

In the absence of such a fee, a comparison of past measures between sources provides suggestions of where additional contributions to nitrogen reduction could come from. Among the sources discussed, centralized wastewater treatment facilities have achieved the most quantifiable reductions in nitrogen inputs and loads. In response to concerns in the Wekiva Study Area, FDEP has adopted new rules that will require further upgrades in treatment. Data in FDEP's 2004 report suggest that the cost is at least $5 per pound of nitrogen removed for an upgrade of existing wastewater treatment facilities. Nitrogen reduction by providing sewer for additional people appears to be one to two orders of magnitudes more expensive.

The effectiveness of fertilizer best management practices is more difficult to assess without in-depth study. The decrease in farm uses of nitrogen is at least partly due to the replacement of farms by residences and other development, without a net reduction in fertilizer sales over the last ten years. A new residential turf rule that will be implemented in 2008 and 2009 aims to change lawn fertilizer compositions and application rates and is expected to result in reductions of primarily phosphorus but also nitrogen inputs by perhaps a quarter.

Onsite sewage treatment and disposal systems in the Wekiva Study Area have so far not contributed to nitrogen reduction practices. The costs of changing design and construction standards appear to be roughly similar to the costs for centralized wastewater treatment facilities. The Department has proposed modifications to onsite sewage rules in the Wekiva Study Area to reduce the nitrogen load of existing and system and decrease the growth in onsite nitrogen loading due to a growing population.

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6 Appendix 1

Summary of Inputs by Land Use/Land Cover Classification

Land Use/Land Cover		Area		Input atm dep.	impervious fraction			net fertil.d fraction	Fertilizer rate	Input fertilizer	Animal waste rate	Input animal waste	Input w/o atm. dep.	Input Total
LU code	Decription	(acre)	(ha)	(kg/yr)	MACTEC, (2007) (-)	DCIA CDM, (2005) (-)	this report (-)	(-)	(kg/ ha year)	(kg/yr)	(kg/ ha year)	(kg/yr)	(kg/yr)	(kg/yr)
1100	Low density Residential	22,645	9,168	77,928	0.147	0.3	0.4	0.36	148	488,472	0	0	488,472	566,400
1200	Medium density Residential	44,361	17,960	152,660	0.278	0.37	0.55	0.27	148	717,681	0	0	717,681	870,341
1300	High density Residential	7,792	3,155	26,815	0.67	0.71	0.7	0.18	148	84,043	0	0	84,043	110,858
1400+ 1480	Commercial and airports	8,470	3,429	29,149	0.9425	0.85	0.94	0.036	200	24,691		0	24,691	53,839
1500	Industrial	2,714	1,099	9,340		0.85	0.85		0	0	0	0	0	9,340
1600	Extractive	634	257	2,183		0.85	0.85		0	0	0	0	0	2,183
1700	Institutional	3,311	1,341	11,396	0.91	0.65	0.91	0.054	200	14,479		0	14,479	25,875
8100	Transportation	3,492	1,414	12,017	0.85	0.01	0.85	0.09	200	25,447	0	0	25,447	37,464
8300	Utilities	2,327	942	8,007	0.85	0.85	0.85	0.09	200	16,957	0	0	16,957	24,964
1800	Recreational, Marinas and fish camps, swimming beaches	1,839	744	6,327	0.015	0.005	0.02	0.588	200	87,534		0	87,534	93,861
1820	Golf Courses	3,174	1,285	10,923	0	0.17	0.17	0.83	175	186,652	0	0	186,652	197,574
2100	Agriculture-Field Crops	59	24	204	0	0.01	0.15	0.8415	150	3,031	0	0	3,031	3,235
2140	Agriculture-Row Crops	693	280	2,384	0	0.01	0.15	0.8415	630	148,664	0	0	148,664	151,047
2150	Agriculture-Field Crops	2,569	1,040	8,839	0	0.01	0.15	0.8415	150	131,266		0	131,266	140,106
2200	Agriculture-Tree Crops	6,016	2,436	20,703	0	0.01	0.15	0.8415	227	465,266	0	0	465,266	485,969
2400	Agriculture-Nurseries	129	52	443	0	0.01	0.15	0.8415	227	9,963		0	9,963	10,407

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2410	Agriculture-Tree Nurseries	83	34	285	0	0.01	0.15	0.8415	227	6,411		0	6,411	6,696
2420	Agriculture-Soc Farms	120	49	414	0	0.01	0.15	0.8415	200	8,198	0	0	8,198	8,612
2430	Agriculture-Ornamentals	5,353	2,167	18,422	0	0.01	0.15	0.8415	227	413,994	0	0	413,994	432,416
2450	Agriculture-Floriculture	21	9	72	0	0.01	0.15	0.8415	200	1,434	0	0	1,434	1,507
2500	Agriculture-Specialty Farms	87	35	298		0.01	0.15	0.8415	200	5,901		0	5,901	6,199
2110	Agriculture-Improved Pasture	13,268	5,372	45,658	0	0.01	0.15	0.8415	63	284,769	41	220,233	505,002	550,660
2120	Agriculture-Unimproved Pasture	4,226	1,711	14,541		0.01	0.15		0	0	41	70,141	70,141	84,682
2130	Agriculture-Woodland pasture	3,280	1,328	11,287	0	0.01	0.15		0	0	41	54,441	54,441	65,728
2300	Agriculture-Feeding Operations	162	66	558		0.01	0.15		0	0	4,150	272,287	272,287	272,845
2510	Agriculture-Horse Farms	2,151	871	7,403	0	0.01	0.15	0.8415	63	46,170	41	35,707	81,876	89,279
2540	Agriculture-Aquaculture	15	6	52		0.01	0.15		0	0	0	0	0	52
1900	open land	2,841	1,150	9,778		0.005	0.15		0	0	0	0	0	9,778
2600	Other open lands rural	266	108	914		0.005	0		0	0	0	0	0	914
3000	Upland nonforested	17,096	6,921	58,831		0.005	0		0	0	0	0	0	58,831
4000	Upland forest	45,169	18,287	155,441		0.005	0		0	0	0	0	0	155,441
5000	Water Body	38,688	15,663	133,136		0.275	0.28		0	0	0	0	0	133,136
6000	Wetlands	52,103	21,094	179,303		0.275	0.28		0	0	0	0	0	179,303
7000	Barren Land	9,428	3,817	32,443		0.005	0		0	0		0	0	32,443
	Totals	304,582	123,313	1,048,157						3,171,023		652,809	3,823,832	4,871,989
	Totals (MT/yr)			1,048						3,171		653	3,824	4,872
	Totals (tons/yr)			1,154						3,492		719	4,211	5,366

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7 Appendix 2

Summary of Groundwater Loads by Land Use/Land Cover

Land Use/Land Cover		Area	Input	Input	GW concentration		GW recharge		GW load		Apparent removal	
			atm. dep.	w/o atm. dep.	Background TN	Impacted TN	recharge	recharge	Background	Additional	Background	additional
LU Code	Descriptive	(ha)	(kg/yr)	(kg/yr)	(mg/L)	(mg/L)	(mm/yr)	(in/yr)	(kg/yr)	(kg/yr)	(-)	(-)
1100	Low Density Residential	9,168	77,928	488,472	0.2	1.3	287	11.3	5,254	28,896	0.93	0.94
1200	Medium Density Residential	17,960	152,660	717,681	0.2	1.3	254	10.0	9,126	50,196	0.94	0.93
1300	High Density Residential	3,155	26,815	84,043	0.2	1.3	267	10.5	1,684	9,263	0.94	0.89
1400+ 1480	Commercial and airports	3,429	29,149	24,691	0.2	1.3	285	11.2	1,952	10,735	0.93	0.57
1500	Industrial	1,099	9,340	0	0.2	0.2	316	12.4	694	0	0.93	n/a
1600	Extractive	257	2,183	0	0.2	0.2	404	15.9	208	0	0.90	n/a
1700	Institutional	1,341	11,396	14,479	0.2	1.3	297	11.7	797	4,383	0.93	0.70
8100	Transportation	1,414	12,017	25,447	0.2	1.3	259	10.2	731	4,021	0.94	0.84
8300	Utilities	942	8,007	16,957	0.2	1.3	259	10.2	487	2,679	0.94	0.84
1800	Recreational, Marinas and fish camps, swimming beaches	744	6,327	87,534	0.2	1.3	228	9.0	339	1,863	0.95	0.98
1820	Golf Courses	1,285	10,923	186,652	0.2	8	259	10.2	666	25,986	0.94	0.86
2100	Agriculture-Field Crops	24	204	3,031	0.2	6	274	10.8	13	382	0.94	0.87
2140	Agriculture-Row Crops	280	2,384	148,664	0.2	23	135	5.3	75	8,605	0.97	0.94
2150	Agriculture-Field Crops	1,040	8,839	131,266	0.2	4	271	10.7	563	10,693	0.94	0.92
2200	Agriculture-Tree Crops	2,436	20,703	465,266	0.2	10	287	**46***	1,006	237,060	0.95	0.49
2400	Agriculture-Nurseries	52	443	9,963	0.2	6	392	15.5	41	1,187	0.91	0.88
2410	Agriculture-Tree Nurseries	34	285	6,411	0.2	6	355	14.0	24	691	0.92	0.89
2420	Agriculture-Sod Farms	49	414	8,198	0.2	4	80	3.2	8	148	0.98	0.98
2430	Agriculture-Ornamentals	2,167	18,422	413,994	0.2	6	347	13.6	1,502	43,566	0.92	0.89
2450	Agriculture-Floriculture	9	72	1,434	0.2	6	221	8.7	4	109	0.95	0.92
2500	Agriculture-Specialty Farms	35	298	5,901	0.2	6	469	18.5	33	954	0.89	0.84
2300	Agriculture-Feeding Operations	66	45,658	505,002	0.2	18	257	10.1	34	77,278	0.94	0.85
2110	Agriculture-Improved Pasture	5,372	14,541	70,141	0.2	5.5	271	10.7	2,916	17,890	0.95	0.74
2120	Agriculture-Unimproved Pasture	1,711	11,287	54,441	0.2	5.5	197	7.8	675	20,006	0.93	0.63
2130	Agriculture-woodland pasture	1,328	558	272,287	0.2	5.5	284	11.2	755	3,003	0.94	0.99
2510	Agriculture-Horse Farms	871	7,403	81,876	0.2	5.5	257	10.1	447	11,857	0.94	0.86
2540	Agriculture-Aquaculture	6	52	0	0.2	6	469	18.5	6	168	0.89	n/a
1900	open land	1,150	9,778	0	0.2	0.2	206	8.1	475	0	0.95	n/a
2600	Other open lands rural	108	914	0	0.2	0.2	216	8.5	46	0	0.95	n/a
3000	Upland nonforested	6,921	58,831	0	0.2	0.2	192	7.6	2,655	0	0.95	n/a
4000	Upland forest	18,287	155,441	0	0.2	0.2	206	8.1	7,522	0	0.95	n/a
5000	Water Body	15,663	133,136	0	0.2	0.2	121	4.8	3,781	0	0.97	n/a
6000	Wetlands	21,094	179,303	0	0.2	0.2	54	2.1	2,278	0	0.99	n/a
7000	Barren Land	3,817	32,443	0	0.2	0.2	72	2.8	551	0	0.98	n/a
	Totals	123,313	1,048,157	3,823,832	0.2		194	7.6	47,348	571,617	0.95	0.85

* based on irrigation

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8 Appendix 3

Summary of Stormwater and Total Loads by Land Use/Land Cover

Land Use/Land Cover			Stormwater runoff					Background				
		Area	DCIA	EMC	Run-off	Load rate	Storm load	Run-off	Storm load	excess storm load	Total Background	Total Excess Load
LU Code	Descriptive	(ha)	(-)	(mg/L)	(in/yr)	(kg/ ha yr)	(kg/yr)	(in/yr)	(kg/yr)	(kg/yr)	(kg/yr)	(kg/yr)
1100	Low Density Residential	9168	0.3	2.29	10.3	6.0	54,742	3.1	9,140	45,602	14,394	74,498
1200	Medium Density Residential	17960	0.37	2.36	12.0	7.2	128,720	3.1	17,905	110,814	27,032	161,010
1300	High Density Residential	3155	0.71	2.42	20.2	12.4	39,110	3.1	3,145	35,965	4,829	45,228
1400+ 1480	Commercial and airports	3429	0.85	2.01	23.5	12.0	41,231	3.1	3,419	37,812	5,371	48,547
1500	Industrial	1099	0.85	1.79	23.5	10.7	11,766	3.1	1,096	10,670	1,790	10,670
1600	Extractive	257	0.85	1.79	23.5	10.7	2,750	3.1	256	2,494	464	2,494
1700	Institutional	1341	0.65	2.29	18.7	10.9	14,598	3.1	1,337	13,261	2,134	17,644
8100	Transportation	1414	0.85	1.79	23.5	10.7	15,137	3.1	1,409	13,728	2,141	17,749
8300	Utilities	942	0.85	1.79	23.5	10.7	10,087	3.1	939	9,148	1,426	11,827
1800	Recreational, Marinas and fish camps, swimming beaches	744	0.005	1.25	3.1	1.0	742	3.1	742	0	1,081	1,863
1820	Golf Courses	1285	0.17	2.32	7.1	4.2	5,396	3.1	1,281	4,115	1,947	30,101
2100	Agriculture-Field Crops	24	0.01	2.48	3.3	2.1	49	3.1	24	25	37	407
2140	Agriculture-Row Crops	280	0.01	2.68	3.3	2.2	622	3.1	280	343	355	8,948
2150	Agriculture-Field Crops	1040	0.01	2.52	3.3	2.1	2,171	3.1	1,037	1,134	1,600	11,827
2200	Agriculture-Tree Crops	2436	0.01	2.05	3.3	1.7	4,136	3.1	2,428	1,707	3,434	238,767
2400	Agriculture-Nurseries	52	0.01	2.3	3.3	1.9	99	3.1	52	47	93	1,235
2410	Agriculture-Tree Nurseries	34	0.01	2.3	3.3	1.9	64	3.1	33	30	57	721
2420	Agriculture-Sod Farms	49	0.01	2.3	3.3	1.9	93	3.1	49	44	56	192
2430	Agriculture-Ornamentals	2167	0.01	2.3	3.3	1.9	4,129	3.1	2,161	1,968	3,663	45,533
2450	Agriculture-Floriculture	9	0.01	2.3	3.3	1.9	16	3.1	8	8	12	117
2500	Agriculture-Specialty Farms	35	0.01	2.34	3.3	1.9	68	3.1	35	33	68	987
2300	Agriculture-Feeding Operations	66	0.01	78.23	3.3	64.8	4,251	3.1	65	4,186	99	7,189
2110	Agriculture-Improved Pasture	5372	0.01	2.48	3.3	2.1	11,033	3.1	5,355	5,678	8,271	82,956
2120	Agriculture-Unimproved Pasture	1711	0.01	2.48	3.3	2.1	3,514	3.1	1,706	1,808	2,381	19,698
2130	Agriculture-woodland pasture	1328	0.01	2.48	3.3	2.1	2,727	3.1	1,324	1,404	2,079	21,410
2510	Agriculture-Horse Farms	871	0.01	2.34	3.3	1.9	1,688	3.1	868	820	1,316	12,676
2540	Agriculture-Aquaculture	6	0.01	2.34	3.3	1.9	12	3.1	6	6	12	174
1900	open land	1150	0.005	1.25	3.1	1.0	1,147	3.1	1,147	0	1,621	0
2600	Other open lands rural	108	0.005	1.25	3.1	1.0	107	3.1	107	0	154	0
3000	Upland nonforested	6921	0.005	1.25	3.1	1.0	6,900	3.1	6,900	0	9,555	0
4000	Upland forest	18287	0.005	1.25	3.1	1.0	18,232	3.1	18,232	0	25,753	0
5000	Water Body	15663	0.275	1.25	9.7	3.1	48,047	9.7	48,047	0	51,828	0
6000	Wetlands	21094	0.275	1.6	9.7	3.9	82,827	9.7	82,827	0	85,105	0
7000	Barren Land	3817	0.005	1.25	3.1	1.0	3,805	3.1	3,805	0	4,356	0
	Totals	123313					520,016		217,165	302,850	264,514	874,468

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9 Appendix 4

Wastewater Inputs and Loads

Category	Units	#	Input/ unit (lbs/yr)	Input (MT/ year)	Input (tons/ year)	Assumed removal (-)	Load (tons/ year	Load (MT/ year)
OSTDS	Systems	55,417	29	730	804	0.4	482	438
WWTF (sewer)	GW discharge			72.6	80	0.4	48	44
	Surface Water discharge			28.8	32	0	32	29
	Reuse discharge			164.7	181	0.7	54	49
	Sum WWTF (sewer)			266	293		134	122

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Florida Passive Nitrogen Removal Study

Experimental Media Evaluation

Final Report

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Division of Environmental Health
Bureau of Onsite Sewage Programs
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4/28/2008

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Section 1 Project Description

The goal of the Florida Passive Nitrogen Removal Study is to evaluate methods that can be used to enhance nitrogen removal in onsite wastewater systems in a passive and cost effective manner. Task 2 of the study entails an experimental evaluation of candidate filter media that can be used to remove nitrogen from septic tank effluent (STE) in passive systems. The purpose of Task 2 is to perform small scale testing to identify candidate media and identify performance, longevity, and other factors pertinent to the design of passive systems and evaluation of their practicality and economics. The Task 2 study plan was described in detail in the Florida Passive Nitrogen Removal Study Laboratory Media Evaluation Quality Assurance Project Plan, finalized on 11/16/2007.

The *Florida Passive Nitrogen Removal Study Literature Review and Database, September 26, 2006,* proposed the development of a two stage filter system for passive removal of total nitrogen from septic tank effluent. The two stage system consisted of an initial unsaturated media filter for ammonification and nitrification, followed in series by a saturated anoxic denitrification filter. The system would be deployed between the septic tank and the soil treatment unit (drainfield) or soil dispersal system of new or existing facilities. Nitrogen in septic tank effluent would be substantially removed before wastewater was directed to the soil for treatment or dispersal.

Following the recommendations of the literature review, an experimental on-site wastewater treatment system was operated and monitored for sixty days to evaluate enhanced nitrogen removal. The purpose of the experimental investigation was to address key issues that have implications to process performance, design, feasibility, longevity, and economics.

This report describes the experimental configuration, filter media, and operation of the treatment systems, presents monitoring data, evaluates system performance, and makes recommendations based on the experimental results.

Section 2 Materials and Methods

A. Project Site

The experimental studies were conducted at Flatwoods Park, 18205 Bruce B. Downs Boulevard, Tampa FL 33592. The park is a day use public recreational facility operated by Hillsborough County. Wastewater is generated by two sources: a lavatory with two hand washing sinks and two flush toilets, and a continuously occupied single family home (ranger residence). The park was open for public use every day during the study period. Park visitation is highest on weekends and on weekday afternoons. Wastewater from the ranger residence and lavatory is collected in a septic tank before being pumped to a mounded onsite sewage and disposal system. The source water for the ranger residence and lavatory is municipal water supplied by the City of Tampa.

B. Experimental Treatment Systems

The filter media that were evaluated are listed in Table 1, along with the estimated bulk density and the range of particle sizes of the material as procured. Stage 1 media included clinoptilolite, expanded clay and tire crumb. These media provided substantial external porosity (> 45%), while clinoptilolite and expanded clay would be expected to exhibit desirable water retention characteristics. Additionally, the clinoptilolite media provides cationic ion exchange capacity (1.5 to 1.8 meq./g) which could enhance sorption and retention of ammonium ions. Tire chips are produced by the cutting up of recycled tires, and are available in particles sized of 5 mm and less that are suitable for use as filter media.

Clinoptilolite media was obtained by the supplier in three particle size gradations: 16x50, 8x16, and 4x8. The 16x50 was passed through a No. 35 mesh sieve to remove the smaller particles; materials retained on the screen were particles of 0.50 to 1.19 mm size. The 8x16 (1.19 to 2.38 mm) and 4x8 (2.38 to 4.76 mm) sizes were used as supplied. Each clinoptilolite size fraction was rinsed eight times before placement in the filter. Livlite and tire crumb media were prepared using dry sieving as follows. Media were initially sieved through a 5 mm square mesh wire screen to remove extraneous larger particles. Materials passing through the 5 mm screen were sieved through a 3 mm square mesh screen. Materials that were retained on the 3 mm screen composed the 3 to 5 mm size material that was used in the upper layer of the filter. Materials passing through the 3 mm screen were sequentially sieved through US Sieve Numbers 10, 18 and 35 (openings of 2.00, 1.00 and 0.500 mm), providing media of 1.0 to 2.0 mm size for the middle filter layer and 0.5 to 1.0 mm size for the lower filter layer. While filter media can be more completely characterized using particle size distribution analysis (PSD), effective diameter (D_{10}), and uniformity coefficient (D_{60}/D_{10}), these data were not available for the materials as procured nor obtained for the size fractions.

The Stage 2 electron donor media was elemental sulfur, which provided an autotrophic denitrification process in the anoxic filter. Crushed oyster shell was used as an alkalinity source, as sulfur-based autotrophic denitrification will consume alkalinity. Expanded shale was included in two Stage 2 columns and provided anion exchange capacity, which would sorb nitrate under non-steady operational conditions.

A schematic of the experimental filter columns is shown in Figure 1. Three filter systems were evaluated, each consisting of an unsaturated filter followed by a saturated filter. Filters were fabricated from PVC pipe, at 3 in. inner diameter for Stage 1 (unsaturated) filters and 1.5 in. inner diameter for Stage 2 (saturated) filters. A 1/8 inch square mesh screen was used for media support and retention at the outlet media end of each column.

A single peristaltic pump with three pump heads (Cole Parmer) was used to dose septic tank effluent to the three Two-Stage Filter systems. Each of the three Stage 1 filters was dosed with a separate pump head; each of the three pump heads was attached to the same pump. A single tube drew STE from an effluent intake manifold in the septic tank that screened STE through 1/16 in. slots. The STE tube branched into three separate tubes prior to the pump heads; each branch tube supplied one peristaltic pump head. The pump head size, dosing tube size, dosing time and pump speed were identical for all three Two-Stage Filter systems.

The media configuration in the six columns is listed in Table 2. Total media depth in the vertical unsaturated Stage 1 columns was 24 in. The Stage 1 filters employed a stratified media configuration, with particle sizes decreasing in the downward direction, with 2 in. of larger particle sized media on the bottom for particle retention. Stratification of media based on particle size was based on the expected progression of biochemical reactions within the filter media. The processes in the upper media layer include adsorption of wastewater particulates and colloids, hydrolysis and release of soluble organics, aerobic utilization of soluble organics, and biomass synthesis. In this region, the biochemical processing of organic matter between doses must keep up with the newly applied wastewater constituents from each

Table 1 Procured Filter Media

Material	Bulk density, lb/ft^3	Particle Size Range
Zeo-Pure AMZ Clinoptilolite	**55**	**0.3 - 4.76 mm**
Livlite Expanded Clay	**41**	**0.4 - > 5 mm**
Tire Crumb	**25**	**0.3 - > 5 mm**
Elemental sulfur	**77**	**2 - 5 mm**
Oyster shell	**82**	**3 - 15 mm**
ACT-MX ESF-450 Utelite	**54**	**0.4 - 4.5 mm**

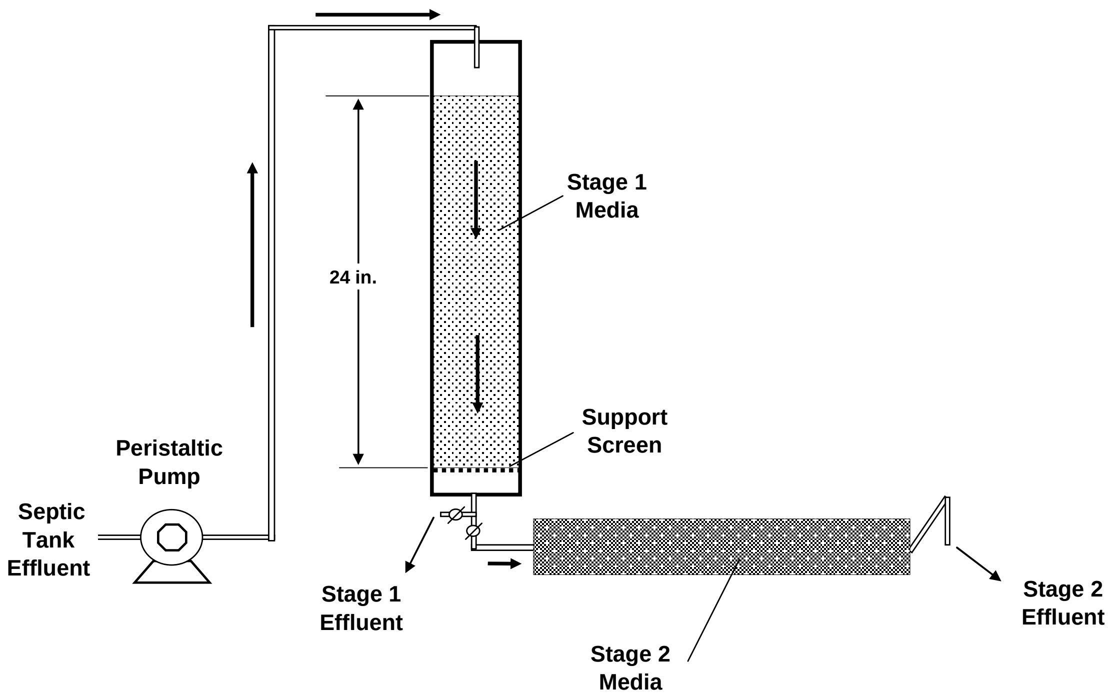

Figure 1 Experimental Filter System Schematic

dose. The greatest accumulation of organic and inorganic mass will occur in the upper layer, and the use of larger particle size media will provide greater space for accumulation of solids. Stratified media should enhance to potential for long term operation while maintaining treatment efficiency. The use of finer particle sizes in lower depths was intended to provide greater surface area for microbial attachment and a finer media for physical filtration, the later which could improve removal of pathogens and other wastewater constituents. The progression of coarser to finer media size through the filter was also intended to enable coarser media to filter out larger particulates and protect the finer media that follows.

Three Stage 2 columns were constructed using unstratified media containing elemental sulfur, crushed oyster shell, and expanded shale (Table 2) of 24 in. total media depth. Each filter contained a 3:1 vol/vol ratio of elemental sulfur to crushed oyster shell. The fraction of expanded shale in Stage 2 media ranged from 0 to 40%. Expanded shale contains anion exchange capacity which can bind nitrate ions, potentially enhancing removal. Higher expanded shale fractions were accompanied by lower elemental sulfur fractions, which would reduce the total surface area of elemental sulfur and possibly the overall sulfur oxidation rate. A lower sulfur oxidation rates could have the positive effect of reducing effluent sulfate levels if sulfur oxidation exceeded the amount needed for denitrification.

The Stage 1 filters were vertically oriented and Stage 2 filters oriented horizontally (Figure 1). The Stage 1 filters were supplied with septic tank effluent by a multi-head peristaltic pump with a timed dosing of once per one half hour (48 doses/day). Wastewater trickled downward through the Stage 1 media, through the support screen, and into a tube that directed Stage 1 effluent to the Stage 2 filter (Figure 1). The water elevation in the tube below the Stage 1 filter provided hydraulic head for passive movement of water through the Stage 2 filter. A valve and sample port (with another valve) was located in the tube below the Stage 1 filter. In normal filter operation, the sample port valve was closed and the valve leading to Stage 2 open, providing passive flow of Stage 1 effluent to and through the horizontal Stage 2 filter. The design of the two stage filter system minimized internal volume within the connecting piping; liquid volumes in the Stage 1 and Stage 2 filters comprised greater than 90% of the total internal volume.

C. Operation and Monitoring

Operation of the experimental treatment systems was commenced on 1/2/2008. The hydraulic loading rate to the Stage 1 filters was 3 gallons of septic tank effluent per square foot of surface area per day. To allow time for establishment of microbial activity, the systems were operated for three weeks before the first liquid samples were collected. Monitoring for wet chemistry parameters was conducted on five separate occasions, on days 22, 33/34, 42/43, 49/50, and 60/62.

Monitoring was conducted at seven monitoring points, consisting of influent septic tank effluent (STE), effluents from each Stage 1 filter, and effluents from each Stage 2 filter. Temperature, pH, and dissolved oxygen (DO) measurements were performed by inserting probes directly into the Stage 2 effluent port, into Stage 1 effluent collection reservoirs, and for STE in a 1 liter sample container immediately after collection. Sulfate and nitrogen samples from the effluents of Stage 1 and Stage 2 filters were collected by routing effluent from the filters directly into prepared sample containers located in an iced cooler. For STE, samples for sulfate, nitrogen, biochemical oxygen demand (BOD), and total suspended solids (TSS) were collected by directly filling prepared sample containers with pumped STE and immediately placing samples containers on ice in a cooler.

Monitoring was generally conducted in the following sequence:

- Stage 2 effluent: temperature, pH, DO, alkalinity
- Stage 2 effluent: sulfate sample collection
- Stage 2 effluent: nitrogen sample collection
- Stage 1 effluent: nitrogen sample collection
- Field blank: preparation
- STE and Stage 1 effluent: temperature, pH, DO, alkalinity
- STE: sulfate and nitrogen sample collection
- STE: BOD and TSS sample collection

Table 2 Configuration of Two Stage Filter Media

Stage	Filter	Column inner diameter, inch	Media depth, inch	Media placement	Media
Stage 1 unsaturated aerobic	1A	3.0	24.0	Stratified	**Clinoptilolite** depth (in.) / diameter (mm) / top 8 / 2.38 - 4.76 8 / 1.19 - 2.38 6 / 0.5 - 1.19 1 / 1.19 - 2.38 1 / 2.38 - 4.76 / bottom
	1B				**Expanded Clay** depth (in.) / diameter (mm) / top 8 / 3 - 5 8 / 1.0 - 2.0 6 / 0.5 - 1.0 1 / 1.0 - 2.0 1 / 3 - 5 / bottom
	1C				**Tire Crumb** depth (in.) / diameter (mm) / top 8 / 3 - 5 8 / 1.0 - 2.0 6 / 0.5 - 1.0 1 / 1.0 - 2.0 1 / 3 - 5 / bottom
Stage 2 saturated anoxic	2A	1.5	24.0	Nonstratified (1 - 3 mm)	75% elemental sulfur 25% oyster shell
	2B				60% elemental sulfur 20% oyster shell 20% expanded shale
	2C				45% elemental sulfur 15% oyster shell 40% expanded shale

D. Analytical Methods

Nitrogen and sulfate analyses were performed by a NELAC certified laboratory (ELAB Inc.). Total kjeldahl nitrogen was performed by digestion and colorimetric determination (EPA 351.2). Ammonia nitrogen was performed by semi-automated colorimetry (EPA 350.1). Nitrate plus nitrite nitrogen was performed by cadmium reduction and colorimetry (EPA 353.2). Sulfate was measured by anion chromatography (EPA 300.0). Quality assurance and control procedures were followed by ELAB Inc. For each sampling event, nitrogen analysis were performed on a field blank; the maximum field blank N value was 0.073 mg/L (App. A).

Temperature, pH and dissolved oxygen (DO) were measured using a Hach 40d multimeter with Intellical glass membrane probe and luminescent Dissolved Oxygen probe. Probes were calibrated according to manufacturer's instructions using three standard solutions (4,7,10) for pH, and for DO, an air saturated water solution and a zero DO (sodium sulfide) solution. The Hach LDO probe (LDO 10103) included a temperature sensor that performed automatic temperature compensation for DO. Total alkalinity was measured by titration with 1.6N sulfuric acid to a bromocresol green-methyl red endpoint.

Section 3 Results and Discussion

A. Applied Hydraulic Loading

Applied hydraulic loadings to Stage 1 filters are summarized in Table 3. Flowrates were measured by collecting and quantifying the cumulative liquid volume exiting the Stage 2 filters (i.e. the final effluent) over time periods of 15 to 40 hours and dividing volume by elapsed time. Hydraulic loading rates over the time of experimental operation are shown in Figure 2. Flowrates were fairly consistent, while the average flowrate to System 3 (tire crumb/45% sulfur media) was somewhat lower than that to Systems 1 and 2. The applied hydraulic loadings to the Stage 1 filters were reasonably close to the loadings that were targeted in the experimental design.

Table 3 Applied Hydraulic Loading Rate

System	Media	Average	Standard Deviation
		gal/ft^2-day	gal/ft^2-day
1	Clinoptilolite / 75% Sulfur	2.71	0.28
2	Expanded Clay / 60% Sulfur	2.95	0.20
3	Tire Crumb / 45% Sulfur	2.51	0.18

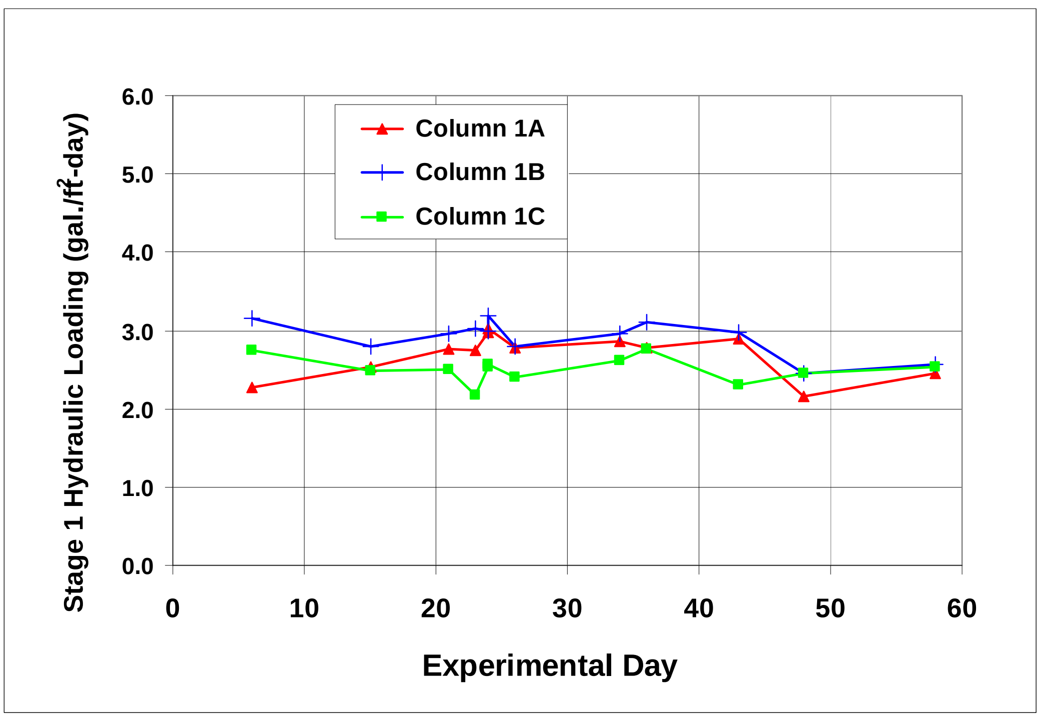

Figure 2 Hydraulic Loading Rate Applied to Stage 1 Filters

B. Septic Tank Effluent

Septic tank water quality parameters are summarized in Table 4. The average Total Nitrogen (TN) of 77.4 mg/L was somewhat higher than typical for single family residences. The high TN may be a reflection of the contribution of day users to the wastewater; typical short term visitation patterns may result in a relatively high proportion of urine which contains the majority of nitrogen in human waste. The nitrogen speciation results show that the majority of STE was ammonia, with the balance generally organic nitrogen. Single values for NH_3 (2.6 mg/L) and NO_x (21 mg/L) appear to be outliers, as suggested by examination of median values; inquiries with the analytical lab and reanalysis did not resolve this issue. The biochemical oxygen demand may be more characteristic of typical single family Florida residences. The TSS was relatively low, which may have reflected the passage of STE through the effluent screen.

Table 4 Septic Tank Effluent Quality
(all values in mg/L except pH)

System	Average	Standard Deviation	Median	Range	n
Total Nitrogen	77.4	6.2	78	69 - 85	5
Total Kjeldahl N	73.2	14.7	78	48 - 85	5
Organic Nitrogen	20.7	28.6	8	3.0 - 71	5
NH_3-N	52.5	30.2	70	2.6 - 74	5
NO_x-N	4.2	9.4	0.04	.028 - 21	5
Total Inorganic N	56.8	30.8	70	2.7 - 74	5
SO_4-S	23	4.7	22	17 - 29	5
C-BOD_5	203	71	190	140 - 180	3
TSS	18.7	5.5	16	15 - 25	3
Temperature	22.5	6.7	24	14.1-30.9	5
DO	0.008	0.1	0.00	0 - 0.02	5
pH	7.39	0.27	7.33	7.11 - 7.85	5
Alkalinity	416	157	455	140 - 381	5

C. Applied BOD and Nitrogen Loading

Applied loadings of BOD and Total Nitrogen (TN) are summarized in Table 5. The variations in applied loading of TN with time (Figure 3) reflect both the variations in flowrate applied to each system and variation in TN of the STE influent. The applied nitrogen loading was fairly consistent (Figure 3). The systems were initially operated for three weeks to allow microbial processes to become established; nitrogen was not monitored during this time.

Table 5 Applied BOD and Nitrogen Loading Rates

System	C-BOD$_5$ (n = 3)		Total Nitrogen (n = 5)		
	Average	Average	Average	Standard Deviation	Average
	gram/m^2-day	lbs./ft^2-day	gram/m^2-day	gram/m^2-day	lbs./ft^2-day
1	22.8	0.0064	8.68	0.70	0.0018
2	24.2	0.0068	9.23	0.74	0.0019
3	20.6	0.0058	7.83	0.63	0.0016

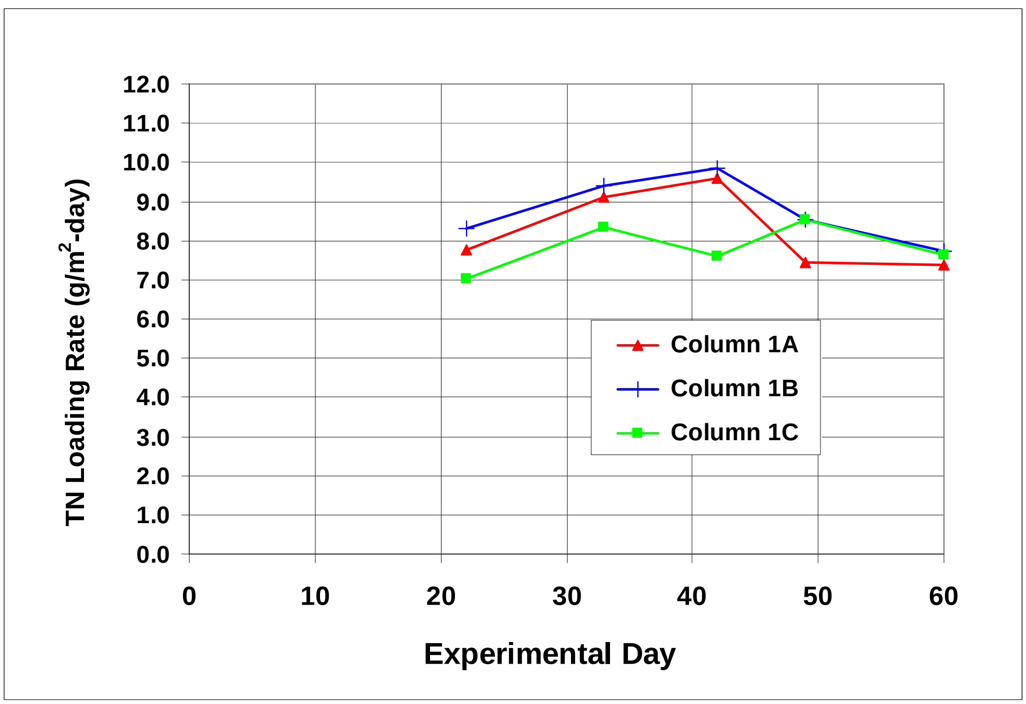

Figure 3 Rate of STE Total Nitrogen Applied to Stage 1 Filters

D. Performance of Two Stage Treatment Systems

Nitrogen species in the influents and effluents of each filter in the two stage systems are summarized in Table 6. Total Nitrogen (TN) removal efficiency of the two stage filter systems is plotted in Figure 4 and summarized in Table 7. TN removal efficiency of Systems 1 and 2 averaged greater than 97%, while System 3 averaged 33%. The low TN removal efficiency is System 3 was in large part because the tire crumb media was less effective in ammonia reduction than clinoptilolite and expanded clay media. Another factor contributing to lower TN removal efficiency of System 3 was the increase in effluent NO_x concentrations from System 3 Stage 2 that occurred as the test progressed. This is discussed below.

Stage 1 effluent ammonia nitrogen concentrations were less than 0.1 mg/l in filters with clinoptilolite and livelite media, and were often near levels of detection. Clinoptilolite and livelite are high water retention media compared to tire crumb, which exhibited higher average effluent ammonia levels (Table 6). It is noted that the average ammonia level for the clinoptilolite column was based on 4 sample results rather than 5. Column 1A effluent ammonia was quite high in the first sample event; this value was considered an outlier and was inconsistent with the fact that ammonia was completely absent in the effluent of anoxic Column 2A which followed the clinoptilolite column. The organic nitrogen in the final effluents from Systems 1 through 3 averaged 1.4 to 2.1 mg/L. For systems with highly efficient removal of Total Inorganic Nitrogen (TIN, the sum of ammonia and NO_x), the effluent nitrogen was dominated by the organic component. The average TIN removal efficiency of Systems 1 and 2 was 99.8 and 98.1 mg/L, and effluent nitrogen was predominantly organic N.

Average values of field monitoring parameters are summarized in Table 8. Each of the three Stage 1 media were effective at increasing dissolved oxygen (DO) from virtually zero in STE to over 7 mg/L in Stage 1 effluent (Figure 5). The low values of DO in Stage 1 effluents on Day 23 may have been due to some transient operational condition, perhaps associated with a characteristic of the feed (STE alkalinity was quite low on Day 23). Another possible explanation for low DO in Stage 1 effluent was the DO probe. Although the probe passed all manufacturer recommended testing and troubleshooting measures, it was replaced the following week. While wastewater DO was increased significantly by passage through the unsaturated Stage 1 filters, it was significantly reduced by passage through Stage 2 media (Figure 6). The change in pH in Stage 1 filters appears to be associated with the process of biochemical nitrification, which consumes 4.57 mg/l alkalinity as $CaCO_3$ per gram ammonia nitrogen nitrified. The highest alkalinity reduction through Stage 1 filters is for Column 1B, which also has the greatest decline in pH. Additionally, Column 1B was highly efficient in removing ammonia. Another factor that affects alkalinity is denitrification. Average NO_x concentrations were lower in Column 1A (clinoptilolite) than in Column 2A (livelite). Trends in average alkalinity changes in Stage 1 filters show some consistency with nitrogen transformation trends.

The STE alkalinity of 1/26/2008 was 140 mg/L (alkalinity/TN of 2.0) and resulted in an effluent alkalinity of zero in all three Stage 1 filters. These zero alkalinity numbers were included in the average values presented in Table 8. The alkalinity results of the 1/26/2008 sampling appear to be valid, and may offer one explanation of high Stage 1 effluent ammonia levels at 1/26/2008. If alkalinity in STE is low, nitrification in Stage 1 filters may be inhibited. In this study, the alkalinity/total nitrogen in STE averaged 5.3 and was greater than the required stoichiometric ratio of 4.57 in all but the first sample event. Nevertheless, alkalinity has the potential to change significantly. The implication to passive nitrogen removal systems are the possible need to supplement alkalinity for one pass systems when the alkalinity/total nitrogen ratio in STE is low. The use of recycle around Stage 1 is also a logical approach that can be integrated into passive nitrogen removal systems.

Table 6 Nitrogen Species In Filter Influents and Effluents
(Average of n=5; all values in mg/L)

Sample Point	Total Nitrogen	Total Kjeldahl Nitrogen	Organic N	NH_3-N	NO_x-N	Total Inorganic Nitrogen
Influent (STE)	77.4	73.2	20.7	52.5	4.2	56.8
Stage 1 Effluent						
1A Clinoptilolite	35.2	8.9	2.2	0.1*	26.3	33.0
1B Expanded clay	56.2	1.0	0.9	0.1	55.2	55.3
1C Tire crumb	65.4	29.0	2.4	26.6	36.4	63.0
Stage 2 Effluent						
2A 75% Sulfur	2.2	2.2	2.1	0.11	0.03	0.14
2B 60% Sulfur	2.1	2.0	1.4	0.61	0.02	0.63
2C 45% Sulfur	43.9	36.6	1.8	34.8	7.3	42.1

*n=4

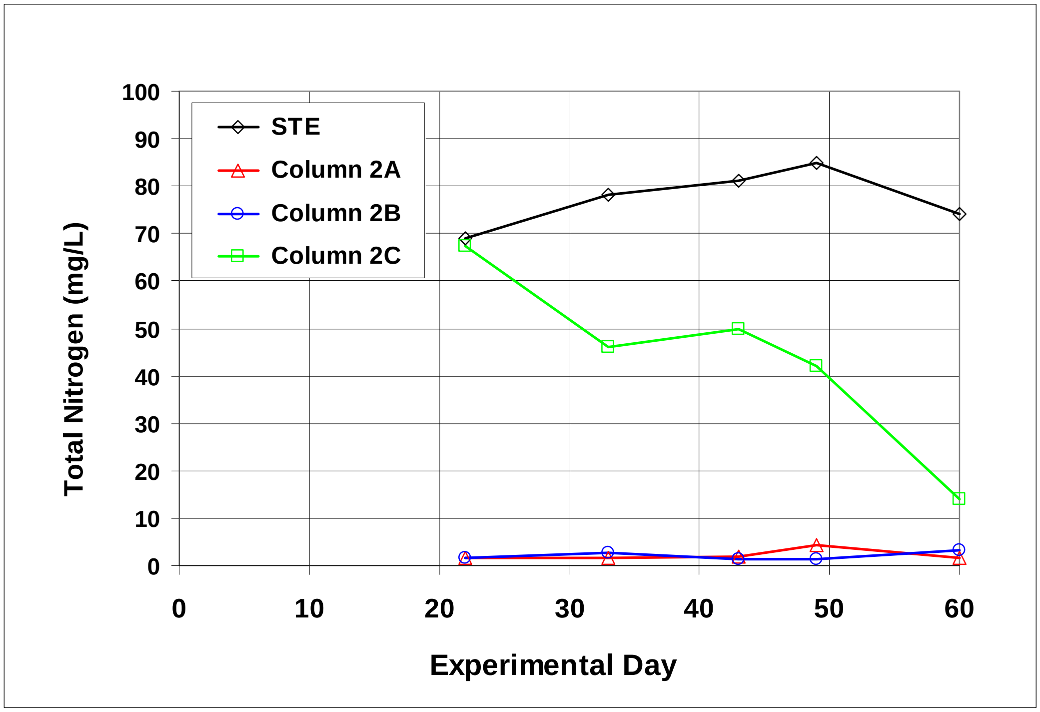

Figure 4 Total Nitrogen in Influent STE and Effluent of Two Stage Filter Systems

Table 7 Two Stage Treatment System Nitrogen Removal Efficiency
(n=5)

System	Media	Total Nitrogen		Total Inorganic Nitrogen	
		Average	Range	Average	Range
1	Clinoptilolite / 75% Sulfur	97.1	94.9 - 97.9	99.8	99.7 - 99.9
2	Expanded Clay / 60% Sulfur	97.7	96.6 - 98.6	98.1	97.5 - 98.7
3	Tire Crumb / 45% Sulfur	33.0	2.2 - 50.6	34.4	2.0 - 52.5

Table 8 Field Parameters In Filter Influents and Effluents
(Average of n=5)

Sample Point	Dissolved Oxygen, mg/L	pH	Alkalinity, mg/L as $CaCO_3$	Alkalinity Change, mg/L as CaCO3
Influent (STE)	0.008	7.49	416	-
Stage 1				
1A Clinoptilolite	7.28	7.65	283	-133
1B Expanded clay	7.27	7.22	86	-330
1C Tire crumb	7.10	7.42	178	-238
Stage 2				
2A 75% Sulfur	0.06	7.02	437	+154
2B 60% Sulfur	0.05	6.97	225	+139
2C 45% Sulfur	0.93	7.25	294	+116

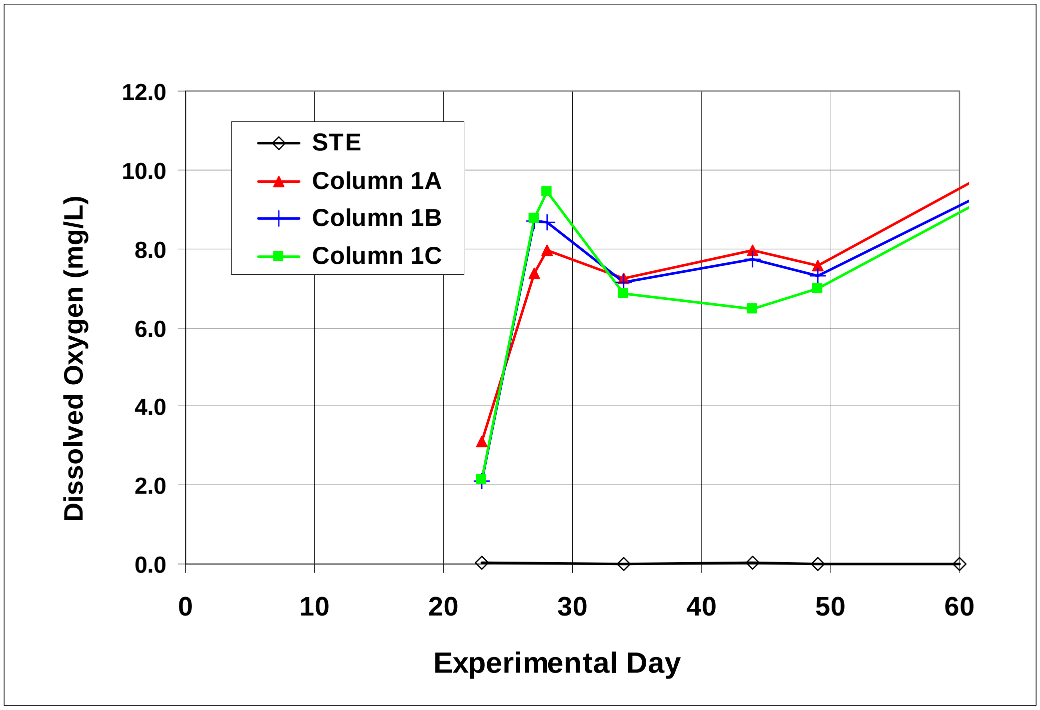

Figure 5 Dissolved Oxygen in Effluent of Unsaturated Filters (Stage 1)

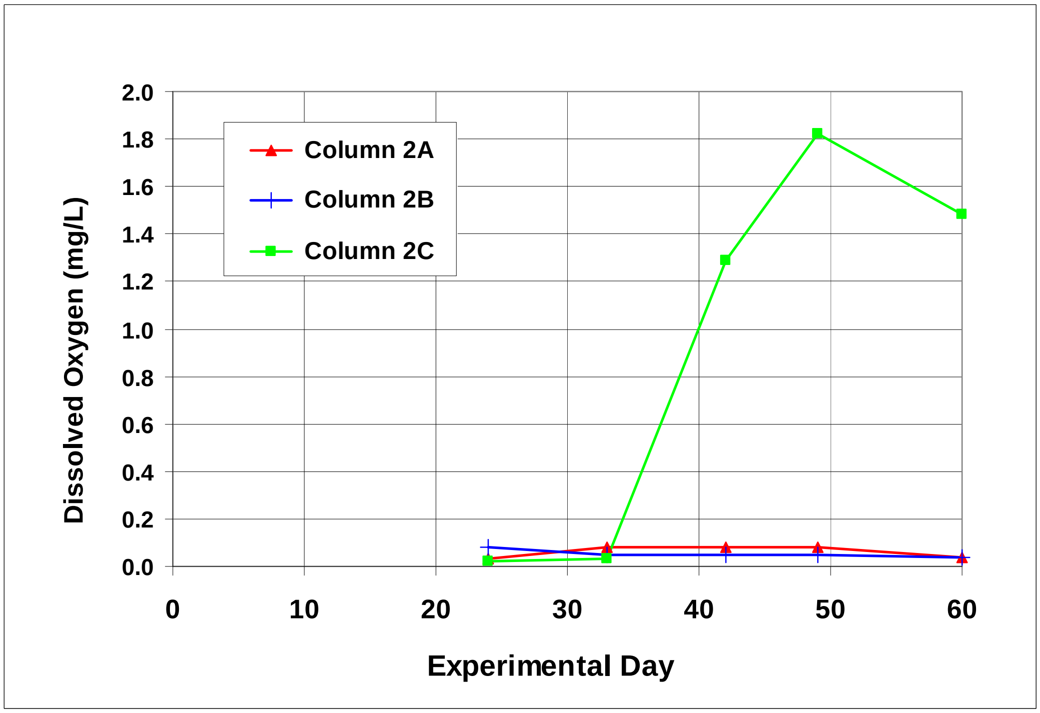

Figure 6 Dissolved Oxygen in Effluent of Denitrification Filters (Stage 2)

E. Performance of Unsaturated Aerobic Filters (Stage 1)

Nitrogen removal performance of the aerobic, unsaturated (Stage 1) filters is summarized in Table 9. Average TN removal was 50% for clinoptilolite media, which was a quite good and unexpected performance considering that the filter operated as single pass at a loading of 2.7 gal./ft^2-day. The expanded clay media was virtually as efficient as clinoptilolite at ammonia and TKN removal but less efficient at NO_x removal. The tire crumb was less efficient than clinoptilolite of expanded clay at ammonia removal, but performance appeared to be improving as the study progressed (Figure 7).

F. Performance of Anoxic Denitrification Filters (Stage 2)

The performance of the three denitrification (Stage 2) filters is illustrated in Figures 8 and 9. For System 1 (clinoptilolite / 75% sulfur) and System 2 (expanded clay/ 60% sulfur), TN removal efficiencies are high and and Stage 2 (final effluent) NO_x concentrations are low. System 3 showed a lower TN removal efficiency, which is partly due to higher Stage 2 effluent NO_x (Figure 8). The System 3 Stage 2 had the lowest fraction of sulfur (45%) of the three Stage 2 filters, which reduced the total sulfur surface area in the filter and may have resulted in insufficient dissolution of electron donor source or contact with wastewater fluid parcels with reactive surfaces. The release of inhibitory materials from the tire media in Column 1C is another possibile explanation for lower NO_x removal efficiency of Column 2C, although this is purely speculative. Dissolved oxygen increased significantly over time of operation in the effluent of the sulfur filter (Column 3B) that followed the tire crumb media. DO remained at levels below 0.1 mg/l in the anoxic filters following clinoptilolite and livelite media. In a sulfur based denitrification filter, consumption of influent DO would be expected to occur preferentially to denitrification. A biochemical equation developed from reaction stoichiometry and energetics indicates that 0.82 grams S are required per removal of 1 gram oxygen, or 5.7 mg/L sulfur for a 7 mg/L influent DO. Although ample sulfur is present in all Stage 2 filters to react with influent DO, the reasons that the one sulfur filter did not reduce DO to very low levesl cannot be explained. More research is needed into sulfur based denitrification filter design and extended operation.

Table 9 Stage 1 Nitrogen Removal Efficiency
(n=5)

System	Media	Total Nitrogen		Ammonia Nitrogen	
		Average	Range	Average	Range
1	Clinoptilolite	50.6	18.8 - 88.1	99.9*	99.9 - 99.9
2	Expanded Clay	26.1	10.2 - 32.9	99.9	99.5 - 99.9
3	Tire Crumb	13.0	0 - 28.2	60.5	35.1 - 87.7

*n=4

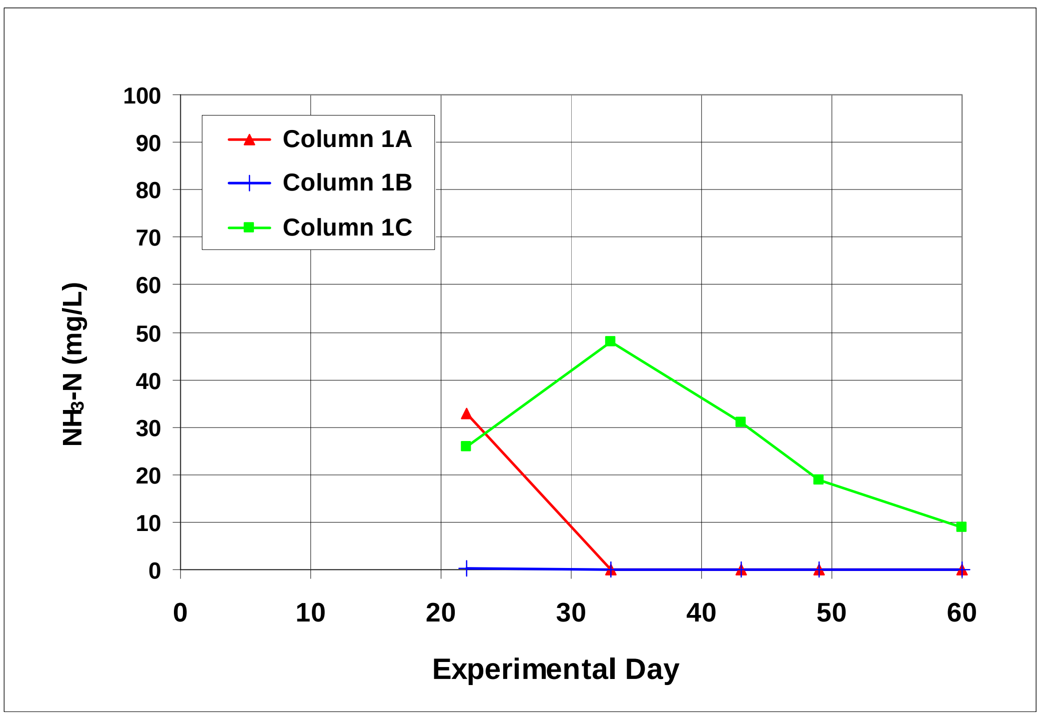

Figure 7 Effluent Ammonia from Unsaturated (Stage 1) Filters

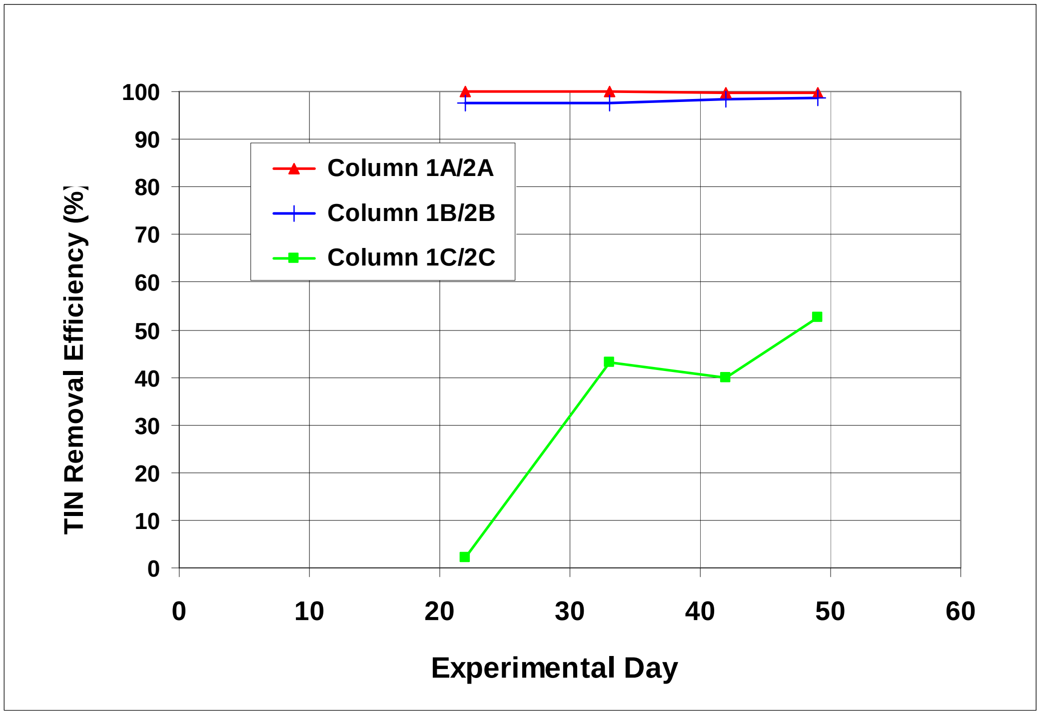

Figure 8 Total Inorganic Nitrogen Removal Efficiencies Two Stage Systems

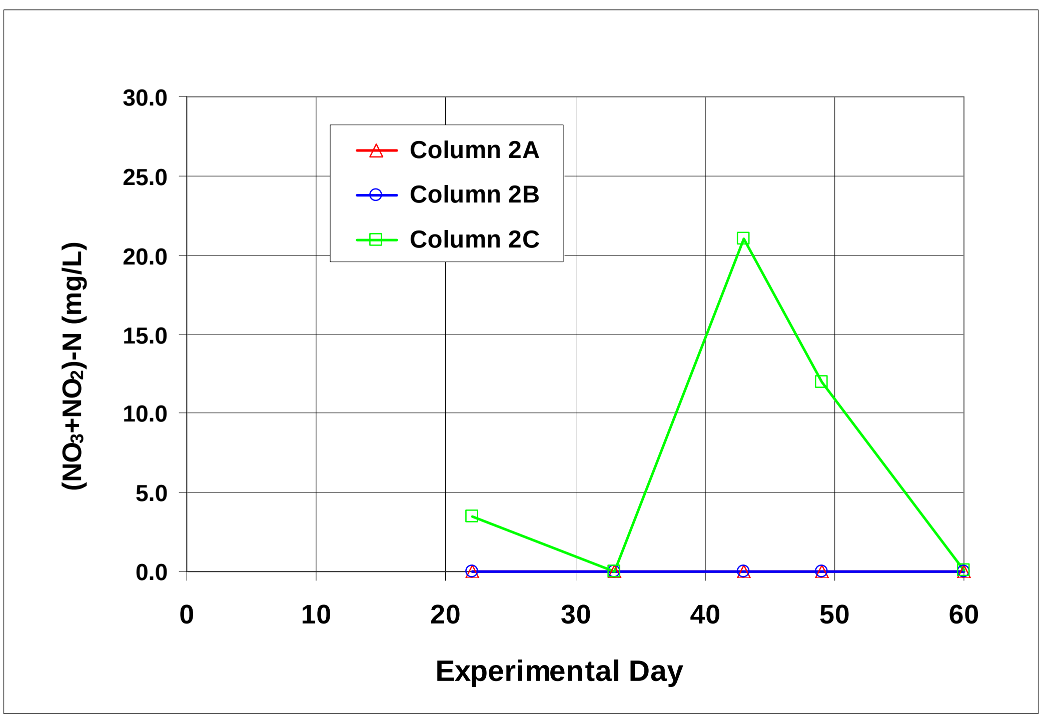

Figure 9 NO_x Concentrations in Stage 2 Filter Effluents

Section 4 Conclusions and Recommendations

Three two stage experimental wastewater systems using media filters were operated in a passive mode to treat septic tank effluent. The systems used no aerators, a single wastewater pump, and otherwise operated in passive mode. The following conclusions are based on five monitoring events conducted over the sixty day experimental period:

- Average hydraulic loading rates of septic tank effluent applied to Stage 1 filters with clinoptilolite, expanded clay, and tire crumb media were 2.71, 2.95 and 2.51 gallons per square foot per day, respectively.
- Average total nitrogen (TN) loading rates were 8.7, 9.2 and 7.8 grams per square meter per day to Stage 1 filters with clinoptilolite, expanded clay, and tire crumb media, respectively.
- The total nitrogen in septic tank effluent averaged 77.4 mg/L with a standard deviation of 6.2 mg/L.
- Septic tank effluent carbonaceous five day biochemical oxygen demand and total suspended solids averaged 203 and 18.7 mg/L, respectively.
- Total Nitrogen (TN) removal efficiencies for Two Stage Systems averaged 97.1, 97.7 and 33.0%, respectively, for clinoptilolite, expanded clay and tire crumb media.
- Effluent Total Nitrogen (TN) concentrations for Two Stage Systems averaged 2.2, 2.1, and 43.9 mg/L, respectively, for clinoptilolite, expanded clay and tire crumb media.
- Total Inorganic Nitrogen (TIN) removal efficiencies for Two Stage Systems averaged 99.8, 98.1, and 34.4 %, respectively, for clinoptilolite, expanded clay and tire crumb media.
- Effluent Total Inorganic Nitrogen (TIN) concentrations for Two Stage Systems averaged 0.14, 0.63 and 42.1 mg/L, respectively, for clinoptilolite, expanded clay and tire crumb media.
- Total Nitrogen (TN) removal efficiencies for Stage 1 Systems averaged 50.6, 26.1, and 13.0%, respectively, for clinoptilolite, expanded clay and tire crumb media.
- Effluent Total Nitrogen (TN) concentrations for Stage 1 Systems averaged 35.2, 56.2 and 65.4 mg/L, respectively, for clinoptilolite, expanded clay and tire crumb media.
- Ammonia Nitrogen (NH_3-N) removal efficiencies for Stage 1 Systems averaged 99.9, 99.9, and 60.5%, respectively, for clinoptilolite, expanded clay and tire crumb media.
- Ammonia Nitrogen (NH_3-N) concentrations for Stage 1 Systems averaged 0.11, 0.61 and 34.8 mg/L, respectively, for clinoptilolite, expanded clay and tire crumb media.
- Average dissolved oxygen in unsaturated (Stage 1) effluents were 7.28, 7.27 and 7.10 mg/L, respectively, for clinoptilolite, expanded clay and tire crumb media.
- Average dissolved oxygen in anoxic filter effluents (Stage 2) were 0.06, 0.05 and 0.93 mg/L, respectively, for sulfur media percentages of 75, 60, and 45%.
- For systems 1,2 and 3, the average decline in total alkalinity as $CaCO_3$ in aerobic filters (Stage 1) was 133 to 330 mg/L, while alkalinity increase in anoxic filters (Stage 2) was 116 to 154 mg/L.
- Clinoptilolite and expanded clay appear to be suitable media for full scale application.

The results from the passive nitrogen removal experimental study suggest that the innovative designs that were employed have potential to be developed into full scale passive nitrogen treatment systems. However, the scope of the ongoing study is quite limited and does not provide the basis for rational engineering design. Significantly more data is needed in order to rationally formulate engineering options for passive nitrogen removal systems. Additional studies are needed to address key issues that have direct implications to process performance, design, feasibility, longevity, and economics.

Based on the results of this study, it is recommended that additional studies be conducted to provide data for rational design of a full scale on-site wastewater treatment process to demonstrate enhanced nitrogen removal using a passive system. Additional studies include:

- **Extend Operation of Current Systems** The time of operation of the experimental systems (60 days) is insufficient and should be extended. Biological systems typically need extended times to fully adapt and establish. Extended operation would provide a longer operational period to ascertain TKN and ammonia removal performance in Stage 1 filters, denitrification that is achieved in Stage 1 single pass filters, and NO_x removal in Stage 2 filters.

- **Operation of Current Systems at Higher Loading Rates** The performance results of the Stage 1 filters with zeolite (Column 1A) and livelite (Column 1B) media suggest that these filters could be operated at higher loading rates. The design loading rate has important implications for Stage 1 filter size, required media volume, and system costs.

- **Operate Unsaturated (Stage 1) Filters in Recycle Mode** Recycle of effluent from the unsaturated filter would and mixing with untreated septic tank effluent would increase total nitrogen reduction in Stage 1 by pre-denitrification. The nitrate loading to the anoxic denitrification filter would decrease, affecting the Stage 2 design and media life.

- **Monitoring of Anoxic Denitrification Filters** The operation of anoxic denitrification filters should be examined through more detailed analysis of nitrate levels, including profiles through the columns. Additional analyses for sulfate, pH, alkalinity, and dissolved oxygen should be used to increase understanding of these filters for more rationally based designs as Stage 2 filters.

- **Examine Other Treatment Aspects** Additional treatment issues should be examined, including removal of biochemical oxygen demand, suspended solids, and pathogens, additional types of media, and residuals management.

Appendix A

NELAC Certified Laboratory Water Quality Data

Sample Event 1

Sample Point	Influent	System 1		System 2		System 3		Field blank
		Stage 1 Effluent	Stage 2 Effluent	Stage 1 Effluent	Stage 2 Effluent	Stage 1 Effluent	Stage 2 Effluent	
TKN	48	37	1.5	1.6	1.7	29	64	0.046
NH_3	45	33	0.02	0.23	0.092	26	68	0.020
(NO_3+NO_2)-N	21	19	0.022	47	0.020	48	3.5	0.042
SO_4	88	-	230	-	810	-	150	-
C-BOD_5	-	-	-	-	-	-	-	-
TSS	-	-	-	-	-	-	-	-

Sample Event 2

Sample Point	Influent	System 1		System 2		System 3		Field blank
		Stage 1 Effluent	Stage 2 Effluent	Stage 1 Effluent	Stage 2 Effluent	Stage 1 Effluent	Stage 2 Effluent	
TKN	78	1.7	1.6	1.2	2.6	52	46	0.046
NH_3	70	0.020	0.062	0.022	0.63	48	42	0.020
(NO_3+NO_2)-N	0.039	33	0.024	52	0.019	12	0.027	0.046
SO_4	52	-	430	-	570	-	160	-
C-BOD_5	280	-	-	-	-	-	-	-
TSS	15	-	-	-	-	-	-	-

Sample Event 3

Sample Point	Influent	System 1		System 2		System 3		Field blank
		Stage 1 Effluent	Stage 2 Effluent	Stage 1 Effluent	Stage 2 Effluent	Stage 1 Effluent	Stage 2 Effluent	
TKN	81	1.7	1.9	0.8	1.4	35	29	0.07
NH_3	74	0.042	0.089	0.020	0.2	31	26	0.020
(NO_3+NO_2)-N	0.028	47	0.026	72	0.021	32	21	0.054
SO_4	61	-	590	-	720	-	220	-
C-BOD_5	190	-	-	-	-	-	-	-
TSS	16	-	-	-	-	-	-	-

Sample Event 4

Sample Point	Influent	System 1		System 2		System 3		Field blank
		Stage 1 Effluent	Stage 2 Effluent	Stage 1 Effluent	Stage 2 Effluent	Stage 1 Effluent	Stage 2 Effluent	
TKN	85	2.5	4.3	0.046	1.2	19	30	0.06
NH_3	71	0.072	0.24	0.097	0.12	19	27	0.020
(NO_3+NO_2)-N	0.04	7.6	0.03	57	0.017	42	12	0.073
SO_4	78	-	590	-	620	-	200	-
C-BOD_5	-	-	-	-	-	-	-	-
TSS	-	-	-	-	-	-	-	-

Sample Event 5

Sample Point	Influent	System 1		System 2		System 3		Field blank
		Stage 1 Effluent	Stage 2 Effluent	Stage 1 Effluent	Stage 2 Effluent	Stage 1 Effluent	Stage 2 Effluent	
TKN	74	1.4	1.6	1.3	3.3	10	14.0	0.046
NH_3	2.6	0.053	0.14	0.041	2.0	9	11.000	0.020
(NO_3+NO_2)-N	0.051	25	0.035	48	0.028	48	0.066	0.040
SO_4	66	-	510	-	630	-	350	-
C-BOD_5	140	-	-	-	-	-	-	-
TSS	25	-	-	-	-	-	-	-

Florida Passive Nitrogen Removal Study

Economic Analysis of Passive Nitrogen Removal Systems

Draft Report

Prepared for:

Florida Department of Health
Division of Environmental Health
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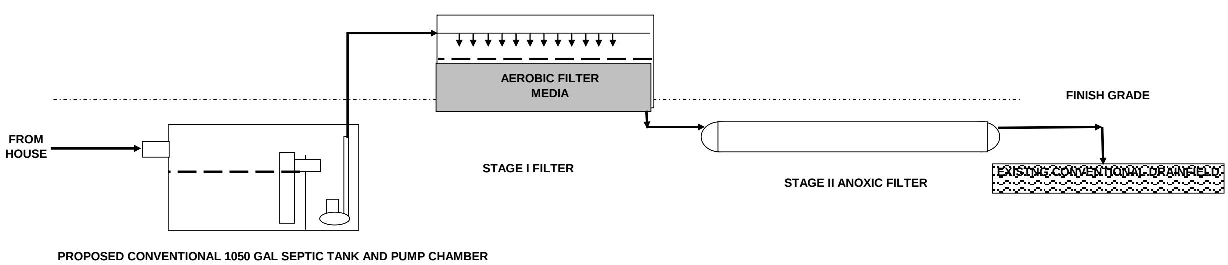
Economic Analysis Objectives The objective of economic analysis is to provide an equitable evaluation of the cost of alternative passive nitrogen removal systems over their entire life. A Life Cycle Cost Analysis (LCCA) was applied (Fuller and Peterson, 1995). LCCA entails estimating all present costs and future costs over the useful life of the system (project life). The LCCA included costs for:

- Equipment, materials, and installation
- Energy, scheduled maintenance, and monitoring
- Media replacement and residuals management
- Salvage and decommissioning values at the end of the project life.

In the LCCA methodology, all present and future costs are combined using the standard accounting techniques of Present Worth (PW) and Uniform Annual Cost (UAC). For the LCCA evaluation of passive nitrogen systems, a 30 year project life, 4% discount rate, and current ENR published cost factors (ENR, 2008) were used to determine the PW and UAC for each alternative configuration.

Design Criteria To perform a Life Cycle Cost Analysis of passive nitrogen removal systems, it is necessary to specify key design criteria. The experimental evaluation that was conducted in Task 2 of the Florida Passive Nitrogen Removal Study provided the basis for design alternatives. The Task 2 experiments evaluated three two stage nitrogen removal systems, provided proof of concept of the passive, two stage nitrogen removal process, and confirmed that high nitrogen reductions could be obtained. A definition sketch of two stage passive nitrogen removal technology for onsite wastewater treatment is shown in Figure 1.

Figure 1 Passive Nitrogen Removal System Schematic



Though the experimental investigations confirmed the ability to reduce nitrogen in passive two stage systems, the scope of the Task 2 studies was limited and did not support a definitive design for a Passive Nitrogen Removal System. Key design features that were not fully delineated in the Task 2 experimental studies include:

- sizing of the aerobic unsaturated Stage 1 filter
- Stage 1 filter media
- media composition of anoxic denitrification filter (Stage 2)

- sizing of the anoxic denitrification filter
- Stage 2 media replacement interval

To perform economic analysis, multiple alternative designs of two stage passive nitrogen removal systems were configured and LCCA was used to evaluate the alternative designs.

The application of passive nitrogen removal technology relies on use of readily available materials, labor skills, and minimal operational controls. The basic design elements of primary treatment, pump, dosing and the Stage 1 filter are shown in Figure 2. The system requires installation of a conventional septic tank and pump chamber with 1 day pump holding capacity. For this evaluation, conventional pre-cast concrete septic tanks, pumps, effluent filters, readily available HDPE Drainage piping products and a conventional drain-field were considered for determining costs. The cost analysis did not include any specialty landscaping or other improvements for the system. It should be noted that the researchers believe that other non-conventional equipment and materials may be incorporated within the design elements to allow for a more site specific customized treatment system. These elements may be defined as more research results and materials investigations are completed.

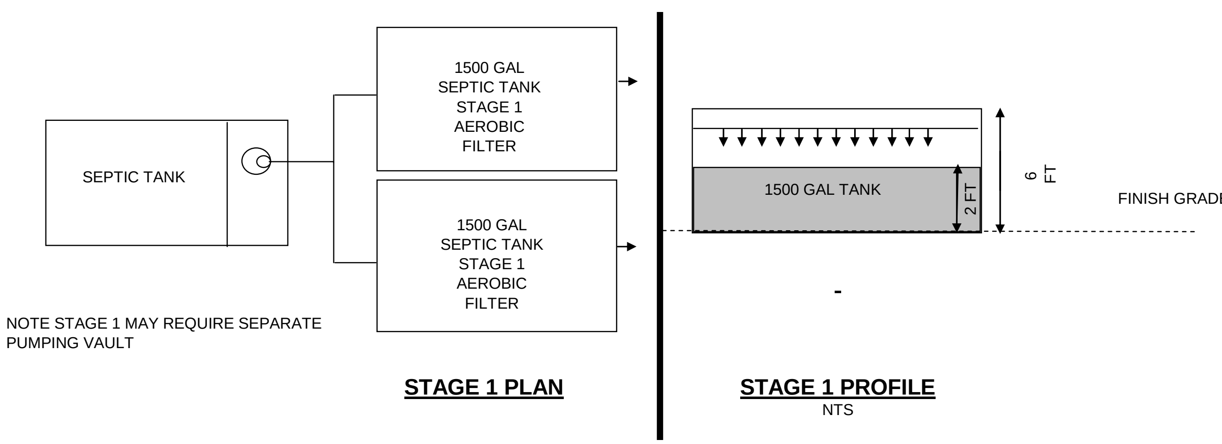


Figure 2 Basic Design Elements of Primary Treatment and Stage 1 Filter

Design of the Stage 2 filter was evaluated based on the anticipated life expectancy of the media and filter size. Several combinations of Stage 2 filter size and media replacement intervals were evaluated. The basic design elements for one Stage 2 filter alternative are shown in Figure 3.

Life Cycle Cost Analysis The Life Cycle Cost Analyses (LCCA) were performed for 12 candidate design configurations of differing Stage 1 filter size, Stage 1 media, and Stage 2 filter size. The candidate designs included all possible combinations of the three individual factors as listed in Table 1. The listed options were considered reasonable and feasible based on the results of the Task 2 experiments. All designs were based on a single family residence with a flowrate of 300 gallons per day. Present worth and annualized cost estimates were prepared for each case. A 30 year project life and a 4% annual discount rate were used in all analyses.

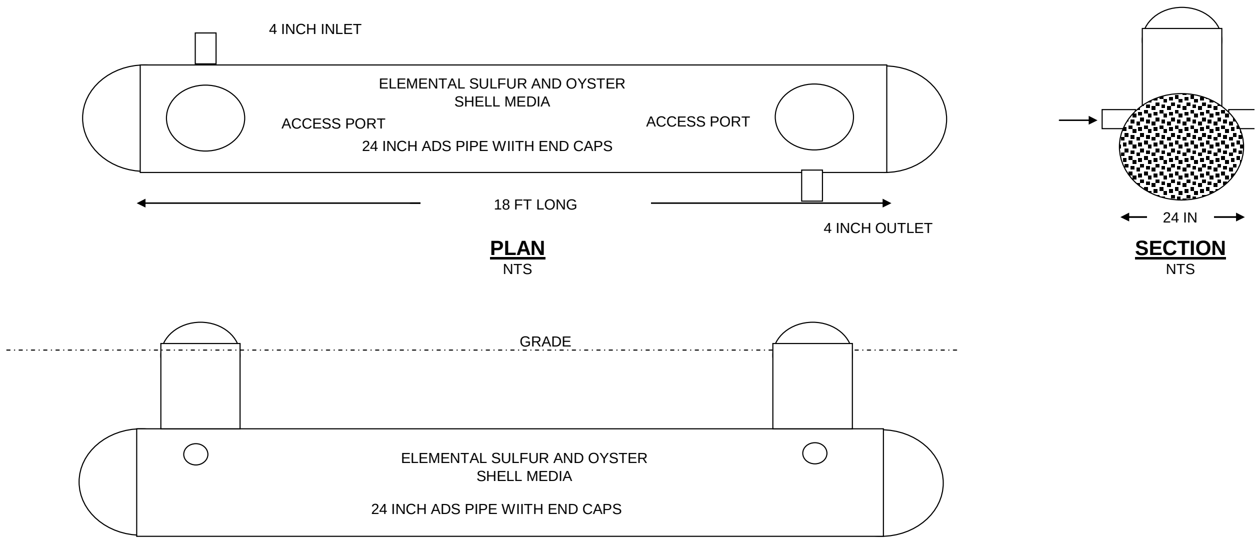


Figure 3 Basic Design Elements of Stage 2 Filter

Table 1 Design Factor Options of Alternatives

Stage 1 Filter Plan Area, ft^2

- 100
- 75

Stage 1 Media

- Clinoptilolite
- Expanded clay

Stage 2 Filter volume/media replacement interval

- 750 gallon, 15 year
- 375 gallon, 5 year
- 75 gallon, 1 year

The options for Stage 1 filter plan area and media are based on the experimental results (Task 2). Complete nitrification and high TKN removal were obtained with both clinoptilolite and livlite media at a hydraulic loading rate (HLR) of 3 gal/ft^2-day under timed pressure dosing of 48 times per day (30 minute cycle). Both clinoptilolite and livlite media are candidate media for Stage 1. The Task 2 experiments were of limited duration; it is difficult to discern a difference in performance between clinoptilolite and livlite in terms of ammonia and TKN removal. It is judged that, with either media, higher HLR could be applied to the Stage 1 filters while still achieving very high ammonia and TKN reductions. The Stage 1 filter area of 100 ft^2 provides 3 gal/ft^2-day, while the second option of 75 ft^2 filter area increases the average HLR to 4 gal/ft^2-day. While it may be possible to increase HLR even further, definitive design guidance should properly be based on actual operating data.

Stage 2 media composition was specified as sulfur and oyster shell at a 3:1 vol./vol. ratio, as was applied in all of the Stage 2 columns in the Task 2 experimental investigation. The economic analysis cases did not include utelite (expanded shale) in the Stage 2 filter media. Incorporating additional media components in any Stage 2 design reduces the space available for the reactive sulfur and theoretically reduces the run time. Since the experimental results did not provide definitive evidence of enhanced performance with the expanded shale component, utelite was not included. It should be noted that the Task 2 experiments were not designed to elucidate all possible operating conditions under which expanded shale could have advantageous properties. Longer term operation of treatment systems would be needed to fully explore this question.

Three options were specified for Stage 2 filter size/media replacement combinations (Table 1). Options range from low volume, frequent media replacement strategy to a designs with larger filter size, longer run time and longer media replacement interval. These options reflect the uncertainty in Stage 2 design due to the lack of demonstrated NO_x removal performance over extended time periods. The smaller filter design with frequent media replacement would be advantageous if performance deterioration was caused by factors such as preferential flow paths (channeling) or chemical precipitation which could occur in shorter times than the useful life of the media. In this case, the NO_x removal effectiveness of the Stage 2 filter would decline, even though the sulfur media was largely unused. Modular media replacement systems could be developed with perhaps renovation and reapplication of spent media.

Hardware Costs Costs for hardware were based on estimates of the installed costs of system components, including: primary treatment tank (i.e. septic tank), septic tank effluent pump, control system, pressure distribution system to Stage 1 filter, Stage 1 filter enclosure and underdrain, and Stage 2 filter enclosure. The estimated costs of treatment hardware are listed in Table 2.

Table 2 Estimated Costs of Treatment Hardware (2008$)

Item	Description	Cost/Unit	No Units	Total
PRIMARY SEPTIC TANK INSTALLATION				
1	1050 GALLON SEPTIC TANK/PUMP CHAMBER	$1,250.00	1	$1,250.00
2	EFFLUENT FILTER	$300.00	1	$300.00
3	PLUMBING	$500.00	1	$500.00
Subtotal				**$2,050.00**
STAGE I INSTALLATION COST				
4	STAGE I TANKS (2 -1050gal -100 SF)	$1,250.00	2	$2,500.00
5	PUMP TIMER	$300.00	1	$300.00
6	PLUMBING	$150.00	1	$150.00
Subtotal				**$2,950.00**
STAGE II INSTALLATION COST (15 YR OPTION)				
7	STAGE II PIPE TANKS (24IN X 18 FT)	$864.00	2	$1,728.00
8	ACCESSWAYS	$200.00	2	$400.00
9	PLUMBING	$300.00	1	$300.00
Subtotal				**$2,428.00**
STAGE II INSTALLATION COST (5 YEAR OPTION)				
10	STAGE II PIPE TANKS (18IN X 18 FT)	$576.00	2	$1,152.00
11	ACCESSWAYS	$200.00	2	$400.00
12	PLUMBING	$300.00	1	$300.00
Subtotal				**$1,852.00**
STAGE II INSTALLATION COST (1 YEAR OPTION)				
13	STAGE II PIPE TANKS (12IN X 18 FT)	$432.00	2	$864.00
14	ACCESSWAYS	$200.00	2	$400.00
15	PLUMBING	$150.00	1	$150.00
Subtotal				**$1,414.00**

Primary Treatment and Final Effluent Disposal All alternatives include identical primary treatment (septic tank) installation of $2,350 and identical final disposal system of a non-mounded drainfield that is gravity fed from the Stage 2 filter.

Media Costs Media costs were based on contacting media vendors and gathering data for at-dock media prices. Shipping costs were obtained from cost estimates provided by shipping firms for whole truckload quantities; whole truckloads would apply for the case of numerous installations of passive nitrogen removal in Florida. Costs were included for media size gradation. Media costs are summarized in Table 3.

Table 3 Estimated Costs of Filter Media (2008$)

Media	Cost at dock	Shipping[1]	Total	Bulk density
	$/lb.	$/lb.	$/lb.	lb/ft^3
Clinoptilolite	$0.25	$0.10	$0.35	55
Livelite	$0.05	$0.05	$0.10	41
Sulfur	$0.35	$0.05	$0.40	77
Oyster shell	$0.35	$0.05	$0.40	82

Operations and Maintenance Costs Operations and maintenance were:

- One maintenance site visit per year;
- One Stage 2 effluent monitoring per year;
- Power (Based on projected pump efficiency, power efficiency, and run times)
- Septic tank pumping, once per five years; and
- Cost of media replacement and residual management at specified intervals.

Estimated costs for operations and maintenance items are shown in Table 4. Energy use was estimated by assuming one ¾ hp pump operating for 2 minutes every one half hour. Stage 2 effluent monitoring included analyses for C-BOD_5, TKN, and NO_x.

Table 4 Estimated Costs of Operations, Maintenance and Stage 2 Media (2008 $)

Item	Unit Cost (2008 $)	Uniform Annual Cost[1] (2008 $)	Present Worth[1] (2008 $)
Operations and Maintenance			
Annual Maintenance, yearly	150	143	4,304
Monitoring Analyses, yearly	70	67	2,009
Electricity, yearly	124	119	3,558
Septic Tank Pumping, 5 year interval	225	43	1,284
Stage 2 Media Replacement[2]			
100 ft^3, once per 15 years	4,304	173	5,198
50 ft^3, once per 5 years	2,152	361	10,844
11 ft^3, once per year	473	452	13,573

[1] 30 year project life, i = 4%.

[2] Includes media, labor, materials, and spent media disposal at $0.05/lb.

LCCA Results The overall cost of the 12 alternatives was compared on a Present Worth (PW) and Uniform Annual Cost (UAC) basis. PW and UAC for the 12 alternatives are listed in Table 5 and plotted in Figures 4 and 5, respectively. The Uniform Annual Costs range from $1,754 to $2,250 (Figure 5). The UAC per volume of effluent treated is shown in Figure 6 and ranges between $0.016 to $0.021. A full summary if the LCCA is shown in Table 6.

The costs that were estimated by LCCA for passive nitrogen technology appear higher than costs that are often expected for onsite wastewater treatment systems. Economic analyses for onsite systems have often not applied full Life Cycle Cost Analysis. For comparative purposes, life cycle costs were estimated for an onsite system using a recirculating sand filter (RSF) with pressure dosing but without a Stage 2 denitrification filter. The RSF system employed a septic tank and drainfield and the cost analysis was otherwise comparable to the passive nitrogen removal systems. The average costs of the two stage passive nitrogen removal systems with clinoptilolite and livlite media are compared to the RSF system in Table 7. The RSF system without Stage 2 denitrification was 67 to 72% of the cost of the passive two stage nitrogen removal systems. The life cycle cost of the clinoptilolite systems was 8% greater than the livlite systems.

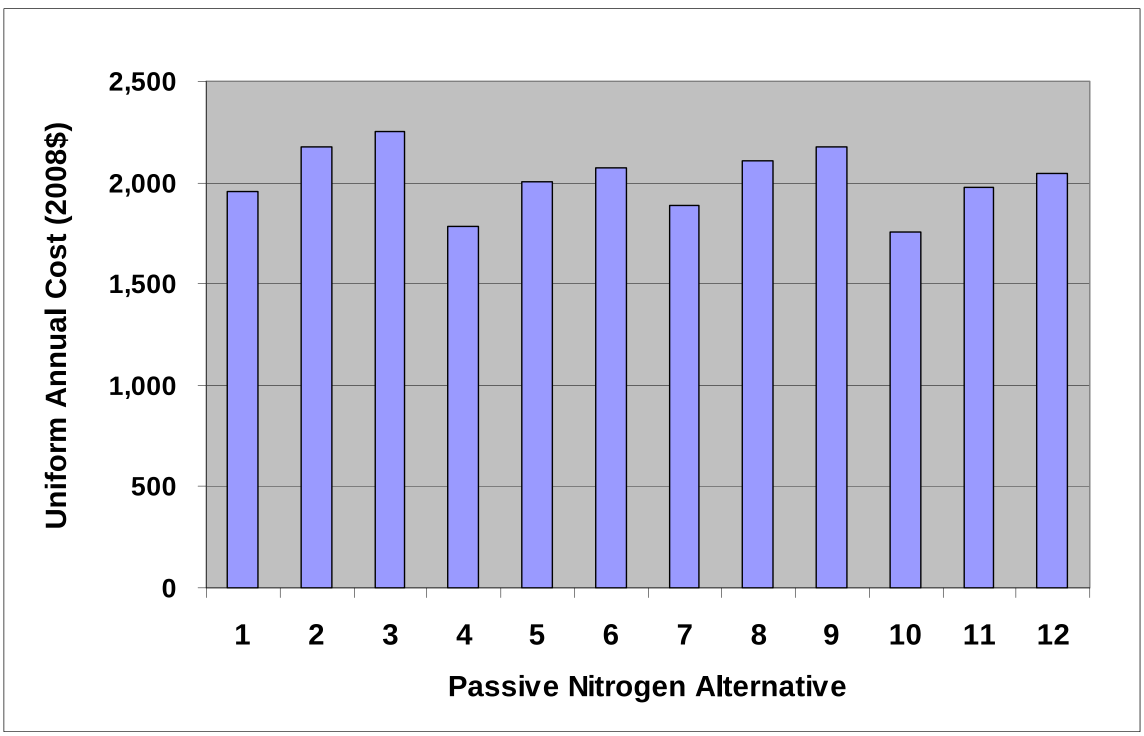


Figure 4 Uniform Annual Cost of Alternative Systems (30 year project life, i = 4%)

Table 5 Uniform Annual Cost and Present Worth of Alternatives[1]

SYSTEM ID	STAGE 1		STAGE 2		LIFE CYCLE COST 30 year, i=4%	
	Media	Plan Area	Media Volume	Replace	Uniform Annual Cost	Present Worth
	Type	ft^2	ft^3	Years	2008 ($)	2008 ($)
1	Clino	100	100	15	1,956	35,007
2	Clino	100	50	5	2,177	38,251
3	Clino	100	11	1	2,250	39,051
4	Clay	100	100	15	1,780	31,977
5	Clay	100	50	5	2,002	35,221
6	Clay	100	11	1	2,075	36,021
7	Clino	75	100	15	1,885	33,794
8	Clino	75	50	5	2,107	37,038
9	Clino	75	11	1	2,180	37,839
10	Clay	75	100	15	1,754	31,522
11	Clay	75	50	5	1,976	34,766
12	Clay	75	11	1	2,048	35,566

Notes: Stage 1 Media: Clino: Clinoptilolite AMZ Clay: Livlite Expanded Clay
Stage 2 Media: 3:1 Elemental Sulfur & Oyster Shell
Total System Costs includes base septic tank and drainfield installation

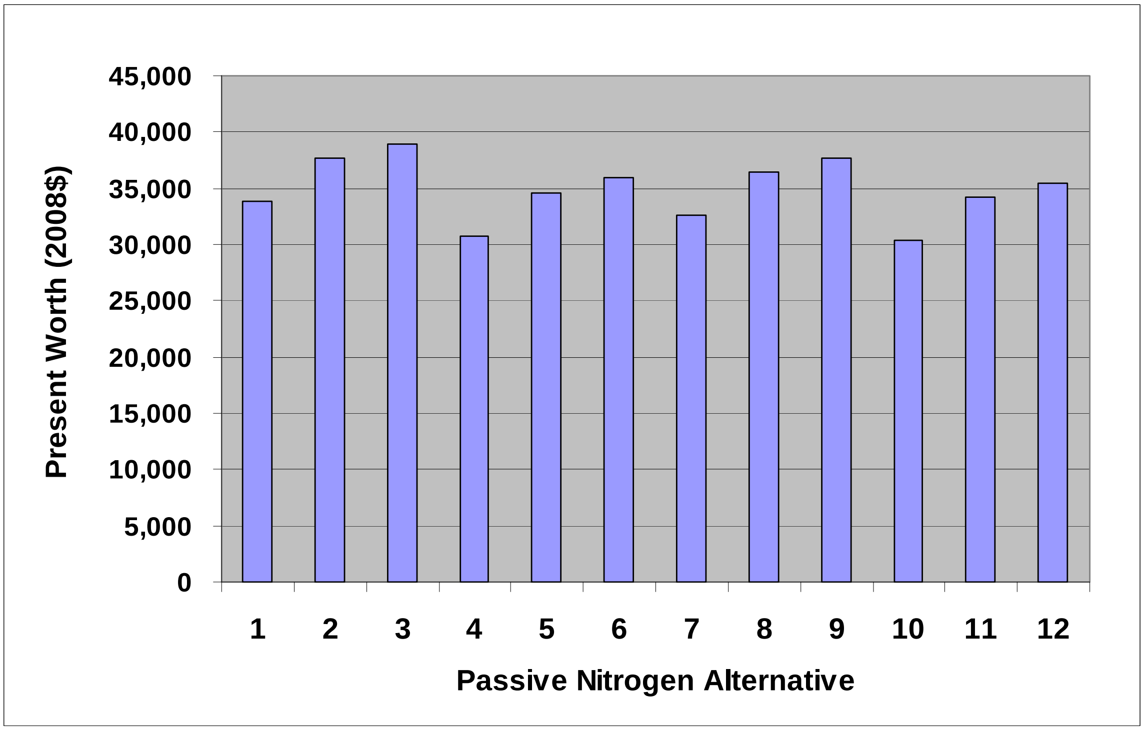


Figure 5 Present Worth of Alternative Systems (30 year project life, i = 4%)

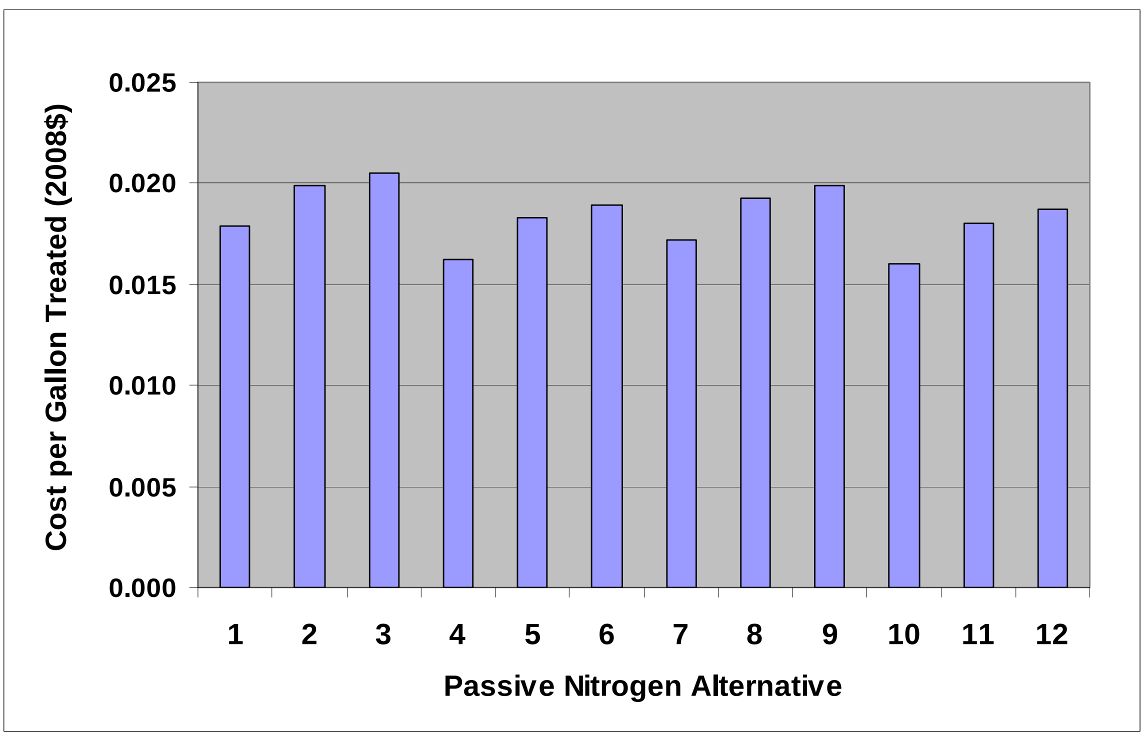


Figure 6 Uniform Annual Cost per Volume Treated (30 year project life, i = 4%)

Table 6 Full Life Cycle Economic Analysis for Passive Nitrogen Removal Systems

SYSTEM ID	STAGE 1							STAGE 2							TOTAL LIFE CYCLE COSTS (30 YRS, i=4%)										
	Media	Plan	Hardware (includes septic tank)	Media Qty.	Cost Opinion	Media Cost	System Cost	Replace	Media Vol.	Hardware	Media Qty.	Cost Opinion	Media Cost	System Cost	Installed System	Standard Drainfield	**Total System Installed**	Maintain and Monitor	Energy	Septic Pumping	**Total O&M**	**Stage II Media Replace**	Salvage Value	Total Life Cycle Cost: Present Worth	Total Life Cycle Cost: Uniform Annual Cost
	Type	ft^2	2008 ($)	lbs	$/lb	2008 ($)	2008 ($)	Years	ft^3	2008 ($)	lbs	$/lb	2008 ($)	2008 ($)	2008 ($)	2008 ($)	2008 ($)	2008 ($)	2008 ($)	2008 ($)	2008 ($)	2008 ($)	2008 ($)	2008 ($)	2008 ($)
1	Clino	100	5,000	11,000	0.35	3,850	8,850	15	100	1,000	7,825	0.40	3,130	4,130	12,980	4,500	17,480	6,313	3,558	1,284	11,155	5,198	0	33,833	1,956
2	Clino	100	5,000	11,000	0.35	3,850	8,850	5	50	750	3,913	0.40	1,565	2,315	11,165	4,500	15,665	6,313	3,558	1,284	11,155	10,844	0	37,664	2,177
3	Clino	100	5,000	11,000	0.35	3,850	8,850	1	11	500	861	0.40	344	844	9,694	4,500	14,194	6,313	3,558	1,284	11,155	13,573	0	38,922	2,250
4	Clay	100	5,000	8,200	0.10	820	5,820	15	100	1,000	7,825	0.40	3,130	4,130	9,950	4,500	14,450	6,313	3,558	1,284	11,155	5,198	0	30,803	1,780
5	Clay	100	5,000	8,200	0.10	820	5,820	5	50	750	3,913	0.40	1,565	2,315	8,135	4,500	12,635	6,313	3,558	1,284	11,155	10,844	0	34,634	2,002
6	Clay	100	5,000	8,200	0.10	820	5,820	1	11	500	861	0.40	344	844	6,664	4,500	11,164	6,313	3,558	1,284	11,155	13,573	0	35,892	2,075
7	Clino	75	4,750	8,250	0.35	2,888	7,638	15	100	1,000	7,825	0.40	3,130	4,130	11,768	4,500	16,268	6,313	3,558	1,284	11,155	5,198	0	32,620	1,885
8	Clino	75	4,750	8,250	0.35	2,888	7,638	5	50	750	3,913	0.40	1,565	2,315	9,953	4,500	14,453	6,313	3,558	1,284	11,155	10,844	0	36,452	2,107
9	Clino	75	4,750	8,250	0.35	2,888	7,638	1	11	500	861	0.40	344	844	8,482	4,500	12,982	6,313	3,558	1,284	11,155	13,573	0	37,710	2,180
10	Clay	75	4,750	6,150	0.10	615	5,365	15	100	1,000	7,825	0.40	3,130	4,130	9,495	4,500	13,995	6,313	3,558	1,284	11,155	5,198	0	30,348	1,754
11	Clay	75	4,750	6,150	0.10	615	5,365	5	50	750	3,913	0.40	1,565	2,315	7,680	4,500	12,180	6,313	3,558	1,284	11,155	10,844	0	34,179	1,976
12	Clay	75	4,750	6,150	0.10	615	5,365	1	11	500	861	0.40	344	844	6,209	4,500	10,709	6,313	3,558	1,284	11,155	13,573	0	35,437	2,048
RSF	SEPTIC SAND	100	5,000	19,800	0.014	277	5,277	na	na	na	na	na	na	na	5,277	4,500	9,777	6,313	7,116	1,284	14,713	na	0	24,490	1,416

Notes: Stage 1 Media: Clino: Clinoptilolite AMZ Clay: Livlite Expanded Clay
Stage 2 Media: 3:1 Elemental Sulfur & Oyster Shell
Total System Costs includes base septic tank installation and drainfield

Table 7 Cost Comparison of Two Stage Nitrogen Systems and RSF

System	Uniform Annual Cost (2008 $)[1]	Present Worth (2008 $)[1]
Two Stage System with Clinoptilolite[2]	2,092	36,200
Two Stage System with Expanded Clay[2]	1,939	33,549
Recirculating Sand Filter, One Stage	1,416	24,490

[1]30 year life, i=4%

[1]Average of 6 alternatives

References

Fuller, S. and S. Peterson (1995) Life Cycle Costing Manual NIST Handbook 135, National Institute of Technology and Standards, U.S. Department of Commerce.

Engineering News Record (ENR) (2008).

Florida Passive Nitrogen Removal Study

Passive Nitrogen Removal Systems Recommendations

Draft Report

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Background

The objective of the Passive Nitrogen Removal Study was to identify “passive” onsite treatment systems that can achieve greater nitrogen reductions than exhibited by conventional septic tank/drainfield systems yet are relatively simple to operate and have low life cycle costs. In this context, “passive” was defined by the Florida Department of Health as employing fixed-film biological nitrogen removal processes that were configured in such a way as to eliminate the need for mechanical aeration equipment and require no more than one pump.

The study’s focus was only on Passive Nitrogen Removal Systems (PNRS) that would be suitable for single family homes, which discharge septic tank effluent (STE) with characteristics typical of single family residences in the U.S. (U.S. Environmental Protection Agency, 2002). From the literature review of passive biological nitrogen removal processes conducted in Task 1, and evaluation of equipment and issues important to practical implementation of PNRS technology, candidate PNRS were identified. What appears to be the most promising type of PNRS is embodied by a two stage system that consists of an above ground, unsaturated (aerobic) gravity nitrification fixed-film reactor (Stage 1) and a horizontally configured saturated (anoxic/anaerobic) fixed-film denitrification reactor (Stage 2). This PNRS is envisioned as a sequence of operations inserted between the septic tank and the drainfield of a conventional onsite treatment system as shown in Figure 1.

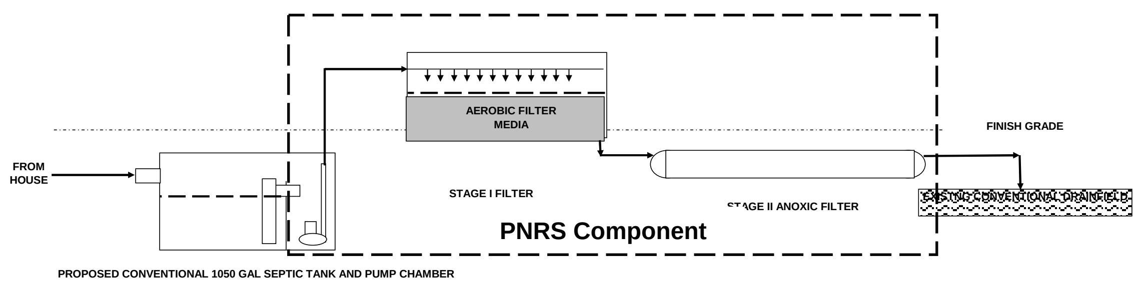

Figure 1 Conceptual PNRS Component Placed within Conventional Onsite System

Wastewater from the home would flow through a septic tank after which the STE enters a flow equalization tank or chamber, the first component of the PNRS. A small pump would be set to operate on regular timed intervals to provide flow equalization. The pump would be used to lift the STE to the Stage 1 filter, which contains a specified granular media. The Stage 1 filter is set at an elevation that achieves gravity flow through the entire PNRS process train and into the subsurface infiltration trenches. This use of the pump, which is the one single pump that is allowed by the criteria established by FDOH, provides the benefits of equalizing the daily flow throughout the day and pressurized distribution to achieve uniform application of the STE over the Stage 1 filter. Uniform areal distribution of limited volume doses is critical to maintaining unsaturated and aerobic conditions in the filter media and effective process performance. These conditions are necessary to support autotrophic bacteria that can nitrify the organic and ammonium nitrogen (Total Kjeldahl N or TKN) in the wastewater and maximize the residence time of the STE as it percolates downward through the filter media. The nitrified filtrate collects at the filter bottom where it flows by gravity into the Stage 2 filter. The Stage 1 filtrate flows through the Stage 2 filter media. The Stage 2 filter media is specific mixture that is submerged below the water surface (i.e. pores are water saturated), which provides the necessary anoxic/anaerobic biochemical conditions to support denitrification of the Stage 1 filtrate. The nitrogen gas produced in this stage is vented to the atmosphere and denitrified filtrate leaves the PNRS and continues to flow by gravity to the subsurface infiltration trenches for dispersal into the soil.

In addition to nitrification and denitrification, the PNRS also would substantially reduce biochemical oxygen demand, suspended solids, and other constituents such as pathogens. Thus, PNRS would have the effect of "lightening the load" to a soil dispersal system. The soil system would shift from a role of a principal treatment component to one of dispersal of the advanced treatment effluent, and in addition, of providing polishing treatment, backup protection and other options for effluent dispersal and reuse.

PNRS can also be configured to be adaptable for a number of additional treatment requirements, some of which are mentioned here. Phosphorus can be reduced by sorption on media with high P affinity. PNRS can be evolved into a Passive Nutrient Removal System (PNuRS) by incorporating P removal media. Since both P removing media and reactive denitrification media have some limited life and will eventually have to be replaced, one logical approach would be to incorporate P sorbing media into the Stage 2 filter. Other options include a separate P removal filter following the Stage 2 denitrification filter, or placing P removal media in a modified soil dispersal system. It is noted that the experimental studies that were conducted in Task 2 of the Florida Passive Nitrogen Removal Study included two Stage 2 denitrification filters that contained expanded shale (utelite) as a media component, which is reported to have an appreciable affinity for inorganic P.

Reduction of pathogens from onsite wastewater is a high priority for environmental and public health protection. The ability of PNRS to remove target pathogens or indicator organisms has not been measured, so there is no specific data with which to support reductions in soil system size. However, the PNRS configuration employs biological filtration through 24 in. of unsaturated filter media (Stage 1) followed by biological filtration through saturated, anoxic filter media (Stage 2). Physical, chemical and biochemical processes within PNRS would reduce the levels of pathogen and indicator organisms. Since PNRS will reduce pathogens, it is possible that the size of soil systems that receive PNRS effluent could be reduced. Measurement of pathogen and indicator organism reductions in PNRS are needed before specific design recommendation can be made for reduction in drainfield area.

PNRS can de adapted for enhanced pathogen destruction using other technologies such as ultraviolet disinfection. PNRS compatible UV systems are available that feature low energy consumption, long life, and minimization of fouling through management of lamp surface temperature. A logical placement of UV in PNRS would be after the anoxic denitrification filter (Stage 2), although placement between the Stage 1 and Stage 1 filters may offer unique advantages.

PNRS effluent is low in dissolved oxygen. Introduction of PNRS effluent into an unsaturated media, such as a sand filled soil treatment layer located above the water table, would result oxygen transfer into the PNRS effluent stream. The same process would occur in natural soil provided that PNRS effluent was introduced into unsaturated media; rate of oxygenation would depend on the aeration capacity of the soil media.

PNRS may have an inherent ability to address emerging contaminants, including a wide variety of substances such as chemical components of personal care products, pharmaceutical products and their metabolic derivatives, consumer food items and their breakdown products, and hormonally active substances. PNRS treatment systems host a great surface area with a highly varied ensemble of microenvironment niches with specific chemical conditions, microbial transformations, and redox environments. A variety of microbial biofilm actions can be brought to bear on specific emerging contaminants, leading potentially to phenomena such as cometabolism and secondary substrate utilization. The ability of PNRS to remove the numerous emerging contaminants is unknown. The use of oxidation technologies, such as ozone, could be incorporated in PNRS for enhanced removal of specific contaminants. Incorporating advanced technologies with PNRS would perhaps be more feasible for cluster treatment and larger systems.

Recommendations

The following sections address pertinent issues of PNRS for onsite treatment and make recommendations for:

- Design
- Permitting
- Installation
- Control
- Maintenance and monitoring
- Replacement of passive treatment media.

A. Design

The PNRS consists of three components; Lift Pump/Flow Equalization, Stage 1 Filter, and Stage 2 Filter. The individual component design requirements are discussed below. However, because the PNRS relies primarily on gravity flow through the process train, these relative elevations of the assembled components of the PNRS and the septic tank and subsurface infiltration trenches are critical. It is essential that the engineering design develop a full, site specific hydraulic profile for the entire onsite treatment system, including the house wastewater drain invert, primary treatment (septic tank), the PNRS components, and the subsurface infiltration trenches (drainfield).

1. Flow Equalization

The flow equalization component of the PNRS should provide the following:

- Screened septic tank influent
- Wet well
- Dosing pump and programmable controls and optional telemetry
- Daily flow measurement
- Securable access

The wet well is a watertight vault that is downstream of the septic tank. It may be a chamber of the septic tank or a separate tank. The influent to the vault (assumed to be STE) should be screened to remove larger particulates in the STE. The screen should be attached to septic tank outlet.

The vault must have a sufficient “working volume” (volume between the pump off level control and the high water alarm level control) to provide storage of wastewater between pumping events to equalize the flow to through the remainder of the PNRS throughout the day. For single family home systems, the volume provided is typically 50% or greater of the average daily wastewater flow.

The dosing pump is a submersible pump that is elevated off the vault floor on a low pedestal to prevent solids that accumulate on the vault floor from being drawn into the pump volute. The pump must be sized to be capable of providing the design discharge rate of the pressure distribution network in the Stage 1 filter and the static lift and force main losses between the pump and the Stage 1 filter distribution network. A 3 or 4 level control system should be used. The level controls include 1) pump off (redundant to the timer), 2) dose enable (level at which a full dose is ensured (volume between the dose enable and pump off control), which is necessary for filling and pressurizing the distribution network), 3) optional override to pump out wastewater in excess of assumed normal operating flow, and 4) high water alarm. A 3 level control system does not include the override control. In addition to the level controls, a timer is used to operate the pump on regularly spaced timed intervals throughout the day.

Daily flow measurement is important to diagnosing performance problems with the PNRS. The capability to record daily flows should be provided. The most simple devices that can provide adequate accuracy are a running time meters for the pump and counter to record the total number of pumping events.

Easy access to the vault and pump must be provided. The vault access should be above grade and securable to prevent unauthorized entry.

2. Stage 1 Filter

Unsaturated filtration is a well established onsite wastewater treatment technology that is widely applied. General design principals used in current onsite practice also apply to the unsaturated PNRS Stage 1 filter. The most widely applied unsaturated filter systems that would meet the FDOH “passive” definition are single pass, intermittent sand filters. Recirculating media filters that can achieve recirculation by gravity would also meet the “passive” definition. PNRS design can employ much of the knowledge and techniques of sand filter designs, which can be found in sources including Anderson, et al., 1985, Crites and Tchobanoglous (1996), Commonwealth of Massachusetts (2002), Jantrania and Gross (2006), and US EPA (2002).

The Stage 1 Filter should provide the following:

- Filter housing that is structurally suitable for above grade installation
- Filter size that can accommodate a minimum of 100 ft^2 of surface area, 24 in. depth of select filter media, and underdrain
- Underdrain
- Select media
- Pressurized distribution system for applying STE over the media
- Removable filter cover providing ports for air ingress;
- Accessible filtrate monitoring port at outlet;
- Gravity flow conveyance to the Stage 2 filter.

The filter housing can consist of a number of materials, but is typically concrete or constructed of landscaping timbers above grade over an impermeable liner below grade. Underdrain piping consisting of a minimum of two 4-in perforated DWV pipe with 0.5 in holes at the 5 and 7 o’clock positions; pipes are laid on the tank floor or impermeable liner. These drains are connected to a common outlet drain that leads to the Stage 2 filter. The underdrain is covered with 6-in of 0.75 to 1.5 in. gravel, which in turn is covered with 3 to 4-in of pea gravel. The select filter media used is clinoptilolite or livlite, which is washed before placement, and placed in stratified configuration as described in Task 2 report (Smith, 2008).

The elevation of the filter housing underdrain outlet invert is a critical to gravity flow within the PNRS. This invert elevation must provide sufficient elevation to maintain complete submergence of the Stage 2 filter media and maintain flow to the infiltration system without backing up into the Stage 1 filter. Fiction losses in the Stage 2 filter media must be considered.

STE is applied to the surface of the filter media by a pressure distribution network (see Converse and Tyler, 2000; Otis, 1982; US EPA, 1980; US EPA, 2002). Small diameter pipe that is perforated with small orifices is used. The goal of the network design is to provide the highest density of orifices as reasonable. However, there is a trade-off; the greater the number of orifices, the larger the pump required. Also, more orifice can mean more piping, which means in turn that dose volumes must increase to achieve pressurization of the network if the distribution is to be uniform. Therefore, the distribution network design must depend on the desired number of daily doses and rate of application. For single pass filters, doses will be limited to 6 or 8 per day. It will be difficult to achieve uniform distribution with a larger number of daily doses without making the orifices very small, which makes them susceptible to plugging. If more doses per day are required, the Stage 1 filtrate should be recirculated (see recirculation below).

The filter surface and distribution piping may be covered with pea gravel and left open to the atmosphere. Odors typically are not a problem. However, rainfall on the filter surface must be accounted for in design. A flat fiberglass cover works well, (if designed to support foot traffic). Venting is required to provide aeration, which can be provided by elevating the cover to create a small gap between the cover and the tank wall. If security is an issue, external vents can be used so that the cover can be locked down.

Recirculation is an option for the Stage 1 filter design. Recirculation offers several advantages. Because doses can be larger, the number of doses per day can be increased to a maximum of 48 per day, thereby increasing residence time of the STE in the filter for more complete nitrification. The increased number of doses maintains a moist environment in the filter to enhance biochemical activity. With recirculation, other studies have shown that 50% of the total nitrogen (TN) in the STE will be removed. If the filtrate is recycled back to the septic tank to utilize the organic carbon in the STE as an electron donor in the biochemical process, the removals can increase to 70%. This will lower the NO_x loading to the Stage 2 filter and theoretically increase the longevity of the reactive denitrification media. Recycle of Stage 1 filter to promote pre-denitrification with will also recover alkalinity that is removed during nitrification, allowing nitrification to proceed without excessive pH decline. This is an important advantage where the STE has a high ratio of TN to alkalinity.

The Stage 1 filtrate can be recycled back to either the flow equalization component or to the septic tank. In either case, the sizing of the tanks must be reviewed to ensure adequate storage for flow equalization and hydraulic residence time in the septic tank is provided.

To properly evaluate the recirculation option, additional experimental studies should be performed. The experimental studies performed in Task 2 were operated in single pass mode, and this remains the basis of the present recommendations.

3. Stage 2 Filter

The Stage 2 filter should provide the following:

- Stage 2 filter housing of fiberglass or plastic pipe with a horizontal orientation
- Select media with a total volume of 50 ft^3 (375 gallon)
- Gas venting
- Accessible monitoring point at outlet

The Stage 2 filter housing is envisioned as a watertight, horizontal fiberglass or plastic 24-in diameter pipe. Its total length is 18 ft to provide a length to width ratio of at least 10:1. The total media volume should be at least 50 ft^3. Header plates, perforated with 0.25-in diameter holes on square matrix at 2 in. centerline spacing, are placed at the inlet and outlet to secure the media and provide flow distribution.

The media consists of a 3:1 volumetric ratio of granular elemental sulfur/oyster shell, as specified in Task 2 report (Smith, 2008). Friction losses through this media must be determined to set the Stage 1 filter outlet invert elevation so that flow through the Stage 2 filter and to the infiltration trenches can be maintained at all flow rates without flooding the Stage 1 filter outlet.

The elevation of the Stage 2 filter outlet invert to the infiltration trenches should be set such that there a small air gap is maintained at the crown of the pipe to vent the off-gases from the media. This gap should be vented into a short length of a buried rock filled trench to scrub odors from the venting gases.

It is recommended that an alternative configuration be tested consisting of short segments, which are loaded in parallel by a common header to reduce the total headloss through the system so the Stage 1 filter can be lowered in elevation. The short segments also would simplify media replacement. However, the parallel configuration will reduce the length to width ratio, which may increase the potential for preferential flow through the media with a concomitant reduction in media contact and residence time that could reduce nitrogen removal performance.

PNRS can be configured using a modular type design approach that is adaptable for larger applications, such as cluster systems, or that is expandable as the required treatment capacity increases. It is envisioned that, with the creation of a sufficient market, vendors would establish regional media inventory, reducing shipping costs and preparing material according to required size gradations. Alternative sources of alkalinity, perhaps local sources, could be explored for their possible advantages in terms of cost, process efficacy, and residuals management.

B. Permitting

Regulation of onsite systems in Florida is governed by the Florida Administrative Code, Chapter 64E-6, *Standards For Onsite Sewage Treatment And Disposal Systems.* These rules allow innovative and alternative systems to be used but require testing and evaluation of the systems prior to general approval. Nothing in these rules would seem to be prohibit PNRS, nor does it appear that special rule provisions are needed to allow PNRS.

The PNRS provide a high level of treatment low in TN, BOD, and TSS, which should qualify for reductions in drainfield size or increases in drainfield hydraulic loadings. However, section 64E-6.009, *Alternative Systems* does not allow downsizing or loading increases if traditional rock-filled trenches are used.

C. Installation

Installation and construction of PNRS share common procedures with traditional onsite treatment systems including the location of the residence, locations of other structures and wells, property boundaries, trees and vegetation, topography and elevations, seasonal high groundwater table elevations, and aesthetic and environmental constraints. Other considerations include available area and elevation differences, electrical supply for the dosing pump, and access for system construction, system maintenance and periodic media replacement. The following installation items are the most critical to the PNRS performance:

- System layout and system hydraulic profile
- Media preparation and placement
- Watertightness testing of all components and piping

A system layout plan that ensures an efficient use of the available area and available elevation must be developed. This includes a hydraulic profile of the complete system from the either the house plumbing stub out or the septic tank outlet invert to the inlet invert of the infiltration system. It is critical to establish the hydraulic profile before construction commences to ensure that flow through the system from the Stage 1 filter outlet can occur by gravity. An important aspect of this profile is to determine the headloss through the Stage 2 filter. If two great for the available hydraulic “fall” across the system, other configurations of the Stage 2 filter should be considered including deep bury to create a high driving head through the filter, multiple modules laid horizontally and plumbed to operate in parallel, multiple modules installed vertically and plumbed in series, or other configurations to allow gravity flow.

Both the Stage 1 and Stage 2 filter media should be repeatedly rinsed with clean water on-site to remove fines prior to placement in the filter housings. Placement of the media must follow the specifications as described in the Task 2 report (Smith, 2008). In the Stage 1 filter, the clinoptilolite or livlite filter media, must be well sorted by size and placed on level within one inch in the specified stratified configuration. The Stage 2 filter sulfur and oyster shell media, must be mixed after rinsing to achieve spatially homogeneity before placement.

D. Control

The rate at which well operating treatment performance is established in a biological treatment system depends on the establishment of microbial populations. Treatment with PNRS should be established at least as quickly as for a suspended growth system. Startup should be rapid and can be accomplished using full strength wastewater and normal hydraulic operation. Control methods are covered previously in the Flow Equalization section.

In the future, distributed treatment infrastructure may be operated and maintained by Responsible Management Entities (RMEs). An RME would have responsibility for managing multiple systems for single family residences, cluster systems, and larger systems. Remote monitoring of onsite treatment systems is one tool which may be used by future RMEs. The PNRS is compatible with remote monitoring, and would transmit signals for power failure, level alarms, or pump failure.

E. Maintenance and Monitoring

Maintenance recommendations for PNRS include:

- Once per six month checking of counters and elapsed-time meters;
- Once per year inspection and servicing of all electrical and mechanical parts, including pump, filters, float assembly, and control panel;
- Once per year flushing and testing of flow distribution system by manual operation and visual observation;
- Once per year process testing by sampling the septic tank, Stage 1 filter, and Stage 2 filter effluents for BOD, DO, and TKN and Nitrate analyses.

F. Replacement of Passive Treatment Media

Stage 2 media composition is specified as sulfur and oyster shell with a 3:1 volumetric ratio, as was applied in all of the Stage 2 columns in the Task 2 experimental investigation. The economic analysis cases in Task 3 did not include utelite (expanded shale) in the Stage 2 filter media. Incorporating additional media components in any Stage 2 design reduces the space available for the reactive sulfur and theoretically reduces the run time. Since the experimental results did not provide definitive evidence of enhanced performance with the expanded shale component, utelite was not included.

The economic analysis included three options for Stage 2 filter size/media replacement combinations, as listed in Table 1. These options span a spectrum from a low reactor volume, frequent media replacement strategy to one of larger filter size with longer run time and less frequent media replacement. These options reflect the lack of continuous operating data for sulfur based denitrification systems. The smaller filter design with frequent media replacement would be advantageous if shorter term performance deterioration was caused by factors such as preferential flow paths (channeling) or chemical precipitation. In this case, the NO_x removal effectiveness of the Stage 2 filter would decline, even though the sulfur media was largely unused. Modular media replacement systems could be developed with perhaps renovation and reapplication of spent media. To fully explore Stage 2 filter design, longer term operation of treatment systems is needed to demonstrate continued NO_x removal performance. The recommended design is the intermediate filter size case, which provides a relative Stage 2 volume and empty bed residence time similar to that of the Stage 2 filters that were operated in the Task 2 experiments.

Table 1 Stage 2 Design Options

Stage 2 Filter volume/media replacement interval

- 750 gallon, 15 year
- 375 gallon, 5 year
- 75 gallon, 1 year

Media replacement recommendations for PNRS include:

- Full media replacement at five year interval;
- Biannual NO_x monitoring of PNRS effluent for possible variation in media replacement intervals;
- Disposal of media in landfill;
- Investigate possible processing of removed media for reapplication;
- Investigate alternative beneficial uses for removed media.

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Evaluation of Passive Nitrogen Removal Systems

Daniel P. Smith[1]

ABSTRACT

Removal of nitrogen from onsite wastewater using "passive" systems has been identified as a significant need by the Florida Department of Health (FDOH). The Florida Passive Nitrogen Removal Study was initiated with the objectives of performing a literature review of passive nitrogen removal systems, identifying conceivable media, elucidating the processes underlying media function, and performing experiments to support recommendations for onsite wastewater treatment applications. This paper describes the experimental approach and results of bench scale testing of passive nitrogen removal media treating septic tank effluent (STE). The operative definition of the term "passive" specified that systems incorporate reactive media, have no aeration pumps, and employ at most a single effluent dosing pump. These constraints required that gravity flow be one facet of system design and influenced design of the bench scale systems. A central modus of bench scale evaluations was a two-stage system of porous media filters, with an initial unsaturated media filter (ammonification and nitrification) followed in series by a saturated anoxic filter with reactive media (denitrification). Parallel two-stage systems contained unsaturated media columns followed in series by saturated media columns. The unsaturated Stage 1 columns received STE and included synthetic and natural media, and sorbing and reactive media. Stage 2 (saturated) columns received Stage 1 effluent and included synthetic and natural media, sorbing media, and reactive media to supply electron donor and alkalinity. Factors that were evaluated include influent and effluent water quality, dosing regime, applied loading rates (hydraulic, organic and nitrogen), and the Total Nitrogen:alkalinity ratio of influent.

Keywords Onsite wastewater Nitrogen reduction Filtration Reactive media

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PASSIVE NITROGEN REMOVAL

There are currently 2.6 million onsite wastewater treatment and disposal systems in the State of Florida, with over fifty thousand new connections in 2006/2007 (FDoH 2008). Technologies that increase nitrogen removal have been identified as one component of a strategy to reduce the potential effects of onsite nitrogen loading to sensitive environments (Roeder, 2007). The Florida Passive Nitrogen Removal Study was established to identify and evaluate passive treatment systems that are capable of achieving greater nitrogen reductions than exhibited by conventional septic tank/drainfield configurations.

The Florida Department of Health (FDOH) provided a definition of "passive" as "*A type of onsite sewage treatment and disposal system that excludes the use of aerator pumps and includes no more than one effluent dosing pump in mechanical and moving parts and uses a* *reactive media to assist in nitrogen removal."* The specific interest is in approaches to onsite treatment that employ filter media, including reactive media, systems that do not employ aerators, and systems that minimize the need for liquid pumping.

In the first task of the Florida Passive Nitrogen Removal Study was a literature review of passive technologies with the potential to enhance nitrogen removal from on-site wastewater treatment systems (Smith and Otis, 2007). The report proposed a coupled two stage filter system for passive removal of total nitrogen from septic tank effluent. The first stage is an unsaturated media filter for ammonification and nitrification. Pumping to the unsaturated filter will allow pressure and timed dosing. The second stage is a saturated anoxic filter with reactive media (denitrification). The two stage configuration is mandated by the obligatory biochemical sequence of aerobic nitrification followed by anoxic denitrification. The use of an unsaturated trickle flow media filter for initial nitrification is necessary because of the constraint that aeration pumps can not be used in the passive system. Flow from Stage 1 to 2 would be by gravity and pumping would not be required. The system would be deployed between the septic tank and the soil treatment/dispersal system of new or existing facilities. Nitrogen in septic tank effluent would be substantially removed before wastewater was directed to the soil for treatment or dispersal.

The second phase of the Florida Passive Nitrogen Removal Study Task 2 is an experimental evaluation of candidate filter media and systems for total nitrogen removal from STE. Small scale testing will be performed to identify candidate media for subsequent evaluation using full scale onsite wastewater treatment systems. To perform the media evaluations, it was desired to conduct studies in a manner that closely resembles the functioning of an actual onsite system. The actual candidate media will be used, placed in appropriate depth and distribution. Dosed filter operation is preferable, similar to what could be established in an operating system.

FILTER MEDIA

Aerobic (Unsaturated) Filters

Media properties significantly affect the performance of unsaturated aerobic filters. Smaller particle size distribution increases the specific surface area available for biofilm attachment and particle retention. Media in the upper filter layer will have a greater need to retain and biodegrade wastewater solids and larger particle size may beneneficial. Water retention capacity is a significant property of unsaturated filter media. High water retention will maximize the length of time that newly applied wastewater remains in the filter and increase contact time with media surfaces and attached microorganisms. Unsaturated media filters are four phase systems: solid media, attached microbial film, trickling wastewater, and the gas phase. The air filled porosity (i.e. gas phase) is that portion of the external porosity that is available for the transport of oxygen to surfaces throughout the media. High air filled porosity is desirable to maintain aerobic biochemical reactions reactions, particularly since higher hydraulic loading rates increase the pore water content and will possibly inhibit nitrification. The characteristics of the specific filter media interact with the design and operation of the unsaturated filter. The average hydraulic loading rate per filter surface area affects the average loading rates of organics and nitrogen. While the rate of wastewater generation is typically highly variable, filtration will be most effective when wastewater is applied continuously or as small frequently applied doses. In an unsaturated filter, a small hydraulic application rate per dose will result in improved trickle flow contact of new wastewater parcels with surfaces and attached biofilms. If the hydraulic application rate is a small fraction of the total water retained within the unsaturated filter, breakthrough of constituents into filter effluent will be minimized.

Anoxic (Saturated) Filters

Anoxic saturated media filters form a second stage in the passive nitrogen removal system. Anoxic filters contain a "reactive" media that provide a slowly dissolving source of electron donor for reduction of nitrate and nitrite by microbial denitrification. Denitrifying microorganisms grow predominantly attached to the media surfaces. Water flows by advection through the media pores, where oxidized nitrogen species are consumed by attached microorganisms. Water saturation of the pores prevents ingress of oxygen, which could interfere with nitrate reduction. Factors influencing the performance of anoxic denitrification filters include hydraulic and nitrogen loading rates, media particle size and surface area, pore size, flow characteristics within the reactor, and length or depth of filter bed. Dissolution of reactive electron donor media must be sufficiently rapid to supply electron equivalents for nitrate/nitrite reduction and possibly other reactions. On the other hand, too rapid a dissolution rate would reduce the longevity of the media and could release higher concentrations of excess dissolution products in the effluent. Another factor influencing denitrification performance is the accumulation of suspended solids within the column, which could result in the development of preferential flow paths, decreased contact time of wastewater with media surfaces, and performance deterioration. Nitrate and nitrite removal over extended operation would be more likely if the anoxic filter received a first stage unsaturated filter effluent with low levels of suspended solids and biodegradable organics (BOD). The aspect ratio of the denitrification filter and flow entrance and withdrawal would also affect flow patterns and potential short circuiting. The effects of flow channeling on performance deterioration could possibly require maintenance or media replacement at time scales appreciably shorter than theoretical longevities based on stoichiometric requirements for denitrification.

Media

Media that will be evaluated are listed in Table 1 and shown in Figures 2 through 7. Zeo-Pure is a clinoptilolite with high water retention characteristics and a total cation exchange capacity for ammonium of 1.8 to 2.0 meq/g (Zeox Corp. 2008). Zeolite media have been shown previously to be highly effective in improving ammonium filtration under both steady and non-steady loadings (Smith et al. 2004). Livelite is an expanded clay which also has high water retention and porosity. Recycled rubber materials are produced by shredding of used tires and particle size reduction, and are available as tire chips and crumb rubber in particle sizes of 5 mm and less. Tire chips have been evaluated for use as a drainfield media, where they would function in an unsaturated filtration mode (Grimes et al. 2003). Elemental sulfur will be used as electron donor media is in the Stage 2, which will establish an autotrophic denitrification process in the anoxic filter. Elemental sulfur has been evaluated as denitrification filter media for onsite wastewater (Sengupta et al. 2006), groundwater (Darbi et al. 2003), aquaculture (van Rijn et al. 2006), and stormwater (Smith, 2008). Crushed oyster shell will be used as an alkalinity source, as sulfur-based autotrophic denitrification is an alkalinity consuming biochemical reaction. Expanded shale is also included in the Stage 2 media mix. Utelite has an anion exchange capacity of 120 meq/100 g (Zhu et al. 1997), which would bind nitrate ions and possibly enhance performance resiliency under non-steady operation.

Table 1 Filter Media

Material	Bulk density, lb/ft^3	Particle Size Range	Supplier
Zeo-Pure AMZ 8/20 Clinoptilolite	55	0.8 - 2.3 mm	Ash Meadows, Armagosa, NV
Livlite Expanded Clay	41	3 to 5 mm	Big River, Alpharetta, GA
Tire Crumb	25	0.3 - 5 mm	Global Tire Recycling, Wildwood, FL
Elemental sulfur	77	2 - 4 mm	Georgia Sulfur, Valdosta, GA
Oyster shell	82	3 - 15 mm	Harold's Supply, Dover, FL
ACT-MX ESF-450 Utelite	54	0.4 - 4.5 mm	ES Filter, Ogden, UT

EXPERIMENTAL SYSTEMS

A schematic of the experimental filter columns is shown in Figure 1. Three filter systems will be evaluated, each consisting of an unsaturated filter followed by a saturated filter. Filters will be fabricated from 3 in. diameter tubing (unsaturated filters) and 1.5 in. diameter tubing (saturated filters), using a 1/8 inch screening for media support and retention. Filter columns will be constructed of materials with high contact angles for water sorption to minimize wall effects. The surface area of the filter media will also be twenty to fifty times that of wall area.

Septic tank effluent will be applied to the surface of the first stage media, resulting in a downward percolation of wastewater over and through the media filter bed. The unsaturated pore spaces in the first stage media will allow air to reach microorganisms attached to the media surfaces, enabling aerobic biochemical reactions to occur. Effluent from the bottom of the first stage filter will be passed through a saturated anoxic horizontal flow filter that contains reactive media that supplies electron donor for denitrification. Following startup, the column systems will be operated for two months and monitored for nitrogen species and other water quality parameters. Of particular interest are the concentrations of nitrate, nitrite and total nitrogen in the second stage effluent.

The configuration of the three 2 Stage filters is listed Table 3. The Stage 1 columns will use clinoptilolite, expanded clay, and tire crumb, with total media depth will of 24 in. Media stratification based on particle size is based on the expected progression of biochemical reactions within the filter media. The processes in the upper media layer include adsorption of wastewater particulates and colloids, hydrolysis and release of soluble organics, aerobic utilization of soluble organics, and biomass synthesis. In this region, the biochemical processing of organic matter between doses must keep up with the newly applied wastewater constituents from each dose. The greatest accumulation of organic and inorganic mass is expected in the upper layer, and the use of larger particle size media will provide greater space for accumulation of solids. Stratified media should enhance to potential for long term operation while maintaining treatment efficiency. The use of finer particle sizes in lower depths will provide greater surface area for microbial attachment and a finer media for physical filtration, the later which could improve removal of pathogens and other wastewater constituents. The progression of coarser to finer media size through the filter will also enable coarser media to filter out larger particulates and protect the finer media that follows.

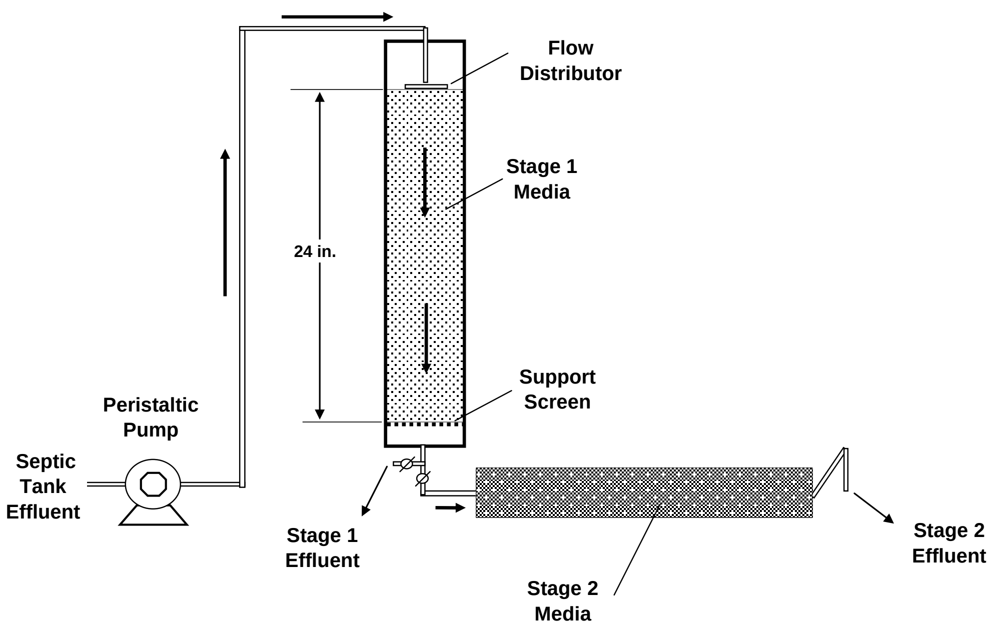

Figure 1 Experimental Filter System Schematic

Three Stage 2 columns will be constructed or unstratified media containing elemental sulfur, crushed oyster shell, and expanded shale (Table 2) of 24 in. media depth. Each filter will contain a 3:1 ratio of elemental sulfur to crushed oyster shell (vol./vol.), which has previously been shown to provide adequate alkalinity. The difference in the Stage 2 media composition is the fraction of expanded shale, which ranges from 0 to 40%. Expanded shale contains anion exchange capacity which can bind nitrate ions, potentially enhancing removal. In addition, higher expanded shale fractions are accompanied by lower fraction of elemental sulfur. The later could reduce the total surface area of elemental sulfur and the overall sulfur oxidation rate, resulting in less complete denitrification. Effluent sulfate levels could be reduced with lower sulfur fractions. The use of three sulfur fractions will allow this issue to be examined.

Table 2 Configuration of Two Stage Filters

Stage	Filter	Column ID, inch.	Total depth, inch	Media placement	Media
Stage 1	1A	3.0	24.0	Stratified	8 in. clinoptilolite (2.3-4.8 mm) 8 in. clinoptilolite (0.8-2.3 mm) 8 in. clinoptilolite (0.5-1.1 mm)
	1B				8 in. expanded clay (3-5 mm) 8 in. expanded clay (0.8-2.3 mm) 8 in. expanded clay (0.5-1.1 mm)
	1C				8 in. tire crumb (3-5 mm) 8 in. tire crumb (1-3 mm) 8 in. tire crumb (0.4-1 mm)
Stage 2	2A	1.5	24.0	Nonstratified	75% elemental sulfur 25% oyster shell
	2B				60% elemental sulfur 20% oyster shell 20% expanded shale
	2C				45 % elemental sulfur 15% oyster shell 40% expanded shale

The Stage 1 filters will be vertically oriented and Stage 2 filters placed horizontally (Figure 1). The Stage 1 filters will be supplied with septic tank effluent by a multi-head peristaltic pump, with a repeat cycle timer for dosing of once per one half hour (48 doses/day). The water elevation in the tube below the Stage 1 filter will provide hydraulic head for passive movement of water through the Stage 2 filter. A valve and sample port (with another valve) will be located in the tube below the Stage 1 filter. In normal filter operation, the sample port valve will be closed and the valve leading to Stage 2 will be open. The design of the filter system minimizes internal volumes within the connecting piping.

Operation will be conducted at a hydraulic loading to the Stage 1 filters of 3 gal./ft^2-day. Operating characteristics of Stage 1 and Stage 2 filters are shown in Tables 3 and 4. At 48 doses per day, a single dose will add a volume that is 6% of the water retained within the Stage 1 filter bed. The estimated average water residence time in the Stage 1 filter is 9 hr. (Table 3). An average water residence time of 12 hr. is provided in the Stage 2 filter (Table 4).

Table 3 Operating Characteristics of Stage 1 Filters (unsaturated)

Diameter, inch	**3.0**
Media depth, inch	**24**
Flow, gpd/ft^2	**3.0**
Doses/day	**48**
Empty bed volume, liter	**2.8**
Resident water volume, liter[1]	**0.21**
Single dose volume / resident water volume	**0.06**
Average residence time, hour	**9.0**

[1]Assumes 50% external porosity, 15% water filled.

Table 4 Operating Characteristics of Stage 2 Filters (saturated)

Diameter, inch	**1.5**
Media depth, inch	**24**
Flow, gpd/ft^2	**12.0**
Empty bed volume, liter	**0.69**
Pore volume, liter[1]	**0.28**
Average residence time, hour	**12.0**

[1]Assumes 40% external porosity.

Operation of columns was initiated on 12/20/2007. The systems will be operated for four weeks before monitoring is initiated. Target parameters are total nitrogen, ammonia, nitrate and nitrite, and other water quality parameters.

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Figure 2 Stage 1 media: Ash Meadows clinoptilolite (0.5 to 1 mm)

Figure 3 Stage 1 media: Livelite expanded clay (0.5 to 1 mm)

Figure 4 Stage 1 media: Rubber crumb (0.5 to 1 mm)

Figure 5 Stage 2 media: Elemental sulfur pastille (0.5 to 1 mm)

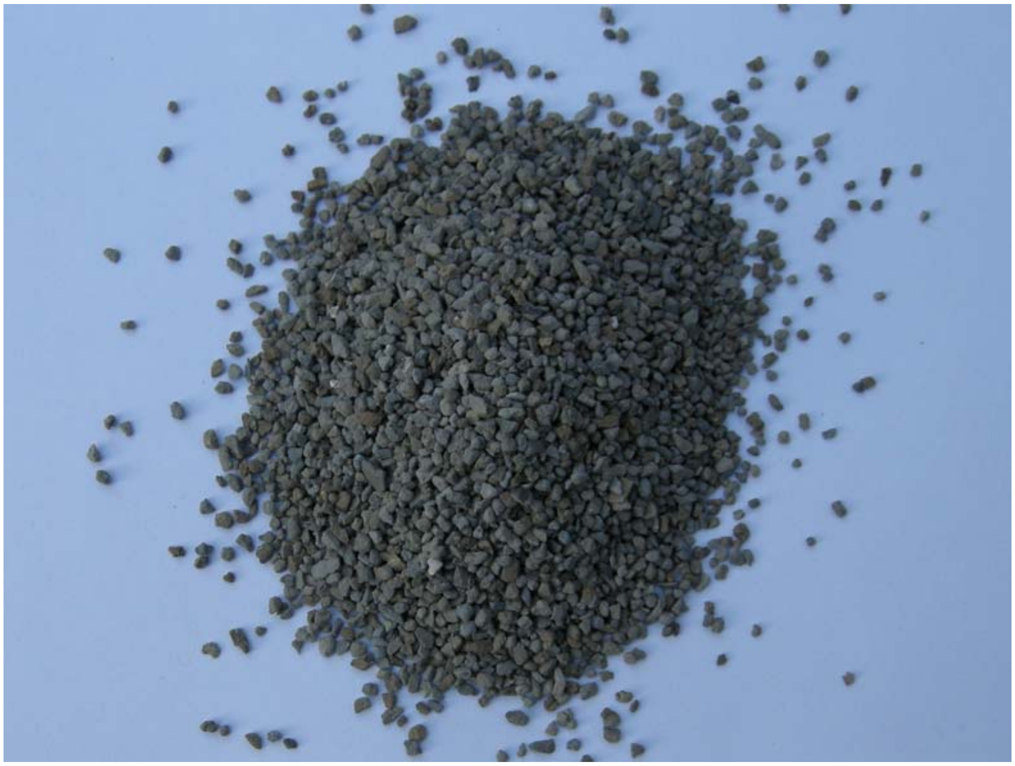

Figure 6 Stage 2 media: Utelite expanded shale (0.5 to 1 mm)

Figure 7 Stage 2 media: Crushed oyster shell (0.5 to 1 mm)

Florida Passive Nitrogen Removal Study

Laboratory Media Evaluation

Additional Sampling Events

Prepared for:

Florida Department of Health
Division of Environmental Health
Bureau of Onsite Sewage Programs
4042 Bald Cypress Way Bin #A-08
Tallahassee, FL 32399-1713

Prepared by:

Applied Environmental Technology
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Prepared and Approved by:

Daniel P. Smith

4/16/2008

Daniel Smith, P.E., Principal Investigator   Date

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Section 1 Project Background

The goal of the Florida Passive Nitrogen Removal Study (DOH Contract CORY6) is to evaluate methods that can be used to enhance nitrogen removal in onsite wastewater systems in a passive and cost effective manner. Task 2 of the study entails an experimental evaluation of candidate filter media that can be used to remove nitrogen from septic tank effluent in passive systems. The purpose of Task 2 is to perform small scale testing to identify candidate media and identify performance, longevity, and other factors pertinent to the design of passive systems and evaluation of their practicality and economics. The Task 2 study plan was described in detail in the Florida Passive Nitrogen Removal Study Laboratory Media Evaluation Quality Assurance Project Plan, finalized on 11/16/2007.

The *Florida Passive Nitrogen Removal Study Literature Review and Database, September 26, 2007*, proposed the development of a two stage filter system for passive removal of total nitrogen from septic tank effluent. The two stage system consisted of an initial unsaturated media filter for ammonification and nitrification, followed in series by a saturated anoxic denitrification filter. The system would be deployed between the septic tank and the soil treatment unit (drainfield) or soil dispersal system of new or existing facilities. Nitrogen in septic tank effluent would be substantially removed before wastewater was directed to the soil for treatment or dispersal.

Following the recommendations of the literature review, an experimental on-site wastewater treatment system was operated and monitored for sixty days to evaluate enhanced nitrogen removal. The treatment system contains three two-stage systems operating in parallel in passive operation, all requiring a single multi-head pump. The system was installed at Flatwoods County Park in Hillsborough County, Florida. The results of the data indicate that passive systems have a potential to achieve high nitrogen removal efficiencies and low effluent concentrations, with several systems removing over 96% of total nitrogen and reducing effluent total nitrogen concentrations to less than 2 mg/L. A report describing the operation and performance of these innovative systems is in preparation as part of DOH Contract CORY6. The results of these additional sampling events will become an appendix to the final report.

The results from the ongoing passive nitrogen removal experimental study are highly encouraging. The purpose of the additional sampling events is to gather additional data on the small-scale laboratory set-up to help address issues regarding process performance, design, feasibility, longevity, and economics.

Section 2 Project Description

A. Project Purpose

The purpose of the present proposal is to perform additional sampling events for the experimental evaluation of media filters for passive nitrogen removal in on-site wastewater treatment that was developed in DOH Contract CORY6.

B. Project Objectives

This work will build upon and expand the ongoing study. This project involves continued operation of currently established systems. The existing experimental system developed in DOH Contract CORY6 will serve as the platform. The following sections describe specific tasks, the rationale and significance to the goal of passive nitrogen removal system development, and types of data that will be collected. A summary schedule and budget are also included.

C. Project Tasks

Project tasks are shown in Table 1. The Florida Passive Nitrogen Removal Study Laboratory Media Evaluation Quality Assurance Project Plan was submitted and approved in DOH Contract CORY6 for this phase of the study and is by reference incorporated and modified into this project.

Table 1 Project Tasks and Projected Schedule

Task/Activity	Start	Projected Completion
Task 1 Extend Operation of Current System	Week 1	Week 17
Task 2 Disassemble sampling stations and evaluate quality of treatment media	Week 18	Week 20
Task 3 Prepare Draft Report	Week 21	Week 23
Task 4 Prepare Final Report	Week 24	Week 26

Task 1 Extend Operation of Current System

The total run time of the currently configured systems will be extended by 5 months. Under the laboratory experiment task in DOH Contract CORY6, the operational time of the experimental systems (< 60 days) is short. Biological systems may need extended times to fully adapt and establish. The extension will at least triple the operational time of monitoring.

There are several performance features of the existing systems that need to be examined by additional monitoring. The unsaturated zeolite filter (Column 1A) appears to exhibiting enhanced denitrification over weeks of operation. Total Nitrogen (TN) removals have increased to 88% at 3 gal/ft^2-day, with an effluent TN of 10 mg/L. This performance, if verified, suggests that the unsaturated zeolite filter could be a highly effective passive treatment filter in itself. High TN removals in a single pass unsaturated filter would substantially reduce nitrate loading to a Stage 2 anoxic denitrification filter and increase longevity. It is important to verify zeolite filter operation. The livelite filter (Stage 1, Column 2A) has produced high ammonia reductions, and very high nitrate accumulations. Adaptation of this filter to denitrification must be examined with longer run times, and verification of existing nitrification performance is needed. The tire crumb filter has taken longer to establish nitrification, which has shown improvement, but is still less than optimal. Longer operation is needed to determine how well this system will perform.

Table 2 summarizes what samples will be taken. Monitoring of STE, Stage 1 effluents and Stage 2 effluents will be conducted five times for nitrogen species and field parameters and will follow the same approach as used in DOH Contract CORY6. In addition, data are needed on removal of carbonaceous biochemical oxygen demand for all of the Stage 1 (unsaturated) systems. High ammonia removal efficiencies are often presumed to imply high reductions in C-BOD_5, but actual C-BOD_5 removals have not been measured in the currently ongoing study. Measurements of the effluent C-BOD_5 from the Stage 1 filters are needed to verify Stage 1 BOD reductions. Monitoring of C-BOD_5 will be conducted three times during the extended study; for each of the three monitoring events, C-BOD_5 analyses will be performed for samples of influent (STE) and each Stage 1 effluent. Analyses that will be performed by the NELAC certified laboratory during the extended study are listed in Table 2.

Deliverables: Field Data, Chain of Custody Forms, and Laboratory Results for each sampling event. Table summarizing weekly site visits, condition of systems and flow-rate information.

Table 2 Extended Operation NELAC Certified Lab Analyses

	Septic tank effluent	Effluent from unsaturated filters	Effluent from saturated anoxic filters	Total number of analyses
TKN	5	5	5	35
NH_3-N	5	5	5	35
(NO_3+NO_2)-N	5	5	5	35
C-BOD_5	3	3	0	12

Task 2 Disassemble sampling stations and evaluate quality of treatment media

The sampling stations will be disassembled and the quality of the treatment media will be examined. A visual inspection of the media will be performed. The bulk mass reductions of Stage 2 (sulfur) media will be estimated by measuring the mass of the materials, taking samples from the Stage 2 columns, and measuring their mass versus fresh sulfur particles.

Deliverables: Documentation that stations have been disassembled, table summarizing measurements of materials

Task 3 Prepare Draft Report

A draft report will be prepared describing experimental methods and procedures, results of the research, discussion and conclusions, and all monitoring data for the additional sampling events. The draft report will be submitted to the Department of Health in Word format.

Deliverables: Draft Report in Word format

Task 4 Prepare Final Report

The Department of Health will be responsible for soliciting comments from the Research Review and Advisory Committee and will submit all comments to the provider within two weeks of receipt of the draft report.

A final report will be prepared for the additional sampling events including comments on the draft report. The final report will be submitted in Word format to the Department of Health as well as 20 paper copies. This report will become an appendix to the final report for DOH Contract CORY6.

Deliverables: Final Report in Word format, 20 paper copies of Final Report

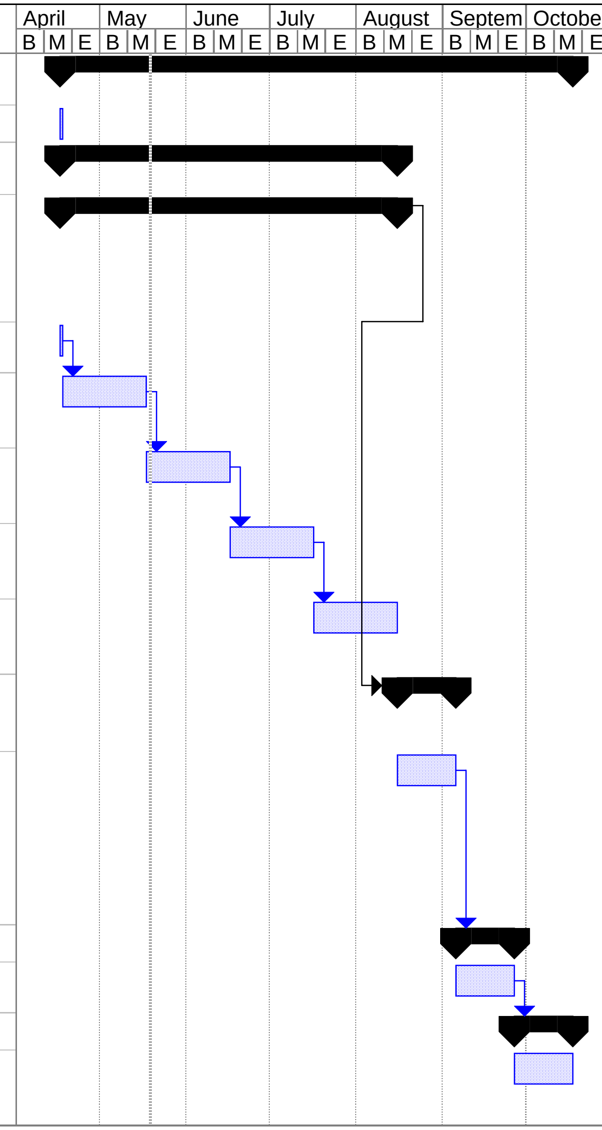
Section 3 Project Budget

A summary budget is attached in Table 2. Total project budget not to exceed $10,320.00.

Table 2 Project Budget

Task 1 Extend Operation of Current System = (Nitrogen Sampling Event Cost x 5) + (BOD Sampling Event Cost x 3) + (Weekly Site Visits x 12) ▪ Nitrogen Sampling Event Cost ▪ BOD Sampling Event Cost ▪ Weekly Site Visits (weeks where there is no sampling event).	$8,220.00 ▪ $1,104.00 ▪ $180.00 ▪ $180.00
Task 2 Disassemble sampling stations and evaluate quality of treatment media	$1,000.00
Task 3 Prepare Draft Report	$400.00
Task 4 Prepare Final Report	$700.00

ID	Task Name	Duration	Start	Finish	Predec	Cost
1	**Passive Nitrogen Additional Sampling Events**	**184 days**	**Thu 4/17/08**	**Sat 10/18/08**		**$10,320.00**
2	Initiate Purchase Order	1 day	Thu 4/17/08	Fri 4/18/08		$0.00
3	**Task 1 Extend Operation of Current System**	**121 days**	**Thu 4/17/08**	**Sat 8/16/08**		**$8,220.00**
4	**Deliverables: Field data, chain of custody forms, and lab results for each sampling event**	**121 days**	**Thu 4/17/08**	**Sat 8/16/08**		**$8,220.00**
5	Sampling Event #1 (analysis only)	1 day	Thu 4/17/08	Fri 4/18/08		$1,284.00
6	Sampling Event #2 (3 weekly visits and analysis)	1 mon	Fri 4/18/08	Sun 5/18/08	5	$1,644.00
7	Sampling Event #3 (3 weekly visits, BOD, and analaysis)	1 mon	Sun 5/18/08	Tue 6/17/08	6	$1,824.00
8	Sampling Event #4 (3 weekly visits and analysis)	1 mon	Tue 6/17/08	Thu 7/17/08	7	$1,644.00
9	Sampling Event #5 (3 weekly visits, BOD, and analaysis)	1 mon	Thu 7/17/08	Sat 8/16/08	8	$1,824.00
10	**Task 2 Disassemble sampling stations and evaluate quality of treatment media**	**21 days**	**Sat 8/16/08**	**Sat 9/6/08**	**4**	**$1,000.00**
11	Deliverables: Documentation that stations have been disassembled, table summarizing measurements of materials	3 wks	Sat 8/16/08	Sat 9/6/08		$0.00
12	**Task 3 Prepare Draft Report**	**21 days**	**Sat 9/6/08**	**Sat 9/27/08**	**11**	**$400.00**
13	Deliverable: Draft report in Word format	3 wks	Sat 9/6/08	Sat 9/27/08		$0.00
14	**Task 4 Prepare Final Reports**	**21 days**	**Sat 9/27/08**	**Sat 10/18/08**	**13**	**$700.00**
15	Deliverable: Final report in Word format, 20 paper copies of final report	3 wks	Sat 9/27/08	Sat 10/18/08		$0.00



Project: SamplingExtensionProject9Ma
Date: Mon 5/19/08

Task		Milestone		External Tasks	
Split		Summary		External Milestone	
Progress		Project Summary		Deadline	

ATTACHMENT I

A. SERVICES TO BE PROVIDED

1. Definition of Terms

a) Bureau= Bureau of Onsite Sewage Programs

b) Department = Florida Department of Health (FDOH), Bureau of Onsite Sewage Programs

c) EPA = Environmental Protection Agency, source of funding for project. Grant was awarded to FDOH

d) QAPP=quality assurance project plan: document guiding the collection, analysis and interpretation of samples, required by EPA

e) Provider=Florida Department of Environmental Protection (FDEP), Tampa District, Charles Kovach, Principal Investigator

2. General Description

a) General statement: The purpose of this project is to build upon previous work to test the feasibility of detecting wastewater inputs to Florida surface waters using optical characteristics such as optical brighteners (OB) from laundry detergents as tracers. This technique takes advantage of (1) the fact that OB in laundry detergent fluoresce at predictable wavelengths, (2) most wastewater from laundry is discharged into municipal wastewater systems or into local onsite sewage treatment and disposal systems (OSTDS), (3) OB are relatively stable in the environment and persist in the water column for weeks up to a month before being photo-degraded, and (4) fresh wastewater appears to possess other optical characteristics distinguishing it from surface water. Using a fluorometer, one can attempt to detect effluent sources by measuring the relative peaks in the wavelengths that correspond to maximal OB fluorescence. This technique has been used in other parts of the world such as northeastern Australia and the northeastern United States with some success. However, fluorescence of certain constituents in natural waters interferes with potential OB signals, especially in Florida where high concentrations of colored dissolved organic matter (CDOM) exist. Additionally, different brands of laundry detergent utilize different types of OB in different concentrations resulting in variations in fluorescence peaks and fluorescence intensities. Previous work conducted by Mote Marine Laboratory and the Florida Department of Environmental Protection in Florida, while limited in the number and types of wastewater samples analyzed, suggested that optical brighteners and wastewater possess distinct optical characteristics and that these characteristics can be isolated. The results further demonstrated that a simple single wavelength approach will not work in Florida waters due to CDOM interference. Additionally, it is unlikely that a simple ratio approach between two wavelengths, one characterizing CDOM and one characterizing CDOM and OB, would provide adequate quantification of OB concentrations. A more thorough understanding of the role CDOM plays in the detection of OB and other wastewater optical characteristics, and the variability among laundry detergents is required and will be a primary focus of this project. The provider will utilize an Emission-Excitation Matrix (EEM) approach in which a sample of water is excited at multiple wavelengths and the fluorescence emission resulting from the excitation at each of those wavelengths is recorded. The resultant matrix is then analyzed using a parallel factor analysis that groups similar excitation-emission peaks to form a “fingerprint” for each sample. The provider will characterize the EEM “fingerprint” of various surface water samples with varying levels and types of OB and wastewater to (a) determine if CDOM interferences can be removed, and (b) evaluate under which conditions a multiple wavelength approach may be feasible.

b) Authority: The Bureau of Onsite Sewage Programs operates under Section 381.0065, Florida Statutes

c) Scope of Service: This work will be conducted under controlled experimental conditions in order to best understand the relationships between OB and CDOM and the likelihood of detecting OB in the presence of these organics. The provider will collect water from several sources along the Florida Gulf Coast to capture different CDOM concentrations, salinities, and wastewater effluents and analyze for selected biochemical parameters. Source waters and mixtures will be optically characterized with and without spikes of optical brightener additions by the provider and/or subcontractors. The provider will draft a final report that will document the results and evaluate the feasibility of using an OB fluorescent technique in Florida waters that will be completed and presented to the Bureau's Research Review and Advisory Committee (RRAC) and the EPA. The provider will revise the report based on comments received. All deliverables shall be completed by September 30, 2008. All major scope changes shall be approved by the project officer of the Environmental Protection Agency, which is funding this contract.

d) Major Program Goals: This project will assess the feasibility of using the fluorescence signature of wastewater, including OB found in most laundry detergents, as a proxy for identifying wastewater effluent inputs in the waters of the Florida Gulf Coast. The project will further evaluate the utility of the EEM-Parallel Factor Analysis approach in mixed water samples, and the feasibility of using a limited number of flow-through fluorometers for the same determination. A secondary goal is the characterization of wastewater with regards to biochemical and optical characteristics.

B. Manner of Service Provision

1. Service Tasks

<u>a) Task List</u>

Task 1 Selection of a Laboratory to perform Excitation-Emission Matrix work

The provider will contract with a laboratory to perform all laboratory experiments associated with the Excitation-Emission Matrix (EEM) technique. The selected facility will have specific experience conducting laboratory and field experiments along the Florida Gulf Coast employing the EEM technique and Parallel Factor Analysis (PARAFAC) as it applies to OB fluorescence in natural waters. This task shall be completed 1 month after contract execution.

Task 2 Experimental Design Development and QAPP Approval

The provider, in coordination with the contract laboratory, will develop and submit a revision of the QAPP previously approved in contract CO0H5 for review and approval by the Bureau and the EPA. QAPP modifications will specifically address the number of samples and locations. No changes to the previously approved analytical method or data quality objectives are planned. The QAPP shall contain a detailed experimental design to address the following objectives:

ATTACHMENT I

- Based on previous work, utilize EEM analyses and PARAFAC modeling for identification of selected optical brighteners and to select target wavelengths for use in a field-based method.
- Characterize the EEM from a variety of sources with variable CDOM, ideally un-impacted by OB. This is a critical step in training the PARAFAC model to identify CDOM signatures prior to the addition of OB.
- Characterize the EEM of effluent from two wastewater treatment facilities, two laundry facilities, and five onsite sewage treatment and disposal systems (OSTDS).
- Characterize the EEM of mixtures by preparing dilution series from various sources of water and mixing them with fixed amounts of OB and/or wastewater.
- Using the PARAFAC model, isolate and validate factors and discrete wavelengths in both the visible and ultraviolet ranges that can be used in field screening instrumentation and possibly future remote sensing applications.

The revised QAPP shall be completed within one month of contract execution, the department will review within two weeks and the final version will be completed within two months of contract execution.

Task 3 Field Sampling and Non-EEM Laboratory Analyses

Based on the final experimental design developed and approved in Task 2, appropriate field sampling sites will be sampled for source water. It is anticipated that a maximum of 34 grab samples will be required to complete all necessary experiments. The anticipated field sampling matrix is as follows:

LOCATION	Sampling Events	Number of Sites	Total N
Wastewater Treatment Plant	2	2	4
Onsite Sewage Treatment and Disposal System	2	5	10
Laundromat	2	2	4
CDOM-Rich Waters	1	15	15
De-ionized Water	1	1	1
		TOTAL N:	**34**

PROPOSED FIELD SAMPLE MATRIX

Wastewater treatment plants, OSTDS, and laundromats will be sampled on two separate occasions to provide an estimate of same site variability at different times. Natural systems will be sampled to maximize the spatial variability of CDOM-rich waters. Five individual water bodies located along the Florida Gulf Coast from the Big Bend to Charlotte Harbor will be selected. Within each water body, three grab samples will be taken along a spatial gradient from the upper reaches down toward the mouth.

All Quality Assurance / Quality Control (QA/QC) procedures will follow FDEP standard operating procedures (SOP). Non-EEM parameters shall be collected at the selected locations to better characterize water samples. Sample parameters include a suite of field parameters including dissolved oxygen, pH, temperature, and conductivity and lab analyses. The following table outlines which parameters will be measured, what reference method will be used, and what the reporting unit will be.

PARAMETER	REFERENCE METHOD	REPORTING UNIT
Nitrate-Nitrite Nitrogen	EPA 353.2 (1983)	mg/L
Total Kjeldahl Nitrogen	EPA 351.2 (1983)	mg/L
Ammonia Nitrogen	EPA 350.1 (1983)	mg/L
Total Phosphorous	EPA 365.4	mg/L
Turbidity	EPA 180.1 (1983)	NTU
Total Suspended Solids	EPA 160.2	mg/L
Methylene Blue Active Substances	SM18 5540C	mg/L
Chlorophyll a	SM 10200H (mod.)	μg/L
Phaeophytin a	SM 10200H (mod.)	μg/L
Fecal Coliform	SM 9222D	CFU
Laboratory Parameters		

Not all sample parameters will be sampled for each station: deionized water (DI) and CDOM-rich samples need not to be analyzed for Methylene Blue Active Substances; wastewater, onsite, laundromat, and DI samples need not be analyzed for Chlorophyll a and Phaeophytin.

All applicable standard methods shall be performed by the FDEP Central Laboratory, FDOH Tampa Laboratory, or another NELAC-approved laboratory. The deliverable for this task will be tabulations of the field observations and measurements during sampling and all non-EEM analytical results. This task shall be completed within four months of contract execution.

Task 4 Laboratory EEM and Parallel Factor Analysis

Characterize the EEM of samples taken during task 3 and run parallel factor analysis (PARAFAC) on them. Characterize the EEM of mixtures by preparing dilution series from the various sources of water and mixing them with fixed amounts of OB and/or wastewater.

Using the PARAFAC model, isolate and validate factors and discrete wavelengths in both the visible and ultraviolet ranges that can be used in field screening instrumentation and possibly future remote sensing applications. The feasibility of identifying individual OB compounds rather than a standardized mixture will be examined. A total of 150 EEM scans are anticipated to complete all laboratory testing. Additional modeling will employ existing data as well as new samples collected. Based on the final experimental design developed and approved in Task 2, source waters, spiked samples and mixtures will be characterized using their excitation-emission matrix in general and the candidate wavelengths in particular. Agreement between dual channel field fluorescence and EEM shall be tested using data collected from a previous project and any obtained during task 3.

Tasks 3 and 4 will occur simultaneously as sample water for this task will be collected under Task 3. Task 4 will be completed within five months of contract execution.

<u>Task 5 Final Report and Presentation of Results</u>

A draft final report shall be submitted to the Bureau and the EPA for review and comment. This report will summarize the results from the laboratory and field experiment and provide recommendations as to the applicability and limitations of using fluorescence as an effluent tracer in Florida waters.

The provider shall submit twenty copies of a draft final report to the department one month prior to delivering the final report and within two months of the last environmental sampling event. The department shall be responsible for soliciting review of the draft final report by interested parties. The draft final report shall be completed within five months of contract execution.

The provider shall present the results of the draft report to the department's RRAC at a location somewhere in central Florida.

In developing the final report, the provider shall consider the comments of the department, the RRAC, and other reviewers that may have commented.

The provider shall submit twenty copies of the final report and also supply the department with at least one electronic copy of the final report and of tabulated analytical results in a format compatible with department software.

Completion of this task shall take place within six months of contract execution.

All reports and brochures developed shall recognize the funding from the U.S. Environmental Protection Agency, and the Florida Department of Health, as applicable.

<u>b) Task Limits</u>

All analysis of routine chemical samples shall be done using laboratories that have been state or federally accredited as having an adequate quality assurance and quality control protocol for the analytes being tested. The provider shall submit evidence of laboratory certification to the department before environmental sampling commences. Preservation methods and holding time requirements of Table II of 40 CFR 136 shall be met.

These requirements shall not apply to the experimental analyses of optical brighteners, and field measurements, for which adequate quality assurance procedures shall be described in the QAPP.

2. Staffing Requirements

<u>a) Staffing levels and qualifications</u>

Provider will have at least one project manager and a qualified person to do field work available on staff. Qualifications shall include an undergraduate degree in science or engineering, and evidence of either work experience or training in use of optical brighteners as tracer of water quality and remote sensing of water bodies. If such staff ceases to be available, provider may substitute staff with equivalent qualifications, provided that the substitute shall be trained on the QAPP by the provider, and the department is given two weeks notice of such a change and the provider's plan for the transition. Subcontractors may be used by the provider; their role shall be described in the QAPP.

3. Service Location and Equipment

The service shall be performed at the provider's laboratory facility and its contract laboratory's facility and at field locations on or near the Florida Gulf Coast. The provider and its contract laboratory shall provide any equipment necessary to perform the tasks but may use funds from this budget for parts and supplies necessary to complete each task. All deliverables shall be delivered to the Department of Health, Bureau of Onsite Sewage Programs.

4. Deliverables

<u>a) Service Tasks</u>

<u>*Task 1 Selection of a Laboratory to perform Excitation-Emission Matrix work*</u>

a) Copy of agreement between laboratory and provider

<u>*Task 2 Experimental Design Development and QAPP Approval*</u>

a) One draft QAPP submitted to the department and EPA Project Officer

b) One QAPP approved by the department and EPA Project Officer.

<u>*Task 3 Field Sampling and Non-EEM Laboratory Analyses*</u>

a) Tabulations of field observations and measurements during sampling and the analytical results

<u>*Task 4 Laboratory EEM and PARAFAC*</u>

a) Raw EEM data files and output and tabulation of results of PARAFAC

<u>*Task 5 Final Report*</u>

a) Twenty copies and one electronic copy of one draft report submitted to the department

b) Twenty copies and one electronic copy of one final technical report accepted by the department

<u>b) Reports</u>:

The provider shall provide an expenditure report for the project together with the final invoice. The expenditure report shall include date, amount, recipient and category of expenditures.

<u>c) Records and Documentation</u>:

Copies of deliverables shall be kept at the provider's facility in electronic and paper format. Field records shall be kept at the provider's office in the format they were obtained. See the provisions of the attached standard contract for length of record keeping.

5. Performance Specifications

a) Outcomes shall be measured in service tasks as specified in 4a. The deliverables will be evaluated for accuracy and percentage completed.

b) Monitoring and Evaluation Methodology. The department shall monitor performance of the provider by review of the deliverables and by attending at least one of the sampling events to observe if sampling procedures outlined in the QAPP are followed. Any observed shortcomings shall be noted to the provider and resolved.

6. Provider responsibility

The provider shall perform the tasks outlined above. The provider shall contract with a separate laboratory facility to generate the EEM data and run the PARAFAC as well as provide interpretation of results.

7. Department responsibility

The department shall facilitate review of QAPP, other deliverables, and reports. Department staff shall also be present for one workday during the field sampling.

C. Method of Payment

1. Payment Clause.

This is a fixed price (unit cost) contract. The department shall pay the provider, upon satisfactory completion of the services outlined in the Attachment I of this contract in accordance with the terms of this contract for a total dollar amount not to exceed $66,680.00 subject to the availability of funds.

Fixed Price Presentation

Deliverables, listed in B4, developed during completion of the tasks described in B1a) shall be paid according to the following schedule:

Deliverable	**Due Date**	**Cost**
Task 1. Selection of a Laboratory to Perform Excitation-Emission Matrix Work		
a) Copy of agreement between laboratory and provider	One month after contract execution	$0
Task 2. Experimental Design Development and QAPP Approval		
a) One draft QAPP submitted to the department and EPA Project Officer	One month after contract execution	$600
b) One QAPP approved by the department and EPA Project Officer	Two months after contract execution	$300
Task 3. Field Sampling and Non-EEM Laboratory Analyses		
a) Tabulations of field observations and measurements during sampling and the analytical results	4 Months after contract execution	$6,680
Task 4. Laboratory EEM and PARAFAC		
a) Raw EEM data files and output and tabulation of results of PARAFAC	5 Months after contract execution	$47,300
Task 5. Final Report		
a) Twenty copies and one electronic copy of one draft report submitted to the department.	5 Months after contract execution	$7,800
b) Twenty copies and one electronic copy of one final	6 Months after	

technical report accepted by the department.	contract execution	$4,000
	Total Cost	$66,680

2. Invoice Requirements.

The provider shall submit an invoice to the contract manager at the address listed in the department's standard contract on a quarterly basis using the form of Attachment II, within 30 days following the end of the period for which payment is being requested. Payment may be authorized only for service tasks on the invoice that are in accord with the above list and other terms and conditions of this contract. Documentation of completion of service tasks shall be submitted to the contract manager prior to, or with the invoice. Partially completed tasks may be invoiced and paid based on the percentage of the service task completed.

D. Special Provisions

1. Federal Funds.

The approximate amount of federal funds contained in the total contract amount is $66,680.00. The catalogue of federal domestic assistance (CFDA) Number is: 66-475 (U.S. Environmental Protection Agency/Gulf of Mexico Program).

Contract Renewal: This contract may be renewable for three years or for the term of the original contract, whichever is longer. Such renewals shall be made by mutual agreement and shall be contingent on satisfactory performance evaluations as determined by the department and shall be subject to the availability of funds. Any renewals shall be in writing and shall be subject to the same terms and conditions as set forth in the initial contract.

Documentation: Provider is required to maintain separate accounting of revenues and expenditures of funds under this contract and each CSFA or CFDA number identified on Exhibit I attached hereto in accordance with generally accepted accounting practices and procedures. Expenditures which support provider activities not solely authorized under this contract must be allocated in accordance with applicable laws, rules and regulations, and the allocation methodology must be documented and supported by competent evidence.

Provider must maintain sufficient documentation of all expenditures incurred (e.g. invoices, canceled checks, payroll detail, bank statements, etc.) under this contract which evidences that expenditures are:

1. allowable under the contract and applicable laws, rules and regulations;
2. reasonable; and
3. necessary in order for provider to fulfill its obligations under this contract.

The aforementioned documentation is subject to review by the department and/or the State Chief Financial Officer and provider will timely comply with any requests for documentation.

Financial Report. The provider shall submit a quarterly financial report stating, by line item, all expenditures made as a direct result of services provided through the funding of this contract to the department within 30 days of the end of each quarter. Each report must be accompanied by a statement signed by an individual with legal authority to bind provider certifying that these expenditures are true, accurate and directly related to this contract.

ATTACHMENT I

Spending Plan. If this contract is funded with federal awards and/or state financial assistance, and the provider is determined to be a recipient or subrecipient pursuant to OMB Circular A-133, §__.105 and/or §215.97(2)(m) and (v), Fla. Stat. (2004), as indicated on Exhibit I, provider must ensure that funding received under this contract in excess of expenditures is remitted to the department within 45 days of the earlier of the expiration of, or termination of, this contract unless, prior to the expiration of this contract, the provider has submitted a Spending Plan to the department which has been approved, in writing, by the department. The Spending Plan must outline the plan for expending the excess funds in a period of no more than 90 days unless a longer period is agreed to by the department and shall be submitted to the department no later than fifteen (15) business days following the identification of the excess amount. The department's approval or disapproval of the Spending Plan will be in writing no later than 10 days after receipt of the Spending Plan. Any excess funds that remain unexpended after the agreed upon time period will be returned to the department forthwith.

This is the end of text.

<u>Attachment 1 for Phase II of Manatee Springs Study</u>

A. SERVICES TO BE PROVIDED

1. Definition of Terms

a) Bureau= Bureau of Onsite Sewage Programs

b) Department = Florida Department of Health, Bureau of Onsite Sewage Programs

c) Effluent = The wastewater that leaves the Nutrient-reducing treatment system (NRTS)

d) Field measurements of water quality = Parameters measured by a probe at the time and location of taking the sample: dissolved oxygen, pH, electric conductivity, and temperature

e) Influent = The wastewater that enters the Nutrient-reducing treatment system (NRTS)

f) Karst Study = Study to investigate impacts of onsite wastewater treatment in the karst area at Manatee Springs State Park, funded by the EPA, Gulf of Mexico Program and the Department. Phase I of this study has recently been completed by the Department and the Provider

g) Nutrient-reducing treatment system (NRTS) =onsite treatment system that reduces the concentration of nitrogen and/or phosphorus in the treated effluent

h) QAPP =quality assurance project plan: document guiding the collection, analysis and interpretation of samples, required by EPA

i) Provider =Florida State University's Department of Oceanography, Dr. Jeff Chanton, Principal Investigator

j) Total Nitrogen = total concentration of nitrogen (mg/L) in a water sample

k) Total Phosphorus = total concentration of phosphorus (mg/L) in a water sample

l) Tracers = The chemical tracers fluorescein and sulfur hexafluoride, which were used in phase I of the Karst Study

2. General Description

a) General statement: Provider will perform environmental sampling of monitoring wells in the vicinity of nutrient-removing onsite sewage treatment and disposal systems and perform sampling to assess the effectiveness of the nutrient-removing systems. Provider will also perform a more detailed assessment of the effectiveness of the nutrient-removing systems over a one-week period. Provider will develop reports and a journal publication summarizing the Karst Study

b) Authority: The Bureau of Onsite Sewage Programs operates under Section 381.065, Florida Statutes

c) Scope of Service: The field work shall take place in Manatee Springs State Park. All deliverables shall be completed within 18 months after installation of the nutrient-reducing treatment systems. The provider shall prepare deliverables using software and hardware applications that are consistent with the Department standards (currently Microsoft software, PC-compatible)

d) Major Program Goals: The study will assess the reduction in environmental impacts obtainable by installation of NRTS. The study will also evaluate the performance of the particular technologies installed on this site.

B Manner of Service Provision

1. Service Tasks

a) Task List

Task 1. Development of QAPP:

The provider shall revise the QAPP of phase I of the Karst Study as needed to guide the field and laboratory work of phase II: a) The provider shall submit the QAPP for review to the department, which in turn will submit it to EPA. b) Provider shall address comments and concerns submitted by the department and EPA in finalizing the QAPP. The final version shall be approved by EPA before field work commences. This task shall be accomplished within the first quarter of the contract period

Task 2. Performance Assessment Sampling:

Provider shall assess the performance of two nutrient-reducing treatment systems, which the Department in coordination with the State Park Service shall cause to be installed. To assess diurnal and daily variability of performance, multiple samples of the influents and effluents shall be taken and analyzed over a one-week period. The week will be determined in coordination with the State Park and the Department. Operating conditions of the treatment systems shall be monitored. Samples shall be analyzed by a DOH-certified lab, field measurements shall be performed by provider's staff. Provider's staff shall be present to monitor and ensure the performance of sampling equipment during the experiment.

The sampling shall occur during seven consecutive 24-hour periods, consisting of:

-Twenty-eight (28) (seven times two influents, two effluents) 24-hour composite samples for influent and effluent of NRTS; analyzed for total nitrogen (nitrite+nitrate, total Kjeldahl nitrogen, total ammonia), and total phosphorous, and including field measurements of water quality and water usage.

-fifty-six (56) (seven times twice two influents, two effluents) sets of shorter-term composite or grab samples; analyzed for total nitrogen (nitrite, nitrate, total Kjeldahl nitrogen, total ammonia), and total phosphorus, including field measurements of water quality and water usage. To address holding time limitations (see B1.b)), up to half of these samples may be analyzed for nitrite+nitrate, instead of nitrite and nitrate separately.

-seven (7) duplicate samples; analyzed for total nitrogen (nitrite, nitrate, total Kjeldahl nitrogen, total ammonia), and total phosphorus.

Details will be determined as described in the QAPP. Results shall be transmitted to the department by e-mail.

Task 3. Environmental Sampling:

The provider shall conduct environmental sampling and analysis approximately every two months in a one-year period for a total of six sampling events. During each sampling event, samples shall be taken at eight monitoring points at the Magnolia site (not sampling M10 and M4), one duplicate at the Magnolia site, five monitoring points at the Hickory site, two background wells, influents and effluents of the two nutrient-reducing treatment systems and one duplicate. One of the sampling events shall be on or immediately following the last day of the performance sampling. Samples shall be analyzed by a DOH-certified lab at least for total nitrogen (nitrite, nitrate, total Kjeldahl nitrogen, and total ammonia), total phosphorous, and fecal coliforms. Provider shall record water usage, water levels in the monitoring wells and field measurements of water quality.

Samples taken during two sampling events shall be analyzed by the provider for the tracers released during phase I.

Details will be determined as described in the QAPP. Results shall be transmitted to the department by e-mail.

Task 4. Report Development:

Based on the tasks 1 through 3 above, the provider shall develop a final report that describes and summarizes the performance assessment and environmental sampling. The provider shall compare the results of this study to Phase I of the Karst Study and to other applicable studies such as the Florida Keys onsite wastewater nutrient reduction systems demonstration project reported on by Ayres Associates in 1998. The report shall include an analysis of the feasibility of nutrient-reduction for water quality in the karst environment studied, and discuss policy options with reference to the Bureau's 2004 and 2007 Wekiva Basin Onsite Sewage Treatment and Disposal System Studies.

a) The provider shall submit twenty copies of a draft final report to the department and the Research Review and Advisory Committee two months prior to delivering the final report and within three months of the last environmental sampling event. The department shall be responsible for convening the Research Review and Advisory Committee for the purpose of reviewing the draft final report. In developing the final report, the provider shall consider the comments of the Research Review and Advisory Committee and other reviewers as may have commented.

b) The provider shall submit twenty copies of the final report and also supply the department with at least one electronic copy of the final report and of tabulated analytical results in a format compatible with department software.

All reports and brochures developed shall recognize the funding from the U.S. Environmental Protection Agency, and the Florida Department of Health, as applicable.

<u>b) Task limits</u>

All analysis of samples for nitrogen and phosphorus and fecal coliforms shall be done using laboratories that have been state or federally accredited as having an adequate quality assurance and quality control protocol for the analytes being tested. The provider shall submit evidence of laboratory certification to the department before environmental sampling commences. Preservation methods and holding times requirements of Table II of 40 CFR 136 shall be met.

These requirements shall not apply to the analysis of tracers performed in the provider's lab and to field measurements of water quality by the provider, for which adequate quality assurance procedures shall be described in the QAPP.

2. Staffing Requirements

<u>a) Staffing levels and qualifications</u>

Provider will have at least one qualified person available to perform the tasks. This staff shall have experience from phase I of this karst study. If such staff ceases to be available, provider may substitute staff with experience in environmental sampling and academic training in an environmental science to the extent of at least a bachelor's degree, provided that the substitute shall be trained on the QAPP by the provider, and the Department is given two weeks notice of such a change and the provider's plan for the transition. Subcontractors may be used by the provider; their role shall be described in the QAPP.

<u>b) Subcontracts</u>

Subcontractors may be used by the provider; their role shall be described in the QAPP.

3. Service Location and Equipment

The service shall be performed at Manatee Springs State Park, the provider's lab and work environment, and the location of the certified lab referred to above. The provider shall provide any equipment necessary to perform the tasks, except as specified in B 7 (Probe). All deliverables shall be delivered to the Department.

4. Deliverables

a) Service Tasks

Task 1: a) one draft QAPP for phase II submitted to the department
b) one final QAPP, approved by the department and EPA project officer

Task 2: Analytical results from samples taken during performance assessment, and tabulations of field measurements and observations.

Task 3: Analytical results from samples taken during environmental sampling, and tabulations of field measurements and observations.

Task 4: a) twenty copies of one draft report submitted to the department
b) twenty copies and one electronic copy of one final report accepted by the department

b) Reports:

The provider shall provide an expenditure report for the project together with the final invoice. The expenditure report shall include date, amount, recipient and category of expenditures.

c) Records and Documentation:

Copies of deliverables shall be kept at the provider's office in electronic and paper format. Field records shall be kept at the provider's office in the format they were obtained. See the provisions of the standard contract for length of record keeping.

5. Performance Specifications

a) Outcomes shall be measured in service tasks as specified in 4 a. The deliverables will be evaluated for accuracy and percentage completed.

b) Monitoring and Evaluation Methodology. The department shall monitor performance of the provider by review of the deliverables and by attending at least one of the sampling events to observe if sampling procedures outlined in the QAPP are followed. Any observed shortcomings shall be noted to the provider and resolved.

6. Provider responsibility

The provider shall perform the tasks outlined above. The provider shall notify Manatee Springs State Park of upcoming sampling events. In scheduling sampling events, the provider shall coordinate with the department in such a way that department staff and/or equipment shall be present.

7. Department responsibility

The department shall cause the NRTS to be installed. The department shall facilitate review of QAPP and reports.

The department offers assistance in performance sampling, in that one staff, upon coordination of schedules, shall be present and assist during the mobilization day and demobilization day of performance sampling. Department staff shall also be present for one additional workday during the performance sampling and transport samples to provider's laboratory on this occasion if needed.

The department offers assistance in environmental sampling, in that one staff, upon coordination of schedules, may accompany or supplement provider's staff during the sampling events.

The department offers to loan to the provider the Bureau's YSI 556 multi-parameter probe and up to three composite samplers for repeated short-term use in field measurements of water quality. Use of this instrument for this project shall be coordinated with the other uses of this instrument in other projects to avoid conflicts. Should the Bureau's YSI 556 cease to be functional, the department shall in other ways provide for the use of probes to measure water quality in the field.

C Method of Payment

1. **Payment Clause**.

This is a fixed price (unit cost) contract. The department shall pay the provider, upon satisfactory completion of the services outlined in the Attachment I of this contract in accordance with the terms of this contract for a total dollar amount not to exceed $24,000.00, subject to the availability of funds.

Fixed Price Presentation

Deliverables, listed in B4, developed during completion of the tasks described in B1a) shall be paid according to the following schedule:

Task 1 (QAPP):		
a) draft	$	
b) final	$	
Subtotal	$	
Task 2 (Performance Assessment Sampling)		
Subtotal	$	
Task 3 (Environmental Sampling)		
Subtotal	$	(12% of this amount for each regular sampling event, 2% additional for each tracer event)
Task 4 (Final Report)		
a) Draft	$	
b) Final	$	
Subtotal:	$	
Total :		$24,000.00

2. **Invoice Requirements.**

The provider shall submit an invoice to the contract manager at the address listed in the department's standard contract at least on a quarterly basis using the form of attachment 4, within 30 days following the end of the period for which payment is being requested. Payment may be authorized only for service tasks on the invoice that are in accord with the above list and other terms and conditions of this contract. Documentation of completion of service tasks shall be submitted to the contract manager prior to, or with the invoice. Partially completed tasks may be invoiced and paid based on the percentage of the service task completed.

D Special Provisions:

1. Federal Funds.

The approximate amount of federal funds contained in the total contract amount is $24,361.00 The catalogue of federal domestic assistance (CFDA) Number is: 66-475 (Gulf of Mexico Program)

2. Termination for Failure to Perform in Unrelated Projects

Part III, Section B, Termination, paragraph 4 of the Standard Contract has added to the last sentence the following text: 'Provided, however, termination under this provision shall not be available to the department for contracts unrelated to this contract by subject matter.'

This is the end of text.

Taylor County Coastal Community Water Quality Monitoring Results of Phase I

Draft Report 05/09/2008

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Taylor County Health Department

Summary

Building on the Healthy Beaches Program of the Florida Department of Health, ten sites at and near the coast of Taylor County were sampled for one year from July of 2004 through June of 2005 approximately weekly for fecal coliform, enterococci and field parameters and monthly for nutrients.

Inventories of onsite systems were gathered in 2001 by a group of local highschool students. No failing drainfield was observed during the field work. These inventories indicated about a third of onsite systems with elevated drainfields. Taking into account the relatively recent age of many houses, sparse settlement and the fact that some of the most seaward houses along with their onsite systems had been destroyed in 1993 during the storm of the century, most onsite systems should represent functioning under modern standards.

Sites were selected to represent a variety of types: beaches with development (Dekle, Keaton, Cedar Island), beaches without development (Adams, Hagens Cove), canals (Dekle, Cedar Island), creeks (Blue and Dekle) and a spring (Sandpiper Springs).

The three developments included consisted predominantly of groups of single family houses with between 50 and 200 onsite systems each. The undeveloped beaches bracketed the study area to the north and the south.

Salinity ranged generally between 15 and 30 ppt at most sites. A freshwater creek and a site where a spring vent mixed with surface water had lower salinity.

Total phosphorus concentrations were below the detection limit of 0.014 mg/L with only one exception (0.2 mg/L at Keaton Beach on 09/13/2004).

Total nitrogen concentration did not show significant differences between sites overall but indicated a trend along the coast from higher concentrations in the north to lower concentrations in the south, and a tendency for higher concentrations at Keaton Beach when current directions was from the north.

Nitrate nitrogen concentrations tended higher during ebb and slack tidal conditions than during flood.

Pathogen indicator counts indicated that the beaches included in the Healthy Beaches assessment were among the less contaminated water bodies investigated. Two of the three developed beaches never exceeded the 400 CFU/100 mL fecal coliform standard.

Site types did not behave the same in all cases. While developed beaches were not significantly different from each other, except salinity, the undeveloped beaches had significant differences between the northern and the southern bracketing site. The two creeks, which varied in salinity, also varied significantly in their enterococci but not their fecal coliform levels, while the spring's site was generally the cleanest of all investigated sites.

The Creek at Dekle, the two canal sites and the northern undeveloped Adams Beach tended to have the highest enterococci counts. For fecal coliform, the fresh Blue Creek joined these sites as among the most contaminated.

Developed beaches (Dekle, Keaton, and Cedar Island) had pathogen indicator counts that were indistinguishable in rank from the southern undeveloped beach, Hagen's Cove,

The fecal coliform to enterococci ratio was significantly elevated at Blue Creek with Dekle Creek in an intermediate position relative to all other sites. Average indicator counts at these other sites showed a very strong log-log relationship with each other.

This consistent pattern and the lack of correlations of indicator counts with salinity and tidal indicators suggested that transport and differences in source types is not important for beach and canal sites. Instead, different intensities of the same local sources may explain observed indicator counts. Creek sites correlations were consistent with downstream transport, in particular of fecal coliform. The spring site correlations were consistent with the concept that sea water with high indicator counts is mixing with relatively clean and fresh spring water.

Rainfall was consistently associated with increased indicator counts. For enterococci the rainfall in the week prior tended to be more important, for fecal coliform the rainfall during the previous day was stronger associated with increased counts. For fecal coliform, cloudy conditions during sampling tended to result in significantly higher counts than sampling during sunny conditions. For enterococci, rainy conditions vs. sunny conditions were significantly higher.

Enterococci counts tended to increase with water temperature and decrease with dissolved oxygen saturation and at several sites tended to decrease with increasing pH.

Building on the importance of rainfall, thresholds were investigated that resulted in high odds ratios for exceeding an enterococci standard of 104 CFU/100 mL, and fecal coliform standards of 400 CFU/100 mL (beach standard) and 14 CFU/100 mL (shellfish standard). For 400 CFU/100 mL fecal coliform, a 24 hour rainfall exceeding 1" was the best threshold. For 14

CFU/100 mL fecal coliform, a 24 hour rainfall exceeding 0.15” was the best threshold. This low threshold is unlikely to lead to runoff and may instead be related to the effect of drop impact or cloudiness. For the 104 CFU/100 mL enterococci standard, the more commonly violated of the two beach thresholds addressed here, a weekly rainfall of more than 1.4” or a 24-hour rainfall of more than 1” result in odd ratios between four and a clean separation.

Exclusion of samples with rainfall beyond the thresholds resulted in a somewhat different pattern of the reduced indicator counts:

Enterococci in the canals and Dekle Creek remained among the highest; most beach sites were now very similar, with Keaton Beach at the lower end. Fecal coliform concentrations were highest in both creeks, intermediate in the canals and lowest on the beaches. Consideration of the water at the spring site as a mixture of seawater and clean fresh groundwater, resulted in a grouping of the seawater together with the other beach data.

Beach sites did not show a differential impact of onsite sewage systems on indicator counts or nutrients. Canal sites had higher indicator counts. The observations that the ratio of enterococci to fecal coliform has the same pattern as at beach sites, the similarity to Dekle Creek concentrations, and the lack of indications of higher contamination during low tide when the impact of OSTDS should be more visible, suggested that onsite systems per se are not the cause for this elevation.

This suggests local sources, regrowth and rainfall as the most prominent sources of indicator organisms. The higher fecal coliform indicators in creeks could be related to the lower decay rate relative to enterococci in freshwater observed by Anderson et al. (2005).

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Introduction

Situation

Taylor County on the Gulf of Mexico Coast of Florida has been a largely rural county. As in other regions, the coastal area has experienced development in recent years, largely residential and vacation homes. This change in land use has the potential to change the quality of water discharged to coastal waters and impact coastal water quality and associated uses. In the area under consideration, the environs of the coastal communities of Keaton Beach, Dekle Beach, and Cedar Island and their environs, recreational contact with water, scalloping and fishing are uses that may be affected. (Figure 1)

The beach water quality sampling program conducted by the County Health Department with funding from the state and EPA since August of 2000 has observed bacteriological quality problems, predominantly with respect to enterococci counts. As a result of these observations, beach advisories have been posted frequently. Health advisories under the “Florida Healthy Beaches Program” are posted (DOH, 2000) if enterococci meet or exceed 104 CFU/100 mL or the 30 day geometric mean exceeds 35/100 mL. Fecal coliform samples lead to an advisory if they exceed 400 CFU/100 mL

The southern half of Taylor County coastal waters including the area under consideration is part of the Big Bend Seagrass Aquatic Preserve and classified as Outstanding Florida Waters. The fecal coliform surface water quality standards for class III waters (recreational use and fish and wildlife health) include (FAC 602-302.530) that less than 10% of sample results over 400 CFU/100mL and not exceed 800 on any one day. Shellfish standards for direct marketing are that fecal coliform not exceed a median of 14 CFU/100mL and not exceed 43CFU/100 mL more than 10% of the time.

Water sampling conducted by the Suwannee River Water Management District (SRWMD, 2000) showed that coliform counts in waters adjacent to the communities of Keaton and Dekle Beach exceeded the marine surface water quality standards with respect to fecal and total coliform counts for recreational uses and maintenance of healthy fish and wildlife. A survey of Southern Taylor County coastal waters by the Florida Shellfish Environmental Assessment Section of the Florida Department of Natural Resources in the winter of 1992/1993 showed mostly moderate and high fecal contamination in near shore areas whereas the offshore water showed low contamination (Smith, 1993).

Possible Sources

The sources of indicator bacteria and nutrients, such as wastewater, domestic animals, cattle or wildlife may exist immediately adjoining the contaminated water or be transported to the impacted water. The possible importance of non-human sources is illustrated by results of ribotyping of E.Coli in Charlotte County surface water, which identified only 5 of 105 cultures in 23 samples as human but 92 as non-human (Vincent, 1999). Centralized wastewater facilities, which did not exist in the study area in the study period, or onsite wastewater, if not properly functioning, may release partially treated wastewater to water. In particular, pre-1983 onsite sewage treatment and disposal systems and unpermitted systems (OSTDS) may be installed into or just above the water table. Newer OSTDS require a 24-inch separation from the wet season water table, which Florida research has shown to be effective in removing pathogen indicators and to a lesser extent, nutrients (Ayres Associates, 1993). The study area has OSTDS infrastructure that is newer than in other parts of the state due to relatively recent growth and due to destruction and subsequent repair of about 57 homes in the "storm of the century" on March 13, 1993, which resulted also in upgrades to onsite systems.

Sediments can constitute a source, as indicator organisms can survive longer and may even regrow (Anderson et al. 2005; Davies et al 1995). For this source to become effective to contaminate the water column, mobilization has to occur, such as during high tides and storm events as has been observed for E.coli (Solo-Gabriele et al., 2000).

Studies elsewhere have suggested that surface runoff from residential areas can also be an important source of coliforms to surface water bodies (U.S. EPA, 2000). Surface runoff is also implicated by findings of association of fecal coliform with rainfall in an area slightly to the South (Smith, 1993) and of fecal indicators and enteroviruses with rainfall, stream flow and temperature (Lipp et al., 2001) and with impervious surface (Mallin et al. 2000). Such runoff may also transport contamination by wildlife (Benton et al., 1983). (Shellfish closing with rainfall)

The purpose of this project was to implement data collection to evaluate source hypotheses for pathogen indicators at beaches in Taylor County. State and local officials were at the same time developing plans to improve sewage waste disposal methods in coastal communities. The Taylor County Water and Sewer Authority received funding to install sewer for two of the three communities (Keaton Beach and Cedar Island Beach) where beaches are monitored. Hookup to sewer occurred only after completion of this study. Thus, this study will present an opportunity for a comparison at a later time to assess the impacts of such a project on water quality.

Project Objectives

Given the variety of possible sources and the need for information to evaluate sewage disposal approaches, the purpose of this project was to conduct expanded environmental monitoring to:

a) measure the characteristics of adjoining surface water that may contribute to beach contamination
b) assess the existence of differences in bacteriological indicators and nutrients between beaches with and without development
c) attempt to find correlations of bacteriological contamination patterns with patterns in other water characteristics
d) establish a baseline to which other small coastal communities can be compared

Methods

Inventories of OSTDS

The environmental studies academy of the local Highschool undertook the task of an inventory of onsite sewage treatment and disposal systems during the summer of 2001. Using property parcels maps as orientation pairs of students visited the lots in each coastal community. They observed the apparent presence or absence of onsite systems and their characteristics. They obtained GPS-coordinates (assumed +/- 5m) using a hand-held GPS-device while standing on or as close to the system as feasible. These observations were recorded on paper forms. Paper records of these observations were transcribed in spring of 2004 at the Bureau of Onsite Sewage Programs.

Experimental Design

This study built upon the existing Florida Healthy Beaches Program in Taylor County. The objective of the experimental design was to allow comparison testing of pathogen and nutrient indicators between sample locations with different characteristics. The experimental design is based on the assumption that different station characteristics can distinguish between several plausible alternatives for sources of pathogen indicators. Results may exclude some of the alternatives and will help focus future analysis on the most promising source scenarios.

The treatments and corresponding site locations (see Figure 1) were:

- Beach in the vicinity of a development (Dekle Beach, Keaton Beach, Cedar Island).
- Beach absent development but accessible to recreational users (Hagen's Cove, Adams Beach). This type allowed assessment of impacts by non-residential sources such as birds and recreational use. Hagen's Cove was included in the assessment by Smith (1993) with a median fecal coliform count of 3.4CFU/100 mL in 8 samples.
- Dead-end canals surrounded by residential development (canals at Dekle Beach and Cedar Island). This type allowed assessment of the combined effect of stagnant water, surface water runoff and OSTDS effluent on surface water quality.
- Water stemming from upstream of the developed area. Three sites were included:
    - A salty creek just upstream from a development, draining a marshy area (furthermore referred to as Dekle Creek). This type allowed assessment of sources in the marsh.
    - Fresh surface water originating further inland (Blue Creek). This creek drains parts of interior Taylor County and forms a reservoir about a mile upstream of the sampling location. This type allowed assessment of further inland sources.
    - Spring originating in the vicinity of the coast (Sandpiper Spring at Cedar Island). This site allowed assessment of a background concentration and groundwater contributions to pathogen indicators presence at the beaches.

Site Locations

The monitoring program included sampling sites at the Coast of Taylor County between Adam's Beach and Hagen's Cove. All sampling site locations were located in the hydrologic unit code (HUC) 3110102. Sites were selected based on accessibility and professional judgment of representativeness for the location type. Each site was recorded with global positioning systems (GPS) technology that is accurate to <5 meters according to manufacturer's information. The locations of the sampling sites are shown in Figure 1 and Table 1. Samples were generally taken in a North-to-South direction, with Adam's Beach as the first sample and Hagen's Cove as the last sample.

Table 1. Sampling Locations

Site #	Name	Latitude	Longitude	Type
1	Adams Beach	29.882118	-83.634375	undeveloped beach
2	Canal at Dekle	29.84925	-83.618833	Canal
3	Creek at Dekle	29.848585	-83.616026	saltwater creek
4	Dekle Beach	29.849275	-83.619743	developed beach
5	Keaton Beach	29.818813	-83.594226	developed beach
6	Blue Creek	29.824808	-83.576323	freshwater creek
7	Canal at Cedar Island	29.811603	-83.580731	Canal
8	Sandpiper Spring	29.816006	-83.583838	Spring
9	Cedar Island Beach	29.815676	-83.587258	developed beach
10	Hagen’s Cove	29.772375	-83.580026	undeveloped beach

a)

b)

c)

Figure 1. Aerial View of the Study Area with Sampling Site Locations

a) overall view
b) Dekle Beach
c) Keaton Beach/Cedar Island

Sampling Methods

Sampling occurred for one year from July 12, 2004 to June 27, 2005. Bacteriological Samples were collected weekly for all sites. This was consistent with the historical record of weekly beach samples. Nutrient samples were collected monthly. The beach sampling program required Taylor County Health Department (TCHD) field personnel to collect samples on Monday or Tuesday of the sampling week.

Samples were grab samples. Where access from land was possible (canals, spring, creeks), samples were taken about 6-12” below the surface from the middle of the stream or as far out as the sampling pole could reach. For beach samples, samplers waded into the water until it was 6-12” deep, and took a sample from the middle of the water column. The sampling container was of PVC material and fastened to a 10 ft sampling pole to avoid sampling sediments stirred by the sampler. The probes were also fastened to the pole and measured field parameters at the depth at which the sample water was taken.

Bacteriological Indicators

The sample container was an empty, sterile Whirl-Pak sampling bag. The collection bag held at least 125 milliliters (mL) but no more than 1000 mL to allow for adequate sampling and mixing. Field sampling personnel did not open the collection bag until just prior to taking the sample in order to protect the bag from contamination. Bacteriological samples were iced or refrigerated at a temperature of 1-4º Celsius (C) and stored in insulated containers to assure proper maintenance of storage temperature during transit to the laboratory, which was generally accomplished within the holding time of six hours. From August 23, 2004 on, 9% of samples were duplicates, one for each sampling event and taken immediately after the regular sample has been taken, rotating between the sampling sites. Fecal Coliform were determined following SM 9222D, Enterococci were determined following EPA 1600. We relied on the QA/QC procedures of the NELAC-certified laboratory’s quality assurance plan.

Nutrients

The DOH-certified laboratory provided the sample containers. Samples were iced or refrigerated with cool packs at a temperature of 1-4º Celsius (C) and stored in insulated containers to assure proper maintenance of storage temperature during transit to the laboratory. Concentrations were determined for Total Phosphorus (EPA 365.3), Nitrate-N (EPA 353.3), Nitrite-N (SM 4500NO2B), Total Kjeldahl N (EPA 351.3), and Total Ammonia Nitrogen (EPA 350.2). We relied on the QA/QC procedures of the NELAC-certified laboratory's quality assurance plan.

Field Parameters

The field parameters water temperature, dissolved oxygen (DO), pH, conductivity and salinity, oxidation-reduction-potential were determined in the field using parameter specific probes (YSI 556). These parameters were measured after two to four minutes in the water based on an initial assessment that this would be sufficient for measured values to stabilize.

Turbidity (Global waters) was only measured beginning with the sampling event of November 01, 2004 because an initial repair of the instrument took longer than expected.

Field Observations

Field observations were recorded, including the general weather conditions (sunny, cloudy, or rainy), rainfall during the last week, three days, and 24 hours (based on a rain gauge installed by Taylor CHD at Keaton Beach Marina), water and air temperatures, tidal conditions (tide1: ebb, slack, or flood; tide2: low, medium, or high), and current direction along the coast line and strength. Additionally, tidal data for the period were obtained for Cedar Key (http://140.90.121.76/cgi-bin/co-ops_qry_direct.cgi?stn=8727520+CEDAR+KEY%2C+GULF+OF+MEXICO+%2C+FL&dcp=1&ssid=WL&pc=W2+-+Hourly+heights&datum=MLLW&unit=0&bdate=20040701&edate=20050630&date=3&shift=1&level=-4&form=0&host=&addr=167.78.4.18&data_type=vwl&format=View+Data) to the South and Shell Point to the North (http://www.ndbc.noaa.gov/data/view_text_file.php?filename=shpf1h2004.txt.gz&dir=/ftp/data/historical/stdmet/). Shell point tidal data were incomplete, existing data suggested that the time of highest tide stage coincided within an hour or so for both stations. Cedar Key water level data and three-hour averages were extracted for the time of sampling at each site.

Data Processing and Analysis

Results were entered from field notes into Excel Spreadsheets after return to the County Health Department, and analytical results were subsequently added. Spreadsheets were transmitted to the Bureau of Onsite Sewage Programs quarterly. QC activities there included verification of data entry on a subset of records, and rechecking of data entries that showed unusual differences between duplicate or related measurements. While analytical results were added correctly, numerous instances of typing and reading errors from the field probe instrument screen were found. Data were processed in Excel, joined with additional tidal data in ACCESS and analyzed in SPSS 12.0. For the purposes of analysis, values below the detection limit were replaced with the value of half the detection limit.

Results

Inventories of OSTDS

During the gathering of the inventory, no failing onsite systems were observed. In Dekle Beach 51 OSTDS were counted, of which 23 were estimated to be above ground systems. In Cedar Island, 128 OSTDS were counted, of which 43 were identified as above ground systems. In Keaton Beach 183 OSTDS were counted, of which 62 were above ground systems. At Blue Creek, no onsite systems were counted but the property appraiser maps indicated 21 occupied lots and one business in this area.

Completeness

Sampling began on 07/12/2004 and ended on 06/27/2005. Of the possible 51 weekly samples 45 were obtained, including 11 sampling events for nutrients. Two sampling events were missed due to hurricanes (Frances 9/6/04 and Jeanne 9/27/04). The other sampling events were missed due to staffing shortages, in particular during holidays (12/27/04; 2/14/05; 4/18/05; 5/30/05). One additional sampling event from one site was precluded due to access problems. During sampling events, two samples for pathogen indicators and one sample for nutrients were lost (completeness 99.6% and 99.1% respectively). Missing field parameters included one event when the probe was not taken into the field, and results from three sampling sites where the field parameters were not recorded or paper records were lost.

Quality Control Measures

39 duplicate samples for pathogen indicators were obtained. Original indicator counts for these samples were not-normal distributed, but the natural log-transformed values for original

samples and duplicates were both approximately normally distributed (Kolmogorov-Smirnov, enterococci a =0.091). The mean difference between natural log-transformed samples and duplicates was not significantly different from zero (means 0.02 for ln fecal coliform, 0.005 for ln enterococci), with a standard deviation of 0.65 and 0.57 for ln fecal coliform and ln enterococci, respectively. 82% and 87% of all duplicates were within a factor of 2 of the original sample for fecal coliform and enterococci, respectively, and 54% of fecal coliform and 41% of enterococci duplicates were within a factor of 1.2.

Differences in natural log-transformed counts did not show differences in precision by site nor an influence of the value of the original sample. A Kruskal-Wallis test for differences by sampler showed no significant differences in the precision of the pathogen samples between the five samplers involved.

The field probe was not regularly recalibrated over the course of the study. Therefore the results are only shown as a relative measure but may not be accurate. In particular, DO measurements showed high variability. Starting with a staff change on 10/25/04 and continuing through subsequent staff changes, measured DO saturations were frequently in excess of 100% saturation, which was only partially alleviated by extending the time until recording the probe reading.

Summary Results by Site

A summary of the results by site are given in tables 2a-c. Significant differences existed between sites for bacteriological indicators but not for nitrogen species (Kruskal-Wallis). Total phosphorus concentrations were below the detection limit of 0.014 mg/L everywhere at all sampling events with only one exception (0.2 mg/L at Keaton Beach on 09/13/2004). Therefore, total phosphorus is not discussed any further.

Results were generally non-normally distributed. Natural-log transformed bacteriological indicator data were normally distributed at most sites, but not at sites 1, 4, and 8 for enterococci and sites 5 and 8 for fecal coliform (alpha =0.05).

Exceedances of beach water quality standards were nearly exclusively of the enterococci standard, for which 44% of the samples exceeded the standard of 104 CFU/100 mL, while fecal coliforms were rarely above 400 CFU/100mL (2.5%). The shellfish fecal coliform standard of 14 CFU/100 mL was exceeded in 62% of the samples.

The highest indicator concentrations occurred in the canal sites, Adams Beach and Dekle Creek, and for fecal coliform in Blue Creek (Figure 2). The lowest indicator concentrations were observed in Sandpiper Springs, the developed beaches and for enterococci in Blue Creek. Hagen's Cove showed low fecal coliform and intermediate enterococci values. The Healthy Beaches sampling sites, where frequent advisories had indicated water quality problems, were among the cleaner sampling sites in this study.

Figure 3 compares median salinity to median indicator and total nitrogen concentrations. The much lower salinity in the fresh Blue Creek compared to other sites suggested that only minor contributions from this freshwater by transport to the other sites occur. On the other hand, the generally intermediate concentrations at the spring location can be understood as a mix between fresher groundwater and more saline surface water. For a mix of fresh groundwater with canal water, salinity would suggest a 50-50 mix.

The roughly decreasing trend for indicator counts at between intermediate and high salinity areas could reflect three scenarios: Either transport with associated dilution and die-off from sources in the reduced salinity areas to the beaches, varying intensities of a common source, or a superpositioning of a common source in all areas with an additional source in lower salinity areas. Each scenario should show different patterns: the first scenario should result in relatively higher concentrations at the high salinity sites when there is more influence from the reduced salinity sites (low tide, lower salinity). The second scenario should result in some commonality of patterns between indicators, while the third scenario should show a common pattern between high and reduced salinity sites as the common saltwater source has more influence (high tide, high salinity).

The variability of results is illustrated by Figure 2, which shows a box plot of bacteriological indicators by site. Median enterococci concentrations were generally a factor of 4 to 7 higher than fecal coliform concentration, except at the two creek sites where both indicators occurred in more even numbers.

Table 2. Summary of results of monitoring by site a) enterococci and fecal coliform concentrations; b) nitrogen species; c) field parameters. Groups are based on significant differences in a multiple comparison Kruskal-Wallis procedure.

Name	Site #	Enterococci (CFU/100mL)			fecal coliform (CFU/100mL)				n
		median	90%	group	Median	90%	max	group	
Adams Beach	1	130	592	ab	24	348	850	abcde	45
Canal at Dekle	2	104	364	ab	32	148	420	abcd	45
Creek at Dekle	3	140	464	a	58	318	900	a	45
Dekle Beach	4	82	300	abc	16	124	430	cde	45
Keaton Beach	5	36	160	bc	9	61	320	de	44
Blue Creek	6	58	234	bc	46	154	460	ab	45
Canal at Cedar Island	7	130	482	a	32	224	460	abc	45
Sandpiper Spring	8	34	178	c	8	43	118	e	45
Cedar Island Beach	9	72	396	abc	18	125	238	bcde	45
Hagen's Cove	10	62	250	abc	11	135	520	cde	44

a)

Name	Site#	Total Nitrogen (mg/L)	Nitrate-N (mg/L)	TKN (mg/L)	Total Ammonia (mg/L)	N
		Median	Median	Median	Median	
Adams Beach	1	1.36	0.039	1.24	0.30	11
Canal at Dekle	2	1.07	0.014	1.07	0.25	11
Creek at Dekle	3	1.02	<0.012	1.02	0.25	11
Dekle Beach	4	0.99	<0.012	0.99	0.20	11
Keaton Beach	5	0.53	0.014	0.51	0.19	11
Bridge at Blue Creek	6	0.88	0.020	0.88	0.17	11
Canal at Cedar Island	7	0.77	<0.012	0.76	0.20	11
Sandpiper Spring	8	0.50	<0.012	0.50	0.28	11
Cedar Island Beach	9	0.55	<0.012	0.55	0.17	11
Hagen's Cove	10	0.72	<0.012	0.72	0.18	10

b)

Name	Site#	Salinity (ppt)	T (F)	pH	ORP (mV)	DO (% sat)	n
		Median	Median	Median	Median	Median	
Adams Beach	1	20.9	69.6	7.9	116	82	44
Canal at Dekle	2	22.5	70.2	8.0	104	66	44
Creek at Dekle	3	19.1	70.1	7.8	105	66	44
Dekle Beach	4	23.5	71.4	8.2	103	98	43
Keaton Beach	5	23.6	70.1	8.2	116	100	43
Bridge at Blue Creek	6	0.1	68.9	7.6	109	66	44
Canal at Cedar Island	7	19.0	71.5	7.9	111	77	44
Sandpiper Spring	8	10.9	72.3	7.6	32	45	44
Cedar Island Beach	9	21.4	75.2	8.1	85	95	44
Hagen's Cove	10	24.2	74.2	8.2	89	100	43

c)

Figure 3 illustrates the median pollutant levels as a function of salinity. The four beaches monitored as part of the Healthy Beaches Program are among the sites with lower concentrations of indicator bacteria. The highest pollutants levels were found in slightly reduced salinities and dropped off in the sites with the higher and lower salinities. Cedar Island Beach, though, showed lower indicator concentrations at intermediate salinities.

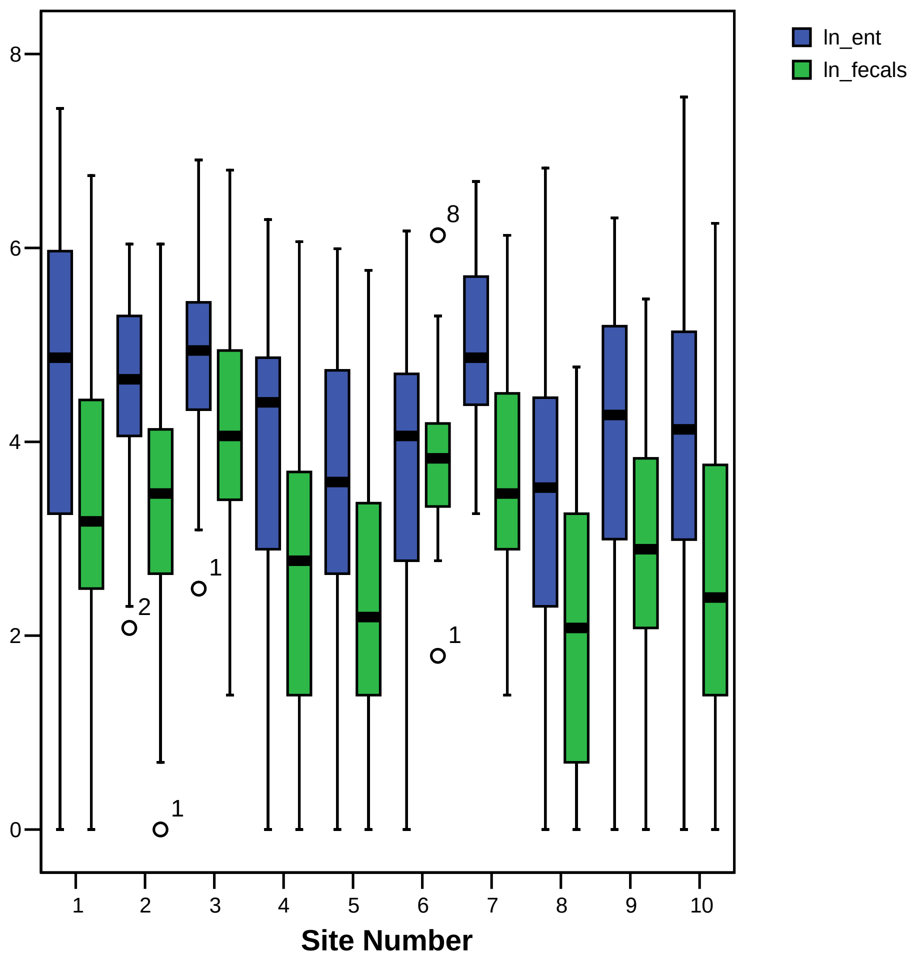


Figure 2. Box plot of ln enterococci and ln fecal coliform concentrations.

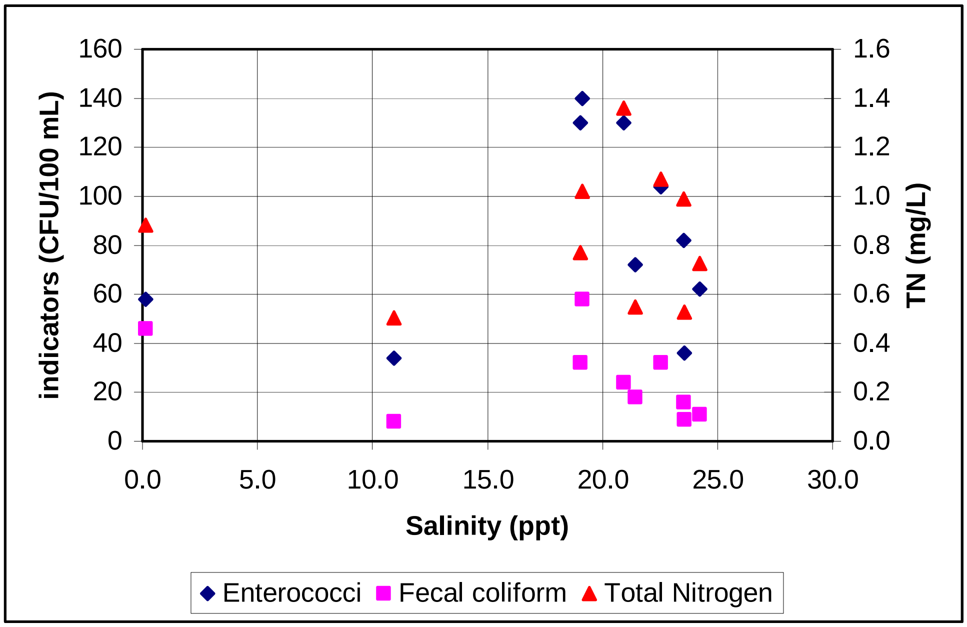


Figure 3. Median indicator and total nitrogen concentrations as a function of salinity.

Tides and Currents

No preferential current direction was observed, with the median observation at all five coastal sites neither direction. Among water quality parameters, salinity (p=0.049) and total nitrogen (p=0.117) were significantly different between current direction observations. Salinity tended to be higher for northerly direction current, while total nitrogen tended to be higher for southerly direction current.

While nitrogen concentrations did not differ significantly between sites, the overall north to south trend for the beach sites was significant (spearman's rho -0.3 between site number and TN and -0.4 between site number and nitrate-nitrogen).

The average tide condition (tide1) was between slack and flood. A comparison of tide conditions with water levels suggested that both ebb and flood conditions tended to be observed at higher water levels than slack conditions. The average tidal stage (tide 2) was medium. A comparison of water level observations vs. tidal stage showed significant differences of water levels between tidal stage observations. While quite variable, generally water levels at high stage were higher than at medium and low tidal stage.

Homogeneity within groups of site types

To assess differences within the groups that were initially planned for the experimental design in a general way, non-parametric Kruskal-Wallis testing was utilized. Fecal coliform, enterococci, total nitrogen, salinity and temperature were compared.

For the background beach sites, Adams Beach and Hagen's Cove, fecal coliform ($p=0.026$), enterococci ($p=0.059$) and total nitrogen ($p=0.014$) were significantly higher at Adams Beach than at Hagen's cove, while salinity, ($p=0.00$), and temperature ($p=0.037$) were higher at Hagen's cove. The temperature difference was likely caused by the generally later time at which Hagen's cove was sampled.

Between the two canal sites, Dekle and Cedar Island, only salinity was significantly different, with the Dekle canal site median 3.7 ppt higher than the canal at Cedar Island ($p=0.00$).

Between the three beach sites close to houses, Dekle, Keaton, and Cedar Island, the only parameter with significant differences was salinity ($p=0.076$), with Cedar Island slightly lower by 2 ppt than the other two sites. This would be consistent with an influence of freshwater inputs from Blue Creek and Sandpiper Springs.

Between the upstream sites, Dekle Creek, Blue Creek and Sandpiper Springs, significant differences ($p=0.00$) existed for fecal coliform, enterococci, and salinity. The differences for total nitrogen and temperature were not significant at the 10% level. Pair wise comparisons showed that Dekle Creek was significantly higher than Blue Creek in enterococci and salinity ($p=0.00$); Dekle Creek showed higher concentrations than Sandpiper Springs in enterococci ($p=0.00$), fecal coliform ($p=0.00$), total nitrogen ($p=0.082$) and salinity ($p=0.05$); Blue Creek showed higher fecal coliform ($p=0.00$) and total nitrogen ($a=0.082$), and lower temperature ($p=0.047$) and salinity ($p=0.00$) than Sandpiper Spring.

These results suggested that the grouping of sites for comparisons was more complex than originally envisioned: 1. Adams Beach (north beach w/o houses); 2. beaches with houses, 3. canals with houses, 4. Dekle Creek (saltwater marsh creek), 5. Blue Creek (freshwater creek), 6. Sandpiper Springs (groundwater), and 7. Hagen's Cove (south beach w/o houses).

More and less contaminated site groupings

A multiple comparison based on Kruskal-Wallis (Cabilio and Masaro, 2001) showed significant differences between these groups for enterococci and fecal coliform, and salinity but not for total nitrogen.

Saltwater creek, canals and the northern beach had relatively high indicator numbers for both enterococci and fecal coliform. While the high counts in the canals could be taken as an indicator of onsite sewage systems, the lack of differences with the other two sites argues against that. The south beach showed also high enterococci counts but lower fecal coliform. In contrast, the freshwater creek had among the highest fecal coliform counts but low enterococci values.

The developed beaches were in an intermediate contaminated group, with significantly fewer enterococci observations than canals and the saltwater marsh, and fewer fecal coliform observations than the saltwater marsh creek and Blue Creek. The developed beaches and Hagen's Cove were neighbors in all rankings and not significantly different from each other. The spring location showed the lowest concentrations of fecal coliform, enterococci and nitrogen.

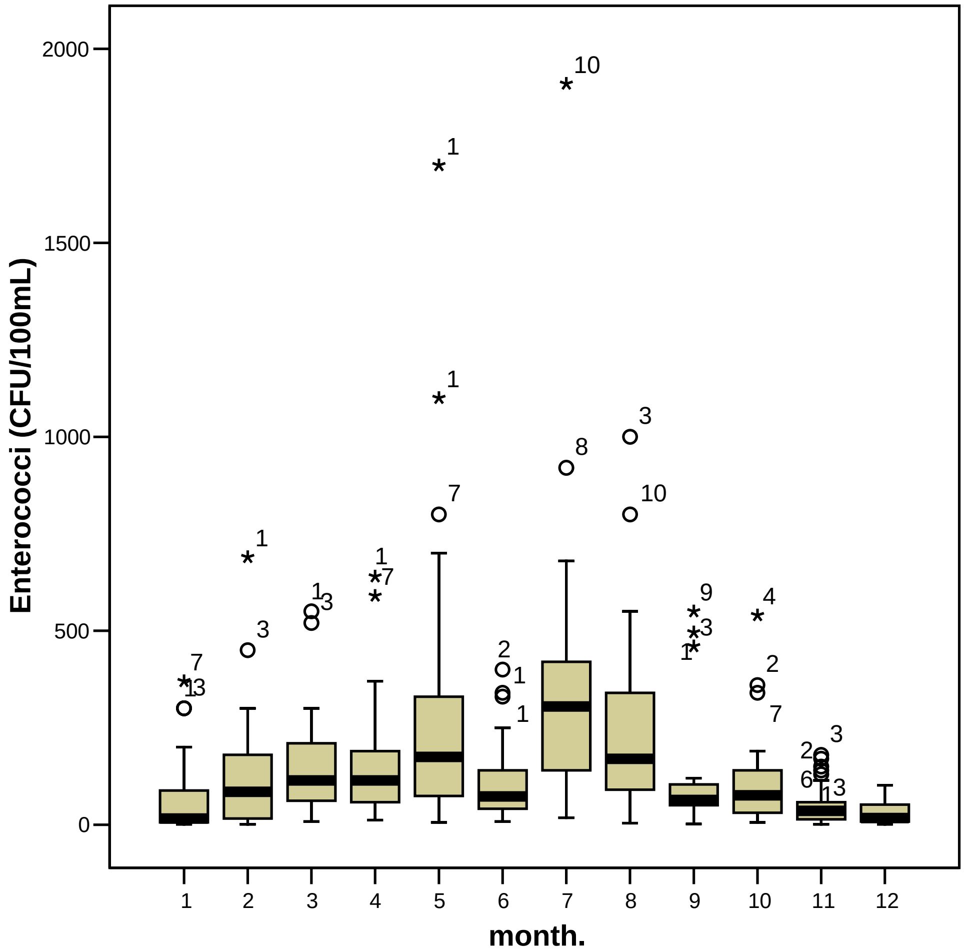
The freshwater Blue Creek had the highest ratio of fecal coliform to enterococci, significantly higher than any other location except the saltwater creek at Dekle, which was intermediate between Blue Creek and all other sites.

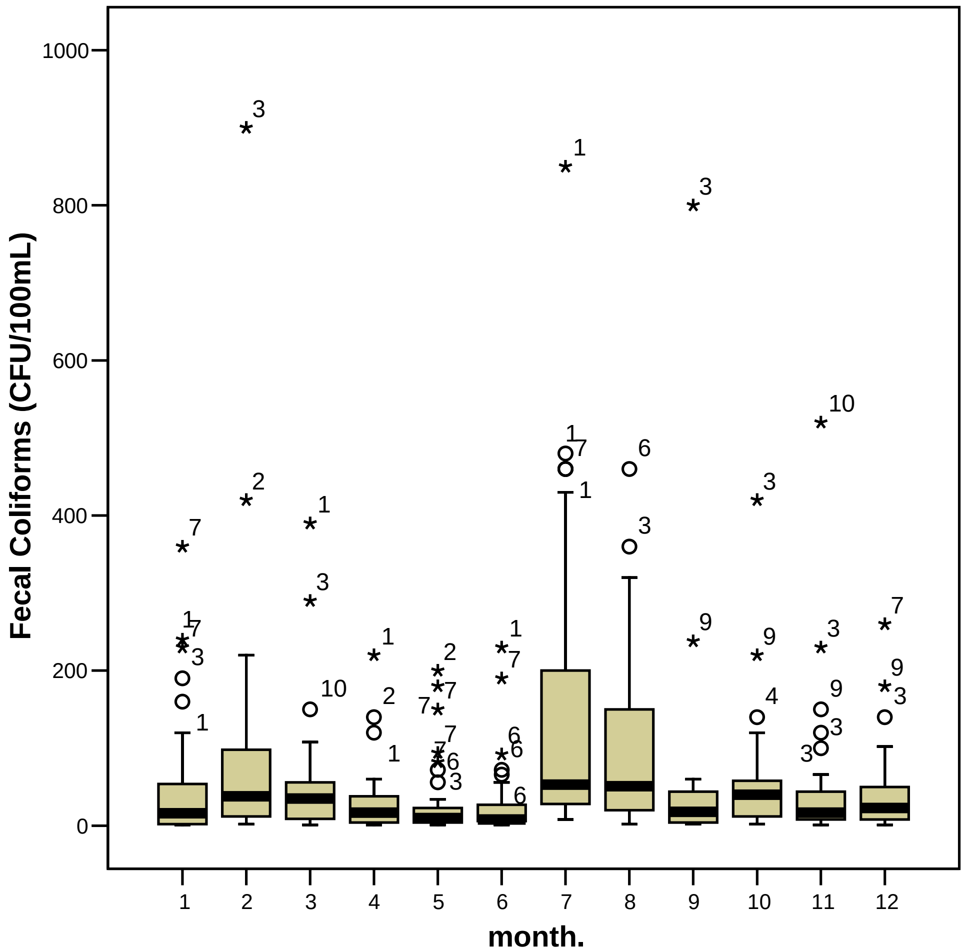
Table 3. Average ranking and significant differences between site type for enterococci, fecal coliform, total nitrogen, and salinity. Cells are filled in the format site type (sites), median, groups without significant differences.

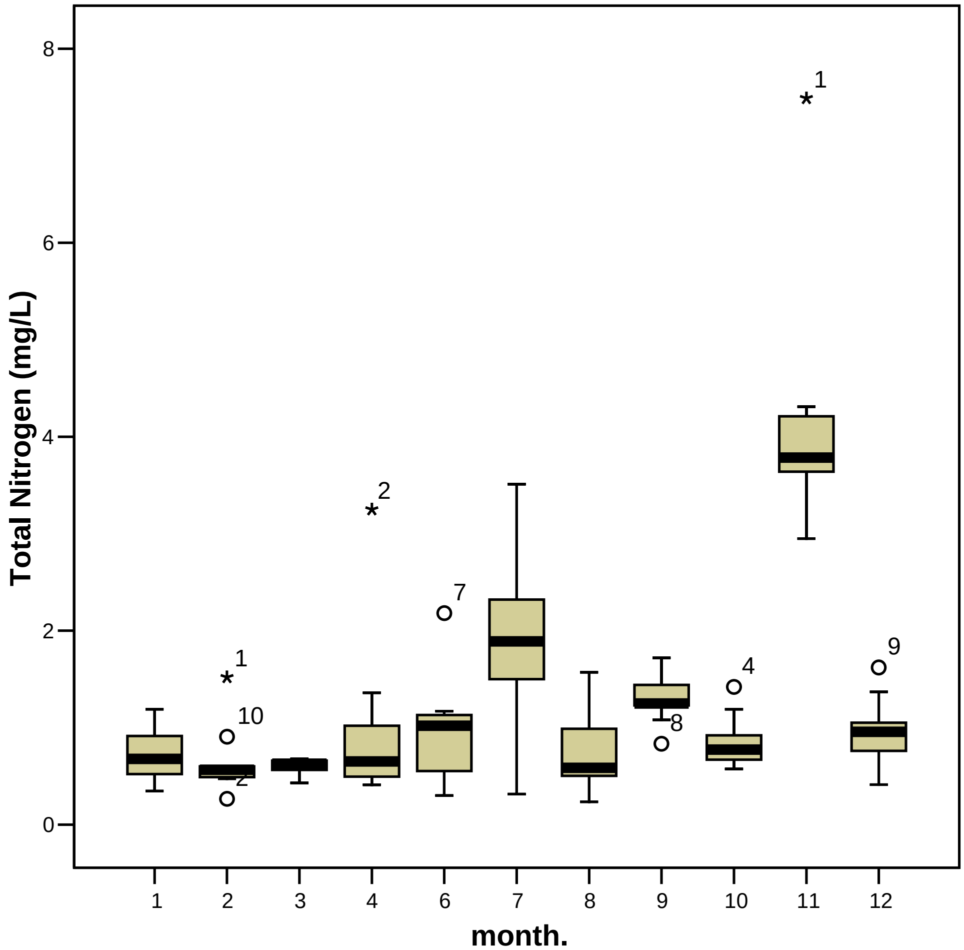
Enterococci (CFU/100mL)	Fecal Coliform (CFU/100mL)	Ratio Fecal Coliform/ Enterococci	Total Nitrogen (mg/L)	Salinity (ppt)
4(3),140,a	4,58,a	5,0.96,a	1,1.36,a	7,24,a
3(2,7),114,a	5,46,a	4,0.53,ab	4,1.02,a	2,22,ab
1(1),130,ab	3,32,ab	1,0.34,b	3,0.96,a	3,20,bc
7(10),62,abc	1,24,abc	2,0.25,b	5,0.88,a	1,20,bc
2(4,5,9),59,bc	2,14,bc	6,0.25,b	7,0.72,a	4,19,cd
5(6),58,bc	7,11,bc	3,0.33,b	2,0.66,a	6,10,d
6(8),34,c	6,8,c	7,0.17,b	6,0.50,a	5,0.1,e

Variability over the course of a year

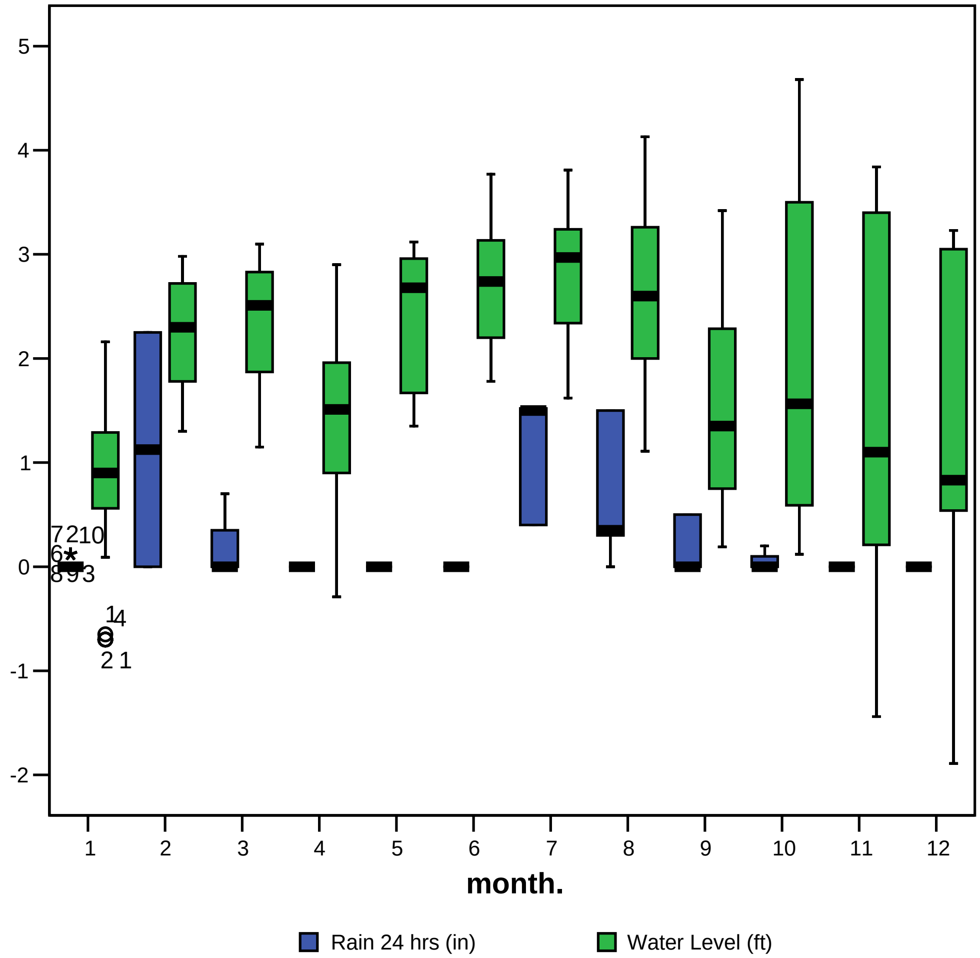
Pollutants and factors show significant variability by month. This variability is not consistent between pollutants. Overall, the highest enterococci values tended to be found in July, August and May, and the lowest in December, January and November. This pattern is similar to the water temperature and water level patterns. The highest fecal coliform values tended to occur in July and August, and the lowest in May and June of the next year. These months correspond to months with and without 24-hour prior rainfall events. Total nitrogen was by far the highest in November, high in July and September and lowest in March, February, January, August and April. Figure 4 shows the monthly variability of observations.




Fecal Coliforms (CFU/100mL)
1000
800
600
400
200
0
1
2
3
4
5
6
7
8
9
10
11
12
month.



Total Nitrogen (mg/L)
8
6
4
2
0
1
2
4
7
1
10
2
9
4
8
1
2
3
4
6
7
8
9
10
11
12
month.



5
4
3
2
1
0
-1
-2
7210
6
893
14
2 1
1
2
3
4
5
6
7
8
9
10
11
12
month.
Rain 24 hrs (in)
Water Level (ft)

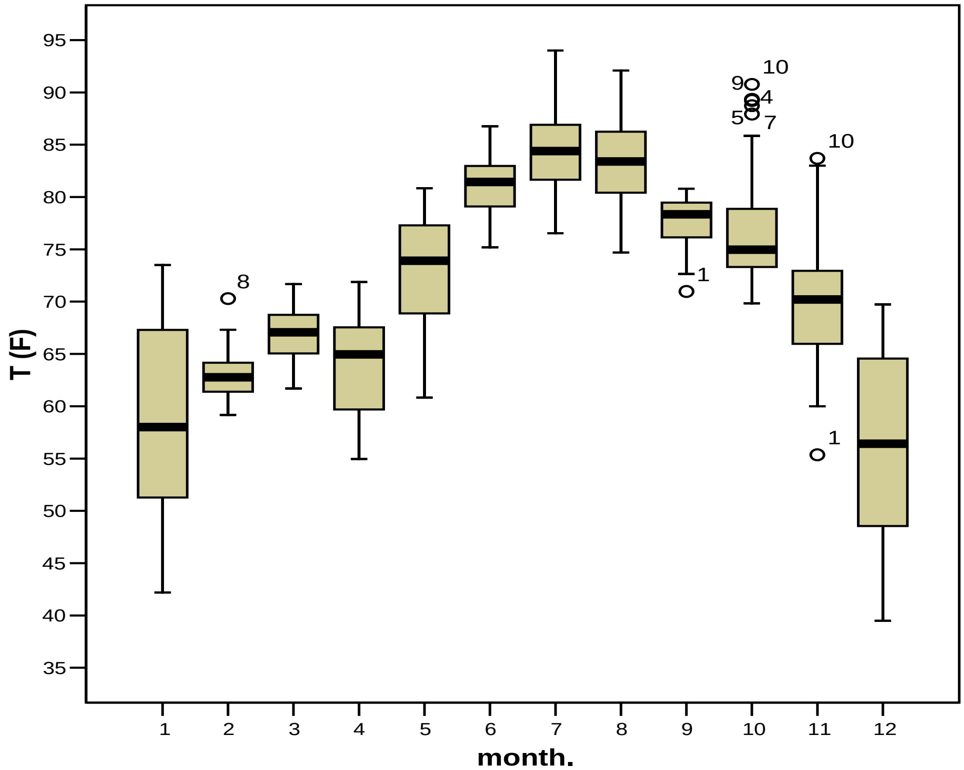


Figure 4. Monthly variation of measurements a) enterococci; b) fecal coliform; c) total nitrogen; d) 24 hr rainfall and water level; e) water temperature. Study period began in month 7 and ended in month 6.

Discussion

Correlation among pollutants

Correlation between pollutants could suggest a common source pattern. To assess such correlations, the Spearman correlation coefficients were determined. TKN and total nitrogen showed close to perfect correlation and will not be discussed separately in the following.

Among all sites, fecal coliform and enterococci correlated strongest (Spearman's rho=0.473) reflecting the overlap of sites that are generally highly and little polluted for each. Only fecal coliform, but not enterococci correlated with total nitrogen (rho=0.315). Ammonia (0.435) and nitrate (0.305) also correlated with total nitrogen. A more detailed examination by site shows that correlation between nitrate and total nitrogen occurs at no site and total and ammonia nitrogen correlate only at three sites (1,4,8). Of these, 8 (Sandpiper Springs) was the site with the highest fraction of ammonia in the total nitrogen. Correlations between bacteriological samples and nitrogen species are sparse and different for enterococci and fecal coliform.

Table 4. Correlations between pollutants. Below the diagonal for all sites, above the diagonal for each site with a significance level of at least 0.05.

	Enterococci (CFU/100mL)	Fecal Coliforms (CFU/100/mL)	Total Nitrogen-N (mg/L)	Nitrate-N (mg/L)	Total Ammonia-N (mg/L)
Enterococci (CFU/100mL)	1	(1:0.4)(3:0.4)(4:0.6)(5:0.4)(6:0.7)(7:0.3)(8:0.6)(9:0.3)(10:0.3)	(3:-0.6)	(1:0.7)	-
Fecal Coliforms (CFU/100mL)	.473(**)	1	(8:0.7)(9:0.8)	-	-
Total Nitrogen (mg/L)	-	.315(**)	1	-	(1:0.7)(4:0.6)(8:0.9)
Nitrate-N (mg/L)	-	-	.305(**)	1	-
Total Ammonia-N (mg/L)	-	-	.435(**)	-	1

Table 5 shows the correlations between pollutants and other field parameters. Air temperature showed very similar but weaker correlation patterns as water temperature; three-day prior rainfall showed fewer correlations than either the one-day or the weekly prior rainfall and both were therefore not included in the table.

While some correlations were consistent across sites, few were strong and few consistent patterns emerge: Enterococci values were consistently positively correlated with temperature, with 24-hour and slightly stronger with weekly rainfall, and negatively with pH and dissolved oxygen saturation. This suggests a mixture of long-lasting rainfall influence and a local temperature dependent source, such as sediments. In contrast, fecal coliforms were consistently and strongest only correlated with 24-hour rainfall, which suggests a rainfall-related source.

Of particular interest are the sites with intermediate salinities that can provide some insight on mixing between different sources.

Blue Creek (6) showed significant correlations for enterococci and to a lesser extent for fecal coliform with water temperature, 24-hour rainfall, and current strength. This suggests that growth and stormwater runoff play an important role.

The spring influenced site (8) had significant positive correlations for enterococci and fecal coliform with salinity, rainfall, and several expressions of water level, suggesting the seaward water as an important source. In contrast, total nitrogen shows no such correlation, which suggests that there was no large concentration difference between groundwater and ocean water but rather relative uniform concentrations. Nitrate-nitrogen is strongly correlated with 24-hour rainfall, suggesting a rainfall source of this nitrogen species.

At Dekle Creek (3) enterococci and fecal coliform showed positive correlation with weekly rainfall and a much stronger negative correlation with pH. Fecal coliform showed some tendency to decrease with salinity and water level suggesting that the upstream marsh rather than the downstream canal system acted a source for these. Total ammonia but not total nitrogen showed some positive correlation with salinity and water level, suggesting that nitrogen speciation is different between the marsh and the canal system.

The canal site at Dekle (2) showed fecal coliforms correlated with 24 hour rainfall and enterococci correlated with weekly rainfall and both correlated negatively with pH. Ammonia was positively correlated with pH. Among the nitrogen species, only nitrate showed a correlation with the weekly rainfall. The canal at Cedar Island (7) showed no correlation for bacterial indicators with rainfall or tide elevation, but fecal coliforms were correlated with turbidity. Only nitrate was correlated strongly with the 24-hour rainfall. This suggested that there was a precipitation related source of nitrate.

The developed beaches all had correlations between bacterial indicators and rainfall, with enterococci more influenced by the weekly rainfall. Salinity was only correlated highly with total nitrogen at Dekle Beach, suggesting a seaward source at this location. Keaton Beach was the only site that showed a correlation with current direction, suggesting higher nitrate and total nitrogen values when the current flows from north to south. This would be consistent with the pattern of higher nitrogen concentration from north to south discussed before.

Similar to the situation at the developed beaches, bacterial indicators at Hagen's Cove were correlated with rainfall. Nitrate-N but none of the other nitrogen species was negatively correlated with several measures of sea levels, this was the only site where this occurred.

At the northern Adams Beach, bacterial indicators were similarly correlated with rainfall. Fecal coliforms were correlated with turbidity. None of the nitrogen species was correlated to any of the observed variables.

In summary, enterococci were generally found elevated correlated with weekly rainfall while fecals were more influenced by the 24-hour rainfall. Correlations at Dekle Creek for fecal coliform and at Blue Creek suggested an element of runoff transport from further inland, while the spring's site indicated a source of contamination in the more saline surface water. The general lack of correlations with salinity and water level, either positive or negative, at beach sites suggested that different intensities of a common source rather than transport or different sources are the cause for differences between sites. Frequent negative correlations with dissolved oxygen saturation and pH and positive correlations with water temperature for enterococci suggested that the relation between source strength and die-off depends on the local water conditions. Canal site patterns were not consistent: while canal site 2 (Dekle) showed correlations of indicators with rainfall and of fecal coliform with pH, ORP and turbidity, canal site 7 (Cedar Island) showed only a negative correlation with DO and pH for enterococci.

The overall total nitrogen concentration gradient from north to south along the coast, as well as the influence of current direction at Keaton Beach suggested an element of transport along the coast and a seaward source for Dekle Beach. The negative correlations of nitrate-N with the tidal direction parameter tide1 at Dekle Creek, Dekle and Keaton Beach, and Hagen's Cove, together with the observation that slack conditions were observed generally at low tidal stage elevations suggest a shoreline process. The positive correlation of nitrate with rainfall overall and at the canal sites, Blue Creek and Sandpiper Springs could be attributed to acid deposition, but this process would raise the question if a similar mechanism of atmospheric deposition is involved as pathogen indicator source.

Table 5. Correlations between pollutants and other observations as indicated by significant (a<0.05) Spearman's rho of 0.3 or larger.

	Enterococci (CFU/100mL)	Fecal Coliforms (CFU/100/mL)	Total Nitrogen-N (mg/L)	Nitrate-N (mg/L)	Total Ammonia-N (mg/L)
All sites	Water T (0.3); Rain 24 hrs (0.3); Rain week (0.3); DO sat (-0.4)	Rain 24 hrs (0.4)	Rain 24 hrs (0.3)	Rain week (0.3); Tide1 (-0.4)	Water T (0.3); WL (0.3)
1	Water T(0.4); Rain 24 hrs (0.4); Rain week (0.4); DOsat (-0.5)	Rain 24 hrs (0.5); turbidity (0.4)	pH (0.7)	ORP (-0.7)	Salinity (0.7)
2	Water T(0.3); Rain week (0.4);	Rain 24 hrs (0.4); pH (-0.4); ORP (0.3); turbidity (0.6)	-	Rain week (0.7)	pH (0.8)
3	Rain week (0.4); pH (-0.6)	Rain 24 hrs (0.3); salinity (-0.4); pH (-0.4); WL (-0.3)	-	Tide1 (-0.7)	Water T (0.7); salinity (0.7); WL (0.7)
4	Rain 24 hrs (0.4); Rain week (0.3) pH (-0.3)	Rain 24 hrs (0.6); pH (-0.4)	Salinity (0.8); WL (0.7)	Tide1 (-0.7)	
5	Rain 24 hrs (0.4); Rain week (0.3); DO sat (-0.6); tide1 (0.3); WL (0.4)	Rain 24 hrs (0.6); tide1 (0.3); WL (0.4)	current dir. (0.7)	Tide1 (-0.7); current dir. (0.6)	pH (0.7)
6	Water T (0.7); Rain 24 hrs (0.4); pH (-0.5); DOsat (-0.6); current str (0.5)	Water T (0.5); Rain 24 hrs (0.3); DOsat (-0.4) ; current str. (0.3)		Rain week (0.7)	Tide2 (0.7); WL (0.8)
7	pH (-0.4); DOsat (-0.4); tide1 (0.4)	-	Water T (0.7)	Rain 24 hrs (0.7)	
8	Water T (0.5); Rain 24 hrs (0.4); salinity (0.5); DOsat (-0.3); tide 1 (0.5); tide2 (0.3); WL (0.5)	Water T (0.3); Rain 24 hrs (0.4); salinity (0.7); tide 1 (0.4); tide2 (0.4); WL (0.7)	Turbidity (0.9)	Rain 24 hrs (0.8)	Water T (0.6)
9	Water T (0.5); Rain 24 hrs (0.4); Rain week (0.6); pH (-0.5); DOsat (-0.4)	Rain 24 hrs (0.3); salinity (-0.3)	-	-	-
10	Water T (0.4); Rain 24 hrs (0.5); Rain week (0.5); pH (-0.4); DOsat (-0.6); WL (0.4)	Water T (0.4); Rain 24 hrs (0.5); Rain week (0.3)	Water T (-0.7); DOsat (1)	Tide1 (-0.6); WL (-0.7)	-

Effect of weather

The weather was recorded as sunny (65%), cloudy (33%) and raining (10 occasions or 2%). Significant differences existed between these categories for fecal coliform (a=0.052) and enterococci (a=0.013) and the 24 hour rainfall (a=0.002). The rainfall was higher (a=0.06) during rainy conditions than during sunny conditions with cloudy conditions in between. While for enterococci the samples obtained during rain were significantly higher than during both sunny and cloudy conditions which were indistinguishable, fecal coliform counts were significantly higher during cloudy conditions than during sunny conditions, but counts during raining conditions were not significantly different from either. This confirmed the difference in the role of rain seen before between the two indicators.

Fecal coliform and enterococci pattern

The correlation analysis had indicated commonalities between fecal coliform and enterococci. Figure 5 shows mean fecal coliform and enterococci concentrations by site. While the variability was considerable, the averages of transformed concentrations appeared to follow a pattern. The designation "creek" increased the fecal coliform concentrations relative to other sites. As discussed previously, there was no significant difference between fecal coliform counts at the two creek sites. All other sites showed a log-log relationship between enterococci and fecal coliform, ranging from Sandpiper Springs to the canal at Cedar Island. While the canals were two of the four most contaminated sites, Adams Beach and Dekle Creek showed very similar concentrations, and the developed Keaton Beach was the second cleanest location, that is, the association between the presence of houses and indicator observations was not uniform. This supported the hypothesis of a common source at different intensities for coastal sites, while the creek sites were enriched in fecal coliform.

Rainfall as risk factor for exceeding water quality standards

When are water quality parameters most likely to be exceeded? To answer this question, the analysis was simplified to cover just the exceedances of enterococci (>= 104) and fecal coliform (>14 and >400) standards. Consideration of the correlation coefficients discussed previously and plots of the frequency of observations with and without exceedances against 24 hr and weekly rainfall suggested thresholds of 24 hr rainfalls of 0.15 in and 1 in and weekly rainfall of 0.3 in and 1 in above which exceedance was more likely. This increased likelihood was expressed as odd ratio

$$OR = \frac{\text{(samples exceeding given rain/samples non-exceeding given rain)}}{\text{(samples exceeding without rain/samples non-exceeding without rain)}} \qquad \textbf{Equation 1}$$

Comparison of the four thresholds and three water quality standards showed that one or two of the thresholds resulted in much higher odd ratios than the others. Table 8 summarizes the impact of rainfall on the water quality.

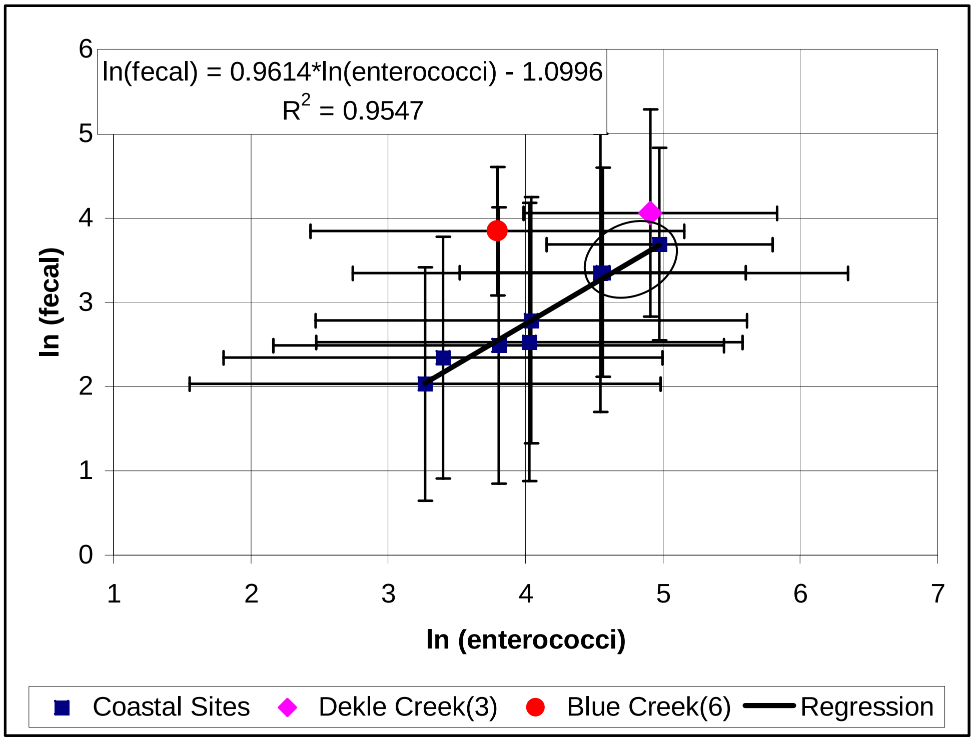


Figure 5. Scatter plot of site means of ln (enterococci) and ln (fecal coliform). Error bars are standard deviation

400 CFU/100mL fecal coliform were rarely exceeded, only 11 times out of the 438 samples for which rainfall data were available. For the fecal coliform >400 standard, sites 5, 8 and 9, two developed beaches and the spring, did not show any exceedance. A 24-hour rainfall of 1" provided the best threshold for increased likelihood of exceedance. This threshold distinguishes the single exceedances at sites 2, 4, 6, and 7, two of three exceedances at site 1; and one of three exceedances at site 3. Three of the four other exceedances (two at Dekle Creek and one at Hagen's Cove) occurred when less than 0.15 in of rain had fallen in the previous 24 hours. Even with this large odd ratio, most of the time, about 80%, the standard is not exceeded, even after a rainfall.

14 CFU/100mL fecal coliform were exceeded more than half of the time. Presence of more than 0.15" of rainfall in the previous 24 hours provided the strongest threshold. Only eight samples of 109 after such a rainfall did not exceed this standard. At sites 2, 3, 4, and 6 all samples after some rain exceeded 14 CFU/100ml. This threshold was least pronounced at site 7, the canal at Cedar Island where samples both with and without rain predominantly exceeded the threshold.

The 104 CFU/100mL enterococci standard was exceeded overall slightly less than half of the time. For some sites, Adams Beach and the canal and creek sites at Dekle, weekly rainfall appeared to be more important, while for others the rainfall in the previous 24 hours results in higher odd ratios. For Dekle and Keaton Beach both thresholds results in somewhat similar odd ratios.

While the impact of a substantial rainfall, such as more than one inch, can be easily visualized as runoff, the significance of a rainfall threshold of 0.15”is less clear. Such a rainfall is less than common interception, depression storage and infiltration values and thus would not expected to induce runoff (Nzewi, 2001: 1066ff.). This suggests that disturbance of surface sediments or a source behavior change associated with such a rainfall could be the causative mechanisms, rather than transport from land to see.

Table 6. Rainfall thresholds for increased exceedances of indicator water quality standards. Odd ratio includes division by zero if no exceedance was observed below the threshold or all observations above the threshold were exceedances.

	Site Number	1	2	3	4	5	6	7	8	9	10	all
Fecal > 400	fraction exceeded	0.068	0.023	0.068	0.023	0.000	0.023	0.023	0.000	0.000	0.023	0.025
r24hrs>1.0”	fraction exceeded given rain	0.40	0.20	0.20	0.20	0.00	0.20	0.20	0.00	0.00	0.00	0.14
r24hrs>1.0”	odd ratio	25.33	None below	4.63	None below	n/a	None below	None below	n/a	n/a	0.00	15.63
Fecal > 14	fraction exceeded	0.59	0.75	0.86	0.52	0.44	0.98	0.77	0.36	0.52	0.40	0.62
r24hrs>0.15”	fraction exceeded given rain	0.91	1.00	1.00	1.00	0.91	1.00	0.91	0.73	0.91	0.90	0.93
r24hrs>0.15”	odd ratio	10.63	All above	All above	All above	25.56	All above	3.75	8.33	15.38	28.13	11.67
Ent≥104	fraction exceeded	0.59	0.52	0.64	0.39	0.28	0.30	0.59	0.20	0.45	0.40	0.44
rwk>1.4”	fraction exceeded given rain	0.83	0.72	0.89	0.61	0.50	0.44	0.67	0.28	0.78	0.71	0.64
rwk>1.4”	odd ratio	**6.82**	**4.16**	**9.33**	5.24	**7.33**	3.36	1.71	2.12	11.67	10.08	4.33
r24hrs>1.0”	fraction exceeded given rain	0.80	0.60	0.80	0.80	0.60	0.80	1.00	0.60	1.00	1.00	0.80
r24hrs>1.0”	odd ratio	3.09	1.43	2.50	**8.00**	4.83	**13.33**	**All above**	**8.25**	**All above**	**All above**	**6.28**

The odd ratios for exceeding the standards in the summer (June, July, August) were significantly higher than one for fecal coliform >400 (3.4) enterococci $\geq$104 (3.3) and fecal coliform >14 (2.1) standards. The winter (December, January, February) odds ratio was only significantly reduced for the enterococci standard (0.3). The odd ratios for the rainfall thresholds were much larger than for other risk factors.

The odds ratios between developed and undeveloped beaches were not statistically different from a value of one for all three standards, even though they suggested on average lower exceedances for the developed beaches.

Patterns after adjustments for rainfall

To assess patterns of indicator concentrations that were not influenced by rainfall, only the data below the rainfall thresholds were analyzed.

For enterococci, samples from sites 1,2,3, and 5 were excluded when weekly rainfalls exceeded 1.4”. Samples from the other sites were excluded when 24 hr rainfall exceeded 1.0”. For fecal coliform, samples where excluded when the 24-hr prior rainfall exceeded 0.15”. Table 9 presents the resulting median concentrations and the reduction in medians compared to the complete data set.

The reduction in counts, along with the reduction in remaining number of samples, decreased the significant differences between sites. Enterococci concentrations were highest in the Creek at Dekle and the Canal at Cedar Island and lowest at Keaton Beach and Sandpiper Springs. Fecal coliform concentrations were highest in the Creek at Dekle, the Canal at Cedar Island and at Blue Creek and lowest at Sandpiper Springs. The reductions in median fecal coliform concentrations were relatively small at the most contaminated sites and relatively large at all beach sites except Hagen’s Cove.

Figure 6 shows the adjusted natural log-transformed indicator counts at each site. While overall there is still a strong linear relationship between fecal coliform and enterococci at the non-creek sites, the points appear to cluster stronger. The two canal sites, along with Dekle Creek, have typical enterococci counts around 100 CFU/100 mL and fecal coliform counts around 40-50. The fresh Blue Creek showed had still high fecal coliform counts but reduced enterococci counts. Most beach sites clustered around an enterococci count of 50-70 with more variable fecal coliform counts between 8 and 14. The cleanest sites were Keaton Beach and

Sandpiper Springs with enterococci counts around 30 and fecal coliform counts around 5. If one assumed based on salinity that about half of the flow at the Sandpiper Springs site consisted of fresh water and this fresh groundwater was free of indicator organisms, the adjusted mean concentrations would fit into the cluster of beach sites. Callahan et al. (2001) found that two springs bracketed the range of indicators of fecal contamination in their seven sites; therefore, this assumption of clean springs may not always be accurate.

Figure 7 shows median fecal coliform and total nitrogen concentrations against median salinity for events without prior rainfall. Median salinity changed less than 2% in either direction between all data and data excluding any rain in the 24 hours prior to sampling, except for Sandpiper Springs, where median salinity decreased from 10.9 to 9.9%. The slight salinity changes indicate that fresh rainwater is never a large volume fraction of the beach sites.

Fecal coliform showed a pattern by site type, with both creeks having high concentrations, the canal intermediate concentrations, and the beach sites still lower median concentrations, with Adams Beach in an intermediate position. Sandpiper Springs had the lowest median fecal coliform concentrations. This pattern appeared related less to salinity and more to the structure of the sampling sites. Sites where the water encounters two banks had higher concentrations than the beach sites with only one land side. Dekle canal with concrete walls had a tendency for lower counts than the Cedar island canal site which had more sloping banks.

The higher fecal coliform indicators in creeks could be related to the lower decay rate relative to enterococci in freshwater observed by Anderson et al. (2005). An inverse relationship of fecal coliform with salinity was also observed by Mallin et al. (2000).

Median total nitrogen under non-rainfall conditions is somewhat lower than including rainfall at most sites with the exception of Hagen’s Cove where the median increased slightly. The largest reductions in the median were at Dekle Beach (27%), Blue Creek (20%), and the Canal at Cedar Island (24%), the latter two in contrast to the small reductions for fecal coliform. While differences in total nitrogen between sites were overall significant, the multiple comparison procedure detected the highest significance in differences between Adams Beach and Keaton Beach only at a level of 0.18. Median total nitrogen ranged from the high at Adam’s Beach (1.3 mg/L) to the low at Keaton Beach (0.5 mg/L).

Table 7. Median concentrations of indicator organisms under dry conditions and reduction of median relative to complete data.

Enterococci for small or no rain						fecal coliform for 24 hr rainfall <0.15"				TN for 24 hr rainfall <0.15"	
Name	Site #	Median (CFU/ 100mL)	reduction in median	group	N	Median (CFU/ 100mL)	reductio n in median	Group	N	TN (mg/L)	N
Adams Beach	1	71	45%	ab	26	14	42%	abc	33	1.3	8
Canal at Dekle	2	89	14%	ab	26	22	31%	ab	33	1.0	8
Creek at Dekle	3	91	35%	a	26	46	21%	a	33	1.0	8
Dekle Beach	4	58	29%	ab	39	8	50%	bc	33	0.7	8
Keaton Beach	5	34	6%	b	25	5.5	39%	bc	32	0.5	8
Bridge at Blue Creek	6	52	10%	ab	39	44	4%	a	33	0.7	8
Canal at Cedar Island	7	104	20%	a	39	32	0%	a	33	0.6	8
Sandpiper Spring	8	30	12%	b	39	4	50%	c	33	0.5	8
Cedar Island Beach	9	58	19%	ab	39	10	44%	abc	33	0.5	8
Hagen's Cove	10	58	6%	ab	38	10	9%	bc	33	0.8	8
All		64	22%		336	16	33%		329		80

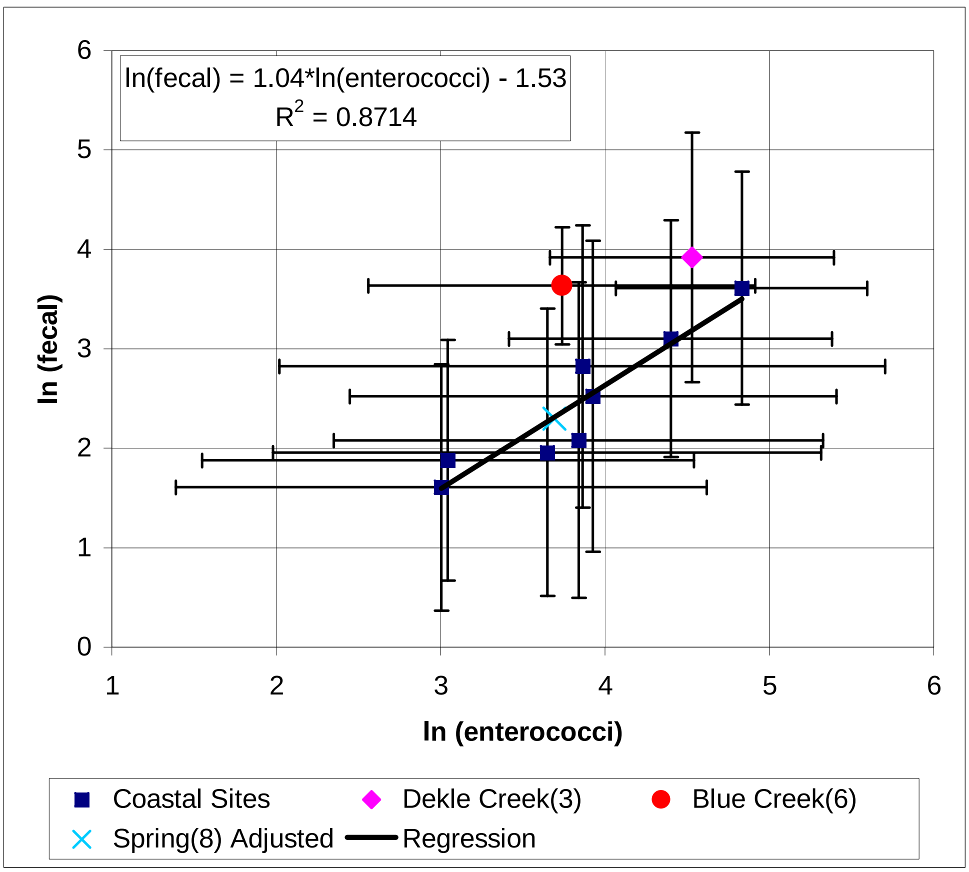


Figure 6. Relationship between mean ln (enterococci) and mean ln (fecal coliform) under dry conditions for each. Error bars are standard deviations.

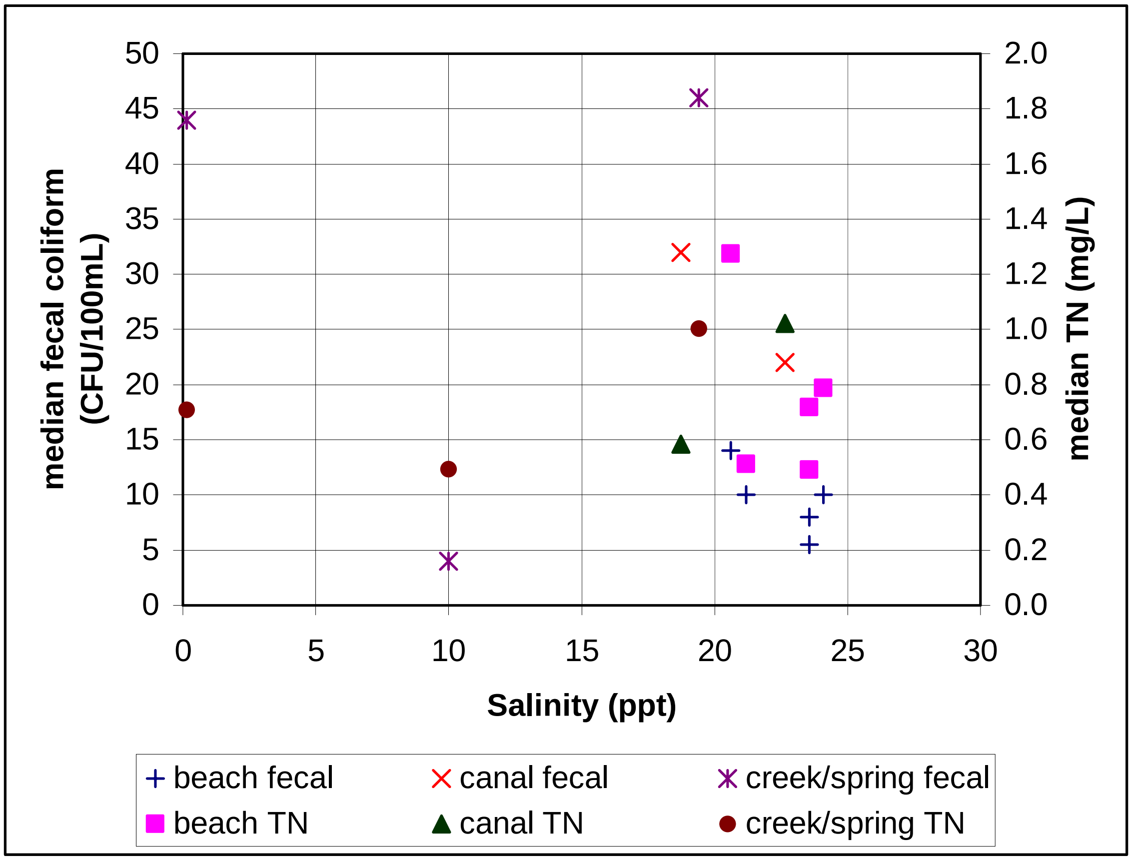


Figure 7. Median salinity by site and median fecal coliform and median total nitrogen concentrations for sampling events with less than 0.15" rain in the previous 24 hours.

Comparison of Indicators

Both enterococci and fecal coliform counts are used as indicators of health risk. Study results indicated that the behavior of the two indicators differs between creeks and all other sites. The fresh Blue Creek showed relatively high fecal coliform concentrations in combination with relatively low enterococci concentrations. The salty Dekle Creek showed a similar level of fecal coliform but significantly higher enterococci concentrations that were similar to enterococci observed in canals. Within the non-creek sites, individual samples showed some correlation and overall means showed a very strong log-log correlation between fecal coliform and enterococci. This correlation indicated for all data that enterococci where by about a factor of 3 higher than fecal coliform and for dry weather conditions that enterococci where by about a factor of 4.6 higher than fecal coliform. While this indicated that both measures were closely related, the conclusions drawn for beach advisories were very different, with fecal coliform standards rarely exceeded and enterococci exceeded in about half the cases.

Conclusions

Building on the Healthy Beaches Program of the Florida Department of Health, ten sites at and near the coast of Taylor County were sampled for one year from July of 2004 through June of 2005 approximately weekly for fecal coliform, enterococci and field parameters and monthly for nutrients.

Inventories of onsite systems were gathered in 2001 by a group of local highschool students. No failing drainfield was observed during the field work. These inventories indicated about a third of onsite systems with elevated drainfields. Taking into account the relatively recent age of many houses, sparse settlement and the fact that some of the most seaward houses along with their onsite systems had been destroyed in 1993 during the storm of the century, most onsite systems should represent functioning under modern standards.

Sites were selected to represent a variety of types: beaches with development (Dekle, Keaton, Cedar Island), beaches without development (Adams, Hagen's Cove), canals (Dekle, Cedar Island), creeks (Blue and Dekle) and a spring (Sandpiper Springs).

The three developments included consisted predominantly of groups of single family houses with between 50 and 200 onsite systems each. The undeveloped beaches bracketed the study area to the north and the south.

Salinity ranged generally between 15 and 30 ppt at most sites. A freshwater creek and a site were a spring vent mixed with surface water had lower salinity.

Total phosphorus concentrations were below the detection limit of 0.014 mg/L with only one exception (0.2 mg/L at Keaton Beach on 09/13/2004).

Total nitrogen concentration did not show significant differences between sites overall but indicated a trend along the coast from higher concentrations in the north to lower concentrations in the south, and a tendency for higher concentrations at Keaton Beach when current directions was from the north.

Nitrate nitrogen concentrations tended higher during ebb and slack tidal conditions than during flood.

Pathogen indicator counts indicated that the beaches included in the Healthy Beaches assessment were among the less contaminated water bodies investigated. Two of the three developed beaches never exceeded the 400 CFU/100 mL fecal coliform standard.

Site types did not behave the same in all cases. While developed beaches were not significantly different from each other, except salinity, the undeveloped beaches had significant differences between the northern and the southern bracketing site. The two creeks, which varied in salinity, also varied significantly in their enterococci but not their fecal coliform levels, while the spring's site was generally the cleanest of all investigated sites.

The Creek at Dekle, the two canal sites and the northern undeveloped Adams Beach tended to have the highest enterococci counts. For fecal coliform, the fresh Blue Creek joined these sites as among the most contaminated.

Developed beaches (Dekle, Keaton, and Cedar Island) had pathogen indicator counts that were indistinguishable in rank from the southern undeveloped beach, Hagen's Cove,

The fecal coliform to enterococci ratio was significantly elevated at Blue Creek with Dekle Creek in an intermediate position relative to all other sites. Average indicator counts at these other sites showed a very strong log-log relationship with each other.

This consistent pattern and the lack of correlations of indicator counts with salinity and tidal indicators suggested that transport and differences in source types is not important for beach and canal sites. Instead, different intensities of the same local sources may explain observed indicator counts. Creek sites correlations were consistent with downstream transport, in particular of fecal coliform. The spring site correlations were consistent with the concept that sea water with high indicator counts is mixing with relatively clean and fresh spring water.

Rainfall was consistently associated with increased indicator counts. For enterococci the rainfall in the week prior tended to be more important, for fecal coliform the rainfall during the previous day was stronger associated with increased counts. For fecal coliform, cloudy conditions during sampling tended to result in significantly higher counts than sampling during sunny conditions. For enterococci, rainy conditions vs. sunny conditions were significantly higher.

Enterococci counts tended to increase with water temperature and decrease with dissolved oxygen saturation and at several sites tended to decrease with increasing pH.

Building on the importance of rainfall, thresholds were investigated that resulted in high odds ratios for exceeding an enterococci standard of 104 CFU/100 mL, and fecal coliform standards of 400 CFU/100 mL (beach standard) and 14 CFU/100 mL (shellfish standard). For 400 CFU/100 mL fecal coliform, a 24 hour rainfall exceeding 1" was the best threshold. For 14

CFU/100 mL fecal coliform, a 24 hour rainfall exceeding 0.15” was the best threshold. This low threshold is unlikely to lead to runoff and may instead be related to the effect of drop impact or cloudiness. For the 104 CFU/100 mL enterococci standard, the more commonly violated of the two beach thresholds addressed here, a weekly rainfall of more than 1.4” or a 24-hour rainfall of more than 1” result in odd ratios between four and a clean separation.

Exclusion of samples with rainfall beyond the thresholds resulted in a somewhat different pattern of the reduced indicator counts:

Enterococci in the canals and Dekle Creek remained among the highest; most beach sites were now very similar, with Keaton Beach at the lower end. Fecal coliform concentrations were highest in both creeks, intermediate in the canals and lowest on the beaches. Consideration of the spring’s water as a mixture of seawater and clean fresh groundwater resulted in a grouping of the seawater together with the other beach data.

Beach sites did not show a differential impact of onsite sewage systems on indicator counts or nutrients. Canal sites had higher indicator counts. The observations that the ratio of enterococci to fecal coliform has the same pattern as at beach sites, the similarity to Dekle Creek concentrations, and the lack of indications of higher contamination during low tide when the impact of OSTDS should be more visible, suggested that onsite systems per se are not the cause for this elevation.

This suggests local sources, regrowth and rainfall as the most prominent sources of indicator organisms. The higher fecal coliform indicators in creeks could be related to the lower decay rate relative to enterococci in freshwater observed by Anderson et al. (2005).

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Appendix

Table A-2. Spearman's rho to assess relationships between pollutants and other observations: a) correlations between pollutants, weather and field parameters by site. b) correlations between pollutants and tide and current indicators by site.

Site	Parameter	Water T (F)	Rain 24 hrs	Rain week	salinity (ppt)	pH	ORP (mV)	DO sat
All	Enterococci (CFU/100mL)	.322(**)	.338(**)	.342(**)	-0.063	-.261(**)	0.019	-.352(**)
All	Fecal Coliforms (CFU/100/mL)	0.082	.388(**)	.160(**)	-.215(**)	-.274(**)	.121(*)	0.013
All	Nitrate-N(mgL)	-0.045	.284(**)	.345(**)	-.213(*)	-.279(**)	-0.041	-0.143
All	Total Ammonia (mg/L)	.309(**)	0.104	-.227(*)	0.16	.215(*)	-.266(**)	-0.192
All	Total Nitrogen (mg/L)	.190(*)	.333(**)	-0.09	-0.087	0.061	-0.123	0.061
1	Enterococci (CFU/100mL)	.425(**)	.361(*)	.426(**)	-0.242	-0.274	-0.158	-.464(**)
1	Fecal Coliforms (CFU/100/mL)	0.218	.527(**)	0.196	-0.083	-0.243	0.285	-0.127
1	Nitrate-N(mgL)	0.278	0.249	0.124	-0.17	-0.559	-.723(*)	-0.511
1	Total Ammonia (mg/L)	0.209	0.11	-0.4	.733(*)	0.588	0.236	-0.055
1	Total Nitrogen (mg/L)	0.218	0.156	-0.069	0.382	.721(*)	0.115	-0.152
2	Enterococci (CFU/100mL)	.324(*)	0.143	.369(*)	-0.112	-.366(*)	-0.189	-.298(*)
2	Fecal Coliforms (CFU/100/mL)	-0.065	.409(**)	0.255	-0.076	-.434(**)	.305(*)	0.269
2	Nitrate-N(mgL)	0.17	0.33	.686(*)	-0.135	0.028	0.228	-0.123

2	Total Ammonia (mg/L)	0.251	0.116	-0.327	0.067	.810(**)	-0.438	-0.383
2	Total Nitrogen (mg/L)	0.409	0.197	0.069	-0.188	-0.067	-0.079	0.079
3	Enterococci (CFU/100mL)	0.213	0.194	.370(*)	-0.229	-.579(**)	-0.104	-0.27
3	Fecal Coliforms (CFU/100/mL)	-0.027	.335(*)	.352(*)	-.378(*)	-.419(**)	0.156	0.09
3	Nitrate-N(mgL)	-0.084	0	0.479	-0.246	-0.216	0.022	-0.38
3	Total Ammonia (mg/L)	.655(*)	0.358	-0.064	.697(*)	0.43	-0.333	-0.309
3	Total Nitrogen (mg/L)	0.333	0.336	0.037	0.152	0.45	-0.164	-0.091
4	Enterococci (CFU/100mL)	0.292	.425(**)	.307(*)	-0.244	-.305(*)	-0.01	-0.223
4	Fecal Coliforms (CFU/100/mL)	0.193	.580(**)	0.12	-0.124	-.373(*)	-0.013	0.041
4	Nitrate-N(mgL)	-0.416	0.255	0.085	0.238	-0.228	-0.218	-0.257
4	Total Ammonia (mg/L)	0.21	0.148	-0.115	0.653	0.577	-0.351	-0.351
4	Total Nitrogen (mg/L)	0.427	0.561	-0.078	.800(**)	0.067	-0.6	-0.35
5	Enterococci (CFU/100mL)	.302(*)	.314(*)	.389(*)	-0.224	-0.212	0.017	-.560(**)
5	Fecal Coliforms (CFU/100/mL)	0.288	.569(**)	0.204	-0.043	-0.212	0.102	-0.237
5	Nitrate-N(mgL)	-0.2	0.249	0.545	-0.407	-0.298	0.291	0.369
5	Total Ammonia (mg/L)	0.336	-0.098	-0.253	0.188	.711(*)	-0.164	0.03
5	Total	0.236	0.595	0.202	-0.079	-0.207	-0.006	0.212

	Nitrogen (mg/L)							
6	Enterococci (CFU/100mL)	.746(**)	.420(**)	0.253	-0.083	-.510(**)	-0.174	-.637(**)
6	Fecal Coliforms (CFU/100/mL)	.526(**)	.308(*)	-0.054	-0.082	-0.214	-0.137	-.384(*)
6	Nitrate-N(mgL)	0.377	0.317	.685(*)	-0.427	-0.439	0.506	-0.244
6	Total Ammonia (mg/L)	0.418	0.081	-0.239	0.179	-0.152	-0.164	-0.236
6	Total Nitrogen (mg/L)	0.4	0.595	-0.124	-0.241	-0.176	-0.152	-0.297
7	Enterococci (CFU/100mL)	0.007	0.28	0.275	-0.229	-.369(*)	0.196	-.388(**)
7	Fecal Coliforms (CFU/100/mL)	-0.171	0.165	-0.04	0.017	0.046	0.143	0.133
7	NitrateN (mg/L)	0.258	.744(**)	0.341	-0.43	-0.531	-0.137	-0.212
7	Total Ammonia (mg/L)	0.455	-0.023	-0.326	0.297	0.413	-0.333	-0.321
7	Total Nitrogen (mg/L)	.700(*)	0.353	-0.345	-0.115	0.298	-0.455	0.055
8	Enterococci (CFU/100mL)	.481(**)	.388(**)	0.227	.475(**)	0.027	0.127	-.319(*)
8	Fecal Coliforms (CFU/100/mL)	.303(*)	.448(**)	0.183	.680(**)	0.151	0.05	0.046
8	Nitrate-N(mgL)	0.355	.788(**)	0.386	-0.034	-0.246	-0.382	-0.041
8	Total Ammonia (mg/L)	.636(*)	0.087	-0.078	0.079	0.139	0.03	-0.042
8	Total Nitrogen (mg/L)	0.612	0.48	-0.12	0.152	0.2	0.067	0.297
9	Enterococci	.519(**)	.412(**)	.563(**)	-0.203	-.455(**)	0.05	-.363(*)

	(CFU/100mL)							
9	Fecal Coliforms (CFU/100/mL)	0.15	.306(*)	0.197	-.340(*)	-0.141	-0.193	0.181
9	Nitrate-N(mgL)	-0.353	0.228	0.058	-0.507	-0.165	-0.555	0.418
9	Total Ammonia (mg/L)	0.364	0.006	-0.405	0.03	0.614	-0.564	-0.152
9	Total Nitrogen (mg/L)	-0.082	0.405	-0.345	-0.152	0.274	-0.345	0.382
10	Enterococci (CFU/100mL)	.412(**)	.478(**)	.496(**)	-0.036	-.377(*)	0.07	-.628(**)
10	Fecal Coliforms (CFU/100/mL)	.352(*)	.513(**)	.310(*)	-0.09	-0.212	-0.017	0.007
10	Nitrate-N(mgL)	-0.455	-0.319	0.159	0.388	0.548	-0.183	0.16
10	Total Ammonia (mg/L)	0.248	0.164	-0.302	0.233	0.4	-0.517	0.25
10	Total Nitrogen (mg/L)	-.721(*)	-0.389	-0.48	-0.35	0.383	-0.05	.950(**)

Site	Parameter	tide1 (1=ebb 2=slack 3=flood)	tide2(3=high 2=medium 1=low)	Current direction (1=L->R 2=neither 3=R->L)	Current strength (3=strong, 2=moderate, 1=weak)	WL
All	Enterococci (CFU/100mL)	.179(**)	0.08	0.005	.159(**)	.216(**)
All	Fecal Coliforms (CFU/100/mL)	0.074	0.028	-0.003	.167(**)	.102(*)
All	Nitrate-N(mgL)	-.405(**)	-0.063	0.105	0.136	-.195(*)
All	Total Ammonia (mg/L)	-0.037	.255(**)	.202(*)	0.076	.346(**)
All	Total Nitrogen (mg/L)	-0.155	.272(**)	.192(*)	0.068	.265(**)
1	Enterococci	0.039	-0.239	0.245	0.025	0.274

	(CFU/100mL)					
1	Fecal Coliforms (CFU/100/mL)	-0.09	0.041	0.192	0.112	0.194
1	Nitrate-N(mgL)	-0.157	-0.345	0.585	0.301	-0.114
1	Total Ammonia (mg/L)	-0.167	0.255	0.078	0	0.491
1	Total Nitrogen (mg/L)	-0.569	0.025	0.272	-0.4	0.355
2	Enterococci (CFU/100mL)	0.093	-0.024	0.034	0.037	0.212
2	Fecal Coliforms (CFU/100/mL)	0.191	0.06	-0.237	-0.247	-0.061
2	Nitrate-N(mgL)	-0.503	0.234	0	-0.152	-0.138
2	Total Ammonia (mg/L)	-0.196	0.171	0.501	0.2	0.378
2	Total Nitrogen (mg/L)	-0.462	0.02	0.4	-0.3	0.164
3	Enterococci (CFU/100mL)	-0.025	-0.094	.	0.17	0.083
3	Fecal Coliforms (CFU/100/mL)	-0.266	-0.171	.	0.2	-.302(*)
3	Nitrate-N(mgL)	-.669(*)	-0.274	.	0.173	-0.354
3	Total Ammonia (mg/L)	-0.058	0.402	.	0.373	.661(*)
3	Total Nitrogen (mg/L)	-0.434	0.045	.	0	0.119
4	Enterococci (CFU/100mL)	0.256	0.112	-0.034	.	0.27
4	Fecal Coliforms (CFU/100/mL)	0.06	-0.021	-0.262	.	0.168
4	Nitrate-N(mgL)	-.723(*)	-0.509	-0.13	.	-0.387
4	Total Ammonia (mg/L)	-0.319	0.179	0.329	.	0.405
4	Total Nitrogen	-0.255	0.366	0.12	.	.627(*)

	(mg/L)					
5	Enterococci (CFU/100mL)	.335(*)	0.204	0.093	0.079	.426(**)
5	Fecal Coliforms (CFU/100/mL)	.325(*)	0.16	0.139	0.264	.409(**)
5	Nitrate-N(mgL)	-.738(**)	-0.1	.638(*)	.	-0.191
5	Total Ammonia (mg/L)	-0.04	0.342	-0.106	.	0.378
5	Total Nitrogen (mg/L)	-0.191	0.558	.729(*)	.	0.588
6	Enterococci (CFU/100mL)	0.14	0.289	.	.463(**)	0.224
6	Fecal Coliforms (CFU/100/mL)	0.017	0.296	.	.349(*)	0.18
6	Nitrate-N(mgL)	-0.591	0.267	.	-0.038	-0.2
6	Total Ammonia (mg/L)	0	.688(*)	.	0.149	.773(**)
6	Total Nitrogen (mg/L)	0.058	0.286	.	0.373	0.082
7	Enterococci (CFU/100mL)	0.243	-0.022	0.147	-0.013	0.064
7	Fecal Coliforms (CFU/100/mL)	.356(*)	-0.054	0.287	0.103	0.037
7	Nitrate-N(mgL)	0.157	0.191	-0.273	.	-0.352
7	Total Ammonia (mg/L)	0.115	0.185	-0.3	.	0.3
7	Total Nitrogen (mg/L)	0.115	0.254	-0.2	.	0.127
8	Enterococci (CFU/100mL)	.490(**)	.348(*)	-0.146	0.258	.472(**)
8	Fecal Coliforms (CFU/100/mL)	.370(*)	.388(**)	-0.151	0.289	.697(**)
8	Nitrate-N(mgL)	0.22	0.191	0	.	0.045
8	Total Ammonia (mg/L)	0.058	0.233	0.337	.	0.173

8	Total Nitrogen (mg/L)	0.404	0.44	0.067	.	0.491
9	Enterococci (CFU/100mL)	0.279	0.243	-0.058	0.19	0.071
9	Fecal Coliforms (CFU/100/mL)	0.029	-0.043	0.067	0.07	-0.103
9	Nitrate-N(mgL)	0.032	0.077	-0.192	0.53	0.075
9	Total Ammonia (mg/L)	0.404	0.221	0.501	0.149	0.236
9	Total Nitrogen (mg/L)	0.173	0.231	0.039	0.224	0.164
10	Enterococci (CFU/100mL)	0.222	0.224	-0.133	.	.382(*)
10	Fecal Coliforms (CFU/100/mL)	0.022	0.071	-0.167	.	0.253
10	Nitrate-N(mgL)	-.640(*)	-0.512	-0.103	.	-.696(*)
10	Total Ammonia (mg/L)	0.25	0.042	0.231	.	0.012
10	Total Nitrogen (mg/L)	-0.083	0.201	0.109	.	-0.043

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"Taylor County Beaches Pathogen and Nutrient Sources Assessment Study: Seasonal Water Quality Impacts"

-FINAL REPORT-

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Laboratories for Engineered Environmental Solutions

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EXECUTIVE SUMMARY

Prior to 2004, beach water quality sampling conducted by the Suwannee River Management District (SRWMD) and the Taylor County Health Department (TCHD) has shown that counts of the pathogen indicators fecal coliform and enterococci frequently exceed the water quality standards for recreationally used surface waters at coastal communities in Taylor County, FL. The ongoing weekly beach monitoring program posts advisories approximately 46% of the time due to high concentrations of indicator bacteria (>400 CFU/100ml for fecal coliforms, >100 CFU/100ml for *Enterococcus*), and between the years of 2004 and 2006, the SRWMD and TCHD monitoring programs found that 94 of 181 samples (52%) failed for enterococci. This has resulted in frequent beach advisories which are significant because these waters are commonly used for recreational fishing (including scallops). Nutrients, while not a part of the regular beach water quality monitoring program, were also of concern. Initially, it was suspected that onsite sewage treatment and disposal systems (OSTDS), in particular pre-1983 and other systems operating without a permit may be a source of the pathogen indicators in these waters. However, this particular area was struck by the "*No-Name Storm*" in March 1993, which destroyed a number of coastal residences with older septic systems and forced the homeowners to rebuild and often update their OSTDS with more modern units.

Of concern are the rapid development and the change from seasonal to full-time residents in the coastal communities of Taylor County, FL, both of which have been identified as potential threats to water quality. Most of the coastal communities have historically relied on OSTDS. Various studies (Meeroff et al. 2005; Morin et al. 2005; Ahmed et al. 2004; Lipp et al. 2001) have investigated the contribution of failing septic tanks on the degradation of water quality, particularly during the seasonal high water table (SHWT) elevation, when septic tanks (that are not constructed to current standards) are expected to operate inefficiently.

There is a need to obtain information on bacteriological and nutrient sources and to evaluate the contribution of OSTDS to the observed water quality problems. The information gathered will be used by state and local officials to address the contamination

of coastal waters, to develop plans to improve sewage treatment and disposal in the coastal communities, and provide data that may be applicable to the management of shellfish in this and other areas. The United States Environmental Protection Agency (USEPA), through the Gulf of Mexico Program, has provided funding for this investigation to the Florida Department of Health, Bureau of Onsite Sewage Programs, which contracted Florida Atlantic University (FAU) to assist in the scientific study to assess possible sources of pathogen indicators and the contribution of OSTDS to coastal surface water quality in Taylor County, FL, by using multiple tracers:

Laboratory Parameters	**Field Parameters**
E. coli and total coliforms	pH
Enterococcus	Conductivity
Total organic carbon (TOC)	Salinity
Total nitrogen (TN)	Temperature
Ammonia-nitrogen	Dissolved oxygen (DO)
Nitrate	Turbidity
Caffeine	Optical brighteners

The objective of this study is to test the hypothesis that OSTDS significantly contribute to the observed water quality degradation and that the problem is aggravated during the SHWT. This hypothesis will be evaluated using pair-wise comparison, intervention analysis, and multiple tracers. The results will be used to assess source tracking hypotheses for nutrients and pathogen indicators so that water quality managers will be able to develop plans for improving water quality in these coastal communities.

The results of the first year of sampling prompted additional questions that could only be addressed by returning for another round of sampling with additional recommended analyses and sampling site density. Additional support was provided by the Florida Department of Health and the Florida Department of Environmental Protection to continue sampling for a second year. By using multiple tracers, including nitrogen isotopic ratios and shallow sediment re-growth experiments, seasonal variability issues were addressed to permit investigators to distinguish between human and non-human sources, functioning OSTDS and surface runoff contributions to pathogen indicators, and nutrient concentrations for identification of significant sources of contamination.

A summary of the results of the five sampling events conducted between 2006 and 2007 indicate the following:

- The SHWT and SLWT determinations were based on historical data from groundwater monitoring well measurements, tidal considerations, precipitation records, river water level stage heights, historical water quality monitoring data, water usage statistics from utility billing records, and soil surveys. The analysis was updated with data from 2006 and 2007, when available, to determine if any changes occurred during sampling. For all indicators, the historical trends were generally observed during the sampling period, with the exception of precipitation, which was lower than expected in 2006. Moreover, none of the sampling activities were conducted within one week of a rain event, which complicated the ability to distinguish directly between storm water and wastewater-related sources.
- The bacteriological results reveal that for both *Enterococcus* and *E. coli*, the microbial densities were generally higher for the SHWT by a factor of 2 – 3, especially for the OSTDS areas. Between 5-10% of all *Enterococcus* samples violated the trigger levels in SLWT, but 30-35% violated in SHWT.
- A general increasing trend in microbial pathogen indicators from upstream to downstream is also apparent. A gradient from higher density of homes toward the beaches is expected for conservative tracers in a conservative water body, particularly given the fact that sampling was timed to coincide with the outgoing tide, which would only magnify this effect. Given the short travel distances in the beach community clusters, the microbiological indicators, which are non-conservative, tended to behave as conservative tracers.
- *Enterococcus* counts are generally higher in OSTDS areas as compared to sewered areas, by a factor of 1.5-2.3, independent of season. In the sewered areas, the enterococci levels were about 40% lower than the sewer background site. In the OSTDS areas, the enterococci densities were higher than background by a

factor of 1.6. At Dekle Beach, the background levels are generally higher than the downstream counts, but the opposite is seen at Steinhatchee.

- However, *E. coli* levels were found to be consistently higher in the sewered areas, which were not expected. *E. coli* violations are nearly four times more frequent at sewered sites compared to those served by OSTDS, an observation that continued into 2007. In sewered areas, the *E. coli* levels were nearly one order of magnitude higher than the background sites and double the concentration observed in the OSTDS sites. The unsewered areas had *E. coli* counts that were 50% higher than their corresponding background sites.
- An analysis of the percentage of violations for dissolved oxygen, *Enterococcus*, and *E. coli* are all higher in the SHWT season. The elevated water table elevation during SHWT allows contaminants to be more rapidly transported through the subsurface to open bodies of water such as coastal canals. This would generally be an indicator that the difference is related to OSTDS, but the results from other indicators suggest other sources can also be attributed to this finding.
- Dissolved oxygen levels generally decreased during the SHWT events. Rainfall would tend to increase DO, but during the lag time after the rainfall events, it is hypothesized that microbial activity increases, so regrowth could have accounted for the observed consumption of dissolved oxygen, even after temperature effects are taken into account. The impact of rainfall could not be directly measured in this study because none of the sampling events happened to coincide with recent rainfall.
- No noticeable differences in ammonia were observed between sites with sewer and sites with OSTDS. Ammonia was generally higher during the May 2006 SLWT sampling events for all sites. However, this was likely due to using a different analytical method for this sampling event compared to the others.
- Elevated TOC and ammonia compared to background sites in the 2006 SLWT (May and December) data may be a symptom of cultural eutrophication, but this requires further research. The nitrogen isotope analysis from May 2007 SLWT sampling events seems to implicate fertilizers at the beach communities, but a

possible industrial source signal could not be discounted upstream at the background site locations.

- On average, nitrate levels were below the concentrations considered high for coastal marine environments. In conjunction with ammonia levels that were higher than background in the SHWT, this finding would seem to direct the source toward runoff of lawns or agricultural operations as both would tend to show high concentrations of ammonia but minimal increases in nitrate. However, from the speciation of nitrogen-containing parameters (ammonia, nitrate, nitrite, and total nitrogen), it was determined that most of the nitrogen detected was in the form of organically bound nitrogen.
- Keaton Beach had isolated samples with extreme microbial contamination recorded during the May 2006 SLWT. The elevated microbial counts were repeated in May 2007 SLWT, which may indicate a persistent local source, such as sediment reservoirs of pathogen indicators or a naturally-occurring regrowth source.
- Within the documented limits of similar microbial ratios, the *E. coli/Enterococcus* ratio (Ec/Es) was evaluated. During the SLWT, only 1 of 6 samples (17%) had Ec/Es ratio values that were indicative of human contributions. However, during the SHWT sampling events, more than 50 percent of the ratios were indicative of human contributions. All of the beach sites showed Ec/Es ratios that were more indicative of human-derived sources of pollution. However, the background sites, with the exception of the Creek at Dekle Beach, consistently produced Ec/Es ratios more indicative of a contribution from non-human sources of pollution. It is has been observed that the ratio may shift with time and distance from the source. Therefore, if environmental conditions between background and downstream sites do not change much with time and distance, the ratio could have been affected by salinity, which did vary between background and upstream or beach sites.
- Molecular techniques were used on 22 of the 309 samples collected in this study, and although a number of the samples collected in this study showed very high levels of bacteria, the samples randomly selected for molecular techniques analysis largely did not. This subset of molecular techniques data indicated that

the analyzed water samples (22 of 309) were not highly contaminated with fecal pollution of human or animal origin. These results are supported by the low IDEXX MPN results for *Enterococcus* and the lack of confluent growth from the samples incubated on the bacterial media, for the subset of 22 samples. Although the selected samples for molecular techniques testing were relatively free of microbial indicators, this was not indicative of the larger sampling campaign. Therefore, it is recommended to expand the number of samples for molecular techniques analysis in future sampling events to better capture the general trend observed with microbial enumeration over the course of the study.

- Studies of shallow sediments were recommended to determine regrowth patterns of microbial indicators. The results from this study were largely inconclusive because of the relatively small sample size (n = 8). The potential for regrowth was recorded in May 2007 SLWT, particularly for Cedar Island Beach and also to a lesser extent at the other three sites (Adams Beach, Dekle Beach, and Keaton Beach) for *E. coli*. However, the results were not reproduced in sediments collected in September 2007 SHWT.
- Sewered areas (Keaton Beach and Cedar Island) have not shown improved water quality in comparison to areas that remain on OSTDS. This finding is supported by the absence of a change in slope in a graph of the bacteriological densities over time at the sewered sites. The slope was expected to change shortly after installation of sewer in these areas. Thus, in sewered areas, the possibility that remnant OSTDS inputs have not been fully flushed from the surficial soils cannot be discounted, particularly given the potential for regrowth. However, the possibility of another source cannot be discounted either, given the unlikelihood of septic effluents continuing to impact water quality for several years after the input.
- For sewered areas, the microbial data, specifically: Ec/Es ratios, enterococci, and *E. coli* levels suggest that sewered areas are more human-influenced compared to the OSTDS sites.
- After tidally influenced transport, the ground water and runoff contributions for a given area do not return to exactly the same water quality level from which they

originated. This daily periodicity is termed as the "slosh" effect by the authors. The slosh effect may play an important role in cycling nutrients as well as pathogen indicators, as Linquist (1993) observed with common soil bacteria under passive and active wave action conditions. A second possibility is that during the SHWT, the soils and canals in the sewered areas may be flushed less effectively, and therefore do not show the same concentrations of bacteria as the septic areas that would tend to leach even more bacteria into the soil, if sited improperly.

- Caffeine was not shown to be an effective tracer.
- Optical brighteners were also ineffective.

Interesting differences in multiple water quality tracers between sewered and non-sewered areas were observed. In terms of microbial pathogen indicators, unexpectedly high *E. coli* counts were found at sewered sites, while higher enterococci levels were found at OSTDS sites. Possible sources evaluated include: 1) legacy and OSTDS sources, 2) recent sewer leaks, 3) sediment reservoirs harboring pathogen indicators, 4) natural sources or runoff contaminated with animal feces, and/or 5) steady upstream contributions, the source of which may or may not be wastewater-related. Some evidence of human-derived input from sewage or OSTDS is found, and from molecular techniques, a dog or bird contribution cannot be discounted. Elevated TOC and higher ammonia levels at the beach communities may indicate recent anthropogenic input from lawn fertilizers or an upstream industrial source. The nitrogen isotope analysis from May 2007 supports this supposition, in particular for the beach communities. Elevated levels of total nitrogen (indicative of organic-N) combined with high enterococci tend to implicate a greater contribution of nutrients to coastal waters from OSTDS, but this combination was not seen consistently. OSTDS are expected to perform more efficiently during the SLWT event, with the likelihood of failure increasing in the SHWT event for older systems. This field study demonstrates that the magnitude of water quality degradation in the area may have a contribution from OSTDS, but outlines other potentially important input sources (see 1-5 this paragraph).

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INTRODUCTION

Taylor County is bordered by Jefferson, Madison, Lafayette, and Dixie Counties and by the Gulf of Mexico. The total area of the County is 789,000 acres (3,191 km^2), of which approximately 15% is comprised of water bodies. Taylor County has four rivers, numerous canals, creeks, and springs, and nearly 60 miles of Gulf of Mexico coastline. The major tourist attractions are fishing and scalloping, particularly from July through September. Half of its southern coast is part of the "*Big Bend Sea Grasses Aquatic Preserve*" and is classified as "*Outstanding Florida Waters*."

The surface water quality criteria to be met for the study sites correspond to Class III waters (recreational use and fish and wildlife health) and are detailed below (FAC 602-302.530):

- Fecal coliform < 10% over 400 MPN per 100 mL, not to exceed 800, on any given day
- Dissolved oxygen not less than 5.0 mg/L in freshwater, never less than 4.0 mg/L in marine waters
- Nutrients (total nitrogen and total phosphorus) limited as needed to avoid imbalance in natural populations
- Turbidity < 29 NTU above natural background concentrations

Prior studies have been conducted by the Suwanee River Water Management District (SRWMD) and the Taylor County Health Department (TCHD) to determine if water quality criteria are being met in Taylor County. An ongoing weekly beach monitoring program posts advisories approximately 46% of the time due to high concentrations of indicator bacteria (>400 CFU/100ml for fecal coliforms, >100 CFU/100ml for *Enterococcus*), and between the years of 2004 and 2006, the SRWMD and TCHD monitoring programs found that 94 of 181 samples (52%) failed for enterococci. Because these waters are used for drinking, recreation, and the harvesting of seafood, it is imperative to maintain the microbiological quality and safety of water. Contamination of these water systems can result in human health risks and significant economic losses due to closures of beaches and shellfish harvesting areas (Scott et al. 2002).

Rapid development and the change from seasonal to full-time residents in the coastal communities of Taylor County, FL have been identified as a potential threat to coastal water quality. Most of the coastal communities historically rely on On-Site Treatment and Disposal

Systems (OSTDS). Moreover, the relatively high density in small lots can be a problem. Various studies (Meeroff et al. 2005; Morin et al. 2005; Ahmed et al. 2004; Lipp et al. 2001) have investigated the contribution of failing septic tanks on the degradation of water quality, particularly during the seasonal high water table (SHWT) elevation, when septic tanks are expected to operate less efficiently, if the proper water table separation distance is not maintained. Initially, it was suspected that onsite sewage treatment and disposal systems (OSTDS), in particular pre-1983 and other systems operating without a permit may be a source of the pathogen indicators in these waters. However, this particular area was struck by the "*No-Name Storm*" in March 1993, which destroyed a number of coastal residences with older septic systems and forced the homeowners to rebuild and often update their OSTDS with more modern units. In two of the sampling locations, Keaton Beach and Cedar Island, the approximately 300 OSTDS were abandoned between November and December 2005 and replaced with sanitary sewer by Taylor Coastal Utilities. A list of dates for each specific septic tank or area is available from Taylor Coastal Utilities.

The Florida Atlantic University (FAU) Laboratories for Engineered Environmental Solutions (Lab.EES) was contracted to conduct a scientific study to assess possible sources of pathogen indicators and the contribution of OSTDS to coastal surface water quality in Taylor County, FL, by using multiple tracers. The results will be used to evaluate source tracking hypotheses for nutrients and pathogen indicators so that water quality managers will be able to develop plans for improving water quality in coastal communities. The results of the first year of sampling prompted additional questions that could only be addressed by returning for another round of sampling with additional recommended analyses and sampling site density. By using multiple tracers, including nitrogen isotopic ratios and shallow sediment re-growth experiments, the proposed plan of work addressed the seasonal variability issues of distinguishing between human and non-human sources, and between functioning OSTDS and surface runoff contributions to pathogen indicators and nutrient concentrations for identification of significant sources of contamination. Table 1 lists the tracers to be analyzed in this study. Table A-1 in the Appendix summarizes the parameters, analytical methods, detection limits, method precision values, trigger levels, expected levels, and encountered ranges.

Table 1 – Summary of parameters analyzed.

Laboratory Parameters	Field Parameters
E. coli and total coliforms	pH
Enterococcus	Conductivity
Total organic carbon (TOC)	Salinity
Total nitrogen (TN)	Temperature
Ammonia-nitrogen	Dissolved oxygen (DO)
Nitrate	Turbidity
Caffeine	Optical brighteners

For 2007 follow-up work, it was desired to increase the site density (i.e. number of sites and distribution) and perform additional experimental work that was recommended to resolve confounding issues discovered in 2006 sampling. These included shallow sediment re-growth, existing infrastructure assessment, and unconventional source tracking tools. In particular, previous work demonstrated a general trend of higher *E. coli* at sewer sites and higher *Enterococcus* at OSTDS sites. This *E. coli* may be from human or natural sources, but if it can survive in the near-shore environment without external inputs, this will complicate source tracking. Thus it was proposed to conduct re-growth studies of shallow sediments in certain key beach sites (Adam's Beach, Dekle Beach, Keaton Beach, and Cedar Island Beach).

The objective of this study is to test the hypothesis that OSTDS significantly contribute to the observed water quality degradation and that the problem is aggravated during the SHWT. This hypothesis will be evaluated using pair-wise comparison, intervention analysis, and multiple tracers. The results will be used to assess source tracking hypotheses for nutrients and pathogen indicators so that water quality managers will be able to develop plans for improving water quality in these coastal communities.

SAMPLING METHODOLOGY

SAMPLING SITES

All sampling sites were selected in order to represent two main groups, sites with central sewer systems and sites served by OSTDS. This was done to allow comparison of the overall water quality between similar neighborhoods, two connected to a public sewer network and the other two served exclusively by septic tanks. Two sewered areas and two non-sewered areas were selected and approved with input from the Taylor County Health Department and the Florida Department of Health Bureau of Onsite Sewage Programs. In each of the four locations, at least three sampling site categories were used, a beach site, a canal/creek (upstream) site, and a background site.

Figure 1 – General location of sampling sites in Taylor County, FL.

The objective of the field study is to distinguish between possible human sources of pollution and various other types of contamination in coastal waterways within Taylor County, FL. Locations were chosen including coastal canals, inland rivers, and beaches. The sampling locations were paired according to OSTDS effects, intervention analysis (before/after sewer installation) effects, beach vs. canal, population density, and upstream effects. Paired sites are summarized in Table 2 and described in more detail in Appendix B.

Table 2 – Breakdown of paired sites for 2006-2007.

Type	Location	Beach	Canal/Creek (Upstream)	Background
Developed without Sewer	Dekle Beach (ρ= low)	A. Dekle Beach JI. Jugg Island Rd	B. Canal at Mexico Rd	C. Creek at Dekle
	Steinhatchee (ρ= high)	J. Main Street Steinhatchee	K. Third Avenue Fork M. Steinhatchee at Airstrip Dr. (L. Boggy Creek @ 51)	N. Steinhatchee Falls
Developed with Sewer Being Installed	Keaton Beach (ρ= medium)	F. Keaton Beach	E. Cortez Road Canal MR. Marina Road (D. Cortez Pump station)	G. Blue Creek at Beach Road
	Cedar Island (ρ= medium)	I. Cedar Island Beach SL. Seahawk Lane	H. Heron Road Canal	G. Blue Creek at Beach Road

Some of the selected sites coincide with sampling sites from previous studies: four beach sites coincide with the Florida Department of Health (FDOH) Beach Monitoring Program sampling points (data from 2000 to 2006), and ten sites coincide with some of the sampling points of a previous FDOH study on water quality in Taylor County (data from 2004 to 2005). Available data collected previous to this study will allow an intervention analysis to evaluate the change in concentration of water quality parameters (e.g. *E. coli*, *Enterococcus*) prior to and after sewer installation.

Two phases of testing were conducted. Based on prior work conducted in Taylor County (Phase one, 2006), additional sampling locations for follow-up sampling (Phase two, 2007) were desired to assist in resolving confounding issues in source tracking hypotheses. In 2006, the three sampling trips used the original 14 sites (see Table 3). In 2007, two additional sampling trips were added along with 7 new sites and the 14 original sites. The new sites were selected based on professional judgment of the representativeness for the location type. The locations were approved by the FDOH project officer by conference call on May 17, 2007 (and in writing on May 18, 2007), and prior to any sampling taking place. All sampling site locations are located in the hydrologic unit code (HUC) 3110102. The overall sampling site list, and which program each site was part of, is listed in Table 3.

Table 3 - Summary of sample site locations (highlighted rows indicate new sites for this study).

Site Code	Name	Location	Hydrology	Residential Development	Healthy Beaches Site?	CHD 04/05 sampling?	FAU 2006 sampling?
PL	Fenholloway at Peterson's Landing	Spring Warrior Beach	Estuary of the Fenhollowav	Developed area without sewer	No	No	No
HS	Hampton Springs Bridge	Perry	Middle of the Fenholloway	Developed area without sewer	No	No	No
FR	Fenholloway River @ 19/Alt27	Perry	Downstream of Buckeye	Developed area with sewer	No	No	No*
AB	Adam's Beach	Adam's Beach	Beach	Undeveloped without sewer	Yes	Yes	No
A	Dekle Beach	Dekle Beach	Beach	Developed area without sewer	Yes	Yes	Yes
JI	Jugg Island Road	Dekle Beach	Beach (downstream)	Developed area without sewer	No	No	No
B	Dekle Beach Canal @ Mexico Road	Dekle Beach	Canal (dead-end)	Developed area without sewer	No	Yes	Yes
C	Creek at Dekle Beach	Dekle Beach	Creek	Upstream, none	No	Yes	Yes
D	Cortez Road Canal (Pump Station)	Keaton Beach	Canal (dead-end)	Upstream, of Blue Creek and developed area with sewer installed**	No	No	Yes
E	Cortez Road Canal Upstream (Jet Skis)	Keaton Beach	Canal (midstream)	Midstream, developed area with sewer installed	No	No	Yes
MR	Marina Road	Keaton Beach	Canal at mouth	Downstream, developed area with sewer installed	No	No	No
F	Keaton Beach	Keaton Beach	Beach	Beach, developed area with sewer installed	Yes	Yes	Yes
G	Blue Creek at Beach Road	Keaton Beach Or Cedar Island	Creek	Upstream, background, no development	No	Yes	Yes
H	Heron Road Canal	Cedar Island	Canal (dead-end)	Developed area with sewer installed	No	Yes	Yes
I	Cedar Island Beach	Cedar Island	Beach	Developed area with sewer installed	Yes	Yes	Yes
SL	Seahawk Lane	Cedar Island	Beach towards the estuary of Blue Creek	Developed area with sewer installed	No	No	No
J	Main Street (Roy's)	Steinhatchee	Estuary of the Steinhatchee	Downstream, developed, high population, OSTDS	No	No (SRWMD data available)	Yes
K	3rd Avenue Fork	Steinhatchee	River	Middle stream, developed, high population, OSTDS	No	No	Yes
L	Boggy Creek at 51	Steinhatchee	Creek	Upstream creek, developed, high population, OSTDS	No	No	Yes
M	Steinhatchee at Airstrip Drive	Steinhatchee	Creek	Upstream creek gradient, developed, high population, OSTDS	No	No	Yes
N	Steinhatchee Falls	Steinhatchee	River	Upstream, background, low density, OSTDS, campground	Yes	No (SRWMD data available)	Yes

*Monitored on one occasion during 2006 sampling
**Historical data show that this is a site with intermediate concentrations

Boundaries of the Study

The monitoring program includes sampling sites located along the "loop" extending from Adams Beach to Steinhatchee in Taylor County, FL. Four beach monitoring sites are identical to those already implemented as part of the Florida Healthy Beaches Program. These include (from north to south): Adam's Beach, Dekle Beach, Keaton Beach, and Cedar Island. A summary of the sampling locations is found in Table 3. The highlighted sites were sampled only in 2007. Global Positioning Systems (GPS) were used to locate all monitoring sites. Some variation in position may occur due to tidal effects, flooding, etc. In some cases (i.e. sites B, C, D, E, and H), tidal variability is expected, because some sampling sites are located in shallow (< 6 in.) water, at times.

The seven new sites were selected to address several confounding issues that arose during the first year of monitoring. The naming scheme was changed from the A, B, C, D, ... pattern to avoid having to change all of the site codes from the 2006 sampling events. More information on the selection process for the new sampling locations is presented in Appendix B.

SAMPLING EVENTS

In a previous study, Morin et al. (2005) suggested that septic tanks do not work properly when the water table is high, since insufficient distance between the drainfield and the groundwater level (<0.6 m) leads to inadequate treatment. In many parts of coastal Florida, the water table is constantly high, often reaching ground level elevations during the wet season. Thus, the drainfield piping network may become submerged, and the wastewater becomes directly connected to the receiving water body. Because of this fact, sampling activities were purposely designed to be conducted during the seasonal high (September) and seasonal low water table elevation (May, December) events. It should be noted that no attempt was made to assess the water table heights during the sampling dates in this study, although this could prove useful, if available.

To determine the SHWT and SLWT, and the timing of sampling events, multiple approaches (ground water levels, tidal periods, rainfall patterns, historical water quality data, etc.) were used to determine the expected seasonality of groundwater table elevation in the coastal areas. First, historical ground water level measurements from three shallow monitoring wells in Taylor County, FL were analyzed. This data was acquired from the Suwanee River Water Management District c/o Warren Zwanka, Hydrogeologist, P.G. (9225 CR49, Live Oak, FL 32060; 800-226-

1066). The data consisted of daily and average monthly ground water level measurements from 1995 until 2005. The wells used were:

1. 020731002 (30°15’48.283’’ N Latitude, 83°39’39.745’’ W Longitude)
2. 020828001 (30°17’21.166’’ N Latitude, 83°32’10.334’’ W Longitude)
3. 030730001 (30°11’45.332’’ N Latitude, 83°40’11.743’’ W Longitude)

For each well, the water level data was compiled as monthly averages for each year, representative of the previous ten year time span for each well and was plotted in Figure 2. Error bars shown indicate the characteristic variability in the aggregate data over the ten year timeframe and correspond to one standard deviation from the mean. From Figure 2, the seasonal high water table typically occurs during the months of March – April. The lowest water table elevation is typically during June. The differences were on the order of one foot. However, these monitoring wells were quite removed from the coast as indicated by their high water table elevations, and may not show the same seasonality as coastal waters. The same wells were analyzed for 2006 and 2007 as shown in Figure 2 (right). The same general trend is followed, although the conditions were uncharacteristically drier than expected from historical values. The 2007 data set is incomplete but is compared to historical values in Table 4. The 2007 data also closely follows the historical averages.

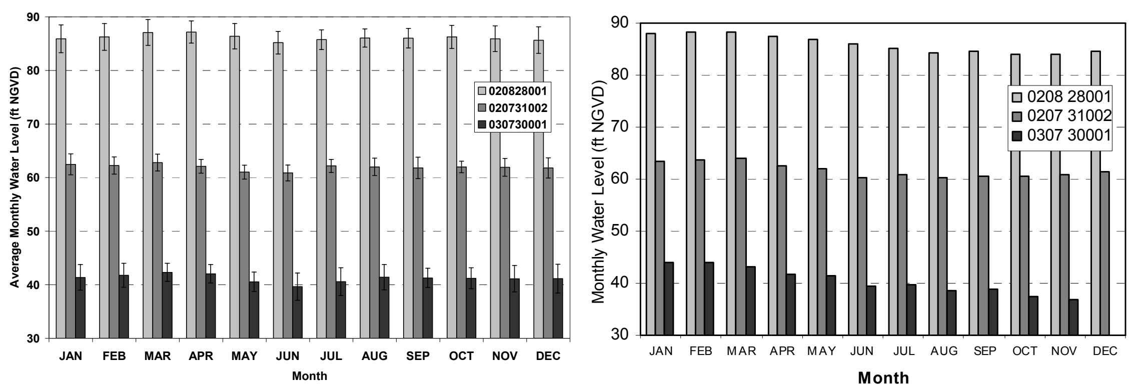


Figure 2 - Determination of seasonal high water table using mean ground water level data from three shallow monitor wells in Taylor County, FL. The graph on the left shows data from 1995 – 2005 (SRWMD 2005). The graph on the right shows the 2006 trend.

Table 4 – Ground water table elevations for 2007 from three shallow monitor wells in Taylor County, FL.

S020731002	2007	1995-2005	s
JAN	63.2	62.5	2.0
FEB	63.3	62.3	1.6
MAR	62.8	62.8	1.6
APR	61.7	62.1	1.3
MAY	60.9	61.0	1.3

S020828001	2007	1995-2005	s
JAN	85.7	85.9	2.6

S030730001	2007	1995-2005	s
JAN	40.8	41.4	2.4

Average precipitation records were also considered for the determination of seasonal water level elevations. Using data provided by the NOAA National Climatic Data Center (NCDC) Weather Station Historical Data Service (http://www.ncdc.noaa.gov/oa/climate/stationlocator.html), the daily rainfall data for three stations in the Taylor County area, were obtained. The stations used were: 1) Perry (30°06'N / 83°34'W; 13.7 m above sea level; in service 1948 – present; COOP ID 087025); 2) Sea Hag Marina (29°40'N / 83°23'W; 1.5 m above sea level; in service 2002 – present; COOP ID 088076); 3) Steinhatchee 6 ENE (29°40'N / 83°24'W; 3.0 m above sea level; in service 1958 - 2001; COOP ID 088565), and Huxford Tower (http://flame.fl-dof.com/fire_weather/observations/dof_rainfall.html). Figure 3 shows the results of this analysis. All stations showed that the wettest months occurred in June through September. Generally drier periods occur in November – December and April – May. The 2006 data was characterized as a dry year, particularly in summer. The 2007 data more closely followed the historical trends.

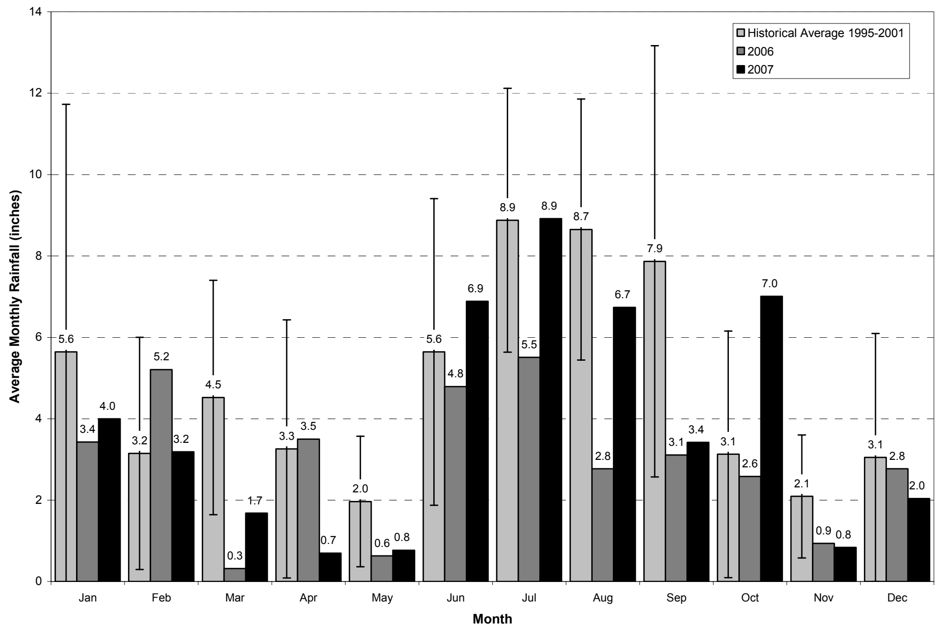


Figure 3 - Average monthly rainfall for Steinhatchee EN, FL station comparing the historical averages from 1995 – 2001 to the actual rainfall records for 2006 and 2007 (error bars represent one standard deviation from the mean).

The SHWT and SLWT determinations based on historical data from groundwater monitor well measurements, tidal considerations, precipitation records, river water level stage heights, historical water quality monitoring data, water usage statistics from utility billing records, and soil surveys generally followed the historical trends during the sampling period, with the exception of precipitation, which was lower than expected in 2006. Furthermore, none of the five sampling activities were conducted within one week of a rain event, which complicated the ability to distinguish between storm water and wastewater-related sources. It is important to note, that seasonality is maintained during 2006-2007 because this will be used in the analysis of source tracking hypotheses.

Sampling events were planned to occur for 3 consecutive days during each sampling trip. Sample collection was timed to coincide with ebb tide, the period in which the water level is falling from high tide to low tide. This was done to get a better representation of the potential contamination contribution from human sources. Samples collected during flood tide, the period when the water level is increasing from low tide to high, tend to underestimate the contribution of inland or terrestrial sources. Under the flood tide condition, most of the water is of marine origin, and thus dilution plays a confounding role in the results. During field sampling events, all samples were collected from downstream to upstream, to avoid possible cross contamination.

FIELD SAMPLING METHODOLOGY

The field sampling protocol basically replicated the May, September, and December 2006 sampling event for the three beach site locations (Dekle Beach, Keaton Beach, and Cedar Island) and Steinhatchee with the additional sampling locations described earlier. Sampling consisted of three consecutive days, collected during outgoing tide. Samples were collected and analyzed according to the previous QAPP or similarly effective methods. Appendix D contains a summary of the trip reports filed for each of the five sampling events.

The following physical parameters were determined in the field:

- pH (YSI 556 probe, FDEP FT1100)
- Conductivity (YSI 556 probe, FDEP FT1200)
- Salinity (YSI 556 probe, FDEP FT1300)
- Temperature (YSI 556 probe, FDEP FT1400)
- DO (YSI 556 probe, FDEP FT1500)
- General weather conditions (sunny, cloudy, or rainy) and wind characteristics
- Ambient air temperature
- Tidal conditions (ebb, flood, or slack; high, medium, or low)
- Current direction and strength

The following parameters were determined in the laboratory and governed by the following SOPs:

- Ammonia and other anions of interest (NOAA seawater protocol)
- *E. coli* and Total Coliforms (FAU LT6100)
- *Enterococcus* (FAU LT6200)
- Total Organic Carbon and Total Nitrogen (FAU LT5200)

SAMPLE HANDLING

Sampling collection, preservation, storage, and analysis followed the field sampling procedures governed according to the previous Quality Assurance Project Plan (QAPP, March 2006) filed and approved for DOH contract number CO0F7: Taylor County Beaches Pathogen and Nutrient Sources Assessment and specific Standard Operation Procedures (SOPs) for each parameter.

Field parameters: pH, conductivity, salinity, temperature, and DO were determined using a YSI 556 multiparameter probe. Turbidity was recorded using a portable nephalometric VWR Model 800 turbidometer. TOC/TN analysis was conducted in the laboratory using an Apollo 9000 TOC/TN analyzer. Bacterial analyses were conducted by the defined substrate fluorescent antibody technique using the IDEXX quanti-tray method. More information regarding sample handling and collection procedures is found in the QAPP.

QUALITY CONTROL

Analysis of total organic carbon, total nitrogen, *E. coli*, *Enterococcus*, total coliforms, field parameters, optical brighteners, and other additional experiments were conducted by the FAU-Lab.EES in accordance with procedures described in the approved QAPP. Analysis of caffeine, ammonia, and nitrate were reported by a certified laboratory, *Florida Environmental Services (FES)*, *US Biosystems*, or *NOAA-AOML's Ocean Chemistry Division*. QA/QC procedures of the certified laboratory's quality assurance plan were inspected and can be made available upon request. Where appropriate, calibration forms, calibration curves, and results for field duplicates, laboratory replicates, and blanks are attached (See Appendix C for more details).

RESULTS AND DISCUSSION

The results herein are organized and presented by sampling site. Sites were grouped into two categories: sites with OSTDS (septic tanks) and sites with sewer.

DEVELOPED SITES WITH SEPTIC TANKS

Dekle Beach

The Dekle Beach location was representative of a low-density developed area served by OSTDS. Measured DO was in the range of 3.1 – 9.1 mg/L. On the first sampling day in September 2006 (SHWT), the beach site (Site A) presented the lowest DO concentration (3.1 mg/L). This value does not meet the Class III criterion for marine waters (>4.0 mg/L). In September, the upstream and background sites also violated the Class III criterion. The violations occurred at the upstream site on the first and second day and at the background site on the first day. It is interesting to note that DO violations only occurred during the SHWT. Table 5 shows the range of DO levels and nutrients noted in the 2006 sampling events. Table 6 summarizes the 2007 results.

Table 5 – Dekle Beach nutrient results for 2006.

			SLWT (May 2006)			SHWT (Sept 2006)			SLWT (Dec 2006)		
Site	Charac.	Parameter	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
A	Beach	DO	8.7	6.7	5.8	**3.1**	4.4	4.5	9.1	7.0	7.1
B	Upstream	(mg/L)	7.6	6.2	5.8	**3.4**	**3.6**	4.3	7.4	6.0	8.5
C	Background		6.5	6.4	5.4	**3.8**	4.6	4.7	8.1	7.5	5.2
A	Beach	NH_4^+	<0.1	0.24	0.13	0.17	0.13	0.08	0.01	<0.01	0.01
B	Upstream	(mg/L as N)	0.67	0.22	0.13	0.12	0.07	0.04	0.04	0.06	0.01
C	Background		0.72	0.28	0.30	0.06	0.07	0.05	0.05	0.05	0.06
A	Beach	NO_3^-	<0.011	<0.011	<0.011	<0.0052	<0.0052	0.020	0.007	0.004	0.003
B	Upstream	(mg/L as N)	<0.011	<0.011	<0.011	<0.0052	0.020	<0.0052	0.007	0.010	0.003
C	Background		<0.011	<0.011	<0.011	0.020	0.020	0.020	0.009	0.009	0.007
A	Beach	TN	0.8	0.3	0.5	0.6	0.7	0.6	0.4	0.3	0.3
B	Upstream	(mg/L as N)	0.6	0.6	0.5	0.6	0.8	0.7	0.3	0.4	0.3
C	Background		0.7	0.4	0.6	0.6	0.7	0.6	0.4	0.4	0.5
A	Beach	TOC	16	11	11	15	20	19	10	11	10
B	Upstream	(mg/L as C)	12	13	11	17	21	18	13	11	10
C	Background		12	10	11	17	21	18	12	12	11

Note: Values in **bold** indicate violations of the trigger level

Table 6 – Dekle Beach nutrient results for 2007.

Site	Charac.	Parameter	SLWT (May 2007)			SHWT (Sept 2007)		
			Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
A	Beach	DO (mg/L)	7.5	6.6	6.8	4.4	4.2	**3.8**
JI	Beach		7.6	6.9	7.1	**2.6**	**2.7**	**2.3**
B	Upstream		7.2	6.7 ± 0.5	7.4	**3.0**	**2.0**	**2.6**
C	Background		6.5	6.0	6.3	**3.9**	4.7	**3.5**
A	Beach	NH_4^+ (mg/L as N)	0.00	0.00	0.00	0.00	0.01	0.01
JI	Beach		0.01	0.01	0.01	0.02	0.02	0.03
B	Upstream		0.06	0.05 ± 0.02	0.00	0.08	0.07	0.14
C	Background		0.06	0.03	0.04	0.07	0.06	0.11
A	Beach	NO_3^- (mg/L as N)	0.00	0.00	0.00	0.00	0.05	0.00
JI	Beach		0.01	0.00	0.00	0.00	0.00	0.01
B	Upstream		0.01	0.00	0.01	0.01	0.01	0.02
C	Background		0.01	0.01	0.01	0.02	0.01	0.03
A	Beach	TN (mg/L as N)	0.5	0.5	0.4	nr	nr	nr
JI	Beach		0.4	0.5	0.5	nr	nr	nr
B	Upstream		0.7	0.6	0.5	nr	nr	nr
C	Background		0.7	0.6	0.5	nr	nr	nr
A	Beach	TOC (mg/L as C)	7.3	7.5	8.4	7.4	10.5	8.2
JI	Beach		5.5	7.3	8.4	8.1	5.7	6.0
B	Upstream		8.9	7.8 ± 0.1	8.6	7.2	10.5	nr
C	Background		9.1	7.9	7.5	5.0	4.9	nr

Note: Values in **bold** indicate violations of the trigger level

In terms of nutrients, analyses were conducted to determine the levels of ammonia, nitrate, total nitrogen, and total organic carbon at the three sites. Figure 4 shows that during the May 2006 event (SLWT), the ammonia was generally higher than in September 2006 (SHWT) or December 2006 (SLWT). The results for the 2007 events were more indicative of the September and December events in 2006, and largely different than the May 2006 SLWT event. This may be an artifact caused by changing the ammonia analysis method (see Quality Control Section – Ammonia).

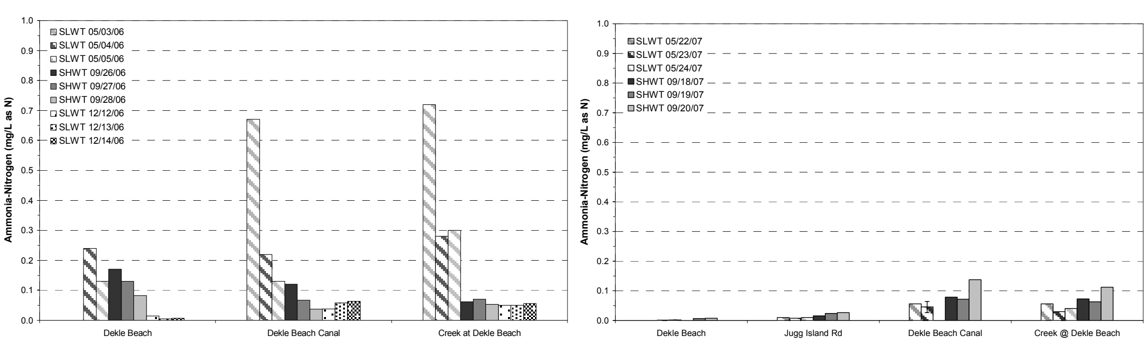


Figure 4 – Results for Ammonia at Dekle Beach for 2006 (left) and 2007 (right).

The ammonia concentration tended to increase as the sampling sites moved upstream in the 2006 SLWT (both in May and, to a lesser extent, in December). The reverse was true in the 2006

SHWT. Since ammonia is an indicator of recent nitrogen contributions, septic tanks could be a potential source. However, if this was the case, the upstream site would tend to be closer to the background concentrations. Since this was not observed, the ammonia increase may be an indication of the application of fertilizers from lawns, as opposed to septic tanks. Since northern Florida is more influenced by frontal systems that tend to carry more rainfall in the spring months compared to South Florida, and since the first fertilizer applications are generally done in the spring, this is a potential source and would explain why the issue does not arise in December when fertilizers are generally not applied. This is further supported by looking at the potable water usage statistics in the area, which indicate that May is the highest usage month on average (Figure 5). It is likely that much of this additional usage is attributable to irrigation, which would result in increased runoff of ammonia-based fertilizers. A more representative background site location might resolve this issue in follow-up testing.

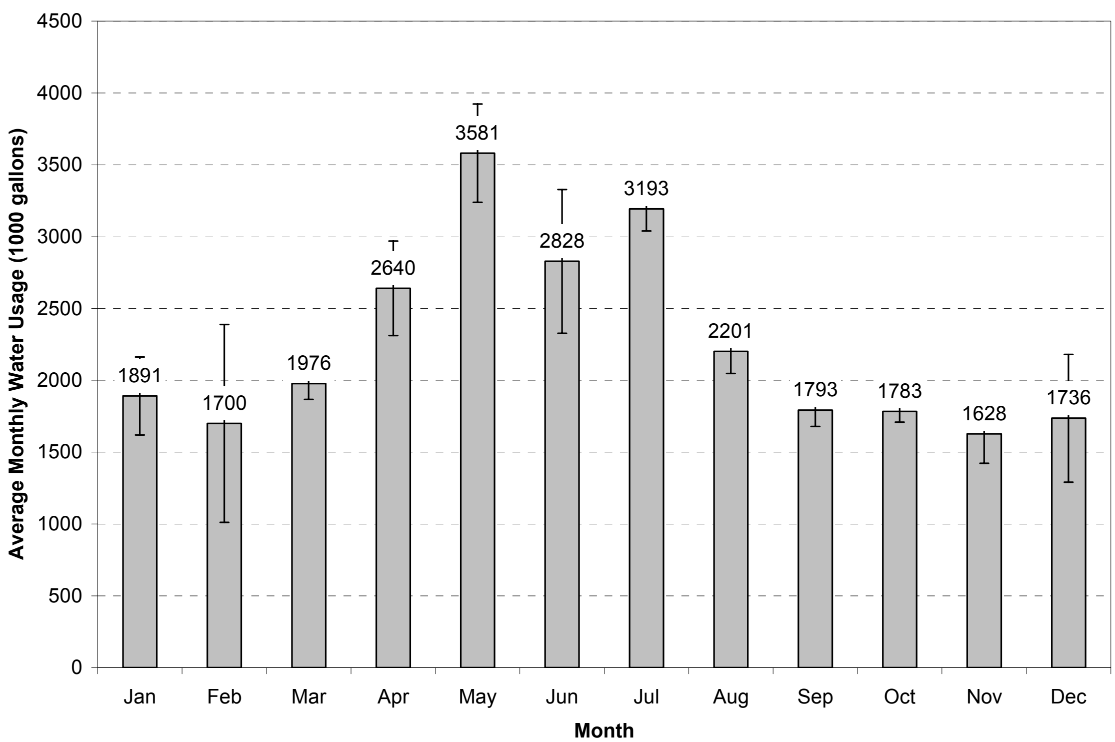


Figure 5 - Monthly water usage in Taylor County over the period including 2000 to 2004.

In 2007, follow-up testing revealed that the ammonia levels were all below 0.14 mg N/L, which is considerably lower than the ammonia levels recorded in 2006, but still double the value considered high in shallow coastal waters (0.07 mg N/L, Zhang 2006). As in 2007, the trend was for the ammonia to increase in the upstream direction. This trend also held true for both seasons, unlike in 2006. The Jugg Island Road site (JI) shows a concentration gradient from upstream to the beach. This site is located downstream of the Dekle Beach site (A) and also downstream of the background site (C). It is a possibility that the ammonia is of terrestrial origin (fertilization practices) from the Dekle Beach community; however, at the JI site, dogs were also observed on all sampling dates in 2007.

Nitrate concentrations were below the detection limit at all sites within the Dekle Beach area during the 2006 SLWT, and although most of the samples were again below the detection limit during the 2006 SHWT, the background site presented a detectable nitrate concentration for all three days. In 2007, the nitrate levels were again very low; however, in the 2007 SHWT, the background site (C) recorded nitrate values that were 2-3 times higher than the previous year. In addition, one (September 19, 2007) of the three days at the Dekle Beach site (A) showed a value of 0.05 mg N/L for nitrate. This nitrate spike was not repeated on either of the other two sampling days at this site.

Figure 6 shows that total nitrogen concentrations were relatively constant throughout the site, regardless of the season for both years. The TN results for the May SLWT events are similar in 2006 and 2007. No data is available for the 2007 SHWT due to an equipment malfunction. This should be re-sampled in the future. TOC (Figure 7) also appeared to have been generally constant across all sampling areas during the 2006 SLWT event periods and the 2006 SHWT events, although the SHWT events had higher concentrations when compared to the SLWT samples, and the December 2006 SLWT values were slightly lower than the May 2006 SLWT events. The May 2007 SLWT results were slightly lower than the December 2006 results. SHWT values were much lower than the 2006 SHWT results. The reasons are unclear, and additional sampling is recommended. Note that all figures for each parameter have been set with the same y-axis range to facilitate comparisons across the four different sampling locations (Dekle Beach, Keaton Beach, Cedar Island, and Steinhatchee).

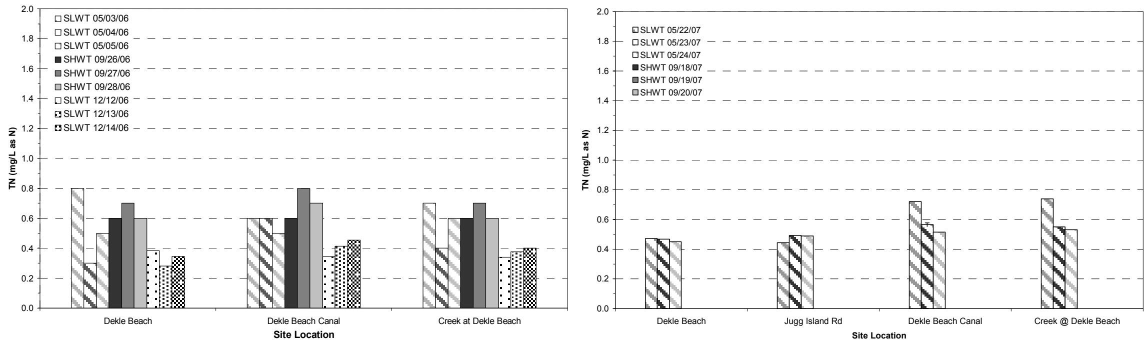


Figure 6 – **Results for Total Nitrogen at Dekle Beach for 2006 (left) and 2007 (right).**

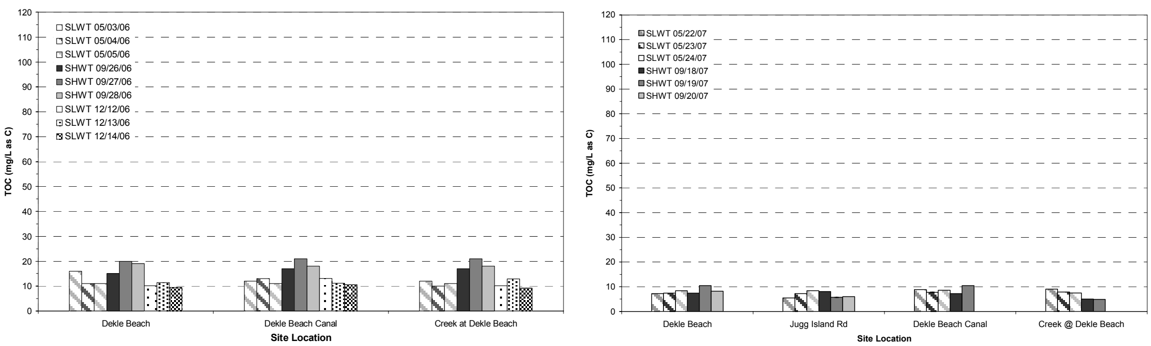


Figure 7 – **Results for TOC at Dekle Beach for 2006 (left) and 2007 (right).**

Table 7 shows the microbial constituents analyzed for the Dekle Beach community in 2006, and Table 8 summarizes the same parameters for 2007. In terms of microbial water quality, concentrations of *Enterococcus*, *E. coli* and total coliforms were compared. *Enterococcus* concentrations were generally lower at the beach than the upstream and background sampling points.

Table 7 – Dekle Beach turbidity and bacterial results for 2006.

Site	Charac.	Parameter	SLWT (May 2006)			SHWT (Sept 2006)			SLWT (Dec 2006)		
			Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
A	Beach	Turbidity	0.8	3.9	0.9	3.5	2.5	2.3	0.4	0.7	0.5
B	Upstream	(NTU)	1.4	1.7	1.1	1.4	1.4	1.2	0.7	1.1	0.7
C	Background		1.8	2.7	2.6	2.5	1.3	1.5	0.4	0.7	0.8
A	Beach	*Enterococcus*	20	<10	<10	52	<10	10	<10	<10	<10
B	Upstream	(MPN/100 mL)	85	**122**	74	**110**	31	**185**	20	20	20
C	Background		75	31	10	**220**	63	20	10	10	10
A	Beach	*E. coli*	**484**	278	390	**620**	**1890**	**1730**	63	98	91
B	Upstream	(MPN/100 mL)	**693**	**1040**	**698**	**808**	**2400**	**8160**	285	187	250
C	Background		**1300**	**1540**	**815**	**1510**	**1550**	**2250**	185	183	97
A	Beach	Total Coliforms	8660	6290	5100	4790	24200	>24200	1178	1000	751
B	Upstream	(MPN/100 mL)	4200	5170	12000	19900	15500	17300	3538	1815	3325
C	Background		6870	5490	12000	13000	17300	>24200	3945	2318	1532
A	Beach		24	56	78	12	378	173	13	20	19
B	Upstream	Ec/Es Ratio	8	9	9	7	77	44	14	9	15
C	Background		17	50	82	7	25	113	19	18	10

Note: Values in **bold** indicate violations of the trigger level

Table 8 – Dekle Beach turbidity and bacterial results for 2007.

Site	Charac.	Parameter	SLWT (May 2007)			SHWT (Sept 2007)		
			Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
A	Beach	Turbidity (NTU)	0.9	1.2	1.5	1.4	1.1	1.3
JI	Beach		1.6	1.9	1.8	5.5	1.9	1.3
B	Upstream		2.3	2.3 ± 0.2	1.8	1.6	1.5	1.8
C	Background		2.1	2.3	2.3	0.7	1.8	1.2
A	Beach	*Enterococcus* (MPN/100 mL)	<10	10	<10	20	<10	10
JI	Beach		<10	<10	10	10	<10	**318**
B	Upstream		<10	15 ± 11	10	63	**213**	**201**
C	Background		31	<10	30	52	20	**169**
A	Beach	*E. coli* (MPN/100 mL)	130	30	71	**24196**	**528**	**6294**
JI	Beach		152	40	97	**17329**	**1510**	169
B	Upstream		162	51 ± 22	70	**1892**	**3968**	**5475**
C	Background		132	82	112	**1993**	**2367**	**15531**
A	Beach	Total coliforms (MPN/100 mL)	12997	19863	9804	>24196	11199	12033
JI	Beach		9804	24196	17329	>24196	9804	5172
B	Upstream		2046	10919	9208	19863	8164	8664
C	Background		12997	19863	14136	24196	7215	>24196
A	Beach	Ec/Es Ratio	26	3	14	1210	106	629
JI	Beach		30	8	10	1733	302	1
B	Upstream		32	4	7	30	19	27
C	Background		4	16	4	38	118	92

Note: Values in **bold** indicate violations of the trigger level

Figure 8 shows that the *Enterococcus* concentrations were below the trigger level (100 MPN/100mL) on the beach for both 2006 and 2007. However, upstream of the beach, the canal site showed concentrations near the trigger level (3 of 6 were above), while further upstream, the quantities diminished below the trigger level except for one sample in the 2006 SHWT and another one in 2007 SHWT. The higher concentrations observed in the upstream site both years were possibly due to fresh input from residential septic tanks, influenced by the timing of the sampling (early morning peak residential flow). Sampling during the December 2006 SLWT with cooler water temperatures showed minimal amounts of *Enterococcus*.

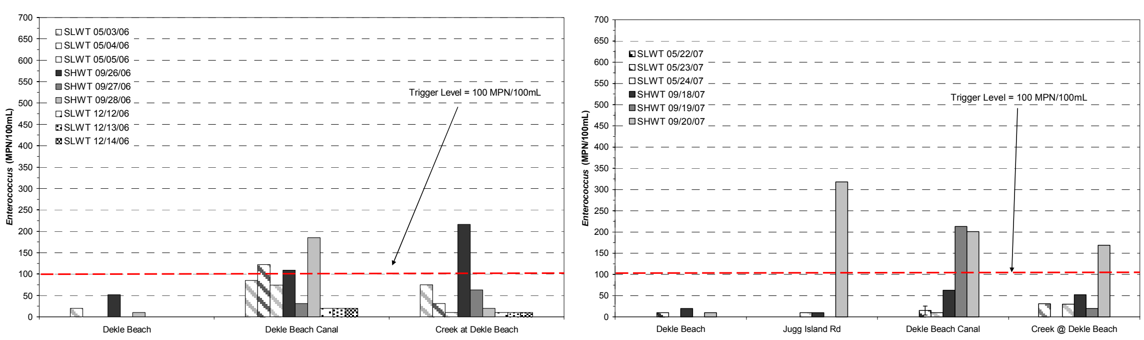


Figure 8 – Results for *Enterococcus* at Dekle Beach for 2006 (left) and 2007 (right).

E. coli densities at the Dekle Beach area were generally higher during the SHWT in both years compared to the same site at SLWT. The SHWT values also consistently violated the trigger level of 400 MPN/100 mL (see Figure 9). The highest value in 2006 (8160 MPN/100mL) was encountered at the upstream site (B), which was expected due to its relative proximity to active septic tanks and less influence from marine dilution. In 2007, the highest value recorded (>24196 MPN/100 mL) was at the beach itself (A). The 2007 SHWT *E. coli* samples were extremely high ($>10^5$/100 mL). In fact, three of the samples taken here were measured beyond the scale of the figure at well above 15,000 MPN/100 mL. Although this was unexpected, the Taylor County Health Department weekly beach sampling program had recorded excessively high values in the preceding weeks as well. The cooler temperatures characteristic of the December 2006 sampling (SLWT) resulted in minimal amounts of measurable *E. coli.*

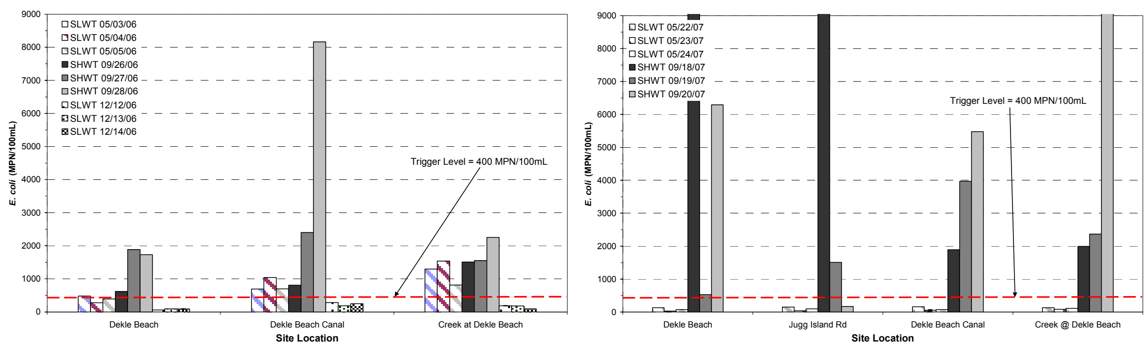


Figure 9 – Results for *E. coli* at Dekle Beach for 2006 (left) and 2007 (right).

Except for the last sampling day in 2007 SHWT at the new Jugg Island Road site (JI), the *E.coli/Enterococcus* ratios were higher than 4.0, suggesting a human source of pollution. The one exception at site JI (Ec/Es = 0.5) is likely due to recent dog inputs. Turbidity was also monitored for the microbial samples since high turbidity may indicate surface runoff or wind-driven mixing of sediments into the water column. This mixing could contribute a legacy source of pathogens protected from the elements within the shallow sediment. This would appear as a higher local concentration of pathogen indicators coming from the release of cells from the sediment floc, if bacterial regrowth is in fact occurring. Since this effect would be wind-driven, background sites would have similar concentrations to upstream sites during outgoing tides, which was observed for the Creek at Dekle and Dekle Beach Canal sites with similar microbial indicators counts. Potential bacterial regrowth will be discussed in more detail later. Overall, turbidity values were generally low, although the SHWT event showed slightly higher levels. None of these samples was overly turbid.

Steinhatchee

The Steinhatchee location was representative of a higher density, developed area served exclusively by OSTDS. The sites sampled were mostly freshwater as opposed to marine or brackish water from the other beach monitoring locations. Table 9 summarizes nutrient values for the Steinhatchee sites in 2006, and Table 10 summarizes the readings for 2007 sampling. In terms of nutrients, analyses were conducted to determine the levels of ammonia, nitrate, total nitrogen, and total organic carbon at the 5 sites. The DO levels were in the range of 1.1 – 9.7 mg/L (Table 9 and Table 10). With the exception of Site J (Main Street-Roy's), during the 2006 SHWT, all the sites consistently violated the Class III criterion for DO (>5.0 mg/L for freshwater). This phenomenon was not repeated in 2007, where no violations were recorded for DO.

Table 9 – Steinhatchee nutrients results for 2006.

Site	Charac.	Parameter	SLWT (May 2006)			SHWT (Sept 2006)			SLWT (Dec 2006)		
			Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
J	Downstream	DO (mg/L)	5.4	5.6	5.1	**4.6**	5.2	5.4	9.7	8.0	7.9
K	Upstream		5.8	6.6	5.8	**3.3**	**1.7**	**1.6**	5.7	**4.5**	5.3
L	Tributary		5.3	6.9	6.1	**3.3**	**2.4**	**2.4**	**1.6**	**0.5**	**3.3**
M	Upstream		6.5	6.6	6.4	**2.7**	**2.6**	**2.8**	**3.3**	**2.6**	**1.4**
N	Background		6.9	6.6	6.5	**1.1**	**1.4**	**1.4**	**2.2**	**2.0**	**1.8**
J	Downstream	NH_4^+ (mg/L as N)	0.23	<0.1	<0.1	0.13	0.02	0.02	0.04	0.04	0.05
K	Upstream		0.23	0.14	0.24	0.01	0.04	<0.010	0.06	0.05	0.05
L	Tributary		0.15	0.15	0.10	0.03	<0.010	0.05	0.03	0.03	0.05
M	Upstream		0.63	0.69	0.51	<0.010	<0.010	<0.010	0.05	0.09	0.10
N	Background		0.24	0.13	<0.1	<0.010	<0.010	<0.010	0.13	0.13	0.13
J	Downstream	NO_3^- (mg/L as N)	<0.011	<0.011	<0.011	0.02	0.02	0.01	0.015	0.025	0.028
K	Upstream		0.04	<0.011	0.03	0.01	0.04	<0.0052	0.129	0.060	0.107
L	Tributary		0.03	0.01	0.01	0.04	0.05	0.05	0.008	0.011	0.005
M	Upstream		<0.011	<0.011	<0.011	0.02	<0.0052	0.02	0.018	0.029	0.024
N	Background		0.02	<0.011	<0.011	0.01	<0.0052	<0.0052	or	or	0.012
J	Downstream	TN (mg/L as N)	0.9	0.4	0.4	0.5	0.4	0.4	0.2	0.4	0.3
K	Upstream		0.1	0.3	0.3	0.2	0.4	0.3	0.3	0.4	0.3
L	Tributary		0.5	0.3	0.4	1.1	1.0	0.8	0.4	0.5	0.6
M	Upstream		1.8	0.8	1.0	0.2	0.3	0.3	0.4	0.3	0.4
N	Background		0.6	0.3	0.3	0.2	0.4	0.3	0.3	0.3	0.3
J	Downstream	TOC (mg/L as C)	10	12	10	18	16	16	7.6	7.6	7.5
K	Upstream		4.0	8.0	8.0	10	16	13	11	11	7.4
L	Tributary		14	13	15	72	79	91	37	33	38
M	Upstream		20	16	13	19	21	21	15	17	18
N	Background		16	17	11	18	20	20	19	5.8	19

Note: Values in **bold** indicate violations of the trigger level
or = out of range

Table 10 – Steinhatchee nutrients results for 2007.

Site	Charac.	Parameter	SLWT (May 2007)			SHWT (Sept 2007)		
			Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
J	Downstream		5.7	5.5	6.0	5.6	6.5	5.9
K	Upstream	DO	6.2	6.0	6.8	6.8	6.5	7.5
L	Tributary	(mg/L)	8.6	8.2	9.1	9.5	9.5 ± 0.2	9.5
M	Upstream		8.1	7.8	8.7	8.9	9.0	9.2
N	Background		8.5	8.3	9.5	9.2	9.2	9.3
J	Downstream		0.03	0.04	0.03	0.04	0.02	0.06
K	Upstream	NH_4^+	0.04	0.04	0.04	0.05	0.05	0.08
L	Tributary	(mg/L as N)	0.09	0.07	0.07	0.02	0.02	0.05
M	Upstream		U	0.01	0.01	0.03	0.03	0.04
N	Background		0.04	0.08	0.08	0.04	0.04	0.06
J	Downstream		0.01	0.01	0.01	0.02	0.13	0.02
K	Upstream	NO_3^-	0.05	0.06	0.05	0.06	0.06	0.08
L	Tributary	(mg/L as N)	0.01	0.02	0.02	0.04	0.05	0.09 ± 0.01
M	Upstream		0.01	0.01	0.02	0.04	0.04	0.06
N	Background		0.01	0.00	0.01	0.02	0.03	0.03
J	Downstream		0.6	0.4	0.4	nr	nr	nr
K	Upstream	TN	0.7	0.4	0.4	nr	nr	nr
L	Tributary	(mg/L as N)	0.7	0.7	0.6	nr	nr	nr
M	Upstream		0.4	0.4	0.3	nr	nr	nr
N	Background		0.4	0.3	0.4	nr	nr	nr
J	Downstream		9	6	4	nr	nr	nr
K	Upstream	TOC	8	6	6	8	30	3
L	Tributary	(mg/L as C)	14	16	12	31	34	43
M	Upstream		9	11	11	10 ± 2	8 ± 0.5	9
N	Background		10	8	11	10	9	9

Note: Values in **bold** indicate violations of the trigger level; U = below detection

Figure 10 shows that during the May 2006 event (SLWT), the ammonia was higher at all sites when compared with the September 2006 SHWT. Ammonia concentrations in December 2006 (SLWT) were higher than September 2006 (SHWT), but about half of the values measured in May 2006 (SLWT). The ammonia concentration tended to increase as the sampling sites moved upstream in both SLWTs, with the Boggy Creek site of particular significance in May 2006. This appears to have changed in the later sampling events. Since ammonia is an indicator of recent nitrogen contributions, septic tanks could be a source. However, the upstream site would tend to be more similar to the background levels. In 2007, all sites for both seasons were below 0.1 mg N/L for ammonia. The unexpectedly low ammonia results in 2007 made it difficult to interpret nitrogen isotope ratios collected from the Steinhatchee sites. This will be discussed in more detail later.

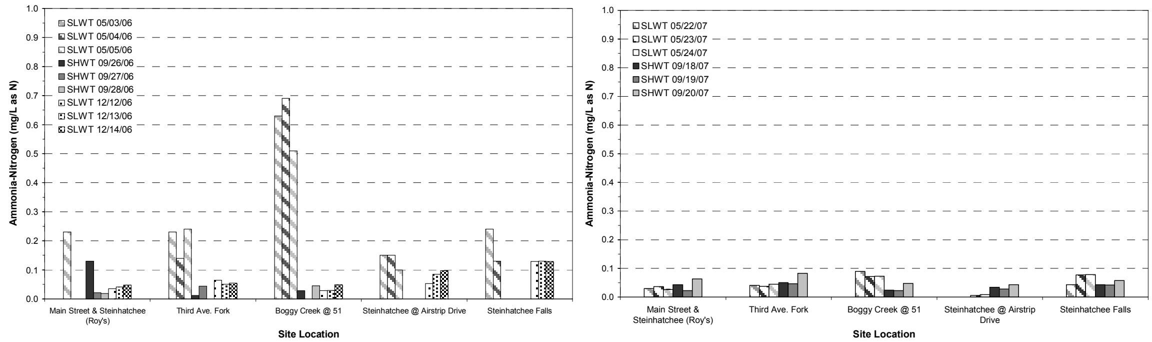


Figure 10 – Results for Ammonia at Steinhatchee for 2006 (left) and 2007 (right).

Nitrate concentrations were below the detection limit at all sites within the Steinhatchee area during the 2006 SLWT (May 2006). During the 2006 SHWT (September 2006), although most of the samples were again below the detection limit, the background site presented a detectable nitrate concentration for all three days. In 2006, nitrate values were highest in December (2006 SLWT), but only for Site K. Two samples in December 2006 (Site N, days 1-2) were "over range" and could not be re-analyzed due to insufficient sample. In 2007, the SHWT (September) sampling event nitrate levels were generally higher than those recorded in SLWT (May), with some samples at or above 0.1 mg N/L of nitrate-nitrogen.

Figure 11 shows that total nitrogen concentrations were relatively constant throughout Steinhatchee, except at the Boggy Creek site, regardless of the season or year. In 2006, the Boggy Creek site (L) shows generally higher levels of TN in May 2006 and September 2006, but thereafter, the levels return to the average baseline seen at the other Steinhatchee sites. Figure 12 shows that TOC was generally constant across all site locations during the SLWT and SHWT events, except for the SHWT (September).

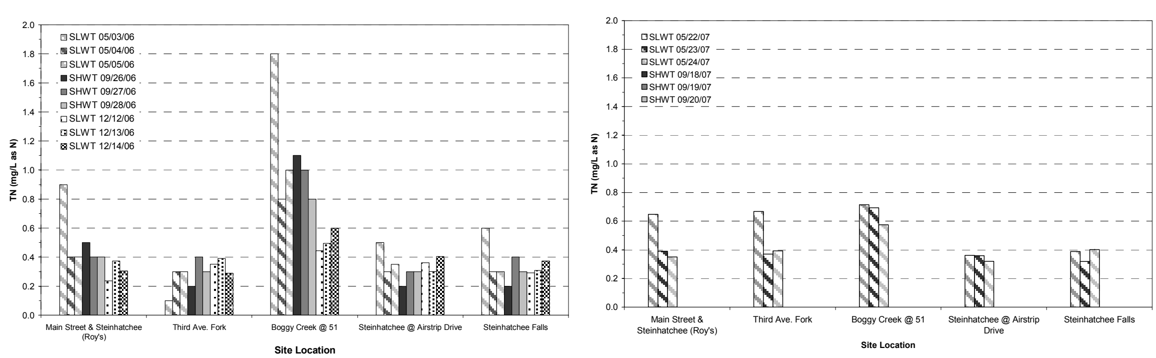


Figure 11 – Results for Total Nitrogen at Steinhatchee for 2006 (left) and 2007 (right).

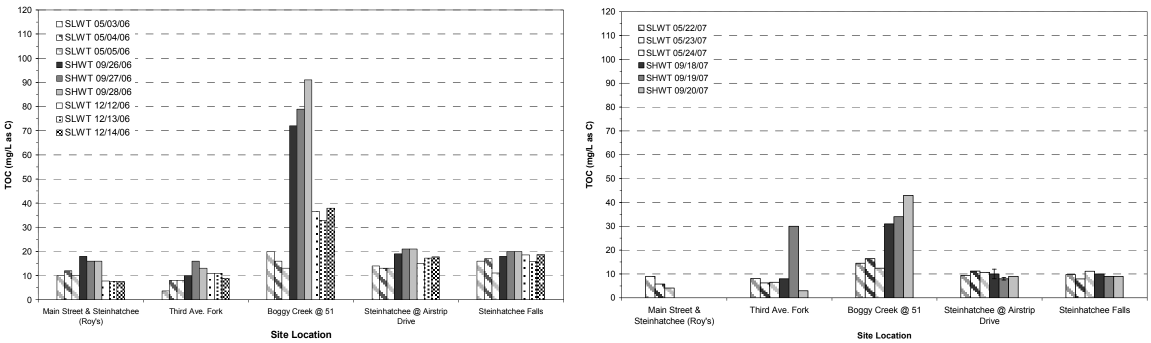


Figure 12 – Results for TOC at Steinhatchee for 2006 (left) and 2007 (right).

All Steinhatchee sampling sites were similar except for the Boggy Creek site (L) September 2006 SHWT that was 4 times higher than the values of the other four sites or compared to the May 2006 SLWT event and double for the December 2006 SLWT event. Boggy Creek has no known point sources with NPDES (treated sanitary sewer) or MS4 (municipal separate storm sewer for populations >50,000) permits. Agricultural animal input is not considered significant in this watershed (USEPA 2003). The undeveloped portion of the watershed comprises greater than 99% of the total area. Thus OSTDS, stormwater runoff, and domestic animal inputs from urban development are not expected to be important. Something else occurs here that deserves further investigation. However, the higher TOC levels at Boggy Creek observed in 2006 were not repeated during the May 2007 SLWT.

According to the Florida Department of Environmental Protection (FDEP) *Basin Status Report for the Suwannee Basin*, part of the Steinhatchee River watershed (more specifically, the Boggy Creek drainage basin) is 98 percent pine flatwoods and wetlands, most of which are used for commercial timber production (FDEP 2001). Thus there is a potential for "agricultural" inputs from tree farming operations. During the three sampling events, evidence of recent human activity was also noted at the Boggy Creek site. In particular, recently deposited litter, fresh tire tracks in the mud, and dead animal carcasses (wild boars). In May 2006, the site showed indications of recent boating/fishing activity and a fresh hydrocarbon sheen was noted streaking across the water surface near the sampling site. The samples collected were characterized by a deep reddish-brown color indicative of humic and fulvic acids and a large amount of decaying vegetation. The reddish-brown water sampled at Boggy Creek indicative of decaying vegetation was reminiscent of the highly colored water collected from the Fenholloway River. This coloration could simply be tannic constituents from the upstream pines and timber activity. In September 2006, a hydrocarbon sheen similar to those recorded in May 2006 was also observed, and in December 2006 the turbidity increased noticeably. On the last sampling day of December 2006, several gun shots were heard in the nearby wetlands just north of the sampling site, presumably from hunters in the area.

Table 11 shows the results for microbial constituents in 2006, and Table 12 summarizes the same parameters in 2007. In terms of microbial water quality, concentrations of *Enterococcus*, *E. coli* and total coliforms were compared. *Enterococcus* concentrations were lower at the beach than the upstream and background sampling points. For 2006, Figure 13 shows that the *Enterococcus* concentrations were generally below the trigger level (100 MPN/100mL) at the rivermouth (5 of 6). However, in the upstream direction, the Third Street Fork site (Site K) showed concentrations above the trigger level approximately 50 percent of the time in both 2006 and 2007. The Boggy Creek site (Site L) showed concentrations above the trigger level most of the time (5 of 6) in the September 2006 (SHWT) and December 2006 (SLWT) sampling events. This trend continued in 2007 SHWT with three out of three days showing violations, and those counts were even higher than those measured in 2006. Further upstream, the quantities diminished below the trigger level in both years except for one sample in the 2006 SHWT (September) at Steinhatchee Falls. The higher concentrations in the upstream site (Site K, Third Avenue Fork) may have been due to fresh input from residential septic tanks.

Table 11 – Steinhatchee turbidity and bacterial results for 2006.

Site	Charac.	Parameter	SLWT (May 2006)			SHWT (Sept 2006)			SLWT (Dec 2006)		
			Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
J	Downstream	Turbidity (NTU)	0.6	1.8	1.1	2.7	1.3	1.2	0.4	0.4	0.4
K	Upstream		1.4	1.6	0.4	1.0	1.0	0.6	1.0	0.7	0.4
L	Tributary		1.3	2.1	1.4	1.0	1.3	1.5	17.8	12.7	0.6
M	Upstream		6.4	8.1	7.4	1.6	1.3	1.5	2.4	1.9	11.8
N	Background		1.7	2.8	1.8	1.9	1.5	1.5	8.7	2.8	2.3
J	Downstream	*Enterococcus* (MPN/100 mL)	<10	52	40	**183**	20	<10	20	31	20
K	Upstream		41	85	**260**	**307**	**169**	41	41	63	ns
L	Tributary		52	15	20	**388**	**120**	**262**	**359**	**235**	31
M	Upstream		10	61	74	52	20	10	10	20	10
N	Background		<10	41	10	41	**146**	84	31	31	20
J	Downstream	*E. coli* (MPN/100 mL)	199	**3280**	**528**	**496**	**411**	241	52	63	97
K	Upstream		30	75	74	324	160	41	75	146	ns
L	Tributary		10	10	**411**	383	109	132	197	341	85
M	Upstream		<10	10	<10	41	20	20	63	26	21
N	Background		20	<10	<10	108	30	10	10	10	10
J	Downstream	Total coliforms (MPN/100 mL)	5170	12000	6870	17300	10500	9210	1515	2878	2851
K	Upstream		10500	15500	11200	10500	9210	8160	14136	15531	ns
L	Tributary		10500	14100	15500	11200	17300	13000	17329	6294	6867
M	Upstream		1330	906	985	3650	2380	2490	1202	1001	639
N	Background		959	1270	1220	4350	2050	3870	>24196	272	471
J	Downstream	Ec/Es Ratio	NA	63.1	13.2	2.7	20.6	NA	2.6	2.0	4.9
K	Upstream		0.7	0.9	0.3	1.1	0.9	1.0	1.8	2.3	ns
L	Tributary		0.2	0.7	20.6	1.0	0.9	0.5	0.5	1.5	2.7
M	Upstream		NA	0.2	NA	0.8	1.0	2.0	3.7	1.3	2.1
N	Background		NA	NA	NA	2.6	0.2	0.1	0.3	0.3	0.5

Note: Values in **bold** indicate violations of the trigger level

Table 12 – Steinhatchee turbidity and bacterial results in 2007.

Site	Charac.	Parameter	SLWT (May 2007)			SHWT (Sept 2007)		
			Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
J	Downstream	Turbidity (NTU)	3.3	1.5	1.6	0.6	1.2	1.7
K	Upstream		1.0	1.5	0.8	0.6	1.1	1.1
L	Tributary		7.3	8.2	9.7	0.8	1.8	1.7
M	Upstream		1.4	2.2	1.7	1.2	2.0	1.9
N	Background		3.1	3.5	3.0	1.2	1.8	1.7
J	Downstream	*Enterococcus* (MPN/100 mL)	<10	52	<10	10	<10	10
K	Upstream		**146**	**132**	97	63	**121**	86
L	Tributary		52	86	42 ± 15	**657**	**345 ± 199**	**233**
M	Upstream		<10	30 ± 18	<10	63	52	30
N	Background		<10	<10	20	41	10	52
J	Downstream	*E. coli* (MPN/100 mL)	80	61	109	**488**	284	**5794**
K	Upstream		50	20	63	148	355	107
L	Tributary		<10	<10	41 ± 14	96	233 ± 79	135
M	Upstream		10	10	<10	<10	15 ± 7	10
N	Background		<10	<10	<10	20	41	10
J	Downstream	Total coliforms (MPN/100 mL)	8164	9804	3873	8164	5475	7270
K	Upstream		19863	6488	17329	10462	24196	11199
L	Tributary		<10	14136	17697	6488	6867	5794
M	Upstream		1376	5830	1421	2909	8960	1860
N	Background		789	432	663	2755	1333	1515
J	Downstream	Ec/Es Ratio	16	1.2	22	49	57	579
K	Upstream		0.3	0.2	0.6	2.3	2.9	1.2
L	Tributary		0.1	0.1	1.0	0.1	0.7	0.6
M	Upstream		2.0	1.2	1.0	0.1	0.3	0.3
N	Background		1.0	1.0	0.3	0.5	4.1	0.2

Note: Values in **bold** indicate violations of the trigger level

E. coli densities for the Steinhatchee area were similar during all sampling events, with the exception of the SLWT (May, Day 2), when the value measured at the mouth of the Steinhatchee River (Site J) violated the trigger level of 400 MPN/100 mL by a factor of 8 (see Figure 14). A similar phenomenon was observed in 2007, but this time the violation occurred in SHWT (September). These two isolated events could have occurred shortly after a large release of waste from the septic tank or might be attributable to the restaurant workers dumping spent wash water from mop buckets as witnessed once in 2006. The *E.coli/Enterococcus* ratios were generally less than 4, suggesting a non-human source of pollution. In 2007, only the rivermouth shows consistent signs of human contribution based on the EC/Es ratio (5 of 6). Turbidity was monitored for the microbial samples, since high turbidity may indicate terrestrial runoff or wind-driven mixing of the overlying water column with the sediments. However, none of these samples was overly turbid, although the December 2006 Boggy Creek values were elevated compared to the other two sampling events that year. Samples collected in 2007 during May and September showed similarly low turbidity as expected from the 2006 results.

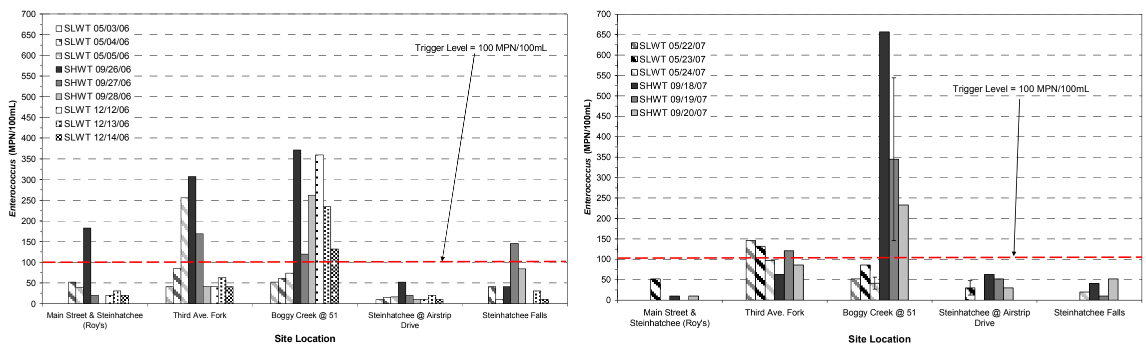


Figure 13 – Results for *Enterococcus* at Steinhatchee for 2006 (left) and 2007 (right).

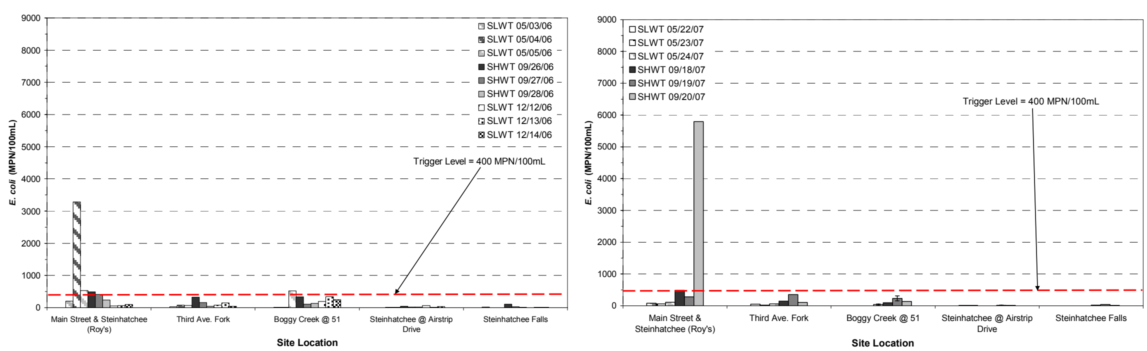


Figure 14 – Results for *E. coli* at Steinhatchee for 2006 (left) and 2007 (right).

One interesting observation from the Boggy Creek site (L) in September 2007 was that on the second sampling day, the field team returned to take a second sample in the afternoon to collect water for the molecular techniques assay. In the early morning sample collected at 7:45 AM just after local high tide, the *E. coli* and total coliform counts were about double compared to those taken in the afternoon at 4:15 PM just after local low tide. The *Enterococcus* counts were almost five times higher. Since the Boggy Creek tributary is too far upstream to show a tidal effect, we believe that this may be attributed to die-off associated with exposure to direct sunlight.

DEVELOPED WITH SEWER RECENTLY INSTALLED

Keaton Beach

The Keaton Beach location was representative of a medium-density, developed area with a sewer system recently installed. The DO values were in a broad range (1.0 – 10.5 mg/L), with generally lower values decreasing from the beach in the upstream direction. The pump station site (Site D) violated the Class III criterion for DO (<5.0 mg/L for freshwater) for all 3 sampling days during both SHWT events in 2006 and 2007. The DO levels for the SLWT (May 2006, 2007 and December 2006) were well above the criterion for both marine and freshwater for all Keaton Beach sampling sites. Near saturation values were encountered during the cooler December 2006 sampling event. Table 13 summarizes the nutrient data for Keaton Beach in 2006, and Table 14 lists the results from 2007. In terms of nutrients, an analysis was conducted to determine the levels of ammonia, nitrate, total nitrogen, and total organic carbon.

Table 13 – Keaton Beach nutrients results for 2006.

			SLWT (May 2006)			SHWT (Sept 2006)			SLWT (Dec 2006)		
Site	Charac.	Parameter	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
F	Beach	DO	6.6	6.1	5.3	4.8	5.2	5.6	10.0	6.3	9.3
E	Midstream	(mg/L)	6.2	5.9	5.4	4.5	4.7	4.9	8.1	8.4	6.2
D	Upstream		5.9	5.7	5.1	**2.6**	**1.7**	**1.9**	10.3	6.6	10.5
G	Background		6.8	6.8	5.7	**4.9**	**4.9**	5.0	8.0	8.9	6.7
F	Beach	NH_4^+	0.28	0.15	0.10	0.08	0.11	0.07	0.01	0.11	0.01
E	Midstream	(mg/L as N)	0.50	0.20	<0.1	0.04	0.06	0.06	0.11	0.04	0.08
D	Upstream		0.44	0.65	<0.1	0.06	<0.010	0.1	0.03	0.12	0.03
G	Background		0.18	<0.1	<0.1	0.03	<0.010	<0.010	0.12	0.01	0.13
F	Beach	NO_3^-	<0.011	<0.011	<0.011	<0.0052	<0.0052	0.02	0.003	0.020	0.004
E	Midstream	(mg/L as N)	<0.011	<0.011	<0.011	0.02	0.02	0.02	0.014	0.010	0.014
D	Upstream		<0.011	<0.011	<0.011	<0.0052	<0.0052	<0.0052	0.004	0.068	0.005
G	Background		0.04	0.05	0.05	<0.0052	<0.0052	0.01	0.050	0.005	0.063
F	Beach	TN	0.3	0.3	0.5	0.5	0.5	0.5	0.3	0.4	0.2
E	Midstream	(mg/L as N)	0.3	0.5	0.4	0.5	0.6	0.9	0.4	0.6	0.3
D	Upstream		0.8	0.7	0.7	0.7	0.7	0.7	0.5	0.4	0.6
G	Background		0.4	0.5	0.4	1	1.1	1	0.3	0.3	0.4
F	Beach	TOC	7	9	10	14	14	14	7	8	5
E	Midstream	(mg/L as C)	7	12	9	18	19	20	11	21	7
D	Upstream		14	22	18	66	69	67	21	15	20
G	Background		5	8	7	72	104	67	12	7	11

Note: Values in **bold** indicate violations of the trigger level

Table 14 – Keaton Beach nutrients results for 2007.

Site	Charac.	Parameter	SLWT (May 2007)			SHWT (Sept 2007)		
			Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
F	Beach	DO (mg/L)	6.5	6.0	6.5	5.0	4.2	**3.6**
MR	Beach		6.3	6.1	6.4	4.5	4.3	5.0
E	Midstream		6.1	5.9	6.3	**3.3**	**3.7**	4.1
D	Upstream		6.1	5.8	6.5	**1.0**	**1.7**	**2.4**
G	Background		8.4	7.9	8.6	5.8	5.9	6.3 ± 0.1
F	Beach	NH_4^+ (mg/L as N)	0.00	0.01	0.00	0.01	0.01	0.02
MR	Beach		0.04	0.05	0.00	0.04	0.04 ± 0.01	0.05
E	Midstream		0.00	0.00	0.00	0.04	0.04	0.11
D	Upstream		0.00	0.00	0.00	0.09	0.08	0.07
G	Background		0.08	0.09	0.08	0.09	0.09	0.10
F	Beach	NO_3^- (mg/L as N)	0.01	0.00	0.01	0.00	0.00	0.00
MR	Beach		0.01	0.01	0.00	0.01	0.01	0.01
E	Midstream		0.00	0.00	0.00	0.01	0.01	0.01
D	Upstream		0.01	0.00	0.01	0.00	0.00	0.01
G	Background		0.02	0.10	0.08	0.06	0.08	0.09
F	Beach	TN (mg/L as N)	0.6	0.3	0.4	nr	nr	nr
MR	Beach		0.6	0.5	0.4	nr	nr	nr
E	Midstream		0.7	0.5	0.5	nr	nr	nr
D	Upstream		1.0	0.7	0.6	nr	nr	nr
G	Background		0.5	0.4	0.4	nr	nr	nr
F	Beach	TOC (mg/L as C)	7.2	5.6	4.7	6.5	6.9	6.2
MR	Beach		6.7	6.4	5.2	5.3	4.2	4.9
E	Midstream		8.0	7.8	6.6	nr	nr	nr
D	Upstream		15	15	10	66	76	82
G	Background		4.9	6.2	4.3 ± 0.6	5.4	4.8	4.8 ± 0.6

Note: Values in **bold** indicate violations of the trigger level

Figure 15 shows that during the May 2006 event (SLWT), the ammonia concentrations were generally higher when compared to the September 2006 event (SHWT) and the December 2006 event (SLWT). Ammonia levels tended to increase as the sampling sites moved upstream in the 2006 SLWT, similar to Dekle Beach, however with generally lower concentrations. In 2007, the generally increasing trend in the upstream direction is repeated but with lower overall concentrations of ammonia. No spikes above 0.1 mg N/L were observed in either season in 2007. Since ammonia is an indicator of recent nitrogen contributions and the area was recently converted to sewer, the results suggest the application of fertilizers from lawns. In December 2006, the Cortez Road and Blue Creek sites showed higher ammonia than in the SHWT event in May 2006. The data is inconclusive for this event.

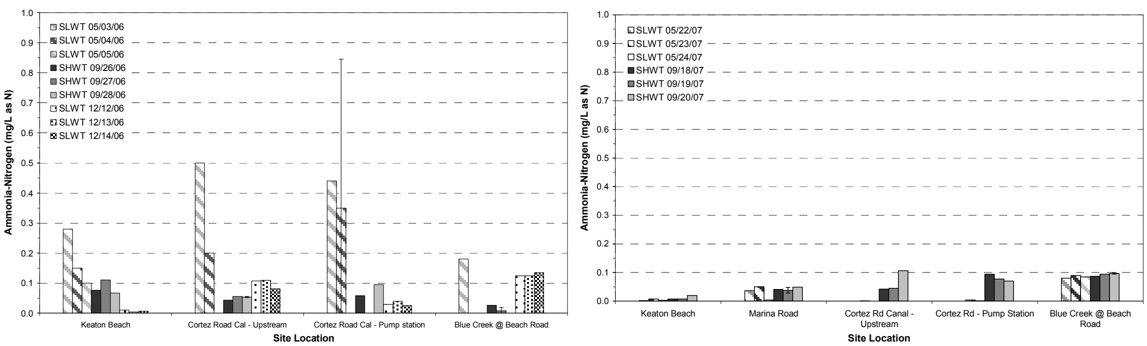


Figure 15 – Results for Ammonia at Keaton Beach for 2006 (left) and 2007 (right).

Nitrate concentrations were at or below the detection limit at all sites within the Keaton Beach area during the 2006 and 2007 SLWT events. Although most of the samples were low, the background site presented a detectable nitrate concentration for all three days in all three SLWT events, generally one order of magnitude higher than the other sites downstream. During the 2006 SHWT, nitrate levels were comparatively very low, with most values below detection. In 2007, the only site within Keaton Beach with high nitrate levels was the background site at Blue Creek (G), which was greater than 0.07 mg N/L on 5 of the 6 sampling days.

Figure 16 shows that total nitrogen concentrations generally increased going upstream, and that the 2006 SHWT results were generally higher than the 2006 SLWT event in 2 of 4 instances. The reasons for this are not clear when looking at the TN data. It is apparent from nitrogen speciation (i.e. ammonia, nitrate, nitrite, and total nitrogen) that most of the nitrogen detected is of organic origin. The 2007 levels are similar to those seen in 2006.

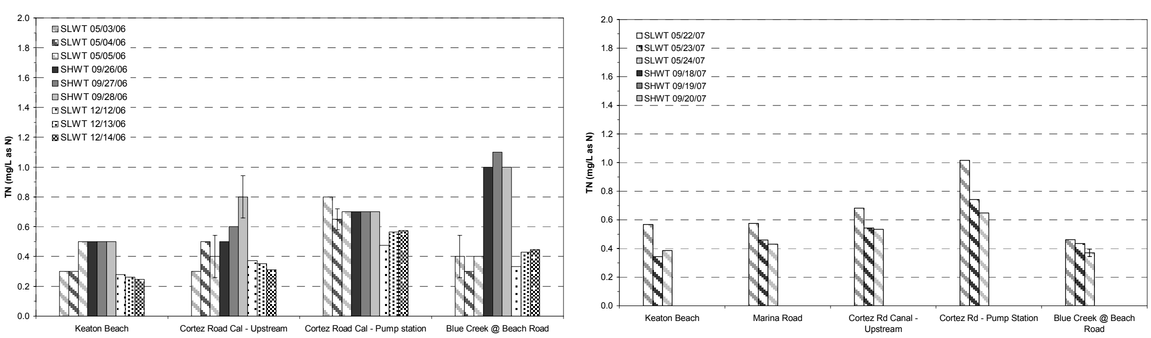


Figure 16 – Results for Total Nitrogen at Keaton Beach for 2006 (left) and 2007 (right).

TOC also increased appreciably (Figure 17) as the sampling moved upstream in 2006. While near the beach, the change in TOC from 2006 SLWT to 2006 SHWT events was about 50 percent, which is similar to trends seen at the OSTDS site locations. However, the difference in the two upstream sites was a factor of 3 to 7 times the SLWT results. This suggests irrigation runoff (rainfall was very scarce during May) or some other discharge. However the recent application of fertilizers is not suggested, since the average ammonia values at these sites remained low, except in May 2006 SLWT. Another remote possibility for this contribution would be a contribution from the wastewater discharge infiltration basins located just southeast of the upstream site (D). An investigation is warranted as to the source of this runoff component and whether an upstream discharge into the water body is also involved. The May 2007 samples were about 50 percent lower than the SLWT events in 2006, and recurrence of the 2006 SHWT spike was not noted in the data.

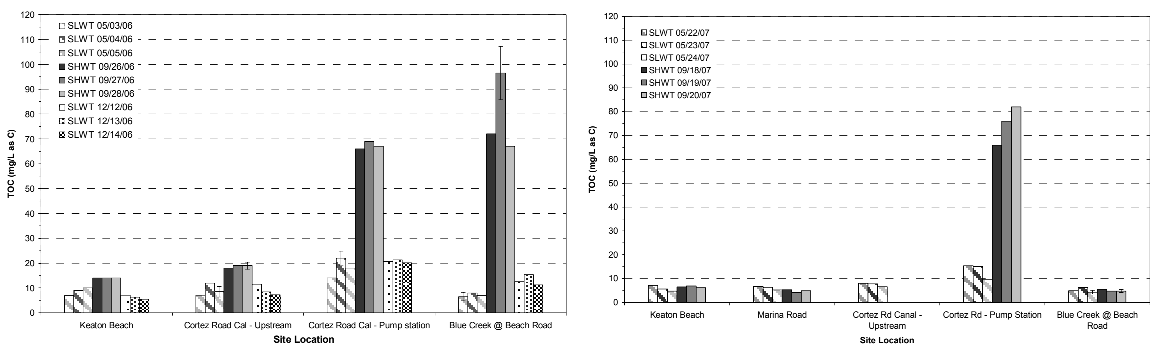


Figure 17 – Results for TOC at Keaton Beach for 2006 (left) and 2007 (right).

Table 15 shows the microbial constituents analyzed for 2006, and Table 16 summarizes those values for 2007 follow-up sampling. In terms of microbial water quality, concentrations of *Enterococcus*, *E. coli*, and total coliforms were compared.

Table 15 - Keaton beach turbidity and bacterial results for 2006.

Site	Charac.	Parameter	SLWT (May 2006) Day 1	Day 2	Day 3	SHWT (Sept 2006) Day 1	Day 2	Day 3	SLWT (Dec 2006) Day 1	Day 2	Day 3
F	Beach	Turbidity	0.6	1.7	1.1	1.1	1.0	0.7	0.5	0.4	0.7
E	Midstream	(NTU)	2.7	5.5	3.7	1.5	1.2	1.2	0.0	1.1	0.4
D	Upstream		2.1	2.0	1.2	2.0	3.5	3.0	0.9	0.5	0.9
G	Background		1.7	2.4	1.5	1.2	1.1	1.3	0.2	2.2	0.6
F	Beach	*Enterococcus*	41	<10	<10	<10	<10	<10	<10	<10	10
E	Midstream	(MPN/100 mL)	**609**	<10	<10	31	62	55	10	20	<10
D	Upstream		10	61	<10	**201**	**158**	**279**	10	10	<10
G	Background		20	10	51	52	35	20	<10	31	<10
F	Beach	*E. coli*	278	**790**	**560**	**1340**	**1600**	**891**	191	31	97
E	Midstream	(MPN/100 mL)	**488**	245	281	**490**	**1180**	**700**	31	52	20
D	Upstream		**4610**	**8160**	**8160**	**2060**	**2360**	**1530**	85	169	63
G	Background		20	41	30	98	20	41	63	199	187
F	Beach	T. coliforms	6590	14100	17300	10500	24200	9800	3430	1066	1532
E	Midstream	(MPN/100 mL)	5490	7270	2040	9800	15500	12000	1732	1892	805
D	Upstream		>24200	>24200	>24200	14100	17300	14100	1201	5247	1664
G	Background		2600	4110	4110	14100	17300	4110	3436	959	5748
F	Beach	Ec/Es Ratio	6.8	158	112	268	320	178	38	6.2	10
E	Midstream		0.8	49	56	16	19	13	3.1	2.6	4.0
D	Upstream		461	134	1632	10	15	5.5	8.5	17	13
G	Background		1.0	4.1	0.6	1.9	0.6	2.1	13	6.4	37

Note: Values in **bold** indicate violations of the trigger level

Table 16 – Keaton beach turbidity and bacterial results for 2007.

Site	Charac.	Parameter	SLWT (May 2007) Day 1	Day 2	Day 3	SHWT (Sept 2007) Day 1	Day 2	Day 3
F	Beach	Turbidity	1.1	1.3	1.6	0.3	0.9	0.7
MR	Beach	(NTU)	2.8	2.3	2.1	2.2	1.1	1.5
E	Midstream		3.4	3.4	4.8	3.3	1.3	1.8
D	Upstream		1.2	1.3	1.4	3.9	3.1	1.5
G	Background		2.3	2.5	2.2 ± 0.1	2.6	3.0	3.2 ± 0.1
F	Beach	*Enterococcus*	10	20	<10	<10	20	10
MR	Beach	(MPN/100 mL)	20	31	31	**120**	41	52
E	Midstream		31	10	<10	41	97	41
D	Upstream		20	31	<10	63	**106**	74
G	Background		26 ± 22	20	**124 ± 57**	**146**	**253**	**149 ± 25**
F	Beach	*E. coli*	91	92	150	**1602**	**1951**	**1565**
MR	Beach	(MPN/100 mL)	111	116	187	**7701**	**1951**	**3169**
E	Midstream		245	71	41	**4569**	**3968**	**737**
D	Upstream		256	256	41	**1576**	**2755**	**1119**
G	Background		85 ± 57	<10	<10	345	**496**	**427 ± 298**
F	Beach	Total coliforms	5475	7701	4160	9208	9804	6488
MR	Beach	(MPN/100 mL)	8664	3076	9804	12997	12997	19863
E	Midstream		8164	9804	8164	10462	12033	14136
D	Upstream		>24196	19863	>24196	9208	15531	7270
G	Background		2305	2247	6310	4884	4352	3904
F	Beach	Ec/Es Ratio	9.1	4.6	30.0	320.4	97.6	157
MR	Beach		5.6	3.7	6.0	64.2	47.6	60.9
E	Midstream		7.9	7.1	8.2	111.4	40.9	18.0
D	Upstream		12.8	8.3	8.2	25.0	26.0	15.1
G	Background		4.3 ± 6.0	0.3	0.0	2.4	2.0	2.7 ± 1.6

Note: Values in **bold** indicate violations of the trigger level

Enterococcus concentrations were generally lower at the beach than the upstream and background sampling points in 2006. This trend was also observed in 2007 for the upstream sites, but unexpectedly the background site enterococci counts were mostly above the trigger level in 2007 (4 of 6). This phenomenon deserves further investigation. Figure 18 shows that the *Enterococcus* concentrations were below the trigger level (100 MPN/100mL) on the beach in both years. However, upstream of the beach (F), the Keaton Beach canal pump station site at Cortez Road (D) showed *Enterococcus* violations during the 2006 SHWT, but did not violate the trigger level during either of the other two sampling events in 2006 (SLWT). The background site was always observed to be below the trigger level for all three sampling events in 2006, but this was not observed in 2007. In conjunction with the prior analysis, a summer sanitary sewer overflow at the pump station in 2006 might be suggested, although this does not resolve the upstream issues with TOC (likely of natural origin).

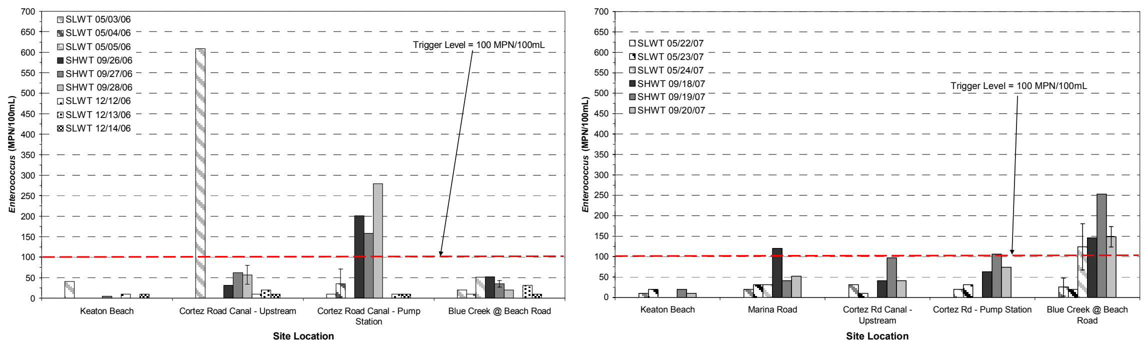


Figure 18 – Results for *Enterococcus* at Keaton Beach for 2006 (left) and 2007 (right).

With regard to *E. coli*, the beach site showed violations of the trigger level except for the December 2006 and May 2006 events. Like the *Enterococcus* results, the pump station site shows a dramatic exceedance of the *E. coli* trigger level of 400 MPN/100 mL (see Figure 19 – the violation is a magnitude higher than the trigger level). The problem is more acute during the May 2006 SLWT event, as there were no significant levels of *E. coli* in December 2006. The 2007 SHWT results at Keaton Beach violated the trigger level for all sites but the background (G). No microbial concerns with *E. coli* were encountered upstream of the pump station at the background site in either year, although the 2007 levels were slightly higher.

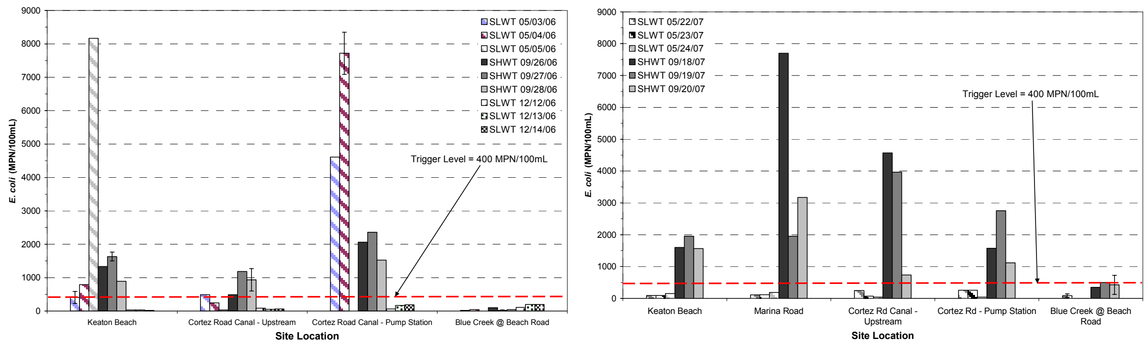


Figure 19 – Results for *E. coli* at Keaton Beach for 2006 (left) and 2007 (right).

This site is characterized by a large population of shore birds, and it is possible that the strong *E. coli* signal may have an important avian contribution. However, the *E. coli/Enterococcus* ratios were generally greater than 4.0 from the pump station, downstream, suggesting a human source of pollution. The upstream ratios were more indicative of mixed natural or animal contributions (<1.0 or between 1.0 and 4.0). Turbidity was also monitored to indicate if runoff from the surface is important. High microbial values coupled with turbidity may indicate runoff of agricultural origin. None of these samples was overly turbid, but some turbidity was present in the upstream samples.

Cedar Island

The Cedar Island community was selected as representative of a medium-density, developed area with a recently installed sewer system. For this set of sites, the general physical water quality parameters fell within expected levels, and conductivity, salinity, and TDS were in accordance with the saltwater/freshwater regime (Note: salinity issues are discussed in detail later). The DO values were in the range of 4.2 – 10 mg/L. During the SHWT, Site G violated the Class III criteria (>5.0 mg/L for fresh water) during the first and second day, and Site H violated the Class III criteria (>4.0 mg/L for marine water) during the first day of the SHWT. Table 17 summarizes the nutrient data for Cedar Island. In terms of nutrients, analysis was conducted to determine the levels of ammonia, nitrate, total nitrogen, and total organic carbon at the 3 sites.

Table 17 – Cedar Island nutrients results for 2006.

Site	Charac.	Parameter	SLWT (May 2006)			SHWT (Sept 2006)			SLWT (Dec 2006)		
			Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
I	Beach	DO	6.1	5.6	5.1	**4.8**	**4.9**	5.3	10	5.9	8.8
H	Upstream	(mg/L)	5.6	5.6	5.0	**4.2 ± 1.5**	**4.3**	**4.3**	7.2	5.9	5.2
G	Background		6.8	6.8	5.7	**4.9**	**4.9**	5.0	8.0	8.9	6.7
I	Beach	NH_4^+	0.31	<0.1	0.45	0.04	0.07	0.07	0.02	0.01	0.05
H	Upstream	(mg/L as N)	0.16	<0.1	0.91	0.06	0.04	0.06	0.06	0.06	0.08
G	Background		0.18	<0.1	<0.1	0.03	<0.010	<0.010	0.12	0.12	0.13
I	Beach	NO_3^-	<0.011	<0.011	<0.011	0.01	0.02	0.01	0.011	0.005	0.017
H	Upstream	(mg/L as N)	<0.011	<0.011	<0.011	0.01	<0.0052	0.01	0.005	0.010	0.012
G	Background		0.04	0.05	0.05	<0.0052	<0.0052	0.01	0.050	0.068	0.063
I	Beach	TN	0.7	0.4	0.5	0.7	0.6	0.5	0.3	0.3	0.2
H	Upstream	(mg/L as N)	ns	0.3	0.3	0.7	0.6	0.8	0.3	0.3	0.3
G	Background		0.4	0.5	0.4	1.0	1.1	1.0	0.3	0.3	0.4
I	Beach	TOC	8.0	9.0	9.0	21	20	14	6.5	7.1	5.6
H	Upstream	(mg/L as C)	ns	9.0	6.0	25	20	18	6.9	5.8	6.7
G	Background		8.0	8.0	7.0	72	104	67	12	7.3	11

Note: Values in **bold** indicate violations of the trigger level
ns = not sampled

Table 18 – Cedar Island nutrients results for 2007.

Site	Charac.	Parameter	SLWT (May 2007)			SHWT (Sept 2007)		
			Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
I	Beach		5.8	5.6	6.1	**4.0**	4.3	5.1
SL	Beach	DO	5.9	5.7	6.4	4.8	5.0	5.4
H	Upstream	(mg/L)	6.1	5.7	6.5	**3.9**	**4.0**	**4.2**
G	Background		8.4	7.9	8.6	5.8	5.9	6.3 ± 0.1
I	Beach		0.00	0.00	0.00	0.01	0.02	0.04
SL	Beach	NH_4^+	0.00	0.01	0.02	0.04	0.06	0.11
H	Upstream	(mg/L as N)	0.05	0.04	0.02	0.03	0.02	0.04
G	Background		0.08	0.09	0.08	0.09	0.09	0.10
I	Beach		0.00	0.00	0.00	0.00	0.01	0.01
SL	Beach	NO_3^-	0.00	0.00	0.01	0.01	0.01	0.01
H	Upstream	(mg/L as N)	0.00	0.01	0.02	0.01	0.01	0.01
G	Background		0.02	0.10	0.08	0.06	0.08	0.09
I	Beach		0.5	0.4	0.4	nr	nr	nr
SL	Beach	TN	0.4	0.4	0.4	nr	nr	nr
H	Upstream	(mg/L as N)	0.5	0.4	0.4	nr	nr	nr
G	Background		0.5	0.4	0.4	nr	nr	nr
I	Beach		6.6	6.0	6.4	nr	nr	nr
SL	Beach	TOC	5.8	4.8	6.7	nr	nr	nr
H	Upstream	(mg/L as C)	5.8	4.7	4.9	nr	nr	nr
G	Background		4.9	6.2	4.3 ± 0.6	5.4	4.8	4.8 ± 0.6

Note: Values in **bold** indicate violations of the trigger level

Figure 20 shows that during the May 2006 event (SLWT), the ammonia levels were higher at all sites when compared with the September 2006 (SHWT) event. The December 2006 events were higher than the September 2006 SHWT, but generally more similar to the May 2006 event. For the May 2006 SLWT, 5 of the 9 samples were high in ammonia including one sample at the Heron Road Canal site (H), which was one order of magnitude higher than the level considered indicative of human pollution. Interestingly, thereafter, there are no further exceedances at site H for the remainder of 2006 and into 2007 sampling events. For the first two sampling events in 2006, the ammonia concentration tended to decrease as the sampling sites moved upstream. The reverse was true in December 2006, where there was a marked increase as the sampling moved in the upstream direction. This trend continued into 2007 for both SLWT and SHWT. In 2007, a beach site located between the Blue Creek estuary and the Cedar Island Beach site was sampled to see if a connection between estuary and the beach could be an important source of nutrients here. If this is indeed the case, then the concentrations would generally decrease from the Blue Creek site level to the Seahawk Lane levels and finally to the Cedar Island Beach site. The Heron Road site is located south of this gradient and would probably not show any effect. After analyzing the ammonia levels, the results from SLWT are inconclusive because the beach levels are below detection, but in SHWT the concentration gradient seems to support this hypothesis. Since ammonia is an indicator of recent nitrogen contributions and the area currently does not have septic tanks, the results suggest the application of fertilizers or some other anthropogenic source.

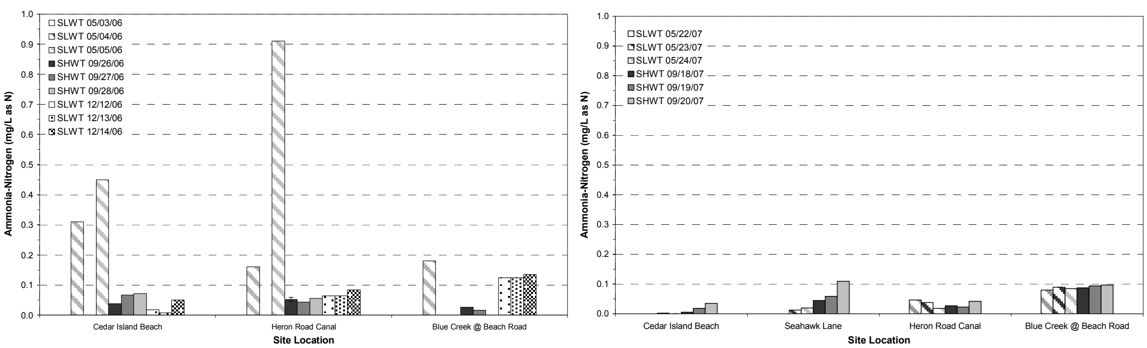


Figure 20 – Results for Ammonia at Cedar Island for 2006 (left) and 2007 (right).

Nitrate concentrations were at or below the detection limit for nearly all Cedar Island sites during all sampling events in both years. During the 2006 SLWT (May and December), although most of the samples were again at or below the detection limit, the background site (G) presented a detectable nitrate concentration for all three days. This was continued into 2007, where the background site nitrate levels exceeded the value considered high for shallow coastal areas for 5 of the 6 samples collected. The only low nitrate value at the background site was on the first day of sampling in 2007 SLWT (May 22, 2007).

Figure 21 shows that total nitrogen concentrations generally decreased going upstream during the May 2006 SLWT event, and increased during the September 2006 SHWT and December 2006 SLWT events. Except for the beach, the September 2006 SHWT results doubled compared to the May 2006 SLWT. The December 2006 concentrations were about half of the May 2006 SHWT event. The 2007 levels were similar, and no differences in TN concentration among the sites upstream (H, G) was observed compared to the beach sites (I, SL).

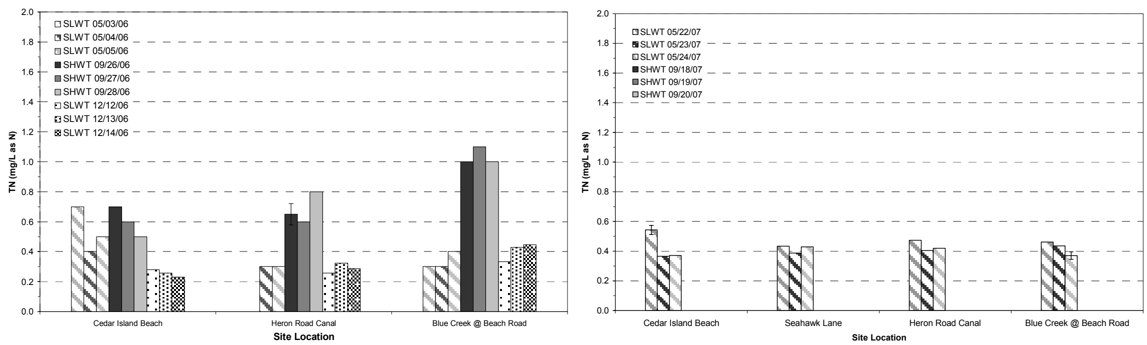


Figure 21 – Results for Total Nitrogen at Cedar Island for 2006 (left) and 2007 (right).

Figure 22 shows that TOC values in 2006 increased significantly as the sampling moved upstream, while near the beach, the change in TOC from SLWT to SHWT events was about 50 percent, which is similar to the other sampling site locations. May 2007 SLWT results mimicked the May and December 2006 SLWT events.

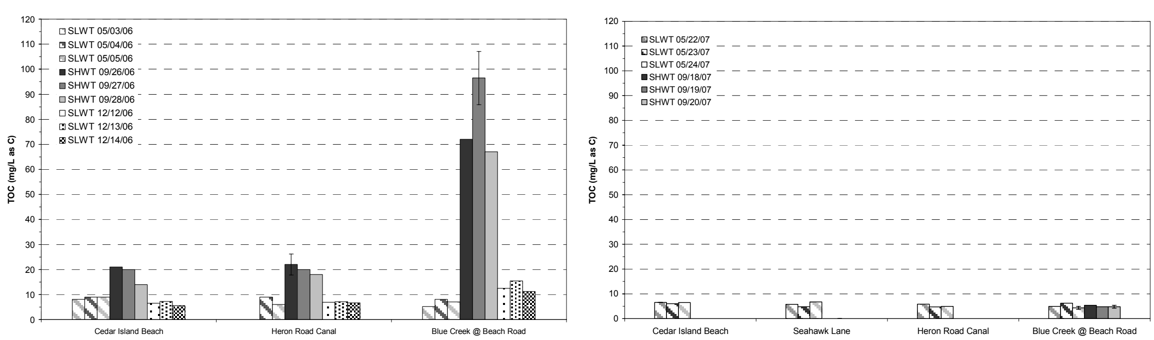


Figure 22 – Results for TOC at Cedar Island for 2006 (left) and 2007 (right).

Table 19 summarizes the microbial results for 2006, and Table 20 lists those for 2007. In terms of microbial water quality, concentrations of *Enterococcus*, *E. coli,* and total coliforms were compared. In 2006, *Enterococcus* concentrations were generally lower at the beach than the upstream and background sampling points. This was also observed in 2007, but the counts at the Cedar Island Beach site (I) were considerably lower than the previous year. Figure 23 shows that the *Enterococcus* concentrations were generally below the trigger level (100 MPN/100mL) on the beach (14 of 15) with the lone violation occurring in SHWT 2006. For both years, results were generally higher in the SHWT compared to the SLWT. The further upstream site (H) was below the trigger level, with one exception which occurred in the 2006 SHWT. None of the December 2006 SLWT samples showed significant amounts of *Enterococcus*, which is not unexpected. The background site (G) showed the most concentrated contamination from *Enterococcus* in SHWT 2007 with all three days violating the trigger level (4 out of 6 overall for both seasons compared to none in 2006).

Table 19 – Cedar Island turbidity and bacterial results for 2006.

Site	Charac.	Parameter	SLWT (May 2006)			SHWT (Sept 2006)			SLWT (Dec 2006)		
			Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
I	Beach	Turbidity	1.5	1.5	1.2	1.9	1.3	1.0	2.1	0.3	4.0
H	Upstream	(NTU)	2.2	3.3	2.4	3.2	1.6	1.5	0.7	1.2	1.5
G	Background		1.7	2.4	1.5	1.2	1.1	1.3	0.2	2.2	0.6
I	Beach	*Enterococcus*	<10	<10	10	**185**	63	20	<10	<10	10
H	Upstream	(MPN/100 mL)	52	20	10	41	**540**	10	<10	10	<10
G	Background		20	10	51	52	35	20	<10	31	<10
I	Beach	*E. coli*	350	**527**	**1340**	**10500**	**>24200**	**5790**	109	145	199
H	Upstream	(MPN/100 mL)	**850**	**956**	**431**	**910**	**4610**	**1840**	163	315	266
G	Background		20	41	30	98	20	41	63	199	187
I	Beach	Total Coliforms	7700	2700	11200	>24200	>24200	>24200	546	1961	1050
H	Upstream	(MPN/100 mL)	9210	12000	1990	1720	14100	17300	3470	4541	4587
G	Background		2600	4110	4110	14100	17300	4110	3436	959	5748
I	Beach		70	105	134	57	384	290	22	29	20
H	Upstream	Ec/Es ratio	16	48	43	22	8.5	184	33	32	53
G	Background		1.0	4.1	0.6	1.9	0.6	2.1	13	6.4	37

Note: Values in **bold** indicate violations of the trigger level

Table 20 – Cedar Island turbidity and bacterial results for 2007.

Site	Charac.	Parameter	SLWT (May 2007)			SHWT (Sept 2007)		
			Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
I	Beach		2.3	2.0	1.4	1.4	1.9	1.7
SL	Beach	Turbidity	2.0	2.1	1.9	2.6	2.7	3.7
H	Upstream	(NTU)	3.1	3.4	4.2	3.7	3.2	2.5
G	Background		2.3	2.5	2.2 ± 0.1	2.6	3.0	3.2 ± 0.1
I	Beach		<10	<10	<10	10	40	52
SL	Beach	*Enterococcus*	<10	<10	<10	74	41	**120**
H	Upstream	(MPN/100 mL)	20	<10	10	20	20	51
G	Background		26 ± 22	20	**124 ± 57**	**146**	**253**	**149 ± 25**
I	Beach		85.5	41	425	**7701**	**1459**	**1782**
SL	Beach	*E. coli*	164	94	104	**2809**	**530**	**852**
H	Upstream	(MPN/100 mL)	143	2909	218	**1345**	**1334**	**1631**
G	Background		85 ± 57	<10	<10	345	**496**	**427 ± 298**
I	Beach		9313	6867	>24196	>24196	7701	11199
SL	Beach	Total coliforms	9208	6131	14136	14136	4352	5794
H	Upstream	(MPN/100 mL)	15531	8164	14136	8414	7701	9208
G	Background		2305	2247	6310	4884	4352	3904
I	Beach		17	8.2	85	770	36	34
SL	Beach	Ec/Es Ratio	33	19	21	38	13	7.1
H	Upstream		7.2	582	22	173	67	32
G	Background		4.3 ± 6.0	0.3	0.0	2.4	2.0	2.7 ± 1.6

Note: Values in **bold** indicate violations of the trigger level

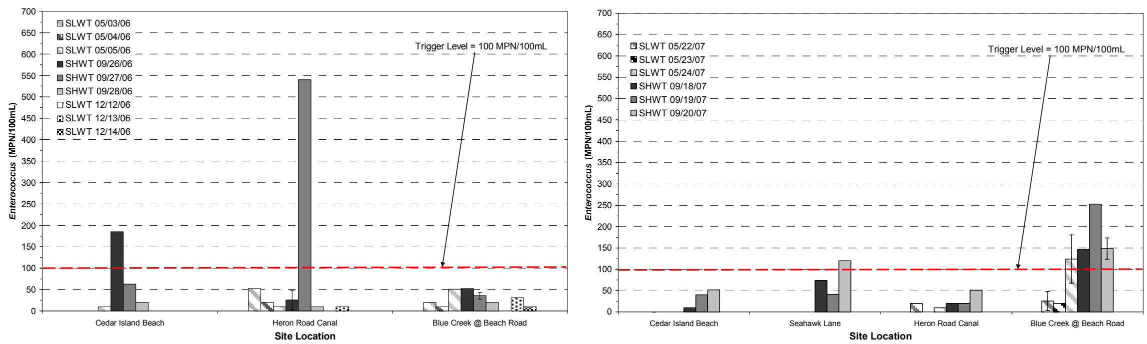


Figure 23 – Results for *Enterococcus* at Cedar Island

Results for *E. coli* generally showed exceedance of the 400 MPN/100mL trigger level on the beach and just upstream of the beach (see Figure 24) during the May 2006 SLWT and more prominently during the September 2006 SHWT sampling event. This trend continued in 2007. Extremely high microbial densities (from 1840 up to >24,200) were recorded during the September 2006 sampling in Cedar Island Beach (I) and the upstream canal site (H). Similarly high counts were found in September 2007 at those sites and also at the new beach site at Seahawk Lane (SL). Values of 10^5 CFU/100 mL are more characteristic of urban or agricultural wastewater inputs than natural levels. It was suspected that the source of this coliform contamination was related to legacy inputs from shallow sediment regrowth either from legacy septic inputs or from the nearby upstream estuary acting as a microbial incubation site. However, we cannot discount the possibility of the nearby boat channel mucky sediments providing a source as well. The purpose of selecting the Seahawk Lane site was to see if a concentration gradient between the estuary and the beach site exists. Additionally, its location is upstream of the boat channel. Therefore, if the mucky sediments are important then the Seahawk Lane site would have considerably less microbial counts compared to the downstream beach site. To account for the tidal sloshing effect, the SL sample was collected in a protected area outside of the strong prevailing bulk current streamline. It is interesting to note that adding the SL site improved the trend showing that higher *E. coli* counts are encountered in the direction inland toward the estuary. The 2007 results indicate a strong *E. coli* signal at the SL site, which would seem to support the estuarine source or local shallow sediment regrowth hypotheses. The December 2006 SLWT event showed generally lower *E. coli* levels compared to the May 2006

SLWT event, presumably due to lower temperatures. There were no recorded violations in December 2006 at any site.

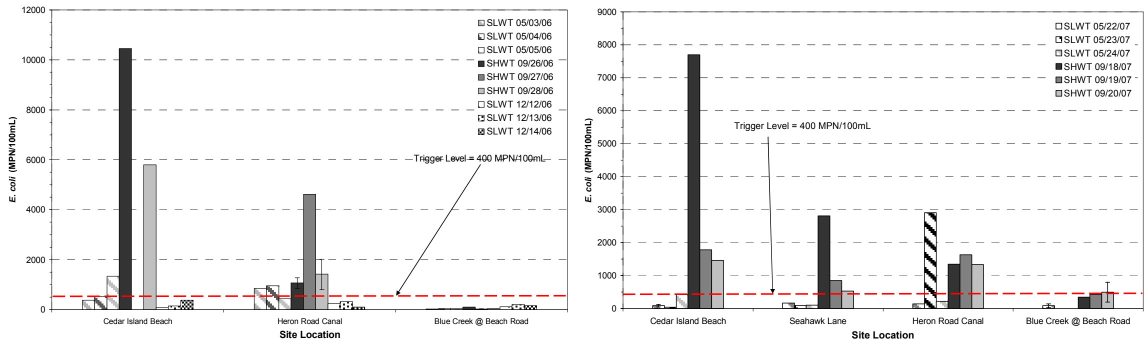


Figure 24 – Results for *E. coli* at Cedar Island for 2006 (left) and 2007 (right).

The *E.coli/Enterococcus* ratios were above 4.0 on the beach and at the site just upstream of the beach, which suggests inputs of human origin. The *E.coli/Enterococcus* ratios were generally below 3.0 for the background site (in May and September for both years), suggesting an important non-human or natural contribution upstream consistent with the other microbial findings. In December, the *Enterococcus* levels were too low to generate accurate Ec/Es ratios. Turbidity was similar to the other sites.

ADDITIONAL SITES

One potential source of nutrient contamination identified earlier is due to industrial release, such as from pulp and paper mills (Health Canada 2001; McMaster et al. 2004). From aerial photography and field reconnaissance, it was determined that a large industrial source discharges into the Fenholloway River upstream of the impacted areas, north of Dekle Beach. It was hypothesized that this source potentially influences the nutrient dynamics of the coastal areas of Taylor County, FL due to the prevailing clockwise current direction (Gyory, Mariano, and Ryan 2008) and the magnitude of the loading. During the dry season, this industrial effluent discharge can constitute up to 80% of the river's volume (Bortone and Cody 1999). The Fenholloway River is 36 miles long, and its watershed drains approximately 392 square miles of mostly rural areas (i.e. forest, wetlands, and natural areas). In 1947, the Fenholloway River was designated as Class V for navigation, utility, and industrial use. In 1997, the designation was changed to Class

III for recreational use, propagation and maintenance of a healthy, well-balanced population of fish and wildlife. Historical water quality data for the river were obtained and are summarized in Table 21.

Table 21 - Water Quality Data for Buckeye Florida Specialty Cellulose Mill.

Parameter	USEPA 2003	FDEP FILES FOR 2004	Proposed TMDL (USEPA 2003)
Flow	43 MGD	44 MGD	
BOD_5	22 mg/L (8200 lb/d)	22 mg/L (8200 lb/d)	1050 – 1255 lb/d
TSS	--	14 mg/L (5000 lb/d)	--
Ammonia	3.3 mg/L (1200 lb/d)	--	37 – 360 lb/d
Total Nitrogen (TN)	5.0 mg/L (1800 lb/d)	7.1 mg/L (2600 lb/d)	10.5 – 1075 lb/d
Total Phosphorus (TP)	2.0 mg/L (750 lb/d)	1.4 mg/L (550 lb/d)	79 – 360 lb/d
Specific Conductance	--	2700 µmho/cm	--
Color	--	1200 PCU	--

During the December 2006 trip, samples were collected at the Fenholloway River downstream from a specialty cellulose mill. The results for ammonia were the highest measured over the course of the study, by a factor of 20. While all other sampling locations were essentially below 0.15 mg/L as N, the Fenholloway samples were all higher than 3.0 mg/L as N (nearly five times higher than the trigger level). The additional nutrients could also be coming from wastewater treatment facilities or septic tanks (discussed in more detail later). Historically, the City of Perry Wastewater Treatment Facility also discharged to the Fenholloway River, but this practice was halted in 2004 when the plant was switched to land treatment. Investigation of the connection between the upstream Fenholloway discharge and its potential impacts along the beaches south of the discharge was beyond the initial scope of this study, but in the follow-up sampling it was desired to investigate its effects. The selection of these additional sites was described earlier, and the nutrient results from 2007 sampling events are summarized in Table 22.

Table 22 – Additional sample site nutrients results for 2007.

Site	Charac.	Parameter	SLWT (May 2007)			SHWT (Sept 2007)		
			Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
FR	Source	DO (mg/L)	4.9	5.1	5.9	5.5	5.7	6.1
HS	Midstream		6.2	6.5	7.5	6.8	7.1	7.3
PL	Discharge		5.5	7.2	7.9	6.4	6.7	7.4
AB	Beach		8.1	7.0	7.4	4.4	7.0	6.1 ± 0.7
FR	Source	NH_4^+ (mg/L as N)	2.49	2.29	2.63	3.21	2.85	2.63
HS	Midstream		1.60	1.59	2.98	2.57	2.52	2.07 ± 0.05
PL	Discharge		0.55	0.39	0.30	0.41	0.44	0.62 ± 0.08
AB	Beach		0.00	0.06	ns	0.00	0.00	ns
FR	Source	NO_3^- (mg/L as N)	0.35	0.29	0.23	0.00	0.52	0.53
HS	Midstream		0.36	0.32	0.30	0.08	0.20	0.26 ± 0.01
PL	Discharge		1.00	0.30	0.42	0.24	0.25	0.38
AB	Beach		0.00	0.01	ns	0.00	0.04	ns
FR	Source	TN (mg/L as N)	6.9	6.5	6.5	nr	nr	nr
HS	Midstream		4.2	4.0	4.4	nr	nr	nr
PL	Discharge		2.5	2.0	1.8	nr	nr	nr
AB	Beach		0.6	0.5	0.5	nr	nr	nr
FR	Source	TOC (mg/L as C)	170	163	161	170	172	163 ± 13.4
HS	Midstream		97	91	119	134	133	nr
PL	Discharge		62	50	40	89 ± 6.4	90	87
AB	Beach		9.1	8.2	8.6	8.7	9.3	9.1

Note: Values in **bold** indicate violations of the trigger level

For this Fenholloway River set of sites, the general physical water quality parameters fell within expected levels, and conductivity, salinity, and TDS were in accordance with the saltwater/freshwater regime. The DO values were in the range of 4.9 – 7.9 mg/L. During the SLWT, the FR site violated the Class III criteria (>5.0 mg/L for fresh water) during the first day. This was the only DO violation recorded in this study. The Adams Beach site (AB) did not have any DO violations.

In terms of ammonia, the three Fenholloway River sites were 1-2 orders of magnitude higher than the 0.07 mg N/L threshold for human contamination in both seasons. Both the near to the industrial discharge and at the midstream site, the ammonia levels were high in the range of 1.6-3.2 mg N/L. Those levels decreased by a factor of 10 at the rivermouth discharge to ocean presumably due to tidal dilution forces. In the distance that the river's nutrient loading reaches the Adams Beach site location, the levels return to background. The nitrate levels follow a similar pattern, but the measured concentrations are not nearly as high as those seen for ammonia. The total nitrogen values are not much different than those seen at other sampling sites in this study, suggesting that the fraction of organic nitrogen may be less than at other sites. The TOC values behave like the ammonia levels for these sites. It is likely that this additional TOC is derived largely from the deep organic color (Figure 25).

Figure 25 – Photograph showing the large variation in apparent color from the Fenholloway River (left), to the Steinhatchee Falls (middle), and the Boggy Creek (right) site.

Table 23 summarizes the microbial results for 2007. In terms of microbial water quality, concentrations of *Enterococcus*, *E. coli,* and total coliforms were compared. Despite the noticeable color content of the river samples, the turbidity levels are not much higher than any of the other sites in this study. Earlier it was hypothesized that the industrial input may contribute nutrients and pathogen indicators as a continuous point source upstream of the beach communities. If this were true, then the microbial indicators would be high at these stations. The *Enterococcus* counts are consistently high for the river sites in both seasons, with 12 of 17 samples violating the trigger level, but the downstream beach site only has one violation from the five samples. It is interesting to note that the *E. coli* counts do not behave the same way. In fact, the river site nearest to the industrial discharge is at or below detection for all six sampling days, but the counts increase in the downstream direction and result in alarmingly high counts at the rivermouth (>10^4 MPN/100 mL) and Adams Beach (>10^5 MPN/100 mL) in SHWT. The large enterococci component suggests an influence from natural or animal sources in the upstream reaches, while the ratio at the rivermouth and downstream beach suggest a human influence, although this could be affected by a differential salinity induced die-off. It is not known if the industrial discharge is disinfected prior to release to the environment; however, the process water may contain chlorine dioxide as a bleaching agent which can act a residual disinfectant during the facultative lagoon treatment process. If this is the case, then a differential resistance exhibited by the two indicator species may be masking the source and acting as a confounding agent.

Table 23 – Additional sample site turbidity and bacterial results for 2007.

Site	Charac.	Parameter	SLWT (May 2007)			SHWT (Sept 2007)		
			Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
FR	Source	Turbidity (NTU)	4.9	5.5	5.6	3.7	3.9	3.6
HS	Midstream		2.8	3.1	3.2	2.3	3.6	2.6
PL	Discharge		2.8	2.3	2.6	1.8	1.8	2.2
AB	Beach		1.6	ns	3.6	3.4	1.7	2.5 ± 0.3
FR	Source	*Enterococcus* (MPN/100 mL)	41	72	ns	**318**	**269**	**228**
HS	Midstream		**164**	31	**106**	**278**	**168**	**119**
PL	Discharge		**144**	97	**187**	**208 ± 18**	**188 ± 60**	72
AB	Beach		<10	ns	<10	73	<10	**330 ± 21**
FR	Source	*E. coli* (MPN/100 mL)	<10	<10	<10	10	<10	<10
HS	Midstream		10	52	<10	63	52	75
PL	Discharge		30	41	40	**1292 ± 208**	**1025 ± 170**	**545**
AB	Beach		657	ns	81	**15531**	**11199**	**3625 ± 485**
FR	Source	Total coliforms (MPN/100 mL)	6910	>24196	>24196	>24196	>24196	10112
HS	Midstream		19863	6893	17329	14136	12033	6586
PL	Discharge		10112	9606	17329	>24196	10112	17329
AB	Beach		10462	ns	9804	>24196	>24197	10112
FR	Source	Ec/Es Ratio	0.1	0.1	ns	0.0	0.0	0.0
HS	Midstream		0.1	1.7	0.0	0.2	0.3	0.6
PL	Discharge		0.2	0.4	0.2	6.2 ± 0.5	5.9 ± 2.8	7.6
AB	Beach		131.4	ns	16.2	213	2240	11 ± 0.8

Note: Values in **bold** indicate violations of the trigger level

Sampling at the Fenholloway River site actually began in December 2006 SLWT. The data from this site including the 2006 and 2007 results is compiled in Table 24. Similar patterns as those already described are seen for the December 2006 SLWT results.

Table 24 – Water quality results monitored the Fenholloway River site (FR) for 2006-2007.

Parameter	SLWT (Dec 2006)			SLWT (May 2007)			SHWT (Sept 2007)		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
DO (mg/L)	7.6	6.3	7.0	4.9	5.1	5.9	5.5	5.7	6.1
Ammonia (mg/L N)	3.9	3.4	3.2	2.5	2.3	2.6	3.2	2.9	2.6
Nitrate (mg/L N)	0.3	0.6	0.6	0.4	0.3	0.2	0.0	0.5	0.5
TN (mg/L N)	ns	ns	ns	6.9	6.5	6.5	nr	nr	nr
TOC (mg/L C)	75	79	78	170	163	161	170	172	163
Turbidity (NTU)	8.7	8.2	7.7	4.9	5.5	5.6	3.7	3.9	3.6
Enterococcus (MPN/100 mL)	31	41	75	41	72	ns	318	269	228
E. coli (MPN/100 mL)	10	<10	10	<10	<10	<10	10	<10	<10
Total Coliform (MPN/100 mL)	>24196	>24196	>24196	6910	>24196	>24196	>24196	>24196	10112
Ec/Es Ratio	0.3	0.1	0.1	0.1	0.1	ns	0.0	0.0	0.0

OTHER TRACERS

Caffeine

During the seasonal low water table, caffeine levels were expected to be below detection, which, in general, they were. Caffeine was only detected in 3 sites during the May SLWT sampling event: Steinhatchee Falls (Site N), Third Avenue Fork (Site K), and Cedar Island Beach (Site I).

When found, caffeine was detected at very low levels (< 0.04 µg/L), all of which were below the 0.10 µg/L trigger level.

During the seasonal high water table event (September 2006), caffeine sampling was conducted more frequently (a total of 20 samples), since caffeine levels were anticipated to be detected more often. This expectation is based on the assumption that the main source of caffeine contamination is from septic tanks, when performance is compromised during the seasonal high water table. However, only one background site (Steinhatchee Falls, Site N) showed detectable levels. This background site was measured at 0.321 µg/L, which is above the threshold value of 0.10 µg/L. Because this sample was the only one (of the 20 collected) to show any detectable levels of caffeine, the sample was re-analyzed and found to be below detection. Therefore, the high value reported may have been a case of laboratory contamination.

Overall, the caffeine testing program did not indicate large amounts of human-derived inputs. Because so few sites had detectable results, it was expected that SLWT in December 2006 would provide no further useful caffeine information. The conclusion is that caffeine may be a useful tracer, but it must be used in an environment much more densely populated than what is present in Taylor County, FL. Follow-up testing in 2007 did not include caffeine as a tracer.

No definitive conclusions can be drawn from these results because at these levels, the dilution in the waterways neither precludes nor excludes the possibility of a wastewater contribution.

Optical brighteners (OB)

Optical brighteners are fluorescent dyes added to laundry detergents as whitening agents. The presence of optical brighteners is an indicator of gray water inputs. In this study, the qualitative method with cotton pads was used to evaluate the presence or absence of optical brighteners. No significant glow was observed on the collected pads during the events of May and September. However, on the second day of sampling during the SLWT, May 4th, 2006, the pad at Site J (Roy's) had a significant glow when passed over with a portable handheld UV lamp. This was most likely due to cleaning and mopping activities around the restaurant that coincided with the time of sample collection.

During the December 12, 2006 sampling event, possible optical brighteners were visually detected at Site L (Boggy Creek @51). This is the first time that visible fluorescence is detected under natural sunlight over the course of the study (see Figure 26). Later that same day, more evidence is discovered at Site N (Steinhatchee Falls). This is shown in Figure 27. In addition, a 55 gallon drum is discovered floating in the upstream portion of the Falls (Figure 28). It is possible that the drum contained a surfactant that could have been responsible for the observed fluorescence. Similar drums are seen elsewhere in Steinhatchee (Figure 29). In the previous sampling trip (September), the team also noticed that the portable restroom facility was being serviced with a large amount of cleaning agent applied by a truck (Figure 30). It is difficult to compare the appearance and morphological characteristics of the floating fluorescence because the lighting inside the portable restroom is not ambient sunlight, but there are similarities (Figure 31).

Figure 26 - During the December 12, 2006 sampling event, possible optical brighteners are visually detected at Site L, Boggy Creek @51, highlighted in the circle.

Figure 27 - During the December 12, 2006 sampling event, more evidence of optical brighteners is discovered at Site N (Steinhatchee Falls), highlighted in the circle.

Figure 28 - A possible explanation for the sudden appearance of optical brighteners at the surface is discovered (12/12/2006).

Figure 29 - Similar drums are discovered at Site J (Main Street - Roy's) on December 13, 2006.

Figure 30 - Photograph of the service truck cleaning the portable restroom facility during September 28, 2006 at Steinhatchee Falls.

Figure 31 - Photographs of the cleaning agent employed in the portable restrooms at Steinhatchee Falls.

Active searching for floating fluorescence, led to documentation of sightings of similar fluorescence on subsequent days during the December sampling event (see Figure 32 and Figure 33). However, without a suitable reference for comparison, it remains difficult to make a definitive evaluation of presence or absence (see Figure 34) in the qualitative test. It remains unclear if in fact these sightings are optical brighteners of human origin, and no obvious pattern to their appearance is determined.

Figure 32 - Floating fluorescence observed at Site G (Blue Creek @ Beach Road) on December 13, 2006. Note the submerged traffic cone to give a sense of scale.

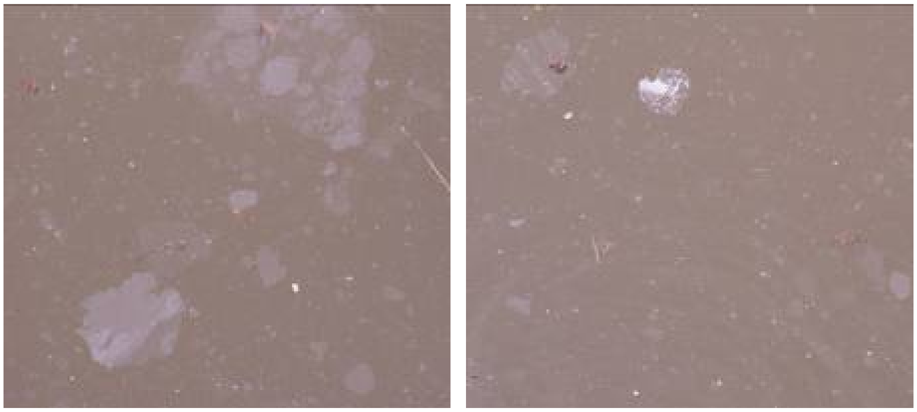


Figure 33 - More floating fluorescence visible under ambient sunlight at Site M (Steinhatchee River at Airstrip Drive).

Optical brighteners have just recently been employed as a tracer, and detection methods are still being evaluated. The absence of optical brighteners does not indicate that the sites are free of wastewater or gray water contribution. The method is still not perfected and is not certified. In addition, a reasonable amount of laundry soap manufacturers are taking optical brighteners out of their formulas, and dilution and solar exposure can mask results.

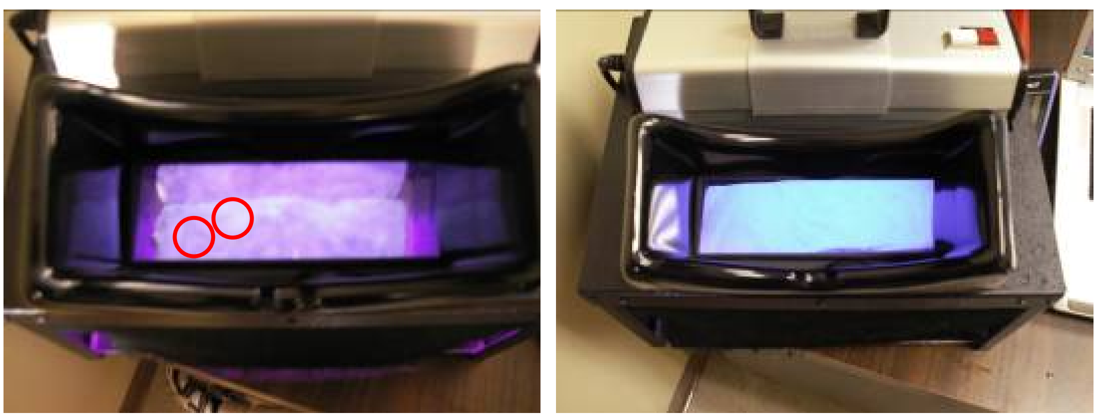


Figure 34 - A field collected optical brightener pad (Dekle Beach collected on 12/14/2006). In the left picture, a clean pad is shown on top to compare with the collected pad on the bottom. Very tiny flakes are visible under ultraviolet illumination (circled). In the right picture, a pad with optical brighteners is shown for comparison. Notice the same blue fluorescence seen for the tiny flakes on the left.

Molecular Techniques

In the 2006 sampling events, both optical brighteners and caffeine were tested as unconventional tracers of human pollution. However, the qualitative method for detection of optical brighteners was not sensitive enough to be considered an effective tracer, and caffeine results were inconclusive due to extremely high dilution and low development intensity. Therefore, neither of these methods was continued in 2007 sampling events. However, given the recent rapid advancements in technology, molecular techniques and nitrogen isotopic ratios were determined to be much more informative as tracers.

The molecular biology research team from the National Oceanographic and Atmospheric Administration Atlantic Oceanographic and Meteorological Laboratories (NOAA-AOML) Ocean Chemistry Division offered to attempt to analyze eight samples collected from the Taylor County study in an effort to determine if molecular techniques could be used as a tracer method. This is an experimental process being developed for NOAA, with no guarantee of success for same. Samples from four sites were collected from the September 2006 SHWT sampling event and another four sites from the December 2006 SLWT sampling event. For each set of four sampling sites, three different molecular based assays were performed, including two sets of DNA analyses, one set of *E. coli* tracer tests, and one set of *Enterococcus* tracer tests.

For each of the sets of four, an additional 2-4 L of samples were collected in sterile Whirl-Pak bags and transported to the temporary field laboratory. The selected sites (4 of 13) were picked based on the previous two days' results for microbiological analyses (i.e. those sites with the highest *E. coli* and *Enterococcus* values were selected). Collection of water in this manner was the best chance to collect sufficient material to be above the detection limit for the assay. For each site, 400-900 mL of sample was filtered in sterilized filter holders. Those samples prepared for *E. coli* and *Enterococcus* typing were filtered using Whatman 7141-104 cellulose nitrate filters, and those prepared for DNA testing were filtered in duplicate using Super®200 0.2 µm filters. The forceps that handled the filters were dipped in ethanol (HPLC grade) and flamed in a Bunsen burner prior to coming in contact with the filters.

For *Enterococcus*, the filter was then transferred to a Petri dish containing M Enterococcus Agar (90003-930 from VWR). For *E. coli* testing, the filter was then transferred to a Petri dish containing MTEC agar (233410 from VWR). Both sets of plates were then incubated at 35-37°C in a VWR 1525 signature series general purpose incubator for 1-2 days and then transported at 0°C until delivered to the NOAA-AOML facility, where they were stored at -80°C until analyzed. The DNA filters were transferred to an Analyslide® Petri dish and frozen immediately, but not at -80°C, without incubation. No positive controls were performed.

The filtered samples were then processed to collect DNA extracts. The environmental microbiology laboratory (Dr. Kelly Goodwin, PI) at the National Oceanographic and Atmospheric Administration (NOAA) Atlantic Oceanographic and Meteorological Laboratories (AOML) performed molecular biological analysis on a subset of samples collected from Taylor County, FL. Molecular analysis was performed for the following, as detailed in Table 25:

- **Fecal indicating bacteria:**
  - *Enterococcus* spp. [Haugland et al. 2005]
- **Source tracking markers:**
  - Human HF8 gene cluster of *Bacteroides* spp. [Bernhard and Field 2000]
  - Human-specific *esp* gene of *Enterococcus faecium* [Scott et al. 2005]
- **Human bacterial pathogens (fecal):**
  - *E. coli* O157:H7 [Maurer et al. 1999]
  - *Salmonella* spp. [Kong et al. 2002]
- **Human bacterial pathogens (nonfecal):**
  - *Staphylococcus aureus* [Mason et al. 2001]

Three replicate water samples were collected from a study site and bacteria and particles were harvested onto membrane filters. The water (2000 - 4000 mL) was filtered onto Whatman 7141-104 cellulose nitrate filters or 0.2 μm Supor-200 filters. The cellulose nitrate filter membranes were incubated either on mTEC agar for enrichment of *E. coli* and *Salmonella* or M Enterococcus agar for enrichment of *Enterococcus* and *Staphylococcus*. Filters were incubated overnight at 35-37°C and then delivered frozen to AOML for processing. The goal was to obtain confluent growth on the membrane filters and to test for markers of human fecal pollution and a variety of pathogens. However, in general, the samples were not confluent growth, which was contrary to what was expected based on the measured microbial densities from the previous days' sampling.

Supor-200 filters were frozen and sent to AOML for later DNA extraction. DNA lysates were prepared from the filters by bead-beating (Haughland et al. 2005) in Qiagen AE buffer with a Qbiogene FastPrep bead beating instrument at speed 6.5 for a total of 40 s. The lysates were diluted 1:5 with fresh AE buffer and stored at -80˚C until analysis. An aliquot (5 μL) of each 1:5

dilution was utilized as template DNA in 50 μL PCR reactions as outlined in Table 25. Standard positive and negative PCR controls were used.

Samples were tested for the presence of amplifiable DNA and for PCR inhibition using primers that amplify a universal region of the bacterial genome (16S rRNA gene). As an additional control, samples were analyzed for the presence enterococci (23S rRNA gene targeting the enterococci group including *E. faecalis, E. faecium, E. durans, E. casseliflavus, E. gallinararum,* and *E. hirae*). The lysates also were analyzed for the presence of several pathogens and markers of human fecal pollution, as detailed in Table 25.

September and December 2006 Results

The goal was to obtain filters with confluent bacterial growth for the PCR analysis, but confluent growth was not obtained. Nonetheless, a subset of the samples received was analyzed by PCR. The results of the PCR assays are summarized in Table 26. All samples were amplifiable and without significant PCR inhibition, as indicated by successful amplification of 16S rDNA. Six out of nine samples contained enough *Enterococcus* DNA to generate successful amplification. The three negative samples had low concentrations of *Enterococcus*, as indicated by the following IDEXX enumeration (MPN/100mL): 20 (Creek at Deckle Beach) and <10 (Keaton Beach). In general, the *Enterococcus* counts were low for all of these samples, with only one sample exceeding the single sample standard for *Enterococcus* (104 MPN/100 mL). *Enterococcus* is the recommended indicator for marine waters (EPA 2004). Positive reactions were not obtained for any of the markers of human fecal pollution or for pathogens, even for the one site exceeding the single sample guideline for *Enterococcus* (sample 070918L Boggy Creek). All positive and negative controls functioned as expected (data not shown).

Growth enrichment was used here to enhance detection of rare pathogen signals, and confluent growth on the incubated filters is anticipated to be needed for adequate sensitivity. None of the filters from this study demonstrated true confluent growth; therefore negative signals are not unexpected. However, lack of confluent growth due to problems with the media cannot be ruled out because plate function was not verified through growth of positive controls.

The PCR assays described here have been adapted from clinical applications and the use of these protocols for environmental samples is in the research phase. These protocols have demonstrated good sensitivity with clinical samples; however, there is a known gap between the sensitivity achieved with assays under clinical conditions and the sensitivity achieved with environmental samples (Baums et al. 2007; Goodwin and Litaker 2007). The magnitude of this gap is currently under investigation. DNA extraction efficiencies were not quantified here, but are currently under investigation.

Overall, the molecular data indicated that the analyzed water samples (only 22 samples of 309) were not highly contaminated with human-derived fecal contamination. These results are supported by the low IDEXX MPN results for *Enterococcus* and the lack of confluent growth from the samples incubated on the bacterial media. However, the possibility of false negative results can not be ruled out because of the possibility of low DNA extraction efficiencies and/or lack of confluent growth due to problems with the bacterial media.

Table 25 - Details of molecular analysis, including, gene target, PCR primer sequences, and thermocycling reaction conditions for 2006.

Target Organism/Gene	Primer Sequences 5' → 3'	PCR Thermocycling Conditions	Ref.
Universal Bacterial 16S rRNA gene	**Unifor:** ACTCCTACGGGAGGCAGC **Unirev:** GACGGGCGGTGTGTACAA	**Per 50uL rxn:** 5uL Finzyme 10X buffer, 1.25uL dNTPs (10mM), 1.5uL BSA (10mg/mL), 2.5uL Unifor primer (10uM), 2.5uL Unirev primer (10uM), 0.75uL Finzyme, Hotstart Taq Polymerase. **Cycling:** 94˚C, 10min; 30 cycles of 94˚C 30s, 58˚C 30s, 72˚C 30s; 70˚C 8min	Zheng et al. 1996
Enterococci group general 23S rRNA gene	**ECST748F:** AGAAATTCCAAACGAACTTG **ENC854R:** CAGTGGTCTACCTCCATCATT	**Per 50uL rxn:** 5uL Finzyme 10X buffer, 1.25uL dNTPs (10mM), 1.5uL BSA (10mg/mL), 4.5uL ECST748F primer (10uM), 1.5uL ENC854R primer (10uM), 0.75uL Finzyme, Hotstart Taq Polymerase. **Cycling:** 94˚C, 10min; 30 cycles of 94˚C 30s, 60˚C 30s, 72˚C 30s; 70˚C 8min	Haugland et al. 2005
Human-specific marker *Enterococcus faecium* *esp* gene	**espF:** TATGAAAGCACAAGTT **espR**: CGTCGAAAGTTCGATTTCC	**Per 50uL rxn:** 5uL Finzyme 10X buffer, 1.25uL dNTPs (10mM), 1.5uL BSA (10mg/mL), 1.5uL espF primer (10uM), 1.5uL espR primer (10uM), 0.75uL Finzyme, Hotstart Taq Polymerase. **Cycling:** 94˚C, 10min; 40 cycles of 94˚C 1min, 58˚C 1min, 72˚C 1min; 70˚C 8min	Scott et al. 2005
Human-specific marker *Bacteroides* HF8 gene cluster	**HF183F:** TCATGAGTTCACATGTCCG **Bac708R:** CAATCGGAGTTCTTCGTG	**Per 50uL rxn:** 5uL Finzyme 10X buffer, 1.25uL dNTPs (10mM), 1.5uL BSA (10mg/mL), 2uL HF183F primer (10uM), 2uL Bac708R primer (10uM), 0.75uL Finzyme, Hotstart Taq Polymerase. **Cycling:** 94˚C, 10min; 40 cycles of 94˚C 30s, 59˚C 30s, 72˚C 30s; 70˚C 8min	Berhnard et al. 2000

Target Organism/Gene	Primer Sequences 5’ → 3’	PCR Thermocycling Conditions	Ref.
Salmonella spp. *IpaB* gene	**IpaBF:** GGACTTTTTAAAAGCGGCGG **IpaBR:** GCCTCTCCCAGAGCCGTCTGG	**Per 50uL rxn:** 5uL Finzyme 10X buffer, 1.25uL dNTPs (10mM), 1.5uL BSA (10mg/mL), 1.5uL IpaBF primer (10uM), 1.5uL IpaBR primer (10uM), 1uL formamide, 0.75uL Finzyme, Hotstart Taq Polymerase. **Cycling:** 94˚C, 10min; 35 cycles of 94˚C 1min, 62˚C 1min, 72˚C 1min; 70˚C 8min	Kong et al. 2002
Staphylococcus aureus clfA gene	**clfAF:** GCAAAATCCAGCACAACAGG AAACGA **clfAR:** CTTGATCTCCAGCCATAATTG GTGG	**Per 50uL rxn:** 5uL Finzyme 10X buffer, 1.25uL dNTPs (10mM), 1.5uL BSA (10mg/mL), 0.5uL ClfAF primer (10uM), 0.5uL ClfAR primer (10uM), 0.75uL Finzyme, Hotstart Taq Polymerase. **Cycling:** 94˚C, 10min; 40 cycles of 94˚C 1min, 55˚C 1min, 72˚C 1min; 70˚C 8min	Mason et al. 2001
Escherichia coli strain O157:H7 *rfb* gene	**0157PF8:** CGTGATGATGTTGAGTTG **0157PR8:** AGATTGGTTGGCATTACTG	**Per 50uL rxn:** 5uL Finzyme 10X buffer, 1.25uL dNTPs (10mM), 1.5uL BSA (10mg/mL), 5.0uL 0157PF8 primer (10uM), 5.0uL 0157PR8 primer (10uM), 0.75uL Finzyme, Hotstart Taq Polymerase. **Cycling:** 94˚C, 10min; 40 cycles of 94˚C 30s, 55˚C 30s, 72˚C 30s; 70˚C 8min	Maurer et al. 1999

Table 26 - Summary of PCR analysis for various markers of human fecal pollution and pathogens for 2006 sampling.

Site ID	Site description	IDEXX fecal indicators* (MPN/100mL)	universal bacterial 16S rRNA gene	enterococci 23S rRNA gene	Human marker, enterococci *esp* gene	Human marker, *Bacteroides* HF8 cluster	*Salmonella* spp., *IpaB* gene	*Staphylococcus aureus*, *clfA* gene	*E. coli* O157:H7, *rfb* gene
060928L	Boggy Creek @ 51	EC = 132 ENT = 262	+	+	-	-	-	-	-
060928I	Cedar Island Beach	EC = 5794 ENT = 20	+	+	-	-	-	-	-
060928C	Creek at Dekle Beach	EC = 2254 ENT = 20	+	-	-	-	-	-	-
060928F	Keaton Beach	EC = 891 ENT = <10	+	-	-	-	-	-	-
061213DI	Cortez Rd Canal – Pump Station	EC = 187 ENT = <10	+	+	-	-	-	-	-

* EC = *Escherichia coli*; ENT = *Enterococcus*

May 2007 Results

During the last two sampling events in 2006, the molecular techniques approach proved to be independent of the previous day's microbial density. In other words, the sampling locations with the highest expected microbial counts were sampled for molecular techniques, and unfortunately, the previous day's counts did not necessarily match the following day's microbial counts for the selected sites (see Table 27). Three of the sites sampled in December 2006 did not have sufficient microbial material for the PCR to amplify. This was largely due to relatively low microbial counts (below trigger levels) and also the limited volume that could be filtered on site (190-300 mL) before filter blinding was achieved. Thus, it was recommended to expand the number of samples from four (4) to nine (9), in order to increase the chances of achieving confluent growth within 24 hours of extraction. In addition, a 1:25 dilution instead of 1:100 was applied to help increase the resolution.

Table 27 - Summary of microbial counts on the day before and the day of sampling for PCR analysis during 2006.

Site ID	Site Description	Detectable PCR?	*E. coli* Day before	*E. coli* Day of sampling	Enterococci Day before	Enterococci Day of sampling
060928L1	Boggy Creek @ 51	Yes	109	132	120	262
060928I1	Cedar Island Beach	Yes	>24,196	5794	63	20
060928C1	Creek at Dekle Beach	Yes	1552	2254	63	20
060928F1	Keaton Beach	Yes	1541	891	10	<10
061213C1	Creek at Dekle Beach	No	188 ± 4	183	10	10
061213DI	Cortez Rd Canal – Pump Station	Yes	63	169	<10	10
061213I1	Cedar Island Beach	No	84	145	<10	<10
061213M1	Upstream Steinhatchee River	No	20	148	41	62

The molecular biology research team from the National Oceanographic and Atmospheric Administration Atlantic Oceanographic and Meteorological Laboratories (NOAA-AOML) Ocean Chemistry Division again offered to attempt to analyze samples collected from the Taylor County study in an effort to determine if molecular techniques could be used as an effective tracer method. This is still an experimental process being developed for NOAA, with no guarantee of success for same. Samples from nine sites were collected. For each, three different molecular based assays were performed, including two sets of DNA analyses, one set of *E. coli* tracer tests, and one set of *Enterococcus* tracer tests.

For each of the nine sites, an additional 2-4 L of samples were collected in sterile Whirl-Pak bags and transported to the temporary field laboratory. The selected sites were picked based on source tracking hypotheses.

- PL1 (Petersons Landing). This site is located at the mouth of the Fenholloway River, which contains the industrial discharge from a specialty cellulose mill. This is the point at which the river empties to the ocean. Any microbial input should appear as natural because there is very little opportunity for human sewage input along the length of this industrial river. This should represent the microorganisms cultured in the treatment facility to remove the BOD in the aerated treatment lagoons of the mill.
- AB1 (Adam's Beach). This site is located downstream of a boat landing. No homes are located nearby. Therefore, human sewage pollution from OSTDS should be minimal. However, this site is characterized by historically high microbial densities.
- A1 (Dekle Beach). This site is located along the beach with a relatively high density of septic tanks nearby and remnants of historic septic tanks destroyed by a storm even last decade. This site should show signs of human sewage indicators.
- C1 (Creek at Dekle Beach). This site is the background site for Dekle Beach. The creek is tidally influenced but should not show strong signals of human sewage because there are no close human settlements upstream.
- F1 (Keaton Beach). This site is located at a public beach with sewer networks recently installed in 2005. This site should show weaker signals of human sewage pollution but may show strong indicators of bird-derived microbial indicators.
- G1 (Blue Creek). This site is the freshwater background site for Keaton Beach and Cedar Island. This site should show no signs of human-derived fecal indicators because no settlements are nearby and the surrounding areas have recently been converted to sewer.
- I1 (Cedar Island Beach). This site is Gulf-front property with historically high microbial indicator density. Sewer was recently installed in 2005, but the old drainfields are now submerged with recent sea level rise and beach erosion in this area. The presence of a

boat marina nearby with historically polluted sediments and muck may influence the readings here, which should theoretically show weak signals in terms of human sewage indicators.

- L1 (Boggy Creek @51). This site is a freshwater tributary of the Steinhatchee River. Over the past three sampling events, we noted unexpected findings even though this is supposed to be a natural background site. We are not sure what to expect here.
- FR1 (Fenholloway River @ 27). This site is the industrial discharge of a specialty cellulose mill located about 1 mile downstream of the plant. This site should show strong indicators of naturally-derived microbial tracers.

Samples were tested for the presence of amplifiable DNA and for PCR inhibition using primers that amplify a universal region of the bacterial genome (16S rRNA gene). As an additional control, samples were analyzed for the presence of Enterococci (23S rRNA gene targeting the Enterococci group including *E. faecalis, E. faecium, E. durans, E. casseliflavus, E. gallinararum,* and *E. hirae*). The lysates also were analyzed for the presence of several pathogens and markers of human fecal pollution.

Table 28 summarizes the PCR data for the May 2007 samples. It is in a similar format to the previous data summary table for the prior samples, except that PCR assays were added for a third human fecal marker (*Bacteroides* HuBac), for a dog marker (*Bacteroides* DogBac), and for an additional pathogenic bacteria (*Campylobacter jejuni*). Note that for environmental detection of human fecal sources, the *Bacteroides* HuBac marker is more sensitive than Enterococci esp or *Bacteroides* HF8 marker, and is potentially carried by a greater proportion of the human population (Sinigalliano 2007 personal communication). However, it may have some slightly greater cross-reactivity with dog fecal sources. For the dog marker, *Bacteroides* DogBac, it is not clear at this time just how prevalent this dog marker actually is among the wider domestic dog population, although studies are on-going. Thus it is not clear yet what sized dog population is required in the fecal input for environmental detection of this marker.

Table 28 - Summary of PCR analysis for various markers of human fecal pollution and pathogens from Taylor County samples collected on May 23, 2007.

Site ID	Site Description	IDEXX fecal indicators* (MPN/100mL)	Universal bacterial 16s rRNA gene	enterococci 23S rRNA gene	Human marker, enterococci *esp* gene	Human marker, *Bacteroides* HF8 gene cluster	Human marker, *Bacteroides* HuBac cluster	Dog marker, *Bacteroides* DogBac cluster	*Salmonella* spp., *ipaB* gene	*Campylobacter jejuni, HipO* gene	*E. coli* O157:H7, *rfb* gene	*Staphylococcus aureus, clfA* gene
070523AB1	Adams Beach	EC=ns ENT=ns Ratio=?	+	+	-	-	+	-	-	-	-	+
070523A1	Dekle Beach	EC=30 ENT=10 Ratio=3.0	+	-	-	-	+	+	-	-	-	+
070523C1	Creek @ Dekle	EC=82 ENT=<10 Ratio=>8.2	+	-	-	-	+	-	-	-	-	+
070523F1	Keaton Beach	EC=92 ENT=20 Ratio=4.6	+	+	-	-	+	-	-	-	-	+
070523G1	Blue Creek	EC=<10 ENT=20 Ratio=<0.5	+	+	+	-	+	+	-	-	-	+
070523I1	Cedar Island Beach	EC=41 ENT=<10 Ratio=>4.1	+	-	-	-	-	-	-	-	-	-
070523L1	Boggy Creek @51	EC=<10 ENT=86 Ratio=<0.1	+	+	-	+	+	-	-	-	-	+
070523FR1	Fenholloway River @98	EC=<10 ENT=72 Ratio=<0.1	+	+	-	-	-	-	-	-	-	-
070523PL1	Peterson's Landing	EC=41 ENT=97 Ratio=0.4	+	+	-	-	-	-	-	-	-	-

*EC = *E. coli*; ENT = *Enterococcus*

Overall, the molecular data of the analyzed water samples do not suggest a fecal source of contamination, although Boggy Creek, Fenholloway River, and Peterson's Landing are certainly over the recommended single-sample full-body contact exposure limit. None of the nine samples met the threshold criteria for using the Ec/Es ratio to check the findings of PCR analysis against this parameter. Only the Peterson's Landing site was close enough to attempt this analysis, and since the ratio is less than 0.4, this would indicate a natural or animal source, and the PCR analysis confirms no signs of human-derived pollution nor domesticated dog fecal markers.

While all three of the more contaminated sites (Boggy Creek, Fenholloway River, and Peterson's Landing) showed Enterococci detection by PCR, only Boggy Creek showed relatively high Enterococci abundance plus evidence of human-source fecal contamination (by two independent human-source *Bacteroides* markers). Thus Boggy Creek (site 070523L1) may warrant some closer scrutiny. Actual human fecal pathogens were not detected in any of the PCR samples. Six of the nine samples again contained enough Enterococci DNA to generate successful amplification, and in general these samples with detectable Enterococci DNA corresponded to the higher concentrations observed by the IDEXX Enterolert assays collected on the other sampling days at these same sites. Unlike the previous SLWT event in December 2006, there was greater evidence of potential human-fecal-source contamination, although it could not be quantified, but one site showed the presence of human-source Enterococci esp gene, another sample showed the presence of human source *Bacteroides* HF8, and six of nine samples contained human-source *Bacteroides* HuBac. In addition, two samples also indicated positive dog-source fecal contamination. It is interesting to note that the same six samples indicating presence of human source HuBac also coincidentally show presence of coagulase-positive *Staphylococcus aureus* clfA gene, which is considered a marker for the potentially antibiotic-resistant skin pathogen *S. aureus*. This is a striking difference from the December 2006 SLWT, as the presence of this putative skin pathogen was not seen in DNA extracts from previous Taylor County sampling events. No other pathogens were detected by PCR. In general (with the exception of Boggy Creek), most of the samples showing the presence of human-source *Bacteroides* were samples that had

Enterococci levels (by IDEXX Enterolert) well below the recommended exposure limits. However, in this assay, the abundance of the *Bacteroides* in the positive detects were not quantified.

September 2007 Results

Given the potentially promising results from the SLWT in December 2006 and May 2007, the source-tracking PCR analysis was also conducted in SHWT in September 2007. The real-time quantitative PCR (polymerase chain reaction) results for the *Bacteriodes* source tracking markers are tabulated in Table 29. For some of these samples, the measured quantities were lower than the prepared standard curve (those values were marked in red), but some of those were still above the levels considered for positive hits (those were marked with an asterisk).

Table 29. Real-time quantitative PCR results for *Bacteriodes* microbial source-tracking markers for September 2007

Site ID	Site Description	Volume filtered(mL)	Dilution	All Bac	HuBac	HF-8	Dog Bact
070919AB1	Adams Beach	300	1:25	440.9	0.0	0.0	3.7
070919A1	Dekle Beach	255	1:25	55.3	0.0	3.9*	4.9
070919C1	Creek at Dekle	350	1:25	40.9	0.9	0.0	2.1
070919F1	Keaton Beach	335	1:25	74.3	0.0	0.0	2.8
070919G1	Blue Creek	355	1:25	3.1*	4.4*	0.0	8.2*
070919I1	Cedar Island Beach	375	1:25	37.7	0.0	0.0	3.9
070919L1	Boggy Creek @51	200	1:25	27.7	3.2*	0.0	36.7
070919FR1	Fenholloway River @98	80	1:25	69.2	0.0	0.0	0.0
070919PL1	Peterson's Landing	135	1:25	32.2	7.6*	0.0	53.8

Note: Values in red indicate that these are below the lowest point on the standard curve.
* Indicates that the qPCR values is below the lowest point on the standard curve but still above the threshold for positive results

Table 30 summarizes the analysis from September 2007 SHWT testing with presence/absence PCR results for human source fecal contamination markers and pathogens. In general, human pathogens were not detected, but some samples were positive for markers suggesting human fecal contamination. Four samples (Fenholloway River, Blue Creek, Boggy Creek, and Petersons Landing) show the presence of some detectable *Enterococcus*, but this is not necessarily of human origin. One sample (Dekle

Beach) had a hit for human-source HF8 for *Bacteroides*. This may suggest the presence of human fecal contamination, another sample (Blue Creek) had a hit for the human-source enterococci esp. gene. In addition, three samples (Blue Creek, Boggy Creek, and Petersons Landing) showed the presence of the human-source *Bacteroides* HuBac marker. Both HF8 and enterococci esp. are relatively rare in the human population, so environmental detection may suggest possible sewage contamination. The finding of HF8 in Dekle Beach is not unexpected since the relatively large density of septic tanks discharges directly in this area. However, it is surprising to find enterococci esp. marker at Blue Creek, which is a background site. Nevertheless, the Blue Creek site is located relatively close to the wastewater infiltration basin, which may be influencing the surface water quality of the Blue Creek headwaters during certain times of the year.

It is interesting to note that the Fenholloway River, Blue Creek, Boggy Creek, and Petersons Landing sites also met the minimum threshold criteria for using the Ec/Es ratio. The Blue Creek site had a ratio of 2.0, which is indicative of transitional pollution with contributions from both animal and human sources. The PCR data confirmed contributions from both human and animal (dog) markers. Both the Boggy Creek and Fenholloway River sites had ratios less than one, which is indicative of a strong animal component. This was also confirmed by PCR analysis with the detection of dog *Bacteriodes*. However, evidence of human-associated *Bacteriodes* was also found. Similar PCR results were noted in Petersons Landing, although the Ec/Es ratio at this site was more indicative of human-derived pollution at 5.5, which is greater than the threshold of 4.0. Because the PCR analysis did not quantify the gene detection, we cannot estimate the relative contribution from each source. Nevertheless, the PCR results do seem to support the findings of the Ec/Es ratio, when applicable in both 2006 and 2007 testing.

Table 30 - Summary of PCR analysis for various markers of human fecal pollution and pathogens from Taylor County samples collected on September 19, 2007.

Site ID	Site Description	IDEXX fecal indicators* (MPN/100mL)	Universal bacterial 16s rRNA gene	enterococci 23S rRNA gene	Human marker, enterococci *esp* gene	Human marker, *Bacteroides* HF8 gene cluster	Human marker, *Bacteroides* HuBac cluster	Dog marker, *Bacteroides* DogBac cluster	*Salmonella* spp., *ipaB* gene	*Campylobacter jejuni, HipO* gene	*E. coli* O157:H7, *rfb* gene	*Staphylococcus aureus, clfA* gene
070919AB1	Adams Beach	EC = 11199 ENT = <10 Ratio = >1120	+	-	-	-	-	-	-	-	-	-
070919A1	Dekle Beach	EC = 528 ENT = <10 Ratio = >53	+	-	-	+	-	-	-	-	-	-
070919C1	Creek @ Dekle	EC = 2367 ENT = 20 Ratio = 118	+	-	-	-	-	-	-	-	-	-
070919F1	Keaton Beach	EC = 1951 ENT = 20 Ratio = 98	+	-	-	-	-	-	-	-	-	-
070919G1	Blue Creek	EC = 496 ENT = 253 Ratio = 2.0	+	+	+	-	+	+	-	-	-	-
070919I1	Cedar Island Beach	EC = 1459 ENT = 40 Ratio = 36	+	-	-	-	-	-	-	-	-	-
070919L1	Boggy Creek @51	EC = 178±78 ENT = 204±199 Ratio = 0.9	+	+	-	-	+	+	-	-	-	-
070919FR1	Fenholloway River @98	EC = <10 ENT = 269 Ratio = <0.1	+	+	-	-	-	-	-	-	-	-
070919PL1	Peterson's Landing	EC = 1025±170 ENT = 188±60 Ratio = 5.5	+	+	-	-	+	+	-	-	-	-

*EC = *E. coli*; ENT = *Enterococcus*

Compared to HF8 and enterococci esp., HuBac is much more common in the human population, so detection may suggest the presence of human fecal contamination, but this may be an individual point-source and might not represent actual sewage contamination (i.e. may be from a single latrine, septic tank, boat toilet, etc, although it may also be found in sewage as well). Keep in mind also, that PCR detection only signals the presence of gene markers, it does not discriminate if the detected cells are actually viable.

None of the PCR assays truly discriminate between septic or sewer as sources of contamination. They are only capable of determining differences between host organism sources, such as distinguishing between human, dog, cow, etc., which can potentially reflect differences in sources, such as terrestrial runoff containing domestic animal/livestock markers versus groundwater seepage contaminated with human-source markers from sewage or septic tanks. Therefore, it must be clearly stated that these molecular assays cannot distinguish if the human source marker in the environment is from a sewered source or a septic tank, or an untreated cesspit, or from baby diapers on the beach, for instance.

Nutrient Isotope Ratio

Nitrogen and oxygen isotope ratio experiments followed the procedures outlined in Heikoop et al. 2000; Sammarco et al. 1999; Risk and Erdmann 2000; Costanzo et al. 2004; among others. Samples for $\delta^{15}N/\delta^{14}N$ and $\delta^{18}O/\delta^{16}O$ were collected in 1.0 L sterile Whirl-Pak bags without preservative. They were field filtered using sterile 0.2 µm cellulose acetate syringe filters (VWR P/N 28145-477) and sterile 30 cc Leur-lok tip syringes (BD #309650, Franklin Lakes, NJ). These were transferred to precleaned 100 mL plastic sample bottles and immediately frozen on dry ice. The samples were transported to: Mark Altabet at SMAST/U Massachusetts Dartmouth, 706 S. Rodney French Blvd., New Bedford, MA 02744-1221. Dr. Altabet has developed a new experimental technique for testing source tracking hypotheses in water samples based on nitrogen/oxygen isotopes in aqueous nitrates and ammonia. These samples were analyzed after the nutrient analyses were completed. Based on the concentration levels in the sample, this provided a starting point to determine which isotopes of nitrogen and oxygen from nitrate/nitrite or just nitrogen from ammonium should be analyzed for.

The results of the May 2007 SLWT sampling event are summarized in Table 31. Briefly, seven (7) reliable analyses of the $\delta^{15}N$ and $\delta^{18}O$ of nitrate were possible. The ammonium analyses were inconclusive for the Fenholloway River and Petersons Landing sites for reasons unknown; however, there was insufficient sample to perform follow up testing, after the initial screening. That is why no values are reported for those two samples, and neither site could be plotted. Although these samples were clearly heavy with humic material, it could not be determined if that might have caused a problem with the analysis. In the future more than 125 mL of sample should be collected.

Table 31 - Summary of isotopic testing for May 2007 SLWT sampling event.

No.	Sample	Site	Reported NH_4^+(μM)	Reported NO_3^- (μM)	Instrument NO_3^- (μM)	NO_3^- $\delta^{15}N$	NO_3^- $\delta^{18}O$
1	070523AB1	Adams Beach	4.10	0.82	6.26	7.66	25.12
2	070523A1	Dekle Beach	0.07	0.07	3.83	8.26	24.66
3	070523C1	Creek at Dekle	2.10	0.48	1.57	3.71	22.01
4	070523F1	Keaton Beach	0.53	0.15	1.26	4.30	21.94
5	070523G1	Blue Creek	6.40	6.93	5.33	-0.63	7.03
6	070523I1	Cedar Island Beach	0.13	0.33	3.01	5.64	24.84
7	070523L1	Boggy Creek @ 51	5.10	1.60	2.01	2.52	15.35
8	070523FR1	Fenholloway River @ 98	163.7	21.0	0.71	na	na
9	070523PL1	Petersons Landing	27.8	21.6	*	na	na

* = insufficient sample volume; na = not analyzed

Deviations in nitrogen to phosphorus ratios are not always due to nitrogen inputs or sinks. Sometimes these differences are due to variations in the stoichiometry of nutrient uptake and re-mineralization. Furthermore, nitrate inputs and losses can partially mask each other if they occur in same water body or if their sources become completely mixed. To address this issue, complementary measurements of $^{15}N/^{14}N$ ratios from nitrate can provide clues as to whether the isotopic enrichment is driven by nitrogen fixation or denitrification. Most of the deep ocean is homogenous with respect to $\delta^{15}N$, typically taken as 5‰ relative to atmospheric nitrogen (Liu and Kaplan 1989; Sigman et al. 2000). Nitrogen fixation reduces the value to -2 to 0‰ relative to atmospheric nitrogen, while denitrification preferentially consumes ^{14}N, which has the effect of producing a marked increase in the ratio into the range of 20-30‰ relative to atmospheric nitrogen (Sigman et al. 2005). However, the N isotopes can suffer from counteracting effects from nitrogen fixation and denitrification, such that for instance, nitrogen fixation in subtropical marine surface waters adds new nitrate and decreases the $\delta^{15}N$, while simultaneous denitrification removes low-$\delta^{15}N$ nitrate from suboxic regions and raises the $\delta^{15}N$ of the residual nitrate. If the two processes occur in the same region or if waters with multiple sources are mixed vigorously, the tracer signals of both processes will be reduced. However, the $\delta^{18}O$ from newly produced nitrate does not depend on the origin (i.e. from newly fixed nitrogen, from biomass, etc.) of the ammonium being nitrified. Thus if the oxygen is fractionated at the same extent as the nitrogen, then denitrification (anoxic process) is dominant. Therefore, Sigman et al. (2005) propose that 1:1 $\delta^{18}O$:$\delta^{15}N$ elevation signals denitrification-dominant sources of nitrate.

As far as interpretation, samples 1 to 4 and 6 (Adams Beach, Dekle Beach, Creek at Dekle, Keaton Beach, and Cedar Island Beach) from May 2007 SLWT have moderate $\delta^{15}N$ and high $\delta^{18}O$. This signature is most consistent with a commercial nitrogen fertilizer source. Their $\delta^{18}O$ when plotted against $\delta^{15}N$ fall near a 1:1 line (Figure 35) showing the variation amongst them is likely due to algal utilization increasing both together (Sigman et al. 2005). Most commercial fertilizer products are around 89% ammonia. Thus, these sites should also have elevated levels of ammonium, if fertilizers are an important source. Note these samples have fairly high (>0.07 mg N/L or >5.0 μM) ammonium levels (refer to Table 31), which is also consistent with relatively recent fertilizer inputs. Samples 5 and 7 (Blue Creek and Boggy Creek) clearly fall below the 1:1 line and are more likely to have nitrate produced from nitrification that is mixing in with the fertilizer source to varying degrees. It is interesting to note, that both of these sites are freshwater compared to the other sites, which are marine. For both of these sites, the $\delta^{15}N$ values are lower, indicating a potential groundwater influence that might be indicative of OSTDS usage (past or present) or excessive fertilization practices.

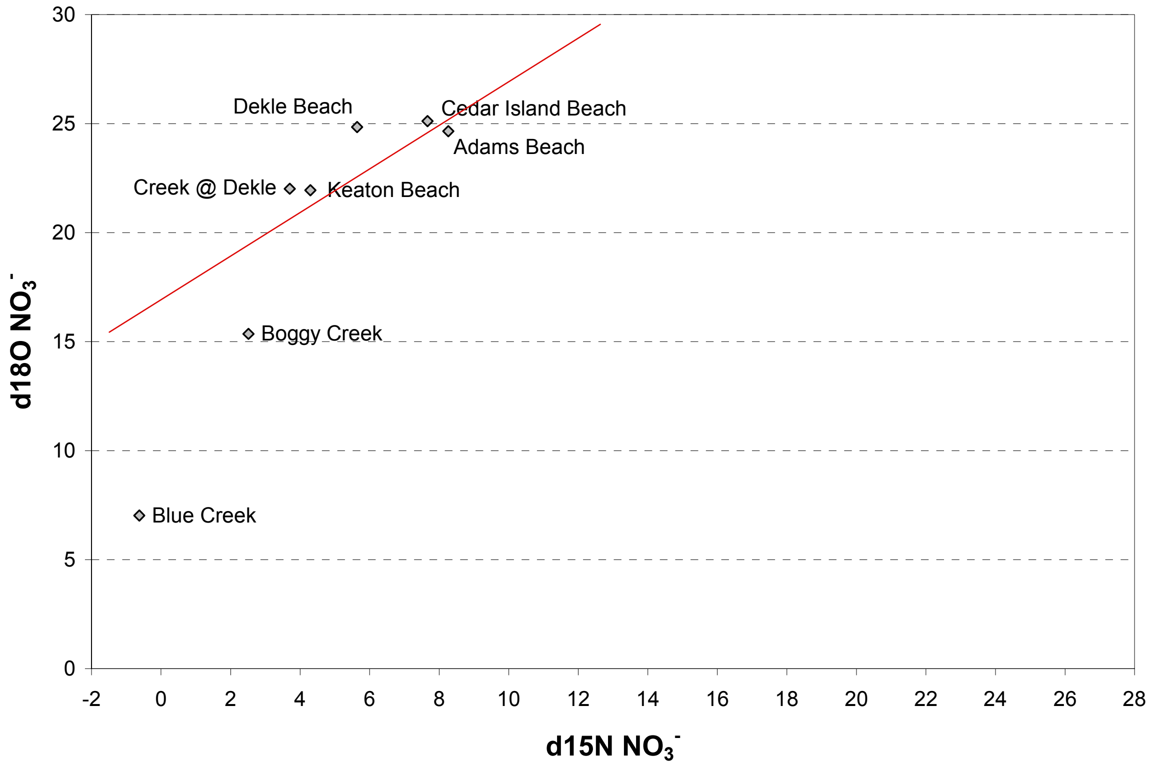


Figure 35 - Plot of $\delta^{18}O$ versus $\delta^{15}N$ for isotopic samples from May 2007 SLWT sampling event.

Isotope testing from May 2007 SLWT also revealed a higher nitrate level than those reported in samples 1-4 (Adams Beach, Dekle Beach, Creek @ Dekle, and Keaton Beach) and 6-7 (Cedar Island Beach and Boggy Creek). Since nitrate contamination in the laboratory and in the instrument was not found, it is possible that the reported nitrate levels were actually lower than what was found in the sample during isotope analysis; however, the nutrient testing was performed with different methods by two different laboratories, which could account for the discrepancy. In addition, a third party analysis of the total nitrogen levels seems to support the reported values. Thus, the enhanced levels found in the isotope analysis may have resulted from column bleed from the colored humic-rich samples, which had nitrogen levels that were 20 times higher than the other samples. This column bleed enrichment could not be verified because of insufficient sample volume remaining after the analysis. During the September 2007 event, this was remedied by using dilutions, blind standards, and replicates.

The results of the September 2007 SHWT sampling event are summarized in Table 32. Briefly, seven (7) reliable analyses of the $\delta^{15}N$ and $\delta^{18}O$ of nitrate were possible. In this event, the Dekle Beach and Keaton Beach nitrate levels were too low to perform the test. Compared to the SLWT event, only two of the samples (Adams Beach and Creek at Dekle) have moderate $\delta^{15}N$ and high $\delta^{18}O$. This signature is most consistent with a commercial nitrogen fertilizer source, just as seen in SLWT for both of these two sites. However, their $\delta^{18}O$ when plotted against $\delta^{15}N$ (Figure 36) fall below the same 1:1 line that was plotted for the May 2007 SLWT data, thus showing that the observed variation is less likely due to algal utilization increasing both together, as was seen in SLWT. These sites show that more nitrogen fixation and assimilation is occurring than was seen in the SLWT in May 2007. Furthermore, if fertilizers are an important source, these sites should also have elevated levels of ammonia-nitrogen. However, only the Creek @ Dekle site shows elevated ammonium (refer to Table 32), which is also consistent with relatively recent fertilizer inputs.

Table 32 - Summary of isotopic testing for September 2007 SHWT sampling event.

No.	Sample	Site	Reported NH_4^+(μM)	Reported NO_3^- (μM)	NO_3^- $\delta^{15}N$	NO_3^- $\delta^{18}O$	NH_4^+ $\delta^{15}N$
1	070523AB1	Adams Beach	0.07	3.15	1.47	19.4	na
2	070523A1	Dekle Beach	0.54	0.15	na	na	na
3	070523C1	Creek at Dekle	8.00	1.86	2.72	12.1	-12.6
4	070523F1	Keaton Beach	1.40	0.17	na	na	na
5	070523G1	Blue Creek	6.90	6.20	-7.17	5.6	-13.1
6	070523I1	Cedar Island Beach	2.50	0.83	-1.42	14.6	na
7	070523L1	Boggy Creek @ 51	3.40	6.65	-15.59*	4.5*	na
8	070523FR1	Fenholloway River @ 98	187.70	38.00	-23.07*	6.8*	-2.1
9	070523PL1	Petersons Landing	44.15	26.80	-6.37	7.1	4.3

* = insufficient sample volume for repeated analysis (values reported are estimates); na = not analyzed

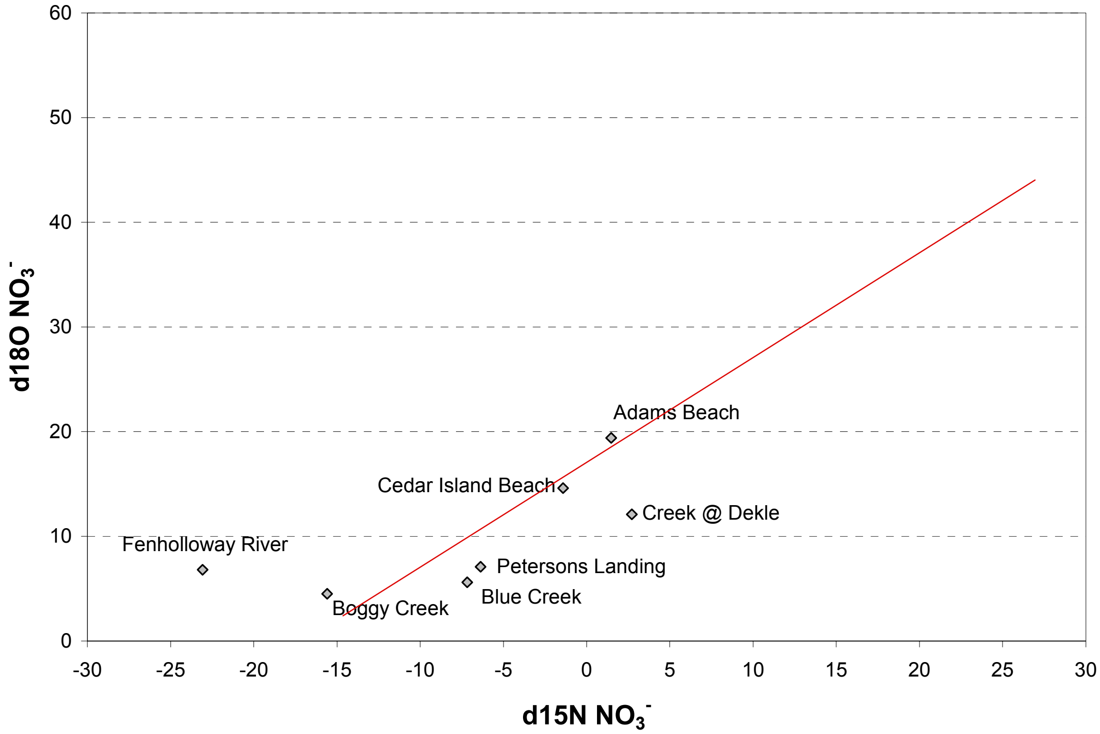


Figure 36 - Plot of $\delta^{18}O$ versus $\delta^{15}N$ for isotopic samples from September 2007 SLWT sampling event

Shallow Sediment Re-Growth Studies

During May 2006 SHWT sampling at Cedar Island, 2 out of 2 samples showed indications of human sources based on the microbial sample analysis. This sample set was too small to draw any meaningful conclusions. However, when all of the data (n = 21) was compiled for Cedar Island for the first three sampling events (May 2006, September 2006, and December 2006), all sites showed signs of human pollution, despite being converted to a sewer network from septic tanks during the course of the study. This potentially indicates that another source, such as possible regrowth in warm, shallow, stagnant waters may be responsible (Solo-Gabriele et al. 2000).

The coastal areas in this study are characterized by Bayvi soil, which is a relatively deep, poorly-drained, sandy soil. This type of material is similar to the soils that have been found to harbor pathogen indicator regrowth in studies conducted in South Florida (Solo-

Gabriele et al. 2000). To identify if shallow regrowth is acting as a legacy reservoir of microbial pathogen indicators, studies of persistence under harsh environmental conditions were conducted.

For shallow sediment studies, microbial indicators were extracted from soil samples using a modified version of the procedure outlined by Van Elsas and Smalla (1997). First suitable soil samples were collected in sterile Whirl-Pak bags. Approximately half of the bag contained sediment and overlying water. Samples were immediately stored at 4°C and kept overnight for analysis the next day. To enumerate the organisms in the sediment samples, two preliminary steps were performed. The first step was to measure the moisture content of the sand by recording the mass difference before and after drying (105°C for 24 h) approximately 50 – 60 g of sample on pre-weighed dishes. Samples were placed in the dessicator for at least one hour prior to measuring the final mass. The second step was to extract the microorganisms from the sand particles to a predetermined volume of sterile water. To accomplish this, 50 – 60 g (1/8 cup) of wet sand was aseptically removed from the sterile sample bag using a stainless steel scoop that was flamed in ethanol for sterilization. This material was placed into a new sterile Whirl-Pak bag using a sterilized sample spoon to remove the sediment from the scoop, as needed. Sterile phosphate buffer solution was prepared in 1.0 Liters of reagent water with the following added to it: 1 mL/L of phosphate buffer solution prepared by dissolving 42.5 g KH_2PO_4 crystals and 1.7 g NH_4Cl in 700 mL of reagent water and adjusting to pH 7.2 with 30% NaOH before diluting to 1.0 L; 1 mL/L of magnesium sulfate solution prepared by dissolving 22.5 g $MgSO_4 \cdot 7H_2O$ in 1.0 L of reagent water; 1.0 mL/L of calcium chloride solution prepared by dissolving 27.5 g of $CaCl_2$ in 1.0 L of reagent water; and 1.0 mL/L of ferric chloride solution prepared by dissolving 0.25 g of $FeCl_3 \cdot 6H_2O$ in 1.0 L of reagent water. After mixing, the solution was sterilized in the autoclave and brought to room temperature. Then 50 mL of sterile phosphate buffer dilution water (PBS) was added to each container using sterile 10 mL serological pipets and manually shaken vigorously for 120 seconds. Then the slurry was decanted into a pre-sterilized coffee filter (#4 grade), which were sterilized using an ultraviolet lamp for 30 minutes on each side (May 2007) or autoclaved after being wrapped in aluminum foil (September 2007).

The filtrate was collected into another sterile Whirl-Pak bag. An additional 50 mL of PBS was used to remove the sand from the container. All of the additional liquid and sand were filtered and combined. The final volume of filtrate was recorded, and this filtrate was analyzed for re-growth of microbial indicators. Samples were stored at 4°C, and the procedure was then repeated again 120-168 hours (5-7 days) later. A summary of the initial results from sediment regrowth studies is found in Table 33 and Table 34.

Table 33 - Summary of sediment regrowth studies for the May 2007 SLWT sampling event.

Sample	Site	Solids content (%)	*E. coli* (MPN/100mL per g dry solids)			*Enterococcus* (MPN/100mL per g dry solids)		
			05/24/07	05/29/07	Ratio*	05/24/07	05/29/07	Ratio*
070523AB	Adams Beach	75.1	3.2	4.7	1.5	3.1	0.5	0.2
070523A	Dekle Beach	77.3	10.5	26.7	2.5	0.7	<0.2	<0.3
070523F	Keaton Beach	82.9	4.0	8.3	2.1	0.2	<0.2	<1.0
070523I	Cedar Island Beach	80.7	3.5	149.5	42.7	0.4	1.1	2.8

*Ratio = 2nd day value divided by initial value

If the ratio of the second day value to the initial value is greater than unity, then the sample exhibits a capability for regrowth. For the May 2007 SLWT event, all four *E. coli* samples showed signs of potential regrowth with ratios greater than one. In particular, the Cedar Island Beach site shows the strongest regrowth with almost 50 times more colonies per gram of dry sediment. In terms of *Enterococcus*, only Cedar Island Beach shows any potential for harboring these pathogen indicators and protecting them from desiccation during the tidal cycle.

For the September 2007 SHWT event, none of the sediments from any of the four sites tested showed signs of regrowth potential for either microorganism indicator. This could suggest a seasonal phenomenon, although the sample size is too small to make a definitive conclusion. Further study is warranted.

Table 34 - Summary of sediment regrowth studies for the September 2007 SHWT sampling event.

Sample	Site	Solids content (%)	*E. coli* (MPN/100mL per g dry solids)			*Enterococcus* (MPN/100mL per g dry solids)		
			09/19/07	09/26/07	Ratio*	09/19/07	09/26/07	Ratio*
070920AB	Adams Beach	82.6	8.8	<0.2	<0.02	<0.2	<0.2	<1.0
070920A	Dekle Beach	71.5	41.9	<0.3	<0.01	0.5	<0.3	<0.5
070920F	Keaton Beach	80.2 ± 0.4	4.6	<0.2	<0.05	<0.4	<0.2	<0.5
070920I	Cedar Island Beach	84.9	1.9	<0.2	<0.12	0.2	<0.2	<1.0

*Ratio = 2nd day value divided by initial value

Existing Infrastructure Assessment

Information regarding the hydraulic regime of the Blue Creek estuary, the existing sewer network and OSTDS in the study area (which may include types of systems, ages, depths to ground water table elevation, catalog of sewer leak events, and septic failures), and any existing upstream industrial wastewater discharges will be collected through literature review, record review at the Taylor County Health Department and Taylor Coastal Utilities, and interviews. This work is ongoing, and results will be forthcoming.

Summary of Wastewater Treatment Plant Site Visit:

On May 25, 2007, the FAU team met with David Morgan (Wastewater Treatment Plant Operator) and drove to the facility located on 18820 Beach Road, Perry, FL, roughly between Keaton Beach and Cedar Island just inland of Beach Road off Spoonbill Road. Mr. Morgan informed us that the collection system consists of two major lift stations (Keaton Beach and Blue Creek church) and a pressurized sewer network with grinder pumps at each household connection. Typical flowrates are on the order of 12,000 gpd with annual maximum daily flows up to 80,000 gpd in summer (Memorial Day weekend). The treatment facility consists of a package activated sludge plant with integrated aeration system, clarifier, and chlorine bleach (NaOCl) disinfection, a holding pond, a spray irrigation field, an office/work-shop, and a back-up power generator.

Figure 37 - Photos from the Taylor Coastal Centralized Wastewater System Phase 1 Wastewater Treatment Facility (May 24, 2007) showing the package activated sludge plant (top left), holding pond (top right), spray field disposal system (bottom left), and office/workshop (bottom right).

According to Mr. Morgan, the sewer networks were installed in the following order during Phase 1 improvements: 1) Keaton Beach, 2) Ezell Beach, and 3) Cedar Island. Phase 2 will address Dekle Beach, Dark Island, and Fish Creek, which remain on OSTDS. The collection network consists of 1-1/4-inch pipe at the home connecting to 3-inch or 4-inch mains within the neighborhoods that connect to larger 6-inch or 8-inch force mains to the plant. In the winter, the package plant is fed with corn due to extremely low flows from few winter residents. Construction of Phase 1 of the conversion-to-sewer process (for about 450 customers) began approximately in January 2006. The engineering consultant for the job is JEA (Jones, Edmunds, and Associates).

Information that is still to be collected includes the following:

1. Timeline of construction and installation activities
2. Number of tanks replaced
3. Number of customers served

On the potable water side, the drinking water source is groundwater from three coastal wells that pump about 92,000 gpd each from the Floridan Aquifer. The Florida Department of Environmental Protection (FDEP) has performed a source water assessment on the system, which indicated no potential sources of contamination near the wells. The assessment results are available on the FDEP Source Water Assessment and Protection Program website at www.dep.state.fl.us/swapp. According to the 2005 Consumer Confidence Report, no violations were detected from 2003 to 2005, although As, Ba, Cr, Ni, Na and nitrate were detected (but below the maximum contaminant level).

FURTHER DATA ANALYSIS

Differences Between OSTDS and Sewer Sites

A premise of the QAPP was that differences will exist between the water quality in Seasonal High Water Table elevation events and Seasonal Low Water Table elevation events. A similar analysis was conducted in urban coastal South Florida, and significant differences were found. A similar trend was expected in the rural Taylor County sites investigated, but not necessarily as significant due to the bimodal distribution of the seasonality. In the first step, sewered and non-sewered sites were compared in both seasons. During the SLWT, sites were expected to have similar bacteriological indicator densities, and during the SHWT, sites served by OSTDS were expected to have higher concentrations. Table 35 shows the definitions of the variables used in the t-Test, and Table 36 summarizes the results and if the tested hypotheses are true or false in a confidence interval (C.I.) of 95%.

Table 35 - Definition of the variables used in the t-Test analysis.

Variables	Description
Var. 1	Parameter mean for sites with sewer
Var. 2	Parameter mean for sites served by OSTDS

Table 36 – Summary of the Student t-Test results for Taylor County according to wastewater treatment and disposal

Hypotheses of equal means	Mean Var. 1	Mean Var. 2	t	df	Result 95% C.I.	Expected
During SLWT:						
E. coli: Var. 1 = Var. 2	228	309	-0.70	56	True	True
Total Coliform: Var. 1 = Var. 2	3886	5039	-1.04	58	True	True
Enterococcus: Var. 1 = Var. 2	12	31	-2.39	44	False	True
During SHWT:						
E. coli: Var. 1 = Var. 2	1473	1087	0.60	20	True	False
Total Coliform: Var. 1 = Var. 2	12918	11622	0.45	18	True	False
Enterococcus: Var. 1 = Var. 2	25	84	-3.07	24	False	False

In Taylor County, during the SLWT, *E. coli* and total coliforms were similar in both sewer and OSTDS sites, as expected. However, *Enterococcus* was significantly higher in the sites served by OSTDS. During the SHWT, the differences were expected to be caused by OSTDS not functioning as efficiently, resulting in an increased load of bacteriological indicators to adjacent waters. Therefore, significant ($p < 0.05$) differences between sewered and non-sewered sites were anticipated. The results in Table 36 show that *E. coli* and total coliforms concentrations were statistically similar in both sites. A possible explanation for this observation is potential re-growth or some other persistent local source (not necessarily of human origin) happening at the sewered sites (Keaton Beach and Cedar Island). *Enterococcus* densities were statistically elevated in the sites with OSTDS, but as OSTDS sites had initially higher concentrations of *Enterococcus* than sewered sites, the ratio of the differences was also analyzed. During the SLWT the ratio between *Enterococcus* at OSTDS sites and sewered sites was 2.6, while during the SHWT the ratio increased, with OSTDS sites having concentrations 3.4 times those with sewer.

Seasonality

A similar statistical analysis was performed focusing on the season factor. The expected result was that bacteriological counts would be higher during the SHWT, particularly in the sites served by OSTDS. Table 37 shows the definitions of the variables used in the t-Test. Table 38 summarizes the statistical findings and states if the tested hypotheses are true or false in the confidence interval (C.I.) of 95% based on a $\log_{10}$-normal distribution of the data (sorting by bins).

Table 37 - Definition of the variables used in the t-Test analysis.

Variables	Description	Distribution
Var. 1	Parameter mean during the SLWT	Log normal
Var. 2	Parameter mean during the SHWT	Log normal

Table 38 - Summary of the Student t-Test results for Taylor County according to season (logs of bacterial counts are used since the underlying distribution was distributed log-normal).

Comparison	Mean	Mean	t	df	Significant	Expected
Variable 1 x Variable 2	**Var. 1**	**Var. 2**			**95% C.I.**	
Sites with sewer:						
E. coli: SLWT x SHWT	2.10	2.95	-4.21	15	True	No
Total Coliform: SLWT x SHWT	3.42	4.01	-4.30	19	True	No
Enterococci: SLWT x SHWT	0.94	1.25	-2.25	14	True	No
Sites with OSTDS:						
E. coli: SLWT x SHWT	1.95	2.47	-2.40	38	True	Yes
Total Coliform: SLWT x SHWT	3.46	3.95	-4.32	53	True	Yes
Enterococci: SLWT x SHWT	1.25	1.66	-3.04	35	True	Yes

In Taylor County, both sewered and non-sewered sites were significantly ($p < 0.05$) affected by season effects. No significant difference was anticipated between seasons in the sewered sites, so any observed increases in bacteriological indicators are believed to be attributable to runoff contributions, higher water temperatures and because no rainfall events coincided with the sampling events, a function of periodic events such as over-fertilization, over-irrigation of lawns caused by drought conditions, or alternatively, some persistent local source. All bacteriological indicators were higher during the SHWT in the sites with sewer. The only increase in counts that were not significant ($p < 0.05$) in the sewered sites was that of *Enterococcus*. In the sites with OSTDS, the expected increase in concentration of bacteriological indicators was observed. This increase was significant ($p < 0.05$) for TC and *Enterococcus*, but not for *E. coli*. Because the effect of sewage treatment and disposal alternative in the bacteriological data is not clear from this analysis, an analysis of variance (ANOVA) should clarify which differences were due to OSTDS or seasonality only. From the results of the t-Test, the effects of OSTDS were not as strong as expected, but two major factors may be influencing the results: drought conditions and potential re-growth of *E. coli* in some of the sewered sites in Taylor County.

In order to confirm the findings suggested by the trend analyses, ANOVA was performed. ANOVA allows the confirmation of the significance of the factors of interest on the concentration of the measured water quality indicators, and to evaluate if interactions between the factors of interest result in cumulative effects. The major factors under study were: sewage treatment and disposal alternative (sewer vs. OSTDS), and season (SLWT vs. SHWT). Before ANOVA could be performed, although, the following steps were taken: data transformation, correlation analysis, and principal component analysis (PCA).

ANOVA assumes that the data analyzed is normally distributed. If data is drastically skewed, then results are compromised. A frequency histogram was plotted for all measured variables to evaluate which parameters violated the normality assumption. If normality was violated by extremely skewed data, transformations were applied. No transformations were necessary for the following physical parameters: water temperature, pH, DO, SC, conductivity, TDS, and salinity. Turbidity, TOC, TN, and all of the bacteriological indicators had skewed distributions. Table 39 shows the skewness of the original data for each transformed parameter and the data skewness after the transformations were applied.

Table 39 – Skewness after data transformation.

Parameter	Original	Log	Inverse	Sq. root
Turbidity	2.98	0.91	1.78	1.86
TOC	3.19	0.73	2.68	0.78
TN	1.19	0.86	9.89	1.02
E. coli	4.97	0.75	4.40	1.40
TC	0.70	1.01	6.70	0.06
Enterococcus	2.04	0.07	1.02	0.07

The transformation that resulted in the lowest skewness for each parameter was selected for use. The following are the applied transformations:

- Log of (Turbidity +1)
- Log of TOC
- Log of (Total nitrogen + 1)
- Log of *E. coli*
- Square root of Total Coliform
- Log of *Enterococcus*

After transformations were applied, total coliform data was normally distributed. Turbidity, TOC, TN, *E. coli* and *Enterococcus* were still not normally distributed, but data were more symmetric after the log transformation.

This analysis was undertaken to evaluate the strength of the correlations between the measured water quality parameters. An important reason to perform correlation analysis is to identify if the analysis of variance (ANOVA) should be performed directly on the original data, or if artifices (i.e. principal components) are needed in order to eliminate highly correlated variables, which complicate further analysis. It must be stated that a significant ($p < 0.05$) correlation does not indicate cause or direction of the relationship. The correlation analyses were done in the following data sets: 1) data grouped by wastewater treatment and disposal alternative, and 2) data grouped by season.

The following eleven variables were studied: pH, SC, conductivity, TDS, salinity, turbidity, TOC, TN, *E. coli*, TC, and *Enterococcus*. The parameters were grouped in eleven components, all of the components representing a different combination of the eleven measured variables. The order of the variables does not affect the results. From Figure 38 it can be seen that most of the variation in this data set can also be accounted for by the first two components: 72% of the data variance.

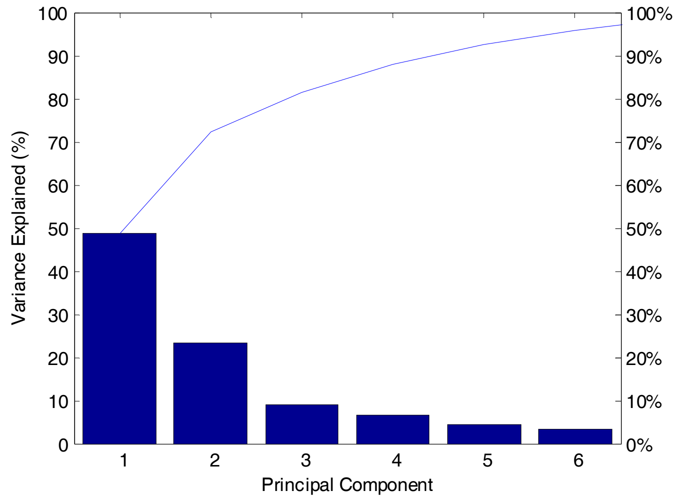


Figure 38 - Individual and cumulative variation for the first 6 principal components out of 11 in Taylor County.

The first two principal components (PCs) were retained to represent the original data set. Below is the general equation that describes the new data set:

$$NewData = a_{ij}x_j + a_{ij}x_j + ... + a_{i,j}x_j + ... \quad \Big\} \quad \text{PC1} = 49\% \text{ of the variance}$$

$$...a_{ij}x_j + a_{ij}x_j + ... + a_{i,j}x_j \quad \Big\} \quad \text{PC2} = 23\% \text{ of the variance}$$

Where $a_{i,j}$ are the estimated coefficients (output of principal component analysis) for the i^{th} principal component of the j^{th} dependent variable. Table 40 shows the calculated coefficients, eigenvalues, and percentage of variance accounted by PC1 and PC2. On the right side is the simplified representation of the PCs retained. To facilitate the interpretation of the components, variables with coefficients higher than half of the maximum coefficient in that component were substituted by a check mark "√". If the coefficient of a variable was below this criterion, the variable was not considered important for the principal component interpretation, and denoted by "nr," similar to the process outlined in Jolliffe (2002).

Table 40 – Principal component analysis results for Taylor County.

	Coefficients (a)	
Variable, X (j)	$a_{1,j}$	$a_{2,j}$
pH (1)	-0.34	-0.02
SC (2)	-0.42	-0.09

	Coefficients (a)	
Variable, X (j)	$a_{1,j}$	$a_{2,j}$
pH (1)	√	nr
SC (2)	√	nr

	Coefficients (a)	
Variable, X (j)	**$a_{1,j}$**	**$a_{2,j}$**
Cond. (3)	-0.42	0.00
TDS (4)	-0.42	-0.09
Salinity (5)	-0.42	-0.10
Turbidity (6)	0.15	0.33
TOC (7)	0.11	0.47
TN (8)	-0.13	0.47
E. coli (9)	-0.30	0.38
TC (10)	-0.17	0.43
Ent. (11)	0.09	0.32
Eingenvalues	5.41	2.49

	Coefficients (a)	
Variable, X (j)	**$a_{1,j}$**	**$a_{2,j}$**
Cond. (3)	√	nr
TDS (4)	√	nr
Salinity (5)	√	nr
Turbidity (6)	nr	√
TOC (7)	nr	√
TN (8)	nr	√
E. coli (9)	√	√
TC (10)	nr	√
Ent. (11)	nr	√
Variance	49.18	22.66

From Table 40, it can be seen that PC1 is a weighted average of physical parameters with a contribution from *E. coli*. The PC2 is a weighted average of turbidity, TOC, TN, *E. coli*, TC, and *Enterococcus* (nutrients and bacteriological parameters). Turbidity was grouped with nutrient and bacteriological data, which is not unexpected because turbidity is likely to be associated with runoff and bacterial counts. In addition, nutrients are also expected to be associated with runoff. Below are the equations for PC1 and PC2.

$$PC1 = -0.34\ pH - 0.42\ SC - 0.42\ Cond. - 0.42\ TDS - 0.42\ Sal. + 0.15\ Turb. \ldots$$

$$\ldots + 0.11\ TOC - 0.13\ TN - 0.30\ \textit{E. coli} - 0.17\ TC + 0.09\ \textit{Ent.}$$

$$PC2 = -0.02\ pH - 0.09\ SC. - 0.09\ TDS - 0.10\ Sal. + 0.33\ Turb. \ldots$$

$$\ldots + 0.47\ TOC + 0.47\ TN + 0.38\ \textit{E. coli} + 0.43\ TC + 0.32\ \textit{Ent.}$$

ANOVA was used to evaluate the stated hypothesis that the major factors significantly ($p < 0.05$) affect the water quality of coastal environments. As stated earlier, the major factors were: sewage treatment and disposal alternative (sewer vs. OSTDS) and season (SLWT vs. SHWT). ANOVA also permits the evaluation of the significance of interactions between factors. When this study was designed, it was expected that both factors and the interactions were important. Analysis of the t-Test results, summary of statistics, data grouping, and trend analysis suggested that not all of the expected effects seemed to be significant. ANOVA was performed, and Table 41 shows the p-values for the significant ($p < 0.05$) effects.

Table 41 – Summary of the results for ANOVA

Comparison	Main Effect (p)		Interactive Effect (p)
	Sewer	Season	Sewer.Season
PC1	ns	ns	ns
PC2	ns	<0.000	ns

Legend: na – not applicable; ns – not significant (p>0.05).

Neither of the two factors had a significant impact on the physical water quality parameters (PC1 = pH, SC, conductivity, TDS, salinity). The trend analysis suggested that season affected the concentration of the physical parameters, but when the physical parameters were grouped together in the PC1, the seasonal impact was not significant. In rural Taylor County, DO values suggested a negative impact of OSTDS on the water quality. While average values of DO in sites with sewer was 6.4 mg/L, the DO average in the sites with OSTDS was 5.1 mg/L. Also, when season is considered, DO values on the sites served by OSTDS suffered a 45% decrease in concentration between the SLWT and SHWT, while in the sewered sites, the decrease was only 33%. Overall, however, wastewater treatment and disposal alternative was not a significant ($p < 0.05$) factor for the pooled physical water quality parameter data.

Since the SHWT exhibited elevated concentrations of nutrients and bacteriological indicators when compared with SLWT during the trend analysis, it was expected that the principal component for these parameters would confirm the difference. For the bacteriological results alone, the t-Test showed that season was an important factor for the bacteriological concentrations in both sewered and non-sewered sites. The only significant factor for data variation in PC2 (nutrients and bacteriological results) was season, and no significant differences were noted between sewer sites and sites served by OSTDS or the interaction term.

As seen in the trend analysis and t-Test, the only bacteriological indicators that suggested OSTDS contribution to increased pollutant loading to adjacent surface waters was

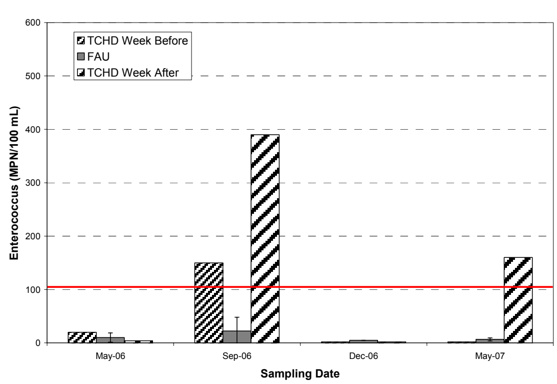
Enterococcus. In the ANOVA, when all the bacteriological parameters and nutrient data were grouped into a single component (PC2), the significance of sewer vs. OSTDS on the data variance with respect to *Enterococcus* was not observed. This can be explained by the following: 1) TN concentrations that were actually higher for the sites with sewer, were inflated by the Blue Creek (Background site) concentrations, 2) *E. coli* and TC that had similar concentrations in both sewered and non-sewered sites suggest potential re-growth in some of the sewered sites or even a persistent local source, and 3) the drought period when the data was collected, which may have halted the expected decline in efficient operation of the OSTDS during the SHWT.

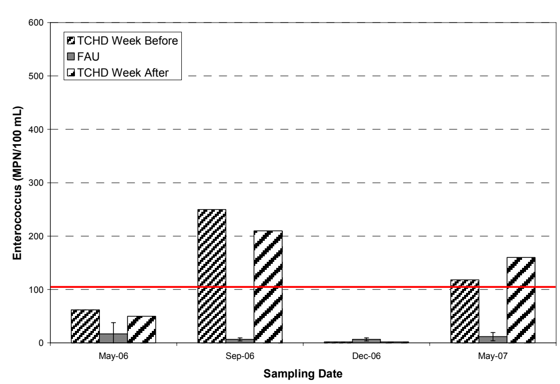
In summary, seasonality was not a significant ($p < 0.05$) factor for the physical water quality parameters, although a decrease in the DO values were observed in both sewered (33%) and non-sewered sites (44%) from the SLWT to the SHWT. For the nutrient and bacteriological data, season was a significant ($p < 0.05$) factor, even though the SHWT contribution due to runoff and OSTDS inputs may have been inhibited by the drought conditions in 2006. TN and TOC concentrations were generally higher during SHWT when compared to the SLWT. Ammonia values, however, were higher during the SLWT. This finding suggests a potentially important contribution from irrigation runoff during the sampling event on May 2006, although closer inspection of the data reveals that this may be an artifact caused by changing analytical methods from May 2006 to the subsequent four sampling events. The bacteriological results, as expected, showed higher microbial indicator densities during the SHWT, average values for both sewered and non-sewered sites together shows that the ratio between SHWT and SLWT concentrations were: 4.4 for *E. coli*, 2.5 for TC, and 2.5 for *Enterococcus*.

For the bacteriological data, *E. coli* concentrations were fairly similar between sites with sewer and sites with OSTDS. TC densities were slightly higher at sites served by OSTDS when compared to the sites with sewer. *Enterococcus* showed a possible impact of OSTDS in the water quality of adjacent surface waters because levels were slightly higher at sites served by OSTDS. During the SLWT, the average concentration of *Enterococcus* in the sites with OSTDS was 2.6 times higher than the average concentration in the sites with sewer. During the SHWT, this ratio increased to 3.4. However, when the bacteriological data is pooled together in the PC2, ANOVA shows that the effect of wastewater treatment and disposal alternative is not significant ($p < 0.05$). Drought conditions in 2006 may have dampened the expected increase in SHWT groundwater table elevation, which may have masked any potential OSTDS contribution to adjacent water bodies. The low population density may be another reason why the impact of OSTDS in the area was not observed to be significant, when compared to findings in coastal urban South Florida.

Comparison of Daily Samples with Florida Healthy Beaches Sampling

During this study, the Taylor County Health Department (TCHD) also collected weekly samples for enterococci as part of the requirements for the Florida Healthy Beaches monitoring program. Since these samples were analyzed using a different methodology, it was suggested that the results might not be comparable. Therefore, the results of TCHD samples were compared to the arithmetic means from testing conducted by FAU-LAB.EES. Those results are summarized in Figure 39 for four of the sampling trips including: May 2006 (SLWT), September 2006 (SHWT), December 2006 (SLWT), and May 2007 (SLWT). The values labeled, "TCHD Week Before" correspond to the Monday before FAU sampling took place (i.e. 5/1/2006, 9/25/2006, 12/11/2006, and 5/21/2007). The values labeled, "TCHD Week After" correspond to the Monday after the FAU sampling event took place (i.e. 5/8/2006, 10/2/2006, 12/18/2006, and 5/30/2007). For purposes of computing the standard deviation error bars, all values below detection (<10 MPN/100 mL) were reported as 5 MPN/100 mL.





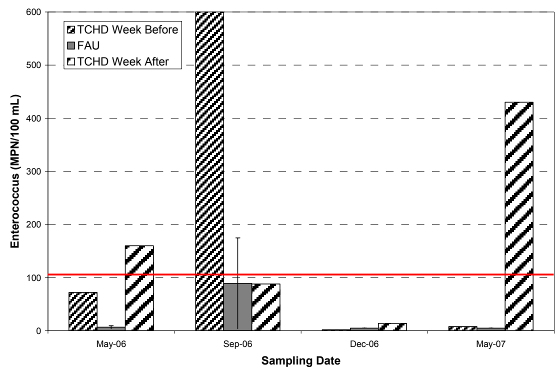


Figure 39 – Comparison of Taylor County Health Department sampling and FAU-LAB.EES sampling for enterococci for Dekle Beach (top left), Keaton Beach (top right), and Cedar Island Beach (bottom).

In general, the May 2006 and December 2006 results seemed to match consistently with the order of magnitude of the TCHD sampling. However, the September 2006 data show differences compared to the Monday before FAU sampling took place, and the May 2007 data seem to correlate better with the Monday before compared to the Monday after sampling. The FAU data does seem to be consistently lower than the TCHD data, which is unexpected, since the collection time for the FAU samples was set according to the tidal cycle to provide the highest measurable concentration (assuming terrestrial sources), while the TCHD samples did not follow a consistent tidal cycle. Although we cannot account for daily variation, it is possible that increased use of the beaches over the weekend might contribute to higher readings on Monday that would tail off during the middle of the week, when FAU samples were collected (Tuesday – Thursday).

E. coli/ Enterococcus Ratio (Ec/Es)

It has been suggested that the quantities of fecal coliform (FC) and fecal streptococci (FS) that are discharged by humans are significantly different from those discharged by animals (Tchobanoglous et al. 2003). Coyne and Howell (1994) reported that the FC/FS ratio is typically less than 0.7 for animal-derived pollution and greater than 4.0 for human-derived pollution, when the fecal streptococcus counts exceed 100 CFU/100 mL. Ratios in the range from 1 to 2 typically indicate a mixed contribution. Use of the FC/FS ratio in the field has been criticized (i.e. Mara and Oragui 1981, Pourcher et al. 1991, Sinton et al. 1998) due to an inconsistent relationship between the FC/FS ratio and pollution sources. The authors admit that these types of ratios have demonstrated limited value as an effective tracer; however, when taken in context with a multiple tracer approach, the ratio may provide some insight. Fecal coliform and fecal streptococci were not analyzed for in this study. However, since *E. coli* is a fecal coliform and *Enterococcus* is a fecal streptococcus, an *E. coli* to *Enterococcus* (Ec/Es) ratio was used. Thus, a variant of the FC/FS ratio, i.e. the Ec/Es ratio is presented here to see if it supports the findings from the multiple tracers discussed in detail earlier.

Coyne and Howell (1994) reported some success with a fecal coliform/fecal streptococcus (FC/FS) ratio method for indicating probable sources of fecal contamination. The technique is not considered definitive; however, the following guidelines are recommended (Geldreich and Kenner, 1969; Coyne and Howell, 1994):

1. The pH range should be between 4.0 and 9.0 because fecal coliforms die off quicker than fecal streptococci in acid or alkaline water.
2. Sampling should occur within 24 hours after waste deposition. The faster die-off rate of fecal streptococci will alter the ratio as time from contamination increases.
3. Sample near the point of discharge or as close as possible to the pollution source. Pollution from several sources can be confounding.
4. FC/FS ratios are of limited value in waters where regrowth can occur.
5. Ratios should not be used when fecal streptococcal counts are less than 100 MPN/100 mL. It becomes difficult to distinguish fecal streptococci in wastes from those that occur naturally in soil and water.

Although fecal coliform and fecal streptococci were not used in this study, the same guidelines stated above are applied for the *E. coli* and *Enterococcus* ratios in this study. For this sampling campaign, criterion number 1 is met.

For the most part, criterion number 1 was met for all sites, criterion number 2 was not possible to control for in this study because it was not possible to determine the time of contamination or the point of discharge without knowing the source ahead of time, criterion number 3 was followed in the selection of sites, criterion number 4 was not possible to control for but was a subject of further study detailed later, and criterion number 5 was followed. For the last criterion, *Enterococcus* densities met the minimum 100 MPN/100 mL value in 14% of the samples (22/153) in 2006 and 24% of the samples (34/139) in 2007.

A large number of samples were below detection for either *E. coli* or *Enterococcus* or both, during the five sampling events. For purposes of estimating the ratio for samples that were outside the analytical range of the method, *E. coli* and *Enterococcus* concentrations that were lower than 10 MPN/100mL were estimated to be equal to 5 MPN/100mL and concentrations higher than 24,196 MPN/100mL were considered to be equal to 24,196 MPN/100mL. Ratio data are included in the attached raw data tables in the appendix. Although, the Ec/Es ratio is not an absolute method due to the difference in the die-off rate of the contaminants, the results can be used for screening purposes by evaluating the frequency with which the ratios fall within certain indicative values. This is believed to be a more accurate predictor of fecal contamination source than the controversial ratio alone. Thus, the Ec/Es ratio data that met the *Enterococcus* >100 MPN/100 mL criterion in 2006 was evaluated in Table 42.

Table 42 – Summary of *E. coli/Enterococcus* ratio data organized by site for 2006.

Site Code	Site Name	Ratio	Source	Frequency/Number
Dekle Beach (OSTDS)				
060504B1	Dekle Beach Canal	8.5	Human	4/4
060926B1	**Dekle Beach Canal**	**7.4**	**Human**	
060928B1	**Dekle Beach Canal**	**44**	**Human**	
060926C1	**Creek at Dekle Beach**	**7.0**	**Human**	
Steinhatchee (OSTDS)				
060926J1	**Main Street & Steinhatchee (Roy's)**	**2.7**	**Mixed**	0/10
060505K1	Third Ave. Fork	0.3	Non Human	
060926K1	**Third Ave. Fork**	**1.1**	**Mixed**	
060927K1	**Third Ave. Fork**	**0.9**	**Non Human**	
060926L1	**Boggy Creek @ 51**	**1.0**	**Non Human**	
060927L1	**Boggy Creek @ 51**	**0.9**	**Non Human**	
060928L1	**Boggy Creek @ 51**	**0.5**	**Non Human**	
061212L1	Boggy Creek @ 51	0.5	Non Human	
061213L1	Boggy Creek @ 51	1.5	Mixed	
061214L1	Boggy Creek @ 51	1.9	Mixed	
060927N1	**Steinhatchee Falls**	**0.2**	**Non Human**	
Keaton Beach (Sewer)				
060503E1	Cortez Road Canal - Upstream	0.8	Non Human	3/4
060926D1	**Cortez Road Canal - Pump station**	**10**	**Human**	
060927D1	**Cortez Road Canal - Pump station**	**15**	**Human**	
060928D1	**Cortez Road Canal - Pump station**	**5.5**	**Human**	
Cedar Island (Sewer)				
060926I1	**Cedar Island Beach**	**57**	**Human**	2/2
060927H1	**Heron Road Canal**	**8.5**	**Human**	
Additional Sites				
None	None	None	Not applicable	0/0

Values in **bold** are from SHWT

It is interesting to note that during the SLWT, only 1 of 6 ratio values were above the human-derived input cut-off (ratio > 4). However, during the SHWT sampling events, more than 50 percent of the ratios were indicative of human contributions, and the sewered communities had a higher percentage of hits indicating human contributions when the two OSTDS sites are combined. The expectation was that during the SLWT there would be fewer human source indicators, and the data support that supposition with only 1 of 6 samples indicating human contributions. In SHWT, when OSTDS are not expected to operate as efficiently, we see a much larger frequency of potentially human contributions, but we also see those at the two sewered areas. These findings suggest a sewage leak or a legacy source, such as shallow sediment regrowth.

In 2006, the majority of the data meeting the threshold correspond to sites with OSTDS. Dekle Beach has 4 out of 4 ratios of human origin during May and September. Steinhatchee however, shows only non-human or mixed ratios for all ten ratios that met the criterion. For the sites that converted to sewer networks (Keaton Beach and Cedar Island), only 6 ratios met the criterion. At Keaton Beach, the three ratios that indicate human sources coincided with the September sampling event in which a pump station leak may have occurred. At Cedar Island, 2 out of 2 samples showed signs of human sources. This sample set is too small to draw any definitive conclusions. However, when all of the ratio data, including those below the 100 MPN/100 mL threshold (n = 21), is compiled for Cedar Island, all sites show signs of human pollution, despite being converted to sewer network from septic tanks during the course of the study. This potentially indicates that another source, such as possible regrowth in warm, shallow, stagnant waters may be responsible (Solo-Gabriele et al. 2000).

Taking a look at the data collected from 2007 follow-up testing in Table 43, it is interesting to note that during the SLWT, none of the seven (7) ratio values were above the human-derived input cut-off (ratio > 4). However, during the SHWT sampling events, 8 of 26 of the ratios (31%) were indicative of human contributions, and the sewered communities had a higher percentage of hits indicating human contributions when the two OSTDS sites are combined, just as seen in 2006. The data also support the supposition that during the SLWT there are fewer human source indicators (0%). In SHWT, when OSTDS are not expected to operate as efficiently, we see a much larger frequency of potentially human contributions, but we also see those at the two sewered areas. These findings are similar to those observed in 2006.

Table 43 – Summary of *E. coli/Enterococcus* ratio data organized by site for 2007.

Site Code	Site Name	Ratio	Source	Frequency/Number
Dekle Beach (OSTDS)				
070920JI1	**Jugg Island Road Canal**	**0.5**	**Non-Human**	3/4
070919B1	**Dekle Beach Canal**	**19**	**Human**	
070920B1	**Dekle Beach Canal**	**27**	**Human**	
070920C1	**Creek at Dekle Beach**	**92**	**Human**	
Steinhatchee (OSTDS)				
070522K1	Third Ave. Fork	0.3	Non Human	0/6
070523K1	Third Ave. Fork	0.2	Non Human	
070919K1	**Third Ave. Fork**	**2.9**	**Mixed**	
070918L1	**Boggy Creek @ 51**	**0.1**	**Non Human**	
070919L1	**Boggy Creek @ 51**	**0.7**	**Non Human**	
070920L1	**Boggy Creek @ 51**	**0.6**	**Non Human**	
Keaton Beach (Sewer)				
070918MR1	**Marina Road**	**64**	**Human**	2/5
070919D1	**Cortez Rd - Pump Station**	**26**	**Human**	
070524G2	Blue Creek @ Beach Road	0.03	Non Human	
070918G1	**Blue Creek @ Beach Road**	**2.4**	**Mixed**	
070919G1	**Blue Creek @ Beach Road**	**2.0**	**Mixed**	
070920G1	**Blue Creek @ Beach Road**	**3.8**	**Mixed**	
070920G2	**Blue Creek @ Beach Road**	**1.6**	**Mixed**	
Cedar Island (Sewer)				
070920SL1	**Seahawk Lane**	**7.1**	**Human**	1/6
070524G2	Blue Creek @ Beach Road	0.03	Non Human	
070918G1	**Blue Creek @ Beach Road**	**2.4**	**Mixed**	
070919G1	**Blue Creek @ Beach Road**	**2.0**	**Mixed**	
070920G1	**Blue Creek @ Beach Road**	**3.8**	**Mixed**	
070920G2	**Blue Creek @ Beach Road**	**1.6**	**Mixed**	
Additional Sites				
070920AB1	**Adam's Beach**	**11 ± 0.8**	**Human**	4/15
070918FR1	**Fenholloway River**	**0.03**	**Non Human**	
070919FR1	**Fenholloway River**	**0.02**	**Non Human**	
070920FR1	**Fenholloway River**	**0.02**	**Non Human**	
070522HS1	Hampton Springs	0.1	Non Human	
070524HS1	Hampton Springs	0.2	Non Human	
070918HS1	**Hampton Springs**	**0.2**	**Non Human**	
070919HS1	**Hampton Springs**	**0.3**	**Non Human**	
070920HS1	**Hampton Springs**	**0.6**	**Non Human**	
070522PL1	Petersons Landing	0.1	Non Human	
070524PL1	Petersons Landing	0.2	Non Human	
070918PL1	**Petersons Landing**	**5.9**	**Human**	
070918PL2	**Petersons Landing**	**6.5**	**Human**	
070919PL1	**Petersons Landing**	**3.9**	**Mixed**	
070919PL1	**Petersons Landing**	**7.9**	**Human**	

Values in bold are from SHWT

In general for both years, the inland background sites had low ratios, many of which were less than 1.0, which is indicative of non-human sources. On the other hand, nearly all of the beach sites for both years showed ratios that were well above 4.0, indicative of human sources. Insufficient data is available to draw a complete conclusion from this phenomenon; however, the authors have postulated some potential reasons that will be investigated in future research. First, in this study, the Dekle Beach site (OSTDS) is located upstream of the Keaton Beach (sewer) and Cedar Island (sewer) sites, along the general bulk flow transport of the prevailing ocean current. As a result, the twice daily tides tend to mix ground water and runoff contributions together, along with tidal oceanic water. As the tides exchange, a portion of the contaminants are lost each tidal cycle to the coastal ocean and transported downstream. The portion which is not lost, is redistributed with the incoming tides, and mixed with ground and surface waters that migrate into the coastal area during low tide. Hence ground water and runoff contributions for a given area do not return to exactly the same water quality level from which they originate. This daily periodicity has been termed as the "slosh" effect by the research team, which may play an important role in cycling nutrients and pathogen indicators in Taylor County. Observations made by the research team in conjunction with NOAA have observed this phenomenon elsewhere in Florida coastal waters and could be confirmed in Taylor County with installation of a current meter and intensive 48-96 hour sampling for bacteria and nutrients. In this study, samples were collected only once per day, so diurnal effects could not be analyzed.

A second possibility is that during the SHWT, the soils and canals in the sewered areas may be flushed less effectively, and therefore do not show the same concentrations of bacteria as the septic areas that would tend to leach even more bacteria into the soil. This may be difficult to test. Affluent communities tend to use more water for landscaping and lawn care (with increased use of fertilizers). If these areas are on sewer network and experiencing heavy water use for irrigation purposes for instance, it is possible that they have maintained the higher water table elevations for the whole year, and thus the adjacent canals may show discernable differences from the sewered and non-sewered test sites during the SLWT.

Analysis of OSTDS vs. Sewer (Horizontal Analysis)

This section summarizes the general comparison of the data for each analyte from areas served by sewers and areas served by OSTDS during both the SHWT and SLWT. The mean values were calculated using results from all sites grouped by classification - sewered sites versus sites served by septic tanks (background sites were included). Results for field duplicates and lab replicates were not included. Geometric means were used for comparison of the bacterial results, since it is the preferred statistic for summarizing microbiological data (Standards Methods 19th edition 1995). This is appropriate because the collected data was constrained as being non-negative and as such, the assumption of normally-distributed, non-skewed data was not possible. The geometric mean is better at showing where most of the data points lie by lowering the weight of outliers. The other parameters, such as nitrate and ammonia, were compared using arithmetic means. When using arithmetic means, values that were recorded to be below detectable levels were assumed to be zero because of widely varying detection limits across the data set. Bacterial results that were recorded higher than the 24,196 MPN/100mL limit were considered equal to 24,196 MPN/100mL, and those results below 10 MPN/100mL were considered equal to 5 MPN/100mL. These comparisons are summarized in Table 44. Standard deviations are also included in the table.

The bacteriological results reveal that *E. coli* counts levels are generally lower in OSTDS areas as compared to sewered areas, but *Enterococcus* counts behave oppositely and were higher by a factor of about 1.5, independent of season. *Enterococcus* counts are generally higher in OSTDS areas as compared to sewered areas, by a factor of 1.5-2.3, independent of season. In the sewered areas, the enterococci levels were about 40% lower than the sewer background site. In the OSTDS areas, the enterococci densities were higher than background by a factor of 1.6, as shown in Table 45 using geomeans. At Dekle Beach, the background levels are generally higher than the downstream counts, but the opposite is seen at Steinhatchee.

Table 44 - Comparison of Results for Sewered and Non-Sewered Sites

	SLWT (May 2006)	SHWT (Sept 2006)	SLWT (Dec 2006)	SLWT (May 2007)	SHWT (Sept 2007)
	E. coli (MPN/100mL)				
Sewer	490 ± 2613	1121 ± 5804	105 ± 100	138 ± 641	1626 ± 1994
OSTDS	127 ± 740	301 ± 1715	72 ± 92	34 ± 49	429 ± 6104
	Enterococcus (MPN/100mL)				
Sewer	15 ± 140	44 ± 136	7.3 ± 6.8	11 ± 10	53 ± 59
OSTDS	28 ± 55	73 ± 106	23 ± 79	14 ± 41	44 ± 147
	TOC (mg/L as C)				
Sewer	10 ± 4.7	37 ± 28	10 ± 5.4	7.3 ± 3.1	22.8 ± 31.5
OSTDS	12 ± 3.4	22 ± 21	15 ± 8.2	8.8 ± 2.7	12.6 ± 11
	TN (mg/L as N)				
Sewer	0.46 ± 0.17	0.70 ± 0.19	0.35 ± 0.11	0.52 ± 0.16	nr
OSTDS	0.57 ± 0.34	0.51 ± 0.25	0.36 ± 0.08	0.50 ± 0.13	nr
	Ammonia (mg/L as N)				
Sewer	0.20 ± 0.24	0.05 ± 0.03	0.06 ± 0.05	0.02 ± 0.02	0.05 ± 0.03
OSTDS	0.26 ± 0.22	0.06 ± 0.05	0.06 ± 0.03	0.03 ± 0.03	0.05 ± 0.03
	Nitrate (mg/L as N)				
Sewer	0.01 ± 0.02	0.01 ± 0.01	0.02 ± 0.02	0.01 ± 0.01	0.02 ± 0.02
OSTDS	0.01 ± 0.01	0.01 ± 0.02	0.03 ± 0.03	0.01 ± 0.02	0.03 ± 0.03

Table 45 - Enterococci Results for Sewered and Non-Sewered Sites Relative to Background

	SLWT (May 2006)	SHWT (Sept 2006)	SLWT (Dec 2006)	SLWT (May 2007)	SHWT (Sept 2007)
	Enterococcus (MPN/100mL)				
Sewer	14 ± 159	42 ± 144	6.6 ± 4.2	9.9 ± 10	36 ± 36
Background	22 ± 21	34 ± 14	12 ± 14	41 ± 63	168 ± 55
OSTDS	33 ± 60	58 ± 126	33 ± 112	16 ± 42	47 ± 156
Background	19 ± 27	51 ± 80	12 ± 8.3	12 ± 13	39 ± 58

However, *E. coli* levels were unexpectedly found to be consistently higher in the sewered areas (see Table 46). In sewered areas, the *E. coli* levels were nearly one order of magnitude higher than the background sites and double the concentration observed in the OSTDS sites. The unsewered areas had *E. coli* counts that were 50% higher than their corresponding background sites.

Table 46 – *E. coli* Results for Sewered and Non-Sewered Sites Relative to Background

	SLWT (May 2006)	SHWT (Sept 2006)	SLWT (Dec 2006)	SLWT (May 2007)	SHWT (Sept 2007)
	E. coli (MPN/100mL)				
Sewer	863 ± 2772	1993 ± 6013	96 ± 108	137 ± 597	1909 ± 1978
Background	29 ± 11	33 ± 38	162 ± 52	8.8 ± 36	392 ± 183
OSTDS	110 ± 704	295 ± 1875	94 ± 96	37 ± 46	465 ± 6149
Background	97 ± 701	235 ± 980	50 ± 83	23 ± 59	290 ± 6072

The *Enterococcus* and *E. coli* densities were plotted as geometric means of the sites in the downstream direction in Figure 40. A general increasing trend from upstream to downstream is apparent. As noted previously, *Enterococcus* counts were higher in the SHWT period when compared to the SLWT, by a factor of 2 – 3. However, *E. coli* was found to be consistently higher in the sewered areas, which was not expected. When taken in context with the *Enterococcus* results, these higher levels of *E. coli* may not be necessarily of human origin.

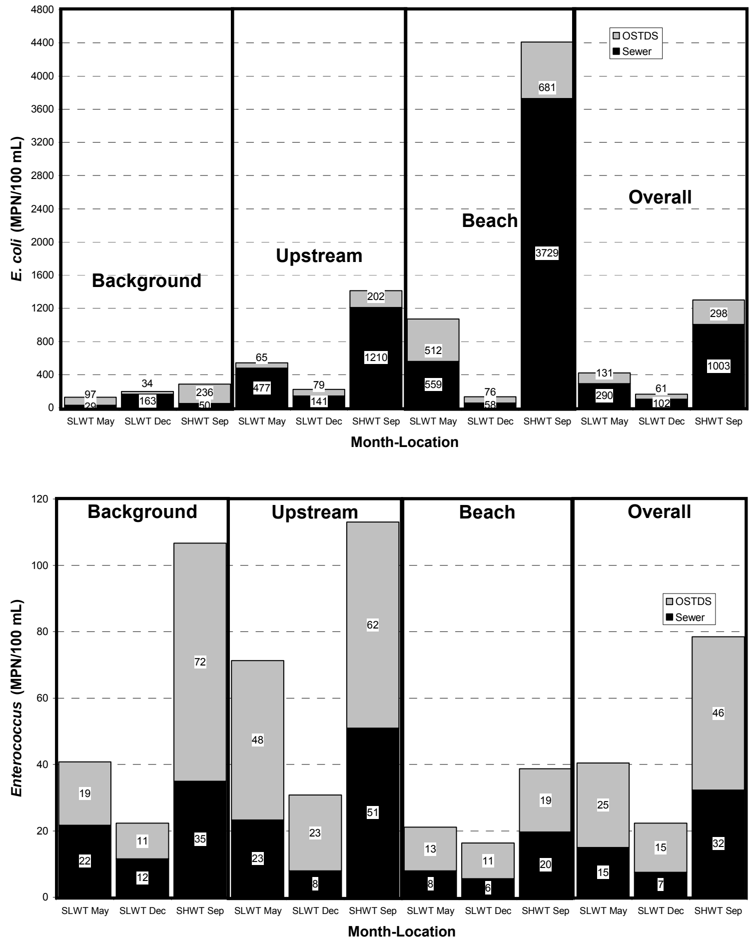


Figure 40 - Spatial trends in bacteriological indicators between OSTDS sites and sewer sites (Top-*E. coli*; Bottom-Enterococci)

The results indicate that the *Enterococcus* and *E. coli* densities correlated as expected with the change from SLWT to SHWT. However, the actual numbers may be misleading due to several very high *E. coli* results (> 5000 MPN/100 mL) from sewered areas that occurred during both seasons at Cedar Island Beach and Dekle Beach Canal.

Comparison of 2006 data from the seasonal high water table event (September 2006) and the seasonal low water table events (May and December 2006) revealed the following:

- *Enterococcus* results are not higher in OSTDS areas when compared to sewered areas. In fact, the values are slightly less but very similar.
- *Enterococcus* results are higher in the SHWT period compared to the SLWT, by at least a factor of two.
- *E. coli* was higher in sewered areas than in non-sewered areas in the SHWT and SLWT events (by a factor of two), except in December when the levels were similar and very low. It should be noted that the *E. coli* results do not indicate that the additional colony forming units are necessarily of human origin.
- TOC values are higher in the SHWT period as opposed to the SLWT events, by a factor of 2-3. December and May (SLWT) were similar to each other.
- Total nitrogen remains fairly constant between all events (slightly lower in December)
- Ammonia is 4 times higher in the May SLWT, but the SHWT and December SLWT events were similar.
- Ammonia levels as a percent of total nitrogen are higher in the SLWT events.
- Nitrate is negligible at all periods.

From this analysis, the total nitrogen and *Enterococcus* parameters would tend to implicate a greater contribution of nutrients to coastal waters from septic systems. However, runoff (TOC and higher ammonia in the SLWT) may also be an important factor.

In 2007:

- *Enterococcus* results are higher in OSTDS areas when compared to sewered areas, by at least a factor of two.
- *Enterococcus* results are higher in the SHWT period compared to the SLWT, by at least a factor of two, similar to what was observed in 2006.
- *E. coli* was higher in sewered areas than in non-sewered areas in the SHWT and SLWT events (by a factor of two), just as seen in 2006.
- Ammonia levels in 2007 behaved similarly as compared to the last two sampling events in 2006.

- Nitrate remains negligible at all periods.

Intervention Analysis

As stated earlier, two of the beach communities (Keaton Beach and Cedar Island) were recently converted (January 2006) to a sewer network from OSTDS. An analysis of the temporal variations between these two sites and the site that remained on OSTDS (Dekle Beach) over the same time period was performed using the microbial indicators *Enterococcus* and *E. coli*. The purpose was to see if a change in the slope of the cumulative densities could be observed. If so, this would indicate factors affecting the concentration trend (i.e. a positive impact from switching to sewer). The plot was created using historical data from the weekly FDOH Beach Monitoring Program. Data were taken from Dekle Beach, Keaton Beach, and Cedar Island, from 2000 to 2007, and normalized to the Dekle Beach data, which remained on OSTDS for the entire time period under investigation.

For the *Enterococcus* sampling (Figure 41), no obvious departure in slope was observed during the period of time coinciding with the retrofit of OSTDS to sewer network at Cedar Island, although there is the possibility of a sharp increase in slope for the Keaton Beach site during the transition period, but shortly thereafter the previous slope appears to be restored. At the tail end of the data set, it appears that the enterococci levels are increasing sharply starting in early summer 2007. Therefore, data extending from July 2007 to December 2007 were plotted in Figure 42, which shows the continuation of this sharp increase until the end of the year, when it appears to have returned back to the original slope. The cause of this departure is unknown, but is in the opposite direction of what is expected after conversion to sewer.

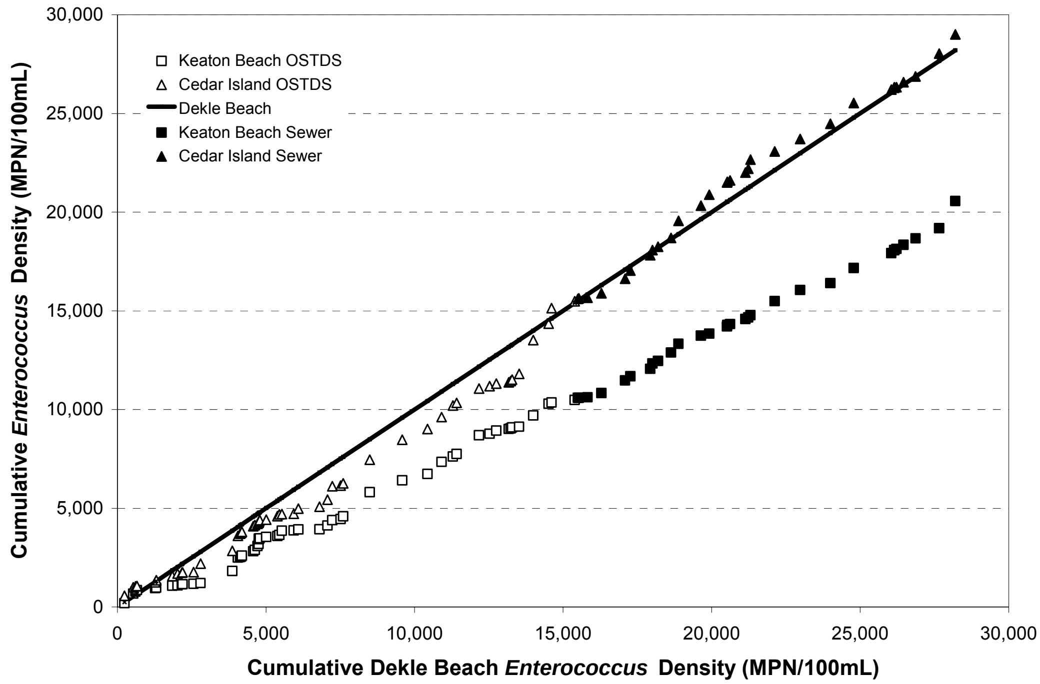


Figure 41 – *Enterococcus* correlation of Keaton Beach and Cedar Island sites with Dekle Beach from January 2000 to July 2007.

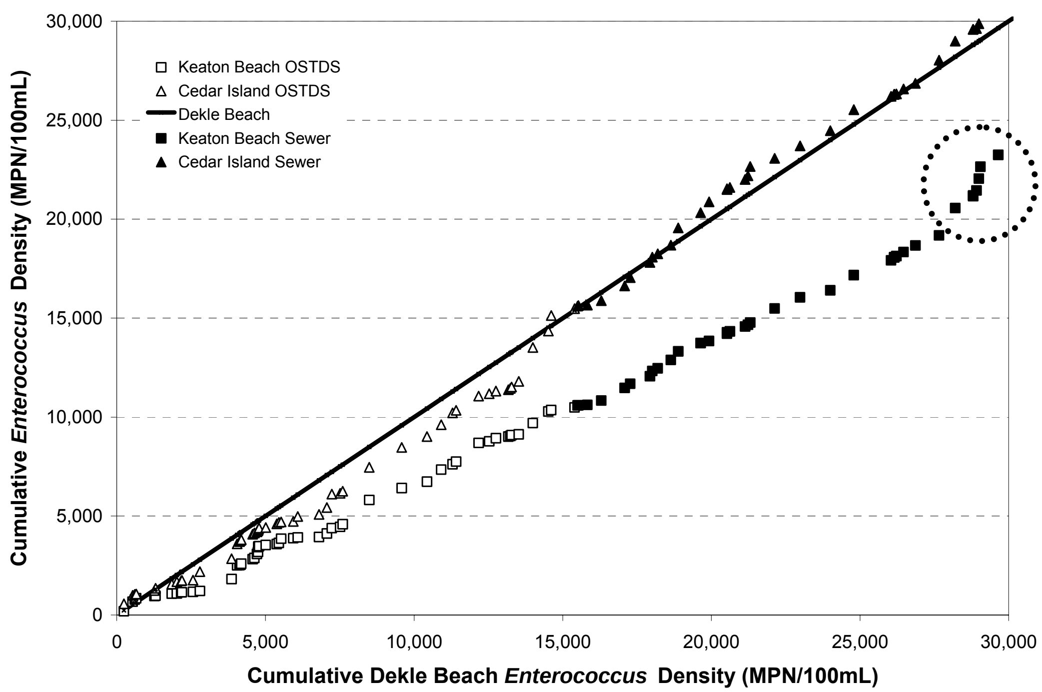


Figure 42 – *Enterococcus* correlation of Keaton Beach and Cedar Island sites with Dekle Beach from January 2000 to December 2007.

However, the fecal coliform data (Figure 43) had an abrupt change in slope detected during the summer of 2003. While this may signal a change in sampling or analytical methodology, an unexplained isolated event of fecal coliform input at Keaton Beach may also have occurred from July 14 – 21, 2003, which is prior to sewer conversion. Also between August 2005 and August 2006, the fecal coliform slope at Keaton Beach is relatively flat, but thereafter returns to mirror the Dekle Beach curve after that period. This change in slope would indicate a marked improvement in fecal coliform levels possibly due to conversion to sewer, but the return of the slope to the pre-sewer level after August 2006 cannot be explained.

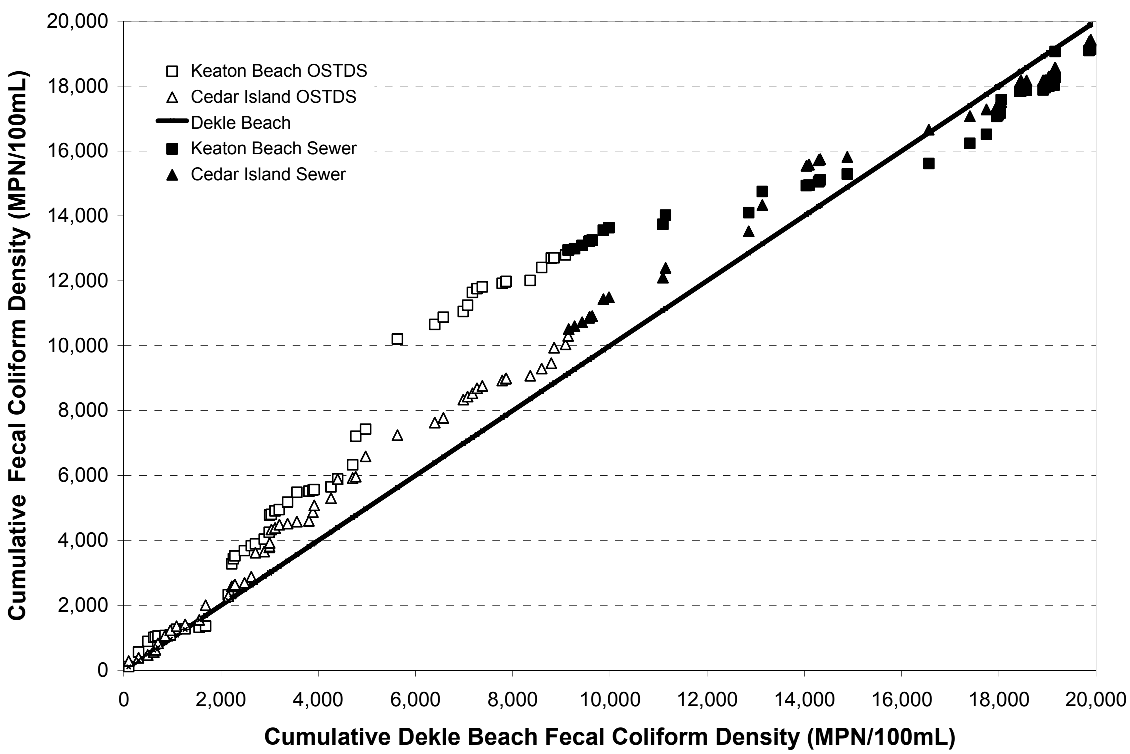


Figure 43 – Fecal coliform correlation of Keaton Beach and Cedar Island sites with Dekle Beach January 2000 to December 2007.

Correlation of Analytes

The strategy in this analytical approach was to compare specific analytes to quantify the strength of their relationship with other parameters. Relationships were tested using scatter plots, in order to confirm correlations by identifying structural patterns in the plots.

Salinity Effects

Although recognized relationships exist between salinity, temperature, and bacteria die-off rate, no observable correlations were seen on the scatter plots when temperature, turbidity, and several other physical parameters were correlated with *Enterococcus*, *E. coli* or the *E. coli/Enterococcus* ratio (Appendix A). However, at first glance, salinity appears to influence some of the bacteriological results. In general, the brackish and marine sites sampled tend to have higher colony counts of *E. coli* and *Enterococcus*. Therefore, box plots of salinity were constructed using all of the available data from 2006 to 2007 to investigate this influence of salinity regime on the bacteriological parameters (Figure 44). The box plot of *Enterococcus* densities showed that microbial densities were slightly higher on average for the sites with freshwater (<10‰) compared to sites with brackish/salt water (<10‰), but overall differences were minimal. The same plot for *E. coli* densities showed the opposite effect. The *E. coli* counts were higher at the sites with higher salinity, even though salinity is expected to increase the die-off rate of *E. coli*. It is possible that this trend is artificial given the fact that most of the freshwater sites were characterized as background. As such, *E. coli* inputs were expected to be lower at these sites. Also most of the developed area is along the coast, which would likely have higher levels of *E. coli* near the higher salinity regimes of the beaches.

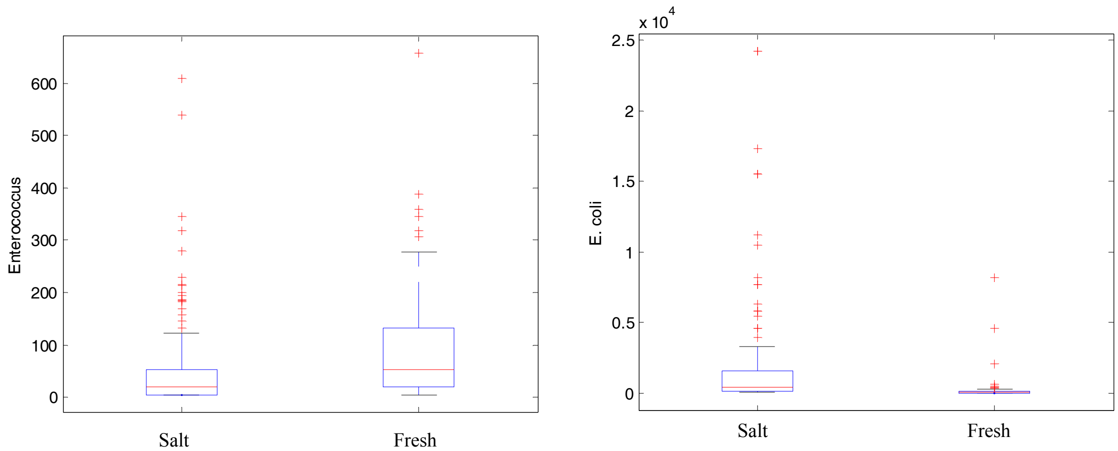


Figure 44 – Box plots of microbial concentration in fresh and salt waters (*Enterococcus* on left and *E. coli* on right).

The box plot of the *E. coli/Enterococcus* ratio shows a larger ratio for brackish/marine environments compared to the freshwater sites. In Figure 45, the *E. coli/Enterococcus* ratio was plotted against salinity using only those data points that met the minimum *Enterococcus* threshold (>100 MPN/100 mL). Freshwater sites (background sites) tend to be closer to zero (<1), suggesting a natural or animal contribution, while the average ratio is higher for samples collected in marine environments. Again, this effect is likely enhanced by the fact that most of the freshwater sites (inland) were classified as background, but an increased die-off of *E. coli* with respect to *Enterococcus* would tend to skew the ratios downward. However, the higher salinity sites (brackish/salt water) showed a higher ratio. Thus, the expected salinity induced die-off is not observed. This could be caused by microbial acclimation to local salinity conditions, or it could be the result of recent or legacy inputs of pathogens and nutrients. Thus, the implication is that either the pollution is of marine origin or, more likely, the majority of the coastal pollution originates from the shore because this is where the more dense human population lives.

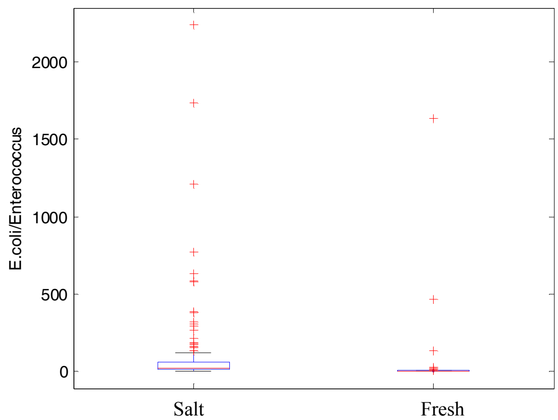


Figure 45 – *E.coli/Enterococcus* ratio in fresh and salt waters.

Using 2006 data, *E. coli* densities do not seem to be correlated with salinity over the broad range tested (0.1 – 37‰) using linear and log scale plots (Figure 46). However, when the freshwater and brackish water outliers are removed, the data no longer appear randomly scattered and the *E. coli* counts decrease in the direction of the higher salinity regime. All the data points except for two fit in the 95% confidence interval for the generally decreasing trend line ($r^2 = 0.435$)

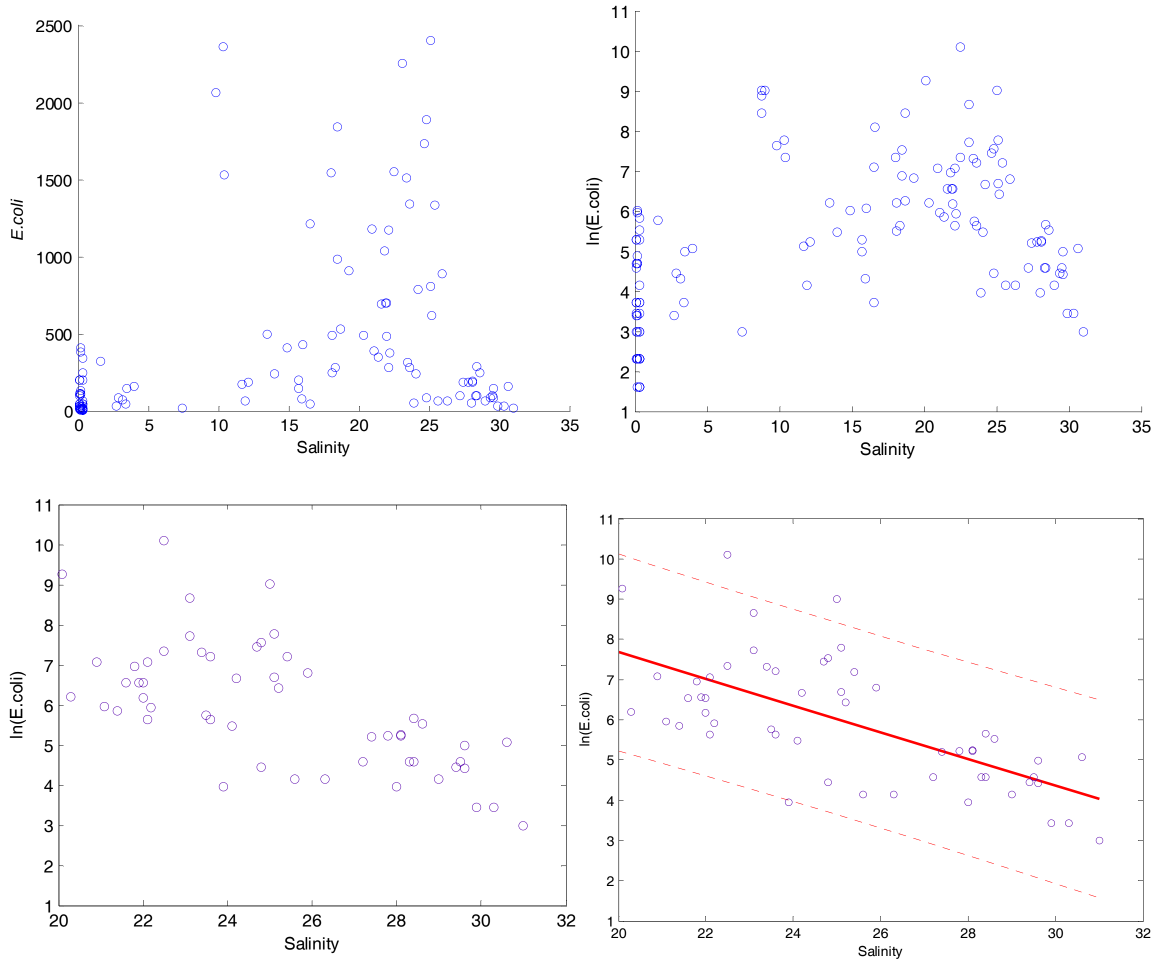


Figure 46 – Plots of *E. coli* with salinity for 2006. Linear scatter plot of *E. coli* density vs. salinity (top left). Log scatter plot of *E. coli* density vs. salinity (to right). Log scatter plot of *E. coli* density vs. salinity for all sites with salinity > 20 ‰ (bottom left). Log scatter plot of *E. coli* density vs. salinity for all sites with salinity > 20 ‰ with a plot of linear regression (r^2 = 0.435) and the 95% confidence interval plotted (bottom right).

A decreasing trend supports the existence of a salinity induced die-off for *E. coli*, only if the slope for a similar plot of *Enterococcus* is different. Therefore, the same analysis is performed for *Enterococcus* in Figure 47. When the log of microbial density is plotted against salinity >10‰, both *E. coli* (m = -0.084) and *Enterococcus* (m = -0.081) have very similar slopes, indicating a similar die-off from brackish to marine environments. Although, if the salinity regime is limited to values greater than 20‰, the slope for *E. coli* increases to m = -0.33 becoming more steep (greater die-off), whereas the corresponding *Enterococcus* slope for this same salinity range does not change as much (m = -0.19).

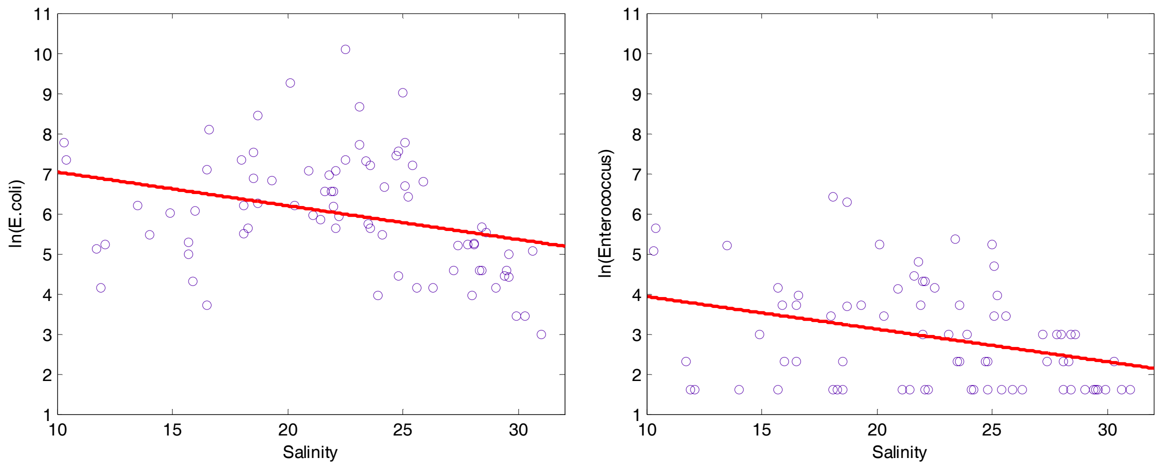


Figure 47 – Plot of *E. coli* (left) and *Enterococcus* (right) with salinity for 2006. Values are plotted as the natural log (*ln*) of microbial density versus salinity >10‰.

Given that *Enterococcus* is generally recommended for marine waters due to a lower salinity die-off compared with the fecal coliform group or *E. coli* (USEPA 1986), the previous analysis initially appears to yield unexpected results. However, one needs to take into account the fact that the majority of the homeowners live closer to the saltwater and therefore, the freshwater appears more pristine in terms of microbial densities. Thus, rather than use either *E. coli* or *Enterococcus* as a tracer, it is suggested that a ratio between *E. coli* and *Enterococcus* be used. In Figure 48, the Ec/Es ratio was also plotted against salinity using only those data points that the met the minimum enterococci threshold (>100 MPN/100 mL). The plot is generally increasing towards higher salinity. Combining the data from 2006 and 2007 does not change the slope of the curve noticeably. Thus, the general trend holds true for both years. The implication here is that either the *E. coli* is of marine origin transported from some upstream source or, more likely, the majority of the homeowners (particularly those with OSTDS) live along the shore, although shallow sediment re-growth without external input cannot be discounted.

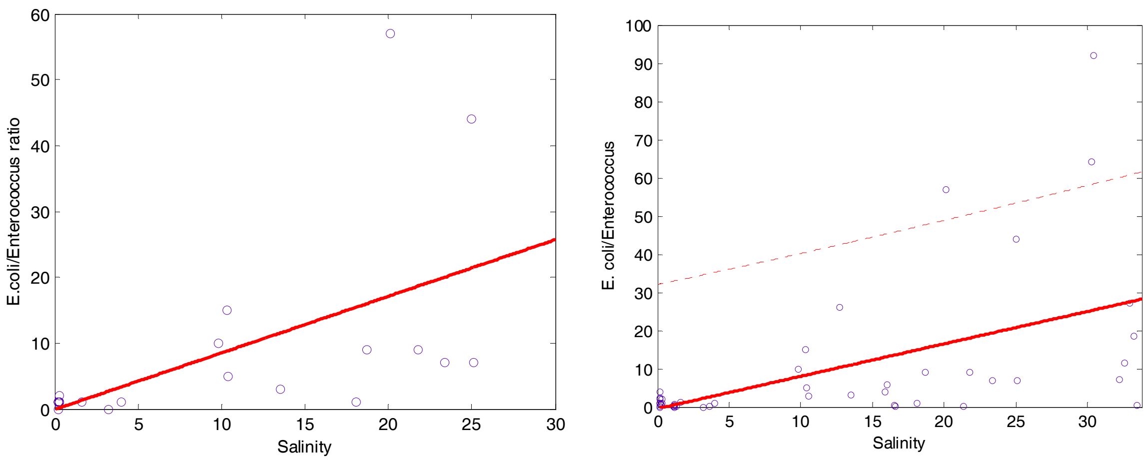


Figure 48 – Plot of the *E. coli/Enterococcus* ratio against salinity for 2006 (left) and all data (right). Note that only the ratios with *Enterococcus* > 100 MPN/100 mL were plotted.

Now when all of the data is combined from 2006 and follow-up testing in 2007, and we repeat the previous analysis with the scatter plots, the previous relationship and the slope disappears (Figure 49).

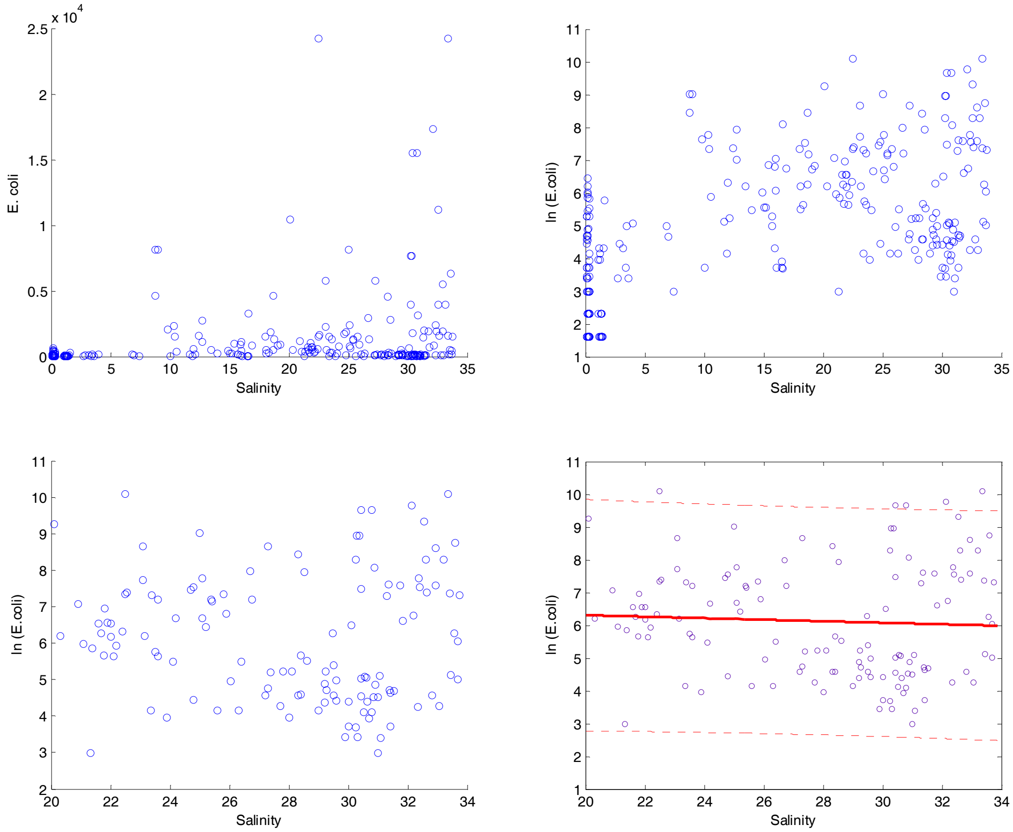


Figure 49 – Plots of *E. coli* with salinity for 2006 and 2007 combined. Linear scatter plot of *E. coli* density vs. salinity (top left). Log scatter plot of *E. coli* density vs. salinity (to right). Log scatter plot of *E. coli* density vs. salinity for all sites with salinity >20‰ (bottom left). Log scatter plot of *E. coli* density vs. salinity for all sites with salinity >20‰ with a plot of linear regression (r^2 = 0.003) and the 95% confidence interval plotted (bottom right).

Furthermore, the slopes of the lines for the *E. coli* and *Enterococcus* plots with salinity are actually different. When the two years worth of sampling is compiled we see that the *E. coli* appear to have acclimated to the salinity conditions, while the enterococci show less environmental resistance, although the correlations are not strong for either data set ($r^2 < 0.1$).

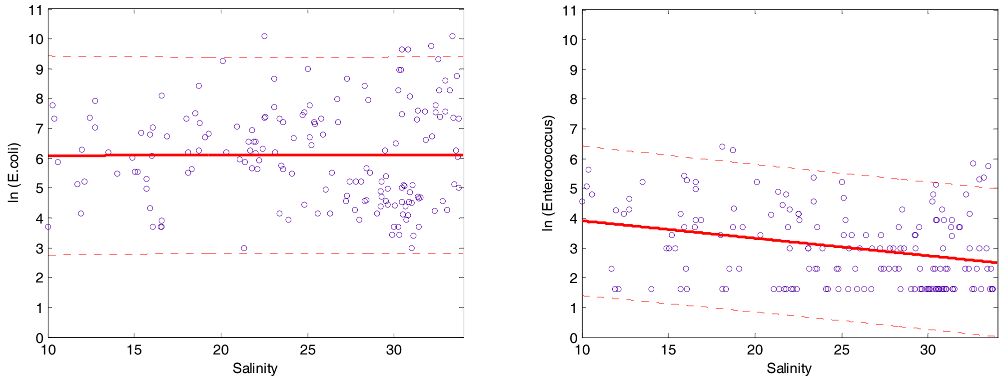


Figure 50 – Plot of *E. coli* (left) and *Enterococcus* (right) with salinity for 2006 and 2007 combined. Values are plotted as the natural log (*ln*) of microbial density versus salinity >10‰.

Nutrients

From the speciation of nitrogen containing parameters (ammonia, nitrate, nitrite, and total nitrogen), it was determined that most of the nitrogen detected was in the form of organic nitrogen. If this nitrogen was mostly incorporated in microbial or algal biomass, it would correlate closely with TOC. Thus, TOC and TN are plotted together in Figure 51. The combined data set from 2006 and 2007 (r^2 = 0.75) correlates more closely than the 2006 data alone (r^2 = 0.40).

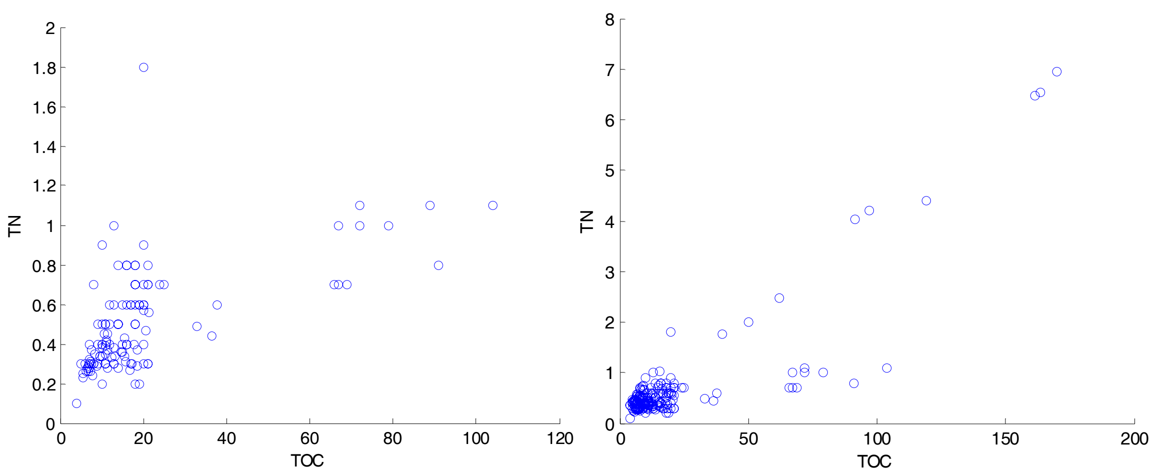


Figure 51 – Plot of Total Nitrogen (TN in units of mg N/L) against Total Organic Carbon (TOC in units of mg C/L). Data for 2006 only is plotted on the left and the combined data from 2006 and 2007 is plotted on the right.

If the TOC/TN is indeed found mostly in the form of biomass, it will also correlate closely with microbial parameters. Thus, both *E. coli* and *Enterococcus* were plotted against TOC in Figure 52. Neither of the microbial indicators correlated well with TOC. What is more likely is that the TOC/TN correlation is more the product of natural organic color than microbial biomass.

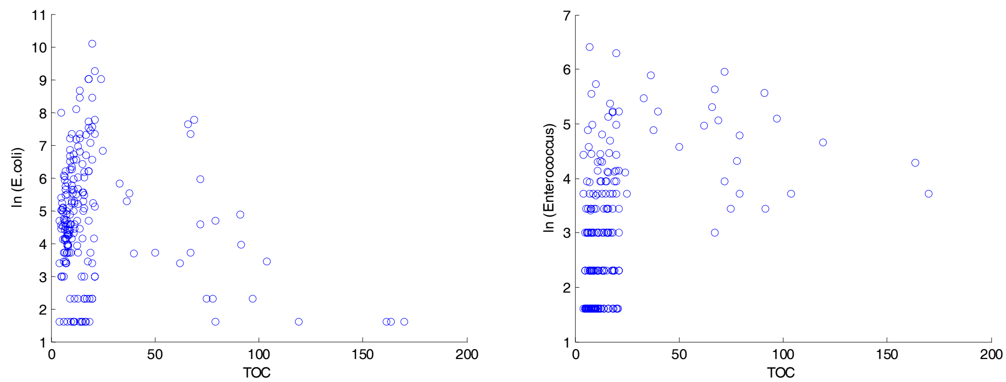


Figure 52 – Plot of *E. coli* (left) and *Enterococcus* (right) with TOC using both 2006 and 2007 data sets. Values are plotted as the natural log (*ln*) of microbial density versus total organic carbon content in mg C/L.

CONCLUSIONS

In general, the measured physical parameters fell within the expected ranges (see Table 47). A summary of the water quality trigger levels with the range of results collected for each parameter is found in Table 47 and Table 48.

Table 47 – Summary of field results for 2006 and 2007 sampling events.

Parameter	Analytical Method/SOP	Trigger Level	Expected Level	Encountered Range
pH	FDEP FT1100	N/A	6.0 – 8.5	7.0 – 8.6
Conductivity	FDEP FT1200	N/A	5 – 55 mS/cm	0.1 – 51 mS/cm
Salinity	FDEP FT1300	N/A	9,000 – 40,000 mg/L	100 – 41,000 mg/L
Temperature	FDEP FT1400	N/A	15 – 25°C	11 – 30°C
Dissolved oxygen	FDEP FT1500	< 4.0 mg/L	< 9.0 mg/L	0.5 – 10.5 mg/L
Turbidity	FDEP FT1600	>29 NTU	< 10 NTU	0.1 – 21.1 NTU
Optical Brighteners	FAU LT9200	N/A	Absent	Absent – Inconclusive

Table 48 – Summary of laboratory results for 2006 and 2007 sampling events.

Parameter	Analytical Method/SOP	Trigger Level	Expected Level	Encountered Range
E. coli **(& Total coliforms)**	Standard Methods SM9223B FAU LT6100	> 400 CFU/100 mL	BDL – 800 CFU/100 mL	BDL – 24000 CFU/100 mL
Enterococcus	Standard Methods SM9223C FAU LT6200	> 104 CFU/100 mL	BDL – 2,000 CFU/100 mL	BDL – 610 CFU/100 mL
Caffeine	FLEnviro SOP	> 0.10 µg/L	BDL	BDL – 0.32 µg/L
Nitrate	EPA 353.2 (FLEnviro SOP)	None*	< 5.0 mg/L	BDL – 1.0 mg/L
Ammonia-nitrogen	EPA 350.1 (FLEnviro SOP)	9.15 mg/L** @pH 7.9, T = 25°C	< 5.0 mg/L	BDL – 3.2 mg/L
TOC	EPA 415.1 FAU LT5200	None	1 – 200 mg C/L	BDL – 170 mg/L
TN	EPA 415.1 FAU LT5200	None	< 10.0 mg/L	BDL – 7.0 mg/L

*0.07 mg/L as N (nitrate and ammonia) has been suggested as a human-impacted threshold level by NOAA-AOML
**From National Ambient Water Quality Criteria for Saltwater (www.epa.gov/waterscience/criteria/wqcriteria.html), EPA 440/5-88-004

The trigger levels for only three of the parameters were violated in this study. These were dissolved oxygen, *Enterococcus*, and *E. coli*. These were investigated for seasonal effects in Table 49, which lists the percentage of violations by season for sewered and non-sewered sites as compared to the background stations (Creek at Dekle, Blue Creek, and Steinhatchee Falls). As expected, the percentage of violations for dissolved oxygen, *Enterococcus*, and *E. coli* are all higher in the SHWT season, even for the background sites. For non-sewered areas, 8% of the *Enterococcus* samples violated the trigger levels in SLWT, but 35% violated in SHWT. Similarly *E. coli* violations increased from SLWT (14%) to the SHWT (19%). Keaton Beach had isolated cases of extreme microbial contamination recorded during the May 2006 SLWT, which skewed the average results but did not mask the general trend because this occurrence was repeated in May 2007 SLWT.

Unexpectedly, *E. coli* violations are nearly four times more frequent at sewered sites compared to those served by OSTDS and also the background sites. Even more alarming is that the number of *E. coli* violations for the sewered sites was much higher in 2007 compared to 2006. Since the sewer system was only just recently installed, water quality conditions monitored may still reflect previous contamination from older OSTDS, recent sewer leaks, sediment reservoirs harboring pathogen indicators from natural sources or runoff contaminated with animal feces, or steady upstream contributions, the source of which may or may not be wastewater-related. However, since the frequency of violations increased in 2007, it is more likely that microbial regrowth in warm, shallow, stagnant waters may be causing this signal, but the original source of these microorganisms is not clear.

Table 49 – Summary of trigger level violations from sampling events in 2006 and 2007.

	Dissolved oxygen*				Enterococci**				*E. coli****			
	SLWT		SHWT		SLWT		SHWT		SLWT		SHWT	
	Frequency	Percent	Frequency	Percent	Frequency	Percent	Frequency	Percent	Frequency	Percent	Frequency	Percent
OSTDS	10/75	13%	26/51	51%	6/74	8%	18/51	35%	10/74	14%	13/69	19%
Sewer	0/69	0%	25/48	52%	3/69	4%	14/48	29%	24/51	47%	40/48	83%
Background	3/27	11%	8/18	44%	1/27	4%	6/18	33%	3/27	11%	8/18	44%

*Dissolved oxygen: Class III waters, marine > 4.0 mg/L; freshwater > 5.0 mg/L
***Enterococcus*: > 104 MPN/100 mL
****E. coli*: > 400 MPN/100 mL (fecal coliforms)

Results for nutrients such as ammonia and nitrate were all below regulatory trigger levels as seen in Table 48; however, many individual results were considered high for marine environments (see Table 50). Nitrate can be an indicator of a runoff contribution, but in this study, the average nitrate readings were measured at below elevated levels at the paired sites for both seasons. On the other hand, ammonia is a better indicator of more recent nutrient inputs, and in this study, many individual ammonia results were considered high for coastal marine environments. In general, elevated nutrient levels indicative of human activities (> 0.07 mg/L as N) were encountered more often at sites considered as background, compared to sewer or OSTDS sites. Nitrates are typically considered as an indicator of fertilizer runoff, but nitrates were not elevated at sewered sites or OSTDS sites, save for December 2006 (SLWT) at the Japanese Garden site (K), which is likely attributable to a recent application of fertilizer because the levels at this site were low for the other four sampling trips. Ammonia levels can be indicative of fresh OSTDS inputs or sewer leaks. Only in SHWT does the frequency of elevated ammonia concentrations at the OSTDS sites exceed the frequency from the background sites, although the values are similar.

Table 50 – Summary of frequency of elevated nitrogen levels from sampling events in 2006 and 2007.

	Ammonia				Nitrate			
	SLWT		SHWT		SLWT		SHWT	
	Frequency	Percent	Frequency	Percent	Frequency	Percent	Frequency	Percent
OSTDS								
Dekle Beach	6/20	30%	8/15	53%	0/20	0%	0/15	0%
Steinhatchee	6/21	29%	2/18	11%	3/30	10%	1/18	6%
Sewer								
Keaton Beach	13/30	43%	7/21	33%	0/30	0%	0/21	0%
Cedar Island	7/21	33%	3/15	20%	0/21	0%	0/15	0%
Background								
Dekle Beach	3/9	33%	3/6	50%	0/9	0%	0/6	0%
Steinhatchee	8/18	44%	0/12	0%	3/18	17%	1/12	8%
Keaton/Cedar	8/9	89%	3/6	50%	1/9	11%	2/6	33%

The Fenholloway River set of samples showed elevated nutrient levels that were 1-2 orders of magnitude higher than those measured at the paired sites. Further investigation into the significance of the nitrogen species levels is warranted to determine if a water quality impact is in fact occurring and if the source is related to the industrial discharge from the Fenholloway River.

Compared to the SLWT, water quality (as evidenced by violations in DO and microbial pathogen indicators) decreased during the SHWT as expected. In addition, more pressure was put on the assimilation capacity of the environment during the SHWT because the end of summer coincides with the peak of the scalloping season, when the tourist population at the sampling sites tends to increase and more wastewater is generated. Water temperatures are also higher in September, which increases bacterial growth rates and reduces DO. Although the DO exhibited large decreases during SHWT, microbial activity generally increased simultaneously. This may have accounted for the observed dissolved oxygen depletion and frequency of trigger level violations.

Higher TOC and ammonia in the SLWT indicate that runoff may be considered an important input in the region. The nitrogen isotope analysis from May 2007 supports this supposition for the beach communities. Differences in water quality parameters measured between sewered and non-sewered areas were also observed in terms of microbial pathogen indicators. Elevated levels of total nitrogen (which was indicative of organic-N) and enterococci tend to implicate a greater contribution of nutrients to coastal waters from septic systems, but this combination was not seen consistently. OSTDS are expected to perform better during the SLWT event, with the likelihood of failure increasing in the SHWT event. This field study demonstrates that the magnitude of water quality degradation in the area may have a contribution from OSTDS, but indicates other potentially more important inputs which may be human-derived. It is suggested that further monitoring of these rural coastal developments continue, so that the results can be compared to other parts of the United States to determine if the methods employed here are universally applicable.

In summary, two years of sampling events in Taylor County have provided the following:

1) There is a difference between the concentration of contaminants in the SLWT and SHWT events as *E. coli*, *Enterococcus*, and dissolved oxygen all showed higher failures rates during the SHWT when compared to regulatory standards (See Table 49).
2) There is a difference between sewered areas when compared to OSTDS areas, and the background sites. Ammonia, dissolved oxygen, and *Enterococcus* levels demonstrated higher regulatory failure rates at the OSTDS sites than the sewer

sites. The reverse was true for *E. coli* (Table 49). The background sites showed no particular variation from the OSTDS and sewered sites except that excluding the Creek at Dekle Beach site, the background sites consistently produced *E. coli/Enterococcus* ratios below approximately 1.0, a possible indication of a contribution from non-human sources of pollution. Conversely, nearly all of the beach sites showed *E. coli/Enterococcus* ratios that were well above 4.0, indicative of human-derived sources of pollution, within the documented limits of this parameter.

3) While OSTDS installations may have an impact to the coastal waters of Taylor County, there are potential sources of contamination in addition to OSTDS, including fertilizer, industrial pollution, lawn irrigation runoff, sewer leaks, and dog feces.
4) Variability within the base load versus seasonal changes is largely temperature, fertilizer, and precipitation driven. As a result, further study is required to determine base loading differences because none of the sampling events over the two-year period coincided with wet events due to the prolonged drought conditions. However, regrowth of bacteria are indicated in two areas as a result of higher nutrient and temperature conditions combined with the appropriate soils, in the SHWT.

Other, more specific results of the five sampling events indicate the following:

- Although the DO exhibited large decreases during September 2006 SHWT, microbial activity generally increased during this period, which could have accounted for the observed consumption of dissolved oxygen, even after temperature effects are taken into account. The opposite occurred during the December 2006 SLWT event (i.e. DO increased dramatically and microbial activity was lower than observed in the other two sampling events of 2006).
- During the September 2006 SHWT event, ammonia levels were substantially lower in comparison to the May 2006 SLWT, but nearly one-quarter of the samples were

considered high for coastal marine environments (>0.07 mg/L as N). The December 2006 SLWT event showed very low ammonia levels.

- The lowest ammonia levels were encountered in Steinhatchee during the SHWT, but during the SLWT, Steinhatchee had some of the highest ammonia readings measured. Ammonia is an indicator of recent nutrient inputs. However, no noticeable differences in ammonia trends are observed between sites with sewer and sites with OSTDS.
- On average, nitrate levels were below the concentrations considered high for coastal marine environments for the OSTDS and sewered paired sites for all sampling events in both years.
- *Enterococcus* and *E. coli* correlated with the change from SLWT to SHWT. However, the actual microbial densities appear to be misleading due to several very high *E. coli* results from sewered areas that occurred during both seasons, at Cedar Island Beach, Cortez Road Pump Station and Dekle Beach Canal. The high *E. coli* densities were replicated during both SHWT and SLWT (May) in 2006 at the Cortez Road site (Site E). Further investigation of this phenomenon is suggested to determine if a sewer leak is responsible.
- For both *Enterococcus* and *E. coli*, the microbial densities were generally higher for the SHWT, especially for the OSTDS areas, but this was also largely true of the newer sewered areas as well. As noted above, Keaton Beach had isolated cases of extreme microbial contamination recorded during the 2006 SLWT, which skewed the average results but did not mask the general trend and were repeated in May 2007 SLWT.
- Although sewer sites presented higher *E. coli* concentrations, it is worth noting that the sewer system was just recently installed and conditions monitored may still reflect previous contamination, particularly at Cedar Island, where the findings suggest microbial regrowth in warm, shallow, stagnant waters as a possible source rather than an external input.
- Between 5-10% of all *Enterococcus* samples violated the trigger levels in SLWT, but 30-35% violated in SHWT. A similar pattern was observed for *E. coli*.
- TOC and higher ammonia in the 2006 SLWT (May and December) data may indicate anthropogenic background sources from lawn fertilizers or an industrial source, but

this requires further research. The nitrogen isotope analysis seems to implicate fertilizers at the beach communities, but a possible industrial source signal could not be discounted upstream at the background site locations in May 2007.

- Sewered areas (Keaton Beach and Cedar Island) have not shown improved water quality in comparison to areas that remain on OSTDS. Thus, in sewered areas, the possibility that remnant OSTDS inputs have not been fully flushed from the surficial soils cannot be discounted. This finding is also supported by the absence of a change in slope in the bacteriological densities over time at the sewered sites.
- None of the five sampling events experienced any significant rainfall. Therefore, no recent wet-event-related activity was measured. If we combine this finding with the detection of dog *Bacteriodes*, high ammonia concentrations, and high water usage in summer months, we may have a contribution from over-irrigation of lawns that may have been over-fertilized also. There is strong evidence for this fertilizer signal from the nitrogen isotope data at many of the sites in SLWT (May 2007) but much less so in SHWT (September 2007).
- Molecular techniques were used on 22 of the 309 samples collected in this study, and although a number of the samples collected in this study showed very high levels of microbial pathogen indicators, the samples randomly selected for molecular techniques analysis largely did not. The subset of molecular techniques data indicated that the analyzed water samples (only 22 of 309) were not highly contaminated with fecal pollution of human or animal origin. These results are supported by the low IDEXX MPN results for *Enterococcus* in this data set.
- Overall, the molecular data of the analyzed water samples do not suggest a fecal source of contamination, although Boggy Creek, Fenholloway River, and Peterson's Landing are certainly over the recommended single-sample full-body contact exposure limit for certain pathogens (*Enterococcus*, *Bacteriodes*, and *Staphylococcus*).
- The molecular data did find that the Boggy Creek site and the Adams Beach site showed indications of human inputs from enterococci, human-associated *Bacteriodes*, and human-associated antibiotic resistant forms of *Staphylococcus*. Both of these sites would be considered upstream background compared to the sewered or septic

communities downstream and would not be considered large point sources. It is possible that these signals are related to a recent individual event, particularly since the pattern did not repeat in subsequent sampling.

- At Dekle Beach and Keaton Beach, the molecular data found that both the beach sites and the Creek at Dekle site have indications of human source *Bacteriodes* and *Staphylococcus* along with enterococci and *E. coli*. In addition, dog *Bacteriodes* is also found in Dekle Beach, both at the beach and the background sites. The human source material is generally indicative of fecal pollution, and the *Staphylococcus* could be interpreted as coming from skin of swimmers or alternatively, it could indicate gray water releases from bathing inside the home that is subsequently released to the environment via OSTDS. The dog indicator is not surprising, since during sampling it was observed that a majority of the homeowners in this community owned dogs. The human source *Bacteriodes* signals were strongest in May 2007 (SLWT) and seen again in September 2007 SHWT, but the *Staphylococcus* hits were not repeated in September 2007 SHWT. The dog *Bacteriodes* signals are also strong in SHWT, but at the background sites only, not at the beaches as seen in SLWT (May 2007).
- Caffeine was not shown to be an effective tracer for Taylor County, since very little material was detectable. High dilution and low development intensity are suspected as reasons for this result.
- Similarly, optical brighteners were also ineffective for the same reasons as caffeine. The qualitative method is not refined enough to be as sensitive as required to be considered an effective tracer.

RECOMMENDATIONS

Over the course of the investigation, a great deal of information has been collected and analyzed. The findings indicate that to resolve the different sources of pollution to the coastal Taylor County communities, the following additional work is recommended:

1. Monitor sewered areas with respect to OSTDS areas for a longer time period to see if the system stabilizes to a point in which water quality improvements are observable. Indications from the December 2006 SLWT sampling are that this may be happening, but the conditions were found to degrade again in 2007. To better accomplish this, it is recommended to add more representative background sites, particularly for Dekle Beach, and to go further upstream for Blue Creek since the background sites for both areas exhibited water quality violations during all five events, the source of which could not be determined. In particular, the Creek at Dekle site is probably more representative of an upstream site than a background site, mainly due to salinity regime and proximity to the other sites at Dekle Beach. For Blue Creek, during all sampling events, signs of recent human activity were observed at this site, including microbial genes associated with human activity (*Bacteriodes* and *Staphylococcus*) related to fecal matter and swimming activities. Also the new spray-field discharge site for the wastewater treatment facility is located just upstream of the sampling point in addition to the being less than 10 meters from the forcemain crossing. Furthermore, indications that a bird rookery is located just upstream were observed.
2. Monitor during the secondary SHWT (March-April). Taylor County has four seasonal events (i.e. two SHWT and two SLWT events) with a bimodal distribution over the course of the year. In this study, only the primary SHWT, which occurs in September was monitored (twice), while both the primary (December) and secondary (May) SLWT events were monitored. Some differences were noted between the primary and secondary SLWT events, and it would add to the completeness of the study, to evaluate if differences can be observed between the primary and secondary SHWT.

3. More station density is required at the beach communities to help resolve upstream – downstream influences. An increasing trend from upstream to downstream was apparent for the microbial indicators. This would implicate a terrestrial source, and more station density would allow the investigators to potentially triangulate the possible location of the source.
4. Sewer leaks in the newly installed areas must be cataloged to remove this possibility as a confounding factor.
5. Studies of shallow sediments are recommended to determine regrowth patterns of microbial indicators. The results from this study were largely inconclusive because of the relatively small sample size. The potential for regrowth was recorded in May 2007 but the results were not reproducible in sediments collected in September 2007.
6. Monitor the Fenholloway River input with respect to proposed new industrial treatment upgrades and pipelines coming on line (intervention analysis).
7. Investigate the water quality from the coastal estuary downstream of Blue Creek. Keaton Beach and Cedar Island are located on opposite sites of the estuary into which Blue Creek discharges. The estuary consistently contained high *Enterococcus* counts. As a result, further analysis of Blue Creek inputs should be undertaken to determine the contributions to the estuary caused by potential anthropogenic activities upstream of the estuary. Two potentially important issues exist with the estuary: 1) east of the Blue Creek sampling point is the location of the discharge field for the new wastewater treatment facility, which may potentially be supplying nutrients to the Blue Creek watershed, and 2) the estuary could be an important bird rookery, which could act as a source of nutrients to the watershed, mainly from bird droppings. Hydraulic studies could be utilized to determine how currents transport nutrients in the estuary. This would allow the researchers to determine if the nutrients generated from the estuary are stimulating the growth of microorganisms in the Cedar Island and Keaton Beach communities. This will also help identify other potential sources of contamination in this region and identify limitations to flushing these nutrients out of the estuary, such as improperly placed marine structures that promote stagnant waters.

8. To improve the resolution of the caffeine testing, it is recommended to collect much larger sample sizes and perform sample extraction/concentration in the field.
9. To improve the optical brighteners technique, it is proposed to investigate specific wavelength matrices that can act as spectrophotometric fingerprints of optical brighteners using a flow-through fluorometer system with multiple wavelength scanning capabilities.
10. It is recommended that nitrogen isotopic ratios be monitored to separate fertilizer inputs from OSTDS inputs. In May 2007, runoff was implicated at the beach sites, but the upstream background sites showed a possible contribution from an industrial source. More data is needed to make a stronger conclusion. The resolution can be improved by providing duplicate samples: one filtered with pre-combusted GFF filter disks and acidified with HCl prior to freezing and the other syringe-filtered and frozen. This will allow the assay to determine if preservation methods are causing changes in the results.
11. Molecular techniques require much larger sample sizes than first anticipated. It is recommended to attempt additional tests with greater sensitivity to help resolve the human vs. animal input issue.
12. The first several sample sets for molecular techniques conducted in this study focused on enterococci sp., HF8, and most recently added in May 2007, HuBac and DogBac from direct DNA filter extracts. One way to potentially improve sensitivity would be to move the assays from a PCR/electrophoresis detection system (which were used for all samples in this study) to a fluorescent real-time qPCR detection system. The drawback is that reagents for qPCR are more expensive than for regular PCR and gel electrophoresis. It may be possible in the future to add independent qPCR assays based on commercially available primers for another human enterococci marker, a dog enterococci marker, a human *Bacteroides* marker, a cow *Bacteroides* marker, and a dog *Bacteroides* marker. These additional tests may be costly due to the proprietary nature of these newly available markers.
13. Direct DNA filters used in molecular techniques allow for the testing of a wide range of targets from the same filters, but it also limits detection sensitivity, especially if targets are in low abundance in relation to a large background microbial assemblage.

Sensitivity can potentially be increased with culture pre-enrichment before extraction (this is basically the approach with the MFC and mEI media filters). Basically, in addition to direct DNA filters, MFC filters and mEI filters, two more filters could be collected. One from an azide dextrose broth culture incubated overnight to enrich for enterococci (while limiting enzyme inhibition due to media dyes as can happen with mEI), and the other from a filter that is incubated under anaerobic conditions on BBE plates to enrich for *Bacteroides*.

14. Another recommendation to improve the sensitivity of molecular techniques would be to consider using media enrichment filters in addition to direct DNA extraction filters. For instance, a *Bacteroides* specific media filter could be added, although this would require anaerobic incubation. This can be accomplished inexpensively in the field using small disposable GasPak EZ pouches.
15. Expanding the microbial screening to include other known human pathogens such as *Giardia*, *Cryptosporidium*, and viruses could potentially be added to the investigation, but these tests are progressively more expensive and labor intensive. *Giardia* and *Cryptosporidium* testing requires filtering on site with a pump filter rig for water volumes ranging from 60 to 100 liters, then the filters are analyzed for IMS/IMF capture and enumeration. Tissue culture *Cryptospordium* viability/infectivity analysis is required after enumeration to determine how many of the oocysts are actually alive. Screening for viruses also involves filtering a large volume of water sample; however, qPCR enumeration of viruses does not take into account infectivity. Enumeration for noroviruses, enteroviruses, human adenovirus, and Hepatitis A can be done simultaneously. However, the expense and labor for these tests is partly why protozoans and viruses are not routinely measured in environmental water quality monitoring programs.

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Appendix A: Analytical Parameters and Sampling Sites

Table A-1- List of Parameters and Analytical Method Information.

Parameter	Method/SOP	Analyzed By	Detection Range	Method Precision
E. coli (& Total coliform)	Standard Methods SM9223B FAU LT6100	Lab-EES	10 – 24,190 MPN/100 mL	2 MPN/100 mL or see published 95% confidence limits
Enterococcus	Standard Methods SM9223C FAU LT6200	Lab-EES	10 – 24,190 MPN/100 mL	2 MPN/100 mL or see published 95% confidence limits
Caffeine	(FLEnviro SOP)	FLEnviro	2.5 – 5000 ng/L	--
Nitrate	EPA 353.2 (FIU SOP)	FLEnviro US-Biosystems NOAA- AOML	0.003 – 10 mg N/L	See QAPP
Ammonia-nitrogen	EPA 350.1 (FIU SOP)	FLEnviro US-Biosystems NOAA- AOML	0.01 – 2.0 mg N/L	See QAPP
TOC	EPA 415.1 FAU LT5200	Lab-EES	Medium: 20 – 750 mg C/L Low: 1.0 – 25 mg C/L	± 3% or ± 50 ppb/C whichever is greater
TN	EPA 415.1 FAU LT5200	Lab-EES	Medium: 1.0 – 20 mg N/L Low: 0.06 – 1.2 mg N/L	± 3% or ± 25 ppb/N whichever is greater

Table A-2 – Sampling sites information list

Site Cod	Site Name	Category	Latitude	Longitude	Sewer/ Septic tank	Beach Monitoring	Health Dept.	Beach Advisories*
AB	Adam's Beach	Beach	29° 52 53.0	83° 38 09.5	Septic tank		X	
A	Dekle Beach	Beach	29° 50 56.8	83° 37 20.6	Septic tank	X	X	155
JI	Jugg Island Road	Beach	29° 50 51.7	83° 37 05.6	Septic tank			
B	Dekle Beach Canal	Upstream	29° 50 56.7	83° 37 07.3	Septic tank	X		
C	Creek at Dekle Beach	Background	29° 50 55.0	83° 36 57.6	Septic tank	X		
F	Keaton Beach	Beach	29° 49 06.7	83° 35 37.3	Sewer	X	X	133
MR	Marina Road	Beach	29° 49 14.7	83° 35 32.1	Sewer			
E	Cortez Road Canal – Upstream	Upstream	29° 49 31.3	83° 35 29.7	Sewer			
D	Cortez Road Canal - Pump station	Upstream	29° 49 45.4	83° 35 29.3	Sewer			
G	Blue Creek @ Beach Road	Background	29° 49 28.9	83° 34 34.9	Sewer	X		
I	Cedar Island Beach	Beach	29° 48 57.2	83° 35 14.4	Sewer	X	X	147
SL	Seahawk Lane	Beach	29° 48 59.7	83° 35 10.3	Sewer			
H	Heron Road Canal	Upstream	29° 48 42.7	83° 34 50.4	Sewer	X		
J	Main Street & Steinhatchee (Roy's)	Downstream	29° 40 23.2	83° 23 42.2	Septic tank			
K	Third Ave. Fork	Upstream	29° 40 09.0	83° 22 00.3	Septic tank			
L	Boggy Creek @ 51	Upstream	29° 44 00.8	83° 21 32.9	Septic tank			
M	Boggy Creek @ Airstrip Drive	Upstream	29° 43 29.9	83° 20 47.5	Septic tank			
N	Steinhatchee Falls	Background	29° 44 47.6	83° 20 33.5	Septic tank			
FR	Fenholloway River	River Source	30° 03 56.6	83° 33 28.6	Sewer			
HS	Hampton Springs	Upstream	30° 04 16.7	83° 39 44.3	Sewer			
PL	Petersons Landing	Downstream	29° 59 45.8	83° 46 34.9	Septic Tank			

* Number of Beach Advisories posted from August 1, 2000 to July 9, 2007.

Table A-3 – Results of comparison field measurements on September 28, 2006.

Site Name	Time	FAU Lab-EES					FDOH					Comparison				
		Water Temp.	pH	SC	Salinity	DO	Water Temp.	pH	SC	Salinity	DO	Water Temp.	pH	SC	Salinity	DO
		00010 °C	00400	00094 mS/cm	00480 ppt	00299 mg/L O_2	00010 °C	00400	00094 mS/cm	00480 ppt	00299 mg/L O_2	00010 °C	00400	00094 mS/cm	00480 ppt	00299 mg/L O_2
Dekle Beach	6:11	23.09	8.3	38.7	24.7	4.5	23.05	8.1	41.5	25.7	4.1	0.2%	2.7%	-6.9%	-4.0%	10.4%
Dekle Beach Canal	6:25	25.41	8.2	39.3	25.0	4.3	25.50	8.3	45.3	25.7	4.2	-0.4%	-1.3%	-14.3%	-2.7%	1.6%
Creek at Dekle Beach	6:37	23.84	8.5	36.9	23.1	4.7	23.69	8.3	39.0	24.8	4.6	0.6%	2.0%	-5.3%	-7.2%	1.9%
Keaton Beach	7:00	24.79	8.4	41.0	25.9	5.6	24.72	8.5	42.6	27.3	5.4	0.3%	-1.1%	-3.8%	-5.2%	3.9%
Cortez Road Canal - Upstream	7:25	26.06	8.2	34.9	21.9	4.8	26.21	8.4	36.7	23.2	5.1	-0.6%	-3.3%	-4.9%	-5.4%	-5.7%
Cortez Road Canal - Pump station	7:40	25.27	7.8	17.6	10.4	1.9	25.24	8.0	18.2	10.8	1.7	0.1%	-3.0%	-3.5%	-3.8%	15.4%
Blue Creek @ Beach Road	7:57	23.00	7.6	0.3	0.1	5.0	22.94	7.7	0.3	0.1	4.4	0.3%	-1.4%	-3.8%	-8.0%	13.0%
Cedar Island Beach	8:17	25.00	8.2	36.5	23.1	5.3	24.92	8.3	37.8	24.0	4.7	0.3%	-1.3%	-3.6%	-3.7%	12.1%
Heron Road Canal	8:35	25.04	8.0	30.0	18.5	4.3	25.02	8.2	31.0	19.2	4.3	0.1%	-2.9%	-3.3%	-3.7%	0.6%
Main Street & Steinhatchee (Roy's)	9:00	24.61	7.9	23.1	14.0	5.4	24.63	8.0	24.1	14.6	5.7	-0.1%	-1.6%	-4.0%	-4.2%	-5.1%
Third Ave. Fork	9:13	24.36	7.4	6.2	3.4	1.6	24.30	7.8	6.3	3.5	1.5	0.2%	-4.4%	-2.5%	-2.8%	5.8%
Boggy Creek @ 51	9:50	21.00	7.6	0.4	0.2	2.4	20.96	7.3	0.4	0.2	2.7	0.2%	3.8%	-2.8%	-4.9%	-13.0%
Boggy Creek @ Airstrip Drive	9:40	21.91	7.4	0.5	0.3	2.8	21.91	7.7	0.6	0.3	2.7	0.0%	-2.7%	-3.2%	-3.8%	3.0%
Steinhatchee Falls	10:08	21.77	7.3	0.5	0.2	1.4	21.78	7.4	0.5	0.2	1.5	-0.1%	-1.6%	-3.5%	-4.3%	-9.2%
										Correlation		1.00	0.88	1.00	1.00	0.98

49.54	1.58	32.39	100.80	44.30	3.01	0.08 5.60	0.01 0.51	0.01 0.62	0.00 0.11	0.31 11.20	0.00 0.00 J3	7.2	45	21	10	1892	10:55 1:09	49	46	10	19863	6	0	10	63	11:00 1:09	30.0
48.18	0.71	31.37	101.40	56.80	3.94	0.07 5.20	0.02 1.22	0.02 1.40	0.00 0.18	0.28 9.80	2.48 0.08 J3	5.0	44	26	10	1993	11:02 1:12	49	47	10	24196	4	1	10	52	11:02 1:12	38.3
49.81	0.30	32.64	97.70	71.10	5.01	0.01 0.52	0.00 0.24	0.00 0.27	0.00 0.03	0.00 0.00	0.00 0.00 J3	6.5	42	24	10	1602	11:10 1:15	49	37	10	9208	0	0	10	<10	11:09 1:15	320.4
46.87	2.24	30.28	100.55	65.05	4.48	0.04 2.90	0.01 0.53	0.01 0.65	0.00 0.12	0.42 15.10	0.31 0.01 J3	5.3	49	34	10	7701	11:52 1:18	49	42	10	12997	9	2	10	120	11:49 1:18	64.2
a	na	na	na	na	na	na	na	na	na	na	na J3		na	na	na	na	na na	na	na	na	na	na	na	na	na	na na	na
43.94	3.32	28.30	103.60	48.40	3.31	0.04 3.00	0.01 0.56	0.01 0.70	0.00 0.14	0.58 20.80	5.58 0.18 J3		48	31	10	4569	11:08 1:22	49	39	10	10462	2	2	10	41	11:06 1:22	111.4
20.81	3.88	12.43	111.20	13.30	0.99	0.09 6.70	0.00 0.31	0.01 0.48	0.00 0.17	2.87 102.10	0.00 0.00 J3		45	15	10	1576	11:05 1:25	49	37	10	9208	6	0	10	63	11:03 1:25	25.0
0.31	2.61	0.15	109.60	68.80	5.84	0.09 6.20	0.06 4.34	0.06 4.50	0.00 0.16	2.62 93.40	25.40 0.82 J3		24	2	10	345	11:13 1:30	49	26	10	4884	12	1	10	146	11:12 1:30	2.4
46.70	1.35	30.37	102.40	56.75	3.96	0.01 0.38	0.00 0.22	0.00 0.31	0.00 0.09	0.17 5.90	34.07 1.10 J3		49	34	10	7701	11:23 1:33	49	48	10	>24196	1	0	10	10	11:21 1:33	770.1
44.25	2.55	28.52	100.90	67.50	4.75	0.04 3.20	0.01 0.48	0.01 0.59	0.00 0.11	0.55 19.50	1.86 0.06 J3		47	25	10	2809	11:54 1:40	49	43	10	14136	6	1	10	74	11:53 1:40	38.0
39.86	3.40	25.41	107.90	55.20	3.87	0.10 0.96 -101%	0.01 0.49 28%	0.01 0.56 24%	0.00 0.07 0%	0.55 19.70 -1%	4.34 0.14 J3		43	13	10	1281	11:17 1:43	49	35	10	8164	2	0	10	20	11:15 1:43	64.1
39.91	4.00	25.43	103.20	55.70	3.93	0.04 2.90	0.01 0.37	0.01 0.44	0.00 0.07	0.56 19.90	1.86 0.06 J3		42	19	10	1408 -9%	11:20 1:46	49	36	10	8664	0	0	10	<10 67%	11:18 1:46	281.6
37.05	0.63	23.15	-190.10	80.50	5.55	0.04 3.10	0.02 1.35	0.02 1.60	0.00 0.25	1.14 40.50	12.39 0.40 J3		28	6	10	488	11:27 1:49	49	35	10	8164	0	1	10	10	11:25 1:49	48.8
11.95	0.61	6.82	nr	87.90	6.84	0.05 3.60	0.06 4.55	0.07 4.80	0.00 0.25	1.87 66.70	17.04 0.55 J3		13	0	10	148	11:28 1:52	49	39	10	10462	6	0	10	63	11:30 1:52	2.3
0.62	1.20	0.30	nr	103.90	8.88	0.03 2.30 -12%	0.04 2.79 -4%	0.04 2.90 -3%	0.00 0.11 0%	2.63 93.50 5%	1.24 0.04 J3		0	0	10	<10	11:37 2:00	49	17	10	2909	5	1	10	63	11:35 2:00	0.1
a	na	na	na	na	na	0.04 2.60	0.04 2.89	0.04 3.00	0.00 0.11	2.50 89.10	6.50 0.21 J3		na	na	na	na	na na	na	na	na	na	na	na	na	na	na na	na
0.44	0.82	0.21	nr	108.90	9.47	0.02 1.70	0.04 2.77 O	0.04 3.00 O	0.00 0.23	3.48 124.00 O	1.86 0.06 O, J3		7	2	10	96	11:34 2:04	49	31	10	6488	33	8	10	657	11:32 2:04	0.1
0.56	1.15	0.27	nr	106.40	9.17	0.04 3.10	0.02 1.58	0.02 1.70	0.00 0.12	2.40 85.40	6.81 0.22 J3		1	1	10	20	11:40 2:08	49	15	10	2755	4	0	10	41	11:37 2:08	0.5
2.65	3.72	1.36	nr	73.60	5.54	3.21 229.40	O	O	0.48 34.60	5.47 194.90	1554.88 50.20 J3		1	0	10	10	11:58 2:12	49	48	10	>24196	21	4	10	318	11:55 2:12	0.0
2.37	2.33	1.21	nr	85.40	6.78	2.57 183.20	0.08 5.55	0.19 13.85	0.12 8.30	5.31 189.00	1369.04 44.20 J3		5	1	10	63	12:01 2:23	49	43	10	14136	17	6	10	278	11:59 2:23	0.2
26.30	1.80	16.04	nr	87.00	6.40	0.41 29.40	0.24 17.10	0.37 26.10	0.13 9.00	2.56 91.20	607.09 19.60 J3		43	9	10	1145	12:05 2:27	49	48	10	>24196	13	4	10	195	12:03 2:27	5.9
26.30	1.80	16.04	nr	87.00	6.40								45	12	10	1439 -23%	12:07 2:30	49	48	10	>24196	14	5	10	221 -13%	12:06 2:30	6.5
49.69	1.66	32.55		100.10	7.02	0.00 0.07 J3	0.04 3.15	0.04 3.20	0.00 0.05	0.51 18.10	0.62 0.02	9.3	49	40	10	11199	2:43 4:33	49	48	10	>24197	0	0	10	<10	2:40 4:42	2239.8
51.07	1.12	33.58	96.40	59.40	4.21	0.01 0.46 J3	0.05 3.87	0.05 3.90	0.00 0.03	0.27 9.60	0.93 0.03	10.5	27	10	10	528	11:57 12:49	49	40	10	11199	0	0	10	<10	11:57 12:53	105.6
51.30	1.94	33.74	96.30	38.00	2.65	0.02 1.70 J3	0.00 0.20	0.00 0.25	0.00 0.05	0.33 11.60	4.34 0.14	5.7	43	19	10	1510 Q	1:11 1:12	49	38	10	9804 Q	0	0	10	<10 Q	1:10 1:50	302.0
50.66	1.46	33.20	99.20	29.50	2.00	0.07 5.10 J3	0.01 0.40	0.01 0.52	0.00 0.12	0.57 20.40	0.62 0.02	10.5	48	28	10	3968 Q	12:50 12:52	49	35	10	8164 Q	16	2	10	213 Q	12:47 12:53	18.6
49.47	1.81	32.37	97.00	68.00	4.66	0.06 4.50 J3	0.01 1.00	0.02 1.10	0.00 0.10	0.42 14.80	0.93 0.03	4.9	44	32	10	2367 Q	1:33 1:33	48	41	10	7215 Q	2	0	10	20 Q	1:32 1:34	118.4
50.22	0.90	32.94	95.30	58.50	4.20	0.01 0.53 J3	0.00 0.12	0.00 0.16	0.00 0.04	0.08 2.80	0.31 0.01	6.9	45	22	10	1951	1:07 4:27	49	38	10	9804	2	0	10	20	1:05 4:40	97.6
48.44	1.10	31.75	98.80	61.70	4.27	0.03 2.20 J3, 37%	0.01 0.78 20%	0.01 0.87 16%	0.00 0.09 -11%	0.34 12.00 14%	0.62 0.02 0%	4.2	45	22	10	1951	2:45 4:33	49	42	10	12997	4	0	10	41 Q	2:45 4:46	47.6
a	na	na	na	na	na	0.04 3.20 J3	0.01 0.64	0.01 0.74	0.00 0.10	0.29 10.40	0.62 0.02		na	na	na	na	na na	na	na	na	na	na	na	na	na	na na	na
46.60	1.33	30.24	100.60	53.90	3.68	0.04 3.20 J3	0.01 0.71	0.01 0.82	0.00 0.11	1.89 67.20	1.86 0.06		48	28	10	3968 Q	2:01 4:33	49	41	10	12033 Q	8	1	10	97 Q	2:00 4:30	40.9
21.27	3.14	12.73	109.80	23.20	1.74	0.08 5.50 J3	0.00 0.35	0.01 0.55	0.00 0.20	2.99 106.60	0.00 0.00		49	16	10	2755	1:48 4:33	49	44	10	15531	6	4	10	106	1:46 4:30	26.0
0.31	3.04	0.15	106.70	69.60	5.91	0.09 6.70 J3	0.08 5.45	0.08 5.50	0.00 0.05	2.67 95.20	22.92 0.74		29	5	10	496	1:12 4:27	49	24	10	4352	17	4	10	253	1:10 4:40	2.0
47.96	1.92	31.30	100.00	59.70	4.31	0.02 1.30 J3	0.01 0.48	0.01 0.58	0.00 0.10	0.29 10.40	or or or		41	23	10	1459	1:21 4:33	49	34	10	7701	3	1	10	40	2:20 4:40	36.5
45.36	2.67	29.47	100.50	69.70	4.95	0.06 4.20 J3	0.01 0.52	0.01 0.59	0.00 0.07	0.49 17.50	2.17 0.07		39	8	10	530	2:52 4:43	49	24	10	4352	4	0	10	41	2:50 4:46	12.9
41.57	3.22	26.76	100.70	57.27	4.04	0.02 1.60 J3	0.01 0.51	0.01 0.58	0.00 0.07	0.53 19.00	5.27 0.17		44	12	10	1334	2:18 4:27	49	34	10	7701	2	0	10	20	2:17 4:40	66.7
34.75	1.16	21.77	nr	90.50	6.54	0.02 1.60 J3	0.13 9.25	0.13 9.40	0.00 0.15	1.07 38.10	8.98 0.29		15	9	10	284	12:48 1:00	49	28	10	5475	0	0	10	<10	12:48 12:57	56.8
18.53	1.14	10.58	nr	85.90	6.49	0.05 3.30 J3	0.06 4.38	0.06 4.60	0.00 0.22	1.97 70.20	15.80 0.51		23	4	10	355 Q	1:45 1:45	49	47	10	24196 Q	10	1	10	121 Q	1:45 4:30	2.9
0.62	1.98	0.30	nr	104.60	8.96	0.03 2.00 J3	0.04 2.88 -7%	0.04 3.00 -6%	0.00 0.12 18%	2.50 88.9 -6%	7.43 0.24 -29%		2	0	10	20 Q	1:52 4:33	47	14	10	1850 Q	5	0	10	52 Q	1:50 4:30	0.4
0.62	1.92	0.30	nr	104.30	8.95	0.03 2.00 J3	0.04 3.10	0.04 3.20	0.00 0.10	2.64 94.1	9.91 0.32		0	1	10	10 67%	1:57 4:33	47	10	10	16070 -159%	5	0	10	52 Q	1:55 4:30	0.2
0.45	1.80	0.22	nr	107.90	9.35	0.02 1.60 J3	0.05 3.33	0.05 3.60	0.00 0.27	2.09 74.30	0.93 0.03		14	6	10	233 Q	2:30 4:33	49	32	10	6867 Q	21	6	10	345 Q	2:25 4:42	0.7
0.46		0.22	nr	110.30	9.63								49	22	10	3873	17:20 17:21	11	0	10	122	5	2	10	63	5:21 5:21	61.5
0.56	1.80	0.27	nr	106.50	9.18	0.04 3.00 J3	0.03 1.81	0.03 1.90	0.00 0.09	2.33 82.80	0.31 0.01		4	0	10	41 Q	2:35 4:33	46	7	10	1333 Q	1	0	10	10 Q	2:33 4:42	4.1
2.63	3.92	1.35	nr	75.50	5.74	2.85 203.50 J3	0.52 37.40	1.12 80.00	0.60 42.60	6.29 223.80	1338.07 43.20		0	0	10	<10 Q	3:01 4:43	49	48	10	>24196 Q	18	4	10	269 Q	3:00 4:46	0.0
2.38	3.64	1.22	nr	88.10	7.08	2.52 179.70 J3	0.20 14.60	0.34 24.60	0.14 10.00	5.30 188.80	1406.21 45.40		5	0	10	52	3:03 4:43	49	41	10	12033	11	4	10	168	3:02 4:47	0.3
26.15	1.78	15.90	nr	89.60	6.66	0.44 31.40 J3	0.25 17.70	0.36 25.40	0.11 7.70	2.54 90.40	591.60 19.10		42	30	10	905	3:11 4:43	49	47	10	>24196	13	7	10	230	3:10 4:47	3.9
a	na	na	na	na	na	na	na	na	na	na	na		43	9	10	1145 -23%	3:15 4:43	49	48	10	10112 82%	11	2	10	145 45%	3:13 4:47	7.9
49.76	2.48	32.60	100.50	85.55	6.08	O	O	O	O	O	O	9.1	48	28	10	3968	1:55 2:15	49	48	10	10112	21	6	10	345	1:53 2:31	11.5
a	na	na	na	na	na	na	na	na	na	na	na		48	24	10	3282 19%	1:58 2:15	49	48	10	10112 0%	20	5	10	315 9%	1:57 2:31	10.4
51.10	1.31	33.60	98.00	53.80	3.84	0.01 0.54	0.00 0.15	0.00 0.15	0.00 0.00	0.10 3.40	0.62 0.02 J3	8.2	48	38	10	6294	12:30 2:11	49	41	10	12033	1	0	10	10	12:26 2:08	629.4
50.88	1.30	33.44	99.70	31.40	2.26	0.03 1.90	0.01 0.37	0.01 0.37	0.00 0.00	0.53 18.90	5.89 0.19 J3	6.0	12	3	10	169	11:49 2:00	49	27	10	5172	21	4	10	318	1:30 2:28	0.5
50.26	1.78	32.95	98.20	36.80	2.56	0.14 9.80	0.02 1.24	0.02 1.30	0.00 0.06	0.58 20.80	1.55 0.05 J3		49	28	10	5475	12:39 2:02	49	36	10	8664	16	1	10	201	12:38 2:08	27.2
46.79	1.22	30.43	96.05	49.70	3.53	0.11 8.00	0.03 1.86	0.03 1.90	0.00 0.04	0.51 18.30	4.65 0.15 J3		49	44	10	15531	12:44 2:02	49	48	10	>24196	12	3	10	169	12:42 2:08	91.9
50.86	0.70	33.41	96.00	51.10	3.64	0.02 1.40	0.00 0.17	0.00 0.17	0.00 0.00	0.12 4.20	0.00 0.00 J3	6.2	46	12	10	1565	1:09 2:11	49	31	10	6488	1	0	10	10	1:07 2:26	156.5
47.42	1.47	30.89	97.70	71.10	5.04	0.05 3.50	0.01 0.67	0.01 0.67	0.00 0.00	0.26 9.30	2.17 0.07 J3	4.9	47	28	10	3169	1:43 2:15	49	46	10	19863	4	2	10	52	1:41 2:28	60.9
48.71	1.78	31.84	97.30	59.13	4.12	0.11 7.60	0.01 0.77	0.02 1.10	0.00 0.33	0.71 25.30	4.34 0.14 J3		38	21	10	737	1:02 2:02	49	43	10	14136	4	0	10	41	1:00 2:26	18.0
21.30	1.52	12.76	107.45	31.35	2.39	0.07 5.00	0.01 0.62	0.01 0.68	0.00 0.06	2.92 103.80	0.00 0.00 J3		44	6	10	1119	12:49 2:02	49	33	10	7270	6	1	10	74	12:47 2:08	15.1
0.31	3.26	0.15	104.40	73.00	6.21	0.10 7.00 3%	0.09 6.40 6%	0.09 6.40 6%	0.00 0.00	2.36 84.00 4%	n/a n/a		22	1	10	638	1:16 2:21	49	26	10	4884	10	5	10	166	1:13 2:24	3.8
0.31	3.19	0.15	107.90	74.80	6.39	0.10 6.80	0.08 6.00	0.08 6.00	0.00 0.00	2.26 80.60	13.63 0.44 J3		17	2	10	216 99%	1:23 2:21	47	26	10	2924 50%	9	3	10	131 24%	1:19 2:26	1.6
46.80	1.72	30.44	100.40	71.45	5.13	0.04 2.50	0.01 0.83	0.01 0.83	0.00 0.00	0.40 14.20	26.33 0.85 J3		46	16	10	1782	1:32 2:21	49	40	10	11199	5	0	10	52	1:30 2:28	34.3
48.63	3.67	32.19	96.70	77.00	5.42	0.11 7.80	0.01 0.75	0.01 0.75	0.00 0.00	0.98 34.80	0.00 0.00 J3		33	18	10	852	1:50 2:15	49	29	10	5794	9	2	10	120	1:48 2:31	7.1
35.74	2.45	22.55	104.80	56.50	4.17	0.04 3.00	0.01 0.59	0.01 0.59	0.00 0.00	0.96 34.10	1.55 0.05 J3		44	19	10	1631	1:29 2:21	49	37	10	9208	3	2	10	51	1:25 2:28	32.0
42.44	1.65	27.29	nr	86.30	5.94	0.06 4.50	0.02 1.70	0.02 1.70	0.00 0.00	1.13 40.20	19.51 0.63 J3		49	29	10	5794 Q	1:37 2:21	49	33	10	7270 Q	1	0	10	10	11:48 2:34	579.4
12.22	1.13	6.99	nr	94.40	7.51	0.08 5.90	0.08 5.49	0.08 5.50	0.00 0.01	1.53 54.50	20.13 0.65 J3		7	3	10	107	11:54 2:00	49	40	10	11199	8	0	10	86	11:51 2:34	1.2
0.62	1.90	0.30	nr	106.20	9.15	0.04 3.10	0.06 4.20	0.06 4.20	0.00 0.00	2.39 85.10	7.12 0.23 J3		1	0	10	10	11:58 2:00	48	11	10	1860	2	1	10	30	11:55 2:34	0.3
0.46	1.68	0.23	nr	109.60	9.54	0.05 3.40 0%	0.09 6.20 -14%	0.09 6.20 -14%	0.00 0.00	1.92 68.40 ####	9.29 0.30 J3, 79%		12	0	10	135	12:02 2:00	49	29	10	5794	14	6	10	233	12:00 2:34	0.6
a	na	na	na	na	na	0.05 3.40	0.10 7.10	0.10 7.10	0.00 0.00	2.03 72.20	4.03 0.13 J3		na	na	na	na	na na	na	na	na	na	na	na	na	na	na na	na
0.56	1.70	0.27	nr	107.30	9.28	0.06 4.10	0.03 2.23	0.04 2.50	0.00 0.27	2.11 75.10	8.36 0.27 J3		1	0	10	10	12:10 2:11	46	11	10	1515	4	1	10	52	12:04 2:34	0.2
2.59	3.60	1.33	nr	78.80	6.08	2.63 187.70	0.53 38.00	1.15 82.20	0.62 44.20	5.66 201.60	###### 41.00 J3		0	0	10	<10	12:12 2:11	48	48	10	10112	17	2	10	228	12:09 2:34	0.0
2.31	2.58	1.18	nr	89.90	7.27	2.10 150.00 3%	0.27 19.30 5%	0.45 31.80 5%	0.18 12.50 7%	4.98 177.30 3%	###### 38.90 ####		7	0	10	75	12:17 2:11	48	39	10	6586	8	3	10	119	12:15 2:08	0.6
a	na	na	na	na	na	2.03 145.00	0.26 18.40	0.42 30.10	0.16 11.70	4.83 172.00	###### 39.20		na	na	na	na	na na	na	na	na	na	na	na	na	na	na na	na
20.09	2.19	11.96	nr	95.00	7.36	0.67 48.00 17%	0.38 26.90 1%	0.50 35.70 0%	0.12 8.80 -3%	3.18 113.20 2%	768.15 24.80 5%		29	8	10	545	12:15 2:11	49	45	10	17329	3	4	10	72	12:20 2:08	7.6
a	na	na	na	na	na	0.56 40.30	0.37 26.70	0.50 35.80	0.13 9.10	3.12 111.00	730.98 23.60		na	na	na	na	na na	na	na	na	na	na	na	na	na	na na	na
58.44	0.08	39.09	95.85	93.50	6.75	0.00 0.30	0.00 0.21	0.00 0.21	0.00 0.00	0.00 0.00	0.93 0.03		0	0	10	<10	2:02 2:15	47	20	10	2310	0	0	10	<10	2:01 2:31	1.0

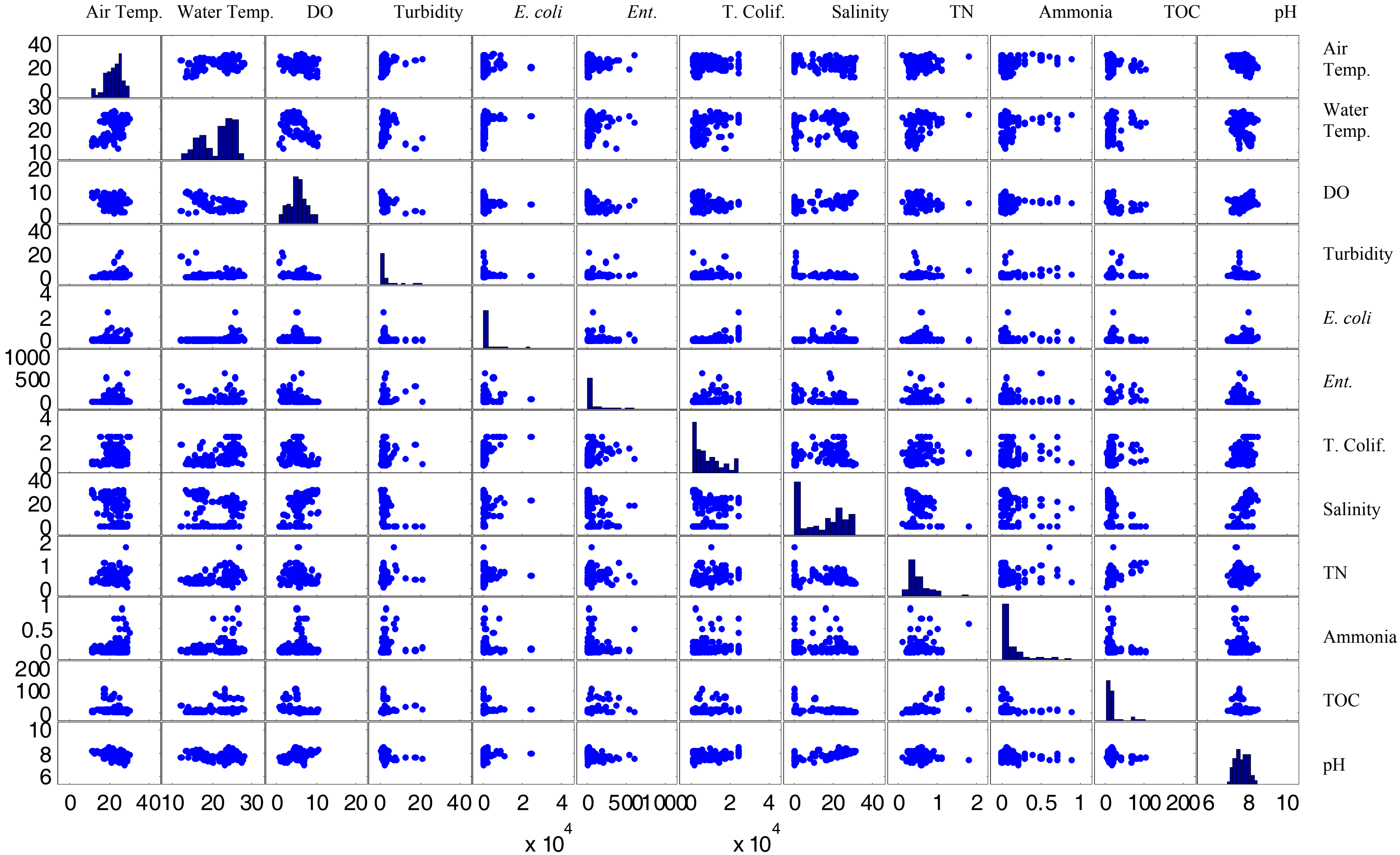
9	2.81	29.27	17.00	87.40	6.32	0.04 2.60	0.01 0.94 J3	0.02 1.10 J3	0.00 0.16 J3	0.71 25.40	0.01 0.36 J3	6.75	0.58 J3i	1	10	10	111		3:05	3:17	49	36	10	8664		2	0	10	20		3:05	3:17	5.6	
8	3.38	26.41	18.20	84.30	6.13	0.00 0.00 U	0.00 0.00 U	0.00 0.00 U	0.00 0.17 ?	0.62 22.10	0.00 0.14 J3	8.03	0.68 J3i	1	23	10	245		2:23	2:55	49	35	10	8164		3	0	10	31		2:21	2:55	7.9	
6	1.15	15.04	17.40	79.50	6.05	0.00 0.00 U	0.01 0.36 J3	0.01 0.48 J3	0.00 0.12 J3	0.63 22.50	0.01 0.18 J3	15.40	1.02 J3i	1	24	10	256		2:11	2:55	49	48	10	>24196	L	1	1	10	20		2:09	2:55	12.8	
8	2.29	0.13	28.90	94.90	8.37	0.08 5.70	0.02 1.11 J3	0.02 1.30 J3	0.00 0.19 J3	0.54 19.10	0.04 1.20 J3	4.94	0.46 J3i	7	1	10	85		2:35	2:55	49	19	10	3255		1	0	10	10		2:34	2:55	8.5	
a	na	na	na	na	na	na na	na na	na na	na na	na na	na na	na	na	0	0	10	<10	U	2:37	3:17	49	20	10	1355		2	2	10	41		2:32	2:55	0.1	K
2	2.25	30.78	18.50	82.00	5.77	0.00 0.00 U	0.00 0.20 J3	0.00 0.34 J3	0.00 0.14 J3	0.22 7.90	0.04 1.20 J3	6.61	0.52 J3i	0	6	10	60		2:51	3:17	49	35	10	8164		0	0	10	<10	U	2:49	3:17	12.0	
5	2.26	30.80	14.80	81.70	5.74	0.00 0.00 U	0.00 0.06 J3	0.00 0.16 J3	0.00 0.10 J3	0.24 8.70	0.01 0.26 J3	6.56	0.57 J3i	1	10	10	111			3:17	49	39	10	10462		0	0	10	<10	U	2:54	3:17	22.2	
2	1.97	31.06	17.00	83.40	5.89	0.00 0.00 U	0.00 0.29 J3	0.01 0.40 J3	0.00 0.11 J3	0.39 14.00	0.02 0.50 J3	5.76	0.43	2	14	10	164		3:25	3:48	49	37	10	9208		0	0	10	<10	U	3:25	3:48	32.8	
1	3.10	26.05	18.10	84.00	6.12	0.05 3.30	0.00 0.24 J3	0.01 0.39 J3	0.00 0.15 J3	0.76 26.90	0.01 0.34 J3	5.81	0.47	2	12	10	143		2:44	3:17	49	44	10	15531		2	0	10	20		2:41	2:55	7.2	
3	3.25	29.21	8.30	80.15	5.65	0.03 2.10	0.01 0.88 J3	0.02 1.10 J3	0.00 0.22 J3	0.51 18.30	0.01 0.22 J3	9.00	0.65 J3i	0	8	10	80		3:01	3:17	49	35	10	8164		0	0	10	<10	U	2:59	3:17	16.0	
8	1.03	16.56	17.60	81.10	6.16	0.04 2.90	0.05 3.69 J3	0.06 4.00 J3	0.00 0.31 J3	1.17 41.50	0.01 0.38 J3	8.17	0.67	1	4	10	50		3:10	3:17	49	46	10	19863		12	1	10	146		3:10	3:17	0.3	
5	1.40	0.30	24.50	92.70	8.10	0.00 0.00	0.01 0.90 J3	0.01 1.00 J3	0.00 0.10 J3	2.38 84.60	0.01 0.40 J3	9.37	0.36	1	0	10	10		3:17	3:48	46	8	10	1376		0	0	10	<10	U	3:18	3:48	2.0	
1	7.31	0.25	3.50	97.00	8.63	0.09 6.40	0.01 0.73 J3	0.01 0.94 J3	0.00 0.21 J3	0.17 6.10	0.01 0.36 J3	14.50	0.71	0	0	10	<10	U	3:13	3:48	0	0	10	<10	O	5	0	10	52		3:14	3:48	0.1	O
6	3.14	0.27	21.90	95.80	8.49	0.04 3.10	0.01 0.65 J3	0.01 0.74 J3	0.00 0.09 J3	2.62 93.40	0.01 0.38 J3	9.78	0.39	0	0	10	<10	U	3:20	3:48	39	4	10	789		0	0	10	<10	U	3:21	3:48	1.0	K
4	4.86	1.36	16.50	64.40	4.93	2.49 177.80	0.35 25.30 J3	0.50 35.40 J3	0.14 10.10 J3	5.27 187.70	1.44 46.40 J3	169.78	6.95	0	0	10	<10	U	3:29	3:48	47	48	10	6910		2	2	10	41		3:27	3:48	0.1	K
5	2.76	1.10	7.20	76.10	6.19	1.60 114.60	0.36 25.70 J3	0.42 29.90 J3	0.06 4.20 J3	4.67 166.20	1.19 38.40 J3	97.20 J3i	4.21	1	0	10	10		3:35	3:48	49	46	10	19863		9	6	10	164		3:33	3:48	0.1	
2	2.75	3.61	6.60	70.60	5.51	0.55 39.20	1.00 71.30 J3	1.09 77.90 J3	0.09 6.60 J3	3.36 119.80	0.82 26.60 J3	62.21 J3i	2.48	0	3	10	30		3:39	3:48	49	48	10	10112		10	3	10	144		3:37	3:48	0.2	
3	ns	30.19	10.40	94.80	7.02	0.06 4.10 J3	0.01 0.82 J3	0.01 1.00 J3	0.00 0.18 J3	0.35 12.40 J3	0.00 0.11 J3	8.20 J3i	0.52	ns	ns	ns	ns		ns	ns	ns	ns	ns	ns	O	ns	ns	ns	ns	O	ns	ns	ns	O
9	1.23	31.09	14.30	90.70	6.58	0.00 0.07 J3	0.00 0.07 J3	0.00 0.13 J3	0.00 0.06 J3	0.08 2.80 J3	0.00 0.04 J3	7.46 J3i	0.47	0	3	10	30		3:00	3:38	49	46	10	19863		1	0	10	10		2:58	3:36	3.0	
1	1.88	30.24	16.80	93.60	6.90	0.01 0.54 J3	0.00 0.00 U	0.00 0.15 J3	0.00 0.21 ?	0.30 10.70 J3	0.01 0.22 J3	7.27 J3i	0.49	1	3	10	40		3:26	3:38	49	47	10	24196		0	0	10	<10	U	3:23	3:36	8.0	
4	1.97	30.71	16.30	91.20	6.67	0.04 2.50 J3	0.01 0.44 J3	0.01 0.55 J3	0.00 0.11 J3	0.40 14.30 J3	0.00 0.01 J3	7.77 J3i	0.56	2	3	10	51			3:38	49	38	10	9804		1	0	10	10		3:29	3:36	5.1	
3	2.58	30.81	16.10	92.10	6.73	0.06 4.00 J3	0.00 0.19 J3	0.01 0.38 J3	0.00 0.19 J3	0.30 10.60 J3	0.00 0.06 J3	7.82 J3i	0.57	2	3	10	51		3:09	3:38	49	41	10	12033		2	0	10	20		3:05	3:36	2.6	
4	2.26	30.02	14.90	84.50	6.03	0.03 2.10 J3	0.01 0.48 J3	0.01 0.54 J3	0.00 0.06 J3	0.41 14.50 J3	0.00 0.02 J3	7.90	0.55	3	5	10	82		3:37	3:49	49	46	10	19863		0	0	10	<10	U	3:34	3:36	16.4	
6	1.25	30.85	13.90	84.90	6.04	0.01 0.53 J3	0.00 0.15 J3	0.00 0.16 J3	0.00 0.01 J3	0.14 5.10 J3	0.00 0.01 J3	5.62	0.34	3	6	10	92		3:40	3:49	49	34	10	7701		2	0	10	20		3:39	3:49	4.6	
2	2.30	27.29	16.70	84.30	6.11	0.05 3.60 J3	0.01 0.95 J3	0.02 1.20 J3	0.00 0.25 J3	0.99 35.20 J3	0.01 0.19 J3	6.37	0.46	6	5	10	116		4:06	4:16	49	18	10	3076		3	0	10	31		4:07	4:42	3.7	
2	3.36	27.73	12.40	82.00	5.87	0.00 0.04 J3	0.00 0.09 J3	0.00 0.14 J3	0.00 0.05 J3	0.78 27.70 J3	0.01 0.36 J3	7.76	0.54	1	6	10	71		3:47	3:49	49	38	10	9804		1	0	10	10		3:44	3:49	7.1	
8	1.32	15.20	10.60	7.73	5.83	0.00 0.29 J3	0.00 0.13 J3	0.00 0.18 J3	0.00 0.05 J3	0.54 19.10 J3	0.00 0.01 J3	14.99	0.74	1	24	10	256		4:11	4:18	49	46	10	19863		3	0	10	31		4:10	4:40	8.3	
9	2.47	0.14	15.30	90.50	7.86	0.09 6.40 J3	0.10 6.93 J3	0.10 7.00 J3	0.00 0.07 J3	1.95 69.40 J3	or, L	6.23	0.44	0	0	10	<10	U	3:57	4:15	49	12	10	2247		1	1	10	20		3:56	4:44	0.3	K
7	2.01	31.41	12.20	80.20	5.58	0.00 0.13 J3	0.00 0.33 J3	0.00 0.33 J3	0.00 0.00 U	0.27 9.50 J3	0.03 1.00 J3	6.01	0.37	4	0	10	41		4:44	4:54	49	32	10	6867		0	0	10	<10	U	4:41	5:03	8.2	
0	2.12	30.42	14.50	81.20	5.71	0.01 0.88 J3	0.00 0.32 J3	0.01 0.47 J3	0.00 0.15 J3	0.35 12.60 J3	0.03 0.87 J3	4.78	0.39	5	4	10	94		4:59	5:02	49	30	10	6131		0	0	10	<10	U	4:55	5:08	18.8	
4	3.44	26.69	13.80	80.10	5.74	0.04 2.70 J3	0.01 0.44 J3	0.01 0.47 J3	0.00 0.03 J3	0.63 22.60 J3	0.01 0.24 J3	4.74	0.40	49	17	10	2909		4:15	4:20	49	35	10	8164		0	0	10	<10	U	4:14	4:40	581.8	
7	1.48	30.52	14.30	78.90	5.49	0.04 2.60 J3	0.01 0.60 J3	0.01 0.63 J3	0.00 0.03 J3	0.52 18.40 J3	or, L	5.74 J3i	0.39 J3i	3	3	10	61		3:53	4:10	49	38	10	9804		4	1	10	52		nr	4:44	1.2	
9	1.48	21.34	15.60	80.90	5.98	0.04 2.70 J3	0.06 4.49 J3	0.06 4.60 J3	0.00 0.11 J3	1.07 38.10 J3	0.02 0.57 J3	6.21 J3i	0.37 J3i	2	0	10	20		4:50	5:01	49	31	10	6488		10	2	10	132		4:48	5:03	0.2	
3	2.15	0.30	17.60	90.30	7.82	0.01 0.40 J3	0.01 0.66 J3	0.01 0.66 J3	0.00 0.00 U	2.17 77.30 J3	0.00 0.07 J3	11.22 J3i	0.36	0	1	10	10		4:28	4:31	49	27	10	5172		0	0	10	<10	U	4:20	4:39	2.0	
a	na	na	na	na	na	na na	na na	na na	na na	na na	na na	na	na	0	1	10	10		4:29	4:32	49	31	10	6488		2	1	10	30		4:23	4:39	0.3	
1	8.16	0.25	9.80	93.00	8.15	0.07 5.10 J3	0.02 1.60 J3	0.02 1.70 J3	0.00 0.10 J3	0.42 15.10 J3	0.00 0.15 J3	16.41 J3i	0.69	0	0	10	<10	U	4:39	4:54	49	43	10	14136		8	0	10	86		4:38	4:38	0.1	K
6	3.48	0.27	18.80	94.30	8.30	0.08 5.50 J3	0.00 0.29 J3	0.00 0.29 J3	0.00 0.00 U	2.26 80.30 J3	0.00 0.05 J3	7.92	0.32	0	0	10	<10	U	4:35	4:37	29	1	10	432		0	0	10	<10	U	4:33	4:38	1.0	K
8	5.45	1.37	15.00	66.00	5.10	2.29 163.70 J3	0.29 21.00 J3	0.40 28.30 J3	0.10 7.30 J3	5.30 188.80 J3	1.42 45.90 J3	163.48	6.53	0	0	10	<10	U	5:08	5:09	49	48	10	>24196	L	4	3	10	72		5:06	5:08	0.1	K
8	3.06	1.11	17.00	78.80	6.48	1.59 113.80 J3	0.32 22.90 J3	0.37 26.60 J3	0.05 3.70 J3	4.44 158.20 J3	1.13 36.50 J3	91.41 J3i	4.03	4	1	10	52	Q	2:55	3:38	48	40	10	6893	Q	3	0	10	31	Q	2:52	3:36	1.7	Q
2	2.32	10.05	17.30	88.20	7.17	0.39 27.80 J3	0.30 21.60 J3	0.37 26.40 J3	0.07 4.80 J3	1.81 64.60 J3	0.33 10.80 J3	50.12 J3i	2.00	2	2	10	41		2:49	3:38	48	47	10	9606		8	1	10	97		2:48	3:36	0.4	
0	3.57	30.64	-4.70	105.90	7.37	ns O	ns O	ns O	ns O	ns O	ns O	8.64 J3i	0.50 J3i	1	8	10	81		3:33	3:24	49	38	10	9804		0	0	10	<10	U	3:32	3:21	16.2	
9	1.45	33.05	4.30	97.50	6.84	0.00 0.11	0.00 0.31	0.00 0.35	0.00 0.04	0.14 5.00	0.00 0.06 J3	8.44 J3i	0.45 J3i	2	5	10	71		3:37	3:23	49	38	10	9804		0	0	10	<10	U	3:33	3:21	14.2	
0	1.80	32.82	5.60	99.60	7.05	0.01 0.67	0.00 0.00 U	0.02 1.50	0.04 2.60 ?	0.21 7.60	0.44 14.30 J3	8.44	0.49	1	8	10	97		3:40	3:54	49	45	10	17329		0	1	10	10		3:38	3:54	9.7	
9	1.82	32.33	6.40	103.00	7.39	0.00 0.00	0.01 0.81	0.01 0.90	0.00 0.09	0.32 11.50	0.00 0.06 J3	8.63	0.51	0	7	10	70		3:42	3:54	49	37	10	9208		1	0	10	10		3:41	3:54	7.0	
6	2.29	31.39	4.60	91.00	6.31	0.04 2.87	0.01 1.01	0.02 1.10	0.00 0.09	0.48 17.10	0.00 0.06 J3	7.50	0.53	2	9	10	112		3:46	3:54	49	43	10	14136		2	1	10	30		3:44	3:54	3.7	
8	1.56	33.70	5.90	94.40	6.53	0.00 0.19	0.01 0.94	0.01 0.95	0.00 0.01	0.21 7.50	0.00 0.02 J3	4.75	0.39	7	7	10	150		3:50	3:54	48	28	10	4160		0	0	10	<10	U	3:48	3:54	30.0	
1	2.14	29.25	7.20	91.00	6.38	0.00 0.31	0.00 0.33	0.01 0.44	0.00 0.11	0.81 28.80	0.00 0.11 J3	5.22	0.43	9	8	10	187		3:58	4:03	49	38	10	9804		3	0	10	31		3:57	4:03	6.0	
5	4.81	30.00	6.80	90.20	6.28	0.00 0.00 U	0.00 0.17	0.00 0.25	0.00 0.08	0.70 24.90	0.01 0.40 J3	6.62	0.53	2	2	10	41		3:55	3:54	49	35	10	8164		0	0	10	<10	U	3:51	3:54	8.2	
9	1.40	16.06	4.20	86.80	6.47	0.00 0.09	0.01 0.59	0.01 0.65	0.00 0.06	0.48 17.20	0.00 0.04 J3	9.76	0.65	1	3	10	41		4:01	4:03	49	48	10	>24196	L	0	0	10	<10	U	4:00	4:03	8.2	
0	2.25	0.14	9.00	100.70	8.64	0.09 6.10	0.08 5.85	0.08 6.00	0.00 0.15	2.03 72.20	0.02 0.62 J3	3.93	0.35	0	0	10	<10	U	4:04	4:03	49	30	10	6131		6	2	10	84		4:02	4:03	0.1	K
0	2.15	0.14	10.80	100.70	8.64	0.08 6.00	0.08 5.67	0.08 5.80	0.00 0.13	1.96 69.70	or, L	4.74	0.39	0	0	10	<10	U	4:06	4:03	49	31	10	6488		9	6	10	164		4:05	4:03	0.0	K
8	1.41	33.69	4.20	89.30	6.06	0.00 0.00 U	0.00 0.24	0.00 0.24	0.00 0.00 U	0.20 7.20	0.02 0.75 J3	6.44 J3i	0.37 J3i	1	40	10	425		4:09	4:18	49	48	10	>24196	L	0	0	10	<10	U	4:08	4:11	85.0	
8	1.88	31.42	3.70	92.50	6.44	0.02 1.40	0.01 0.47	0.01 0.65	0.00 0.18	0.49 17.60	or, L	6.74 J3i	0.43 J3i	4	6	10	104		4:33	4:29	49	43	10	14136		0	0	10	<10	U	4:32	4:28	20.8	
7	4.20	29.51	5.40	92.10	6.48	0.02 1.30	0.02 1.36	0.02 1.40	0.00 0.04	0.52 18.50	0.01 0.17 J3	4.91	0.42	8	12	10	218		4:28	4:29	49	43	10	14136		1	0	10	10		4:26	4:25	21.8	
7	1.55	31.54	6.70	87.90	6.02	0.03 1.90	0.01 0.89	0.01 0.95	0.00 0.06	0.50 17.70	0.01 0.29 J3	4.15	0.35	9	1	10	109		4:25	4:22	49	22	10	3873		0	0	10	<10	U	4:23	4:25	21.8	
7	0.81	23.36	5.00	93.10	6.81	0.04 3.20	0.05 3.22	0.05 3.40	0.00 0.18	0.99 35.10	0.06 1.80 J3	6.50	0.39	5	1	10	63		4:12	4:18	49	45	10	17329		8	1	10	97		4:11	4:11	0.6	
6	1.65	0.32	10.40	101.30	8.70	0.01 0.58	0.02 1.60	0.02 1.60	0.00 0.00 U	2.40 85.50	0.00 0.10 J3	10.69 J3i	0.32 J3i	0	0	10	<10	U	4:20	4:22	46	9	10	1421		0	0	10	<10	U	4:18	4:25	1.0	K
4	9.68	0.35	-16.80	105.00	9.14	0.07 5.20	0.02 1.39	0.02 1.50	0.00 0.11	0.44 15.60	0.00 0.12 J3	12.42	0.58	3	2	10	51		4:16	4:18	49	44	10	15531		4	1	10	52		4:13	4:11	1.0	
a	na	na	na	na	na	0.07 5.20	0.02 1.39	0.02 1.50	0.00 0.11	0.44 15.70	0.00 0.06 J3	na	na	3	0	10	31		4:17	4:18	49	46	10	19863		3	0	10	31		4:14	4:25	1.0	
9	3.03	0.28	10.70	107.80	9.47	0.08 5.60	0.01 0.47	0.01 0.47	0.00 0.00 U	2.39 85.10	0.00 0.02 J3	11.16	0.40	0	0	10	<10	U	4:22	4:22	35	5	10	663		1	1	10	20		4:21	4:25	0.3	K
3	5.60	1.46	6.90	76.00	5.87	2.63 188.20	0.23 16.40	0.29 20.60	0.06 4.20	5.25 186.90	1.32 42.70 J3	161.48	6.48	0	0	10	<10	U	4:39	4:29	49	48	10	>24196	L	ns	ns	ns	ns	O	ns	ns	ns	O
1	3.21	1.18	-0.90	91.30	7.52	2.98 213.10	0.30 21.10	0.34 24.00	0.04 2.90	4.54 161.60	1.18 38.10 J3	119.16	4.41	0	0	10	<10	U	10:16	10:45	49	45	10	17329		6	4	10	106		10:17	10:45	0.0	K
9	2.61	16.57	6.80	100.70	7.85	0.30 21.20	0.42 30.00	0.46 32.60	0.04 2.60	2.02 72.10	0.12 3.80 J3	39.86	1.76	1	3	10	40		10:14	10:45	49	45	10	17329		15	1	10	187		10:13	10:45	0.2	
7	0.24	40.02	6.40	111.10	7.81	0.00 0.00 U	0.00 0.32	0.01 0.42	0.00 0.10	0.01 0.24	0.00 0.05 J3	0.40	0.07	0	0	10	10		nr	4:29	1	0	10	10		0	0	10	<10	U, J4	4:31	4:29	2.0	

13.0	11.9	10.3	0.2	0.029	0.004	0.005	0.001	1.109	0.005	20.6		0.47			6	0	10	63	1:10	3:36	47	30	10	3436	0	0	10	<10	1:12	3:36	13
0.2	0.1	8.0	2.1	0.125	0.050	0.052	0.001	1.298	0.021	12.5		0.33			9	1	10	109	1:28	3:41	31	5	10	546	0	0	10	<10	1:30	3:42	22
29.7	29.6	10.0	0.4	0.018	0.011	0.012	0.001	1.140	0.002	6.5		0.28			6	2	10	84	1:34	3:45	38	40	10	1768	0	0	10	<10	1:36	3:44	17
24.7	24.1	7.2	0.9	0.064	0.005	0.006	0.001	1.202	0.009	6.9		0.26			17	3	10	241	1:37	3:42	49	27	10	5172	0	0	10	<10	1:39	3:44	48
28.2	28.0	9.7	0.4	0.035	0.015	0.015	0.001	1.163	0.006	7.7		0.24			5	0	10	52	1:46	3:46	46	11	10	1515	2	0	10	20	1:47	3:47	3
16.8	15.9	5.7	1.0	0.064	0.129	0.132	0.002	1.264	0.028	10.8		0.35			7	0	10	75	1:52	3:47	49	43	10	14136	4	0	10	41	1:54	3:48	2
8.4	7.4	6.0	1.0	0.088	0.047	0.050	0.004	1.264	0.020	14.6		0.36			2	0	10	20	1:56	3:53	49	34	10	7701	4	0	10	41	1:57	3:53	0
0.4	0.3	1.6	17.8	0.028	0.008	0.009	0.001	1.781	0.012	36.5		0.44			14	3	10	197	2:04	3:49	49	45	10	17329	24	3	10	359	2:05	3:51	1
0.4	0.3	1.6	17.8	ns	ns	ns	ns	ns	ns	ns		ns			13	1	10	160	2:10	3:56	49	44	10	15531	28	0	10	395	2:12	3:56	0
0.4	0.3	3.3	2.4	0.053	0.018	0.020	0.001	1.870	0.013	15.0	h	0.36			6	0	10	63	2:16	3:59	47	16	10	1989	1	0	10	10	2:17	4:00	6
0.3	0.3	2.2	2.4	0.129	?	0.015	or	1.646	0.031	18.6		0.29			0	0	10	<10	2:18	3:57	26	4	10	414	0	0	10	<10	2:19	3:58	1
1.6	1.3	7.6	8.7	3.881	0.324	0.642	0.318	0.941	1.666	74.8	Q, g	nr			1	0	10	10	2:19	4:00	49	48	10	>24196	3	0	10	31	2:19	4:00	0
34.8	34.8	11.7	0.0	U	0.001	0.001	U	0.567	0.002	1.6		0.06			0	0	10	<10	1:05	3:34	6	2	10	84	0	0	10	<10	1:07	3:34	1
28.5	28.4	7.0	0.7	0.005	0.004	0.004	U	1.239	0.002	11.4		0.28			9	0	10	98	12:57	4:14	33	25	10	1000	0	0	10	<10	12:59	4:15	20
28.1	27.8	6.0	1.1	0.057	0.010	0.011	U	1.328	0.003	11.2		0.42			15	1	10	187	1:03	4:16	41	32	10	1815	2	0	10	20	1:05	4:17	9
27.7	27.4	7.5	0.7	0.050	0.009	0.009	U	1.123	0.006	12.8		0.38			13	3	10	183	1:06	4:19	43	35	10	2318	1	0	10	10	1:07	4:18	18
29.9	29.9	8.3	0.4	0.004	0.007	0.007	U	1.045	0.002	6.2		0.26			3	0	10	31	1:10	4:25	33	28	10	1066	0	0	10	<10	1:11	4:23	6
24.5	23.9	6.3	1.1	0.109	0.020	0.021	0.001	1.401	0.009	8.4		0.35			5	0	10	52	1:15	4:23	45	21	10	1892	2	0	10	20	1:16	4:23	3
12.7	11.7	8.4	0.5	0.039	0.010	0.010	U	1.208	0.006	21.3		0.56			12	3	10	169	1:18	4:22	48	34	10	5247	1	0	10	10	1:19	4:21	17
0.2	0.1	6.6	2.2	0.125	0.068	0.069	U	1.230	0.012	15.4		0.43			15	2	10	199	1:24	4:19	41	7	10	959	3	0	10	31	1:25	4:20	6
29.7	29.6	8.9	0.3	0.007	0.005	0.005	U	1.269	0.002	7.3		0.26			11	2	10	145	1:28	4:28	42	32	10	1961	0	0	10	<10	1:29	4:27	29
24.1	23.5	5.9	1.2	0.064	0.010	0.010	U	1.494	0.007	7.1		0.32			20	5	10	315	1:33	4:26	47	37	10	4541	1	0	10	10	1:34	4:26	32
24.1	23.5	5.9	1.2	ns	ns	ns	ns	ns	ns	5.8		0.27			ns	ns	na	ns	na	na	ns	ns	na	ns	ns	ns	na	ns	na	na	ns
26.0	25.6	8.0	0.4	0.041	0.025	0.025	U	0.708	0.007	7.6		0.37			6	0	10	63	1:38	4:29	46	30	10	2878	3	0	10	31	1:39	4:30	2
16.6	15.7	4.5	0.7	0.050	0.060	0.060	0.001	1.500	0.025	10.9		0.39			12	1	10	146	1:42	4:33	49	44	10	15531	5	1	10	63	1:43	4:34	2
4.2	3.5	4.9	1.0	0.057	0.063	0.063	U	1.550	0.034	15.4		0.34			13	0	10	148	1:45	4:33	49	29	10	5794	4	2	10	62	1:43	4:32	2
0.4	0.3	0.5	12.7	0.029	0.011	0.011	U	2.165	0.009	32.9		0.49			23	3	10	341	1:59	4:36	48	38	10	6294	15	5	10	235	2:00	4:36	1
0.4	0.3	0.5	12.7	ns	ns	ns	ns	ns	ns	ns		ns			21	2	10	292	2:03	4:41	45	35	10	2933	18	1	10	231	2:03	4:39	1
0.4	0.3	2.6	1.9	0.085	0.029	0.029	U	1.870	0.013	17.3		0.30			1	0	10	10	1:50	4:31	41	9	10	1014	2	0	10	20	1:51	4:30	1
0.4	0.3	2.5	1.9	0.090	0.025	0.025	U	1.960	0.015	16.8		0.27			4	0	10	41	1:53	4:35	41`	8	10	987	2	0	10	20	1:54	4:34	2
0.5	0.3	2.0	2.8	0.130	?	0.028	or	1.500	0.347	15.8		0.31			1	0	10	10	2:06	4:41	19	3	10	272	3	0	10	31	2:07	4:42	0
1.6	1.2	6.3	8.2	3.364	0.604	0.933	0.329	1.185	1.524	79.2	Q, g	nr			0	0	10	<10	2:10	4:43	49	48	10	>24196	4	0	10	41	2:10	4:43	1
29.5	29.5	7.8	0.5	0.007	0.003	0.003	0.001	1.020	0.002	9.5		0.34	J3i	few bright spots	8	1	10	97	2:58	2:42	30	13	10	657	0	0	10	<10	2:59	2:43	19
29.5	29.4	8.5	0.7	0.006	0.003	0.004	0.001	0.961	0.002	9.6		0.34	J3i	ns	7	1	10	85	3:03	3:24	36	12	10	845	0	0	10	<10	3:04	3:24	17
28.8	28.6	5.2	0.8	0.063	0.007	0.009	0.002	0.851	0.002	10.6		0.45	J3i	half the pad with low intensity britheness and fev	16	5	10	250	3:07	3:26	45	39	10	3325	2	0	10	20	3:08	3:25	13
28.5	28.3	7.0	0.7	0.056	0.012	0.013	0.001	1.143	0.004	9.2		0.40	J3i	not detectable	8	1	10	97	3:09	3:27	41	25	10	1532	1	0	10	10	3:10	3:28	10
30.9	31.0	9.3	0.4	0.007	0.004	0.004	U	1.039	U	5.5		0.25	J3i	few bright spots	2	0	10	20	3:15	3:29	29	23	10	805	0	0	10	<10	3:16	3:28	4
26.7	26.3	6.2	0.9	0.081	0.014	0.015	0.001	1.093	0.005	7.2		0.31	J3i	low intensity britheness	5	1	10	63	3:18	3:30	47	11	10	1664	0	0	10	<10	3:19	3:31	13
13.1	12.1	10.5	0.6	0.025	0.005	0.007	0.001	0.946	0.003	20.2		0.57	J3i	some spots of low intensity bightners	15	1	10	187	3:22	3:33	48	36	10	5748	0	0	10	<10	3:23	3:31	37
0.2	0.1	6.7	4.0	0.134	0.063	0.064	0.002	1.275	0.009	11.3		0.45	J3i	nr	15	2	10	199	3:29	3:33	43	6	10	1050	1	0	10	10	3:30	3:34	20
30.5	30.6	8.8	1.7	0.050	0.017	0.018	0.001	1.157	0.002	5.6		0.23	J3i	not detected	12	2	10	158	3:33	3:36	39	13	10	1010	0	0	10	<10	3:35	3:34	32
22.9	22.2	5.2	1.4	0.084	0.012	0.013	0.001	1.446	0.003	6.7		0.29	J3i	low intensity britheness and very few very bright	24	4	10	373	3:42	3:39	49	35	10	8164	0	0	10	<10	3:43	3:39	75
27.5	27.2	7.9	0.4	0.048	0.028	0.029	0.002	1.390	0.005	7.5		0.30	J3i	not detected	8	1	10	97	3:37	3:36	48	21	10	2851	2	0	10	20	3:38	3:37	5
27.5	27.2	7.9	0.4	ns	ns	ns	ns	ns	ns	7.4		0.29	J3i	ns	ns	ns	na	ns	na	na	ns	ns	na	ns	ns	ns	na	ns	na	na	ns
17.4	16.5	5.3	0.5	0.055	0.107	0.109	0.002	1.438	0.021	8.8		0.29	J3i	not detected	2	2	10	41	3:46	3:41	49	46	10	19863	3	1	10	41	3:45	3:40	1
3.5	2.9	4.9	0.6	0.053	0.055	0.057	0.002	1.227	0.016	14.0		0.28	J3i	low intensity britheness and few very bright spot:	7	1	10	85	3:49	3:42	49	32	10	6867	3	0	10	31	3:50	3:42	3
0.4	0.3	3.3	1.7	0.049	0.005	0.006	0.001	2.112	0.004	37.8		0.60	J3i	low intensity britheness and few very bright spot:	20	0	10	249	3:55	3:44	48	39	10	6586	10	2	10	132	3:57	3:46	2
0.4	0.3	1.4	21.2	0.098	0.024	0.025	0.002	1.775	0.007	17.7		0.40	J3i	nr	3	0	10	31	3:53	3:44	39	5	10	813	1	0	10	10	3:54	3:43	3
0.3	0.3	1.8	2.3	0.129	0.011	0.013	0.001	1.767	0.018	18.6		0.37	J3i	low intensity britheness and few very bright spot:	0	1	10	10	3:59	3:47	29	3	10	464	1	0	10	10	4:00	3:46	1
0.3	0.3	1.8	2.3	ns	ns	ns	ns	ns	ns	ns		ns	J3i	ns	0	1	10	10	4:02	3:48	30	2	10	471	2	0	10	20	4:04	3:48	1
1.6	1.3	7.0	7.7	3.175	0.632	0.982	0.350	1.135	1.852	77.7	Q, g	nr	J3i	ns	1	0	10	10	4:08	3:49	49	48	10	>24196	7	0	10	75	4:09	3:49	0

32.2	31.8	20.9	20.1	4.8	1.9	0.038		0.014	I, Q	0.014	U	U	21	J	0.7	h	nr	49	39	10	10462	12:52	12:58	49	48	10	>24196	14	2	10	185	12:52	12:58	57
31.3	32.3	20.3	19.3	3.2	3.2	0.057		0.014	I, Q	0.014	U	ns	25	J	0.7	h	nr	38	11	10	910	12:47	12:58	49	6	10	1720	4	0	10	41	12:48	12:58	22
27.0	28.3	17.5	16.5	5.3	2.5	0.047		0.014	I, Q	0.014	U	ns	19	J	0.6	h	nr	40	18	10	1212	12:42	12:52	49	42	10	12997	1	0	10	10	12:43	12:52	121
22.5	22.6	14.6	13.5	4.6	2.7	0.13	I	0.023	I, Q	0.023	U	ns	18	J	0.5	h	nr	29	5	10	496	12:56	1:04	49	45	10	17329	13	3	10	183	12:57	1:04	3
3.0	3.0	2.0	1.6	3.3	1.0	0.012	I	0.044	I, Q	0.044	0.0005	U	10	J	0.2	h	nr	19	7	10	324	1:01	1:04	49	39	10	10462	11	16	10	307	1:01	1:04	1.1
0.4	0.4	0.3	0.2	3.3	1.0	0.028		0.033	I, Q	0.033	U	ns	72	J,g	1.1	h	nr	23	6	10	383	1:05	1:12	49	40	10	11199	24	5	10	388	1:06	1:12	1.0
					1.5											h		19	4	10	285	1:10	1:12	49	41	10	12033	23	4	10	355	1:11	1:12	0.8
0.6	0.5	0.4	0.3	2.7	1.6	U		0.017	I, Q	0.017	U	ns	19	J	0.2	h	nr	3	1	10	41	1:16	1:19	49	21	10	3654	4	1	10	52	1:17	1:19	0.8
0.5	0.5	0.3	0.2	1.1	1.9	U		0.0073	I, Q	0.0083	0.001	ns	18	J	0.2	h	nr	8	2	10	108	1:23	1:31	49	24	10	4352	4	0	10	41	1:24	1:31	3
						U		U	Q	U	U	ns	1	J	0.8	h	nr	0	0	10	<10	1:27	1:31	0	0	10	<10	0	0	10	<10	1:27	1:31	1
38.8	37.4	25.2	24.8	4.4	2.5	0.13		U	Q	U	U	ns	20	h	0.7		nr	39	40	10	1887	11:55	12:50	49	47	10	24196	0	0	10	<10	11:52	12:50	377
39.3	40.1	25.6	25.1	3.6	1.4	0.067		0.018	I, Q	0.018	U	ns	21	h	0.8		nr	47	21	10	2400	12:02	12:50	49	44	10	15531	3	0	10	31	11:57	12:50	77
35.6	35.0	23.2	22.5	4.6	1.3	0.07		0.015	I, Q	0.015	U	ns	21	h	0.7		nr	43	20	10	1552	12:05	12:59	49	45	10	17329	6	0	10	63	12:04	12:59	25
40.4	41.2	26.3	25.8	5.2	1.0	0.11		U	Q	U	U	ns	14	h	0.5		nr	44	17	10	1541	12:03	12:59	49	47	10	24196	1	0	10	10	12:06	12:59	154
																		45	18	10	1726	12:08	1:05	49	47	10	24196	0	0	10	<10	12:09	1:05	345
33.4	34.4	21.7	20.9	4.7	1.2	0.056		0.017	I, Q	0.017	U	ns	19	h	0.6		nr	40	17	10	1182	12:13	1:05	49	44	10	15531	4	2	10	62	12:11	1:05	19
17.4	17.8	11.3	10.3	1.7	3.5	U		U	Q	U	U	U	69	h,g	0.7		nr	49	13	10	2359	12:16	1:12	49	45	10	17329	12	2	10	158	12:13	1:12	15
0.3	0.2	0.2	0.1	4.8	1.0	U		U	Q	0.015	0.01	ns	104	h,g	1.1		nr	3	0	10	31	12:19	1:12	49	27	10	5172	3	1	10	41	12:18	1:12	1
0.3	0.2	0.2	0.1	4.9	1.1	0.016	I	U	Q	0.015	0.01	ns	89	h,g	1.1		nr	1	0	10	10	12:23	1:17	49	22	10	3873	2	1	10	30	12:21	1:17	0
35.8	36.1	23.3	22.5	4.9	1.3	0.067		0.015	I, Q	0.015	U	ns	20	h	0.6		nr	49	48	10	>24196	12:28	1:24	49	48	10	>24196	5	1	10	63	12:27	1:24	384
30.2	30.6	19.7	18.7	4.3	1.6	0.043		U	Q	U	U	ns	20	h	0.6		nr	49	25	10	4611	12:52	1:30	49	43	10	14136	19	23	10	540	12:52	1:30	9
24.5	24.4	15.9	14.9	5.2	1.3	0.021		0.015	I, Q	0.015	U	U	16	h	0.4		nr	23	8	10	411	12:45	1:24	49	39	10	10462	1	1	10	20	12:47	1:24	21
7.2	7.2	4.7	4.0	1.7	1.0	0.044		0.047	I, Q	0.053	0.006	U	16	h	0.4		nr	13	1	10	160	1:02	1:40	49	37	10	9208	12	3	10	169	1:03	1:40	1
0.4	0.4	0.3	0.2	2.4	1.3	U		U	Q	U	U	ns	79	h,g	1.0		nr	9	1	10	109	12:57	1:30	49	45	10	17329	9	2	10	120	12:55	1:30	1
0.5	0.5	0.4	0.3	2.6	1.3	U		U	Q	U	U	ns	21	h	0.3		nr	2	0	10	20	12:32	1:17	48	17	10	2382	2	0	10	20	12:33	1:17	1
0.5	0.5	0.3	0.2	1.4	1.5	U		U	Q	U	U	ns	20	h	0.4		nr	2	1	10	30	1:01	1:30	49	10	10	2046	12	1	10	146	1:00	1:30	0
38.7	37.3	25.2	24.7	4.5	2.3	0.082		0.023	I	0.023	U	U	19		0.6	J,h	lot recovere	41	30	10	1730	11:42	2:20	49	48	10	>24196	1	0	10	10	11:41	2:20	173
39.3	39.6	25.5	25.0	4.3	1.2	0.037		U		U	U	U	18		0.7	J,h	Negative	49	35	10	8164	11:48	2:20	49	45	10	17329	14	2	10	185	11:46	2:20	44
36.9	36.1	23.6	23.1	4.7	1.5	0.053		0.017	I	0.017	U	U	18		0.6	J,h	Negative	46	23	10	2254	11:55	2:31	49	48	10	>24196	2	0	10	20	11:54	2:31	113
41.0	40.8	26.7	25.9	5.6	0.7	0.067		0.018	I			U	14		0.5	J,h	Negative	35	16	10	891	11:57	2:31	49	38	10	9804	0	0	10	<10	11:56	2:31	178
34.9	35.6	22.7	21.9	4.8	1.2	0.055		0.020	I	0.02	U	U	20		0.9	J,h	Negative	32	12	10	700	12:01	2:36	49	41	10	12033	2	2	10	41	12:00	2:36	17
35.1	35.9	22.8	22.1	5.0	1.2	0.05		0.02	I	0.02	U	U	18		0.7	J,h	Negative	39	19	10	1174	12:04	2:36	49	47	10	24196	6	1	10	74	12:03	2:36	16
17.6	17.7	11.4	10.4	1.9	3.0	0.095		U		U	U	U	67	g	0.7	J,h	Negative	45	14	10	1529	12:13	2:40	49	43	10	14136	21	1	10	279	12:13	2:40	5
0.3	0.2	0.2	0.1	5.0	1.3	U		0.0057	I			U	67	g	1.0	J,h	Negative	4	0	10	41	12:11	2:40	49	23	10	4106	1	1	10	20	12:09	2:40	2
36.5	36.5	23.8	23.1	5.3	1.0	0.072		0.014	I			U	14		0.5	J,h	Negative	49	29	10	5794	12:17	2:44	49	48	10	>24196	1	1	10	20	12:16	2:44	290
30.0	30.0	19.5	18.5	4.3	1.5	0.056		0.011	I			U	18		0.8	J,h	Negative	46	17	10	1842	12:28	2:48	49	45	10	17329	1	0	10	10	12:25	2:48	184
																J,h		39	12	10	984	12:33	2:48	49	41	10	12033	0	0	10	<10	12:30	2:48	197
23.1	23.0	15.1	14.0	5.4	1.2	0.019	I	0.012	I			U	16		0.4	J,h	Negative	17	3	10	241	12:24	2:44	49	37	10	9208	0	0	10	<10	12:21	2:44	48
6.2	6.1	4.0	3.4	1.6	0.6	U		0.051				U	13		0.3	J,h	Negative	3	1	10	41	12:40	2:52	49	35	10	8164	3	1	10	41	12:39	2:52	1
0.4	0.4	0.3	0.2	2.4	1.5	0.045		0.014	I			U	91	g	0.8	J,h	ıresced a li	10	2	10	132	12:44	2:55	49	42	10	12997	20	1	10	262	12:42	2:55	1
0.5	0.5	0.4	0.3	2.8	1.5	U		0.015	I			U	21		0.3	J,h	ıresced a li	2	0	10	20	12:38	2:52	48	18	10	2489	1	0	10	10	12:34	2:52	2
0.5	0.4	0.3	0.2	1.4	1.5	U		U				0.321	20		0.3	J,h	Negative	1	0	10	10	12:47	2:55	49	22	10	3873	6	2	10	84	12:47	2:55	0

00	slack	nr	7.54	29.2	19.0	28.1	2.7	18.1	6.2	0.5	U	ns	7	K	0.3	K	negative	28	6	10	488	3:39	3:43	48	35	10	5493	32	7	10	609	3:39	3:43	1
00	slack	nr	7.83	15.0	9.8	14.9	2.1	8.8	5.9	0.4	U	ns	14	K	0.8	K	negative	49	25	10	4611	3:47	3:46	49	48	10	>24196	1	0	10	10	3:47	3:46	461
00	slack	out	7.40	0.3	0.2	0.3	1.7	0.1	6.8	0.2	0.043	ns	5	K, d	0.3	K, d	negative	2	0	10	20	3:53	3:51	48	19	10	2603	2	0	10	20	3:53	3:51	1
00	slack	out	7.40	0.3	0.2	0.3	1.7	0.1	6.8	ns	ns	ns	8	K	0.5	K	ns	ns	ns	na	ns	na	na	ns	ns	na	ns	ns	ns	na	ns	na	na	ns
00	nr	out	7.86	33.6	21.8	32.4	1.3	21.1	6.1	0.4	U	ns	ns	K	ns	K	negative	22	8	10	391	4:01	3:57	49	30	10	6131	0	0	10	<10	4:01	3:57	78
00	nr	out	7.69	34.1	22.1	32.7	1.6	21.4	6.1	0.3	U	ns	8	K	0.7	K	negative	19	9	10	350	4:09	4:03	49	34	10	7701	0	0	10	<10	4:09	4:06	70
00	nr	out	7.47	27.5	17.9	27.3	2.2	16.9	5.6	0.2	U	ns	ns		ns		negative	34	16	10	850	4:16	4:12	49	37	10	9208	5	0	10	52	4:14	4:16	16
00	ebb	out	7.77	25.8	16.8	25.8	0.6	15.7	5.4	0.2	U	ns	10	K	0.9	K	negative	15	2	10	199	4:22	4:17	49	27	10	5172	0	0	10	<10	4:21	4:19	40
00	ebb	out	7.65	5.0	3.2	5.0	1.4	2.7	5.8	0.2	0.039	ns	4	K	0.1	K	negative	2	1	10	30	4:28	4:26	49	39	10	10462	4	0	10	41	4:26	4:27	1
00	ebb	nr	7.43	0.5	0.4	0.5	6.4	0.2	5.3	0.6	U	ns	20	K	1.8	K	negative	1	0	10	10	4:25	4:23	49	39	10	10462	5	0	10	52	4:24	4:24	0
00	ebb	out	7.37	0.6	0.4	0.6	1.3	0.3	6.5	0.2	0.026	ns	14	K	0.5	K	negative	0	0	10	<10	4:30	4:30	46	7	10	1333	1	0	10	10	4:30	4:32	1
00	nr	out	6.99	0.5	0.3	0.3	1.7	0.2	6.9	0.2	0.016	ns	16	K	0.6	K	negative	2	0	10	20	4:33	4:33	40	9	10	959	0	0	10	<10	4:33	4:33	4
00	ebb	out	7.75	35.0	22.8	32.8	3.9	22.1	6.7	0.2	U	ns	11		0.3		negative	13	11	10	278	2:26	3:41	48	38	10	6294	0	0	10	<10	2:26	3:42	56
00	ebb	out	7.62	34.6	22.5	33.2	1.7	21.8	6.2	0.2	U	ns	13		0.6		negative	40	12	10	1039	2:31	3:45	49	27	10	5172	11	0	10	122	2:31	3:46	9
00	ebb	out	7.49	29.1	18.9	27.8	2.7	18	6.4	0.3	U	ns	10	Q, Y	0.4	Q, Y	negative	44	17	10	1541	2:37	3:49	48	35	10	5493	3	0	10	31	2:37	3:50	50
00	nr	nr	7.70	38.1	24.8	36.8	1.7	24.2	6.1	0.2	U	ns	9		0.3		negative	28	24	10	790	2:43	3:52	49	43	10	14136	0	0	10	<10	2:43	3:53	158
00	slack	out	7.54	29.3	19.0	28.6	5.5	18.1	5.9	0.2	U	ns	12		0.5		negative	14	7	10	245	2:46	3:55	49	33	10	7270	0	0	10	<10	2:46	3:56	49
00	slack	out	7.54	29.3	19.0	28.6	5.5	18.1	5.9	ns	ns	ns	12		0.5		ns	ns	ns	na	ns	na	na	ns	ns	na	ns	ns	ns	na	ns	na	na	ns
00	slack	out	7.92	15.2	9.9	15.1	2.1	8.8	5.7	U	U	ns	24		0.7		negative	49	35	10	8164	2:49	3:58	49	47	10	24196	3	3	10	61	2:49	3:59	134
00	slack	out	7.91	15.2	9.9	15.1	1.8	8.8	5.8	0.7	U	ns	20		0.6		negative	49	33	10	7270	2:51	4:00	49	48	10	>24196	1	0	10	10	2:53	4:01	727
00	slack	out	8.01	0.3	0.2	0.3	2.4	0.1	6.8	U	0.05	ns	8		0.3		negative	3	1	10	41	2:55	4:15	49	23	10	4106	1	0	10	10	2:55	4:16	4
00	slack	out	7.71	34.5	22.4	34.0	1.5	21.7	5.6	U	U	ns	9		0.4		negative	25	13	10	527	2:59	4:19	47	24	10	2700	0	0	10	<10	2:59	4:19	105
00	nr	out	nr	25.2	16.4	25.1	3.3	15.4	5.6	U	U	ns	9		0.3		negative	31	27	10	956	3:03	4:22	49	41	10	12033	2	0	10	20	3:03	4:22	48
00	ebb	out	7.70	27.1	17.6	27.0	1.8	16.6	5.6	U	U	ns	12		0.4		negative	48	24	10	3282	3:09	4:24	49	41	10	12033	4	1	10	52	3:09	4:25	63
00	slack	nr	7.68	2.9	1.9	2.9	1.6	1.5	6.6	0.1	U	ns	8		0.3		negative	7	0	10	75	3:11	4:27	49	44	10	15531	7	1	10	85	3:11	4:28	1
00	slack	weak	7.37	0.5	0.3	0.5	8.1	0.2	6.9	0.7	U	ns	16	Q, Y	0.8	Q, Y	negative	1	0	10	10	3:25	4:33	49	43	10	14136	3	3	10	61	3:25	4:33	0
00	ebb	out	7.25	0.6	0.4	0.5	2.1	0.3	6.6	0.2	0.011	ns	13	Q, Y	0.3	Q, Y	negative	1	0	10	10	4:09	4:38	41	5	10	906	1	0	10	10	4:09	4:39	1
00	ebb	out	7.25	0.6	0.4	0.5	2.1	0.3	6.6	ns	ns	ns	ns		ns		negative	0	0	10	<10	3:21	4:30	47	10	10	1607	2	0	10	20	3:21	4:32	0
00	nr	out	7.24	0.5	0.3	0.5	2.8	0.2	6.6	0.1	U	ns	17		0.3		negative	0	0	10	<10	3:27	4:35	45	8	10	1274	3	1	10	41	3:27	4:36	0
00	ebb/high	out/moderate	7.75	33.7	21.9	32.9	0.9	21.1	5.8	0.1	U	U	11	J3	0.5	J3	negative	19	12	10	390	3:51	3:48	47	40	10	5099	0	0	10	<10	3:51	3:34	78
00	ebb/high	out/moderate	7.75	33.7	21.9	32.9	0.9	21.1	5.8	ns	ns	ns	ns		ns		negative	17	15	10	393	6:08	4:46	47	40	10	5099	na	na	na	ns	na	na	ns
00	ebb/high	out/weak	7.70	34.9	22.7	34.1	1.1	22	5.8	0.1	U	U	11	J3	0.5	J3	negative	22	29	10	698	3:56	3:35	49	41	10	12033	6	1	10	74	3:56	3:39	9
00	ebb/high	weak	7.45	30.6	20.0	30.6	2.6	19.1	5.4	0.3	U	U	11	J3	0.6	J3	negative	32	18	10	815	4:00	3:41	49	41	10	12033	1	0	10	10	4:00	3:42	82
00	ebb/high	moderate	7.86	35.5	23.1	22.4	1.1	22.4	5.3	0.1	U	U	10	J3	0.5	J3	negative	18	26	10	560	4:03	3:43	49	45	10	17329	0	0	10	<10	4:03	3:44	112
00	ebb	nr	7.49	29.6	19.2	29.6	3.7	18.3	5.4	U	U	U	10	J3	0.5	J3	negative	18	5	10	281	4:10	3:45	46	20	10	2035	0	0	10	<10	4:08	3:50	56
00	ebb	nr	7.49	29.6	19.2	29.6	3.7	18.3	5.4	ns	ns	ns	7	J3	0.3	J3	ns	ns	ns	na	ns	na	na	ns	ns	na	ns	ns	ns	na	ns	na	na	ns
00	slack	nr	7.99	15.5	10.1	15.9	1.2	9	5.1	U	U	U	18	J3	0.7	J3	negative	49	35	10	8164	4:07	3:51	49	48	10	<24196	0	0	10	<10	4:09	3:52	1633
00	slack	nr	7.75	0.3	0.2	0.3	1.5	0.1	5.7	U	0.048	U	7	J3	0.4	J3	negative	2	1	10	30	4:13	3:52	49	23	10	4106	3	2	10	51	4:12	3:53	1
00	ebb/high	out/moderate	7.68	37.2	24.2	37.5	1.2	23.6	5.1	0.5	U	0.04	9	J3	0.5	J3	negative	38	27	10	1338	4:15	3:54	49	40	10	11199	1	0	10	10	4:15	3:56	134
00	high	out/weak	7.34	26.2	17.0	26.8	2.4	16	5.0	0.9	U	U	6	J3	0.3	J3	negative	24	8	10	431	4:20	3:56	47	16	10	1989	1	0	10	10	4:18	3:58	43
00	ebb/high	out/weak	7.70	30.1	19.6	30.6	1.1	18.7	5.1	U	U	U	10	J3	0.4	J3	negative	29	7	10	528	4:24	3:58	49	32	10	6867	1	3	10	40	4:24	4:00	13
00	ebb/high	stagnant	7.47	6.0	3.9	6.0	0.4	3.2	5.8	0.2	0.027	0.03	8	J3	0.3	J3	negative	6	1	10	74	4:28	4:00	49	40	10	11199	18	3	10	256	4:28	4:01	0
00	ebb/high	stagnant	7.55	0.5	0.3	0.5	7.4	0.2	6.1	0.5	U	U	13	Q, Y, J3	1.0	Q, Y, J3	negative	23	8	10	411	4:48	4:40	49	44	10	15531	6	1	10	74	4:47	4:36	6
00	ebb/high	stagnant	7.55	0.5	0.3	0.5	7.4	0.2	6.1	ns	ns	ns	ns		ns	J3	negative	33	6	10	620	6:11	4:49	49	46	10	19863	ns	ns	na	ns	na	na	ns
00	ebb/high	out/stagnant	nr	0.6	0.4	0.6	1.4	0.3	6.4	0.1	0.01	U	15	J3	0.4	J3	negative	0	0	10	<10	4:41	4:12	40	10	10	985	1	1	10	20	4:41	4:10	0
00	ebb/high	out/stagnant	nr	0.6	0.4	0.6	1.4	0.3	6.4	ns	ns	ns	ns		ns		negative	2	0	10	20	4:44	3:37	45	5	10	1162	2	0	10	20	4:52	4:42	1
00	ebb/high	ou/stagnant	7.26	0.6	0.4	0.5	1.4	0.3	6.4	U	0.012	U	11	Q, Y, J3	0.3	Q, Y, J3	negative	1	0	10	10	4:41	4:38	39	4	10	789	1	0	10	10	4:44	4:14	1
00	na	na	7.55	0.0	0.0	0.0	0.1	0.1	or	0.2	U	ns	0	Q, Y, J3	U	Q, Y, J3	negative	0	0	10	<10	4:34	4:09	0	0	10	<10	0	0	10	<10	4:31	4:07	1
00	ebb/high	weak	7.16	0.5	0.3	0.5	1.8	0.3	6.5	U	U	0.03	11	Q, Y, J3	0.3	Q, Y, J3	negative	0	0	10	<10	4:53	4:42	44	9	10	1223	0	1	10	10	4:50	4:40	1

Table A-8. Correlation Matrix of Water Quality Parameters



Appendix B: Sampling Site Information

Developed with Septic Tank

Dekle Beach

The Dekle Beach area is classified as "developed" and is currently served by on site sewage treatment and disposal systems (OSTDS). The FDOH Beach Monitoring Program has conducted water quality analyses on this beach (Site A) since 2000. From the beginning of the program until October 2006, 139 beach advisories have been posted. The Taylor County Health Department has conducted bacterial analyses on the beach sites since 2004. The sampling sites for Dekle Beach are summarized in Table B-1.

Table B-1 – Sampling site designations for Dekle Beach

Code	Site Name	Latitude	Longitude
A	Dekle Beach	29^o 50.948'	83^o 37.178'
JI	Jugg Island Road		
B	Dekle Beach Canal	29^o 50.944'	83^o 37.119'
C	Creek at Dekle Beach	29^o 50.908'	83^o 36.964'

A) Dekle Beach: Along the shore, the remains of old septic tank units were observed during all sampling events. These treatment structures served residences that were destroyed during the "*No Name Storm*" event in March 1993. The beach is narrow and located just at the side of the road. Across the road, along the east side, the residences rely on septic tanks for disposal of their wastewater. Samples were collected by walking into the water up to knee high and using a pole sampler. The probe readings were done by attaching the probe to the pole.

JI) Jugg Island Road: This beach site is located along the border of the Dekle Beach canal inlet and south of the Dekle Beach site (A). This site has a private cluster of four single family homes bordering the ocean. There are two very long piers that extend out into the ocean from the two of the homes, and a small dock on the canal side of the shallow inlet. This site was added in 2007, and the samples were collected by reaching the pole outwards from one of the small stepped piers. The probe readings were done by dropping the probe in the water from the edge of the pier.

B) Dekle Beach Canal: This site is located along the border of the Mexico Road canal in an empty lot between two single family residences. The canal is directly connected to the ocean and surrounded by homes on both sides. Some neighbors have boats in the canal. The samples were collected by reaching the pole towards the middle of the canal. The probe readings were done by dropping the probe in the water right at the edge of the canal.

C) Creek at Dekle Beach: A background site, this creek connects a marsh area to the end of the canal. A two-lane bridge passes over the creek. The water seemed stagnant during the first sampling trip in May (SLWT) although strong outgoing movement was noticed in September on all sampling days. The samples were collected from the road using the pole. The probe readings were done by dropping the probe directly into the water.

Steinhatchee

Steinhatchee is a fairly developed area served by OSTDS, with approximately 1,500 inhabitants, according to OnBoard LLC (http://www.onboardllc.com). The sampling sites for the Steinhatchee basin are summarized in Table B-2.

Table B-2 – Sampling site designations for Steinhatchee

Code	Site Name	Latitude	Longitude
J	Main Street & Steinhatchee (Roy's)	29° 40.385'	83° 23.705'
K	Third Ave. Fork (Japanese Garden)	29° 40.161'	83° 22.011'
L	Boggy Creek @ 51	29° 44.004'	83° 21.542'
M	Steinhatchee @ Airstrip Drive	29° 43.503'	83° 20.789'
N	Steinhatchee Falls	29° 44.792'	83° 20.542'

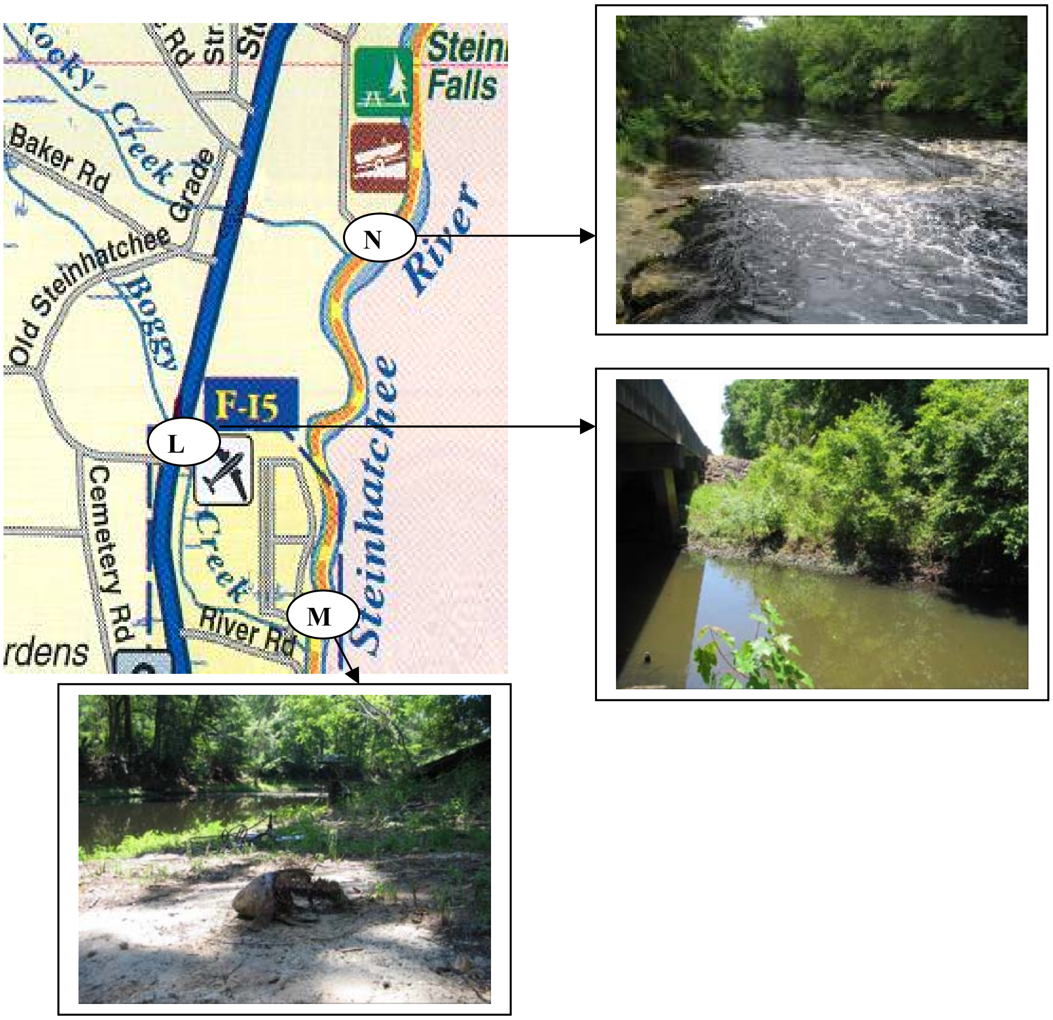
J) Main Street & Steinhatchee (Roy's): This site is located along the north side of the mouth of the Steinhatchee River, at Main Street, just east of the restaurant known as Roy's. There is a large septic tank on the property, with an infiltration field and mound located on the other side of the road. During the first trip in May, the samples were collected from the first pier close to the restaurant. During the second trip in September, this pier was in very shallow water, so the samples were collected from a second pier a few meters east of the previous sampling point. Samples were collected using the pole sampler. The probe readings were done by dropping the probe directly into the water from the edge of the pier.

K) Third Ave. Fork (Japanese Gardens): This site is located at the end of the street along an isolated finger of the Steinhatchee River. The site is close to private property. It was noted that some people go to the site during lunch time to eat. The samples were collected using a pole sampler. The probe readings were done by dropping the probe directly into the water.

L) Boggy Creek @ 51: This site represents the tributary structure upstream of the Steinhatchee River. The samples were collected from the bridge at highway 51 using the pole sampler. The probe readings were done by dropping the probe directly into the water.

M) Steinhatchee @ Airstrip Drive: This site is located in a small community development, with no sewer network. The owners of the nearby homes do not inhabit them year-round, and several homeowners were not present in the area for the entire sampling event. The samples were collected at the Steinhatchee River from a concrete boat ramp using the pole sampler. The probe readings were done by dropping the probe directly into the water, or by attaching to the pole sampler during periods of low flow.

N) Steinhatchee Falls: This site represents the natural background condition for the Steinhatchee River basin. The water velocity is rapid at this site due to a relatively important elevation drop and narrow channel. The samples were collected in the Steinhatchee Falls Park just upstream from the falls using the pole sampler. The probe readings were done by dropping the probe directly in the water.


Stein
Falls
Baker Rd
Creek
Old Steinhatchee Grade
Boggy
F-15
Cemetery Rd
Creek
River Rd
rdens
Steinhatchee
River
N
L
M

Developed with Sewer Being Installed

Keaton Beach

Keaton Beach is a developed residential community with a sewer system installed during 2005/2006. The FDOH Beach Monitoring Program has been sampling at this site (Site F) since 2000, and 115 advisories have been posted since the beginning of the sampling program. The Taylor County Health Department also has data for these sites dating back to 2004. The sampling sites for the Keaton Beach location are summarized in Table B-3.

Table B-3 – Sampling site designations for Keaton Beach

Code	Site Name	Latitude	Longitude
F	Keaton Beach	29^{o} 49.130’	83^{o} 35.610’
MR	Marina Road		
E	Cortez Road Canal – Mid-stream	29^{o} 49.524’	83^{o} 35.520’
D	Cortez Road Canal - Pump station	29^{o} 49.749’	83^{o} 35.492’
G	Blue Creek @ Beach Road	29^{o} 49.485’	83^{o} 34.579’

F) Keaton Beach: The beach has calm waters and is located in a delta. Across the delta is the Cedar Island Beach site, approximately 0.70 km in a straight line. The samples were collected in shallow waters along the north side of the pier, by walking out to knee-high depth and using the pole sampler. The probe readings were done by attaching the probe to the end of the pole and dropping it into the water in front of the sampler.

MR) Marina Road: This site is located at the ocean end of the Keaton Beach canal that runs parallel to Marina Road and Cortez Road. To the south across the bay is Cedar Island and east is the Blue Creek estuary. This site was added in 2007 and is located south of the Keaton Beach site (F). Samples were collected using the pole sampler from a floating double dock. The probe readings were done by dropping the probe directly into the water.

E) Cortez Road Canal: This site is located in the middle of a canal that leads out to the ocean, directly below a residential pier. There were two jet skis stored hanging out of the canal in May and a small motor boat in September. At no point during the sampling effort, did the team encounter any of the residents of the home, suggesting that it may be a holiday house. The water is shallow and mucky. The samples were collected using the pole sampler. The probe readings were done by dropping the probe directly into the water.

D) Cortez Road Canal: This site is located at the inland end of the Cortez Road Canal, less than 100 feet from a new pump station. According to our analysis of the collection system layout, no sewer pipes are located within a 100 ft distance from the sampling site. The water is shallow and looks stagnant. The samples were collected using the pole sampler. The probe readings were done by dropping the probe directly into the water, or by attaching to the pole sampler during periods of extremely shallow depth.

G) Blue Creek @ Beach Road: This is a background site, with freshwater flow. It allows assessment of further inland sources. The water has a dark tea color, presumably from humic, fulvic, and tannic substances. Samples were taken from the Beach Road bridge using the sampling pole and placing the probe directly into the water.


Ezell Beach Dr
ISLAND
Blue Creek
Keaton Beach Dr
Cortez Rd
Ellis Moore Dr
Pine Isle Dr
Marina Rd
Gulf Rd
Beach Villa Dr
Keaton
Beach
G
D
E
MR
F
TAYLOR COASTAL CENTRALIZED
WASTEWATER SYSTEM - PHASE 1
USDA

Cedar Island

Cedar Island is a residential community development, and during this study the sewer system was in the process of installation. The background site for these points is also Blue Creek @ Beach Road (Site G). The FDOH Beach Monitoring Program has conducted sampling there, and 129 advisories have been posted since 2000. It is also a site studied by the Taylor County Health Department. The sampling sites for the Cedar Island location are summarized in Table B-4.

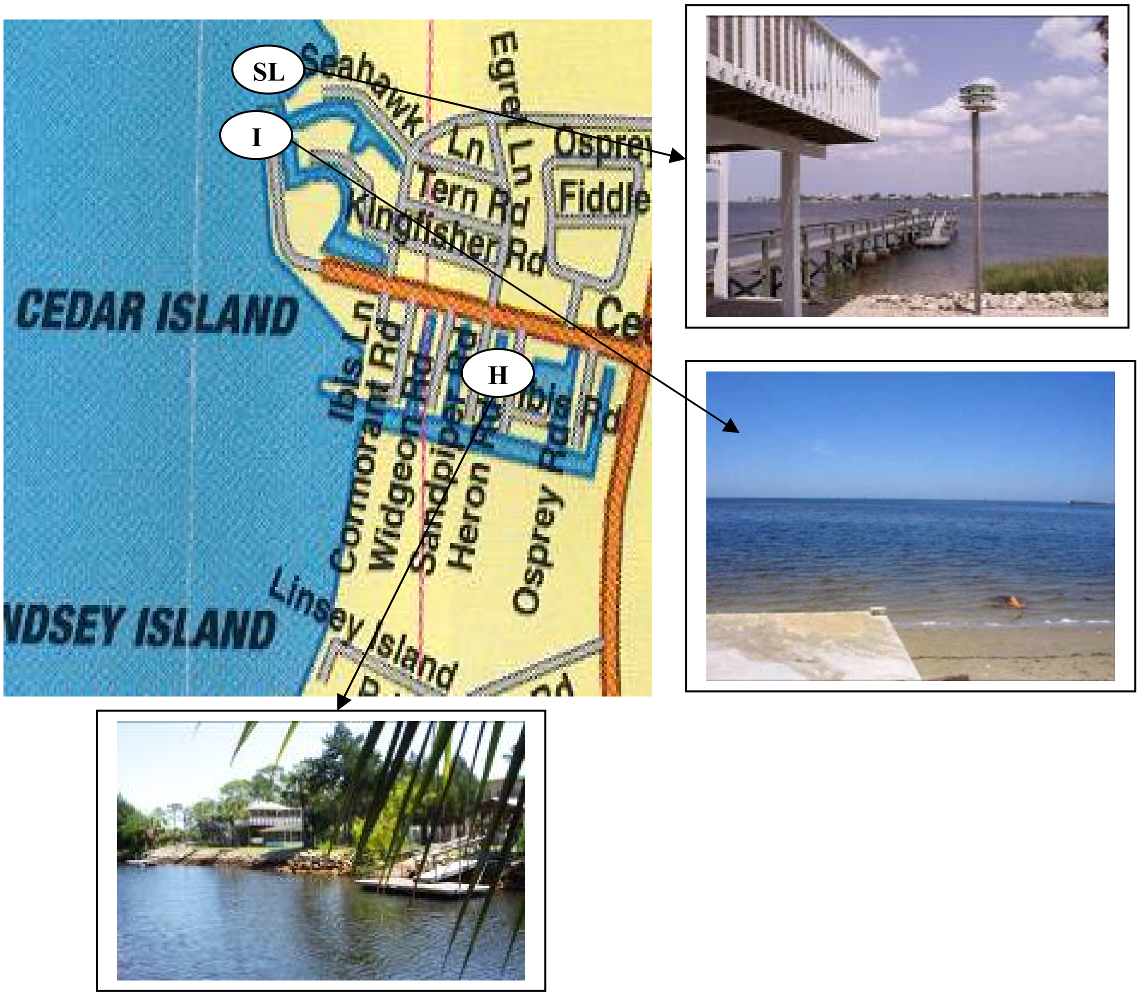
Table B-4 – Sampling site designations for Cedar Island

Code	Site Name	Latitude	Longitude
I	Cedar Island Beach	29° 48.946'	83° 35.238'
SL	Seahawk Lane	29° 48.995'	83° 35.172'
H	Heron Road Canal	29° 48.708'	83° 34.839'

I) Cedar Island Beach: This site has very calm shallow water and resembles a bay. The site is well protected, and the width of the channel leads to seemingly stagnant water. The site is on the beach in front of the concrete foundation of a beach house located at the end of a road adjacent to an inlet. The beach house is now connected to a sewer network. Samples were taken with a sampling pole after wading out to a knee-high depth.

SL) Seahawk Lane: This site is located on the ocean just east of the Cedar Island Beach site (I) and west of the Blue Creek estuary (G). This site was added in 2007, and the samples were collected from a private pier of an unoccupied home. The pier extends out into the bay between Keaton Beach and Cedar Island. Samples were collected using the pole sampler from the end of the pier. The probe readings were done by dropping the probe directly into the water.

H) Heron Road Canal: This site is located on a dead-end canal surrounded by residential development. The sampling location was a small dock accessed through the yard of an unoccupied home. The channel is shallow even during high tide. The bottom is usually visible, and the soil is muck suitable for mangroves.


SL
I
H
Seahawk
Egret Ln
Ln
Tern Rd
Fiddle
Kingfisher Rd
CEDAR ISLAND
Ibis Ln
Cormorant Rd
Widgeon Rd
Heron
Ibis Rd
Osprey Road
Linsey Island

Rationale of New Site Selection

The Fenholloway River set of sites (FR, HS, and PL) attempted to follow-up on the findings from the December 2006 SLWT event. Using aerial photography and field reconnaissance, it was determined that a large industrial source discharges into the Fenholloway River upstream of the impacted areas, north of Adam's Beach. It was hypothesized that this source potentially influences the nutrient dynamics of the coastal areas of Taylor County due to the prevailing current direction and the magnitude of the loading.

To investigate the river's effect, the thought process was to follow the effluent from near the original discharge (FR) to the middle stream and Hampton Springs (HS) and finally to where the river exits into the Gulf of Mexico at Peterson's Landing (PL). The FR site is located approximately one mile downstream of the industrial discharge of a specialty cellulose mill (Buckeye Florida). The HS site is located about midway from the mill to the ocean along the Fenholloway River. The site is underneath an abandoned bridge with almost no development nearby. It is downstream of a golf course and upstream of the Taylor Correctional Institute and the Perry sanitary landfill. The PL site is located at a boat landing near the mouth of the Fenholloway River, where it discharges to the Gulf of Mexico.

Once the Fenholloway River exits to the ocean, the prevailing clockwise (north to south) current (Gyori, Mariano, and Ryan 2008 and references therein) should take the pollutant load towards the impacted beach communities. This hypothesis was investigated by including Adam's Beach (AB) as an additional sampling point between Peterson's Landing and Dekle Beach to potentially determine a concentration gradient in the flow of bulk transport. Adam's Beach was one of the previously sampling sites in prior studies of beach water quality conducted by the Health Department. It showed historically high levels of microbial indicators. No homes or septic tanks are located nearby, but it is a boat landing with evidence of frequent human activity. The landing is extremely shallow and requires the sampler to walk a substantial distance before reaching knee-high water levels.

At Dekle Beach, the May 2006 SLWT showed high ammonia readings. The ammonia also increased in the upstream direction, unexpectedly. Historically, May is also the highest average water usage month. This was attributed to irrigation, which would result in increased runoff of ammonia-based fertilizers. It was determined that a more representative background site might resolve this issue in follow-up testing. However, site reconnaissance did not reveal a suitable or accessible alternative to the Creek at Dekle Beach site. Therefore, it was determined to monitor an upstream beach location at Jugg Island Road (JI), which is also connected to the discharge of the upstream creek (site C)

At Keaton Beach, unexpectedly high ammonia and microbial indicators during May 2006 SLWT indicated the possibility of a sewer leak, which masked any differences between Dekle Beach (OSTDS) and Keaton Beach (sewer). It was hypothesized that remnant OSTDS inputs have not had sufficient time to completely flush out of the subsurface and surficial soils. More station density was desired to resolve spatial variability due to potential sewer leaks. It was determined to sample near the end of Marina Road (MR), which is located upstream of the beach site (F) and downstream of the Cortez Road canal site (E) along the open end, which serves to address the issue of the Blue Creek estuary as well as the concentration gradient downstream of the pump station (D).

At Cedar Island, we recorded extremely high microbial densities (1840 – 24,200 MPN/100 mL) and ammonia levels (0.3 – 0.5 mg/L as NH_3-N) in May 2006 SLWT. These observations are more indicative of impacts associated with urban or agricultural wastewater than natural levels. It was hypothesized that this may be attributed to either re-growth in the shallow sediments or inputs from contaminated sediments in the nearby boat marina. An additional sampling location at Seahawk Lane (SL) was proposed to address these issues as well as assist in resolving the issue of the Blue Creek estuary. The Seahawk Lane site is located in between the Blue Creek estuary and the Cedar Island Beach (I) site upstream of the boat marina and downstream of Sandpiper Spring.

Appendix C: Quality Assurance Quality Control Data

QA/QC Information

Analysis of caffeine, ammonia, and nitrate were reported by a certified laboratory, *Florida Environmental Services (FES)*, *US Biosystems*, or *NOAA-AOML's Ocean Chemistry Division*. QA/QC procedures of the certified laboratory's quality assurance plan were inspected and can be made available upon request. Where appropriate, calibration forms, calibration curves, and results for field duplicates, laboratory replicates, and blanks are attached (See Appendix C for more details).

Caffeine

The contract laboratory used for this project is not NELAC certified for the analysis of caffeine. Certification was not critical since the caffeine sampling was conducted for screening purposes only. Dr. Piero Gardinali of Florida International University (whose research team developed specific analytical procedures for caffeine in surface waters) suggested a GC/MS method of detection that was adopted by the contract laboratory. This method was approved by Lyle Johnson and Maria Castellanos with Florida Environmental Systems. The lowest possible detection limit of 0.01 µg/L was used. Dr. Gardinali suggested from his research that if the caffeine samples yielded results greater than 0.10 µg/L, this was likely to be indicative of human-derived inputs.

According to the approved sampling plan, caffeine analysis was scheduled to be collected at each sampling site once per event (a total of 14 samples per event). During the 2006 seasonal high water table event (September 2006), caffeine sampling was conducted more frequently (a total of 20 samples), since caffeine levels were anticipated to be detected more often. This expectation was based on the assumption that the main source of caffeine contamination would be from septic tanks, if performance is compromised during the seasonal high water table. Mainly because of the method detection limit and the lack of detectable results, sampling for caffeine was discontinued for December 2006, May 2007 and September 2007.

Ammonia

For the ammonia testing, preserved (pH<2 with H_2SO_4) samples were analyzed by a contract laboratory. The analytical detection limit was 0.1 mg/L as N, and the laboratory practical quantitation limit was 0.3 mg/L as N during the SLWT (May 2006). Results varied from below detection to 0.9 mg/L as N. This corresponds to the low range for the analytical technique. Field duplicate analysis for ammonia showed differences of 0.1, 0.6, and 0.1 mg/L as N for each of the three sampling days in May 2006 (SLWT). However, an irregularity was found in the trip blank which showed 0.2 mg/L as N. This may indicate a possible contaminated sample or an analytical error. Changing the analytical method was suggested, and a different certified laboratory (US Biosystems) analyzed the ammonia samples for the September 2006 (SHWT) sampling event. Comparison testing conducted by the *National Oceanic and Atmospheric Administration - Atlantic Oceanographic and Meteorological Laboratory* confirmed that the trip blank readings were false positives related to the dilution water used. Replacing the dilution water with reagent water treated by double reverse osmosis and subsequent deionization yielded similar false positives for ammonia in trip blanks prepared for testing in Boynton Beach, FL. This was remedied by substituting the trip blanks with sterile low-nutrient marine samples collected from the Gulfstream current for the December 2006 sampling event (SLWT) and all other subsequent sampling events. During the 2006 SHWT (September), the analytical detection limit was 0.01 mg/L as N, and the practical quantitation limit was 0.02 mg/L (see Table C-1). In the 2006 SHWT event, the trip blank for ammonia was below detectable limits, and results for samples varied from below detection to 0.13 mg/L as N, which were generally higher during the May 2006 (SLWT) event. For the December 2006 SLWT event and subsequent 2007 sampling events, ammonia and other nutrient analyses were conducted by NOAA-AOML. Samples were preserved using $CHCl_3$ and analyzed using an ammonia method developed specifically by NOAA-AOML for marine and brackish systems.

Table C-1 – MDLs and PQLs for ammonia testing. All values are in units of mg/L as N.

Sampling Event	MDL	PQL	Range	Laboratory
SLWT (05/2006)	0.1	0.3	ND – 0.9	Florida Env.
SHWT (09/2006)	0.01	0.02	ND – 0.13	US Biosystems
SLWT (12/2006)	0.004	0.010	ND – 0.13*	NOAA AOML
SLWT (05/2007)	0.00056	0.0017	ND – 0.09**	NOAA AOML
SLWT (09/2007)	0.00098	0.0042	ND – 0.14***	NOAA AOML

*Additional samples collected from Fenholloway River were measured at 3.18 – 3.88 mg N/L
**Additional samples collected from the Fenholloway River were measured at 0.297 – 2.983 mg N/L
*** Additional samples collected from the Fenholloway River were measured at 0.412 – 3.212 mg N/L

Nitrate

For nitrate testing, samples were collected unpreserved and analyzed by a contract laboratory. The method used had a short hold time of 48 hours (unpreserved). If any sample violated the hold time, the results were flagged. However, during the September 2006 sampling event (SHWT), when hold times were violated, samples were analyzed for nitrate + nitrite (which remained within hold) rather than nitrate only. These samples were then analyzed for nitrite (also within hold), and the nitrate value was determined by difference. Samples that did not violate the hold time were not flagged and were analyzed for nitrate using the direct nitrate analytical method. Of the 45 samples tested in this manner in September 2006 (SHWT), nitrite data and nitrite + nitrate data is included in the raw data table in the appendix.

During the May 2007 SLWT sampling event, three of the samples (070522E1, 070523JI1 and 070524JI1) were flagged as "?-data rejected" because the nitrate plus nitrite results were lower than the nitrite results. Even after re-examining the raw data, no possible errors were discovered to explain the analytical result. No contamination was found, calibration checks passed, no sample injection error was found, but the sample duplicate for two of the three runs was found to violate the +/- 20% criterion. In addition, five samples in May 2007 (and 8 overall) were listed as out of range (OR). The explanation for samples flagged as "or" is that when an analytical run is initiated, the nutrient detector is set to the appropriate full scale absorbance range (AUFS). This is determined by the highest standard used in the linear calibration curve regression. The highest standard is chosen based on the lowest value that will determine all or most of the unknown samples based on previous experience. The reasoning for this is that the higher the standard (and the AUFS range) the lower the sensitivity. When a sample has values that exceed the detector's AUFS, the peak and its value are off scale and are not quantified. To determine the appropriate value for the sample, it is then diluted and re-analyzed. Sometimes the value is so high that several dilutions are required. However, there comes a point when no aliquot is left for dilution, and in that case the value is listed as out of range (OR) on the data sheet. Another flag that occurred "O-sampled but analysis lost or not performed" was used when a sample vial from either a defect in the test tube (a hair line crack) or more likely the test tube was filled to the top resulting in the tube cracking during the freezing process. The provided test tubes are filled to about 0.5 mL from the top in the field, but this is not measured accurately and can vary significantly. Sample leaks can also occur when the chloroform, which is added for preservation of NH4+ samples, accidentally touches the side of the plastic test tube. Chloroform will melt the polystyrene tubes very easily and will also erase the sample label markings. In this case, samples are listed as "not available" for analysis because the test tube identification labels are unreadable. This occurred only once during the entire sampling program and was remedied by marking the test tube cap and side with a simple numbering scheme that followed the order as listed on the appropriate chain of custody form.

During the May 2006 SLWT, the analytical detection limit was 0.011 mg/L as N, and the laboratory practical quantitation limit was 0.033 mg/L as N (see Table C-2). Results varied from below detection to 0.05 mg/L as N. Two field duplicates and the trip blanks were below detection, the last day's duplicates were recorded at 0.012 and 0.014 mg/L as N, respectively. During the September 2006 SHWT, the analytical detection limit was 0.0062 mg/L as N, and laboratory practical quantitation limit was 0.05 mg/L as N. Results, again, varied from below detection to 0.05 mg/L as N. The trip blanks were all below detection in 2006. In 2007, the May trip blank nitrate concentration was 0.00448 mg/L as N, which was just above the practical quantitation limit of 0.004 mg/L as N, and the September 2007 trip blank nitrate level was 0.00294 mg/ L as N, which is just below the practical quantitation limit of 0.003 mg/L as N. As previously described, the trip blanks for nutrient sampling were collected in the open ocean by NOAA personnel and is actually a low nutrient seawater sample rather than an ammonia-free sample. This was done as requested by the AOML laboratory technicians because of consistent sample blank failures in past analyses. Therefore, it is not possible to control the nitrate levels in this type of open ocean trip blank. Nevertheless, these values were on the extremely low end of the analytical scale of the instrument, on both occasions. Overall very low levels of nitrate were found during all events, save for the Fenholloway River samples.

Table C-2 – MDLs and PQLs for nitrate testing. All values are in units of mg/L as N.

Sampling Event	MDL	PQL	Range	Laboratory
SLWT (05/2006)	0.011	0.033	ND – 0.050	Florida Env.
SHWT (09/2006)	0.0062	0.050	ND – 0.050	US Biosystems
SLWT (12/2006)	0.0010	0.003	ND – 0.129*	NOAA AOML
SLWT (05/2007)	0.0008	0.004	ND – 0.097**	NOAA AOML
SLWT (09/2007)	0.0017	0.003	ND – 0.1295***	NOAA AOML

*Additional samples collected from Fenholloway River were measured at 0.324 – 0.632 mg N/L
**Additional samples collected from Fenholloway River were measured at 0.230 – 0.998 mg N/L
***Additional samples collected from Fenholloway River were measured at 0.078 – 0.532 mg N/L

Concerning QA/QC data for nutrients analyzed by NOAA-AOML, regressions, blanks, standard ranges, and slopes for the different nutrients groups for each run are provided in the appendix. The reported blank is a reagent blank. No Laboratory Fortified Blanks (LFBs) were analyzed. Certified reference materials are not available for the sweater nutrient suite due to the inherent instability of the nutrient species. Thus, standards were made from scratch prior to each analysis run. International inter-laboratory comparisons are conducted, and NOAA-AOML participates biannually.

For each field operation, several analytical runs are preformed. This is due to the low number of samples analyzed. The samples are run this way to minimize baseline drift and micro-air bubble formation. It also provides a measure of protection against contaminated reagents or a poorly performing cadmium column. During each analysis, a regression is performed, along with an instrument blank, several washes, and additional blanks. Therefore, out of a run of 30 samples there are approximately 20 additional injections for QA/QC purposes. A source that one might review to help explain in detail the procedures and methods used for the nutrients analysis conducted by NOAA-AOML is the following: "A Suggested Protocol for Continuous Flow Automated Analysis of Seawater Nutrients (Phosphate, Nitrate, Nitrite, and Silicic Acid) in the WOCE Hydrographic Program and the Joint Global Ocean Fluxes Study (Gordon, L.I. et al. 1994). Another source of information would be the USEPA methodology papers. The detection limits from the method papers are described in Table C-3.

Table C-3 – Summary of published analytical ranges for nutrient samples analyzed by NOAA-AOML.

Parameter	USEPA Method	Low Detection Limit	High Linear Limit
Nitrate + Nitrite	353.4	0.075 µg N/L	5.0 mg N/L
Ammonium	349.0	0.300 µg N/L	4.0 mg N/L
Silicic Acid	366.0	0.0012 mg Si/L	6.0 mg Si/L
Ortho-Phosphate	365.5	0.0007 mg P/L	0.39 mg P/L

FAU-Lab.EES TESTING

For the tests performed at the FAU Laboratories for Engineered Environmental Solutions, one field duplicate (FD) was collected for each sampling day, and a lab replicate (LR) was analyzed for microbiological parameters and TOC/TN. Once per sampling event, a field trip blank was analyzed. For the total organic carbon (TOC) and total nitrogen (TN) tests, one calibration standard verification, one calibration check verification, and one blank check verification were also analyzed for approximately every 20 samples. Duplicates, standards, and calibration checks have to meet the acceptable criteria described in the applicable SOP (i.e. duplicate and replicates should fall within 20%, calibration checks and standards should fall within 15%, for both TN and TOC). Data are flagged if otherwise.

TOC/TN

Prior to sampling, the expected results for TOC were between the range 1 – 200 mg/L, and the results for TN were expected to be between 0 – 10 mg/L, the first set of samples collected on May 3, 2006 (SLWT), were analyzed with the instrument adjusted to the medium range of detection (0-750 mg/L TOC, 0-20 mg/L TN). However results later showed that the majority of TOC levels were below 20 mg/L and TN levels were below 2 mg/L. Since the calibration curve was based on values much higher than the concentration range found, the samples analyzed from May 3, 2006 thus received a flag "K" signifying that the values were computed using the middle range of sensitivity. The samples could not be re-analyzed due to the lack of sufficient sample volume, and dilutions could not be analyzed due to limit of instrument detection and the amount of time that the samples were exposed to temperatures higher than 4°C. Subsequent analyses for September and December events were performed using the most sensitive setting (0-20 mg/L TOC, 0-1 mg/L TN). Samples with concentrations higher than the specified range were diluted and re-run using additional sub-samples collected.

Calibration standards, calibration checks, duplicates, and trip blanks were analyzed. The TOC/TN calibration curves are attached (see Appendix C). Data was flagged when the results were not found to be within the requirements stated in the QAPP or the individual parameter SOP. Calibration checks were expected to fall within 15% relative error and field duplicates and laboratory replicates within 20%. In May 2006, during transport, some sample bottles (n = 7) broke prior to analysis due to temperatures falling below 0°C during storage. The resulting expansion of the ice, in glass bottles collected with no head space, caused the containers to rupture. As a result, unpreserved samples (without acid) for each of these instances were analyzed instead. These samples were flagged "Y" since they violated the preservation protocol. This did not occur during any of the other sampling events. TN data is not available for September 2007 SHWT events due to a detector malfunction.

Total Coliforms

Samples were diluted to a proportion of 1:10 with sterile dilution water obtained through double reverse osmosis and autoclave sterilization. This was done to limit salinity effects across sample sites. Field duplicates and laboratory replicates were performed once each sampling day. Trip blanks were collected once for every sampling event. Trip blanks were all below detectable limits, as expected. Field duplicates and lab replicates fell within the range of +/- 20% in about 50% of the samples. The raw data tables in Appendix A include the individual results as well as duplicates and replicates. Summary QA/QC information is tabulated in Appendix D.

Total coliforms generally indicate the presence of soil-associated bacteria and result from natural influences on a water body, such as rainfall runoff or wastewater inflows. In this study, the total coliform levels were generally high and were not particularly useful as an indicator or source tracking parameter, by itself. However, total coliforms were evaluated as a part of a suite of parameters.

E. coli

The IDEXX Colilert method allows detection of total coliforms simultaneously with *E. coli*. Samples were diluted to a proportion of 1:10 with sterile dilution water obtained through double reverse osmosis and autoclave sterilization. Field duplicates and laboratory replicates were performed once each sampling day. Trip blanks were collected once for every sampling event. Trip blanks were all below detectable limits, as expected, and 26% of the field duplicates and 46% of the laboratory replicates fell within a range of +/- 20%. The target value of 20% is a stricter criterion than typically reported for microorganism counts, which contributes to inflating the number of samples that exceeded this target. It was also noticed that relatively low concentrations corresponded to higher differences between samples and duplicates (or replicates). This criterion may have been adversely affected by the dilution, since results were already close to detectable values in some instances. The raw data tables in Appendix A include the individual results as well as duplicates and replicates. Summary QA/QC information is tabulated in Appendix D.

Enterococcus

Samples were diluted to a proportion of 1:10 with sterile dilution water obtained through double reverse osmosis and autoclave sterilization. Field duplicates and laboratory replicates were performed once each sampling day. Trip blanks were collected once for every sampling event. Trip blanks were all below detectable limits, as expected, and 13% of the field duplicates and 7% of the laboratory replicates fell within a range of +/- 20%. As seen with the *E. coli* results, low concentrations corresponded to higher differences between samples and duplicates (or replicates). The raw data tables in Appendix A include the individual results as well as duplicates and replicates. Summary QA/QC information is tabulated in Appendix C.

Field Calibration Log (May 2006)

FIELD INSTRUMENT RECORDS

INSTRUMENT (MAKER/MODEL#): YSI 556

STANDARDS:

Standard A: 7.03
Standard B: 4.00
Standard C: 10.07
Standard D: 50.00

DATE (yy/mm/dd)	TIME (hr:min)	PARAMETER (pH, Cond., DO)	STD (A,B,C,D)	VALUE	DEV %	CALIBRATED (Yes, No)	INSTRUMENT RESPONSE	SAMPLER ID
06/05/05	8:25	pH	A	7.07		YES	GOOD	TB
06/05/05	8:25	pH	B	4.00	–	YES	GOOD	TB
06/05/05	8:25	pH	C	10.05		YES	GOOD	TB
06/05/05	8:45	Conduct.	D	49.26		YES	GOOD	TB

Field Calibration Log (September 2006)

FIELD INSTRUMENT RECORDS

INSTRUMENT (MAKER/MODEL#): YSI 556

STANDARDS:

Standard A: 7.01
Standard B: 10.03
Standard C: 4.00
Standard D: 54.60

DATE (yy/mm/dd)	TIME (hr:min)	PARAMETER (pH, Cond., DO)	STD (A,B,C,D)	VALUE	DEV %	CALIBRATED (Yes, No)	INSTRUMENT RESPONSE	SAMPLER ID
06/09/25	17:00	pH	A	7.00		YES	GOOD	EB
06/09/25	17:00	pH	B	10.03	–	YES	GOOD	EB
06/09/25	17:00	pH	C	3.88		YES	GOOD	EB
06/09/25	17:15	Conductivity	D	50.05		YES	GOOD	EB
06/09/26	13:45	pH	A	7.08		YES	GOOD	EB
06/09/26	13:45	pH	B	9.97		YES	GOOD	EB
06/09/26	13:45	pH	C	3.97		YES	GOOD	EB
06/09/26	14:00	Conductivity	D	54.70		YES	GOOD	EB
06/09/27	14:30	pH	A	6.98		YES	GOOD	DM
06/09/27	14:30	pH	B	10.00		YES	GOOD	DM
06/09/27	14:30	pH	C	4.00		YES	GOOD	DM
06/09/27	14:30	Conductivity	D	54.24		YES	GOOD	DM

Field Calibration Log (December 2006)

FIELD INSTRUMENT RECORDS

INSTRUMENT (MAKER/MODEL#): YSI 556 PROBE

STANDARDS:

Standard A: 9.47 (06/12/11), 9.51 (06/12/12), 9.33 (06/12/13)
Standard B: 7.00
Standard C: 4.00
Standard D: 10.00

DATE (yy/mm/dd)	TIME (hr:min)	PARAMETER (pH, Cond., DO)	STD (A,B,C,D)	TEMP (°C)	VALUE	DEV %	CALIBRATED (Yes, No)	INSTRUMENT RESPONSE	SAMPLER ID
06/12/11	14:50	Cond.	A	22.20	9.06	9.4	YES	GOOD	DM
06/12/11	14:55	pH	B	22.24	7.14	2.0	YES	GOOD	DM
06/12/11	14:58	pH	C	22.07	3.78	5.5	YES	GOOD	DM
06/12/11	15:05	pH	D	21.60	9.96	0.4	YES	GOOD	DM
06/12/11	15:10	DO	100%	23.13	106%	6.0	YES	GOOD	DM
06/12/12	18:00	Cond	A	22.66	9.56	0.5	YES	GOOD	DM
06/12/12	18:06	pH	B	22.60	7.06	0.9	YES	GOOD	TB
06/12/12	18:11	pH	C	22.66	3.92	2.0	YES	GOOD	TB
06/12/12	18:15	pH	D	22.84	9.89	1.1	YES	GOOD	TB
06/12/12	18:20	DO	100%	22.95	95.2%	4.8	YES	GOOD	TB
06/12/13	15:44	Cond.	A	21.50	9.48	1.6	YES	GOOD	TB
06/12/13	15:50	pH	B	21.82	7.05	0.7	YES	GOOD	TB
06/12/13	15:53	pH	C	21.98	3.99	0.3	YES	GOOD	TB
06/12/13	15:56	pH	D	21.21	10.01	0.1	YES	GOOD	TB
06/12/13	16:00	DO	100%	21.88	98%	2.0	YES	GOOD	TB

Field Calibration Log (May 2007)

INSTRUMENT (MAKER/MODEL#): YSI 556 MPS probe

STANDARDS:

Standard A: 4.0 pH (A7051 x 2/2011)
Standard B: 7.0 pH (2606742 x 6/2008)
Standard C: 10.0 pH (2606743 x 12/2007)
Standard D: 10 mS/cm (YSI 3163 Lot 06F1911 x 06/2007)

DATE (yy/mm/dd)	TIME (hr:min)	PARAMETER (pH, Cond., DO)	STD (A,B,C,D)	VALUE	DEV %	CALIBRATED (Yes, No)	INSTRUMENT RESPONSE	SAMPLER ID
07/05/22	05:00	COND	D	10.19	+1.9%	Yes	Good	AR
07/05/22	05:30	pH	A	4.01	+0.3%	Yes	Good	AR
07/05/22	05:36	pH	B	7.03	+0.4%	Yes	Good	AR
07/05/22	05:39	pH	C	10.00	0	Yes	Good	AR
07/05/22	05:48	DO	100%	100.5%	+0.5%	Yes	Good	AR
07/05/24	08:00	COND	D	10.0	0	Yes	Good	AR
07/05/24	08:15	pH	A	4.01	+0.5%	Yes	Good	AR
07/05/24	08:17	pH	B	7.00	0	Yes	Good	AR
07/05/24	08:21	pH	C	10.00	0	Yes	Good	AR
07/05/24	08:30	DO	100%	87.7%	-13%		Recalibrated - out of range DM	
07/05/24	08:40	DO	100%	101.2%	+1.2%	Yes	Good	

Field Calibration Log (September 2007)

FIELD INSTRUMENT RECORDS

INSTRUMENT (MAKER/MODEL#): YSI Model 556 MPS probe #A

STANDARDS:

Standard A: 7.01 pH
Standard B: 10.03 pH
Standard C: 4.00 pH
Standard D: 10 mS/cm

DATE (yy/mm/dd)	TIME (hr:min)	PARAMETER (pH, Cond., DO)	STD (A,B,C,D)	VALUE	DEV %	CALIBRATED (Yes, No)	INSTRUMENT RESPONSE	SAMPLER ID
07/09/18	21:35	COND	D	10.00	0	Yes	GOOD	AR
07/09/18	21:44	pH	A	6.96	-0.7%	Yes	GOOD	AC
07/09/18	21:58	pH	B	10.01	-0.2%	Yes	GOOD	AC
07/09/18	21:59	pH	C	4.00	-0.3%	Yes	GOOD	AC
07/09/18	22:15	DO	100%	100.2%	+0.2%	Yes	GOOD	AC
07/09/19	20:44	COND	D	10.15	+1.5%	Yes	GOOD	AC
07/09/19	20:59	pH	A	7.00	0	Yes	GOOD	AC

FIELD INSTRUMENT RECORDS

INSTRUMENT (MAKER/MODEL#): YSI 556 MPS probe #B

STANDARDS:

Standard A: 7.01 pH
Standard B: 10.03 pH
Standard C: 4.00 pH
Standard D: 10 mS/cm Conductivity

DATE (yy/mm/dd)	TIME (hr:min)	PARAMETER (pH, Cond., DO)	STD (A,B,C,D)	VALUE	DEV %	CALIBRATED (Yes, No)	INSTRUMENT RESPONSE	SAMPLER ID
07/09/18	21:30	COND	D	10.00	0	Yes	GOOD	AR
07/09/18	21:40	pH	A	7.02	+0.1%	Yes	GOOD	AR
07/09/18	21:42	pH	B	9.98	-0.7%	Yes	GOOD	AR
07/09/18	21:52	pH	C	4.01	+0.3%	Yes	GOOD	AR
07/09/18	21:59	DO	100%	100.2%	+0.2%	Yes	GOOD	AR
07/09/19	20:42	COND	D	10.12	+1.2%	Yes	GOOD	AR
07/09/19	20:49	pH	A	7.05	+0.7%	Yes	GOOD	AR

Laboratory Calibration Curves (TOC)

Standards (mg/L)	Raw data			
	May 2006 Med. Range	May 2006 Low Range	Sept. 2006	Dec. 2006
TOC 0	26114	28902	15638	45901
TOC 0	36127	27894	16138	47215
TOC 0	24700	28118	17826	45527
TOC 2 (10)	(443362)	222659	127949	147639
TOC 2 (10)	(419725)	219415	126248	138558
TOC 2 (10)	(411890)	209785	128424	138237
TOC 5 (250)	(2647386)	531953	265617	275935
TOC 5 (250)	(2535373)	531147	257699	279470
TOC 5 (250)	(2549455)	537043	260987	290070
TOC 10 (500)	(5911233)	1020014	494926	550447
TOC 10 (500)	(5692625)	1015825	484313	562799
TOC 10 (500)	(5720239)	1046196	486045	663978
TOC 20 (750)	(9021675)	1704599	979327	819496
TOC 20 (750)	(9174521)	1732395	959557	832820
TOC 20 (750)	(9037390)	1722255	961031	833148
TOC 25	-	-	1241020	-
TOC 25	-	-	1230676	-
TOC 25	-	-	1235555	-
Slope	11706.199	84941.331	48073.430	54009.769
Intercept	338072.646	76647.484	19295.420	34088.114
Correlation	0.995	0.990	0.999	0.997

Laboratory Calibration Curves (TN)

Standards (mg/L)	Raw data			
	May 2006 Med. Range	May 2006 Low Range	Sept. 2006	Dec. 2006
TN 0	-8215	17477	41776	27582
TN 0	-13254	20751	51903	30511
TN 0	4666	40856	48596	23422
TN 0.1 (5)	(842273)	191717	238642	254989
TN 0.1 (5)	(844085)	201831	280106	245572
TN 0.1 (5)	(850000)	177926	237925	
TN 0.2 (10)	(1677074)	324844	395354	537441
TN 0.2 (10)	(1660912)	390106	367380	570448
TN 0.2 (10)	(1653877)	421179	413070	456515
TN 0.3 (15)	(2557918)	-	496664	-
TN 0.3 (15)	(2533799)	-	548890	-
TN 0.3 (15)	(2519171)	-	495322	-
TN 0.4	-	-	-	876527
TN 0.4	-	-	-	884558
TN 0.4	-	-	-	
TN 0.5	-	919428	808822	-
TN 0.5	-	898600	779485	-
TN 0.5	-	913781	792148	-
TN 0.7	-	-	-	1539769
TN 0.7	-	-	-	1603082
TN 0.7	-	-	-	1632136
TN 1	-	1688402	125837	2219708
TN 1	-	1691493	83679	2256257
TN 1	-	1671675	72529	2264578
Slope	149041.500	1664940.665	1346747.430	2208641.962
Intercept	-6234.500	38625.761	117461.790	39074.616
Correlation	1.000	0.998	0.990	0.999

QA/QC Tables for Microbial Parameters and TOC/TN

Matrix	Surface Water
Analytical Parameter	*Enteroccocus*
Analytical SOP	FAU LT6200 (Standard Methods SM9223C)
Number of sampling locations	14 (2006); 21 (2007)

Field QC:	**Frequency/Number**	**Method SOP QC Acceptance Limits**	**Results Fail/Number**	**Corrective Action (CA)**
Trip Blanks	5/252	< MDL = 10	0/5	Not applicable
Method Blanks	-	-	-	-
Field Duplicates	15/252	<20%	2/15	None taken
Laboratory Replicates	15/252	<20%	1/15	None taken
Cal Checks (ICV)	-	-	-	-

Matrix	Surface Water
Analytical Parameter	Total Coliform
Analytical SOP	FAU LT6100 (Standard Methods SM9223C)
Number of sampling locations	14 (2006); 21 (2007)

Field QC:	**Frequency/Number**	**Method SOP QC Acceptance Limits**	**Results Fail/Number**	**Corrective Action (CA)**
Trip Blanks	5/252	< MDL = 10	5-Jan	None taken
Method Blanks	-	-	-	-
Field Duplicates	15/252	<20%	2/15	None taken
Laboratory Replicates	15/252	<20%	1/15	None taken
Cal Checks (ICV)	-	-	-	-

Matrix	Surface Water
Analytical Parameter	*E. coli*
Analytical SOP	FAU LT6100 (Standard Methods SM9223C)
Number of sampling locations	14 (2006); 21 (2007)

Field QC:	**Frequency/Number**	**Method SOP QC Acceptance Limits**	**Results Fail/Number**	**Corrective Action (CA)**
Trip Blanks	5/252	< MDL = 10	0/5	Not applicable
Method Blanks	-	-	-	-
Field Duplicates	15/252	<20%	9/15	None taken
Laboratory Replicates	15/252	<20%	7/15	None taken
Cal Checks (ICV)	-	-	-	-

Matrix	Surface Water
Analytical Parameter	TN
Analytical SOP	FAU LT5200 (EPA415.1)
Number of sampling locations	14 (2006); 21 (2007)

Field QC:	**Frequency/Number**	**Method SOP QC Acceptance Limits**	**Results Fail/Number**	**Corrective Action (CA)**
Trip Blanks	4/189	< MDL	0/4	Not applicable
Method Blanks	14/189	< MDL	0/14	Not applicable
Field Duplicates	12/189	<20%	1/12	Data flagged
Laboratory Replicates	4/189	<20%	0/4	Not applicable
Cal Checks (ICV)	14/189	<15%	4/14	Ran a new cal check, otherwise data flagged

Matrix	Surface Water
Analytical Parameter	TOC
Analytical SOP	FAU LT5200 (EPA415.1)
Number of sampling locations	14 (2006); 21 (2007)

Field QC:	**Frequency/Number**	**Method SOP QC Acceptance Limits**	**Results Fail/Number**	**Corrective Action (CA)**
Trip Blanks	4/208	< MDL	1/4	
Method Blanks	12/208	< MDL	2/12	Ran a new blank, otherwise data flagged
Field Duplicates	12/208	<20%	2/12	Data flagged
Laboratory Replicates	4/208	<20%	0/4	Not applicable
Cal Checks (ICV)	14/208	<15%	1/14	Ran a new cal check, otherwise data flagged

QA/QC Summary Tables for Nutrient Analyses Conducted by NOAA-AOML

Ammonia

Field QC:	Frequency/Number	Acceptance Limits	Results Fail/Number	Corrective Action (CA)
Trip Blank	3/175	< MDL	0/3	na
Lab Replicates	7/175	< MDL	0/7	na
Field Duplicate	6/175	<20%	1/6	Data flagged

Nitrate+Nitrite

Field QC:	Frequency/Number	Acceptance Limits	Results Fail/Number	Corrective Action (CA)
Trip Blank	3/174	< MDL	0/3	na
Lab Replicates	7/174	< MDL	0/7	na
Field Duplicate	6/174	<20%	3/6	Data flagged

Nitrite

Field QC:	Frequency/Number	Acceptance Limits	Results Fail/Number	Corrective Action (CA)
Trip Blank	3/174	< MDL	0/3	na
Lab Replicates	7/174	< MDL	0/7	na
Field Duplicate	6/174	<20%	2/6	Data flagged

Silicate

Field QC:	Frequency/Number	Acceptance Limits	Results Fail/Number	Corrective Action (CA)
Trip Blank	3/175	< MDL	0/3	na
Lab Replicates	7/175	< MDL	0/7	na
Field Duplicate	6/175	<20%	1/6	Data flagged

Phosphate

Field QC:	Frequency/Number	Acceptance Limits	Results Fail/Number	Corrective Action (CA)
Trip Blank	3/169	< MDL	0/1	na
Lab Replicates	7/169	< MDL	4/7	Data flagged
Field Duplicate	6/169	<20%	5/6	Data flagged

Nitrate

Field QC:	Frequency/Number	Acceptance Limits	Results Fail/Number	Corrective Action (CA)
Trip Blank	3/174	< MDL	0/3	na
Lab Replicates	5/174	< MDL	0/5	na
Field Duplicate	3/174	<20%	1/3	Data flagged

Appendix D: Trip Summaries

SLWT (May, 2006)

The first sampling trip was conducted during the first week of May 2006 (SLWT). The following is a daily summary of events.

May 1st – (Monday)	The FAU research team left the University Campus in Boca Raton, FL around 09:00 AM and arrived in Perry, FL at 08:00 PM. Along the way, the team evaluated the conditions at the proposed sampling site locations.
May 2nd – (Tuesday)	Meeting with James Rachal at 08:00 AM at the Taylor County Health Department (TCHD) office. A room with a sink was made available to be used as a temporary laboratory facility for the FAU research team. After all equipment was installed, the 14 sampling sites were visited (with James Rachal) to define the precise location of each sampling point.  Temporary lab
May 3rd – (Wednesday)	**First Sampling Day:** Dr. Eberhard Roeder met the FAU research team at 07:00 AM at the Days Inn Hotel. The first sample was collected at Dekle Beach at 08:16 AM, predicted time of ebb high tide. The field activity finished at 01:45 PM at Steinhatchee Falls. *The first two samples, Dekle Beach and Dekle Beach Canal, violated the holding time of 6 hours for the bacteriological tests, by less than one hour.*

May 4^{th} – (Thursday)	**Second Sampling Day:** The first sample was collected at Dekle Beach at 08:30 AM, predicted time of ebb high tide. The field activity finished at 01:05 PM at Steinhatchee Falls. All samples met the appropriate holding times, and the readings for the previous day's bacteriological tests were recorded. Turbidity tests were conducted at the TCHD rather than at the field due to battery issues with the field turbidometer.
May 5^{th} – (Friday)	**Last Sampling Day:** The first sample was collected at Dekle Beach at 10:05 AM, predicted time of ebb high tide. The field activity finished at 02:30 PM at Steinhatchee Falls. All samples met the appropriate holding times, and the readings for the previous day's bacteriological tests were recorded. Turbidity tests were conducted at the TCHD once again.
May 6^{th} – (Saturday)	Readings of the bacteriological results for the last sampling day were recorded. All equipment was packed up for return to Boca Raton. Biohazardous waste was disposed of with the TCHD. The FAU research team arrived at University Campus in Boca Raton at 02:00 AM on May 7, 2006.

SHWT (September, 2006)

The second sampling trip was conducted during the last week of September 2006 (SHWT). The following is a daily summary of events.

Sept. 25th – (Monday)	The FAU research team left the University Campus in Boca Raton, FL around 06:00 AM and arrived at the Taylor County Health Department in Perry, FL, at 04:00 PM. All equipment was installed in the temporary laboratory, which was a storage/office without a sink on this occasion.
Sept. 26th – (Tuesday)	**First Sampling Day:** The first sample was collected at Dekle Beach at 06:12 AM, predicted time of ebb tide. The field activity finished at 11:05 AM at Steinhatchee Falls. All samples met the appropriate holding times. Turbidity tests were conducted at the TCHD.
Sept. 27th – (Wednesday)	**Second Sampling Day:** The first sample was collected at Dekle Beach at 06:14 AM, during ebb tide. The field activity finished at 10:00 AM at Steinhatchee Falls. All samples met the appropriate holding times, and the readings for the previous day's bacteriological tests were recorded. Turbidity tests were conducted at the TCHD.
May 28th – (Thursday)	**Last Sampling Day:** Dr. Eberhard Roeder and Ms. Elke Ursin met the FAU research team at 05:30 AM at the Days Inn Hotel. The first sample was collected at Dekle Beach at 06:11 AM, during ebb tide. The field activity finished at 10:00 AM at Steinhatchee Falls. All the samples met the appropriate holding times, and the readings for the previous day's bacteriological tests were recorded. Turbidity tests were conducted at the TCHD, and samples for DNA analysis were filtered upon returning to the TCHD in the afternoon.
Sept. 29th – (Friday)	Readings of the bacteriological results for the last sampling day were recorded. All equipment was packed up for return to Boca Raton. Biohazardous waste was disposed of with TCHD. The FAU research team arrived at University Campus in Boca Raton at 12:10 AM on Saturday, September 30, 2006.

SLWT (December, 2006)

The third sampling trip was conducted during the second week of December 2006 (SLWT). Although it did rain during the month of December 2006, all of the rainfall in the month occurred after the sampling event (December 12-14, 2006) was completed, so no stormwater related inputs were expected. The following is a daily summary of events.

Dec. 11th – (Monday)	The FAU research team left the Boca Raton Campus around 06:00 AM and arrived at the Taylor County Health Department in Perry, FL, at 04:00 PM. All equipment was installed in the temporary laboratory, which was the same storage/office used during the September trip.
Dec. 12th – (Tuesday)	**First Sampling Day:** The first sample was collected at Dekle Beach at 06:52 AM, predicted time of ebb tide. Two new sites were sampled during this trip, a new site along the Steinhatchee River (middle river) and one at Fenholloway River. The field activity finished at 11:58 AM. All samples met the appropriate holding times. Turbidity tests were conducted at the TCHD. Ammonia and nitrate samples were prepared in the field laboratory and shipped by FedEx to NOAA-AOML for analysis.
Dec. 13th – (Wednesday)	**Second Sampling Day:** The first sample was collected at Dekle Beach at 07:57 AM, near the predicted time of ebb high tide. The field activity finished at 12:15 PM. All samples met the appropriate holding times, and the readings for the previous day's bacteriological tests were recorded. Turbidity tests were conducted at the TCHD, and samples for DNA analysis were filtered upon returning to the TCHD in the afternoon. Ammonia and nitrate samples were prepared in the field laboratory and shipped by FedEx to NOAA-AOML for analysis.

Dec. 14th – (Thursday)	**Last Sampling Day:** The first sample was collected at Dekle Beach at 10:10 AM, near the predicted time of ebb high tide. The field activity finished at 02:00 PM. All samples met the appropriate holding times, and the readings for the previous day's bacteriological tests were recorded. Turbidity tests were conducted at the TCHD. Ammonia and nitrate samples were prepared in the field laboratory and shipped by FedEx to NOAA-AOML for analysis.
Dec. 15th – (Friday)	Readings of the bacteriological results for the last sampling day were recorded. All equipment was packed up for return to Boca Raton. Biohazardous waste was disposed of with TCHD. The FAU research team arrived at the University Campus in Boca Raton at 12:20 AM Saturday morning (December 16, 2006).

SLWT (May, 2007)

The fourth FAU sampling trip was conducted during the SLWT during the third week of May 2007. The following is a daily summary of events.

May 21 – (Monday)	FAU research team left the University Campus in Boca Raton, FL around 03:00 AM and arrived at the Taylor County Health Department (TCHD) office in Perry, FL at 11:30 AM. All equipment was installed in the temporary laboratory, which was the same storage/office space used during the last sampling campaign in December 2006. Afterwards, several new sites were visited in preparation for sampling on the following day. Seven new sites were selected and sampled during this trip, these included three sites along the Fenholloway River (Peterson's Landing, Hampton Springs Bridge, and Fenholloway River at 19/Alt27), Adam's Beach, Goodtime Drive (Dekle Beach), Marina Road (Keaton Beach), Sandpiper Lane (Cedar Island).
May 22 – (Tuesday)	**First Sampling Day:** The first sample was collected at Adams Beach at 08:21 AM, predicted time of ebb tide (7:51 AM). The field activity finished at 12:58 PM at Fenholloway River. All samples met the appropriate hold times. Samples of shallow sediments were collected for four representative coastal sites. Once the team returned to the TCHD lab, two members returned to the field to collect the final two sites (Peterson's Landing and Hampton Springs Bridge). Turbidity tests were conducted in the TCHD laboratory rather than in the field. Nutrient samples were prepared in the field laboratory and shipped by FedEx to NOAA-AOML for analysis.

May 23 – (Wednesday)	**Second Sampling Day:** The first sample was collected at Hampton Springs Bridge at 07:56 AM, near the predicted time of ebb high tide (8:51 AM). The field team vehicle was temporarily stuck in the dry sand at the first site and required assistance to pull the vehicle out and back on the road. This resulted in an unanticipated 75-minute delay. The field activity finished at 2:03 PM at Fenholloway River. Nine sites were selected for molecular tracers. All samples (except Hampton Springs Bridge) met the appropriate hold times, and the readings for the previous day's bacteriological tests were recorded. Turbidity tests were conducted in the TCHD laboratory rather than in the field. Nutrient samples were prepared in the field laboratory and shipped by FedEx to NOAA-AOML for analysis. Molecular tracer samples were prepared from 3:00 PM to 11:30 PM in the TCHD laboratory.
May 24 – (Thursday)	**Last Sampling Day:** The first sample was collected at Hampton Springs Bridge at 09:00 AM, near the predicted time of ebb high tide (09:50 AM). While two members of the sampling team prepared the shallow sediment samples for re-growth analysis (*n* = 4), the other two collected the Peterson's Landing and Hampton Springs samples. The team met at the TCHD laboratory to prepare the bacteriological tests for the six samples, and then resumed field collection at Adam's Beach at 10:53 AM The field activity finished at 03:06 PM at Fenholloway River. All samples met the appropriate hold times, and the readings for the previous day's bacteriological tests were recorded. Turbidity tests were conducted in the TCHD laboratory rather than in the field. The molecular tracer samples that were incubated the day before were prepared for shipment to NOAA-AOML. The nitrogen isotope samples were filtered and frozen under dry ice. Nutrient samples were prepared in the field laboratory and shipped by FedEx to NOAA-AOML for analysis, along with the molecular tracer samples (*n* = 36).

May 25 – (Friday)	Readings of the results for the last sampling day for bacteriological tests were recorded. At 1:30 PM, the team visited the Taylor Coastal Utilities Wastewater Treatment facility near Cedar Island, FL. After returning to the TCHD, all equipment was packed up for return to Boca Raton. Biohazardous waste was disposed of with TCHD personnel. The FAU research team left Perry at 04:30 PM and arrived at the University Campus in Boca Raton at 02:15 AM Saturday morning (May 26, 2007).

SHWT (September, 2007)

The fifth FAU sampling trip was conducted during the SHWT during the third week of September 2007. The following is a daily summary of events.

September 17 (Monday)	FAU research team left the University Campus in Boca Raton, FL around 06:00 AM and arrived at the Taylor County Health Department (TCHD) office in Perry, FL at 1:30 PM. All equipment was installed in the temporary laboratory, which was the same storage/office space used during the last sampling campaign in May 2007. Afterward, the second sampling vehicle was picked up locally in town.
September 18 (Tuesday)	**First Sampling Day:** The field activity was split between two teams of two samplers, as described earlier. **(Team A, L. Hess and A. Ruffini):** The first sample was taken at Roy's Restaurant (site J) at 6:25 AM, near predicted time of ebb tide (5:15 AM). The field activity finished at 9:41 AM at Petersons Landing (site PL). Meteorological data was not collected at sampling time because the second portable weather station was not functioning. **(Team B, D. Meeroff and H. Hashimoto):** The first sample was taken at Adams Beach (site AB) at 6:09 AM, near predicted time of ebb tide (5:15 AM). The field activity finished at 9:45 AM at Heron Road (site H). All samples from both teams met the appropriate hold times. Turbidity tests were conducted in the TCHD laboratory rather than in the field.

September 19 (Wednesday)	**Second Sampling Day:** The field activity was split between two teams of two samplers, as described earlier. **(Team A, L. Hess and A. Ruffini):** The first sample was taken at Roy's Restaurant (site J) at 6:48 AM, near predicted time of ebb tide (5:51 AM). The field activity finished at 10:38 AM at Adams Beach (site AB). Due to an incorrect number of sample bags collected at Boggy Creek, a re-sampling was undertaken at 4:15 PM (between low and high tide) and noted in the log (sample data was not impeded by tides because the Boggy Creek site is not tidally influenced). In addition to the typical samples collected on day 1, sediment samples were taken from: Adams Beach for regrowth studies. Also four additional surface water samples were taken from: Adams Beach, Petersons Landing, Fenholloway River, and Boggy Creek for molecular techniques testing. **(Team B, D. Meeroff and H. Hashimoto):** The first sample was taken at Deckle Beach (site A) at 6:15 AM, near predicted time of ebb tide (5:51 AM). The field activity finished at 9:15 AM at Heron Road (site H). In addition to the typical samples from day 1, sediment samples were taken from: Deckle Beach, Keaton Beach, and Cedar Island Beach for sediment regrowth testing. Also four additional surface water samples were taken from: Deckle Beach, creek at Deckle Beach, Keaton Beach, Blue Creek at Beach Road, and Cedar Island Beach for molecular techniques testing. 11 of 23 samples did not meet the appropriate hold times for bacteriological testing due to the amount of time required to collect the additional sediment and water samples. None of the hold violations exceeded 60 minutes. The readings for the previous day's bacteriological tests were recorded. Turbidity tests were also conducted in the TCHD laboratory rather than in the field. Nutrient samples were prepared in the field laboratory. Also, molecular tracer samples were prepared using additional sample bags, and shallow sediment re-growth samples were prepared using additional sediment samples. These last two sets of tests were conducted at the TCHD laboratory.

September 20 (Thursday)	**Last Sampling Day:** The field activity was split between two teams of two samplers, as described earlier. All isotope analysis samples were collected on this day. **(Team A, L. Hess and A. Ruffini):** The first sample was taken at Roy's Restaurant (site J) at 7:30 AM, near predicted time of ebb tide (6:50 AM). The field activity finished at 10:23 AM at Petersons Landing (site PL). **(Team B, D. Meeroff and H. Hashimoto):** The first sample was taken at Deckle Beach (site A) at 7:20 AM, near predicted time of ebb tide (6:50 AM). The field activity finished at 10:25 AM at Adams Beach (site AB). The trip blank sample was performed at Adams Beach (site AB) at 10:47 AM. All samples met the appropriate hold times, and the readings for the previous day's bacteriological tests were recorded. Turbidity tests were conducted in the TCHD laboratory rather than in the field. The molecular tracer samples that were incubated the day before were prepared for shipment to NOAA-AOML. The nitrogen isotope samples were filtered and frozen under dry ice. Nutrient samples were prepared in the field laboratory and shipped by FedEx to NOAA-AOML for analysis, along with the molecular tracer samples (n = 36).
September 21 (Friday)	Readings of the results for the last sampling day for bacteriological tests were recorded by 2:30 PM. All equipment was packed up for return to Boca Raton. Biohazardous waste was disposed of with TCHD personnel. The FAU research team left Perry at 03:30 PM and arrived at the University Campus in Boca Raton at 1:15 AM Saturday morning (September 22, 2007).

FLORIDA KEYS
ONSITE WASTEWATER
Sampling Procedures Evaluation

SAMPLING PROTOCOL

Draft 05/14/2008 E. Roeder

Wastewater Sampling Protocol

Florida Keys Onsite Wastewater Sampling Procedures Evaluation

1. INTRODUCTION AND SCOPE

This sampling protocol has been developed from the Florida Department of Environmental Protection's Standard Operating Procedure for Wastewater Sampling (DEP-SOP-001/01), manufacturer's owner's manuals, and various other publications listed in the reference section. The Monroe County Health Department developed these procedures in collaboration with the Florida Department of Health, Bureau of Onsite Sewage Programs.

This protocol will be used during sampling and field evaluations of interim, Keys standard and other performance-based treatment systems during county health department inspections.
The objectives of this protocol are:

- Standardize field inspection procedures
- Compare different sampling procedures
- Further evaluate differences between time-composite sampling and grab sampling
- Evaluate agreement between field methods and laboratory analytical methods

2. ORGANIZATIONAL OVERVIEW

All **persons** conducting field investigations shall be knowledgeable of the protocols contained in this document, and shall receive field training prior to the commencement of the sampling and evaluation regime.

An upper keys sample team and a lower keys sample team shall be assembled.
Each team is responsible for chain of custody requirements, laboratory submissions, data collection, and data entry. Analysis of the data will be performed by the Bureau of Onsite Sewage Programs at the conclusion of the assessment project.

Each **treatment unit** shall be evaluated for the unique design parameters that will affect sample points. Sample points shall be identified prior to commencement of the sampling regime. Influent samples from the settling tank prior to treatment shall be preferred. Influent sampling locations in the treatment tank can be used according to the specific manufacturer's recommendation. Influent sampling ports will be installed if there is no other viable alternative.
Take precautions when taking samples from inside a treatment unit so as not to disturb equipment or the treatment process.

Chain of custody forms (see sample in Appendix) will be used by all samplers. At the end of each system's 24 hour sampling cycle, the completed forms will be faxed to a central location. (Tavernier Environmental Health). Data can then be reviewed and entered into a central database.

3. EXPERIMENTAL DESIGN

3.1. Further evaluation of grab vs. time-composite sampling. To expand the treatment technologies and number of systems evaluated, approximately 10 new volunteer sites will be recruited from interim systems. To be included in the study, systems will have to fulfill the following criteria: residential system, current maintenance contract, permanent residency in the Keys (homestead exemption). Interim systems will be randomly selected from the total population of interim systems in the Florida Keys that were not previously contacted. These sites will be evaluated by time-composite and multiple grab samples according to the previous protocol.

3.2. Comparison of field and laboratory methods of effluent quality assessment. To evaluate the agreement between field and lab assessments of effluent quality, grab samples of effluent will be characterized to compare:

3.2.1. color and odor vs cBOD5 and TSS

3.2.2. residual chlorine, color and odor vs fecal coliform

3.2.3. Hach-kit and test strip results vs laboratory analysis for nitrate, ammonia, and phosphorus

3.2.4. Taylor kit vs laboratory analysis for alkalinity and pH

3.3. Evaluation of field inspection protocols. To evaluate differences between sampling in the presence and absence of maintenance entities and the incremental time needed for different intensities of inspections, a sample of systems will be visited by the CHD in conjunction with a maintenance visit by the maintenance entity. The CHD will initially or on the day prior inspect and sample the system according to the CHD-inspection protocol. The maintenance entity will then indicate where the CHD should take any additional samples according to manufacturer's instruction and perform their maintenance visit.

3.4. Evaluation of effluent quality during CHD-inspections. To assess the effluent quality of the overall population of systems, the CHD will sample systems on the occasion of the annual inspection visit.

4. SAMPLING EQUIPMENT

Three **auto samplers** manufactured by Global Water Instrumentation Inc. shall be employed. These are suction-lift samplers using a peristaltic pump to collect wastewater. The maximum rated lift is 22 feet in the neoprene ¼ inch inside diameter hose. This meets FDEP requirements.

Two of the auto-samplers are model WS300 samplers. These units will be used for influent and effluent time-composite samples. A 150 ml sample will be collected each hour for 24 hours to form a 3600 ml composite. One unit, model WS700, will be used to collect discrete 500 ml grab samples at designated times.
The auto-samplers shall be calibrated, maintained and cleaned according to the manufacturer's specifications. (See user manuals in Appendix)

As alternatives for grab samples, sampling by hand pump and container from free-fall locations (where accessible during joint sampling with the maintenance entity) will be evaluated.

While composite samples are being gathered, the collected sample shall be maintained at 4°C. To accomplish this, bags of ice shall be used in the cooler compartment, around the sample container.

MEMORANDUM of AGREEMENT

BETWEEN

THE FLORIDA DEPARTMENT OF HEALTH
AND
THE UNIVERSITY OF SOUTH FLORIDA

DRAFT

WHEREAS, The Florida Department of Health (DOH) is the State Agency authorized by the Legislature to maintain a comprehensive regulatory program with regard to onsite sewage treatment and disposal systems (OSTDS) in the State of Florida.

WHEREAS, DOH's statutory responsibilities are delineated in part in section 381.0065, Florida Statutes, which includes authority to supervise research on the environmental and public health impact of OSTDS used in Florida. §381.0065(3)(j), Fla. Stat.

WHEREAS, through its Colleges of Public Health and Engineering, the University of South Florida (USF) is involved in the study of wastewater treatment and the environmental fate and transport of wastewater.

WHEREAS, in 1988 a cooperative agreement was in place between DOH's predecessor agency, the Florida Department of Health and Rehabilitative Services (HRS), and USF allowing for a lysimeter station (the Station) to be established on the USF campus. The Station was used to perform experiments through contracts between HRS and selected providers until 1998. The Station has been out of service for approximately 10-years.

NOW THEREFORE, in consideration of the mutual obligations of the parties as set forth above, DOH and USF agree to the following:

I. STATUTORY AUTHORITY

This Memorandum of Agreement (Agreement) is entered into pursuant to the provisions of sections 381.0011, and 381.0065(3)(j), Florida Statutes.

II. PURPOSE

The purpose of this Agreement is to establish the general conditions and joint processes that will enable DOH and USF to collaborate in the operation, modification and upkeep of the Station on the USF campus, which includes disposal of effluent onsite.

III. SCOPE OF THE AGREEMENT:

A. USF AGREES

1. The research facility will be accessible to the DOH and any contract provider that has been approved by DOH to utilize the facility.

2. The facility can be used for USF sponsored projects subject to availability.

3. To provide a wastewater source for the facility.

B. DOH AGREES:

1. To properly abandon the research facility before this MOA or a successor MOA expires, and reconnect the sewage source to the existing central sewerage system.

C. DOH and USF MUTUALLY AGREE TO THE FOLLOWING CONDITIONS FOR EACH PROJECT

1. Wastewater that has passed through the research facility will either be pumped back into the sewer line or discharged into a drainfield; surface discharge will not be permitted and is considered a sanitary nuisance.

2. The location where the effluent is released shall meet rule and statute requirements. Specifically, this includes, a minimum seventy-five foot separation from the boundaries of any surface water body, a minimum of two-hundred feet from a public drinking water well, a minimum twenty-four inch separation from the estimated wet season high water table, avoidance of exposure of the effluent to the public either directly or via vectors, and not to exceed 2,500 gallons per day per acre.

3. To provide the other party with copies of final project reports.

4. To establish a contingency plan outlining procedures for shutting down any failing component and bypassing into the sewer system.

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IV. AGREEMENT MANAGERS:

The following persons are hereby authorized to coordinate between the agencies and act as managers to this Agreement:

DEPARTMENT OF HEALTH:

name and position
Florida Department of Health
4052 Bald Cypress Way, Bin
Tallahassee, Florida 32399-
(850)
e-mail

UNIVERSITY OF SOUTH FLORIDA:

V. TERM OF AGREEMENT:

This Agreement shall begin on the date on which it is signed by both parties, and shall expire five (5) years from execution unless an extension is agreed to by the parties.

VI. REVIEW AND MODIFICATION:

Upon request of either party, both parties will review this Agreement in order to determine whether its terms and conditions are still appropriate. Modifications to the Agreement shall be valid upon execution of a formal written amendment to the Agreement.

VII. TERMINATION:

This Agreement may be terminated at any time upon mutual consent of the parties, or unilaterally by either party upon no less than (30) days notice.

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VII. SIGNATURES:

By signing below, both parties agree to the terms and conditions contained herein:

FLORIDA DEPARTMENT OF HEALTH:

_______________________________________ _______________________
Dr. Lisa Conti, D.V.M., M.P.H., Dipl. ACVPM, CEHP date
Director, Division of Environmental Health

UNIVERSITY OF SOUTH FLORIDA:

_______________________________________ _______________________
date

DRAFT

Request for Qualifications (Draft 05/13/2008)

Characterization of post-manufacturing deformation in plastic tanks and development of recommendations for protocols for structural proof testing, installation, monitoring and maintenance.

A Background:

Onsite sewage treatment receptacles, more commonly referred to as septic tanks, can be manufactured from a variety of materials. The focus of this study will be on polyethylene as a material. The research review and advisory committee for the onsite sewage program has recommended that a study be undertaken to develop insights into the deformation behavior of tanks made out of such material. Deformation can be expected to occur due to at least two factors: elastic or plastic deformation during installation; and long-term deformation under continuous load ("creep"). The Florida Department of Health is requesting qualifications for the task of "Characterization of post-manufacturing deformation in plastic tanks and development of recommendations for protocols for structural proof testing, installation, monitoring, maintenance and assessment of deformation."

The cost of this task shall not exceed $20,000. The work shall be completed by June 30, 2009.

Qualifications are requested for the following services to proceed with the study. This will be a fixed-price purchase order. Vendors must be registered in MyFloridaMarketPlace before purchase order can become effective, unless exempted, and assume any associated fees.

Vendors are invited to submit a bid addressing the qualification requirements by 2 pm July 2, 2008 to:

Florida Dept. of Health,
Bureau of Onsite Sewage Programs
Attn: Eberhard Roeder   Eberhard_roeder@doh.state.fl.us; fax 850-922-6969
Re: Post-Manufacturing Tank Deformation
4052 Bald Cypress Way, Bin A-08
Tallahassee FL 32399-1713

B Services Needed:

1.) Summarize current knowledge of the extent and predictability of installation deformation and creep in buried tanks. While the focus will be on polyethylene, include a comparison of characterizing parameters, such as modulus of elasticity and creep with other construction material (concrete, fiberglass, polypropylene, steel)
2) Review and summarize standards developed by other organizations, such as ASTM, IAPMO, UL relative to polyethylene tanks intended for burial.
3) Gather information on the experience in Florida with polyethylene tanks from manufacturers, installers, and other sources, and summarize this information.

4) Based on 1) and 2) evaluate under what conditions deformation is most likely to result in changes that can impact volume, operation or serviceability of tanks and develop a protocol for field assessment of such deformation
5) Arrange for access agreements to a number of tanks, identified based on factors such as permit records, installer or manufacturer knowledge, and prior investigations by the Department. Tanks shall be in two groups, some that have been recently buried and some that have been buried for long enough that measurable creep can be expected. Apply the field assessment protocol. Tabulate the results and evaluate the observations in regard to the need to include post-manufacturing deformation in approval standards.
6) Develop recommendations for the design, structural proof testing, approval installation, monitoring, operation and maintenance of tanks made from polyethylene and similar materials.
7) Present interim and final results to the Research Review and Advisory Committee on two occasions at meetings of this committee in central Florida.
8) Prepare first and second draft reports for tasks 1 through 4, prepare first drafts for tasks 5 and 6. The second draft for task 4 must be completed and approved by the Department, before the field assessment protocol is applied.

Note: The offeror shall prepare deliverables using software and hardware applications that are consistent with the Department Standards (currently Microsoft software, PC-compatible)

<u>C Qualifications to be submitted:</u>

The offeror will submit a resume, curriculum vitae or qualification statement, which shall include information on length of experience with related projects and publications.
The offeror will also submit an outline of the approach and a cost proposal for the task, which will give prices for the tasks listed in B. The total price will not exceed $20,000.
The offeror has to be principally located in Florida.

<u>D Evaluation of Proposals</u>

To determine the best qualified offeror principally located in Florida whose cost proposal does not exceed the indicated amount, the qualifications will be awarded points according to the following criteria:

1) Length of experience in evaluation of post-manufacturing tank deformations: 0-1 years=0, 1-5 years=1; 6-10 years=2; 10+ years=3
2) Projects addressing onsite sewage treatment receptacle structural design and approval: yes=1; no=0
3) Projects addressing buried tank structural design and structural approval in applications other than onsite sewage: yes=1; no=0
4) Projects addressing post-manufacturing deformation of polyethylene structural components: yes=2; no=0

4) Publications addressing polyethylene deformation, buried tank structural design, for each: 1 in peer reviewed journals or books; 0.3 in trade journals; 0.1 engineering reports
5) Quality of approach outline: Convincing that offeror can accomplish services effectively = 3; persuasive that offeror can build on experience to accomplish services =1; missing, or steep learning curve ahead =0

While DOH reserves the right to select any or none of the offerors, it expects to begin negotiation with the offeror with the most points first.

SECTION 3.0 INTRODUCTORY MATERIALS

3.1 Project Background

Central sewer and wastewater treatment plants (WWTP) are frequently proposed and installed as a solution to water quality problems that are linked to insufficient onsite sewage treatment and disposal systems. Low water quality is usually well documented before the installation of a WWTP. However, fewer studies are available that compare the actual improvement of water quality to the expected result. The Florida Department of Health (DOH) proposes to perform a follow-up study for a wastewater infrastructure project in the Town of Suwannee in Dixie County. It is hypothesized that as central sewer replaces onsite systems, the change in water quality can be measured, and this change can then serve as a measure of the previously present impact of onsite systems.

The Town of Suwannee replaced septic systems with a central sewer and WWTP in 1997. With funding from the Environmental Protection Agency in 1996, DOH evaluated the effects of this conversion using results from background and canal samples for two months before and at the end of sewer installation. The main source of Salmonella was found to come from upstream of the Town of Suwannee. During the sampling period in 1997 there was a period of extensive rains that obscured some of the fecal coliform concentration results making it difficult to see any trends in the values. The 1997 sampling results provided baseline information that can be used to determine any subsequent trends.

The successful provider will take eight sets of weekly samples at locations as close as possible to the original sampling sites from 1997. Use of the same sites and methods will allow an evaluation of water quality changes in sewered areas compared to the Suwannee River upstream and downstream of the town and a monitoring well.

The results of this project will help to determine whether the installation of the WWTP was an enhancement to the Town of Suwannee, and will provide a case study of such a determination which can be used as a model for other local governments facing similar issues. By establishing the environmental effects of sewering this area, this project will also contribute to evaluating potential cost and benefits of protecting water quality. Moreover, the methodology and results can be readily transferred to other locations in which historical monitoring data exist. Results will be publicized to onsite wastewater government programs and communities at conferences, and by circulating the final report among Florida resource agencies on the web and through possible publication in a technical journal.

3.2 Statement of Purpose

The Florida Department of Health (DOH), Division of Environmental Health, Bureau of Onsite Sewage seeks a provider to conduct the Evaluating Environmental Impacts of Onsite Sewage Systems in the Town of Suwannee Study. The provider will conduct sampling in the Town of

Suwannee to evaluate the environmental impact of sewering a community that was previously served by onsite sewage treatment and disposal systems (OSTDS). Basic components of the project are described in Section 4. Up to $65,000.00 in funding may be provided through the Florida Department of Health Bureau of Onsite Sewage Programs. The total cost of the contract will not exceed $65,000.00.

3.3 Definitions

- DOH – Florida Department of Health
- FAC – Florida Administrative Code
- NOAA – National Oceanic and Atmospheric Administration
- OSTDS – Onsite Sewage Treatment and Disposal System
- QAPP – Quality Assurance Project Plan
- RRAC – Research Review and Advisory Committee, committee established in Chapter 381 Florida Statutes to guide the department on priorities for new research projects, assist in selection of contract providers, and review of draft and final project reports.

3.4 Term

The initial term of the contract resulting from this ITN shall be for one year from the date of contract execution.

SECTION 4.0 TECHNICAL SPECIFICATIONS

4.1 Scope of Service

The successful offeror will provide research services to the Florida Department of Health to conduct sampling in the Town of Suwannee to evaluate the environmental impact of sewering a community previously served by OSTDS (Section 3.2). The Florida Department of Health, Division of Environmental Health, Bureau of Onsite Sewage Programs, obtained historical sampling data from 1996 and 1997 on pre-construction and immediately post-construction of the sewer infrastructure. By sampling the same locations again, this project will help to evaluate the long-term effect of sewering on water quality. The successful offeror will develop a quality assurance project plan, gather supplemental data from other agencies, perform sampling, conduct data analysis of sampling results, and write a final project report which will include management recommendations.

The successful offeror must prepare deliverables using software and hardware applications that are consistent with Department standards (currently, Microsoft software, PC-compatible hardware).

4.2 Programmatic Authority

The Bureau of Onsite Sewage Programs operates under Section 381.0065 et seq. of the Florida Statutes. 381.0065(3)(c) directs the Department to "develop a comprehensive program to ensure that onsite sewage treatment and disposal systems … are sized, designed, constructed, installed, … operated, and maintained … to prevent groundwater contamination and surface water contamination". 381.0065(3)(j) specifically directs the Department of Health to award research projects "through competitive negotiation, using the procedures provided in s. 287.057, to public or private entities that have experience in onsite sewage treatment and disposal systems in Florida and that are principally located in Florida".

4.3 Major Program Goals

The goal of the Evaluating Environmental Impacts of Onsite Sewage Systems in the Town of Suwannee Study is to evaluate the environmental effects of sewering an area that was previously served by OSTDS. Ensuring that OSTDS are not causing harm to public health and the environment is important to the mission of the Bureau of Onsite Sewage Programs, "Protecting the public health and environment through a comprehensive onsite sewage program".

4.4 Task List

The successful offeror shall perform at least the following tasks. The Department will approve all deliverables when completed to the Department's satisfaction.

4.4.1 Development of a Quality Assurance Project Plan (QAPP)

4.4.1.1. Objective: Provide a QAPP meeting DOH, DEP, and NOAA requirements to provide a sound scientific methodology for subsequent activities.

4.4.1.2. Activities: Development of a Quality Assurance Project Plan (QAPP) in coordination with DOH and the Department's Research Review and Advisory Committee (RRAC) to provide a sound scientific methodology for subsequent activities, including methodology and quality assurance/quality control procedures In this document the offeror will plan and describe the approach, sampling schemes, analytical methods, and quality control procedures guiding the project. A minimum of one site visit will be required during this task.

4.4.1.3. Deliverables: Draft QAPP submitted to the Department within one month of execution of contract. Finalized QAPP acceptable to the Department, DEP, and NOAA submitted within two months of execution of contract. Cost documentation.

4.4.2 Gather supplemental data from other agencies

4.4.2.1. Objective: Compile data from other sources that relate to water quality, OSTDS, and any other related activity in the study area.

4.4.2.2. Activities: The offeror will gather additional monitoring data and construction data by other agencies, such as the Florida Department of Environmental Protection, the Florida Department of Agricultural and Consumer Services, and the Suwannee River Water Management District. This data will relate to the overall objectives of this project.

4.4.2.3. Deliverables: Compiled data set submitted to the department within one month of submittal of draft QAPP. Cost documentation.

4.4.3 Perform Sampling

4.4.3.1. Objective: Perform environmental sampling at specific locations to provide another set of data.

4.4.3.2. Activities: Eight weekly samples will be taken during November and December 2008 at locations as close as possible to the original sampling locations shown in Figure 1. Use of largely the same sites and methods will allow an evaluation of water quality changes in sewered areas (five stations) compared to the Suwannee River upstream and downstream of the town (four stations) and a monitoring well. The parameters that will be sampled are outlined in Table 1. Ten percent of the laboratory samples will have quality assurance / quality control samples taken to verify the accuracy of the results. Department of Health staff shall attend at least one sampling event.

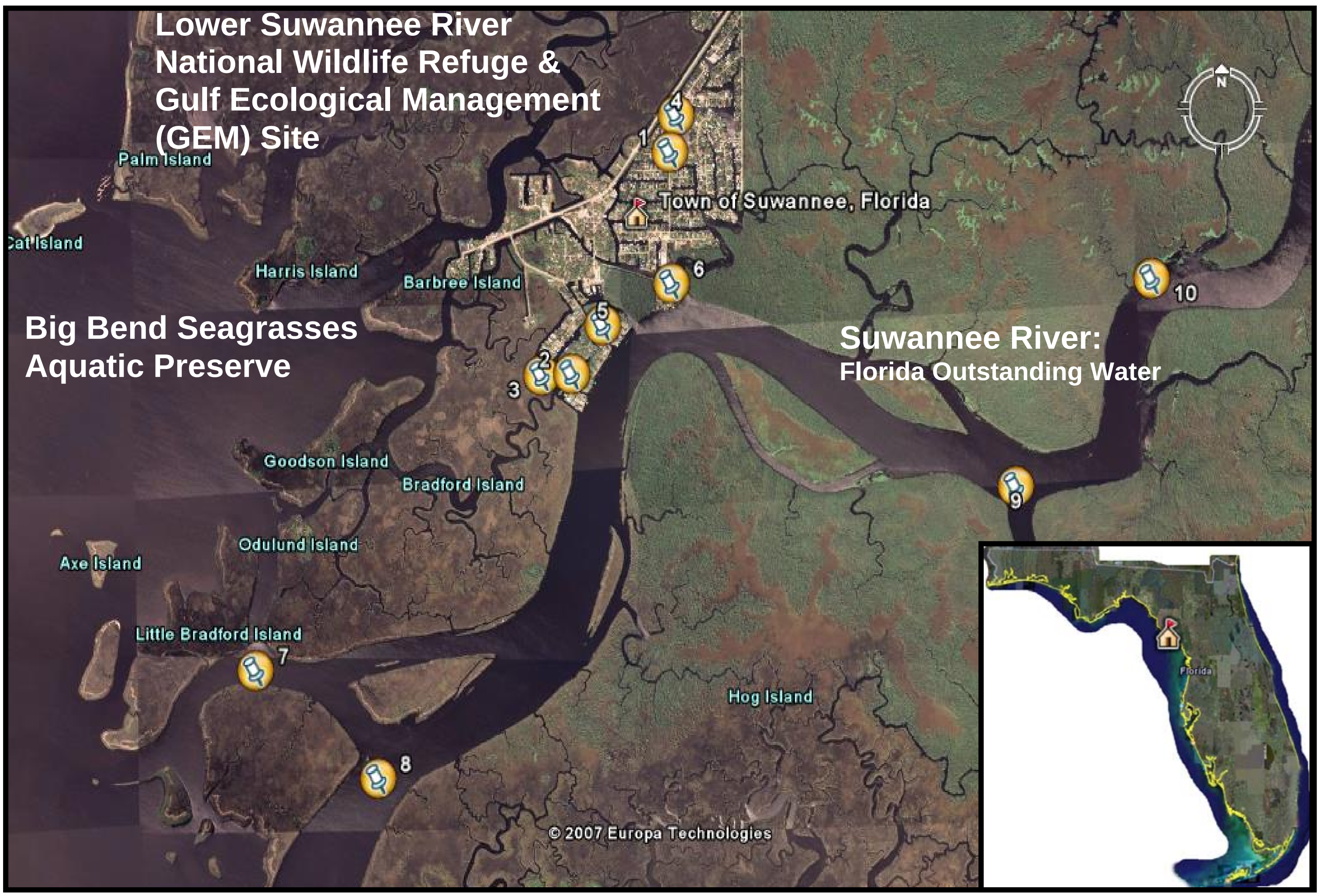

Figure 1. Sample site locations of the 1996/1997 study, Town of Suwannee, Dixie County, Florida

Parameter	Laboratory / Field Sample	Pre-construction 1996	Post-construction 1997	Post-construction 2008
Total Coliform	Laboratory Sample	x	x	x
Fecal Coliform	Laboratory Sample	x	x	x
Enterococci	Laboratory Sample			x
Salmonella	Laboratory Sample	x	x	x
Total Nitrate + Nitrite	Laboratory Sample	x	x	x
Total Kjeldahl Nitrogen	Laboratory Sample	x	x	x
Total Phosphorus	Laboratory Sample			x
Water Temperature	Field Sample	x	x	x
Conductivity	Field Sample	x	x	x
pH	Field Sample	x	x	x
Dissolved Oxygen	Field Sample	x	x	x

Table 1. Sampling parameters

4.4.3.3. Deliverables: Tabulated sample data, raw field sample data, lab reports, and chain of custody reports submitted within four months of executed QAPP. Cost documentation.

4.4.4 Data analysis of sampling results

4.4.4.1. Objective: Analyze the data collected in 4.4.3. Compare the data with previous DOH data sets and other related data sets found in 4.4.2.
4.4.4.2. Activities: Data analysis will address the following question: How much has water quality been affected relative to pre-construction and background stations? This will, at the same time, evaluate what the contribution was from septic systems to the water quality.
4.4.4.3. Deliverables: Draft report submitted to the Department within three months of the date of the last environmental sample. Cost documentation.

4.4.5 Final Report Development

4.4.5.1. Objective: summarize work, interpret obtained data, and develop conclusions
4.4.5.2. Activities: Using results of the data analysis (Section 4.4.4), a final project report will summarize the environmental effects of the sewer projects. The report will be produced in two formats. One format will be a journal manuscript targeted to a municipal engineering audience. The second format will be a report containing project description, data sets, procedures, and summary of the results. This report will be reviewed by the RRAC and DOH. The report will include:

a) Introduction - provides background information about the project
b) Methodology - Describe the approach and analytical techniques used
c) Results - Summarize results of sampling
d) Discussion - Develop recommendations

e) Conclusions - Summarize differences or similarities between areas served by onsite systems and areas served by centralized sewer systems

f) Literature citations - List all references

g) Appendices - include all data, charts, graphics etc.

4.4.5.3. Deliverables: Offeror will submit a draft final report within seven months of contract execution for review and comment by the Department's Research Review and Advisory Committee (RRAC), Department's contract manager and other parties. Offeror will consider comments by these parties in developing the final project report, which will be submitted within eight months of contract execution. Both draft report and final project report, will be submitted in 20 paper copies and electronic format.

4.5 Task Limits

The successful offeror shall not perform any tasks related to the project other than those described in Section 4.4 without the express written consent of the Department.

4.6 Staffing Levels

Each prospective offeror shall include its proposed staffing for technical support. The offeror will maintain at least the following: a project manager to provide oversight and management of the project and to serve as the contact person for the offeror; qualified technical staff for activities to be performed by offeror as outlined in Section 4.4; adequate administrative organizational structure and support staff sufficient to discharge its contractual responsibilities. In the event the Department determines that the successful offeror's staffing levels do not conform to those promised in the proposal, it shall advise the successful offeror in writing and the successful offeror shall have 30 days to remedy the identified staffing deficiencies.

The successful offeror shall replace an employee whose continued presence would be detrimental to the success of the project as determined by the Department with an employee of equal or superior qualifications. The Department's contract manager will exercise exclusive judgment in this matter.

4.7 Professional Qualifications

The offeror shall be one of "public or private entities that have experience in onsite sewage treatment and disposal systems in Florida and that are principally located in Florida", per Florida Statute 381.0065 (3) (j). Offeror must have a project manager on staff that has experience in the execution of projects similar to this. Experience will be judged based on quality and quantity of past projects as indicated on the ITN questionnaire (attachment II). Technical staff shall be trained in the methodology proposed by offeror.

This provision does not abrogate any statutory provision(s) that may require professional licensure, certification, or registration to perform duties associated with this contract.

Florida Department of Health
Development of a Comprehensive Database of Advanced Onsite Sewage Treatment and Disposal System (OSTDS) in the State of Florida

Purpose: The successful provider shall work with the Florida Department of Health, Division of Environmental Health, Bureau of Onsite Sewage Programs to develop a comprehensive database of advanced Onsite Sewage Treatment and Disposal Systems (OSTDS) in the State of Florida. This is to be accomplished by developing and populating a database with all of the state parcels that have onsite system permit information and include at least one of the following types of systems:

- PBTS (of which nutrient reducing and innovative systems are a subset)
- ATUs (including engineer-designed ATUs with drip irrigation)
- Engineer-designed sand filters and other alternative systems

The database will join all known permits, including information in the centralized Department of Health Environmental Health Database, any other electronic database file with this type of information (individual County Health Department databases, Carmody Systems, etc.), and historical hard-copy permits that are not in electronic format. An automated tool will be developed to allow the new database to be periodically updated with information from the Environmental Health Database. The end product will be a new database of all advanced OSTDS with permit information in the State of Florida which will be maintained by the Florida Department of Health for long term use.

The database will contain information about permit records, system types, property locations, contact information, components used, maintenance, monitoring, inspection and sampling results, performance specifications, and site locations of systems. Data fields will be based largely on the existing databases: the statewide permitting databases, CENTRAX and CENTRAX-Rehost, and the Carmody Program maintenance database, which is capable of receiving data from CENTRAX. The project database will be compatible with these databases in so far as it will be capable of receiving suitably formatted data dumps from them.

Information will be extracted from these database sources by querying for the system types of interest. The result of merging these records and supplementing the information with any additional records provided by county health department staffs will be a database of all advanced systems identified at the time of completion of the database. All addresses shall be geocoded to the best extent possible in order to allow for mapping and trip planning. Comparison of the results from different databases with each other and with the project database will allow an assessment of relative completeness of records and data fields.

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Eligible Providers: Potential providers shall have experience in database development and data warehousing. The eligible provider must submit a proposal to the Department of Health which meets the intent of this Request for Proposal by the closing date of the posting. Specific criteria for the proposal are outlined in the Other Requirements section. The total cost of the services shall not exceed $6,000.00

Deliverable: An accurate, comprehensive, and up-to-date electronic database of all advanced OSTDS in the State of Florida with permit information, for the use of the Florida Department of Health, populated with existing FDOH permit information and the ability to add additional record fields as needed. Specific tasks required through this contract are outlined below in the Method of Delivery section. All work shall be completed within four months of start date.

Method of Delivery: The selected provider(s) will accomplish the following tasks:

- Meet with DOH to discuss what data fields will be appropriate for the project. At a minimum, Parcel ID number, 911 Address, date of approval, type of permit, system manufacturer, and characteristics of the system shall be required.
- Design a database with the appropriate fields in Access format, or any other FDOH IT approved Windows based database program that has the capability of storing a minimum of 500,000 individual records.
- Configuration of an automated upload of data from the Environmental Health Database into the new database.
- Development of a minimum of five reports approved by DOH written as part of the database development.
- Data entry or import of the existing electronic permit records for advanced OSTDS from the state's Environmental Health Database system. DOH will provide access to the data.
- Data entry or import of existing electronic data for advanced OSTDS from other sources (Carmody, individual databases in each County Health Department, etc.) shall be coordinated by the provider with assistance from DOH as needed.
- Provide end user training to DOH staff on data entry and database management.
- Provide DOH with the full final functional database.
- Provide technical support for one year from completion date
- If needed, provide hardware/server space for data storage that is FDOH IT approved.
- Consult with DOH IT staff for compliance with the FDOH IT policy.
- A description of the advanced systems database shall be provided. This description shall include a list of fields, their relationship, and the database structure.

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FDOH to Provide – The FDOH will provide, at no cost to the provider, the following support and materials:

- Access to the FDOH Environmental Health Database system
- A point contact to answer programmatic questions

Other Requirements

Travel, Lodging and Subsistence – Costs of travel as well as lodging, ground transportation, and subsistence, shall be the responsibility of the provider.

Period of Performance / Completion Date – The period of performance will be four months from the executed start date, or sooner if work is completed.

Proposal – A proposal must be submitted addressing the scoring criteria on the attached scoring criteria worksheet. The total cost of the project shall not exceed $6,000.00. The submitted proposal should include a description to perform the tasks. A budget shall be submitted outlining the number of hours and cost per hour to complete each task.

Proposals are due by ______________. The proposal shall be submitted in writing to or via email to:
Florida Department of Health
Bureau of Onsite Sewage Programs
4052 Bald Cypress Way, Bin #A-08
Tallahassee, FL 32399-1713

Attn: Elke Ursin Elke_Ursin@doh.state.fl.us
Attn: Eberhard Roeder Eberhard_Roeder@doh.state.fl.us

Remittance – Payment for services will be made at least monthly by the State of Florida warrant payable to provider upon receipt of an acceptable invoice from the provider for services rendered.

Provider Selection Criteria – The submitted proposal will be scored by DOH based on the attached scoring sheet to select the most qualified provider with the highest score. There is the option to negotiate any portion of a submitted proposal.

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Development of a Comprehensive Onsite Sewage Treatment and Disposal System (OSTDS) Database in Alachua County

Scoring Criteria Worksheet

This sheet will be used by evaluators to assign scores to all written proposals. Evaluators will judge the presence and quality of each response in assigning a score.

The provider with the highest score will be awarded the project.

Scoring Criteria	Maximum Score	Score
Qualifications/ Organizational Capacity How well does the offeror address issues such as: -qualification of staff -relevance of past performance to this project	10	
Description of Approach to Performing Tasks Required	50	
Project and Workload Management How well does the offeror address issues such as -organization and assignment of staff -project and quality management -potential for conflicts of interests	10	
Willingness to meet time and budget constraints How well does the offeror address the question if this project can be completed within the time and within budget?	30	
Sums	**100**	
	POSSIBLE	

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FINAL 4/18/2008

FLORIDA DEPARTMENT OF ENVIRONMENTAL PROTECTION

Division of Water Resource Management, Bureau of Watershed Management

CENTRAL DISTRICT • MIDDLE ST. JOHNS BASIN

TMDL Report

Nutrient TMDLs for the Wekiva River (WBIDs 2956, 2956A, and 2956C) and Rock Springs Run (WBID 2967)

Xueqing Gao

April 18, 2008

Acknowledgments

This TMDL was based on the Pollutant Load Reduction Goal (PLRG) for nitrate and total phosphorus developed by the St. Johns River Water Management District. The Florida Department of Environmental Protection (the Department) appreciates the instrumental help provided by Rob Mattson, and valuable suggestions from Ed Lowe, Larry Battoe, and Eric Marzolf. Special thanks also go to Dr. Xufeng Niu, who helped to define the nitrate target using the change-point analysis. We deeply appreciate the kind help provided by the Department's Central District Office staff, especially Chris Ferraro, Dennise Judy, Pat Young, and Ali Kazi, who helped greatly in providing data, valuable insight, and understanding of the point sources identified in the Wekiva River drainage basin. We also deeply appreciate the help from Sarah Jozwiak (NPDES Stormwater Section of the department) in providing information related to MS4 permits, and CDM, Inc for providing information about septic tanks for the Wekiva River Drainage basin.

Editorial assistance provided by
Daryll Joyner and Jan Mandrup-Poulsen

For additional information on the watershed management approach and impaired waters in the Middle St. Johns Basin, contact
Jennifer Gihring
Florida Department of Environmental Protection
Bureau of Watershed Management
Watershed Planning and Coordination Section
2600 Blair Stone Road, Mail Station 3565
Tallahassee, FL 32399-2400
Email: Jennifer.Gihring@dep.state.fl.us
Phone: (850) 245-8418; Suncom: 205-8418
Fax: (850) 245-8434

Access to all data used in the development of this report can be obtained by contacting
Xueqing Gao
Florida Department of Environmental Protection
Bureau of Watershed Management
Watershed Assessment Section
2600 Blair Stone Road, Mail Station 3565
Tallahassee, FL 32399-2400
Email: xueqing.gao@dep.state.fl.us
Phone: (850) 245-8464, SunCom: 205-8464
Fax: (850) 245-8434

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Web sites

Florida Department of Environmental Protection, Bureau of Watershed Management

Total Maximum Daily Load (TMDL) Program

http://www.dep.state.fl.us/water/tmdl/index.htm

Identification of Impaired Surface Waters Rule

http://www.dep.state.fl.us/water/tmdl/docs/AmendedIWR.pdf

STORET Program

http://www.dep.state.fl.us/water/storet/index.htm

2004 305(b) Report

http://www.dep.state.fl.us/water/docs/2004_Integrated_Report.pdf

Criteria for Surface Water Quality Classifications

http://www.dep.state.fl.us/water/wqssp/classes.htm

Basin Status Reports

http://www.dep.state.fl.us/water/tmdl/stat_rep.htm

Water Quality Assessment Reports

http://www.dep.state.fl.us/water/tmdl/stat_rep.htm

Allocation Technical Advisory Committee (ATAC) Report

http://www.dep.state.fl.us/water/tmdl/docs/Allocation.pdf

U.S. Environmental Protection Agency

Region 4: Total Maximum Daily Loads in Florida

http://www.epa.gov/region4/water/tmdl/florida/

National STORET Program

http://www.epa.gov/storet/

Chapter 1: INTRODUCTION

1.1 Purpose of Report

This report presents the nutrient Total Maximum Daily Loads (TMDLs) for Rock Springs Run (RSR) and the Wekiva River, in the Middle St. Johns River Basin. These waters were verified as impaired due to total phosphorus (TP) and nitrate based on evidence of imbalance in aquatic flora provided by the St. Johns River Water Management District (SJRWMD) and were added to the Verified List of impaired waters for the Middle St. Johns River Basin by Secretarial Order on January 3, 2007. The purpose of this TMDL is to establish the allowable loadings of pollutants to RSR and Wekiva River that would restore these waterbodies such that they meet their applicable water quality criteria for nutrients.

1.2 Identification of Waterbody

The Wekiva River Study Area ("WSA"; **Figure 1-1**) is located in central Florida. The boundary of the area was delineated in the Wekiva Parkway and Protection Act (WPPA, 2004) and encompasses 473 square miles. The river is a major hydrological feature of the WSA, which receives discharges from thirty springs and several major tributaries, including Little Wekiva River from the south, Rock Springs Run in the middle, and Blackwater Creek from the north, and discharges into the St. Johns River downstream of Lake Monroe. The eastern and southern parts of the WSA are highly urbanized areas occupied by several municipalities, including the City of Orlando, Winter Park, Casselberry, Winter Spring, Lake Mary, and Sanford. More rural areas are located in the northwestern part of the WSA.

The Wekiva River and RSR and their headsprings currently provide a variety of recreational opportunities, including canoeing, swimming, snorkeling, tubing, boating, and fishing. In addition to being protected under the WPPA, the Wekiva River system (including the main stem and Rock Springs Run) is designated as an Outstanding Florida Water (OFW). The river is also in the Wekiva River Aquatic Preserve, first designated by the Florida Legislature (Chapter 258.36, FS) in 1975. Most recently, the Wekiva River and portions of its major tributaries were designated Florida's third National Wild and Scenic River system (U.S. House of Representatives Bill H. R. 3155). This is a federal designation applied to rivers considered worthy of protection due to their ecological and aesthetic attributes and recreational value.

The Wekiva River basin is also part of the SJRWMD's Surface Water Improvement and Management (SWIM) program for the Middle St. Johns River Basin.   The SJRWMD was required to develop a pollutant load reduction goal (PLRG) for the river, and the PLRG development schedule was expedited per the WPPA.

The area that includes the Wekiva River surface water basin and "springshed" lies within portions of the Marion Upland, Orlando Ridge, and Osceola Plain physiographic regions (Schmidt 1997). Land elevations range from 175 feet above mean sea level (MSL) in the western part of the region to about 10 feet above MSL at the confluence of the Wekiva River and Blackwater Creek (WSI 2004). In the western and southern portions of the region, the land is a series of high (>75 feet above MSL) terraces and ridges, with well-draining sandy soils. In the eastern and northeastern portions of the region, land elevations are generally < 25 feet above MSL, with poorly draining wetland and flatwoods soils.

The Wekiva River basin and springshed lie in a karst-influenced landscape. The Floridan Aquifer is the main water source for springs that discharge to the Wekiva River and its tributaries (Toth and Fortich 2002; Osburn et al. 2002). The principal recharge areas to the Floridan Aquifer are in the western and southern portions of the region (Osburn et al. 2002), with recharge of up to 12" of rainfall annually. Much of the lengths of the Wekiva River and Rock Springs Run lie within the discharge zone of the Floridan Aquifer, resulting in a predominance of spring flow in these streams. Based on Hupalo et al. (1994), spring flow represents at least 67% of the base flow of the Wekiva River. Because of the predominance of spring flow in these streams, the water quality in the springs exerts a major influence on water quality in the spring-run streams.

More detailed information regarding the physiogeography, hydrology, hydrogeology, and climate of the WPPA area can be obtained by consulting the "*Wekiva River and Rock Springs Run Pollutant Load Reduction Goals*" developed by the SJRWMD (Mattson, et al. 2006).

For assessment purposes, the Department has divided the Middle St. Johns Basin into water assessment polygons with a unique **w**ater**b**ody **id**entification (WBID) number for each watershed or stream reach. This TMDL report addresses four WBIDs: WBIDs 2956 and 2956A for the main stem of the Wekiva River, WBID 2956C for Wekiva Spring, and WBID 2967 for Rock Springs Run. **Figure 1.1** shows the locations of the WBIDs covered in this TMDL report.

1.3 Background

This report was developed as part of the Department's watershed management approach for restoring and protecting state waters and addressing TMDL Program requirements. The watershed approach, which is implemented using a cyclical management process that rotates through the state's 52 river basins over a 5-year cycle, provides a framework for implementing the TMDL Program–related requirements of the 1972 federal Clean Water Act and the FWRA.

A TMDL represents the maximum amount of a given pollutant that a waterbody can assimilate and still meet water quality standards, including its applicable water quality criteria and its designated uses. TMDLs are developed for waterbodies that are verified as not meeting their water quality standards, and provide important water quality restoration goals that will guide restoration activities.

This TMDL report will be followed by the development and implementation of a Basin Management Action Plan, or BMAP, to reduce the amount of nutrients that caused the verified impairment of Wekiva River and RSR. These activities will depend heavily on the active participation of the SJRWMD, local governments, businesses, and other stakeholders. The Department will work with these organizations and individuals to undertake or continue reductions in the discharge of pollutants and achieve the established TMDLs for impaired waterbodies.

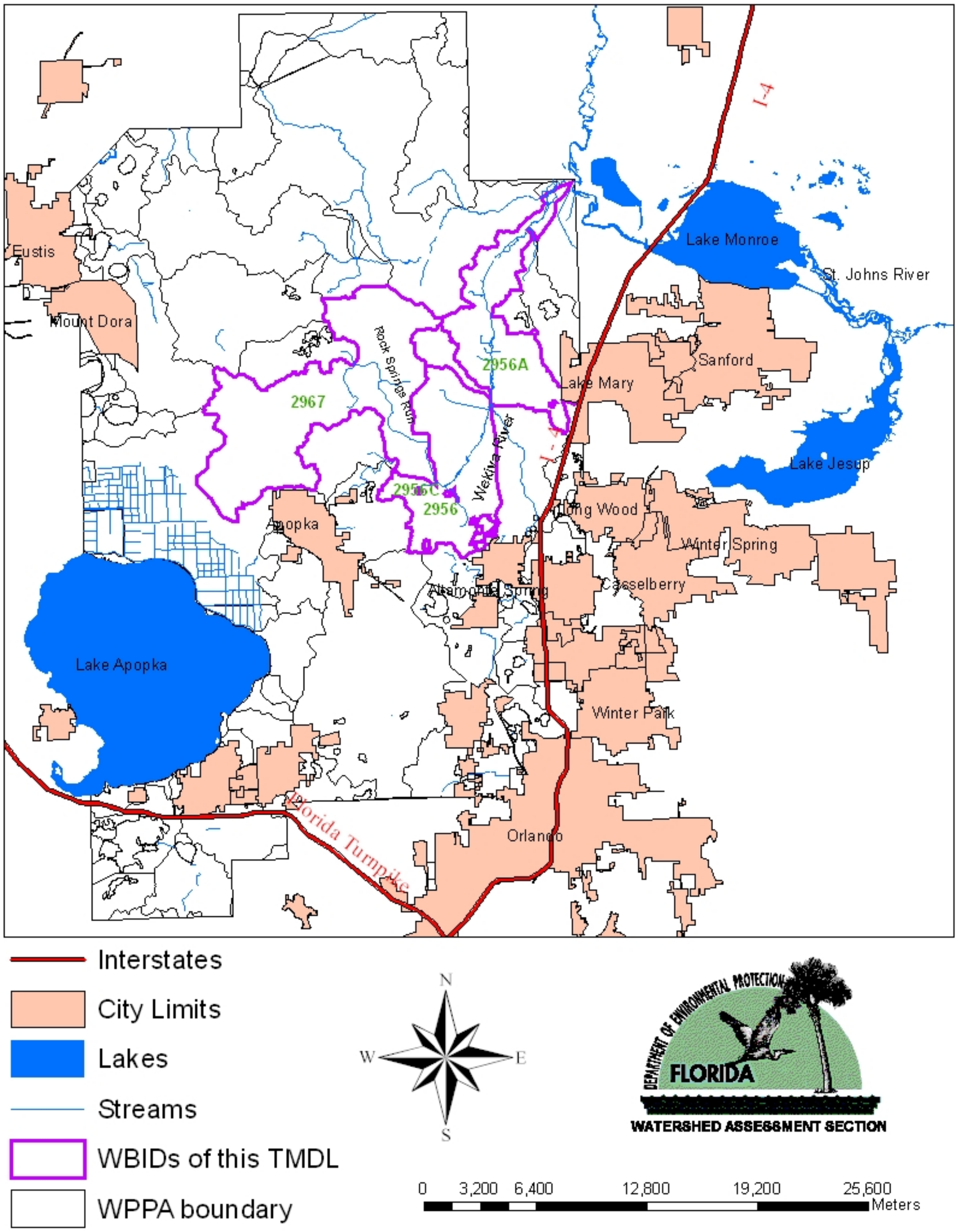


Figure 1.1. Locations of Wekiva Parkway Protection Area (WPPA), main stem of the Wekiva River and Rock Springs Run, waterbody IDs (WBIDs) covered in this TMDL, and major cities located around the waterbodies under question.

Chapter 2: DESCRIPTION OF WATER QUALITY PROBLEM

2.1 Statutory Requirements and Rulemaking History

Section 303(d) of the Clean Water Act requires states to submit to the EPA a list of surface waters that do not meet applicable water quality standards (impaired waters) and establish a TMDL for each pollutant source in each of these impaired waters on a schedule. The Department has developed these lists, commonly referred to as 303(d) lists, since 1992. The list of impaired waters in each basin is also required by the FWRA (Subsection 403.067[4)] Florida Statutes [F.S.]), and the list is amended annually to include updates for each basin statewide.

Florida's 1998 303(d) list included 22 waterbodies in the Middle St. Johns River Basin. However, the FWRA (Section 403.067, F.S.) stated that all previous Florida 303(d) lists were for planning purposes only and directed the Department to develop, and adopt by rule, a new science-based methodology to identify impaired waters. After a long rulemaking process, the Environmental Regulation Commission adopted the new methodology as Chapter 62-303, Florida Administrative Code (F.A.C.) (Identification of Impaired Surface Waters Rule, or IWR), in April 2001, and modified in 2006. The list of waters for which impairments have been verified using the methodology in the IWR is referred to as the Verified List.

2.2 Information on Verified Impairment

The Wekiva River (WBIDs 2956 and 2956A for the main stem and WBID 2956C for Wekiva Spring) and Rock Springs Run (WBID 2967) were not on the Verified List signed by Secretarial Order on May 27, 2004 because annual average *Chl a* concentrations of these WBIDs did not exceed the 20 µg/L threshold for eutrophication assessment at the time these waters were assessed. However, the Florida Impaired Waters Rule (Chapter 62-303, F.A.C) allows the use of information other than *Chl a* concentration to verify nutrient impairment. Recently, the SJRWMD provided to the Department several lines of evidence, indicating that these waters are impaired for nutrients, with the causative pollutants being nitrate[1] and phosphorus compounds.

According to the basin rotation schedule, when waterbodies in the Middle St. Johns River basin are assessed in the second water quality assessment cycle in 2008, these waters could be included on the Verified Listed, based on the evidence provided by the SJRWMD. However, the Florida Legislature passed the Wekiva Parkway and Protection Act (WPPA) in 2004 (Chapter 369, Part III, FS), which requires that:

> *"By December 1, 2005, the St. Johns River Water Management District shall establish pollution load reduction goals for the Wekiva Study Area to assist the Department of Environmental Protection in adopting total maximum daily loads for impaired waters*

[1] Nitrate is typically measured and reported as NO3+NO2. However, because the majority of this measurement is in the form of nitrate, NO3+NO2 is referred to as nitrate in this report.

within the Wekiva Study Area by December 1, 2006" (Chapter 369.318 [8], FS)

Because Rock Springs Run and the Wekiva River main stem are both located within the boundary of the Wekiva Study Area defined by the WPPA, based on the expedited timeline required by the WPPA, the Department conducted an off-cycle water quality assessment for these waterbodies using the recent information provided by the SJRWMD and verified their impairments for nutrients (TP and nitrate). According to the 1999 Florida Watershed Restoration Act (FWRA), Chapter 99-223, Laws of Florida, once a waterbody is included on the Verified List, a TMDL must be developed.

One way that the SJRWMD and its contractors used to identify the impairment was to compare the distribution of periphyton in the Wekiva River and Rock Springs Run with periphyton in two reference spring creeks [Alexander Springs Creek (ASC) and Juniper Creek (JC)]. These two reference creeks are located about 32 to 54 km (20 to 33 miles) north of the Wekiva River System in south Marion and north Lake Counties. Juniper Creek receives inflows from Juniper Springs and Fern Hammock Springs and travels for about 9 km (5.6 miles) before entering the southwest corner of Lake George. Alexander Springs Creek receives inflow from Alexander Springs and travels for about 17 km (10.5 miles) before entering the St. Johns River near Lake Dexter. **Figure 2.1** shows the location of these two creeks. **Table 2.1** shows the landuse categories in the watershed of these two creeks. The dominant landuse in watersheds of both reference creeks is upland forest. Development is insignificant in these watersheds.

Table 2.1. Landuse of Juniper Creek and Alexander Springs Creek basins.

Landuse Description	Juniper Creek		Alexander Springs Creek	
	Acreage	Percent Area	Acreage	Percent Area
Urban and Built-Up	98	0.2%	197	1.7%
Rangeland	488	1.1%	4.0	0.0%
Upland Forest	39495	85.5%	7679	68.1%
Water	457	1.0%	131	1.2%
Wetlands	5603	12.1%	3261	28.9%
Barren Land	3	0.0%	0	0.0%
Transportation, Communication, and Utilities.	23	0.1%	0	0.0%
Total	46,167	100.0%	11,272	100.0%

The SJRWMD compared the percent of stream bottom covered by periphyton[2] in the Wekiva River and Rock Springs Run to the two reference creeks and found that periphyton was more abundant in the Wekiva River and Rock Springs Run than in the two reference creeks. Specifically, periphyton (including benthic algae, filamentous algae, Nitella sp., and etc.) covered about 22.5% and 10.1% of the creek bottom in the Wekiva River and Rock Springs Run, respectively, while the percent of stream bottom covered by these algae was only about 1.8% in the two reference creeks (WSI, 2005). While some amount of periphyton growth is ecologically beneficial, excessive periphyton growth can inhibit the growth of submerged aquatic vegetation (SAV), which is considered the most important aquatic habitat for fish and other

[2] Periphyton refers to the community of attached organisms on submerged surfaces, which may include microsopic algae such as diatoms, green algae, blue-green algae, euglenophytes, as well as filamentous macrophytic algae (Mattson et al. 2006).

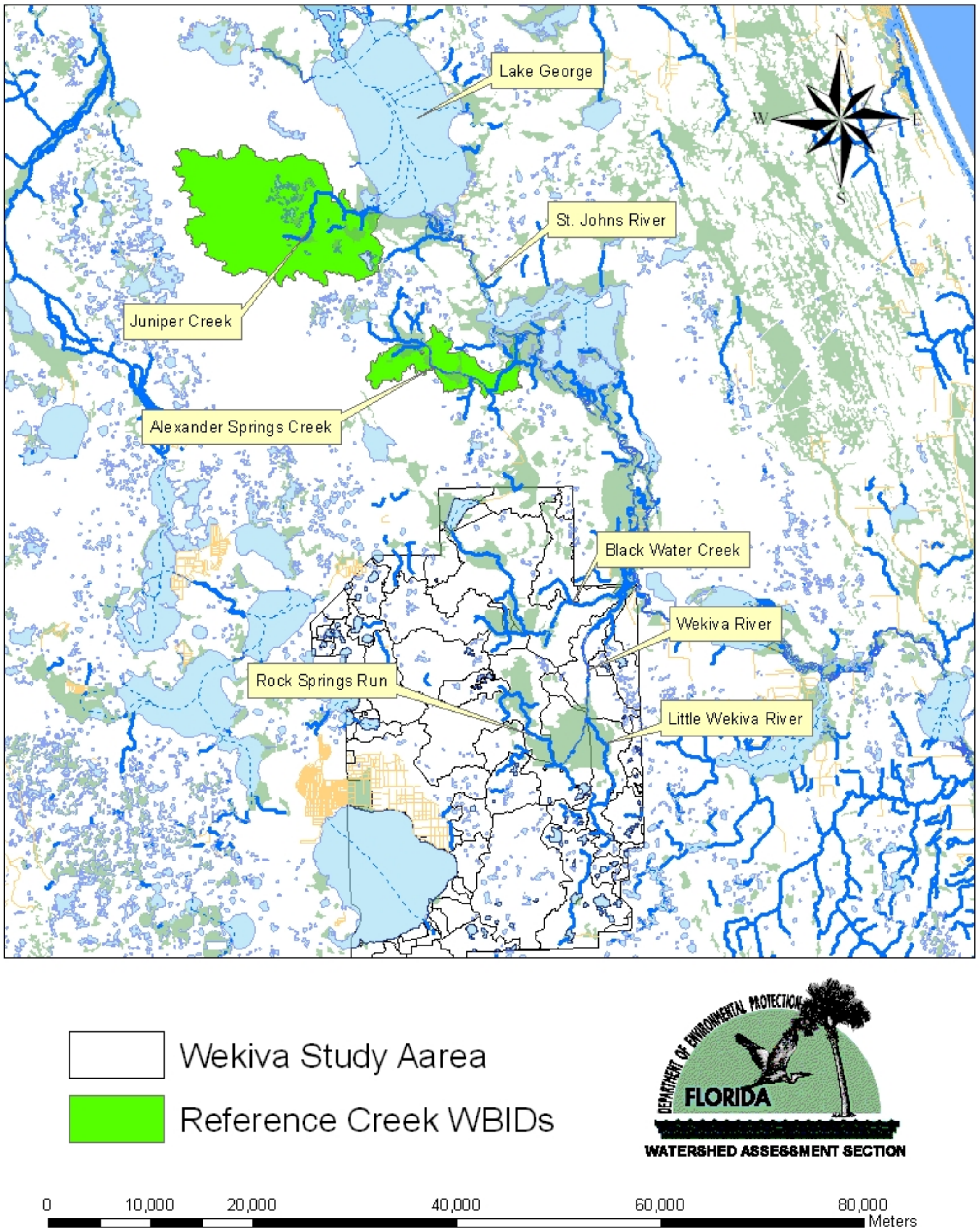


Figure 2.1. Locations of the two reference creeks used in this TMDL.

aquatic organisms in these spring creeks. For the case of the Wekiva River and Rock Springs Run, these high values indicate an imbalance in the flora of the creeks under question.

Another focus of the SJRWMD's studies was to examine the existence of algal species in the creeks that may cause harmful effects to fish and wildlife populations or human health concerns. One algal group of major concern in Florida springs is the filamentous Cyanobacteria, primarily in the genus *Lyngbya*. Studies have shown that, with increased nutrient loading to a waterbody, it is frequently the Cyanobacteria that respond most prolifically (Komarek 2003, Bronmark and Hansson 1998). Increases in *Lyngbya* biomass is a management issue in many Florida Springs (Stevenson et al. 2004). *Lyngbya*, and other Cyanobacteria, produce toxins that may be associated with skin reactions and respiratory distress in humans. Large mats of filamentous Cyanobacteria may also adversely affect macroinvertebrate and fish habitat, water quality and SAV.

Based on data collected by the Green Lab, Inc (contracted by the SJRWMD), Cyanobacteria were found to be the dominant or second largest contributors in the attached algal communities at the majority of the Wekiva River sites examined. The major Cyanobacteria species identified in the study were *Heteroleibleinia sp, Lyngbya wollei,* and *Phormidium sp.1*. Dominance of the attached algal communities by Cyanobacteria was also observed in Rock Springs Run, which was due primarily to the presence of *Lyngbya wolli* (Mattson, 2006). These observations are consistent with the conclusion that the Wekiva River and Rock Springs Run were impaired and the aquatic flora in these streams were imbalanced.

Information from an ecosystem level study conducted by the Wetland Solution, Inc (WSI, 2005) also suggested that the Wekiva River and Rock Springs Run aquatic flora were impaired. In this study, several ecosystem level indices, including gross primary productivity, net primary productivity, system respiration rate, and ecological efficiency, were measured for segments of Wekiva River and Rock Springs Run and compared to those measured in the two reference creeks. It was found that all these system indices were depressed in the Wekiva River and Rock Springs Run compared to the reference creeks, suggesting impairment on the ecosystem functions of the streams under question.

The SJRWMD provided several lines of evidences to show that nitrate and phosphorus compounds are the causative pollutants for observed impairments:

1. Laboratory experiments (Cowell and Dawes, 2004) and field studies (Hornsby et al. 2000) indicated that *Lyngbya* biomass and the biomass and diversity of Cyanobacteria population increased with elevated nitrate concentration, especially when the nitrate concentration increased above 0.30 mg/L.
2. Field studies indicated that the percent biovolume of Cyanobacteria in the algal community increased with increased total phosphorus (TP) concentrations, especially when the TP concentration was higher than 0.090 mg/L (Potapova and Charles, 2005).
3. The mean nitrate and TP concentrations in the Wekiva River and Rock Springs Run range between 0.60-0.70 mg/L and 0.12-0.14 mg/L, respectively, which are significantly higher than the threshold nitrate and TP concentrations identified in the above studies.
4. The percent stream bottom covered by algae was higher and overall ecosystem metabolic activities were lower in the Wekiva River and Rock Springs Run where nitrate and TP concentrations were higher compared to the two reference creeks where nitrate and TP concentrations were lower.

5. The highest biomass of attached algae was found at sites in and around the springs of both the Wekiva River and Rock Springs Run. Filamentous Cyanobacteria (particularly *Lyngbya wollei* and *Phormidium*) were most abundant in terms of biomass at spring vent sites. These spring vents typically have significantly higher nitrate and TP concentrations than sites farther downstream of the vent.

The above evidence indicated that the Wekiva River and Rock Springs Run are impaired and the causative pollutants are nitrate and phosphorus compounds.

Chapter 3. DESCRIPTION OF APPLICABLE WATER QUALITY STANDARDS AND TARGETS

3.1 Classification of the Waterbody and Criteria Applicable to the TMDL

Florida's surface waters are protected for five designated use classifications, as follows:

Class I	**Potable water supplies**
Class II	**Shellfish propagation or harvesting**
Class III	**Recreation, propagation, and maintenance of a healthy, well-balanced population of fish and wildlife**
Class IV	**Agricultural water supplies**
Class V	**Navigation, utility, and industrial use (there are no state waters currently in this class)**

Both the Wekiva River and Rock Springs Run are Class III waterbodies, with a designated use of recreation, propagation, and maintenance of a healthy, well-balanced population of fish and wildlife. The Class III water quality criteria applicable to the impairment addressed by this TMDL are for nutrients.

3.2 Applicable Water Quality Standards and Numeric Water Quality Target

3.2.1 Interpretation of Narrative Nutrient Criterion

Florida's nutrient criterion is narrative only—i.e., nutrient concentrations of a body of water shall not be altered so as to cause an imbalance in natural populations of aquatic flora or fauna. Accordingly, a nutrient-related target was needed to represent levels at which an imbalance in flora or fauna is expected to occur. A threshold commonly used for assessing the nutrient impairment in streams is the annual average *Chl a* concentration of 20 µg/L, which is defined in the Impaired Waters Rule (IWR, 62-303 F.A.C). In addition, the IWR also allows the use of other information indicating imbalance in flora or fauna due to nutrient enrichment, including, but not limited to, algal blooms, excessive macrophyte growth, decrease in the distribution (either in density or areal coverage) of seagrasses or other submerged aquatic vegetation, changes in algal species richness, and excessive dial oxygen swings.

As indicted in SJRWMD's PLRG (Mattson et al., 2006), the impairments of the Wekiva River and Rock Springs Run were primarily manifested through elevated algal biomass, dominance of benthic algal communities by blue-green algae such as *Lyngbya wolli* , and depressed ecosystem metabolic activities and that the impairments were due to the elevated nitrate and phosphorus concentrations. As such, target nitrate and TP concentrations are needed to address the imbalances of these structures and functions of aquatic communities.

3.2.1.1 Setting the nitrate target

The target nitrate concentration for the Wekiva River and Rock Springs Run was established based on several lines of evidence, including 1) laboratory nutrient amendment bioassays, 2) examining the relationship between periphyton biomass and cell density and the nitrate concentration in flow-through systems similar to the Wekiva River and Rock Springs Run, and 3) comparing metabolic rates, specifically, ecological efficiency, of aquatic communities in the Wekiva River and Rock Springs Run with those in reference creeks.

1) **Laboratory nutrient amendment bioassays**

The nutrient amendment bioassay work was conducted by Cowell and Dawes (2004), who examined the required nitrate concentration in the Rainbow River, Marion County, Florida to achieve a reduction of biomass of *Lyngbya wollei*. *L. wollei* is a nuisance blue-green benthic algal species that dominates the Rainbow River due to elevated nitrate concentrations, and is also frequently observed in the Wekiva River and Rock Springs Run where elevated nitrate concentrations exist (Mattson et al. 2006). Using *Lyngbya* cultures incubated in a series of nitrate amendments, Cowell and Dawes (2004) found that, at the end of the nutrient amendment experiments, both the biomasses and growth rates were low in treatment groups with nitrate concentration at or below 300 µg/L, while the growth rates and biomass were significantly higher in treatments with nitrate concentrations at or higher than 600 µg/L. In addition, the experiment also showed that the biomass and growth rate in 300 and 70 µg/L treatment groups were similar, suggesting that further reduction of nitrate concentration below the 300 µg/L level probably would not achieve dramatic further reduction of *L. wollei*. A nitrate concentration of 300 µg/L should be appropriate in controlling *L. wollei*.

2) **Relationship between periphyton biomass and cell density and nitrate concentration**

The nitrate target suggested by the Rainbow River study was corroborated by the findings of Hornsby et al. (2000), who evaluated periphyton and water quality data collected from the Suwannee River and two tributaries including the Withlacoochee River and Santa Fe River. Much of the length of the Suwannee River was heavily influenced by spring inflow, and the algal communities appeared to be generally similar in composition to those in the Wekiva River and Rock Springs Run. Therefore, results from this river were considered applicable to the Wekiva River and Rock Springs Run (Mattson et al., 2006). This study showed positive correlations for both periphyton biomass versus nitrate concentration and cell density versus nitrate concentration. The functional relationships of periphyton biomass (represented as ash free dry mass, or AFDM) versus nitrate concentration and cell density versus nitrate concentration are shown in **Figures 3.1-A**, and **–B,** respectively. Data presented in these figures represent long-term average biomass, cell densities, and nitrate concentrations measured at 13 stations across the Suwannee River system (including the Withlacoochee River and Santa Fe River). **Figure 3.2** shows locations of these stations. As shown in **Figures 3.1-A** and **–B,** both periphyton biomass and cell density per unit increase of nitrate concentration significantly increased when nitrate concentration reached a level between 200 – 300 µg/L.

To narrow down the nitrate concentration that may significantly impact the periphyton biomass and cell density per unit increase of nitrate concentration, the Department contracted Professor Xufeng Niu of the Department of Statistics, Florida State University, to conduct a change-point analysis. The applied method fits a step function through observed data by examining the probability of each data point as the change-point. A nitrate concentration change point was

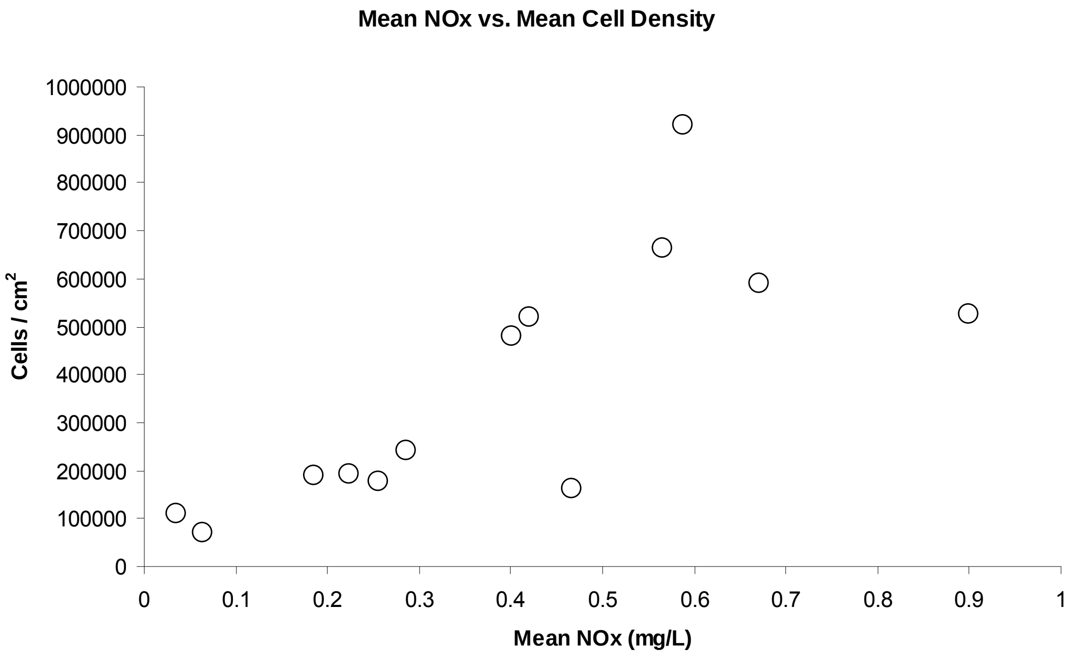


Figure 3.1-A Relationship between mean nitrate concentration and mean periphyton cell density from sampling sites on the Suwannee, Santa Fe, and Withlacoochee Rivers (Mattson et al. 2006).

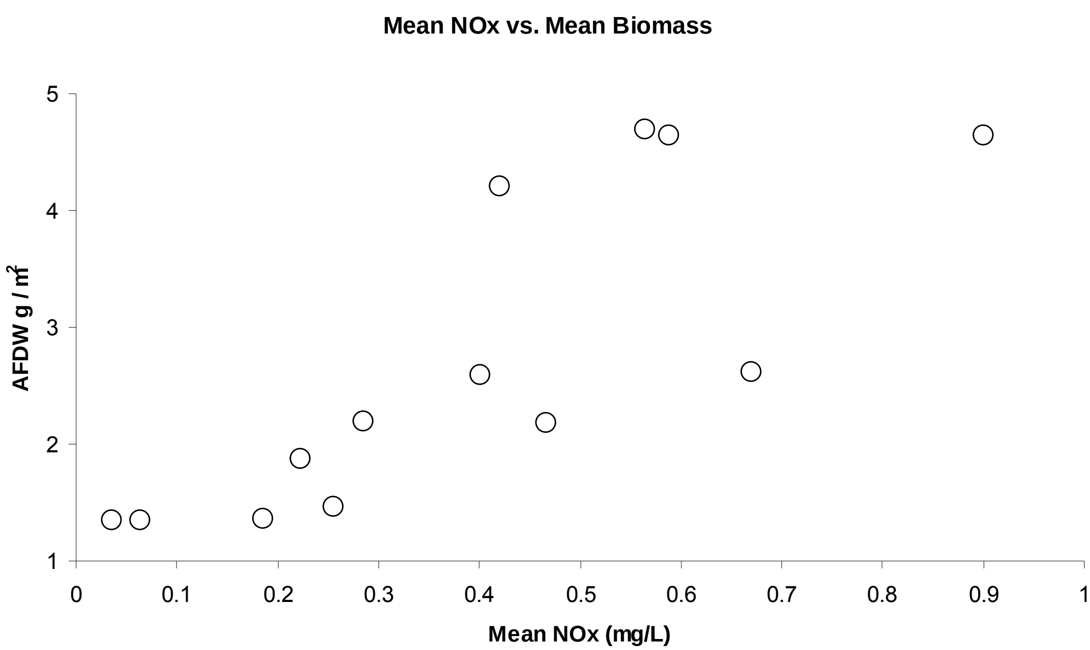


Figure 3.1-B Relationship between mean nitrate concentration and mean periphyton biomass from sampling sites on the Suwannee, Santa Fe, and Withlacoochee Rivers (Mattson, 2006).

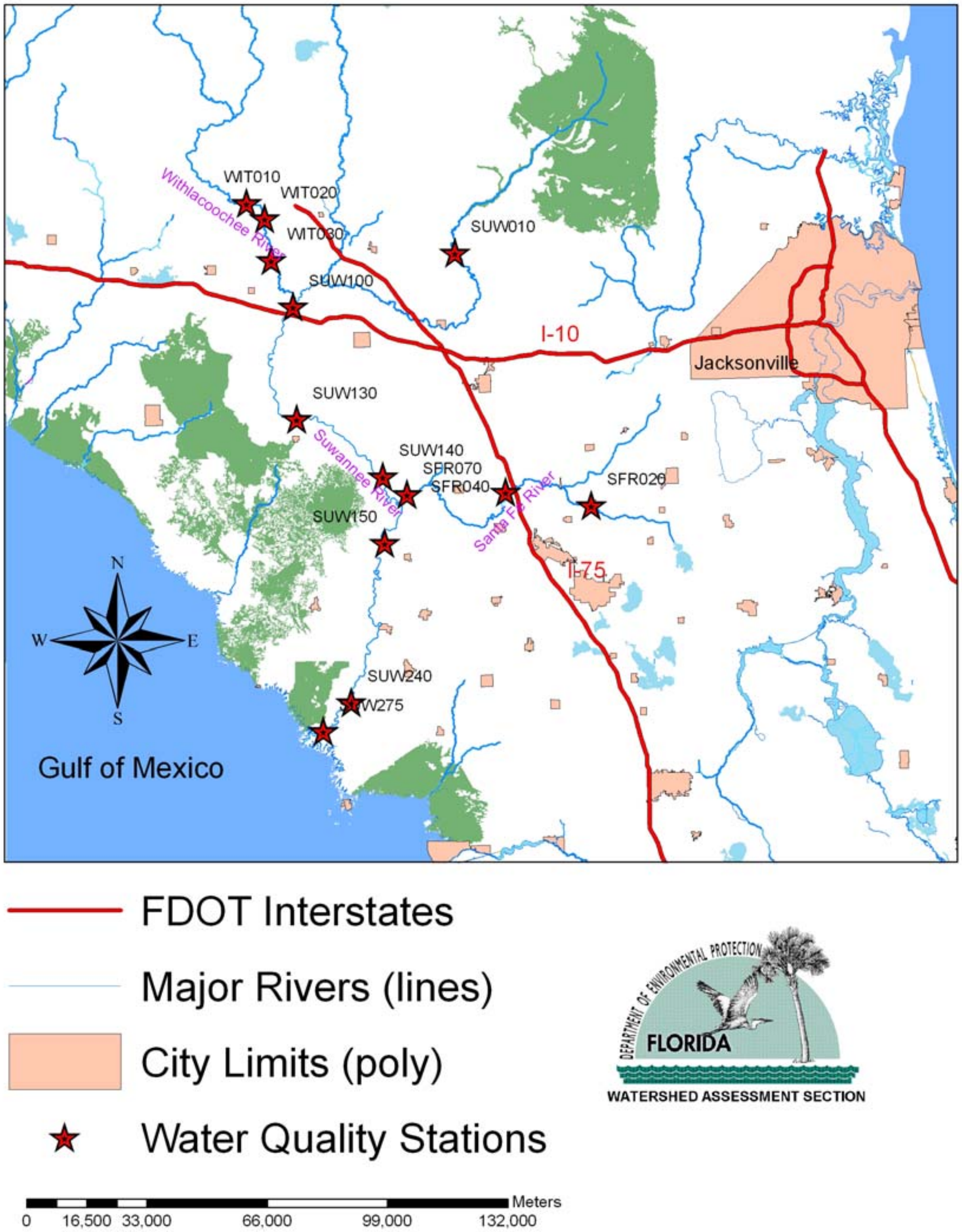


Figure 3.2. Locations of water quality stations from which nitrate and periphyton abundance samples were collected and used for the change-point analysis

identified at a 5% significant level if the change of cell density or periphyton biomass caused by the nitrate concentration was 3.5 times higher (the T test critical value) than the standard error of the change of cell density or periphyton biomass The identified step function (the change-point model) was also compared to linear regression and non-linear regression models for its goodness-of-fit and the extent of over-fitting based on the Bayesian Information Criterion (SBC). For both periphyton cell density and periphyton biomass, change-point step functions were shown to be the best model among the models tested. This supports the use of the change-point model identified in the T test. Details of the change-point analyses are provided in **Apendix B**.

For the functional relationship between cell density and nitrate concentration, the change-point step function identified two cell density levels (**Table 3** in **Apendix B**). One level is about 162,998 cells/cm^2 (P = 0.009), and the other is about 162,998 + 453,295 = 616,293 cells/cm^2 (P = 0.0001). In this study, the 162,998 cells/cm^2 was considered as the baseline condition under which no significant nitrate impact was detected. The nitrate concentration that significantly changed the cell density level from 162,998 cells/cm^2 to 616,293 cells/cm^2 was identified by the change-point step function as 0.401 mg/L (**Table 2** in **Apendix B**), indicating that, to prevent the periphyton cell density from switching to the higher level, the nitrate concentration should not exceed 0.401 mg/L. In addition, based on **Table 2** and **Figure 4** of **Appendix B**, the cell density switch occurred when the nitrate concentration reached between 0.286 mg/L and 0.401 mg/L. Although the nitrate concentration that started the cell density switch could be any nitrate concentration between 0.286 mg/L and 0.401 mg/L, 0.286 mg/L was chosen in this TMDL as the nitrate target to control periphyton cell density to make the nitrate target conservative.

For the functional relationship between periphyton biomass and nitrate concentration, the change-point step function identified two biomass levels (**Table 5** in **Apendix B**). One level is about 1.73 g/m^2 (P= 0.00), and the other level is about 1.73 + 2.42 = 4.15 g/m^2 (P = 0.0001). In this study, the 1.73 g/m^2 was considered as the baseline condition under which no significant nitrate impact was detected. The nitrate concentration that significantly changed the biomass level from 1.73 g/m^2 to 4.15 g/m^2 was identified by the change-point step function as 0.420 mg/L (**Table 4** in **Apendix B**), indicating that, to prevent the periphyton biomass from switching to the higher level, the nitrate concentration should not exceed 0.420 mg/L. In addition, based on **Table 4** and **Figure 6** of **Appendix B**, the highest observed nitrate concentration that allowed the biomass baseline condition was between 0.401 mg/L and 0.0.420 mg/L. Although the nitrate concentration that started the biomass switch could be any nitrate concentration between 0.401 mg/L and 0.420 mg/L, 0.401 mg/L was chosen in this TMDL as the nitrate target to control periphyton biomass to make the nitrate target conservative.

Based on the above discussion, nitrate concentrations lower than 0.286 mg/L and 0.401 mg/L should be appropriate to maintain periphyton cell density and biomass at baseline conditions, respectively. Because 0.286 mg/L is lower than 0.401 mg/L, 0.286 mg/L was selected for the TMDL target to maintain both cell density and biomass at baseline conditions. Choosing the lower nitrate concentration between 0.286 and 0.401 mg/L also adds to the margin of safety of the nitrate TMDL.

3) **Relationship between ecological efficiency and nitrate concentration**

Wetland Solutions, Inc (WSI, 2005) studied the effects of nutrient concentrations on the community metabolic rates in the Wekiva River, Rock Springs Run, and two reference creeks

(ASC and JC). The gross community primary production, community respiration, net primary production, and ecological efficiency were measured and examined. The community metabolic parameter shown to have a significant functional relationship with nutrient concentrations was ecological efficiency, which is defined as the quotient between the rate of gross primary productivity (GPP) and the incident photosynthetically active radiation (PAR) during a specified time interval. It is an ecosystem-level property that estimates the overall efficiency of an aquatic ecosystem to utilize incident solar radiation.

To examine the effect of nitrate concentrations on the ecological efficiency, experimental sites were set up in streams with different nitrate concentrations. **Figures 3.3 - 3.5** show locations of these segments. Two test stream segments were set up in both the Wekiva River and Rock Springs Run, and one test stream segment was set up for each of Alexander Springs Creek (ASC) and Juniper Creek (JC). As shown in **Figure 3.3,** the test segment RSR-SEG1 (one of the test segments for Rock Springs Run) is located close to the spring vent where high nitrate concentrations are typically observed. Segment RSR-SEG2 is a downstream site located close to the confluence of Rock Springs Run and the Wekiva River, and the average nitrate concentration at this site was lower than 300 µg/L at the time the study was conducted.

For the Wekiva River, WR-SEG1 is the test segment located close to the Wekiva Spring, where high nitrate concentrations were observed. Segment WR-SEG2 is the downstream segment where nitrate concentration decreased to about 300 µg/L at the time the study was conducted.

Table 3.1 lists ecological efficiency results and nitrate+nitrite concentrations from these test segments.

Table 3.1. Ecological efficiencies and nitrate concentrations at test segments in WRMS, RSR, JC, and ASC.

Test Segments	Ecological Efficiency (g O_2/mol)	Nitrate+Nitrite (mg/L)	Water Color (PCU)	Period of Record
WR-SEG1	0.11	1.09	8.75	4/29/2005 5/27/2005
WR-SEG2	0.22	0.300	40.0	4/28/2005 5/26/2005
RSR-SEG1	0.08	1.15	26.3	4/1/2005 4/20/2005
RSR-SEG2	0.05	0.270	193	4/1/2005 4/21/2005
ASC-SEG1*	0.36	0.009	58.8	4/29/2005 5/24/2005
JC-SEG1**	0.47	0.015	38.8	3/31/2005 4/18/2005
WR-SEG1	0.11	1.24	21.9	8/10/2005 8/31/2005
WR-SEG2	0.14	0.365	96.3	8/9/2005 9/1/2005
RSR-SEG1	0.05	1.03	77.5	7/12/2005 8/3/2005
RSR-SEG2	0.07	0.045	350	7/12/2005 8/4/2005
ASC-SEG1*	0.23	0.018	150	8/9/2005 8/29/2005
JC-SEG1**	0.26	0.023	65.0	7/11/2005 8/1/2005

*Test segment in Alexander Springs Creek.
**Test segment in Juniper Creek.

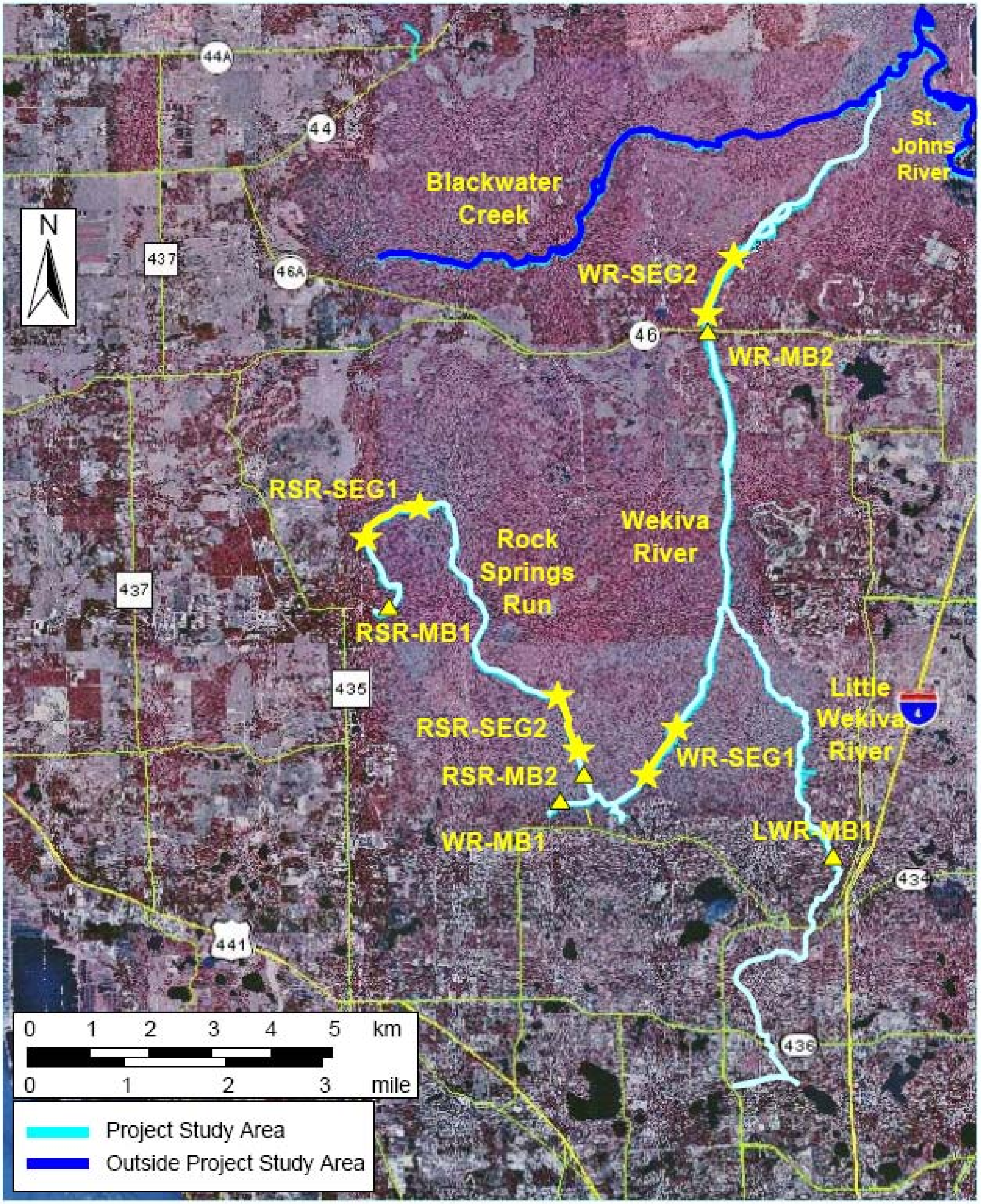


Figure 3.3. Wekiva River and Rock Springs Run stream segments for ecological evaluations. This figure was cited from Wetland Solution, Inc. (2005).

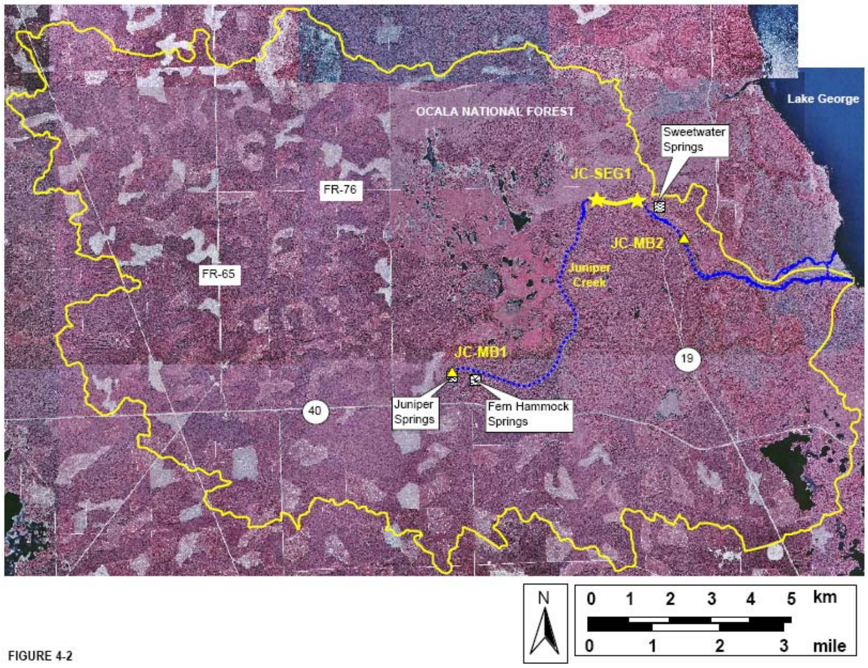


Figure 3.4. Juniper Creek stream segments for ecological evaluations. This figure was cited from Wetland Solution, Inc. (2005).

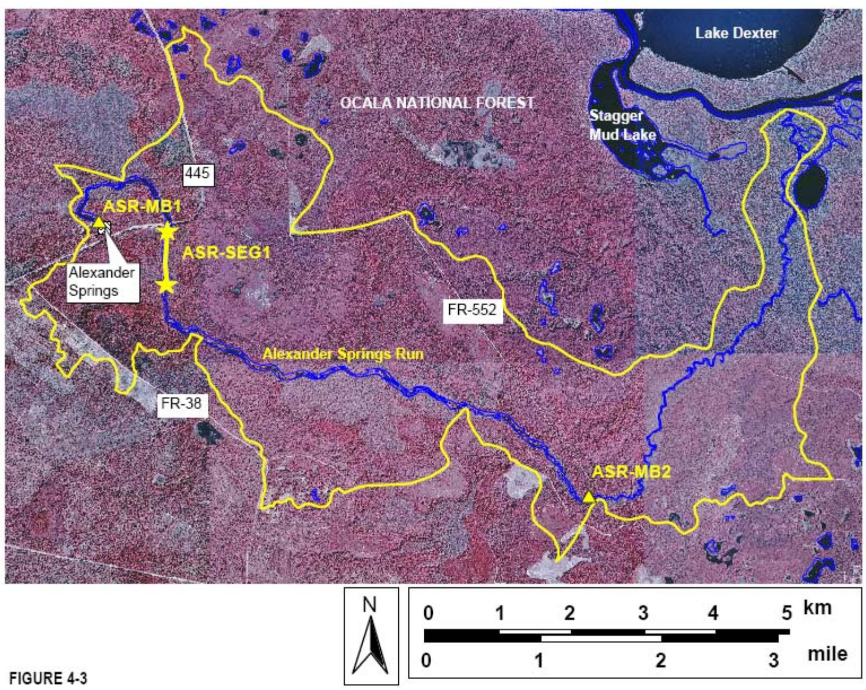


Figure 3.5. Alexander Springs Run stream segments for ecological evaluations. This figure was cited from Wetland Solution, Inc. (2005).

Table 3.1 shows a general trend that the ecological efficiencies of the test segments with high nitrate concentration, e.g. WR-SEG1 and RSR-SEG1 were significantly lower than test segments of reference streams, e.g. ASC-SEG1 and JC-SEG1. For the Wekiva River, ecological efficiency significantly increased at the downstream test segment WR-SEG2 where nitrate concentrations significantly decreased. However, similar increases in ecological efficiency was not observed at the downstream segment (RSR-SEG2) located in Rock Springs Run even when the nitrate concentration at this segment also significantly decreased. One possible reason for the suppressed ecological efficiency at RSR-SEG2 is the high water color. As shown in **Table 3.1**, water color at RSR-SEG2 was dramatically higher for both test events than color levels observed for the other segments. High color at RSR-SEG2 suggested that the gross primary production (GPP) at this segment could be depressed due to the low light availability and the community could be dominated by heterotrophic organisms. Therefore, the amount of organic carbon that can be fixed by the unit photosynthetically active radiation (PAR) could become naturally lower even when the nitrate concentration was not high.

Another explanation for the low observed ecological efficiency at RSR-SEG2 could be the type of aquatic flora dominating the community in this segment. **Table 3.2** lists the percent stream bottom covered by different vegetation types in different test segments. These data were collected by Wetland Solutions, Inc (2005). The plant community data were aggregated into several different functional groups, including benthic algae (ALG), emergent aquatic plants (EMA), floating aquatic plants (FAP), submerged aquatic plants (SAV), shrubs (SHR), and vines (VINE).

Table 3.2. Percent stream bottom covered by vegetation of different functional groups at different test segments.

Functional Vegetation Groups	Test Segments					
	WR-SEG1	WR-SEG2	RSR-SEG1	RSR-SEG2	ASC-SEG1	JC-SEG1
ALG	37.73	7.32	20.21	0.00	1.77	1.82
EMA	5.03	9.13	10.00	37.11	19.16	1.22
FAP	4.77	8.11	10.73	26.65	8.50	1.56
SAV	1.45	27.87	4.49	0.00	48.58	4.21
SHR	1.09	0.07	0.00	1.22	0.08	1.13
VINE	0.00	0.22	0.08	0.00	0.02	0.00

As shown in **Table 3.2**, unlike at any other sites, no benthic algae (ALG) or submerged aquatic vegetation (SAV) were observed at RSR-SEG2, the downstream site of the Rock Springs Run. This could be caused by the high water color observed at this segment. The dominant vegetation communities at RSR-SEG2 were emergent (EMA) and floating aquatic plants (FAP). As the measurement of ecological efficiency was based on measuring the water column dissolved oxygen concentration, and even if EMA and FAP can fix a significant amount of organic carbon using the PAR, less oxygen will be released into the water column than if the community is dominated by ALG and/or SAV. Therefore, it is not totally unexpected that low ecological efficiency was observed at RSR-SEG2. The low ecological efficiency observed at RSR-SEG2 might not be directly related to the nitrate concentration of this segment.

Based on the above discussion, data measured at RSR-SEG2 were excluded when analyzing the relationship between ecological efficiency and nitrate concentration. **Figure 3.6** shows the correlation between the ecological efficiency and nitrate concentration.

The target ecological efficiency for this TMDL was chosen as the average of summer time ecological efficiency measurements taken from the reference sites [ASC-SEG1 (8/9/2005 – 8/29/2005) and JC-SEG1 (7/11/2005 – 8/12/2005)] in the two sampling events (**Table 3.1**). As shown in **Table 3.1**, ecological efficiency varied greatly in the two reference creeks even when nitrate concentrations were low. Defining the target ecological efficiency based on summer data would address the influence of pollutant loadings from both springs and surface runoff (influence from nonpoint source loading is more significant in the summer rainy season than in the winter dry season). The target ecological efficiency defined using this method is 0.25 g O_2/mol. Using the ecological efficiency – nitrate concentration equation defined in **Figure 3.6**, the target nitrate concentration is 0.293 mg/L or 293 µg/L.

The target nitrate concentration based on ecological efficiency is very close to the target nitrate concentrations suggested by the results from the Rainbow River and Suwannee River studies, which are about 300 µg/L and 286 µg/L, respectively. Therefore, the Department established a final target concentration of 286 µg/L, which is the nitrate target derived from the change – point analysis based on periphyton data. This target is lower than both 293 µg/L and 300 µg/L and therefore is the most conservative target. It is protective of the structure and function of aquatic flora in the Wekiva River and Rock Springs Run and adds to the margin of safety (MOS) of this TMDL.

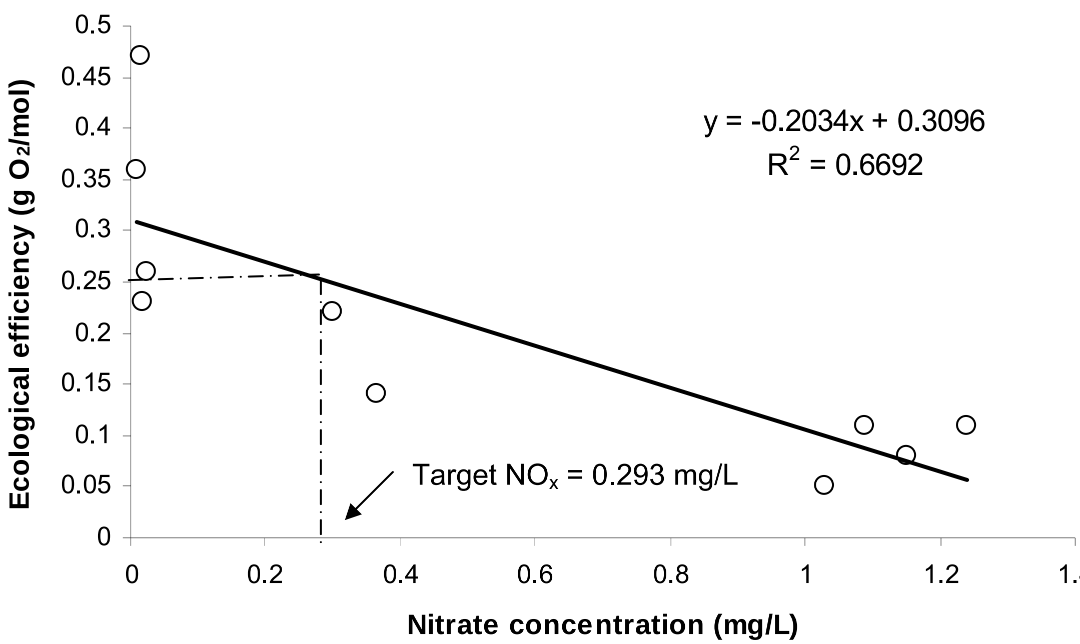


Figure 3.6. Correlation between ecological efficiency and nitrate concentration in WR, RSR, ASC, and JC.

The SJRWMD proposed a biologically effective nitrate concentration of 250 µg/L based on the Rainbow River *Lyngbya wollei* nutrient amendment experiment. Given the natural variation of nitrate concentrations in ambient water, the SJRWMD, when proposed 250 µg/L as the nitrate effective concentration, conducted a statistical analysis to determine a nitrate target concentration that would ensure that the 250 ug/L target would be met the majority of the time. The SJRWMD concluded that the targets should ensure compliance with the 250 ug/L target at least 90% of the time and the statistical analysis indicated that the nitrate target should be 216 µg/L for Wekiva River and 221 µg/L for Rock Springs Run. A detailed description of the statistical method used by the SJRWMD is provided by Mattson et al. (2006).

After carefully reviewing all the above studies from which the SJRWMD's nitrate target (250 µg/L) was derived, the Department believes that it is not necessary to limit the percentage of time the 250 µg/L target can be exceeded and instead established the target as a monthly average concentration. This is mainly because the changes in aquatic vegetation biomass do not respond to the change of nutrient concentration in an instantaneous manner. Therefore, short-term exceedence of this target concentration may not produce negative biological or ecological effects. The 286 µg/L nitrate range obtained from the Suwannee River study was based on the correlation between the long-term average nitrate concentration and long-term average cell density and biomass. Therefore the 286 µg/L should be considered as a long-term average target instead of an instantaneous value. The nitrate range suggested by the *Lyngbya* study was from a nutrient amendment experiment. The value can be considered as a threshold. However, the significant differences in growth rate and biomass between the above 600 µg/L treatment groups and below 300 µg/L treatment groups were not observed until 8 to 12 days after the nutrient amendment study started. This apparently suggested a time lag between the change of the nitrate concentration and the response from *Lyngbya*. In addition, the Lyngbya nutrient amendment study was conducted under the tightly controlled laboratory condition with no competition from other periphyton and plants, no grazing from aquatic animals, no removal effects from the shearing force of the stream flow, and no light attenuation from changing of water color. These factors are very common in natural stream systems such as the Wekiva River and RSR. All these natural processes could significantly slow down the response of *Lyngbya* to the change of nitrate concentration and further elongate the response time delay. Therefore, treating the 300 µg/L nitrate concentration obtained from the *Lyngbya* study as an exact instantaneous value is not necessary.

The same concept also applies to the target nitrate value obtained from the correlation between the ecological efficiency and the nitrate concentration. The ecological efficiency shown in **Table 3.1** and **Figure 3.6** are average values for a period from three to four weeks (WSI, 2005). The nitrate target value derived from an equation, based on average ecological efficiency, should not be treated as an exact instantaneous value. It is more appropriate that the target number be treated as an average target, over a certain time period.

Based on above discussions, the Department established the 286 µg/L threshold as a monthly average target concentration. To address the temporal variation of nitrate concentration in the Wekiva River, the Department analyzed the monthly variation of nitrate concentration in a Wekiva River WBID with high nitrate concentrations (WBID 2956) using the data collected during 1999 through 2005. The monthly variation of nitrate concentration in this WBID is shown in **Figure 3.7**. It appears that the high nitrate concentrations typically appear during the winter, early spring, and later fall, while concentrations are typically lower during the summer season. This observation is not a surprise because one of the major nitrate contributors to the Wekiva River is springs, and summer months typically have higher rainfall and dilution by the surface runoff could play an important role in reducing the in-stream nitrate concentration. Expressing the target as a monthly average provides a margin of safety because restoration activities designed to address the higher winter nitrate concentrations should help ensure that summer nitrate concentrations are even lower.

Toxicity effects of nitrate concentration in the Wekiva River and Rock Springs Run were also discussed by the SJRWMD in the nutrient PLRG. However, all the discussions were based on literature published nitrate toxicity studies conducted in other waterbodies. All these studies concentrated on the acute toxicity effects of nitrate, and chronic effects were only implied by multiplying the acute toxicity results by various safety factors. At the time this TMDL was developed, no information or measurements specific to the Wekiva River and Rock Springs Run indicated that the existing nitrate concentration has caused any toxic effects to aquatic fauna in these waterbodies. In contrast, results from 10 Stream Condition Index (SCI) conducted in the upper reach of Wekiva River (WBID 2956) from 1999 through 2004 showed that benthic macroinvertebrate communities were in good or excellent condition. Results from 2 BIORECON and 8 SCIs for the Rock Springs Run (WBID 2967) in 1997, 1999, and 2002 through 2004 also showed benthic macroinvertebrate communities were in good or excellent condition. Apparently, even under the existing high nitrate concentrations, no direct toxic impacts were observed with the benthic macroinvertebrates communities, which are typically considered sensitive index organisms to toxic materials. The 286 µg/L target nitrate concentration therefore should be sufficient to protect the aquatic fauna in the Wekiva system.

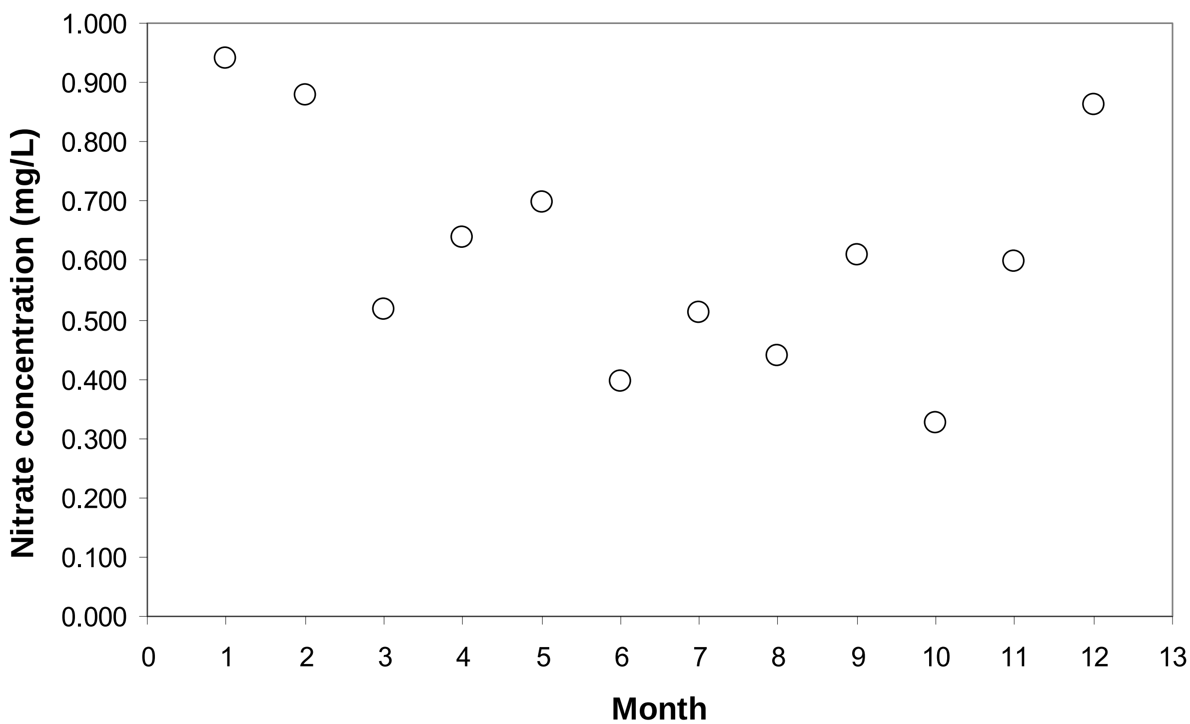


Figure 3.7. Monthly variation of nitrate concentration in upstream WRMS

In conclusion, based on the information currently available, the Department believes that a monthly average nitrate concentration of 286 µg/L should be sufficiently protective of the aquatic flora and fauna in the Wekiva system. This target concentration is higher than long-term median nitrate concentrations of the two reference streams – ASC and JC, which are 50 and 80 µg/L, respectively (Mattson et al. 2006). However, TMDL targets are designed to identify the threshold above which impairment is expected to occur, and natural aquatic systems have assimilative capacities for nutrients. An elevated pollutant concentration in the system alone does not necessarily constitute impairment as long as there is no negative response from the local aquatic flora or fauna. Based on information provided above, 286 µg/L nitrate is the target concentration that will not cause an imbalance in the aquatic flora and fauna in the Wekiva River and Rock Springs Run.

3.2.2.2. Setting the total phosphorus target

The total phosphorus target (TP) for the Wekiva River and Rock Springs Run was established based on the studies on the attached algal communities conducted by the GreenWater Labs (2005) and the ecosystem metabolism studies conducted by the Wetland Solution, Inc. (2005).

The GreenWater Labs collected attached algal samples from 15 sampling sites along the Wekiwa Spring/Wekiva River and 9 sites along Rock Springs/Rock Springs Run. Each site was sampled in winter during December 2004-January 2005 and in summer in June of 2005. At each site, nutrient (include nitrogen and phosphorus) samples were collected at the same time that attached algal samples were collected and analyzed. **Figure 3.8** shows the locations of these sampling sites in the Wekiva River and Rock Springs Run.

The SJRWMD analyzed the relationship between *Chl a* concentration, algal biovolume, ash free dry mass of algal samples, and percent blue-green and green algal biovolume in the total algal biovolume and TP concentration. These analyses yield a threshold relationship between percent biovolume of blue-green and green algae and TP concentration (**Figure 3.9**). High percent biovolumes of blue-green and green algae were observed when the TP concentration increased above 90 µg/L.

The Department also analyzed the relationship between the ecological efficiency and TP data collected by the Wetland Solutions, Inc. **Table 3.3** lists the ecological efficiency and TP data used in this analysis and test stream segments from which these data were collected. Again, because of the high color and the vegetation communities dominated by emergent and floating aquatic vegetation at RSR-SEG2, data from this sampling site were excluded from the analysis. **Figure 3.10** shows the correlation between the ecological coefficient and TP concentration at these test segments. Again, 0.25 g O_2/mol was chosen as the target ecological efficiency. Based on this target and the correlation equation shown in **Figure 3.10**, the target TP concentration should be 65 µg/L. Because the 65 µg/L TP target is lower than the 90 µg/L concentration derived from GreenWater labs attached algal studies, it should addresse both the ecological efficiency and blue-green and green algae dominance issues. Therefore, 65 µg/L was chosen as the final TP target for this TMDL.

There was no seasonal pattern for the percentage blue-green and green algae indicating a critical season or month. There were not enough data to determine when would be the critical season for the low ecological efficiency. Analysis on the monthly distribution of TP concentration in the Wekiva River indicated that high TP concentrations were typically observed during the summer months, which is the typical growth season. Therefore, there is not enough information to determine whether establishing the 65 µg/L as the annual average target would be sufficiently protective. As the 65 µg/L target was derived from the ecological efficiency studies and these studies had a time scale of close to one month, theTP threshold of 65 µg/L was expressed as a monthly target for this TMDL.

Figure 3.8. Sampling stations used by the GreenWater Labs for attached algal studies in the Wekiva River and Rock Springs Run (GreenWater Labs, 2005)

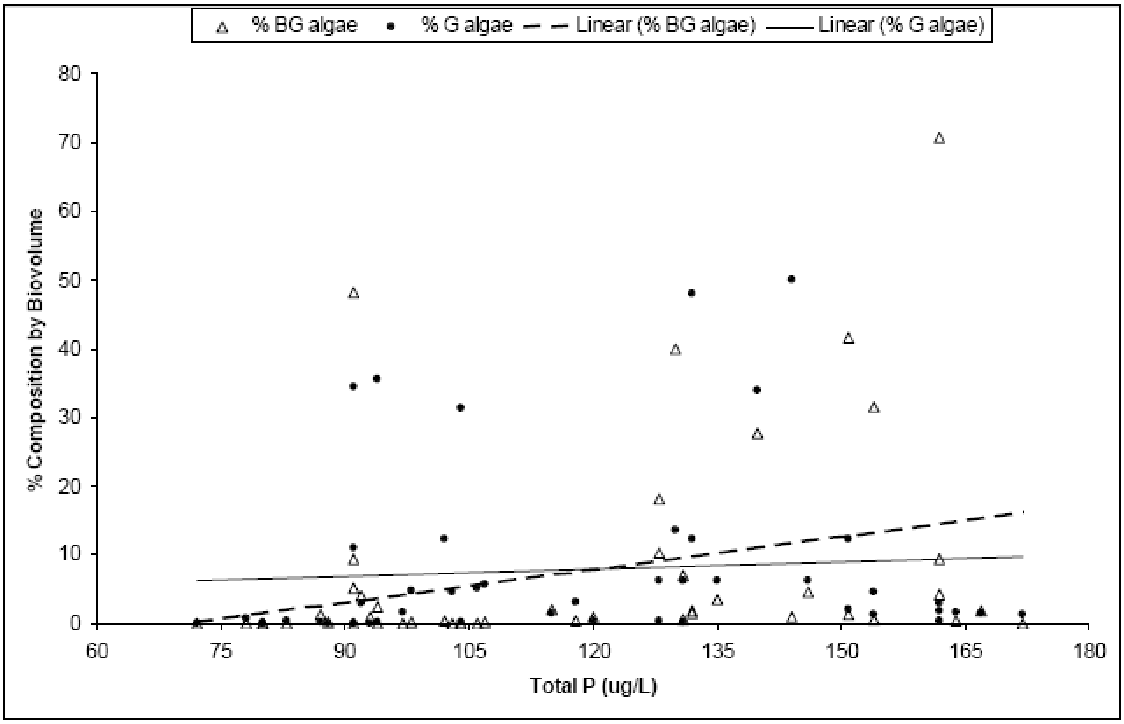


Figure 3.9. Relationship between TP concentration and percent biovolume of blue-green and green algae in the Wekiva River and Rrock Springs Run (Mattson et al. 2006)

Table 3.3. Ecological efficiencies and TP concentrations at test segments in theWekiva River (WR), Rock Springs Run (RSR), Juniper Creek (JC), and Alexander Springs Creek (ASC)

Test segments	Ecological efficiency (g O_2/mol)	TP concentration (mg/L)	Water color (pcu)	Period of Record	
WR-SEG1	0.11	03125	8.75	4/29/2005	5/27/2005
WR-SEG2	0.22	0.118	40.0	4/28/2005	5/26/2005
RSR-SEG1	0.08	0.091	26.3	4/1/2005	4/20/2005
RSR-SEG2	0.05	0.096	193	4/1/2005	4/21/2005
ASC-SEG1*	0.36	0.045	58.8	4/29/2005	5/24/2005
JC-SEG1**	0.47	0.024	38.8	3/31/2005	4/18/2005
WR-SEG1	0.11	0.158	21.9	8/10/2005	8/31/2005
WR-SEG2	0.14	0.141	96.3	8/9/2005	9/1/2005
RSR-SEG1	0.05	0.097	77.5	7/12/2005	8/3/2005
RSR-SEG2	0.07	0.180	350	7/12/2005	8/4/2005
ASC-SEG1*	0.23	0.055	150	8/9/2005	8/29/2005
JC-SEG1**	0.26	0.025	65.0	7/11/2005	8/1/2005

*Test segment in Alexander Springs Creek.
**Test segment in Juniper Creek.

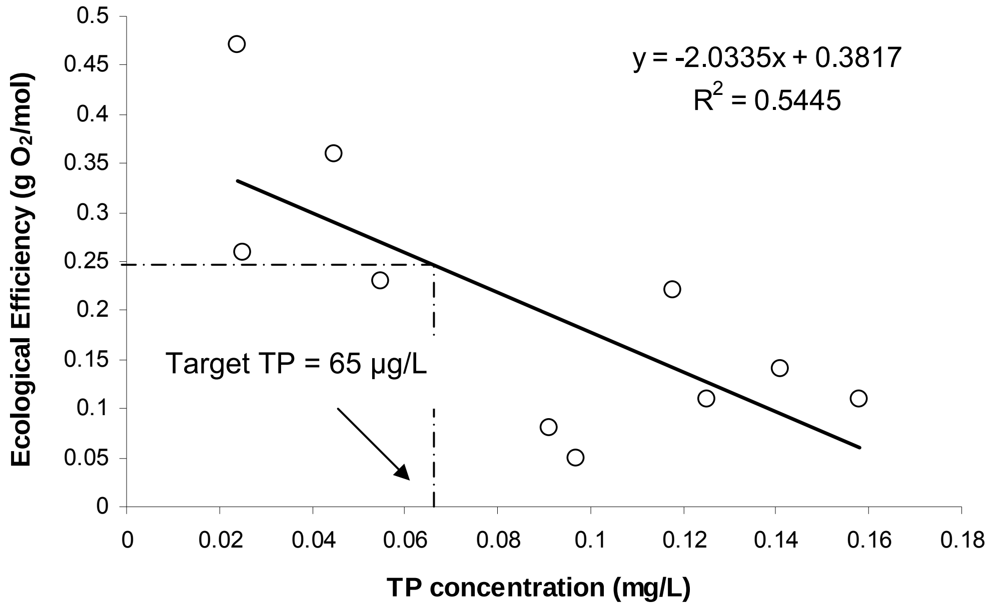


Figure 3.10. Correlation between ecological efficiency and TP concentration in the Wekiva River, Rock Springs Run, Alexander Springs Creek, and Juniper Creek.

3.2.2.3. Summary on the water quality targets

Based on above analyses, the nutrient targets for this TMDL are monthly averages of 286 µg/L for nitrate and 65 µg/L for TP. These water quality targets apply to both the Wekiva River and Rock Springs Run. These targets will protect the structure and functions of the aquatic vegetation communities and also protect the aquatic fauna based on historic SCI and BIORECON results for benthic macroinvertebrates.

Chapter 4: ASSESSMENT OF SOURCES

4.1 Types of Sources

An important part of the TMDL analysis is the identification of pollutant source categories, source subcategories, or individual sources of the pollutant of concern in the target watershed and the amount of pollutant loading contributed by each of these sources. Sources are broadly classified as either point sources or nonpoint sources. Historically, the term "point sources" has meant discharges to surface waters that typically have a continuous flow via a discernable, confined, and discrete conveyance, such as a pipe. Domestic and industrial wastewater treatment facilities (WWTFs) are examples of traditional point sources. In contrast, the term "nonpoint sources" was used to describe intermittent, rainfall-driven, diffuse sources of pollution associated with everyday human activities, including runoff from urban land uses, agriculture, silviculture, and mining; discharges from failing septic systems; and atmospheric deposition.

However, the 1987 amendments to the Clean Water Act redefined certain nonpoint sources of pollution as point sources subject to regulation under the EPA's NPDES Program. These nonpoint sources included certain urban stormwater discharges, including those from local government master drainage systems, construction sites over five acres, and a wide variety of industries (see **Appendix A** for background information on the federal and state stormwater programs).

To be consistent with Clean Water Act definitions, the term "point source" is used to describe traditional point sources (such as domestic and industrial wastewater discharges) **AND** stormwater systems requiring an NPDES stormwater permit when allocating pollutant load reductions required by a TMDL (see **Section 6.1** on **Expression and Allocation of the TMDL).** However, the methodologies used to estimate nonpoint source loads do not distinguish between NPDES and non-NPDES stormwater discharges, and as such, this source assessment section does not make any distinction between the two types of stormwater.

4.2. Potential Sources of pollutants in Watersheds of the Wekiva River and RSR

4.2.1 Point Sources

4.2.1.1 Wastewater Point Sources

There are eight wastewater facilities that are authorized to discharge to surface waters within the drainage basin of the Wekiva River. No NPDES permitted facilities were identified in the subbasin of Rock Springs Run. **Table 4.1** lists the permit numbers, names, business types, and permit types of these facilities, and **Figure 4.1** shows the locations of these facilities. Five of the facilities are concrete batch plants and operate under generic permits. These facilities may discharge wastewater with high pH, high dissolved and total suspended solids, but elevated nitrate and TP concentrations from these facilities are typically not expected. The other three are domestic wastewater facilities: the Wekiva Hunt Club WWTF (FL0036251), Altamonte Springs Regional Water Reclamation Facility (FL0033251), and SCES/Yankee Lake WRF

(FL0042625). These facilities have the potential to contribute nitrate and TP to Wekiva River and, as such, are described in more detail below, however they are not considered significant sources of nutrient loading to the Wekiva River

Table 4.1. NPDES permitted facilities identified in the drainage basin discharge to Wekiva River.

Permit Number	Facility Name	Wastewater Type	Permittee
FL0042625	SCES/Yankee Lake WRF	Domestic Wastewater	Seminole County Environmental Services
FL0033251	Altamonte Springs Regional Water Reclamation Facility	Domestic Wastewater	City of Altamonte Springs
FL0036251	Wekiva Hunt Club WWTF	Domestic Wastewater	Sanlando Utilities Corporation
FLG110301	Florida Rock Industries – Carder Road CBP	Industrial Wastewater	Florida Rock Industries
FLG110557	Rinker Materials – Lockhart Concrete Batch	Industrial Wastewater	Rinker Materials
FLG110231	Action Ready Mix CBP	Industrial Wastewater	Action Ready Mix CBP
FLG110672	CEMEX – Lockhart CBP	Industrial Wastewater	CEMEX – Lockhart CBP
FLG110464	Inland Materials – Orlando CBP	Industrial Wastewater	Inland Materials

SCES/Yankee Lake WRF is located in Sanford, close to the most downstream segment of Wekiva River (**Figure 4.1**). It has a 2.5 MGD annual average daily flow (AADF) permitted capacity, with advanced wastewater treatment facilities, including influent screening, grit removal, anoxic basin followed by aeration chamber, chemical feed, clarification, tertiary filtration, disinfection by chlorination, and aerobic digestion of residuals. Residuals of the facility are hauled to the SCES Greenwood Lakes WWTF for additional treatment.

The majority of the treated wastewater from SCES/Yankee Lake WRF is reused. The facility has a slow rate land application system with an anticipated capacity of 3.707 MGD AADF, consisting of a reclaimed water transmission/distribution system for public access irrigation of recreational areas, residential lawns, golf course, urban landscapes, road medians, nurseries and citrus groves within its Reuse Service Area. Wet-weather or reject flows are sent to three backup systems including 1) a 0.35 MGD AADF permitted capacity slow-rate restricted public access system that consists of a 72.6 acre sprayfield, 2) a 0.36 MGD AADF permitted capacity rapid infiltration basin system that consists of five rapid infiltration basins (RIBs) with a total wetted area of 800,000 square feet, and 3) a 0.75 MGD AADF permitted capacity wet-weather back-up discharge to an upland/receiving wetland system.

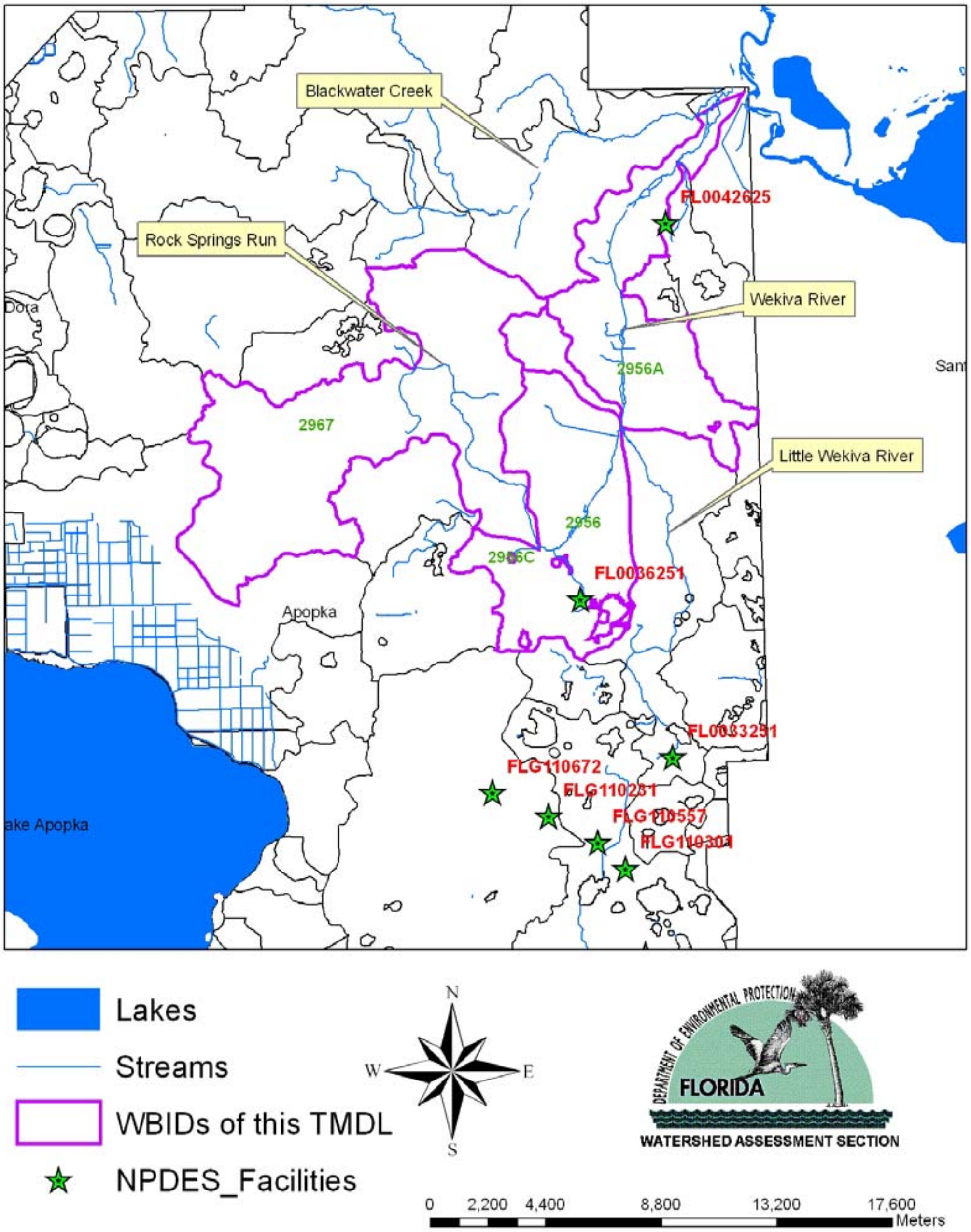


Figure 4.1. NPDES permitted facilities located in the Wekiva River drainage area.

The upland/receiving wetland system is not anticipated to discharge to other surface waters except under extreme wet-weather conditions, which may create intermittent flow through Sulfur Creek, which discharges from Yankee Lake through the receiving wetlands. This infrequent discharge may eventually reach the lower reach of the Wekiva River close to its confluence with St. Johns River. The permit issued to the facility requires the permittee to monitor the water quality in Sulfur Creek monthly when there is flow through Sulfur Creek to ensure that the wetland operation does not adversely impact the water quality of the creek. Based on the information from the Department's Central District Office, there have only been four months when there was flow in Sulfur Creek since July 2005, when the facility started to discharge to the upland/receiving wetland system. **Table 4.2** shows monitoring results in Sulfur Creek upstream and downstream of the effluent point and at the effluent point during the four months period.

Table 4.2. Results of water quality monitoring in Sulfur Creek upstream and downstream of the effluent point and at the effluent point when flow appeared in the creek.

Parameter	August 3, 2005			October 5, 2005			November 9, 2005			March 1, 2006		
	Upst	Downst	Efflu	Upst	Downst	Efflut	Upst	Downst	Efflu	Upst	Downst	Efflu
ammonia, mg/L	0.055	0.052	0.026	0.037	0.036	0.024	0.027	0.023	0.043	0.026		0.043
TKN, mg/L	1.1	0.97		1.1	1.2		1	0.93		0.81		
TN, mg/L	1.1	0.97	0.840	1.1	1.2	1.2	1	0.93	1.400	0.81		2.5
nitrite+nitrate, mg/L	0.020 U	0.020 U		0.0075 U	0.0075 U		0.0075 U	0.0075 U		0.0075 U	0.0075 U	
nitrate			0.200	0.056	0.048	0.46			0.560			1.636
TP, mg/L	0.050 U	0.050 U	0.320			0.22	0.09	0.071	0.180	0.017	0.012 U	0.15
Flow, day MGD			1.757			1.588			0.512			0.853

As shown in **Table 4.2**, in no cases for the four monitoring events were the nitrate and TP concentrations downstream of the effluent point significantly higher than those upstream of the effluent point, indicating that the discharge did not have any significant negative impact on Sulfur Creek water quality. This also indicates that the occasional backup discharge from the SCES/Yankee Lake WRF into the upland/receiving wetland system is not a significant source of nitrate or TP to the WR and RSR.

<u>The Altamonte Springs Regional Water Reclamation Facility</u> is located in the southeastern part of the Wekiva Study Area. The facility currently has a 12.5 MGD AADF permitted discharge to Little Wekiva River (**Figure 4.1**). However, the majority of the treated wastewater from the facility has been directed to reuse by the City of Altamonte Springs for purposes of irrigation within the 5,900 acre Reuse Service Area consisting of city parks, street, and highway medians, city-owned nurseries, residential and commercial lawns. Reclaimed water is also used for street cleaning, dust control, fire protection, water-to-air heat pumps, chillers (cooling water towers), and at automatic car washes. The actual discharge from the facility to the Little Wekiva River is significantly lower than the permitted 12.5 MGD (**Table 4.3**), with a long-term annual average discharge of 1.2 MGD. In addition, the facility also has a planned project that would take the excess reclaimed water to the City of Apopka for reuse and recharge, which will further decrease the discharge to the surface water. **Table 4.3** lists the long-term monthly average daily discharge rate from the facility to the Little Wekiva River, the monthly average TP

concentration of the discharge, and monthly TP loadings from the facility to surface waters. This summary was based on the discharge rate measured in 1999 through 2006, and TP concentrations in 2000 through 2002. No routine monitoring results of nitrate for the facility were available to the Department at the time this TMDL was developed.

Table 4.3. Long-term monthly average daily discharge rate, TP monthly average concentrations, and long-term TP monthly average loading for Altamonte Springs Regional Water Reclamation Facility.

Month	Monthly Average Daily Flow (MGD)	Monthly flow (million gallons/month)	TP (mg/L)	TP loading (lbs/month)
January	0.80	24.88	1.39	287.9
February	0.87	24.40	1.24	252.3
March	1.54	47.82	1.46	583.4
April	1.08	32.25	1.53	412.4
May	1.15	35.61	1.71	508.8
June	1.04	31.07	1.70	440.6
July	1.08	33.44	1.51	422.0
August	1.41	43.67	1.44	524.3
September	2.03	60.86	1.35	686.6
October	1.45	45.08	1.67	630.4
November	0.89	26.57	1.52	337.5
December	1.40	43.53	1.52	554.2
Mean	1.23	38.07	1.50	470.0

Influence of the discharge on the TP concentration of the Little Wekiva River is shown in **Figure 4.2**. TP concentrations measured at water quality stations upstream and downstream of the discharge point were analyzed. As shown in **Figure 4.2**, upstream of the WRF discharge point, the long-term average TP concentration of Lake Lotus, which is located within the Little Wekiva Canal basin and is close to the confluence of Little Wekiva Canal and the Little Wekiva River, is about 70 µg/L. This TP concentration is very close to the TP concentration measured from a water quality station (21FLORANLWD) located close to the inlet of the lake, which is about 60 µg/L. On the Little Wekiva River side, the long-term average TP concentration of Spring Lake, which is also located upstream of the WRF discharge point, is about 40 µg/L. The TP concentrations from all the stations upstream of the discharge point are significantly lower than the TP concentrations of the WRF's efflluent, which is about 1500 µg/L (**Table 4.3)**. Immediately downstream of the discharge point, the long-term average TP concentrations of the Little Wekiva River increases to about 190 µg/L (based on data from 21FLSEM WET, **Figure 4.2**), indicating a significant impact of the discharge on the river TP concentration. This high TP concentration, plus high TP concentrations in the discharges from several springs (**Table 4.11** of this report), including Starbuck Springs (a second magnitude spring, with a long-term average discharge 14.3 cfs, and TP concentration 160 µg/L), Sanlando Springs (a second magnitude spring, with a long-term average discharge 19.6 cfs, and TP concentration 180 µg/L), and Palm Springs (a third magnitude spring, with a long-term average discharge 6.88 cfs, and TP concentration 120 µg/L), result in a long-term average TP concentration of 170 µg/L measured from a station (21FLSJWMLW-WUR) located at the outlet of the Little Wekiva River into the main stem of the Wekiva River, indicating the influence of the WRF discharge on the TP concentration of the main stem of the Wekiva River.

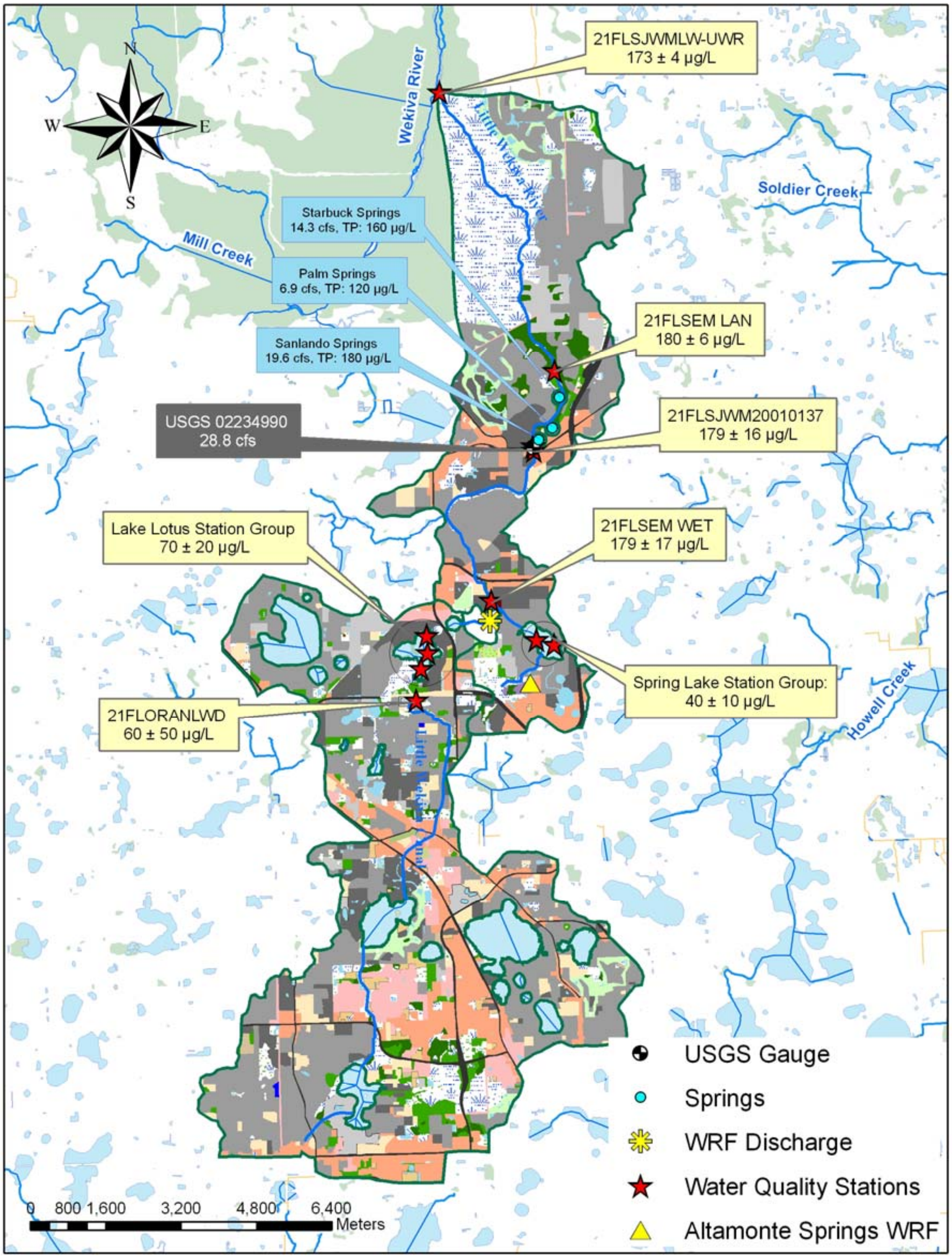


Figure 4.2. Influence of the Altamonte Springs Regional WRF on the spatial distribution of TP concentration in the Little Wekiva River.

Subsequent removal of TP discharged from the WRF during stream transport may not be significant. As was shown in **Figure 4.2**, from the station immediately downstream of the effluent point (21FLSEM WET) to the outlet of the Little Wekiva River to the Wekiva River main stem, the TP concentration did not change significantly. It appears that, to achieve the TP target of the Wekiva River main stem, TP loading from the WRF needs to be reduced significantly.

Wekiva Hunt Club WWTF is located about 1.3 miles east of the confluence of Rock Springs Run and the Wekiva River. The facility has a 2.9 mgd AADF permit to Sweetwater Creek, which in turn discharges to Cove Lake in the northwest, and then to the Wekiva River about 0.4 mile downstream of the Rock Springs Run and the Wekiva River confluence. The facility currently does not have effluent limits for either TN or nitrate. The TP effluent limits for the facility are 0.4 mg/L monthly average and 0.5 mg/L for any single sample.

Long-term monthly average nitrate and TP concentrations of the effluents from the facility were analyzed based on effluent data for the period of 1999 through 2006 retrieved from the Permit Compliance System (PCS) database and tabulated in **Table 4.4**. Compared to the annual average nitrate target and the monthly average TP target established for the Wekiva River and Rock Springs Run, discharge concentrations from the facility are considered very high. **Table 4.4 also** lists the long-term monthly average nitrate and TP loadings from the facility.

While the mean nutrient loads discharged are fairly significant, the discharge nitrate and TP concentrations can be significantly attenuated in the process of transport through the Sweetwater Creek – Cove Lake system before it reaches the Wekiva River. Based on information from the Department's Central District Office (Chris Ferraro, personal communication), the SJRWMD has been working on restoration projects to clean up Cove Lake. The SJRWMD funded a project where a deep area south of Cove Lake and Wekiva Springs Road was dredged deeper to create a sedimentation basin to allow eroded materials from Sweetwater Creek to settle out before the flow reaches Cove Lake. The project may further attenuate the nitrate and TP concentrations of the discharge from Wekiva Hunt Club WWTF.

Table 4.4. Long-term monthly average nitrate and TP concentrations and loadings for the effluent discharge from Wekiva Hunt Club WWTF in the period from 1999 through 2006.

Month	Average monthly flow (million Gallon/month)	Nitrate concentration (mg/L)	TP concentration (mg/L)	Nitrate loading (lb/month)	TP loading (lb/month)
January	51	10.43	0.16	4445	68
February	46	10.67	0.21	4102	81
March	41	10.12	0.19	3467	65
April	39	12.12	0.19	3950	62
May	38	9.46	0.19	3004	60
June	39	11.35	0.24	3699	78
July	47	8.88	0.12	3488	47
August	48	8.45	0.19	3390	76
September	55	7.96	0.24	3659	110
October	47	9.74	0.20	3826	79

November	42	9.85	0.18	3457	63
December	52	9.12	0.17	3963	74
Mean	45	9.85	0.19	3704	72

To analyze the possible attenuation rates for nitrate and TP along the path from the facility discharge point to the WR, the Department examined the water quality data collected from several monitoring sites along the transport path. These sites include:

- Site #1: Background Sweetwater Creek, upstream of the outfall to Sweetwater Creek;
- Site #2: 2400 feet downstream of the outfall to Sweetwater Creek, and about 200 feet upstream of the southernmost tributary that enters from the creek from the east;
- Site #3: 1000 feet upstream of the Wekiva Springs Road bridge;
- Site #4: Downstream side of the Wekiva Springs Road bridge, 200 feet downstream of the northernmost tributary that enters from the west;
- Site #5: North end of Cove lake at the discharge culvert to Wekiva Marina;
- Site #6: Weir structure located at River Bend Road;
- Site #7: Miami Springs Road bridge, upstream of confluence with Sweetwater Creek;
- Site #8: Wekiva River, downstream of Sweetwater Creek discharge.

Figure 4.3 shows the locations of these water quality sites. **Tables 4.5** and **4.6** show measured nitrate and TP concentrations at each sampling site, respectively, during the period from January, 2005 through April, 2006. Nitrate and TP concentrations of the effluent discharge are also included in **Tables 4.5** and **4.6**. **Figures 4.4-A** and **-B** show the long-term average spatial trend of nitrate and TP concentrations at these sampling sites.

Table 4.5. Nitrate concentrations at different sampling sites along the Sweetwater Creek – Cove Lake system

Unit: mg/L

Sites	1/05	2/05	3/05	5/05	6/05	7/05	8/05	9/05	10/05	11/05	1/06	2/06	4/06	mean
Site #1	1.53	0.358	0.247	0.329	0.291	1.13	0.432	0.245	0.199	0.424	0.254	0.361	0.289	0.468
Effluent	11.00	7.050	4.740	6.600	9.050	4.300	7.800	8.700	8.600	6.350	9.060	10.080	N/A	7.778
Site #2	1.19	0.687	0.51	0.577	0.401	1.64	0.349	0.338	0.23	0.28	4.13	0.424	0.345	0.854
Site #3	1.05	0.815	0.459	0.589	0.202	1.23	0.374	0.35	0.201	0.286	4.04	0.424	0.389	0.801
Site #4	1.04	0.816	0.476	0.563	0.227	1.23	0.373	0.367	0.155	0.26	3.89	0.407	0.119	0.763
Site #5	1.04	1.3	0.327	0.243	0.019	0.385	0.019	0.402	0.178	0.321	0.574	1.74	0.000	0.504
Site #6	0.229	0.606	0.045	0.042	0.019	0.554	0.022	0.22	0.28	0.335	0.742	1.05	0.000	0.319
Site #7	0.641	0.701	0.504	0.569	0.384	0.273	0.838	0.741	0.705	0.398	0.841	0.739	0.881	0.632
Site #8	0.711	0.696	0.509	0.586	0.457	0.273	0.773	0.714	0.686	0.521	0.834	0.76	0.866	0.645

Table 4.6. TP concentrations at different sampling sites along the Sweetwater Creek – Cove Lake system

Unit: mg/L

Sites	1/05	2/05	3/05	5/05	6/05	7/05	8/05	9/05	10/05	11/05	1/06	2/06	4/06	mean
Site #1	0.224	0.096	0.093	0.148	0.22	0.28	0.229	0.208	0.136	0.214	0.064	0.09	0.05	0.158
Effluent	0.097	0.240	0.127	0.220	0.840	0.190	0.380	0.670	0.390	0.210	0.110	0.112	N/A	0.299
Site #2	0.196	0.134	0.134	0.115	0.26	0.202	0.279	0.111	0.122	0.119	0.128	0.124	0.102	0.156
Site #3	0.311	0.117	0.107	0.114	0.147	0.25	0.237	0.155	0.095	0.0988	0.134	0.096	0.12	0.152
Site #4	0.192	0.103	0.11	0.108	0.157	0.94	N/A	0.157	0.0841	0.102	0.148	0.092	0.116	0.192
Site #5	0.192	0.034	0.126	0.087	0.103	0.222	0.156	0.188	0.0686	0.133	0.106	0.062	0.174	0.127

Site #6	0.308	0.069	0.105	0.109	0.115	0.864	0.0686	0.127	0.0719	0.119	0.084	0.136	0.174	0.181
Site #7	0.199	0.065	0.085	0.085	0.074	0.22	0.084	0.02	0.0586	0.108	0.09	0.116	0.112	0.101
Site #8	0.09	0.017	0.098	0.089	0.144	0.22	0.0706	0.02	0.0592	0.114	0.094	0.118	0.104	0.095

As shown in **Table 4.5**, with the exception of one sampling event in January of 2006, nitrate concentrations dramatically decrease by the time the effluent reaches Site #2, which is about 2,400 feet downstream of the effluent point. A similar trend was also observed for TP (**Table 4.6**). In most cases, when the effluent TP concentration was significantly higher than the background condition measured at Site #1, a significant decrease in TP concentration was observed when the discharge reached Site #2. Nitrate concentration in the stream decreased more rapidly than TP, suggesting that nitrate is quickly assimilated by the Sweetwater Creek system, instead of being merely diluted by the flow in the creek.

These downstream trends are readily seen in **Figures 4.4-A** and **–B.** On the long-term average basis, nitrate concentrations decrease from 7.78 mg/L at the effluent point to about 0.319 mg/L at Site #5, while TP concentrations decrease from 0.299 mg/L to 0.127 mg/L at Site #5 (Site #5 is located at the outlet of Cove Lake and close to the discharge point of Sweekwater Creek into WRMS). The decreases in concentration between the effluent point and the outlet of Cove Lake represent attenuation rates of 96% for nitrate and 39% for TP. These attenuation rates will be used in a later chapter to estimate the wasteload allocation for the facility.

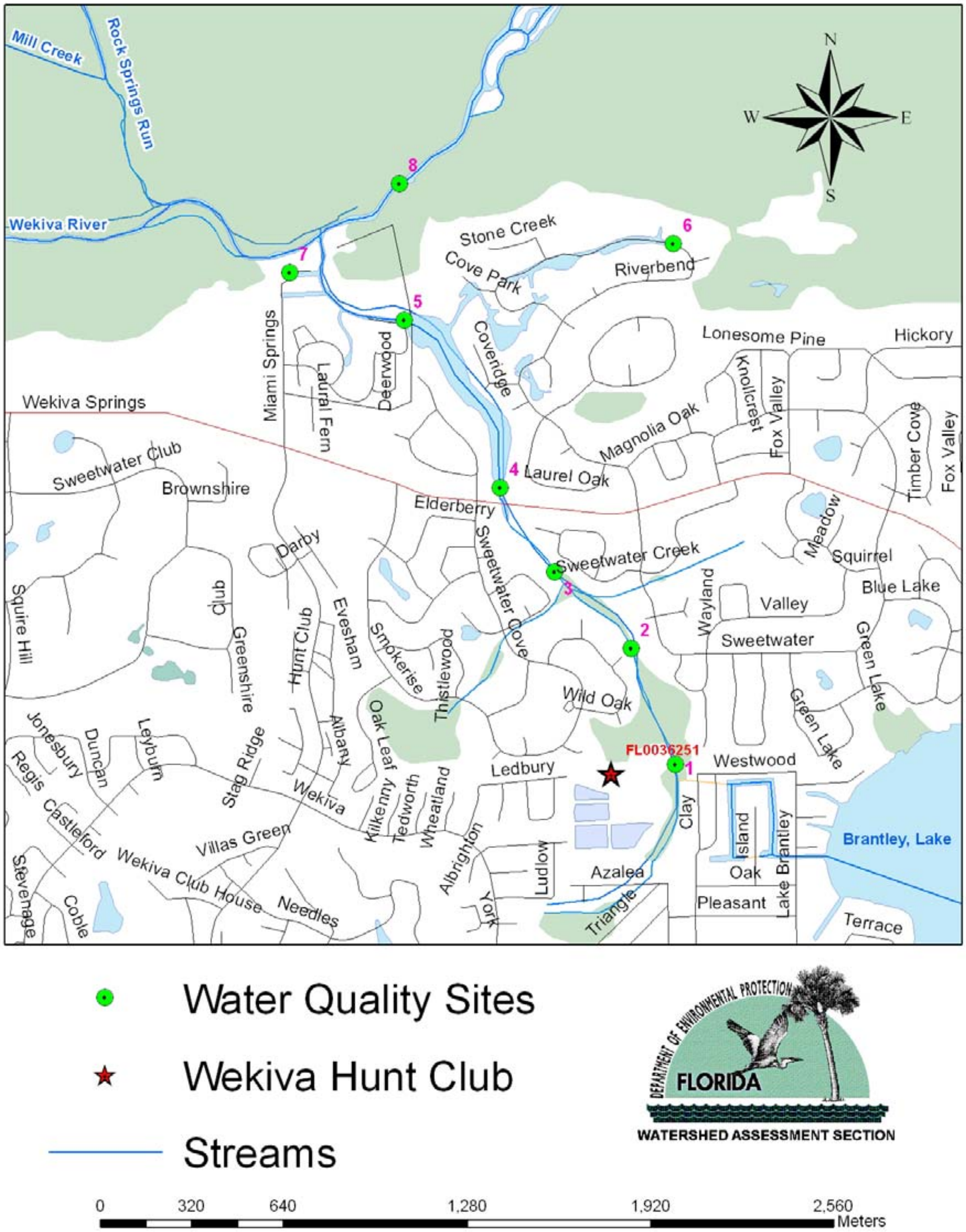
4.2.1.2 Municipal Separate Storm Sewer System Permittees

Within the drainage basin of the Wekiva River System, Orange County has a Phase I MS4 permit (FLS000011). The Florida Department of Transportation (FDOT) District 5 and City of Maitland are co-permittees for this permit. Seminole County also holds a Phase I MS4 permit (FLS000038) with FDOT District 5, City of Altamonte Springs, and City of Lake Mary being co-permittees for this permit. In addition, the City of Orlando holds a Phase I MS4 permit (FLS000014). Lake County does not have MS4 permit within the boundary of the drainage basin.

4.2.2 Nonpoint Sources

Additional nitrate and TP loadings to the Wekiva River system are primarily generated from nonpoint sources in the drainage basin. Major nonpoint sources may include, but are not limited to, loadings from surface runoff and ground water input from the surficial aquifer as stream seepage, as well as spring flows from the Floridan aquifer.

In this analysis, nitrate and TP loadings from the drainage basin to the Wekiva River system were estimated based on annual average rainfall, the area of different landuse categories summarized based on the SJRWMD's year 2000 landuse GIS coverage (scale 1:40,000), and runoff coefficients and event mean concentrations (EMCs) of nitrate and TP for different landuses (Harper, 2003). The nitrate and TP loadings estimated using this method reflect the potential amount of each pollutant that can be generated from different landuses in the drainage basin. These loading estimates did not take into consideration the attenuation during the pollutant transport across the drainage basin. The loading estimates therefore can be higher than the pollutant loadings that eventually reach the Wekiva River systems.


Mill Creek
Rock Springs Run
Wekiva River
Stone Creek
Cove Park
Riverbend
Lonesome Pine
Hickory
Miami Springs
Laurel Fern
Deerwood
Coveridge
Wekiva Springs
Magnolia Oak
Knollcrest
Fox Valley
Timber Cove
Sweetwater Club
Brownshire
Laurel Oak
Elderberry
Darby
Sweetwater Creek
Meadow
Squirrel
Blue Lake
Squire Hill
Club
Greenshire
Hunt Club
Evesham
Smokerise
Thistlewood
Sweetwater Cove
Wayland
Valley
Sweetwater
Green Lake
Wild Oak
Jonesbury
Duncan
Leyburn
Stag Ridge
Albany
Oak Leaf
FL0036251
Westwood
Regis
Castleford
Wekiva
Kilkenny
Tedworth
Wheatland
Ledbury
Clay
Island
Brantley
Brantley, Lake
Villas Green
Wekiva Club House
Albrighton
Ludlow
Azalea
Oak
Lake Brantley
Stevenage
Coble
Needles
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Water Quality Sites
Wekiva Hunt Club
Streams
DEPARTMENT OF ENVIRONMENTAL PROTECTION
FLORIDA
WATERSHED ASSESSMENT SECTION
0
320
640
1,280
1,920
2,560
Meters

Fi **ke**

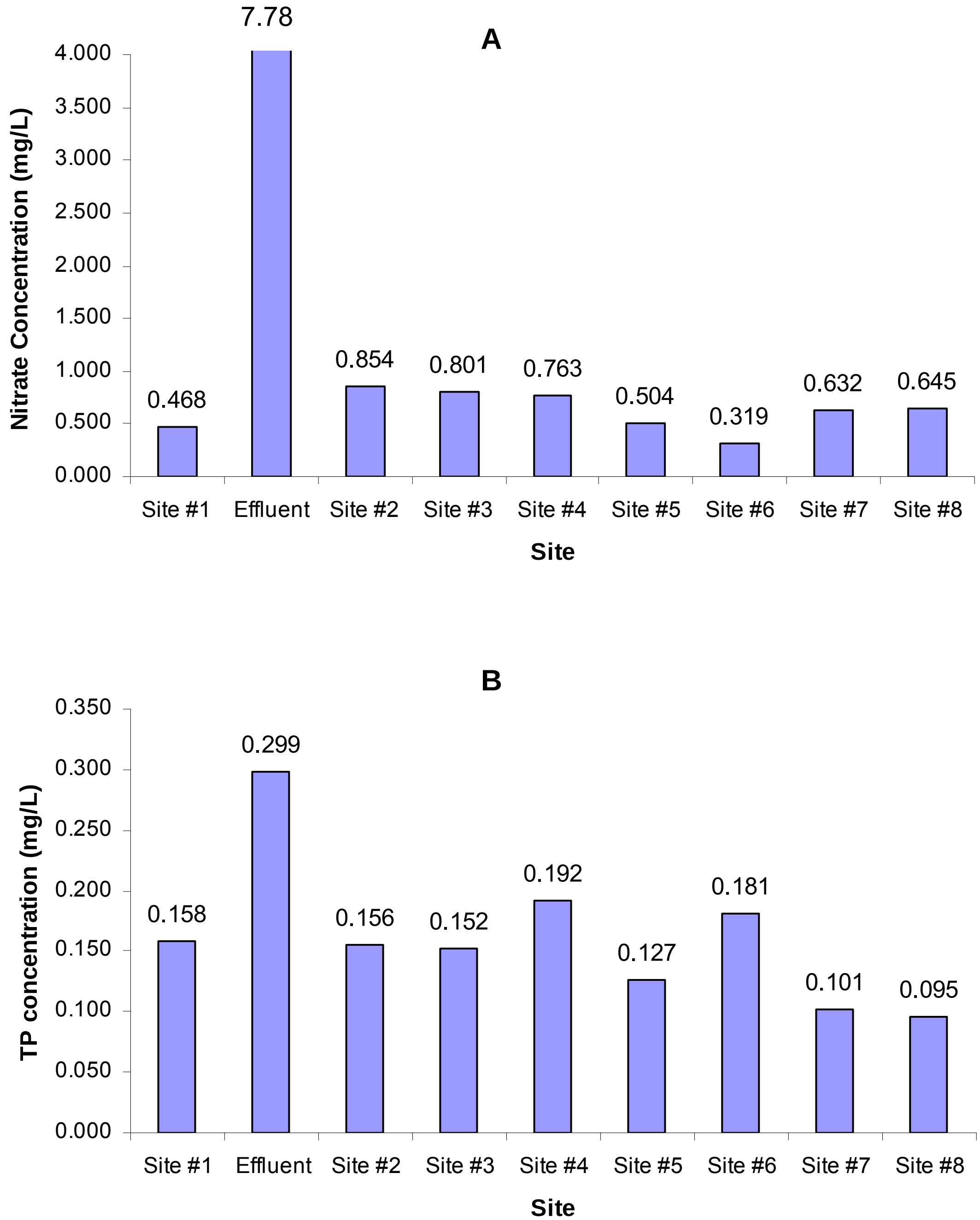

Figure 4.4. Long-term spatial trend of nitrate and TP concentration at different sampling sites along the Sweetwater Creek – Cove Lake system.

4.2.2.1 Land Uses

Based on the drainage basin delineation provided by the SJRWMD, the Wekiva River system drains a basin area of about 240,948 acres. **Figure 4.5** shows the boundary of the drainage basin and the landuse categories (SJRWMD year 2000 landuse GIS coverage) aggregated based on the Florida Landuse Classification Code System (FLUCCS) level one landuse classification. **Table 4.7** summarizes the landuse area from different landuse areas. According to **Table 4.7**, urban and built-up area occupies about 69,374 acres, which accounts for about 28.8% of the total drainage basin and ranks first among all the individual landuse categories for percent landuse acreage. About 32,464 acres of the basin land are used for various agricultural practices. Percent acreage for agricultural landuse is about 13.5%. Natural landuse, including rangeland, upland forest, water, and wetlands, when combined, occupy about 135,904 acres, which accounts for about 56.4% of the total area of the drainage basin. The remaining about 1.0% of the basin landuse is comprised of transportation, communications, and utilities.

Table 4.7. Acreages and percent acreages of different landuse categories for the Wekiva River system draingage basin

Landuse FLUCCS Code	Landuse Description	Acreages	Percent Acreages
1000	Urban and Built-Up	69,374	28.8%
2000	Agriculture	32,464	13.5%
3000	Rangeland	17,362	7.2%
4000	Upland Forest	55,867	23.2%
5000	Water	10,355	4.3%
6000	Wetlands	52,320	21.7%
7000	Barren Land	901	0.4%
8000	Transportation, Communications, Utilities.	2,305	1.0%
Total		240,948	100.0%

For loading estimation purposes, level 1 landuse categories were further divided into the sub-categories listed in **Table 4.8**. In addition, **Table 4.8** listed the acreage, runoff coefficients, nitrate and TP EMCs, and nitrate and TP loadings from these different landuses. A long-term annual average rainfall of 51 inches was used for estimating the long-term annual average pollutant loading. This number was calculated based on the rainfall data collected from a weather station located in Sanford. The period of record is from 1957 through 2002. **Table 4.9** lists the percent nitrate and TP loadings from different landuse categories.

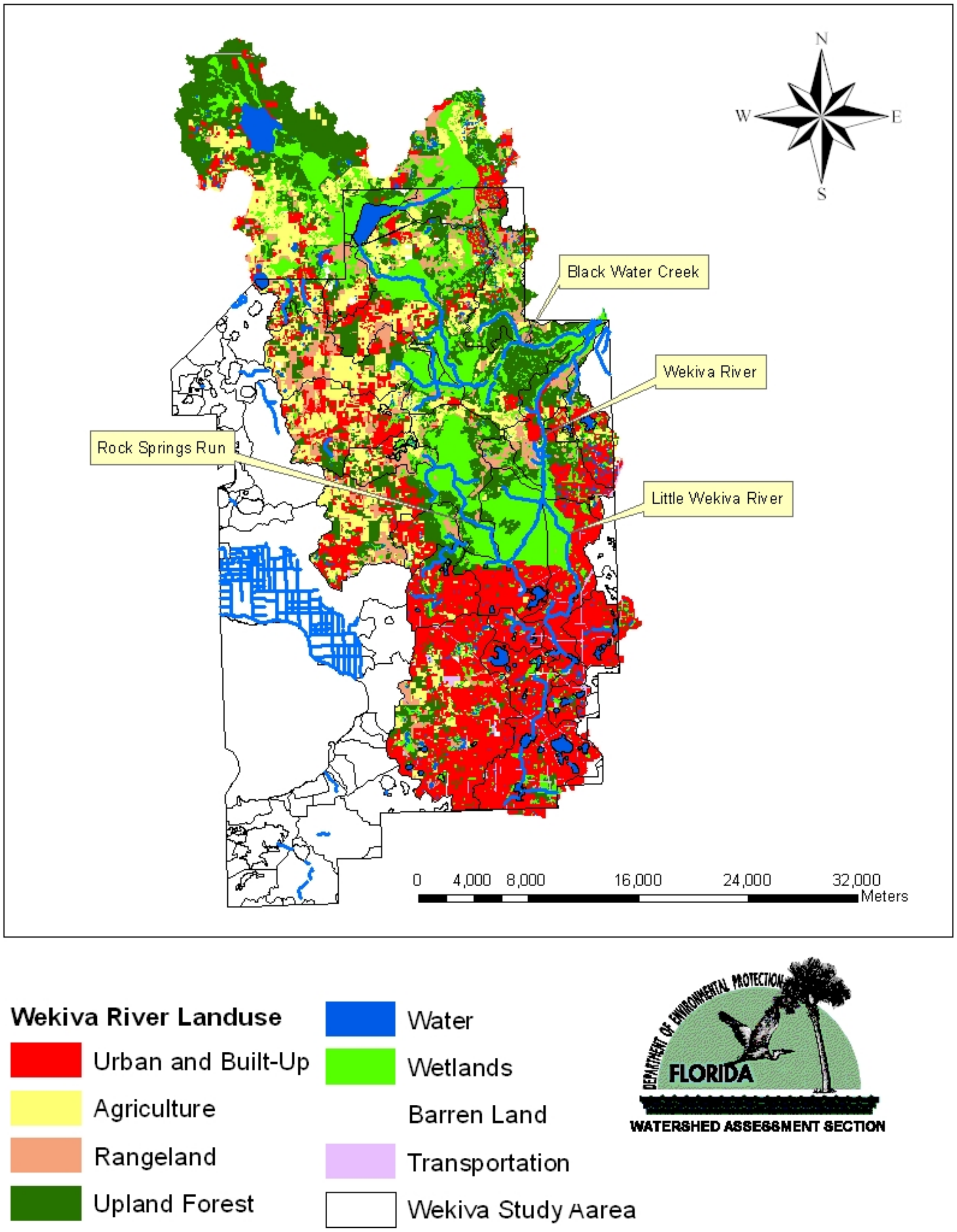

Figure 4.5.   Principal land uses in the drainage basin of Wekiva River.

Table 4.8. Classification of land use categories in the Wekiva River watershed

Laduse	Acreage	Runoff Coefficie.	Annual Runoff (ac-ft/y.)	Monthly Runoff (ac-ft/m)	Event mean concentration (mg/L)		Nitrate loading		TP loading	
					nitrate	TP	Annual (lb/yr.)	Month (lbs/m.)	Annual (lb/yr.)	Monthly (lbs/m.)
Agriculture/ Golf Course	34615	0.3	44134	3678	0.58	0.34	69622	5802	41293	3441
Forest and Rural Open	55867	0.08	18995	1583	0.31	0.05	16015	1335	2583	215
Rangeland	17362	0.12	8855	738	0.4	0.34	9633	803	8188	682
Low-Density Residential	17668	0.27	20274	1690	0.63	0.3	34739	2895	16543	1379
Medium-Density Residential	29098	0.37	45757	3813	0.65	0.4	80893	6741	49780	4148
High-Density Residential	6551	0.68	18932	1578	0.67	0.49	34500	2875	25231	2103
Commercial	5376	0.85	19421	1618	0.67	0.29	35390	2949	15318	1277
Institutional	1532	0.84	5469	456	1.05	0.15	15619	1302	2231	186
Industrial/Utility	2499	0.79	8390	699	0.4	0.31	9128	761	7074	590
Transportation Facilities	2305	0.78	7641	637	0.4	0.34	8313	693	7066	589
Openland	5399	0.16	3671	306	0.31	0.05	3095	258	499	42
Water	10355	0.5	22004	1834	0.19	0.11	11371	948	6583	549
Wetlands	52320	0.23	51143	4262	0.4	0.19	55640	4637	26429	2202
Total	240948		274686	22891			383959	31997	208819	17402

*: Runoff coefficients were cited from Harper (2003).
**: EMCs were provided by the CDM.

Table 4.9. Percent pollutant contribution from different landuse categories

Landuse	Nitrate	TP
Agriculture/Golf Course	18.1%	19.8%
Forest and Rural Open	4.2%	1.2%
Rangeland	2.5%	3.9%
Low-Density Residential	9.0%	7.9%
Medium-Density Residential	21.1%	23.8%
High-Density Residential	9.0%	12.1%
Commercial	9.2%	7.3%
Institutional	4.1%	1.1%
Industrial/Utility	2.4%	3.4%
Transportation Facilities	2.2%	3.4%
Openland	0.8%	0.2%
Water	3.0%	3.2%
Wetlands	14.5%	12.7%
Total	100.0%	100.0%

As shown in **Table 4.9**, the most important nitrate and TP contributors in the drainage basin are urban landuses, which include low, medium, and high density residential, commercial, institutional, industrial/utility, transportation facilities, and other urban open lands. These landuses contribute about 218,583 lbs/year (18,215 lbs/month) and 123,244 lb/year (10270 lbs/month) of nitrate and TP, which account for about 57% and 59% of the total nitrate and TP from the entire drainage basin, respectively. In addition, agricultural landuses contribute about 18% of nitrate and 20% of TP, and wetlands contribute about 15% of the nitrate and 13% of the TP. Total human landuses contribute about 78% nitrate and 83% of TP, while natural landuses, including forest, waters, and wetlands, contribute about 22% of the nitrate and 17% of the TP through surface runoff.

Another possible nonpoint source of nitrate and TP to receiving waters is septic tank discharge. While properly installed and maintained septic tanks can remove phosphorus relatively effectively, significant amounts of nitrate can still be released into the ground water or surface runoff due to the low adsorption rate of nitrate by soil particles.

A GIS shapefile showing locations of septic tank-using land parcels within the boundary of the Wekiva River drainage basin was provided by the CDM (**Figure 4.6**). However, no data on the number of septic tanks located in the drainage basin were available to the Department at the time this TMDL was developed. To estimate the pollutant loading from septic tanks, the number of septic tanks in the basin is required. This number was estimated based on the following information:

(1) The total number of septic tanks located in the Lake County part of the Wekiva study Area was provided by the CDM, Inc., which is 9,286 septic tanks.
(2) The total acreage of the septic tank-using land parcels in Lake County part of the Wekiva Study Area was provided by the CDM, Inc. The total acreage is 20,599 acres.
(3) The number of septic tanks per acre of septic tank-using parcel were estimated as the quotient between the total number of septic tanks in (1) and the total acreage in (2), and is 0.45 septic tanks/per acre
(4) Assuming that septic tanks that contribute significant quantities of nutrients to receiving waters through surface runoff are typically located within 200 meters of receiving waters (Reckhow, 1980), the total acreage of septic tank-using land parcels in the Wekiva River drainage basin that could contribute significant quantities of nutrients to receiving waters through surface runoff is 458 acres.
(5) Multiplying 458 acres in (4) by the 0.45 septic tank/per acre in (3), the total number of septic tanks in the Wekiva River drainage basin that can contribute significant quantities of nutrients to receiving waters through surface runoff is 206 septic tanks.

The nitrate and TP loadings from septic tanks to the Wekiva River system were estimated using the following equation:

$$W = F * N * C * (1-R)$$

Where:

W is the total loading from septic tanks.
F is the number of people per household.
N is the number of septic tank-using households.
C is the per capita pollutant loading.
R is the pollutant removal efficiency.

Based on *Summary Population and Housing Characteristics in Florida* published by the United States Census Bureau in 2000 (http://www.census.gov/prod/cen2000/phc-1-11.pdf), the number

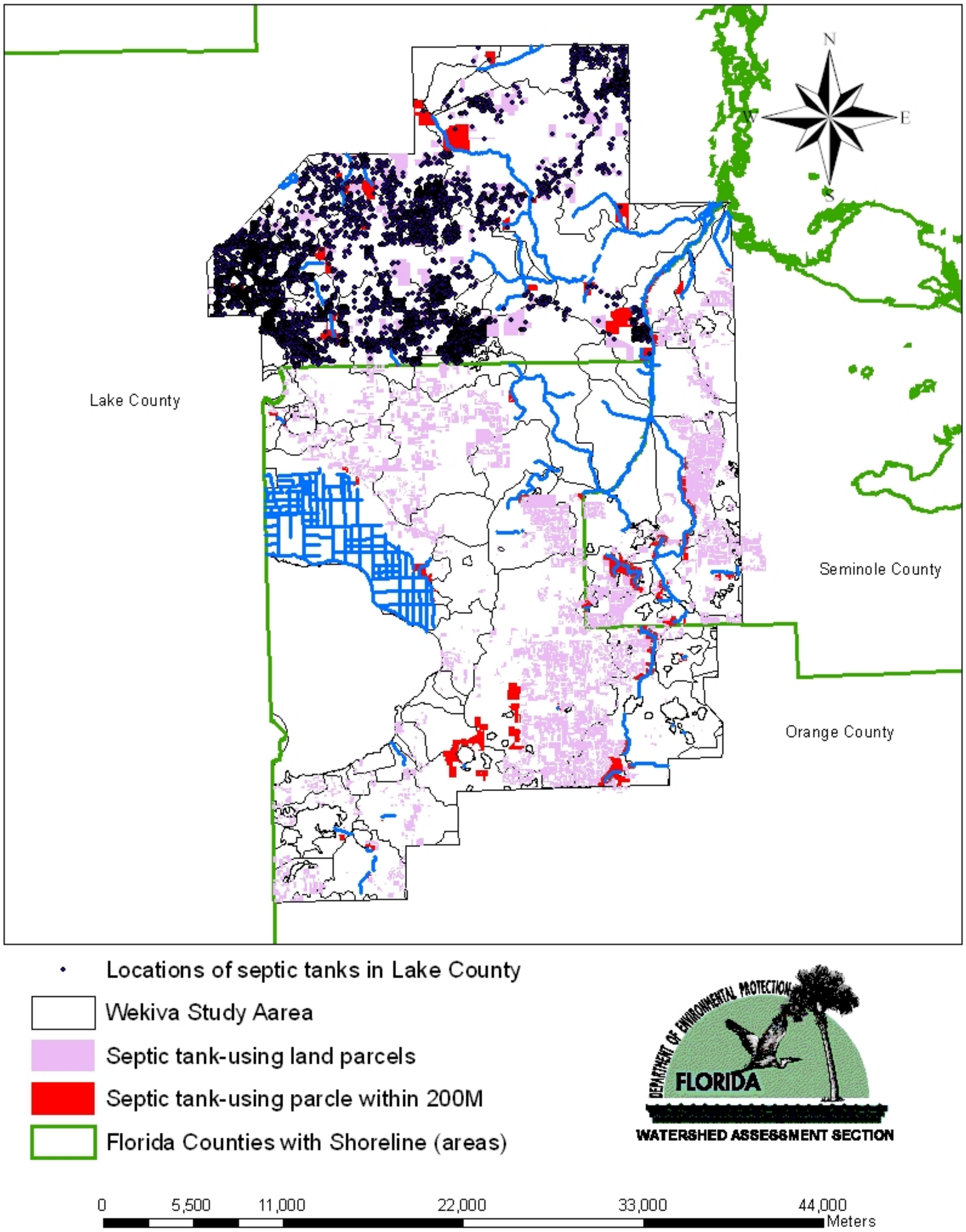

Figure 4.6. Location of septic tank-using land parcels in the Wekiva Study Area.

of people per household in Lake, Orange, and Seminole Counties are 2.34, 2.61, and 2.59, respectively. The average household size (F) for these three counties is 2.51.

The number of septic tank-using households within 200 meters to receiving waters could be considered the same as the number of septic tanks in the same area, assuming that each household uses one septic tank.

Per capita TP loading (**Table 4.10**) for raw sewage was cited from Reckhow (1980). It was a typical value calculated as the mean TP loading from various studies. Nitrate concentration in raw sewage is typically very low. Most of the nitrate appears in the drainage field of conventional septic tanks when organic nitrogen is mineralized through ammonification and ammonia is oxidized through nitrification. Typically, the physical and biochemical processes happening in septic tanks and drainage fields, including precipitation in the septic tank and denitrification in the drainage filed, can remove about 40 to 45% of the total nitrogen (Burton, 1991), and the remaining part of TN was assumed to be in the form of nitrate in this TMDL.

Pollutant removal efficiency (R) is the percent at which TP and nitrate is removed by the septic tank system (including the tank and the soil). In this case, it is assumed that within 200 meters, 70% of TP and 45% of the TN will be removed. The remaining 55% of TN will become nitrate and, together with the remaining 30% of TP, enter surface runoff or ground water.

Based on above information, the TP and nitrate loadings from septic tanks in the Wekvia River drainage basin that are within 200 meters to receiving water are about 499 lbs/year and 2,890 lbs/year, respectively. Compared to nitrate and TP loadings from various landuse categories, loadings from septic tank through surface runoff are relatively small.

Table 4.10. Nutrient load for household wastewater discharged into septic tanks (kilograms/capita/year) (cited from Reckhow, 1980)

TP	TN	Reference
1.49	6.45	Ligman et al., 1974
1.43	5.99	Laak, 1975
N/A	2.65	Bennet and Linstedt, 1975
0.74	4.61	Chan, 1978
1.59	N/A	Ellis and Childs, 1973
1.49	2.15	Siegrist et al., 1976
3	N/A	Bernhard, 1975
0.8	N/A	Otis et al., 1975
N/A	8.2	Walker et al., 1973
1.28	3.2	EPA, 1974
1.46	**4.61**	**Median**

N/A = Not available

The low nitrate and TP loadings from septic tanks estimated using the above method are mainly loadings through surface runoff, which is why a 200 meter distance limit was applied. However, septic tank pollutant loadings can also contribute to the surface water pollution via a ground water pathway, even for those septic tanks located beyond 200 meter distance limit. This is especially true for the Wekiva River drainage basin because this area is underlaid by a Karst geology characterized by limestone or dolostone bedrock with caves and springs. A study conducted by the Florida Department of Health indicated that using the conventional onsite sewage treatment and disposal system in Karst areas could produce nitrate concentrations as high as 60 mg/L in the groundwater adjacent to the drainage field (DOH 2004). The nitrate can be carried into surface waters through either baseflow, or more importantly in the Wekiva River system, through springs discharge.

At the time this TMDL was developed, not enough information was available for the Department to determinethe septic tank pollutant loading through ground water. Therefore, nitrate and TP loadings through spring discharges were estimated. Nitrate and TP loadings through spring discharges can include contribution form septic tanks, other human activities such as agricultural practices, and natural background.

There are about 30 springs located in the Wekiva River drainage area. These springs either discharge directly to the main stem of the Wekiva River or discharge to major tributaries, including RSR, Blackwater Creek (BWC), and Little Wekiva River (LWR), which in turn discharge into the WR. **Figure 4.6** shows the locations of these springs. **Table 4.11** shows the magnitude, discharge rate, and nitrate and TP concentrations of these springs. **Table 4.12** shows the nitrate and TP loadings and percent nitrate and TP loadings from these springs.

Among the 30 springs located in the Wekvia River drainage basin, Rock Springs and Wekiva Springs are the two largest springs. Both of them discharge at more than 50 cfs, while the majority of the other springs discharge at less than 10 cfs. Seminole Springs discharges at a rate of 35.2 cfs, ranking the third in discharge rate. This spring is located in Lake County and discharges into Blackwater Creek. Several other springs, including Messant Spring in Lake County (discharging to Blackwater Creek), Starbuck Springs located in Seminole County (discharging to Blackwater Creek), and Sanlando Springs located in Seminole County (discharging to Little Wekiva River) discharge at a long-term average rate of more than 10 cfs.

According to **Table 4.12**, the total nitrate and TP loading from all the 30 springs are about 511,433 lbs/year and 78,952 lbs/year, respectively. The total nitrate loading from springs is higher than the nitrate loading from surface runoff, which is about 383,959 lbs/year (**Table 4.8**). TP loading from springs are also significant compared to the TP loading from surface runoff, which is about 197,506 lbs/year. These numbers indicate that springs are a very important source of nitrate and TP in the Wekiva River system.

Among all the springs, Rock Springs and Wekiva Springs are the largest nitrate contributors. Combined nitrate loadings from these two springs account for about 70% of the spring nitrate. In addition, these two springs contribute about 59% of the spring TP, indicating that efforts to control nitrate and TP should be put on the recharge areas of these two springs. Other than these two springs, Seminole Spring contributes about 19% of the spring nitrate and 7% of spring TP. Sanlando Spring and Starbuck Springs contribute about 4.5% and 2.1 % of the spring nitrate and 8.8% and 5.7% of the spring TP, respectively. Considering the high nitrate and high TP concentrations observed in these springs, studies on the nitrate and TP loadings in recharge areas of these springs should also be stressed.

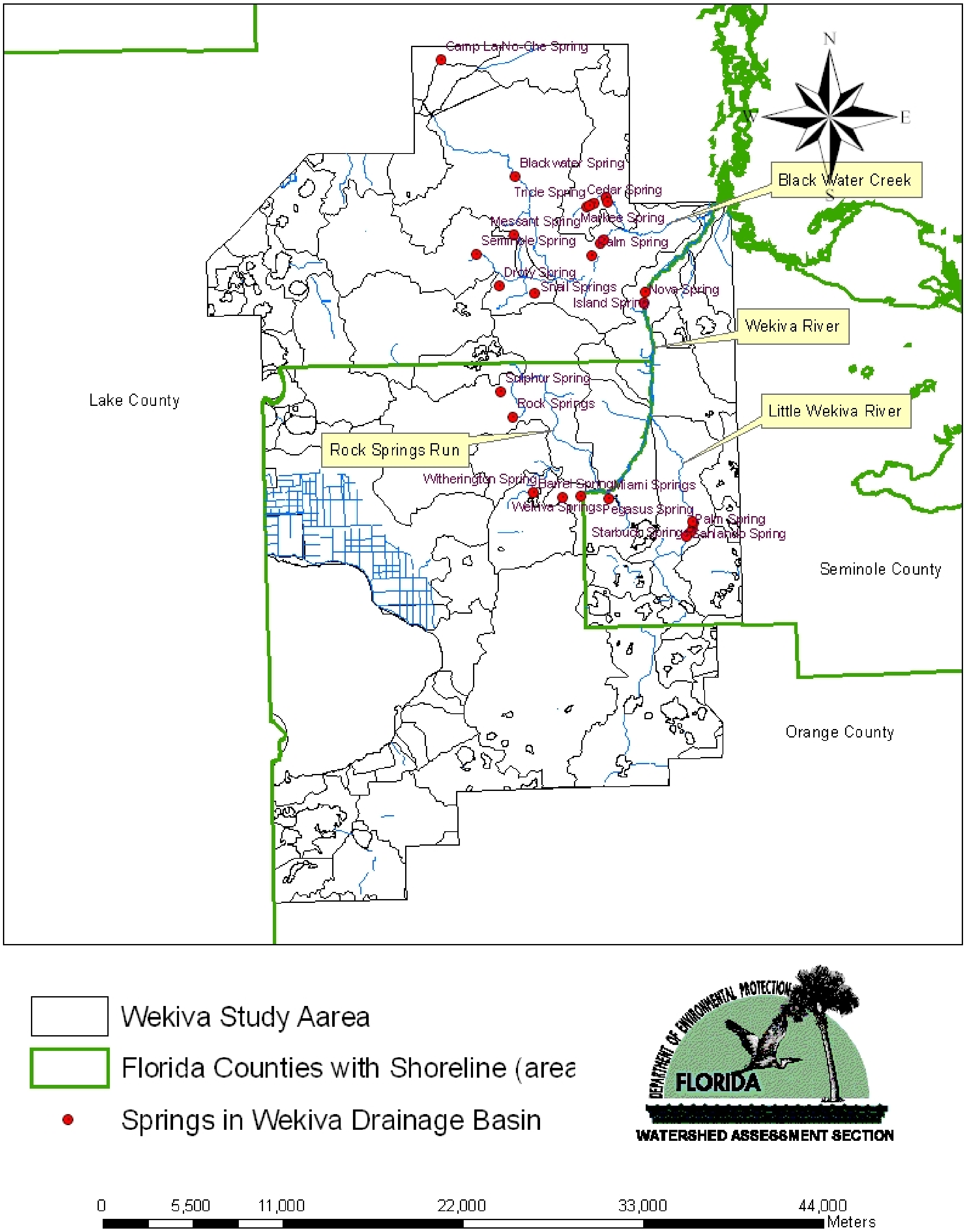

Figure 4.7. Locations of springs in the Wekiva River drainage area.

Table 4.11. Characteristics of springs located in the Wekiva River drainage basin.

Spring Name	Spring Magnitude	Discharge (cfs)	NO3/NO2 (mg/L)	TP (mg/L)
Lake County				
Blackwater Spring	3rd	1.4	0.92	N/A
Blue Algae Boil	5th	0.14	0.03	0.05
Blueberry Spring	5th	0.07	0.03	N/A
Boulder Spring	5th	0.19	0.08	0.18
Camp La-No-Che Spring	4th	0.7	N/A	0.06
Cedar Spring	5th	0.03	0.03	0.05
Droty Spring	4th	0.62	0.03	0.08
Green Algae Boil	5th	0.14	N/A	0.06
Markee Spring	4th	0.25	0.02	0.06
Messant Spring	2nd	14.7	0.01	0.04
Mocassin Spring	4th	0.29	0.01	N/A
Palm Spring	4th	0.63	0.01	0.02
Seminole Spring	2nd	35.2	1.37	0.08
Sharks Tooth Spring	5th	0.15	0.09	0.05
Snail Springs	5th	0.09	0.02	0.15
Tricle Spring	N/A	N/A	N/A	N/A
Orange County				
Barrel Spring	4th	0.25	0.05	N/A
Rock Springs	2nd	57.9	1.5	0.08
Sulphur Spring	4th	0.74	0.02	0.03
Tram Springs	N/A	N/A	N/A	N/A
Wekiva Springs	2nd	67.1	1.43	0.28
Witherington Spring	3rd	4.7	0.38	N/A
Seminole County				
Gingar Ale Springs	5th	0.11	N/A	0.04
Island Spring	3rd	7.83	0.01	0.51
Miami Springs	3rd	5.05	0.17	0.12
Nova Spring	3rd	8.52	0.12	0.14
Palm Spring	3rd	6.88	0.69	0.12
Pegasus Spring	3rd	2.8	0.54	0.22
Sanlando Spring	2nd	19.6	0.59	0.18
Starbuck Springs	2nd	14.3	0.39	0.16

Data cited from SJRWMD's Springs website (http://sjrwmd.com/programs/plan_monitor/gw_assess/springs/).

Table 4.12. Nitrate and TP loadings from springs located in the Wekiva River drainage basin and percent loadings from these springs

Spring Name	Loading		Percent loading	
	NO3/NO (lbs/year)	TP (lbs/year)	NO3/NO	TP
Lake County				
Blackwater Spring	2536		0.5%	
Blue Algae Boil	8	14	0.0%	0.0%
Blueburry Spring	4		0.0%	
Boulder Spring	30	67	0.0%	0.1%
Camp La-No-Che Spring		83		0.1%
Cedar Spring	2	3	0.0%	0.0%
Droty Spring	37	98	0.0%	0.1%
Green Algae Boil		17		0.0%
Markee Spring	10	30	0.0%	0.0%
Messant Spring	289	1158	0.1%	1.5%
Mocassin Spring	6		0.0%	
Palm Spring	12	25	0.0%	0.0%
Seminole Spring	94967	5546	18.6%	7.0%
Sharks Tooth Spring	27	15	0.0%	0.0%
Snail Springs	4	27	0.0%	0.0%
Tricle Spring				
Orange County				
Barrel Spring	25		0.0%	
Rock Springs	171032	9122	33.4%	11.6%
Sulphur Spring	29	44	0.0%	0.1%
Tram Springs				
Wekiva Springs	188959	36999	36.9%	46.9%
Witherington Spring	3517		0.7%	
Seminole County				
Gingar Ale Springs		9		0.0%
Island Spring	154	7864	0.0%	10.0%
Miami Springs	1691	1193	0.3%	1.5%
Nova Spring	2013	2349	0.4%	3.0%
Palm Spring	9349	1626	1.8%	2.1%
Pegasus Spring	2978	1213	0.6%	1.5%
Sanlando Spring	22773	6948	4.5%	8.8%
Starbuck Springs	10983	4506	2.1%	5.7%
Total	511433	78952	100.0%	100.0%

Table 4.13 summarizes the possible nonpoint source contributors of nitrate and TP loadings in the Wekiva River drainage system considered in this TMDL. The contribution of nitrate and TP loads from septic tanks located with 200 meters to receiving water through surface runoff is not a major source. Nitrate loadings from different landuses through surface runoff and springs are comparable, and springs contribute slightly more nitrate than the surface runoff. The major contributor of TP apparently is the surface runoff from the drainage area. Spring TP contribution appears to be less important than the surface runoff.

Table 4.13. Summary of nitrate and TP loadings from possible nonpoint sources in the Wekiva River drainage areas.

Sources	Loading		Percent Loading	
	NO3/NO2 (lbs/year)	TP (lbs/year)	NO3/NO2	TP
Surface runoff from landuses	383,959	208,819	43%	73%
Spring contribution	511,433	78,952	57%	27%
Surface runoff from septic tanks	2,890	499	0%	0%
Total	898,282	288,270	100%	100%

Chapter 5: DETERMINATION OF ASSIMILATIVE CAPACITY

Ideally, existing loading and target loading reaching a given waterbody should be simulated using hydrologic and water quality models. However, there was not adequate time available to develop this TMDL using a modeling approach, and an alternative approach was used to estimate the existing and target loadings.

Existing stream loading can be estimated by multiplying the measured stream flow by the measured pollutant concentrations in the stream. To estimate the pollutant loading this way, flow measured at the outlet of each stream segment under question is required. Several USGS gauging stations were identified in the Wekiva River main stem and its major tributaries. However, none of these stations were located at outlets of the stream segments under question. The Department considered the feasibility of using the available flow measurements to estimate the flow at each segment outlet based on the drainage area ratio among these stream segments. This method would normally provide an approximation of flow estimates at the stream segment outlets. However, because of the ubiquitous existence of springs in the Wekiva River drainage area, flow estimation based on drainage area ratio will not give an accurate result. Therefore, the loads of nitrate and TP were not explicitly calculated. Instead, the percent load reduction required to achieve the nitrate and TP concentration targets were calculated assuming the percent loading reduction would be the same as the percent concentration reduction.

The percent reduction required to achieve the water quality target was calculated using the following formula:

[(existing mean concentration – target concentration) / existing mean concentration] x 100

As discussed in Chapter 3, both the nitrate target and the TP target were established as monthly averages in this TMDL. Therefore, long-term monthly average concentrations were calculated for each month for each parameter based on measured concentrations for the period of record. To make sure that the monthly average concentrations will meet the concentration target even under the worse case scenario, the highest monthly average nitrate and TP concentrations were used as existing monthly mean concentrations to calculate the percent reduction required to achieve the nitrate and TP targets. This approach adds to the margin of safety of the TMDL.

To make sure that the estimated existing mean concentrations represent the existing conditions of the stream, only recent data were used to estimate the existing mean concentration. Because the Verified Period for the Wekiva River basin water quality assessment started at 1996, no data earlier than 1996 were used in this analysis.

Because different nitrate and TP concentrations were observed in different stream segments, percent reduction requirements were calculated separately for each segment. The Wekiva

River was divided into three segments, including Wekiva Spring (WS, WBID 2956C), upstream Wekiva River (UWRMS, WBID 2956), and downstream Wekiva River (DWRMS, 2956A).

Rock Springs (RS) and Rock Springs Run (RSR) are currently combined in the same WBID (2967). To estimate the existing concentrations of RS, nitrate and TP concentration measurements collected from a water quality station located at the spring boil – station 21FLGW 11395, were excluded from the Rock Springs Run WBID and used specifically for existing nitrate and TP concentrations calculation for the spring.

Because nitrate and TP concentrations for Wekiva Spring (WBID 2956C) were only measured in 2002, 2003, and 2004 during very limited sampling events, monthly averages were not calculated for nitrate or TP for the spring and the highest observed concentrations were used as the existing condition**.** **Tables 5.1** and **5.2** show the existing mean concentrations, target concentration and percent reductions required for the stream segments (springs) mentioned above.

In addition to calculating required reductions in Rock Springs Run (WBID 2967), Wekiva Spring (WBID 2956C), and the Wekiva River (WBIDs 2956 and 2956A), the Department also calculated required reductions in nitrate and TP concentrations for the Little Wekiva River (LWR, WBID 2987), and Black Water Creek (BWC, WBID 2929A).

Table 5.1. Existing and target nitrate concentrations for related stream segments and percent concentration reduction required to achieve the nitrate target.

Unit: (mg/L)

Year	RS	RSR (2967)	WS (2956C)	UWRMS (2956)	DWRMS (2956A)	LWR (2987)	BWC (2929A)
January	1.50	0.62	1.30	0.88	0.54	0.70	0.29
February	N/A	0.65	N/A	0.88	0.38	0.59	0.21
March	N/A	0.52	N/A	0.52	0.41	0.26	0.29
April	1.50	0.58	1.25	0.63	0.39	0.36	0.35
May	1.30	0.77	N/A	0.70	0.33	0.39	0.46
June	N/A	0.77	N/A	0.40	0.35	0.44	0.32
July	1.40	0.57	1.34	0.52	0.30	0.25	0.59
August	N/A	0.08	N/A	0.44	0.24	0.26	0.12
September	N/A	0.57	N/A	0.61	0.29	0.36	0.24
October	1.50	0.53	0.94	0.42	0.27	0.17	0.29
November	N/A	0.43	N/A	0.60	0.47	0.28	0.27
December	N/A	0.73	N/A	0.86	0.30	0.49	0.14
Existing Mean (highest monthly mean)	**1.50**	**0.77**	**1.34**	**0.88**	**0.54**	**0.70**	**0.59**
Target Concentration	**0.286**	**0.286**	**0.286**	**0.286**	**0.286**	**0.286**	**0.286**
Percent Reduction	**81%**	**63%**	**79%**	**68%**	**47%**	**59%**	**52%**

Table 5.2. Existing and target TP concentrations for related stream segments and percent concentration reduction required to achieve the TP target.

Unit: mg/L

Month	RS	RSR (2967)	WS (2956C)	UWRMS (2956)	DWRMS (2956A)	LWR (2987)	BWC (2929A)
January	0.081	0.078	0.181	0.094	0.102	0.163	0.034
February	N/A	0.084	0.106	0.087	0.088	0.292	0.049
March	N/A	0.086	0.065	0.110	0.125	0.179	0.062
April	0.084	0.095	0.117	0.110	0.102	0.117	0.045
May	0.082	0.104	0.160	0.144	0.116	0.254	0.060
June	N/A	0.109	0.029	0.145	0.130	0.161	0.101
July	0.078	0.140	0.118	0.114	0.121	0.151	0.061
August	N/A	0.112	0.048	0.124	0.117	0.135	0.052
September	N/A	0.153	0.081	0.168	0.150	0.141	0.073
October	0.084	0.133	0.107	0.156	0.125	0.127	0.096
November	N/A	0.108	0.014	0.126	0.107	0.178	0.050
December	N/A	0.086	N/A	0.093	0.095	0.238	0.087
Existing Mean (highest monthly mean)	**0.084**	**0.153**	**0.181**	**0.168**	**0.150**	**0.292**	**0.101**
Target	**0.065**	**0.065**	**0.065**	**0.065**	**0.065**	**0.065**	**0.065**
Percent reduction	**23%**	**58%**	**64%**	**61%**	**57%**	**78%**	**36%**

Based on **Table 5.1**, the percent reduction required to achieve the nitrate target of 286 µg/L ranges from 47% for the downstream segment of the Wekiva River (WBID 2956A) to 81% for the spring vent of Rock Spring. The downstream segments of the river typically have relatively low nitrate concentrations, and therefore low percent required reduction. Upstream segments typically have higher nitrate concentrations than the downstream segments, and spring vents have the highest nitrate concentrations. Therefore, high percent reductions are usually required for the upstream segment and spring boil. This spatial trend indicates the importance of springs as the nitrate contributor. In addition to the Wekiva River and Rock Springs Run, 59% and 52% reductions are required Little Wekiva River and Blackwater Creek, respectively, to achieve the nitrate target for the main stem of the Wekiva River.

The required percent reduction for TP ranged from 23% for Rock Spring and 78% for the Little Wekiva River tributary. The spatial trend of the required percent reduction is not as consistent as that of nitrate. While required TP percent reductions differ significantly between Rock Spring and Rock Springs Run, the required TP percent reductions are not dramatically different among Wekiva Spring, the upstream segment of the Wekiva River, and the downstream segment of the Wekiva River. To achieve the TP target for the main stem of the Wekiva River, a 78% reduction is required for the Little Wekiva River tributary, indicating the relative importance of the Little Wekiva River tributary as the phosphorus contributor.

Chapter 6: DETERMINATION OF THE TMDL

6.1 Expression and Allocation of the TMDL

The objective of a TMDL is to provide a basis for allocating acceptable loads among all of the known pollutant sources in a watershed so that appropriate control measures can be implemented and water quality standards achieved. A TMDL is expressed as the sum of all point source loads (wasteload allocations, or WLAs), nonpoint source loads (load allocations, or LAs), and an appropriate margin of safety (MOS), which takes into account any uncertainty concerning the relationship between effluent limitations and water quality:

$$TMDL = \sum WLAs + \sum LAs + MOS$$

As discussed earlier, the WLA is broken out into separate subcategories for wastewater discharges and stormwater discharges regulated under the NPDES Program:

$$TMDL \cong \sum WLAs_{wastewater} + \sum WLAs_{NPDES\ Stormwater} + \sum LAs + MOS$$

It should be noted that the various components of the revised TMDL equation may not sum up to the value of the TMDL because (1) the WLA for NPDES stormwater is typically based on the percent reduction needed for nonpoint sources and is also accounted for within the LA, and (2) TMDL components can be expressed in different terms (for example, the WLA for stormwater is typically expressed as a percent reduction, and the WLA for wastewater is typically expressed as mass per day).

WLAs for stormwater discharges are typically expressed as percent reduction because it is very difficult to quantify the loads from MS4s (given the numerous discharge points) and to distinguish the loads from MS4s from other nonpoint sources (given the nature of stormwater transport). The permitting of stormwater discharges also differs from the permitting of most wastewater point sources. Because stormwater discharges cannot be centrally collected, monitored, and treated, they are not subject to the same types of effluent limitations as wastewater facilities, and instead are required to meet a performance standard of providing treatment to the “maximum extent practical” through the implementation of BMPs.

This approach is consistent with federal regulations (40 CFR § 130.2[I]), which state that TMDLs can be expressed in terms of mass per time (e.g., pounds per day), toxicity, or **other appropriate measure**. TMDLs for the Wekiva River, RSR and other related WBIDs in the drainage basin of the Wekiva River system are expressed in terms of pounds per month, pounds per day, and percent reduction of nitrate and TP, and represent the maximum long-term nitrate and TP loadings the WR and RSR can assimilate and maintain a balanced aquatic flora and fauna **(Table 6.1**). It should be noted that the expression of the TMDL on a mass per day basis is for information purposes only

Table 6.1. TMDL components for RSR, WRMS, and related WBIDs

WBID	Parameter	TMDL (percent reduction)	$WLA_{NPDES\ wastewater}$	$WLA_{NPDES\ Stormwater}$	LA	MOS
RS	Nitrate	81%	N/A	81%	81%	Implicit
RS	TP	23%	N/A	23%	23%	Implicit
RSR (2967)	Nitrate	63%	N/A	63%	63%	Implicit
RSR (2967)	TP	58%	N/A	58%	58%	Implicit
WS (2956C)	Nitrate	79%	N/A	79%	79%	Implicit
WS (2956C)	TP	64%	N/A	64%	64%	Implicit
UWRMS (2956)	Nitrate	68%	2,805 lbs/month	68%	68%	Implicit
UWRMS (2956)	TP	61%	40 lbs/month	61%	61%	Implicit
DWRMS (2956A)	Nitrate	47%	N/A*	47%	47%	Implicit
DWRMS (2956A)	TP	57%	191 lbs/month	57%	57%	Implicit

*While there is no WLA for nitrate for DWRMS, it should be noted that a WLA of 572 lbs/month has been established for Total Nitrogen for the SCES/Yankee Lake WRF (see Section 6.3.1). N/A in this table means not applicable.

Altamonte Springs Regional Water Reclamation Facility does not discharge directly into any of the above segments. However, the loading from the facility influences the main stem water quality through discharging into the Little Wekiva River. Therefore, WLA for TP was allocated to the facility as 26 lbs/month. A discharge limit of 286 µg/L was assigned to the facility for nitrate.

In addition to the percent load reductions described in **Table 6.1** that are needed to achieve the water quality in the main stem of the Wekiva River, nitrate loads from the Little Wekiva River and Blackwater Creek tributaries also need to be reduced by 59% and 52%, respectively. Similarly, TP loadings from the Little Wekiva River and Blackwater Creek should be reduced by 78% and 36%, respectively.

The percent load reductions listed on **Table 6.1** were established to achieve the monthly average nitrate concentration of 286 µg/L and the monthly average TP concentration of 65 µg/L. While these percent reductions are the expression of the TMDL that will be implemented, EPA[3] recommends that all TMDLs and associated load allocations and wasteload allocations include a daily time increment in conjunction with other appropriate temporal expressions that may be necessary to implement the relevant water quality standard. Daily maximum concentrations targets for nitrate and TP were established using the following equation[4], which assumes that the nitrate and TP data distributions are lognormal in Wekiva and Rock Springs Run:

$$MDL = LTA * \exp(Z_p\sigma_y - 0.5\sigma_y^2)$$

$$\sigma_y = \mathrm{sqrt}(\ln(CV^2 + 1))$$

[3] November 2006 U. S. Environmental Protection Agency (USEPA 2006) Memorandum “Establishing TMDL “Daily” Loads in Light of the Decision by the U.S. Court of Appeals for the D.C. Circuit in Friends of the Earth, Inc. v. EPA et. al., No.05-5015 (D.C. Cir. 2006) and Implications for NPDES permits.”
[4] EPA, “Options for Expressing Daily Load in TMDL (The Option),” June, 2007.

Where

LTA = long-term average
Z_p = pth percentage point of the standard normal distribution, which can be obtained from any statistics textbook.
σ = standard deviation
CV = coefficient of variance

For the daily maximum nitrate concentration, it was assumed that the average monthly target concentration should be the same as the average daily concentration. Also, assuming the target data set will have the same CV as the existing measured data set and allowing 10% exceedance, the daily maximum nitrate concentrations for Wekiva Upstream, Wekiva Downstream, and Rock Springs Run would be 0.47, 0.48, and 0.52 mg/L, respectively. The most conservative nitrate daily maximum target was chosen as the final daily maximum nitrate target for the Wekiva River – Rock Springs Run system, which is **0.47 mg/L (470 µg/L).** The means, STDEVs, and CVs of nitrate concentrations of different water segments are listed in **Table 6.2**.

Table 6.2. Daily maximum for target nitrate concentration (mg/L)

Statistics	Wekiva Upstream	Wekiva Downstream	Rock Springs Run
Mean	0.65	0.38	0.59
STDEV	0.33	0.2	0.4
CV	0.51	0.53	0.68
Daily Maximum	**0.47**	**0.48**	**0.52**

This same approach was used to calculate the TP daily maximum concentrations for Wekiva Upstream, Wekiva Downstream, and Rock Springs Run, which are 0.09, 0.09, and 0.11 mg/L, respectively. The most conservative TP daily maximum target was chosen as the final daily maximum TP target for the Wekiva River – Rock Springs Run system, which is **0.09 mg/L (90 µg/L).** The means, STDEVs, and CVs of TP concentrations of different water segments are listed in **Table 6.3**.

Table 6.3. Daily maximum for target TP concentration (mg/L)

Statistics	Wekiva Upstream	Wekiva Downstream	Rock Springs Run
Mean	0.12	0.116	0.107
STDEV	0.039	0.033	0.059
CV	0.33	0.28	0.55
Daily Maximum	**0.09**	**0.09**	**0.11**

It should be emphasized that these daily maximum targets were developed for illustrative purposes. Implementation of the TMDL will be based on the monthly average concentration targets.

6.2 Load Allocation

Because no target load was explicitly calculated in this TMDL report due to the lack of flow data at the outlet of each stream segment, TMDLs are represented as the percent reduction required to achieve the nitrate and TP targets. The percent reduction assigned to all the nonpoint sources areas (LA) are the same as those defined for the TMDL percent reduction. To achieve the annual average nitrate target of 250 µg/L in Rock Springs Run and the Wekiva River, the nitrate loads from the nonpoint source related to RS, RSR (2967), WS (2956C), UWRMS (2956), and DWRMS (2956A) need to be reduced by 81%, 63%, 79%, 68%, and 47%, respectively. In addition, nitrate contributions from nonpoint sources related to the LWR (2987) and BWC (2929A) should also be reduced by 59% and 52%, respectively, to ensure that the nitrate target of the Wekiva River will be met.

The required TP percent reductions to achieve the TP target of 65 µg/L are 23%, 58%, 64%, 61%, and 57% for RS, RSR, WS, UWRMS, and DWRMS, respectively. About 78% and 36% of the TP loadings from the LWR and BWC tributaries, respectively, should also be reduced to achieve the TP target of the main stem. The nonpoint sources covered in this allocation include runoff, septic discharge through surface runoff, and the anthropogenic load contained in the spring discharge. All the nonpoint sources covered in LA are restricted to those sources in non-MS4 areas.

6.3 Wasteload Allocation

6.3.1 National Pollutant Discharge Elimination System Wastewater Discharges

Three NPDES permitted facilities were identified in the Wekiva River system that may potentially contribute significant quantities of nitrate and TP to the Wekiva River. These facilities include SCES/Yankee Lake WRF (FL0042625), Wekiva Hunt Club WWTF (FL0036251), and Altamonte Springs Regional Water Reclamation Facility (FL0033251).

The **SCES/Yankee Lake WRF** discharges very little nutrient load to the WR via a backup upland/receiving wetlands surface water system, with the majority of their AWT treated wastewater going to reuse. As described in Chapter 4, the upland/receiving wetlands system typically does not discharge to any receiving surface water except to Sulfur Creek under wet weather conditions. Although Sulfur Creek discharges to the downstream section of the WR intermittently under wet weather, water quality monitoring results in Sulfur Creek upstream and downstream of the effluent point from SCES/Yankee Lake WRF did not show any significant impact from the facility. Therefore, the Department concluded that the existing permit limits for the wetlands discharge (0.75 mgd discharge, and TP and TN limits of 1.0 mg/l, and 3.0 mg/l) are adequately protective of the Wekiva River.

It should be noted that the facility currently does not have a nitrate effluent limit. The nitrate concentration of the effluent from this facility typically accounts for about 24 to 65% of the TN concentration, with an average percent nitrate concentration of 42% (**Table 4.2**). Because no negative impacts have been observed at the monitoring site downstream of the effluent point, the Department concluded that the a nitrate effluent limit was not needed. However, if the percent nitrate concentration from the effluent significantly increases in the future or negative impacts are detected in Sulfur Creek, the Department may require a permit limit for nitrate.

The wasteload allocation for TN was calculated in place of nitrate as follows:

TN allocation (lbs/month) = 8.36* monthly average TN limit * surface AADF limit *365/12

Where,

8.36 is the conversion factor;
The facility's monthly average TN limit is 3.0 mg/L;
The facility's surface discharge AADF limit is 0.75 mgd;
Three Hundred and sixty five days are assumed for each year; and
The monthly load was calculated by dividing the annual load by 12.

The monthly TN **wasteload allocation** is **572 lbs/month**. This allocation, when represented as daily loading, would be **18.8 lbs/day**, dividing the annual load by 365 days.

The TP wasteload allocation was calculated as follows:

TP allocation (lbs/month) = 8.36 * Annual average TP limit * surface AADF limit *365/12

8.35 is the conversion factor;
The facility's monthly average TP limit is 1.0 mg/L;
The facility's surface discharge AADF limit is 0.75 mgd;
Three hundred and sixty five days are assumed for all years; and
The monthly load was calculated by dividing the annual load by 12.

The monthly **TP allocation** is **191 lbs/month**. This allocation, when represented as daily loading, would be **6.3 lbs/day,** dividing the annual load by 365 days.

The Wekiva Hunt Club WWTF discharges into Sweetwater Creek, which in turn discharges to Cove Lake, and then the Wekiva River. The facility has a surface water discharge AADF of 2.9 mgd and a monthly average TP discharge limit of 0.4 mg/L. The facility does not have any nitrate or TN discharge limit. Based on PCS data, the facility is currently discharging at a long-term annual average discharge rate of 539 million gallon/year, with long-term annual averages of 0.19 mg/L TP and 9.94 mg/L nitrate. The long-term annual average nitrate and TP loadings from the facility are 46,751 lbs/year and 869 lbs/year, respectively. However, based on the surface water monitoring data collected during January of 2005 through April of 2006, the discharge nitrate and TP concentrations have attenuation rates of 96% and 39%, respectively, by the time the effluent reaches the sampling site closest to the Wekiva River (Site #5). Based on these attenuation rates, the long-term annual average nitrate and TP concentrations at Site #5 are:

Nitrate concentration = 9.94 mg/L * (1 – 96%) = 0.398 mg/L

TP concentration = 0.19 mg/L * (1 – 39) = 0.116 mg/L.

To achieve the nitrate and TP targets of 0.286 mg/L and 0.065 mg/L, respectively, at the discharge point of Sweetwater Creek to the Wekiva River, percent reductions required are:

Nitrate percent reduction = (0.398 – 0.286)/0.398 * 100% = 28%
TP percent reduction = (0.116 – 0.065)/0.116 * 100% = 44%.

Applying the nitrate and TP percent reductions to the long-term annual average nitrate and TP loadings discharged from the facility, the final wasteload allocations to the facility are:

Nitrate wasteload allocation = 46, 751 lbs/year * (1 – 0.28) = **33,661 lbs/year = 2,805 lbs/month**;
TP wasteload allocation = 860 lbs/year * (1 – 0.44) = **482 lbs/year = 40 lbs/month**;

When represented as daily loads, the nitrate and TP wasteload allocations are **80.5 lbs/day** and **1.3 lbs/day**, respectively.

The Altamonte Springs Regional Water Reclamation Facility discharges directly into the Little Wekiva River, which in turn discharges into the main stem of the Wekiva River. To achieve the 65 µg/L TP target for the Wekiva River main stem, this TMDL recommends that the discharge from the Little Wekiva River into the main stem meets the 65 µg/L target at the outlet of the Little Wekiva River (roughly at station 21FLSJWMLW-UWR, **Figure 4.2**). The TP concentration at this location is calculated using the following equation:

$$Cout = \frac{(Qusgs - Qwrf)*Clakelotus + Qwrf*Cwrf + Qsanlan*Csanlan + Qpalm*Cpalm + Qstar*Cstar}{(Qusgs - Qwrf) + Qwrf + Qsanlan + Qpalm + Qstar}$$

Where,

C_{out} is the TP concentration at the outlet of the Little Wekvia River,
Q_{usgs} is the flow measured at USGS gauge 02234990. The long-term average is **28.8 cfs**.
$C_{lakelotus}$ is the TP concentration of Lake Lotus, which is about **70 µg/L** and reflects the stream TP concentration from combined contribution of nonpoint sources.
Q_{wrf} is the discharge from the Altamonte Springs WRF. The long-term average is **1.9 cfs**.
C_{wrf} is the long-term average TP concentration of WRF's discharge, which is **1500 µg/L**.
$Q_{usgs} – Q_{wrf}$ is the total stream flow minus the flow from the WRF. The difference primarily represents the flow created by nonpoint sources.
Q_{sanlan} is the long-term average discharge rate from Sanlando Springs, which is **19.6 cfs**.
C_{sanlan} is the long-term average TP concentration in the discharge of Sanlando Springs, which is **180 µg/L**.
Q_{palm} is the long-term average discharge rate from Palm Springs, which is **6.9 cfs**.
C_{palm} is the long-term average TP concentration in the discharge of Palm Springs, which is **120 µg/L**.
Q_{star} is the long-term average discharge rate from Starbuck Springs, which is **14.3 cfs**.
C_{star} is the long-term average TP concentration in the discharge of Starbuck Springs, which is **160 µg/L**.

Substituting the number for each of the items listed above into the equation, the final result of the C_{out} is 163 µg/L, which is close to the long-term average TP concentration of 173 µg/L measured at the outlet of the Little Wekiva River, suggesting that above equation, which represents only the mixing in the Little Wekiva River, reflects accurately the fate of TP in the stream. Loss of TP from the water column to the sediment through net deposition may not be a major factor in controlling the TP dynamics in the system.

Possible reasons for high TP concentrations in the discharge of the three springs listed above was also explored. **Table 4.11** listed nitrate and TP concentrations of all the springs located in the Wekiva Study Area. Nitrate and TP concentrations for the majority of these springs are relatively low. However, most of the springs located in the Seminole County, including the three springs listed above, have relatively high TP concentrations as well as high nitrate concentrations. These observations suggested that a significant portion of the high TP concentrations may have resulted from human activities instead of geological background. Therefore, to achieve the main stem TP target of 65 µg/L, TP concentrations from these springs also need to be reduced.

Assuming that we will reduce the TP concentrations from all the nonpoint sources to 65 µg/L (including the concentration at the outlet of Little Wekiva Canal, which is represented by the TP concentration of Lake Lotus, and TP concentrations of the spring discharges), the TP concentration allowable for the WRF discharge, based on above equation, would be 83 µg/L. If the existing discharge rate of the WRF is retained, the allowable monthly loading of TP from the WRF would be **26 lbs/month**. This represents about 94% reduction from its existing long-term average monthly loading of 470 lbs/month (**Table 4.3**). The daily allowable loading for the WRF would be **0.84 lbs/day**.

To estimate the target nitrate loading from the facility, the Department assumed that in-stream nitrate assimilation in the Little Wekiva River is insignificant comparing to the total watershed and point source loads, which adds to the margin of safety of this TMDL. Therefore, to achieve the nitrate target for the main stem of the Wekiva River, the Department recommend, at this point, to reduce the nitrate concentrations from all the nonpoint and point sources to 286 µg/L. This nitrate target also applies to the effluent of the WRF. Assuming that the facility's existing discharge rate is allowed to be kept, the target nitrate loading is **91 lbs/month** for monthly loading, and **2.9 lbs/day** for daily loading. The facility does not have a routine monitoring requirement for nitrate. Based on two Reclaimed Water Effluent Analyses conducted in 2006, the effluent nitrate concentrations from the facility were 4.66 mg/L and 3.96 mg/L. Assuming that the average of these two numbers (4.31 mg/L or 4310 µg/L) reflects the long-term average concentration of the discharge, under the existing discharge rate (1.23 MGD), the existing nitrate load from the facility is 1371 lbs/month. The target load of 91 lbs/month represents about 93% reduction of nitrate load from the existing condition.

6.3.2 National Pollutant Discharge Elimination System Stormwater Discharges

Because no information was available to the Department at the time this analysis was conducted regarding the boundaries and locations of all the NPDES stormwater dischargers, the exact stormwater nitrate and TP loadings from MS4 areas were not explicitly estimated. Within the Wekiva River drainage basin, Orange County has a Phase I MS4 permit (FLS000011), with the Florida Department of Transportation (FDOT) District 5 and City of Maitland as co-permittees. Seminole County also holds a Phase I MS4 permit (FLS000038) with FDOT District 5, City of Altamonte Springs, and City of Lake Mary being co-permittees for this permit. In addition, the City of Orlando holds a Phase I MS4 permit (FLS000014). Lake County does not have MS4 permit within the boundary of the drainage basin. The wasteload allocations for each of the MS4s are the same percent nitrate and TP reductions required for the LA assigned to the nonpoint sources in the river segments that belong to each county and municipality.

It should be noted that any MS4 permittee is only responsible for reducing the loads associated with stormwater outfalls that it owns or otherwise has responsible control over, and is not responsible for reducing other nonpoint source loads within its jurisdiction.

6.4 Margin of Safety

Consistent with the recommendations of the Allocation Technical Advisory Committee (Department, February 2001), an implicit MOS was used in the development of this TMDL. An implicit MOS was provided by the conservative decisions associated with a number of modeling assumptions, the development of site-specific alternative water quality targets, and the development of assimilative capacity.

The MOS was created in several aspects of the analyses. For example, the nitrate target was established based on the most conservative concentration from the three lines of evidence (Suwannee River periphtyon result, Rainbow Creek Lyngbya result, and Rock Springs Run and the Wekiva River ecological efficiency experiment). Requiring that the 286 µg/L target be met every month should result in the nitrate concentration to be even lower than the target concentration during the summer algal growth season based on seasonal analysis on the nitrate concentration, and therefore adds to the margin of safety. In addition, when estimating the required percent load reduction to achieve the water quality target, the highest long-term monthly averages of measured nitrate concentrations, instead of average long-term monthly averages, were chosen to represent the existing condition. This will make estimating the required percent load reduction more conservative and therefore add to the margin of safety.

Similarly, the TP target concentration was established as a monthly average because no seasonal pattern could be identified for community structure and functions of aquatic flora in the system under question. Typically, if no seasonal pattern can be identified for biological responses to nutrient concentrations of the system, the time scale for the water quality target would be set as an annual average. For this TMDL, the Department found that TP concentrations in the system tend to be higher during the summer growth season and therefore established the TP target as a monthly average. This will not only address the growth season TP concentration properly, but also makes the in-stream TP concentration lower than the target TP concentration in other months of the year, which is more conservative and adds to the MOS. In addition, using the highest long-term monthly average instead of the average of long-term monthly average in calculating the required percent load reduction makes estimating the required percent load reduction more conservative and therefore adds to the margin of safety.

6.5 Recommendations for Further Studies

This TMDL is developed primarily based on the nitrate and TP PLRGs developed by the SJRWMD. Because of time limitations, the identification of impairments and development of water quality targets were based on data collected within one year. However, due to the varying nature of the local weather and hydrology, and the variation in responses from local flora and fauna to the change of nitrate and TP concentrations under different weather, hydrological, and hydraulic conditions, similar studies that could support the further identification of impairment and development of water quality targets should be conducted in the future for a longer time period to verify the conclusions and hypotheses used in this TMDL.

The SJRWMD also proposed future studies to further examine the dynamics of aquatic flora and fauna to environments with changing nitrate and TP concentrations. Proposed studies include studies on the effects of nutrient variation on the spring aquatic communities, the relationship between nutrient concentration and periphytic algal growth under varying conditions of current velocity and light availability, the inhibitory effects of periphyton on SAV growth, and the effects of filamentous algal growth on macroinvertebrate and fish habitat. In addition, the Department recommends that the hydrology, hydraulic, and water quality of the Wekiva River system be simulated using calibrated models to better understand the effects of nitrate and TP pollutants on the in-river nitrate and TP concentrations and influences on the structure and functions of aquatic communities. Because springs are major nitrate contributors for both the Wekiva River and RSR, it would be very important to identify and address the potential pollutant sources of nitrate in the ground water recharge area.

Chapter 7: NEXT STEPS: IMPLEMENTATION PLAN DEVELOPMENT AND BEYOND

7.1 Basin Management Action Plan

Following the adoption of this TMDL by rule, the next step in the TMDL process is to develop an implementation plan for the TMDL, referred to as the BMAP. This document will be developed over the next two years in cooperation with local stakeholders, who will attempt to reach consensus on detailed allocations and on how load reductions will be accomplished. The BMAP will include, among other things:

- Appropriate load reduction allocations among the affected parties,
- A description of the load reduction activities to be undertaken, including structural projects, nonstructural BMPs, and public education and outreach,
- A description of further research, data collection, or source identification needed in order to achieve the TMDL,
- Timetables for implementation,
- Confirmed and potential funding mechanisms,
- Any applicable signed agreement(s),
- Local ordinances defining actions to be taken or prohibited,
- Any applicable local water quality standards, permits, or load limitation agreements,
- Milestones for implementation and water quality improvement, and
- Implementation tracking, water quality monitoring, and follow-up measures.

An assessment of progress toward the BMAP milestones will be conducted every five years, and revisions to the plan will be made as appropriate, in cooperation with basin stakeholders.

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Appendices

Appendix A: Background Information on Federal and State Stormwater Programs

In 1982, Florida became the first state in the country to implement statewide regulations to address the issue of nonpoint source pollution by requiring new development and redevelopment to treat stormwater before it is discharged. The Stormwater Rule, as authorized in Chapter 403, F.S., was established as a technology-based program that relies on the implementation of BMPs that are designed to achieve a specific level of treatment (i.e., performance standards) as set forth in Chapter 62-40, F.A.C. In 1994, the Department's stormwater treatment requirements were integrated with the stormwater flood control requirements of the state's water management districts, along with wetland protection requirements, into the Environmental Resource Permit regulations.

Chapter 62-40, F.A.C., also requires the water management districts to establish stormwater pollutant load reduction goals (PLRGs) and adopt them as part of a SWIM plan, other watershed plan, or rule. Stormwater PLRGs are a major component of the load allocation part of a TMDL. To date, stormwater PLRGs have been established for Tampa Bay, Lake Thonotosassa, the Winter Haven Chain of Lakes, the Everglades, Lake Okeechobee, and Lake Apopka. No PLRG had been developed for Newnans Lake when this report was published.

In 1987, the U.S. Congress established Section 402(p) as part of the federal Clean Water Act Reauthorization. This section of the law amended the scope of the federal NPDES permitting program to designate certain stormwater discharges as "point sources" of pollution. The EPA promulgated regulations and began implementing the Phase I NPDES stormwater program in 1990. These stormwater discharges include certain discharges that are associated with industrial activities designated by specific standard industrial classification (SIC) codes, construction sites disturbing 5 or more acres of land, and master drainage systems of local governments with a population above 100,000, which are better known as municipal separate storm sewer systems (MS4s). However, because the master drainage systems of most local governments in Florida are interconnected, the EPA implemented Phase I of the MS4 permitting program on a countywide basis, which brought in all cities (incorporated areas), Chapter 298 urban water control districts, and the Florida Department of Transportation throughout the 15 counties meeting the population criteria. The Department received authorization to implement the NPDES stormwater program in 2000.

An important difference between the federal NPDES and the state's stormwater/environmental resource permitting programs is that the NPDES Program covers both new and existing discharges, while the state's program focuses on new discharges only. Additionally, Phase II of the NPDES Program, implemented in 2003, expands the need for these permits to construction sites between 1 and 5 acres, and to local governments with as few as 1,000 people. While these urban stormwater discharges are now technically referred to as "point sources" for the purpose of regulation, they are still diffuse sources of pollution that cannot be easily collected and treated by a central treatment facility, as are other point sources of pollution such as domestic and industrial wastewater discharges. It should be noted that all MS4 permits issued in Florida include a re-opener clause that allows permit revisions to implement TMDLs when the implementation plan is formally adopted.

Appendix B. Change Point Analysis of the Suwannee River Algal Data

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Technical Report Submitted to
the Florida Department of Environmental Protection

I. Background

Per the request of the Wekiva Parkway and Protection Act (WPPA) passed by the Florida Legislature in 2004 (Chapter 369, Part III, FS), the Florida Department Environmental Protection (the Department) is developing a nitrate Total Maximum Daily Load (TMDL) for the Wekiva River and Rock Springs Run in the central Florida area. Based on information provided by the St. Johns River Water Management District (SJRWMD), these two waterbodies showed elevated periphyton abundance compared to two reference creeks (Juniper Creek and Alexander Springs Run) located in the same region and were therefore considered impaired. After examining the distribution of various physical, chemical, and biological parameters in the Wekiva River and Rock Springs Run and in reference waterbodies, it was decided that elevated nitrate concentrations in the Wekiva River and Rock Springs Run is one of the major pollutants that caused this impairment. According to the 1999 Florida Watershed Restoration Act (FWRA), Chapter 99-223, Laws of Florida, once a waterbody is verified for impairment, a TMDL must be developed.

Establishing a nitrate target for the Wekiva River and Rock Springs Run is a critical part of the TMDL development. To define this target, a functional relationship between the periphyton abundance and nitrate concentration needs to be characterized. Ideally, the functional relationship would be built upon data collected from the Wekiva River and Rock Springs Run. Unfortunately, because of the limit amount of time available to this project, not enough data were available to establish the relationship in these two waterbodies. Therefore, this study uses nitrate and periphyton data collected from a monitoring network on the Suwannee River, which was established for the Surface Water Improvement and Management (SWIM) program by the Suwannee River Water Management District (Hornsby, et al. 2000). Much of the length of the Suwannee River is heavily influenced by spring inflow, and the algal communities appear to be generally similar in composition to those in the Wekvia River and Rock Springs Run. Therefore, results from the Suwannee River are considered applicable to the Wekiva River and Rock Springs Run (Mattson et al., 2006).

Nitrate and periphyton data were collected concurrently from 13 stations across the Suwannee River and two tributaries (Withlacoochee River and Santa Fe River) during the period from 1990 through 1998. **Figure 1** shows locations of these water quality stations. **Table 1** lists the period of records and number of samples for each station. Periphyton abundance was measured as both the cell density (cells/cm^2) and biomass density (ash free dry mass – AFDM/cm^2). Long-term average nitrate concentrations and periphyton measurements were calculated for each sampling station. Functional relationships between nitrate and periphyton abundance were established by plotting either long-term average cell densities or biomass density to long-term average nitrate concentrations from all water quality stations.

Significant relationships were found between nitrate concentration and algal abundance. **Figure 2** shows the correlation of mean nitrate concentrations versus mean periphyton biomass ($r = 0.84$; $P<0.001$). There is also a significant positive correlation between mean algal density and mean nitrate concentration (**Figure 3**: $r = 0.77$; $P<0.01$), indicating that increasing nitrate concentration in nature streams could result in elevated periphyton biomass (Mattson, 2006). In addition, both **Figures 2** and **3** suggest that there is a threshold nitrate concentration in the range between 0.2 and 0.4 mg/L, above which the increase of periphyton abundance per unit increase of nitrate concentration becomes significantly higher than below this threshold point.

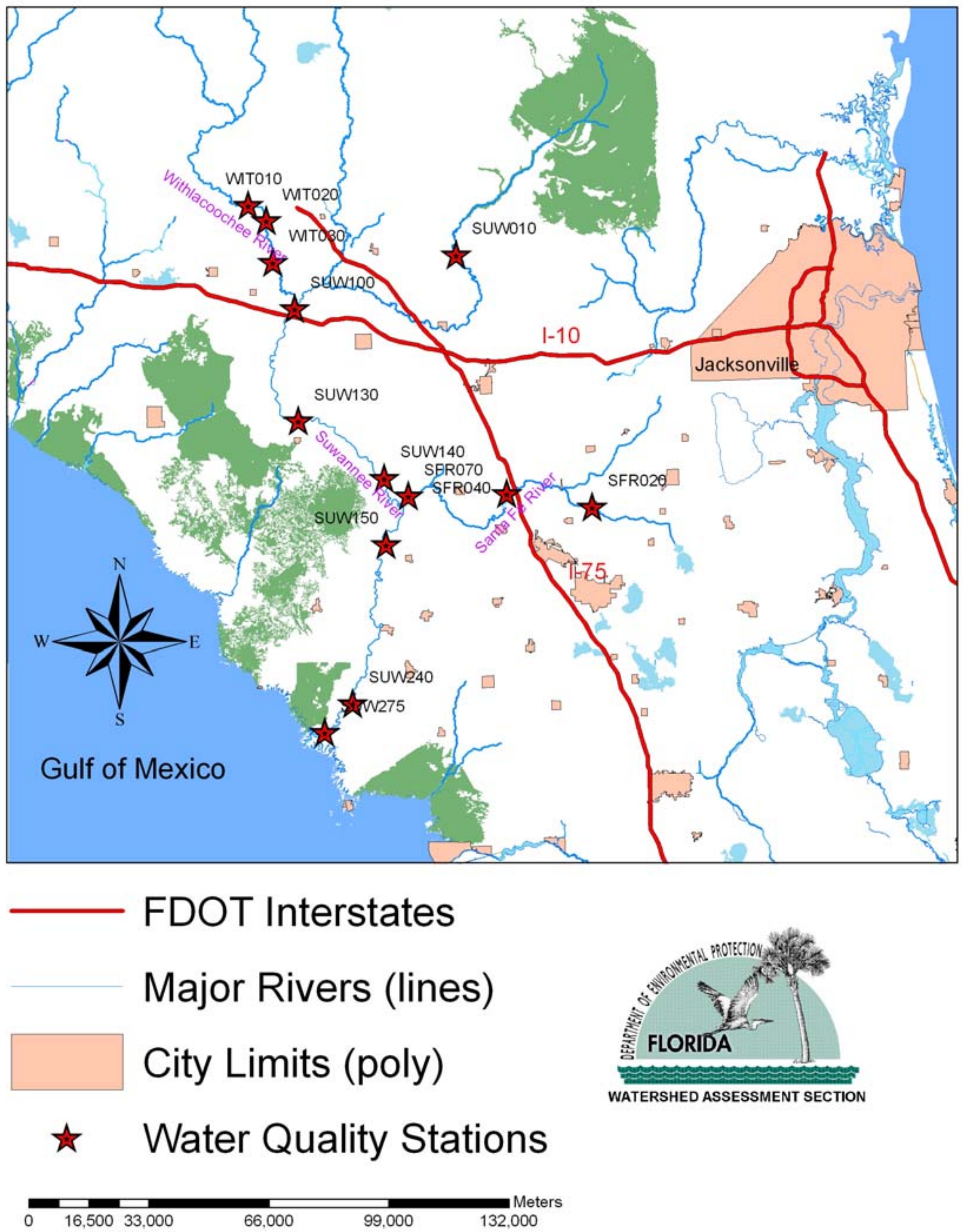

Figure 1. Locations of water quality stations from which measured nitrate and periphyton abundance were used for this analysis.

Table 1. Period of records and number of paired nitrate and periphyton samples from each station

Stations	Period of records	Number of samples
SUW010	06/1990 – 12/1998	24
SUW100	06/1990 – 12/1998	30
SUW130	06/1990 – 12/1998	33
SUW140	06/1996 – 12/1998	8
SUW150	06/1990 – 12/1998	30
SUW240	12/1992 – 12/1998	23
SUW275	03/1990 – 06/1992	10
SFR020	03/1990 – 12/1998	32
SFR040	03/1990 – 12/1998	34
SFR070	03/1990 – 12/1998	33
WIT010	03/1990 – 09/1991	6
WIT020	03/1990 – 09/1991	6
WIT030	03/1990 – 06/1991	5

The purpose of this study is to use change-point statistical analysis to identify the threshold nitrate concentration. This nitrate threshold, once being identified, can be used as the target nitrate concentration for the nitrate TMDL of the Wekiva River and Rock Springs Run.

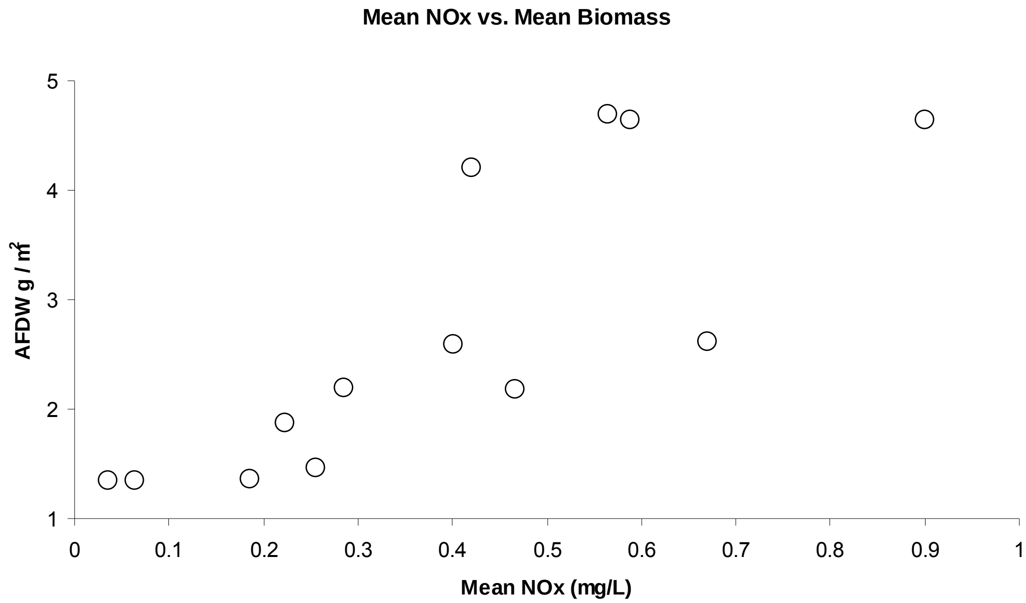

Figure 2. Relationship between mean nitrate concentration and mean periphyton biomass from sampling sites on the Suwannee, Santa Fe, and Withlacoochee Rivers.

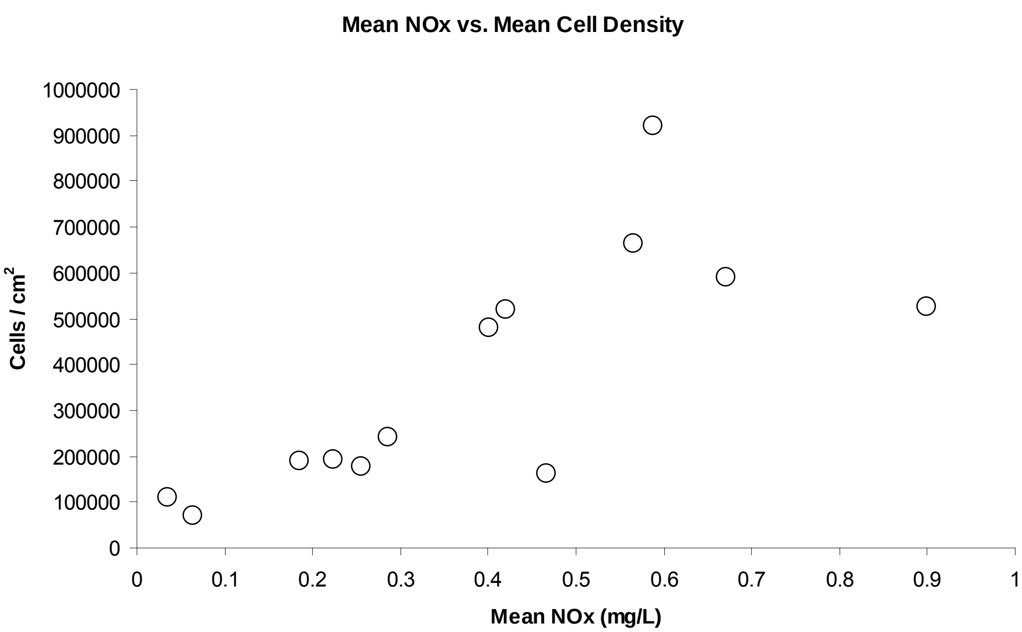

Figure 3. Relationship between mean nitrate concentration and mean periphyton cell density from sampling sites on the Suwannee, Santa Fe, and Withlacoochee Rivers.

The Detection Procedure

Niu et al. (2000) introduced an iterative procedure for detecting and modeling level-shift change points. Niu and Miller (2007) reported the change point analysis and a model comparison procedure for the Stream Condition Index (SCI) and Biological Condition Gradient (BCG) data. The change-point detection procedure in Niuet al. (2000) is similar to that suggested by Chang (1982) and further developed by Chang et al. (1988) for detecting outliers and level shifts in time series analysis. Statistical details of this procedure can also be found in Pankratz (1991, Chapter 8).

For simplicity, let us consider a response variable *Y*, after an appropriate transformation. Suppose that observations $\{(X_i, Y_i),\ i = 1, 2, \cdots, n\}$ are available where *n* is the sample size and *X* is an independent variable. Moreover, we assume that the observations are arranged in the following manner:

- The values $\{X_i,\quad i = 1, 2, \cdots, n\}$ are distinct. If several Y_i's are corresponding to a single *X* value, the median of the Y_i's is taken to be the response value for the *X* value.

- $\{(X_i, Y_i),\ i = 1, 2, \cdots, n\}$ are sorted according to the values of *X* from least to greatest.

For each integer $l > 1$, define the step variable $S_i(l) = 0$ for $i < l$ and $S_i(l) = 1$ for $i \ge l$.

Step 1. Fit the linear regression model:

$$Y_i = \beta_0(l) + \beta_1(l) S_i(l) + \varepsilon_i(l), \qquad i = 1, 2, \cdots, n, \tag{1}$$

where for a fixed *l*, the $\varepsilon_i(l)$'s are assumed to be independent and identically distributed normal random variables with mean zero and variance $\sigma^2(l)$.

Step 2. Calculate the values $\{L(l) = \hat{\beta}_1(l) / se(\hat{\beta}_1(l)),\ l = 2, 3, \cdots, (n-1)\}$ where $se(\hat{\beta}_1(l))$ is the estimated standard error of $\hat{\beta}_1(l)$.

Step 3. Let $L(l_1) = \max\{L(2), L(3), \cdots, L(n-1)\}$ and compare $L(l_1)$ with the critical value *C*=3.0 (or *C*=3.5). The critical value *C*=3.0 (or *C*=3.5) corresponds roughly to $\alpha = 0.10$ (or $\alpha = 0.05$), or the 10% (or the 5%) significance level, based on the simulation results of Chang et al. (1988). If $L(l_1)$ is significant, we conclude that the response *Y* has a change point at X_{l_1} with a level-shift $\hat{\beta}_1(l)$.

Step 4. Let $Y_i^* = Y_i - \beta_1(l_1) S_i(l_1)$. Repeat Steps 1-3 on the new response variable Y_i^* for detecting a possible second change point. Continue the process until no further change point can be identified.

Step 5. Suppose that *k* change points are detected in the response variable *Y* and the corresponding *X* values are $\{X_{l_1}, X_{l_2}, \cdots, X_{l_k}\}$. Fit the model

$$Y_i = \beta_0 + \beta_1 S_i(l_1) + \beta_2 S_i(l_2) + \cdots + \beta_k S_i(l_k) + \varepsilon_i, \qquad i = 1, 2, \cdots, n. \tag{2}$$

Then the estimated coefficients $\{\hat{\beta}_1, \hat{\beta}_2, \cdots, \hat{\beta}_k\}$ will be the *k* estimated level-shift values.

II. Model Comparison

Model (2) fits a step function $\beta_0 + \beta_1 S_i(l_1) + \beta_2 S_i(l_2) + \cdots + \beta_k S_i(l_k)$ to estimate the mean (or median) value of the response variable Y and the predictor variable X. In practice, many other models may be considered to describe the relationship between Y and X. In particular, if the scatter plot of observations $\{(X_i, Y_i),\ i = 1, 2, \cdots, n\}$ shows a straight line or a smooth curve pattern, a linear regression model or a nonlinear smooth-curve model should be fitted to the data instead of the step-function change point model in (2).

For the response variable Y and the predictor variable X, the linear regression model has the form:

$$Y_i = \beta_0 + \beta_1 X_i + \varepsilon_i, \qquad i = 1, 2, \cdots, n. \tag{3}$$

If the relationship between Y and X is nonlinear, many smooth-curve models may be considered. One of the choices is transforming the predictor variable X and fitting a regression model. For example, we may use the natural logarithm transformation log(X) instead of X as the predictor variable and fit the regression model:

$$Y_i = \beta_0 + \beta_1 \log(X_i) + \varepsilon_i, \qquad i = 1, 2, \cdots, n. \tag{4}$$

When different models are fitted to the observations $\{(X_i, Y_i),\ i = 1, 2, \cdots, n\}$, model selection techniques need to be used to decide which model fits the data better. Statistical inferences such as estimation and prediction will then be based on the best model selected. The Bayesian Information Criterion (SBC) suggested by Schwartz (1978) is one of the popular criteria for model comparison. For a fitted model (linear or nonlinear) with *p* parameters, the SBC is defined as

$$\text{SBC(p)} = -2 \ \text{log(maximum likelihood function)} + p \times \text{log(n)},$$

where the likelihood function is based on the distribution assumption of the model such as normal or log-normal or other distribution families, and *n* is the sample size. When the random errors ε_i's have a normal distribution, the SBC(p) has the simplified form:

$$\text{SBC(p)} = n \times \log\left(\sum_{i=1}^{n}(Y_i - \hat{Y}_i)^2 / (n-p-1)\right) + p \times \log(\text{n}), \tag{5}$$

where $\hat{Y}_i$ is the fitted value based on one of the candidate models and $\sum_{i=1}^{n}(Y_i - \hat{Y}_i)^2$ is the **Residual Sum of Squares (RSS)** based on the fitted candidate model.

Intuitively, there are two parts in (5), the first part is

$$n \times \log\left(\sum_{i=1}^{n}(Y_i - \hat{Y}_i)^2 / (n-p-1)\right) = n \times \log \hat{\sigma}^2,$$

which is a measure of the goodness-of-fit of the candidate model. In general, increasing the number of parameters in a model will improve the goodness-of-fit of the model to the data regardless how many parameters are in the **true model** that generated the data. When a model with too many predicators (significant or not significant ones) is fitted to a data set, we may get a perfect fit but the model will be useless for inference such as prediction. In statistics, fitting a model with too many unnecessary parameters is called *over-fitting*. The second part in SBC, $p \times \log(n)$, puts a penalty term on the complexity of a candidate model, which will increase when the number of parameters in a candidate model increases. Thus the criterion SBC requires a candidate model fitting the data well and penalizing the complexity of the model. **For a group of candidate models, the SBC value can be calculated for each of the models and the preferred model is the one with the lowest SBC value.**

III. Change Point Analysis of Suwannee River Algal Data

1. Mean Abundance (Cell Density) vs Mean NOx

a). Change Point Analysis

Table 2 presents the mean NOx and mean abundance data at stations along the Suwannee river and its two major tributaries (Withlacoochee and Santa Fe). The data were collected by the Suwannee River Water Management District (SRWMD). The first column of the table gives the station name. Columns 3 and 4 list the mean NOx and mean abundance at the 13 stations. Among stations with mean NOx above 0.4, it was noticed that station SUW275 reported much lower mean abundance (163243.90). The authors of this report consulted with Mr. Robert Mattson of SRWMD and learned that "the site SUW275 is 'Suwannee River at Gopher River' that is located way, way down on the river." Mr. Mattson considers that SUW275 is the upper, tidal freshwater region of the Suwannee estuary. Current velocities there can be quite strong, and it also may be that the area got a short "shock" of salinity during the drought of 1990-91, even though it is usually a totally freshwater site. Furthermore, Mr. Mattson commented that "the site is a bit different and may not be entirely comparable to upstream, riverine sites such as SUW100 (Suwannee River at Ellaville - at the confluence with the Withlacoochee) and SUW130 (Suwannee River near Luraville, between Ellaville and Branford)."

Based on discussion between the authors of this report and Mr Mattson, we think that the data at station SUW275 is not comparable with those at other stations. Thus the data at SUW275 will be removed from the change point analysis in this report. After removing the data at Station

SUW275, the mean NOx and mean abundance data at the remaining 12 stations of the Suwannee River system are listed in the last two columns in Table 2.

Change point analysis was performed for mean abundance vs mean NOx. One change points was detected at the mean NOx values of 0.401, which was from the station SUW100. The change point has the statistic $L(l_1) = 6.39$ and is significant at the 5% level (95% confidence). Figure 4 presents the fitted step-function regression model to the mean abundance values. The R-square of the regression is 0.803, indicating that the step-function regression model fits the mean abundance values very well.

Table 2. Mean NOx and Mean Abundance Data at the Suwannee River Stations

	Original Data		**Data Used in The Change Point Analysis**	
Station	**Mean NOx**	**Mean abundance**	**Mean NOx**	**Mean abundance**
SUW010	0.035	109810.67	0.035	109810.67
SFR020	0.064	69202.03	0.064	69202.03
SFR040	0.186	189050.82	0.186	189050.82
WIT020	0.223	191812.50	0.223	191812.50
WIT010	0.256	176643.67	0.256	176643.67
WIT030	0.286	241469.20	0.286	241469.20
SUW100	0.401	479615.50	0.401	479615.50
SUW130	0.420	520475.42	0.420	520475.42
SUW275	**0.466**	**163243.90**		
SFR070	0.565	663744.24	0.565	663744.24
SUW150	0.589	920012.14	0.589	920012.14
SUW240	0.671	588875.04	0.671	588875.04
SUW140	0.900	525038.75	0.900	525038.75

Change Points: 1) Mean NOx = 0.401 with the test statistic of 6.39 and confidence level over 95%;
2) Highlighted numbers are the mean NOx-Abundance values at the change point.
3) Notice that the mean NOx value just before the change point is 0.286. Critical changes in mean abundance actually happened as the mean NOx changed from 0.286 to 0.401.

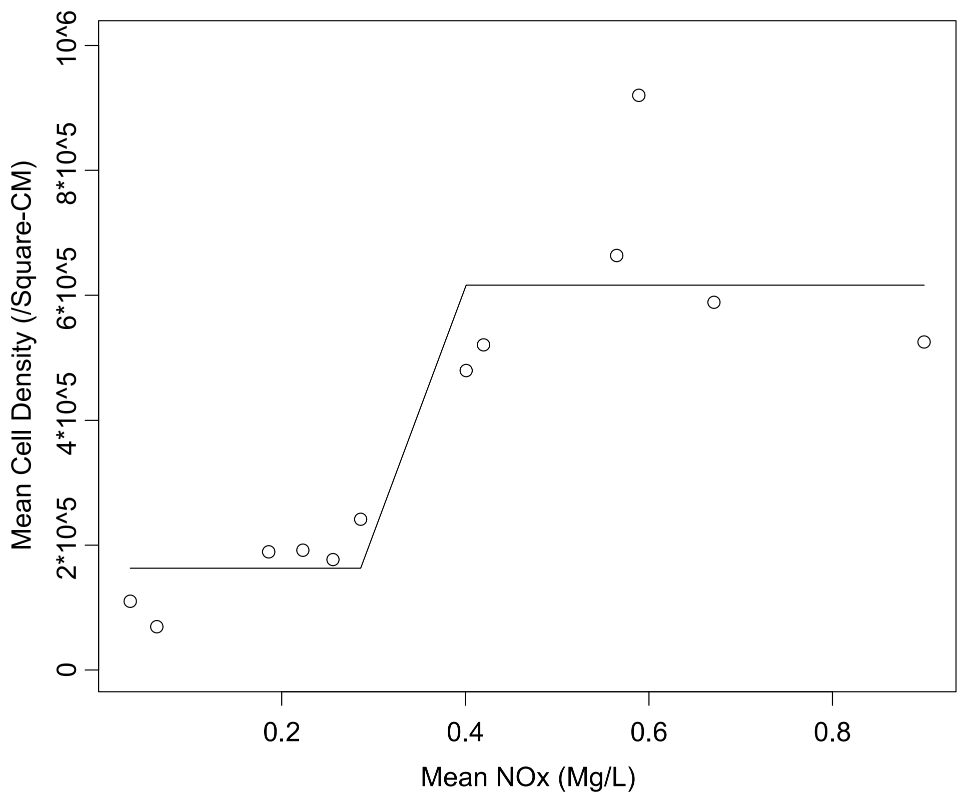

Figure 4. Mean Cell Density vs Mean NOx Change-Point Model (Step Function)

Change Points: Mean NOx=0.401 with the test statistic of 6.39 and confidence level over 95%;

b). Model Comparison

For the purpose of model comparison, two other models, the linear regression model in (3) and the non-linear regression model in (4), were also fitted to the data. Figure 5 presents the two fitted models.

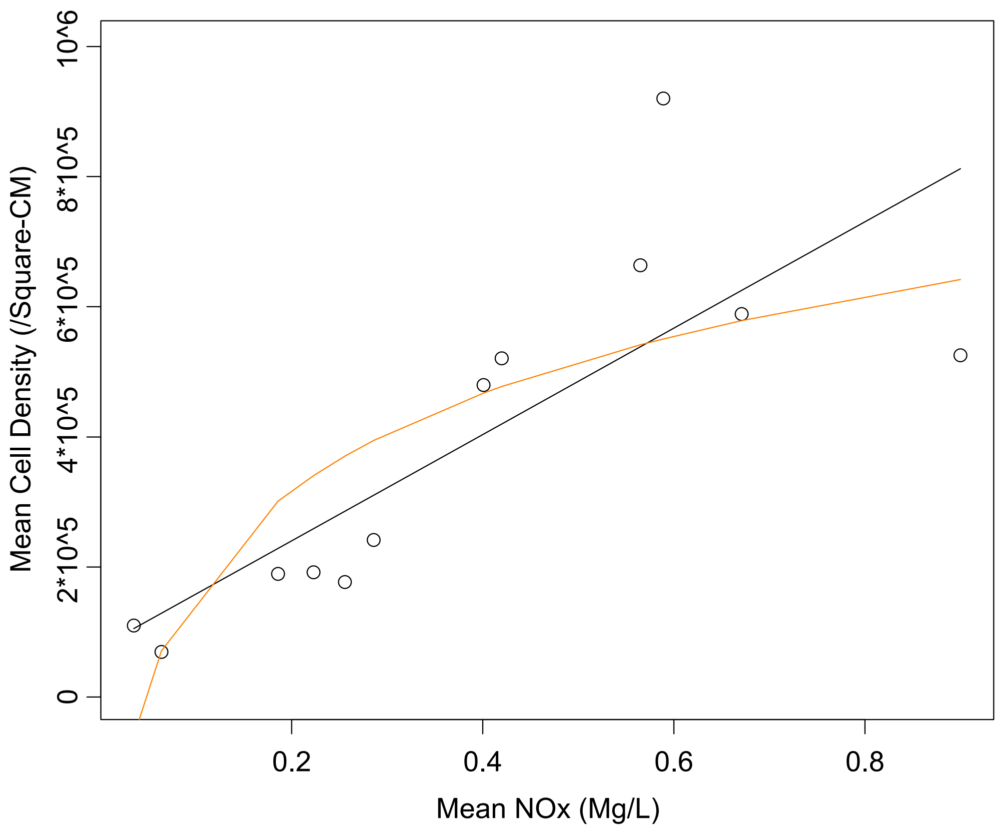

Figure 5. Linear Model (Solid Black), and Non-Linear Model (Mean Cell Density on log(Mean NO), Red)

The three fitted regression models are presented in Table 3. The SBC values for the change-point model, the linear regression model, and the non-linear regression model are 286.22, 293.32, and 294.13, respectively. Thus, the change-point model was the best model among the three models. Based on the fitted change-point model, the change point at Mean NOx of 0.401 is extremely significant (with p-values =0.0001). The mean abundance value at the change point increased 453295.37.

Table 3. Fitted Regression Models

Model 1. Step-Function Regression (Change Point Model) :

```
Coefficients:
                  Value  Std. Error     t value    Pr(>|t|)
(Intercept) 162998.1482  50152.6502      3.2500      0.0087
  NOx_0.401 453295.3680  70926.5581      6.3911      0.0001

Residual standard error: 122800 on 10 degrees of freedom
Multiple R-Squared: 0.8033
F-statistic: 40.85 on 1 and 10 degrees of freedom, the p-value is
   0.00007923
```

SBC Value: 286.22

Model 2. Linear Regression Model:

```
Coefficients:
                  Value  Std. Error     t value    Pr(>|t|)
(Intercept)  76552.0120  87601.4494      0.8739      0.4027
         MN 817477.3373 191903.4375      4.2598      0.0017

Residual standard error: 165100 on 10 degrees of freedom
Multiple R-Squared: 0.6447
F-statistic: 18.15 on 1 and 10 degrees of freedom, the p-value is
   0.001663
```

SBC Value: 293.32

Model 3. Non-Linear Regression Model:

```
Coefficients:
                  Value  Std. Error     t value    Pr(>|t|)
(Intercept) 664741.5001  84092.3831      7.9049      0.0000
        MN1 215985.5866  53485.6084      4.0382      0.0024

Residual standard error: 170800 on 10 degrees of freedom
Multiple R-Squared: 0.6199
F-statistic: 16.31 on 1 and 10 degrees of freedom, the p-value is
   0.002368
```

SBC Value: 294.13

2. Mean Biomass vs Mean NOx

a). Change Point Analysis

Table 4 presents the mean NOx and mean biomass data at stations along the Suwannee river and its two major tributaries (Withlacoochee and Santa Fe). The first column of the table gives the station name. Columns 3 and 4 list the mean NOx and mean biomass at the 13 stations. Based on the discussion between the authors of this report and Mr Mattson, we decided to remove the data at the site SUW275 from the change point analysis in this report. After removing the data at Station SUW275, the mean NOx and mean Biomass data at the remaining 12 stations of the Suwannee River system are listed in the last two columns in Table 4.

Change point analysis was performed for mean biomass vs mean NOx. One change points was detected at the mean NOx values of 0.420, which was from the station SUW100. The change point has the statistic $L(l_1) = 6.10$ and is significant at the 5% level (95% confidence). Figure 6 presents the fitted step-function regression model to the mean abundance values. The R-square of the regression is 0.788, indicating that the step-function regression model fits the mean abundance values very well.

Table 4. Mean NOx and Mean Biomass Data at the Suwannee River Stations

	Original Data		Data Used in The Change Point Analysis	
Station	**Mean NOx**	**Mean abundance**	**Mean NOx**	**Mean Biomass**
SUW010	0.035	1.341	0.035	1.341
SFR020	0.064	1.348	0.064	1.348
SFR040	0.186	1.356	0.186	1.356
WIT020	0.223	1.867	0.223	1.867
WIT010	0.256	1.456	0.256	1.456
WIT030	0.286	2.187	0.286	2.187
SUW100	0.401	2.590	0.401	2.590
SUW130	0.420	4.205	0.420	4.205
SUW275	**0.466**	**2.173**		
SFR070	0.565	4.693	0.565	4.693
SUW150	0.589	4.636	0.589	4.636
SUW240	0.671	2.617	0.671	2.617
SUW140	0.900	4.644	0.900	4.644

Change Points: 1) Mean NOx=0.420 with the test statistic of 6.10 and confidence level over 95%;

2) Highlighted numbers are the mean NOx-Abundance values at the change point.

3) Notice that the mean NOx value just before the change point is 0.401. Critical changes in mean abundance actually happened as the mean NOx changed from 0.401 to 0.420.

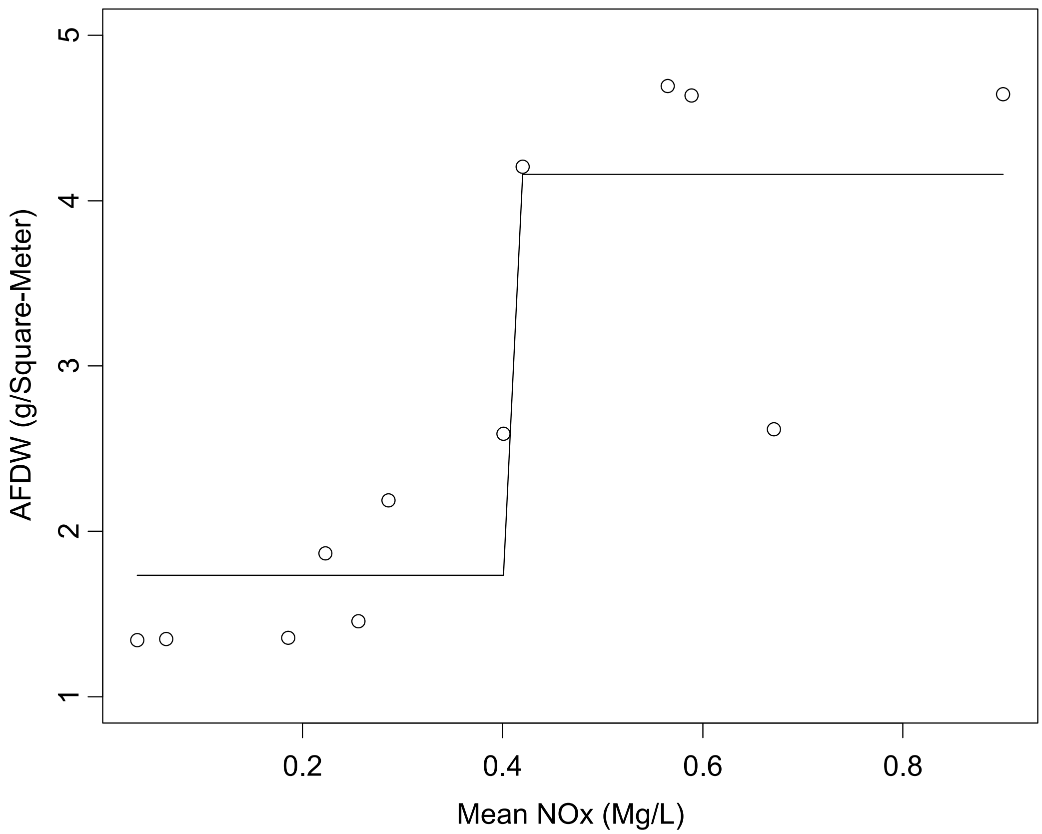

Figure 6. Mean Biomass vs Mean NOx Change-Point Model (Step Function)

Change Points: Mean NOx=0.420 with the test statistic of 6.10 and confidence level over 95%;

b). Model Comparison

For the purpose of model comparison, two other models, the linear regression model in (3) and the non-linear regression model in (4), were also fitted to the data. Figure 7 presents the two fitted models.

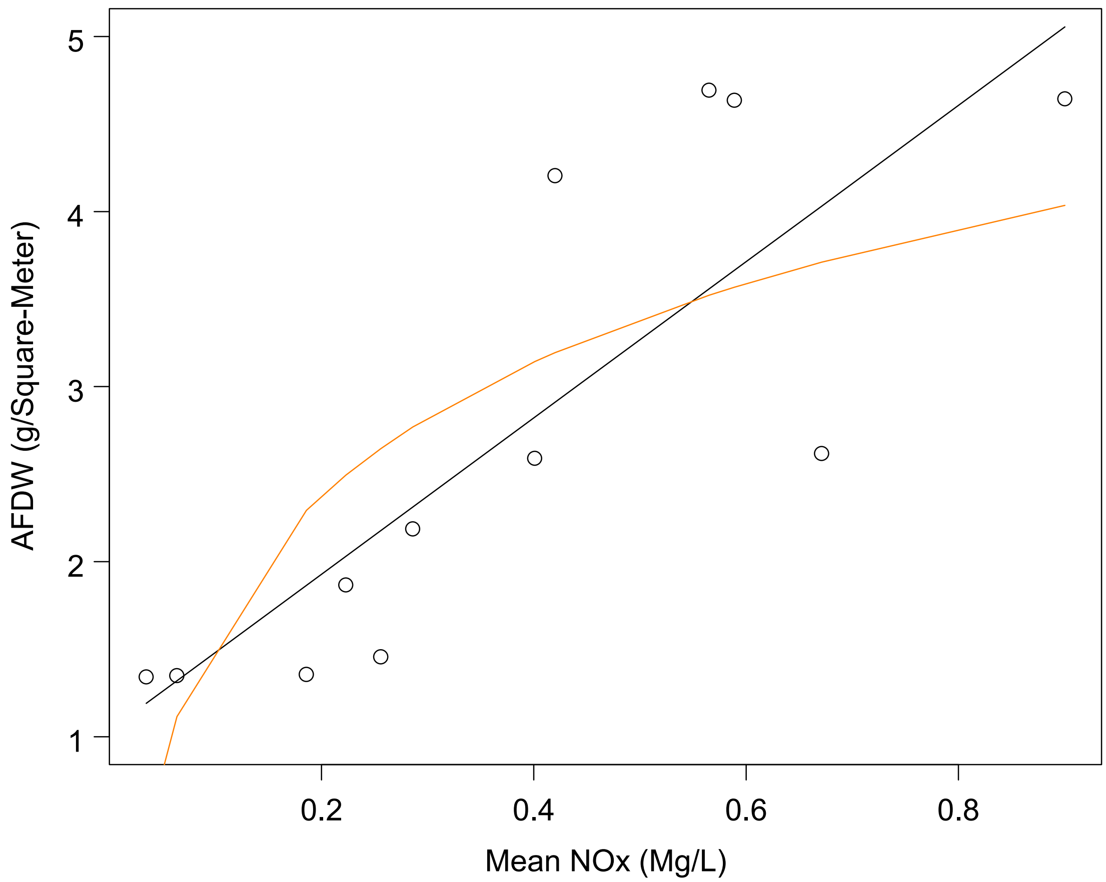

Figure 7. Linear Model (Solid Black) and Non-Linear Model (Mean Biomass on log(Mean NO), Red)

The three fitted regression models are presented in Table 5. The SBC values for the change-point model, the linear regression model, and the non-linear regression model are -4.33, 0.651, and 4.09, respectively. Thus, the change-point model was the best model among the three models. Based on the fitted change-point model, the change point at Mean NOx of 0.420 is extremely significant (with p-values =0.0001). The mean abundance value at the change point increased 2.424.

Table 5. Fitted Regression Models

Model 1. Step-Function Regression (Change Point Model) :

```
Coefficients:
             Value Std. Error t value Pr(>|t|)
(Intercept) 1.7350 0.2565     6.7630  0.0000
         x1 2.4240 0.3974     6.0991  0.0001

Residual standard error: 0.6788 on 10 degrees of freedom
Multiple R-Squared: 0.7881
F-statistic: 37.2 on 1 and 10 degrees of freedom, the p-value is
   0.0001158
```

SBC Value: -4.33

Model 2. Linear Regression Model:

```
Coefficients:
             Value Std. Error t value Pr(>|t|)
(Intercept) 1.0344 0.4432     2.3341  0.0418
         MN 4.4663 0.9708     4.6006  0.0010

Residual standard error: 0.8353 on 10 degrees of freedom
Multiple R-Squared: 0.6791
F-statistic: 21.17 on 1 and 10 degrees of freedom, the p-value is
   0.0009793
```

SBC Value: 0.651

Model 3. Non-Linear Regression Model:

```
Coefficients:
             Value Std. Error t value Pr(>|t|)
(Intercept) 4.1526 0.4746     8.7496  0.0000
        MN1 1.1052 0.3019     3.6611  0.0044

Residual standard error: 0.9639 on 10 degrees of freedom
Multiple R-Squared: 0.5727
F-statistic: 13.4 on 1 and 10 degrees of freedom, the p-value is
   0.004381
```

SBC Value: 4.09

3. Summary and Conclusions

In this report, change point analysis was preformed for the algal data at stations along the Suwannee river and its two major tributaries (Withlacoochee and Santa Fe). **The main findings in this report are the followings:**

1) **For the change point analysis of mean abundance vs mean NOx, one change point was detected at NOx=0.401 that is corresponding to the data at the site SUW100. The change point is significant at the confidence level 95%. Model comparison shows that the change point model fit the data better than the linear regression model and a nonlinear regression model. The mean NOx value just before the change point is 0.286, indicating that critical changes in mean abundance actually happened as the mean NOx changed from 0.286 to 0.401.**

2) **For the change point analysis of mean biomass vs mean NOx, one change point was detected at NOx=0.420 that is corresponding to the data at the site SUW130. The change point is significant at the confidence level 95%. Model comparison shows that the change point model fit the data better than the linear regression model and a nonlinear regression model. The mean NOx value just before the change point is 0.401, indicating that critical changes in mean abundance actually happened as the mean NOx changed from 0.401 to 0.420.**

Based on this analysis, we conclude that the major changes in mean abundance and mean biomass happened at mean NOx around 0.4. Further studies may be needed to confirm this finding.

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Florida Passive Nitrogen Removal Study

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Watermark Engineering Group, Inc.

AET

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- Damann Anderson
- Hillsborough County, Florida

Florida Passive Nitrogen Removal Study

- Objective: evaluate enhanced nitrogen removal from on-site wastewater using *passive systems*
- Influent: septic tank effluent
- Goal: reduce total nitrogen

Florida Passive Nitrogen Removal Study Tasks

- Literature review and database
- Experimental evaluation
- Economic analysis
- Recommendations

Passive Definition

“ A type of onsite sewage treatment and disposal system that *excludes the use of aerator pumps* and includes *no more than one effluent dosing pump* in mechanical and moving parts and uses a *reactive media* to assist in nitrogen removal ”

Technology Constraints

- No aerators: unsaturated filter for Stage 1
- One pump: otherwise gravity flow
  where to place?
- Reactive media:
  electron donor for denitrification
  alkalinity
  ion exchange

Literature Review Recommendation: Two Stage Filter Process to Reduce TN

- Stage 1 unsaturated filter

  aerobic Organic N and ammonia

  produce low effluent TKN

- Stage 2 saturated

  anoxic oxidized N

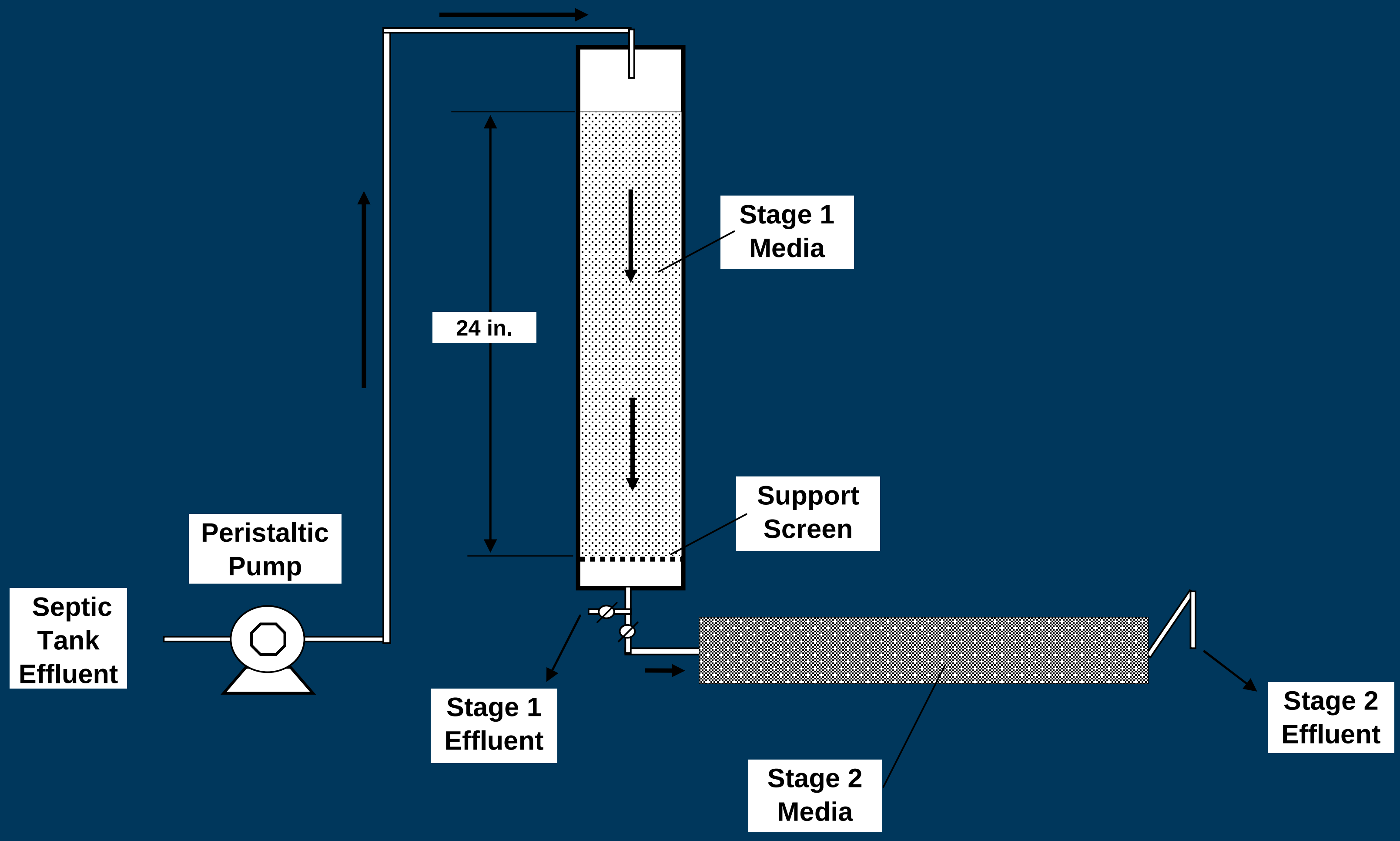
  electron donor for denitrification

  produce low effluent NO_x

Pump Before Stage 1 Filter

- Allows timed dosing
- Nitrification performance of Stage 1 filter
- Potential for Stage 1 recycle
- Flow equalization to entire treatment train
- Gravity flow through second stage filter to dispersal field

Media Evaluation System

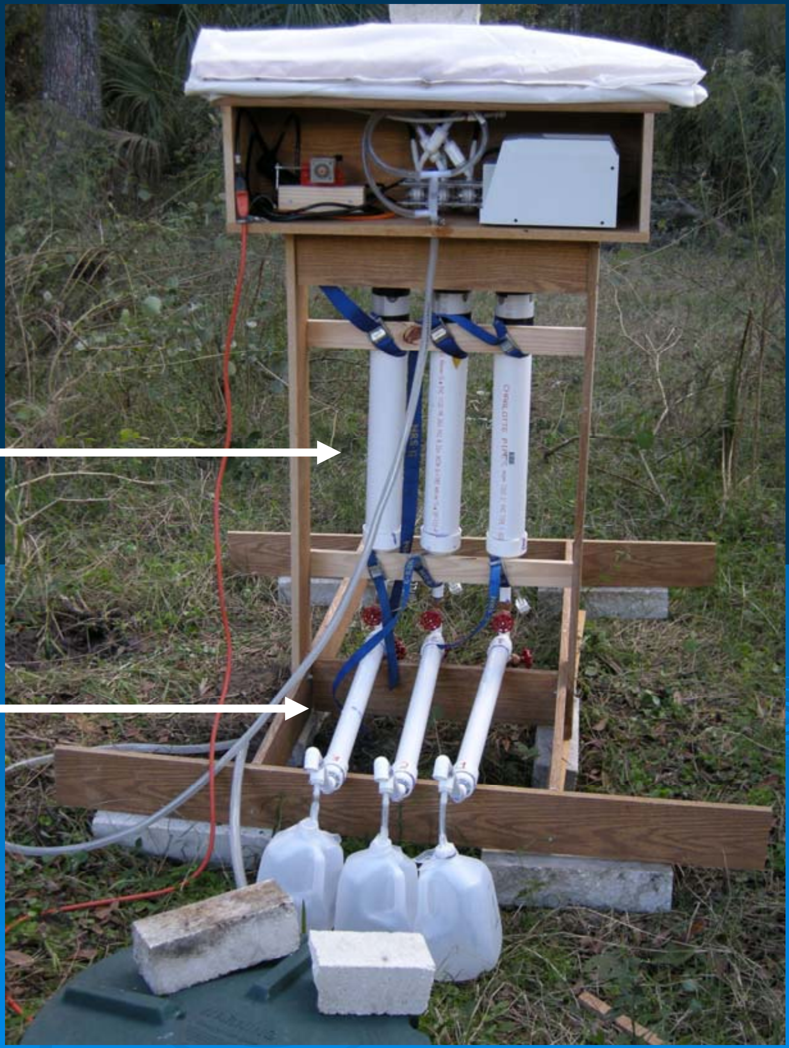


Passive Nitrogen Removal Study

Applied Environmental Technology

Stage 1 (unsaturated)

Stage 2 (saturated)



Media Selection Factors
Stage 1 Aerobic Unsaturated

- Particle size
- Specific surface area
- External porosity
- Air filled porosity
- Water retention capacity
- Cation Exchange Capacity (CEC)

Stage 1 Media (nitrification)

Zeo-Pure clinoptilolite

Expanded clay

Tire crumb

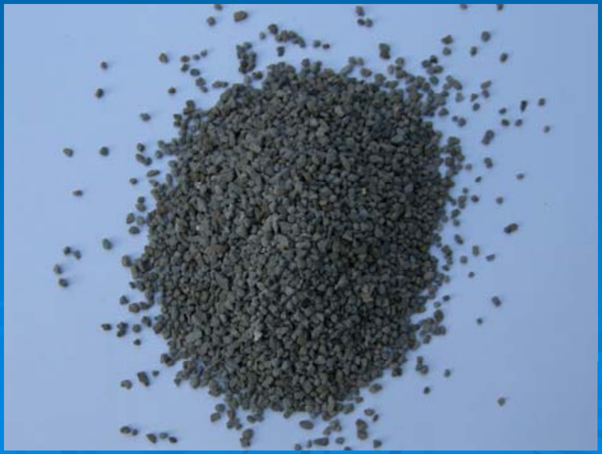
Media Selection Factors
Stage 2 Anoxic Saturated

- Reactivity: electron donor release rate
- Longevity
- Alkalinity supply
- Anion Exchange Capacity (AEC)

Stage 2 Media (denitrification)

Elemental sulfur

Expanded shale



Oyster shell

Autotrophic Nitrogen Reduction

Nitrification

$NH_4^+ + 1.815\ O_2 + 0.1304\ CO_2 \quad = \quad 0.0261\ C_5H_7O_2N + 0.973\ NO_3\text{-} \quad .921\ H_2O + 1.973\ H^+$

Y = 0.21 gX / gN 7.05 g alkalinity / g N

Autotrophic Denitrification

$NO_3^- + 1.002\ S + 0.2205\ CO_2 + 0.65702\ H_2O$

$= \quad 0.0441\ C_5H_7O_2N + 0.4779\ N_2 + 1.002\ SO_4^{-2} + 1.005\ H^+$

Y = 0.36 gX / gN 3.59 g alkalinity / g N

Filter Media

Stage	Filter	Column ID, inch.	Media depth, inch	Media placement	Media
Stage 1 unsaturated aerobic	1A	3.0	24.0	Stratified	**Clinoptilolite** depth (in.) diameter (mm) top 8 3 - 5 8 0.8-2.3 6 0.5-1.1 1 0.8-2.3 1 3 - 5 bottom
	1B				**Expanded Clay** depth (in.) diameter (mm) top 8 3 - 5 8 0.8-2.3 6 0.5-1.1 1 0.8-2.3 1 3 - 5 bottom
	1C				**Tire Crumb** depth (in.) diameter (mm) top 8 3 - 5 8 0.8-2.3 6 0.5-1.1 1 0.8-2.3 1 3 - 5 bottom
Stage 2 saturated anoxic	2A	1.5	24.0	Nonstratified	75% elemental sulfur 25% oyster shell
	2B				60% elemental sulfur 20% oyster shell 20% expanded shale
	2C				45% elemental sulfur 15% oyster shell 40% expanded shale

Expanded shale/oyster shell/elemental sulfur

Test Site: Flatwoods Park, Hillsborough County, Florida

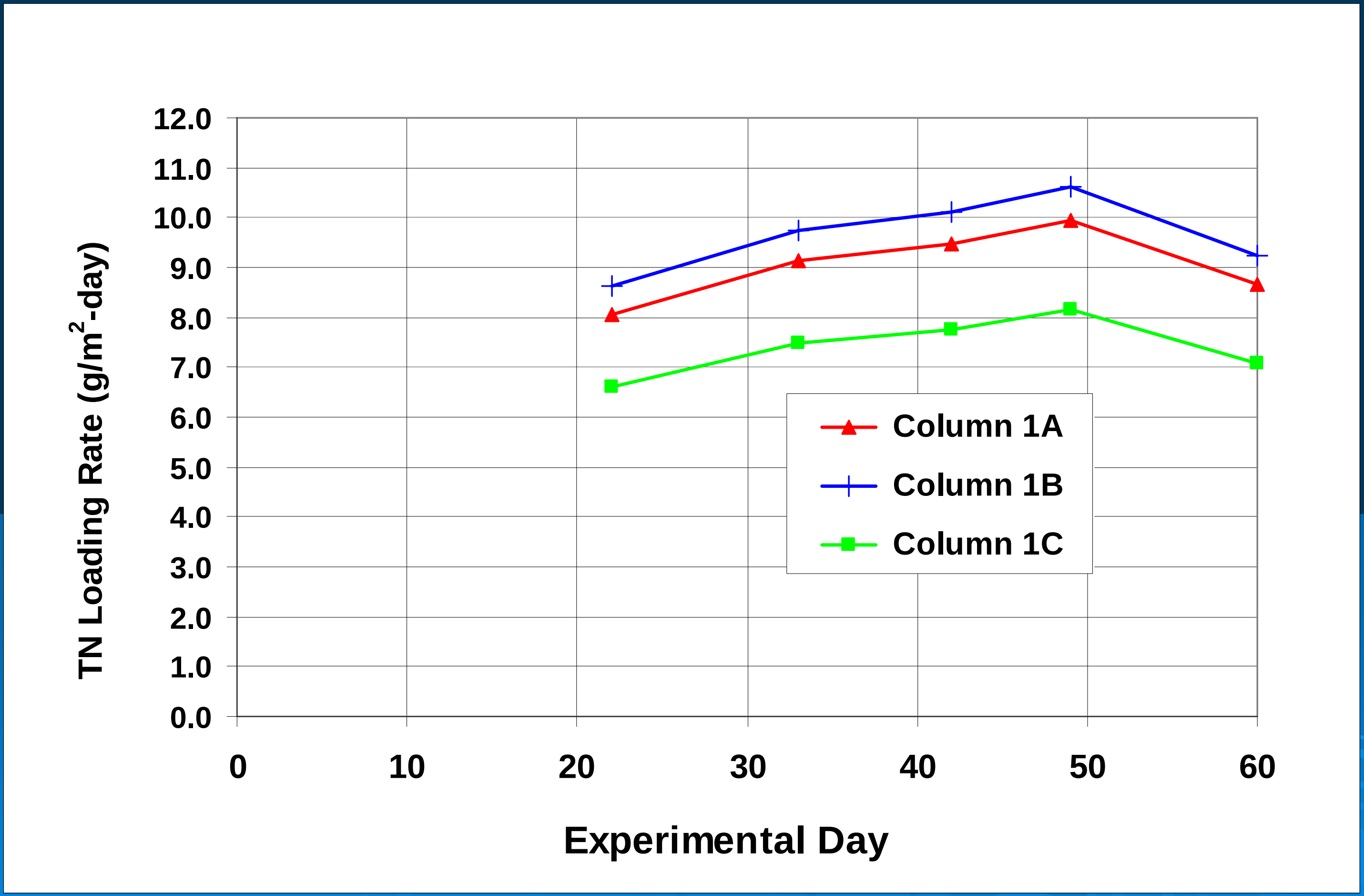
Test Site: Flatwoods Park, Hillsborough County, Florida

Operating Features (one pass)

Feature	Stage 1 Unsaturated	Stage 2 Saturated
Doses/day	48	48
Flow, gpd/ft^2	3.0	12.0
Resident water volume, liter[1]	0.21	0.28
Single dose volume / resident water volume	0.056	0.074
Average residence time, hour	9.0	12.0

[1] Stage 1: 50% external porosity, 15% water filled
Stage 2: 40% porosity

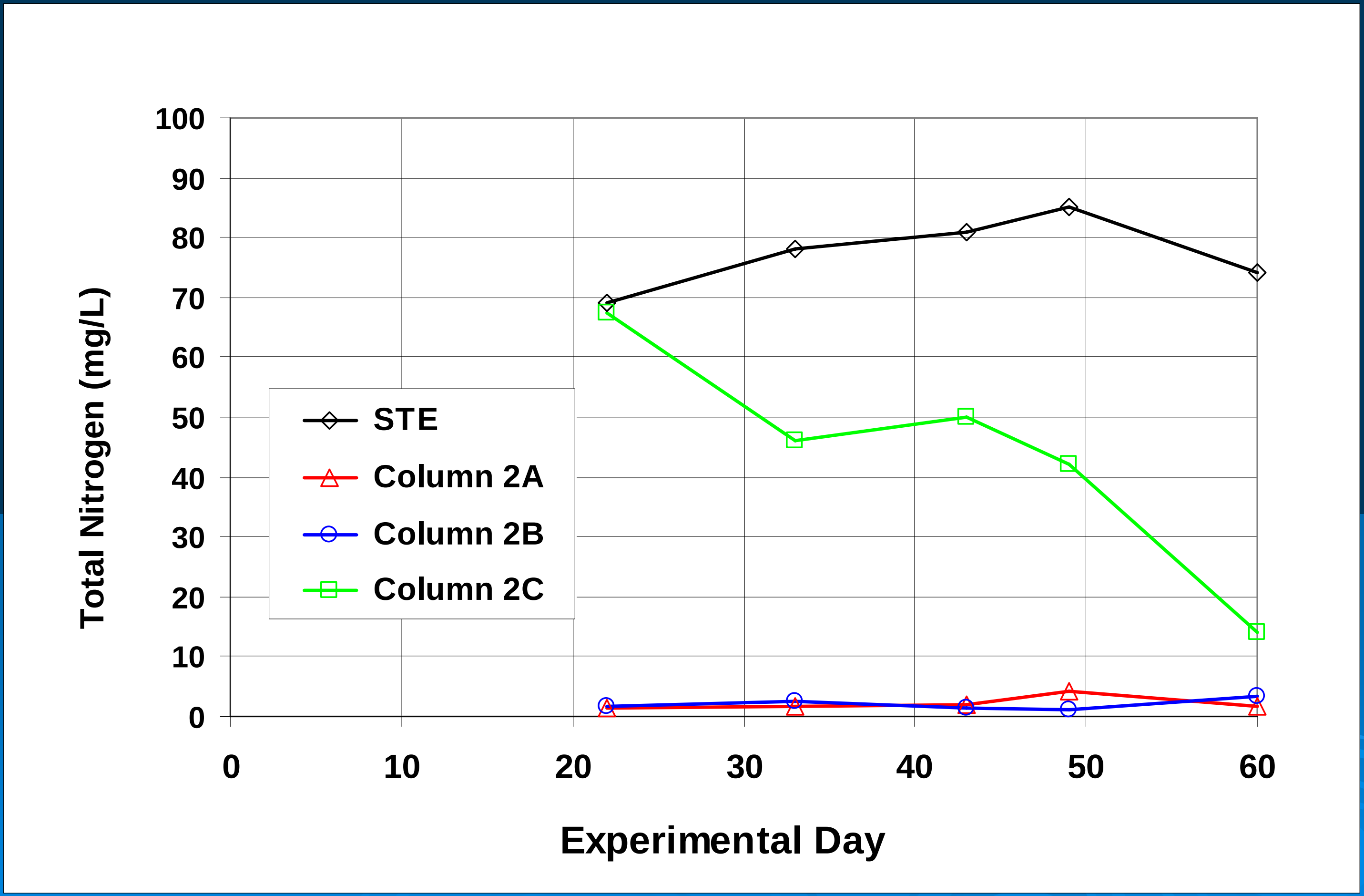
Nitrogen Loading



Feed: Septic Tank Effluent

System	Average	Standard Deviation	Range	n
Total Nitrogen	77.4	6.2	69 - 85	5
Organic Nitrogen	20.7	28.6	3.0 - 71	5
NH_3-N	52.5	30.2	2.6 - 74	5
NO_x-N	4.2	9.4	.028 - 21	5
C-BOD_5	203	71	140 - 180	3
TSS	18.7	5.5	15 - 25	3

2 Stage System: Effluent TN



Nitrogen: Average (n=5)

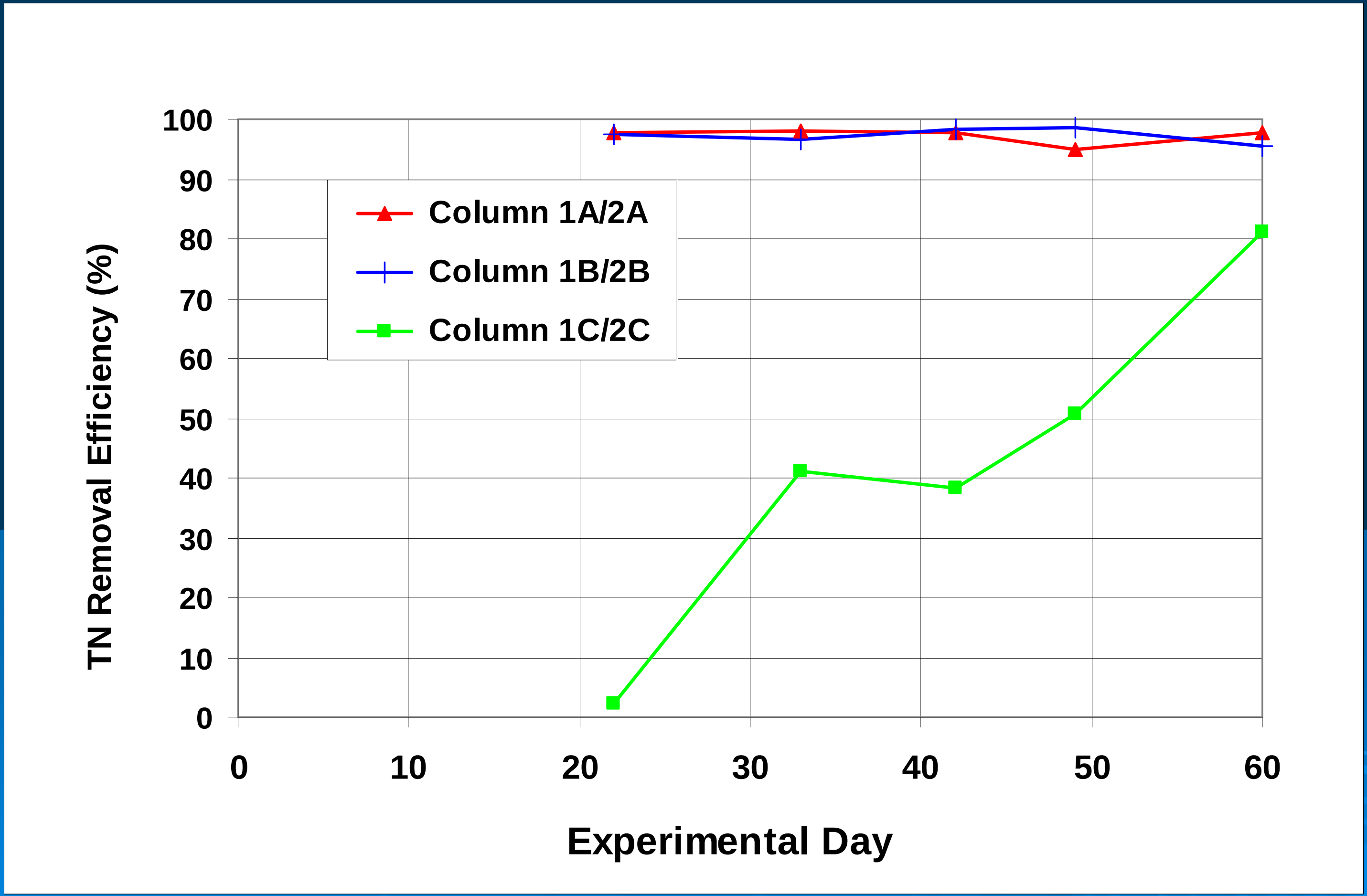
Sample Point	Total Nitrogen	Organic N	NH_3-N	NO_x-N
Influent (STE)	77.4	20.7	52.5	4.2
Stage 1 Effluent				
1A Clinoptilolite	35.2	2.2	0.1*	26.3
1B Expanded clay	56.2	0.9	0.1	55.2
1C Tire crumb	65.4	2.4	26.6	36.4
Stage 2 Effluent				
2A 75% Sulfur	2.2	2.1	0.11	0.03
2B 60% Sulfur	2.1	1.4	0.61	0.02
2C 45% Sulfur	43.9	1.8	34.8	7.3

* n = 4

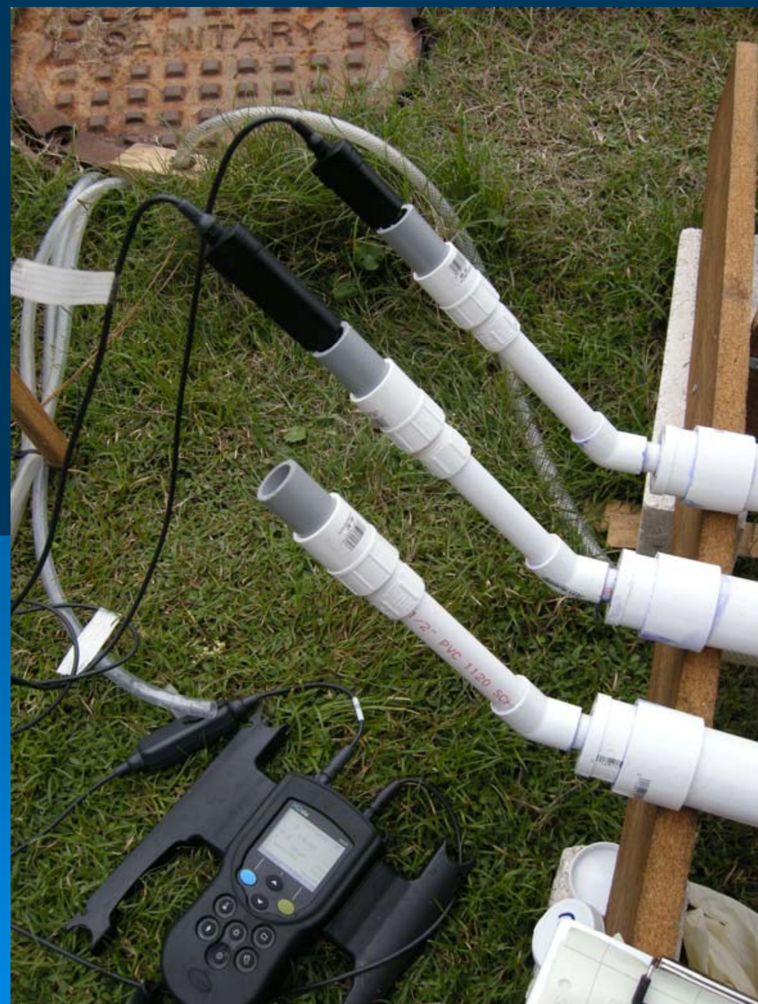
Two Stage Removal Efficiency

System	Media	Total Nitrogen		Total Inorganic Nitrogen	
		Average	Range	Average	Range
1	Clinoptilolite / 75% Sulfur	97.1	94.9 - 97.9	99.8	99.7 - 99.9
2	Expanded Clay / 60% Sulfur	97.7	96.6 - 98.6	98.1	97.5 - 98.7
3	Tire Crumb / 45% Sulfur	33.0	2.2 - 50.6	34.4	2.0 - 52.5

Total Nitrogen Removal Efficiency



Stage 2 Effluent LDO and pH Probes



Results: Field Parameters

Sample Point	Dissolved Oxygen, mg/L	pH	Alkalinity, mg/L as $CaCO_3$	Alkalinity Change, mg/L as CaCO3
Influent (STE)	0.008	7.49	435	-
Stage 1				
1A Clinoptilolite	7.28	7.65	283	-133
1B Expanded clay	7.27	7.22	86	-330
1C Tire crumb	7.10	7.42	178	-238
Stage 2				
2A 75% Sulfur	0.06	7.02	437	+154
2B 60% Sulfur	0.05	6.97	225	+139
2C 45% Sulfur	0.93	7.25	294	+116

Stage 1 Removal Efficiency

System	Media	Total Nitrogen	Ammonia Nitrogen
		Average	Average
1	Clinoptilolite	50.6	99.9*
2	Expanded Clay	26.1	99.9
3	Tire Crumb	13.0	60.5

* n = 4

Example System

- Stage 1 Aerobic

  100 ft^2 x 24 in. expanded clay

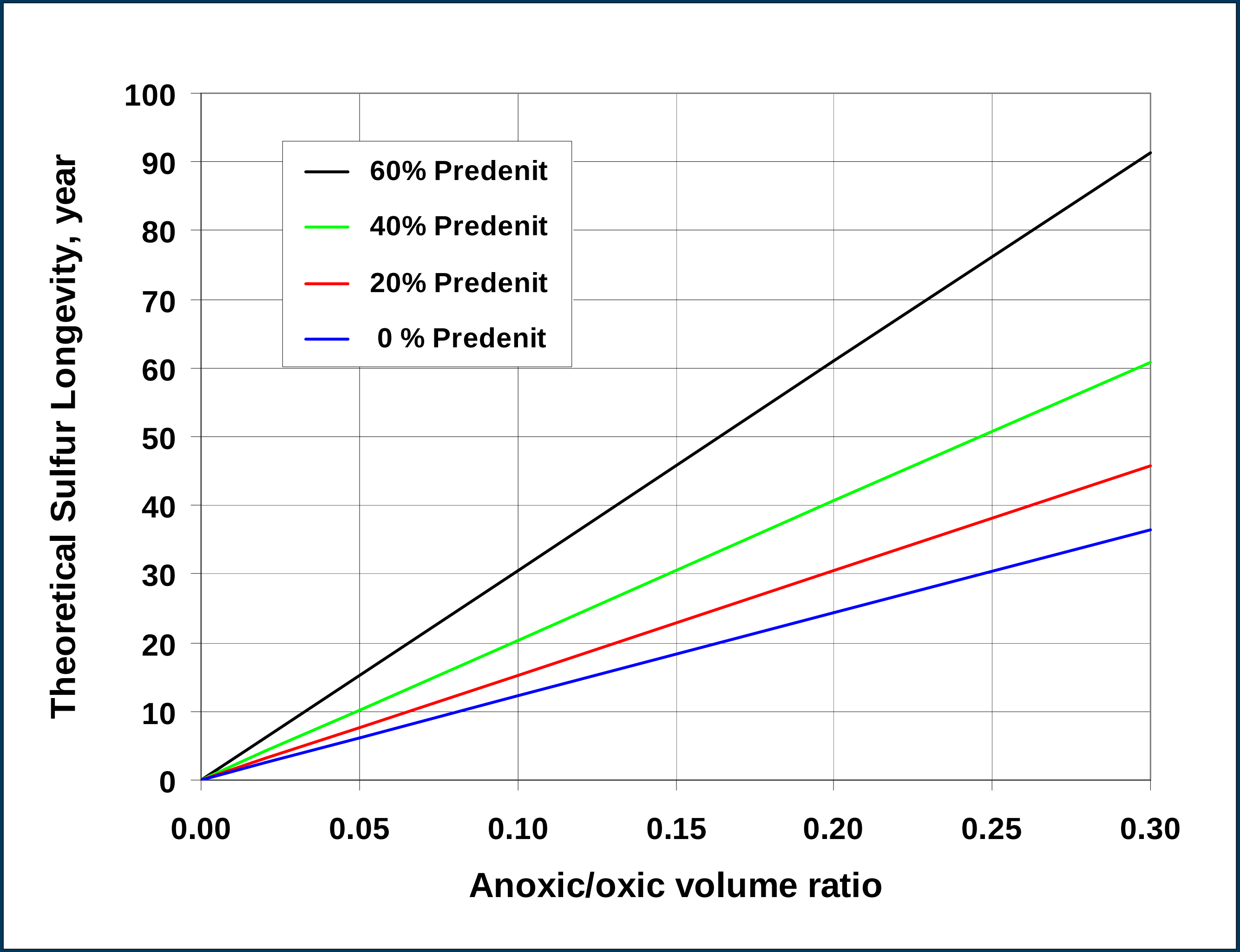
- Stage 2 Anoxic

  Sulfur/oyster/shale 60/20/20 volume

  $V_{anoxic} = 1/4 \; V_{aerobic}$

- Media cost: Stage 1 $700 / Stage 2 $825

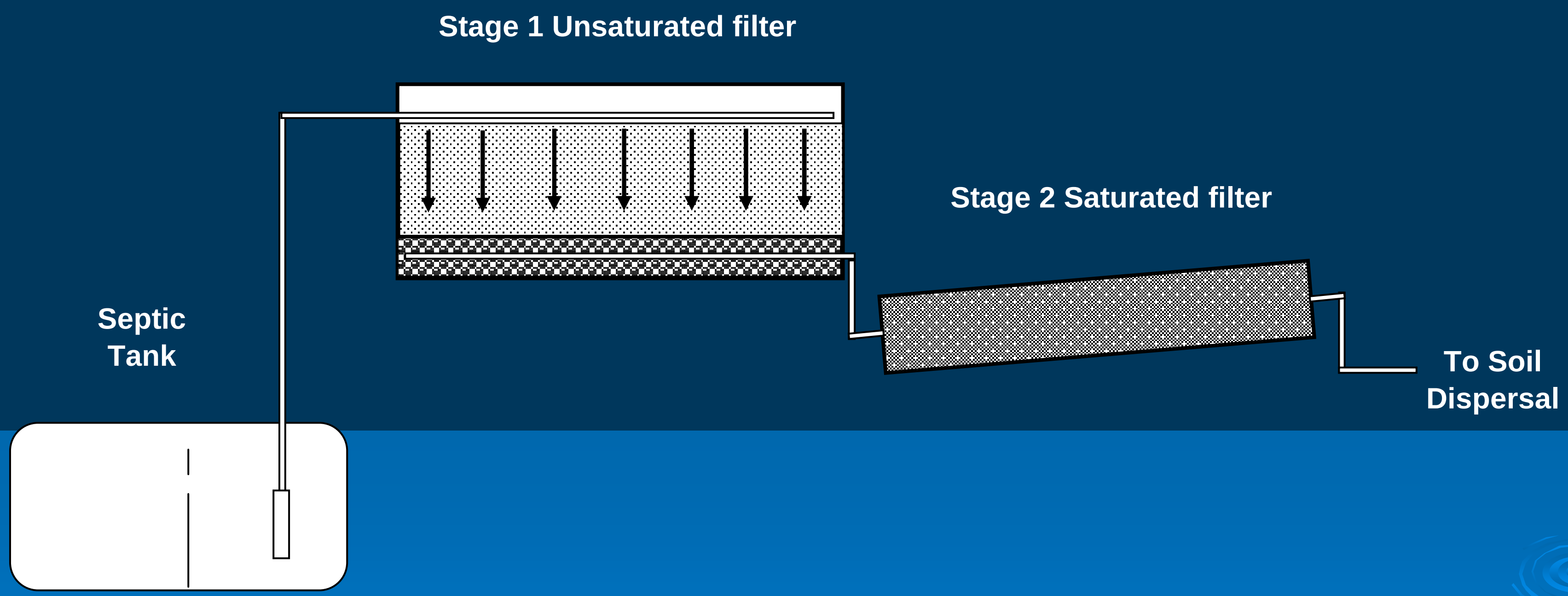
Theoretical Longevity of Electron Donor
4 persons @ 13 gram N/capita-day



Conclusions

- Proof of Two Stage Filter concept for Passive N Removal
- Single pass at 3 gal./ft^2-day
- Total N removal of 97%
- Total N reduced from 77 to 2 mg/L
- Longer term operation needed

Example On-Site Configuration



Economic Analysis

- Objective: Life Cycle Cost Analysis (LCCA)
- Provide equitable comparison of alternatives
- Include costs for:

  Equipment, materials, installation

  Energy, maintenance, monitoring

  Media replacement and residuals
- Base case: 300 gallon per day Florida STE

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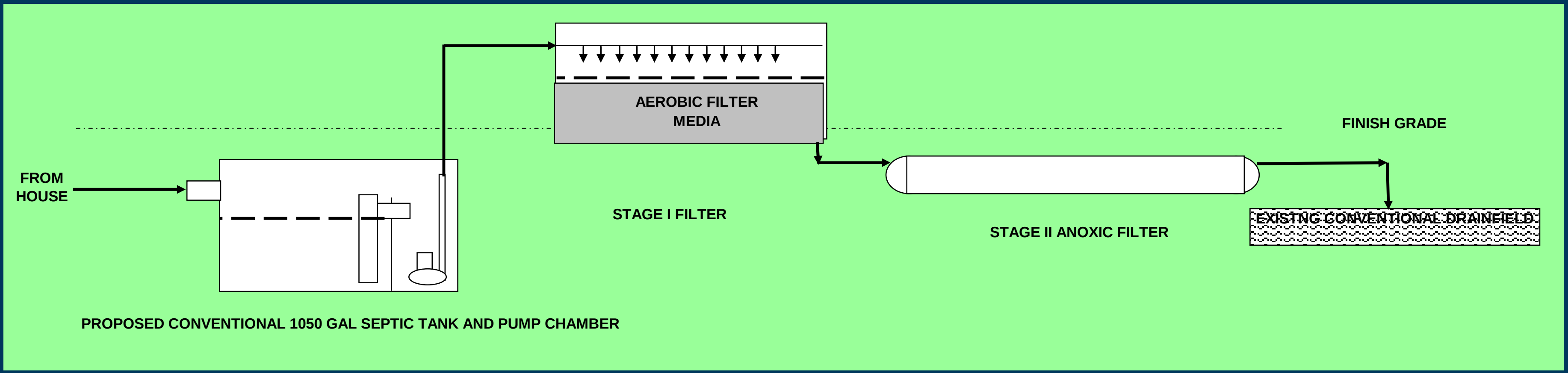
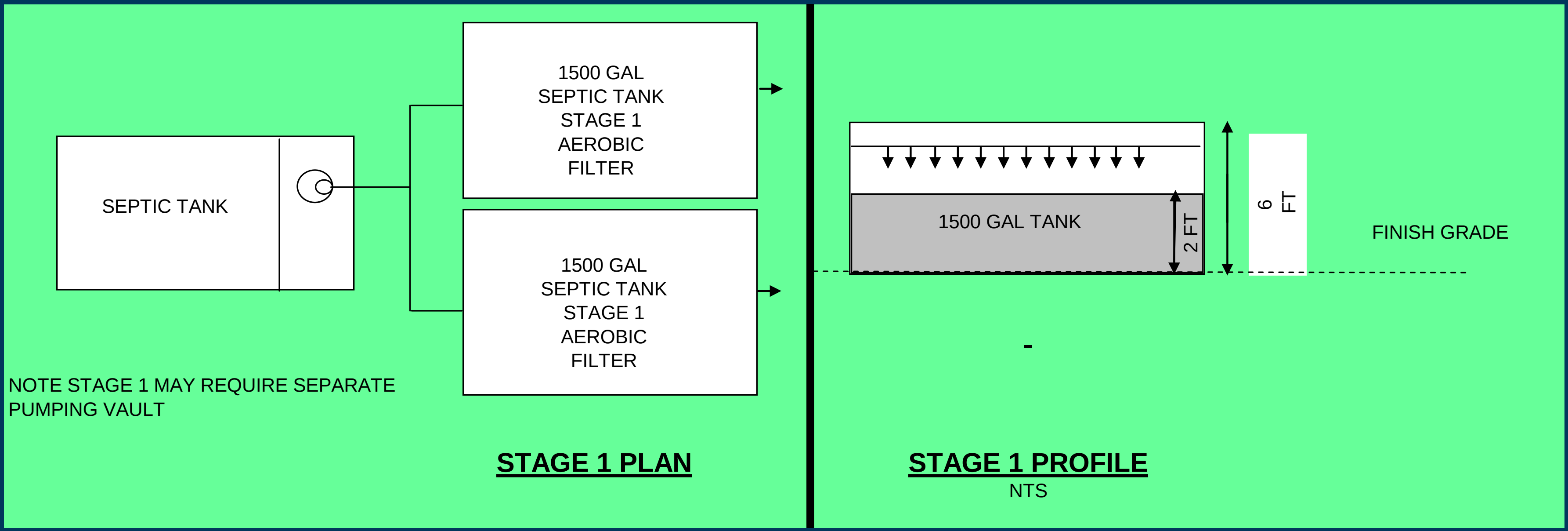
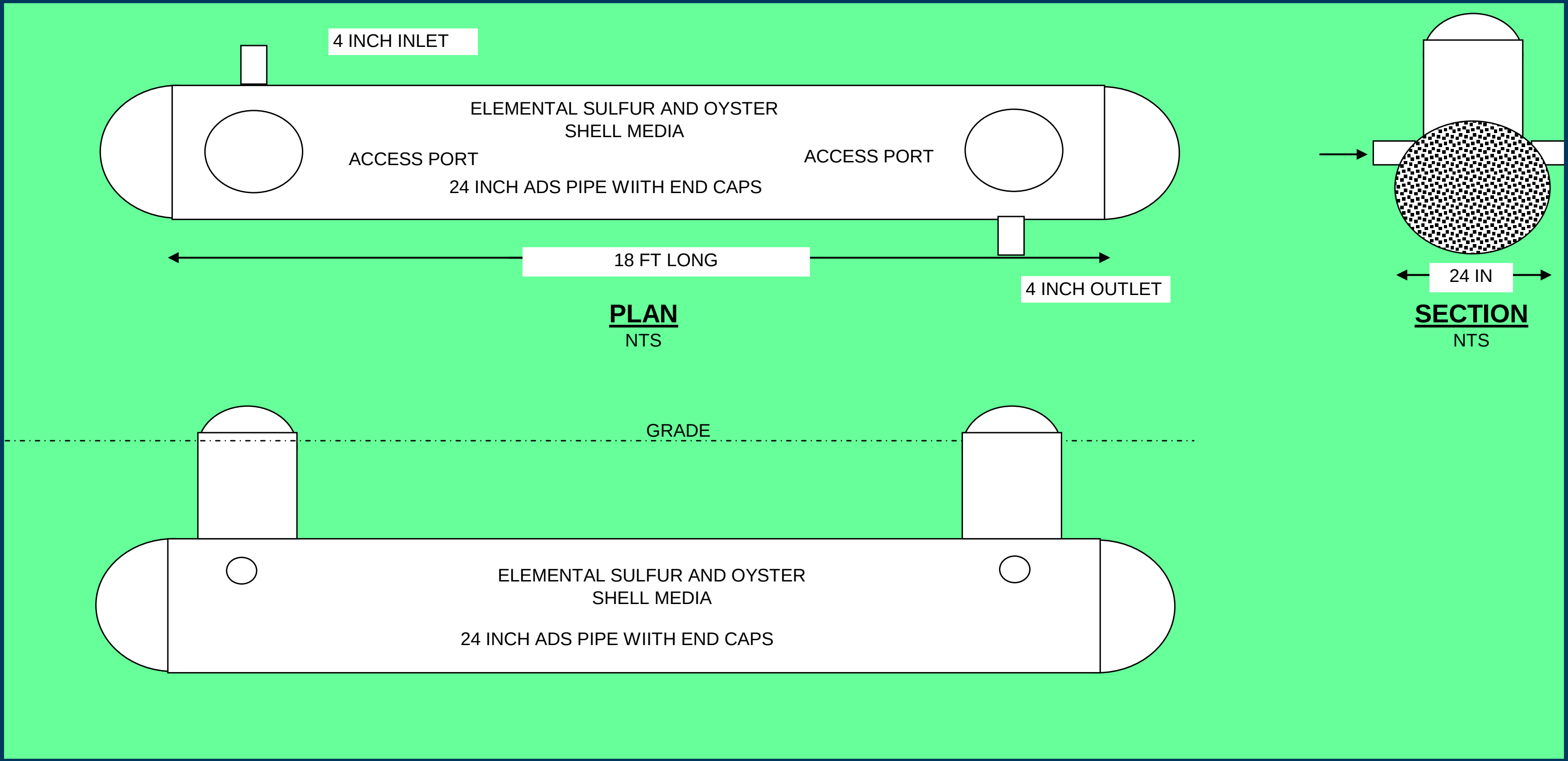
PNRS Schematic



Design Factor Alternatives (300 gpd)



Design Factor Alternatives (300 gpd)



Design Factor Alternatives (300 gpd)

Stage 1 Filter Plan Area, ft^2

- 100
- 75

Stage 1 Media

- Clinoptilolite
- Expanded clay

Stage 2 Filter volume/media replacement interval

- 750 gallon, 15 year
- 375 gallon, 5 year
- 75 gallon, 1 year

O& M and Media Replacement

Item	Unit Cost (2008 $)	Uniform Annual Cost[1] (2008 $)	Present Worth[1] (2008 $)
Operations and Maintenance			
Annual Maintenance, yearly	250	239	7,174
Monitoring Analyses, yearly	100	96	2,870
Electricity, yearly	124	119	3,558
Septic Tank Pumping, 5 year interval	225	43	1,284
Stage 2 Media Replacement[2]			
100 ft^3, once per 15 years	4,304	173	5,198
50 ft^3, once per 5 years	2,152	361	10,844
11 ft^3, once per year	473	452	13,573

[1] 30 year project life, i = 4%.

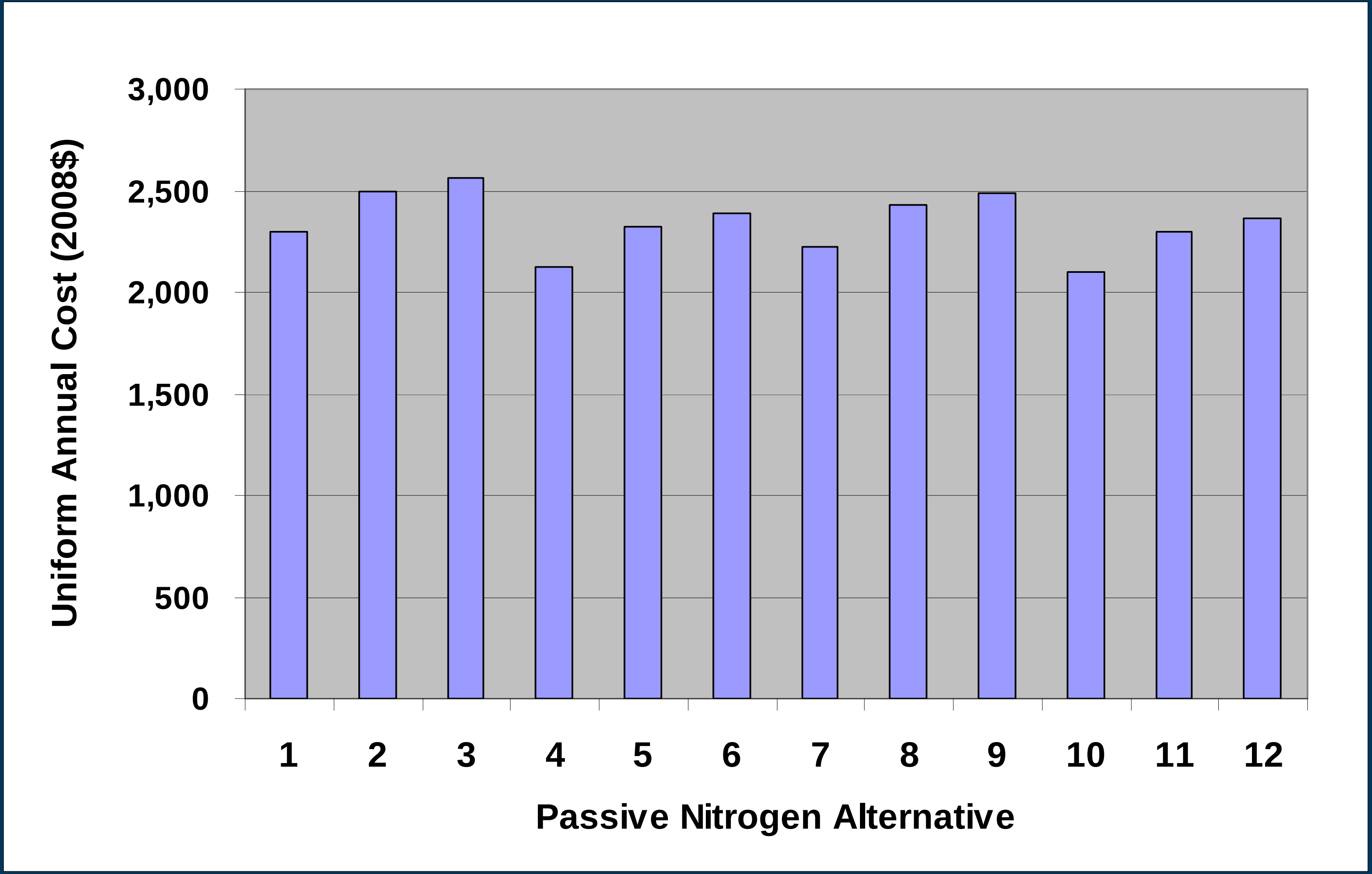
[2] Includes media, labor, materials, and spent media disposal at $0.05/lb.

Alternatives Costs (30 year, 4%)

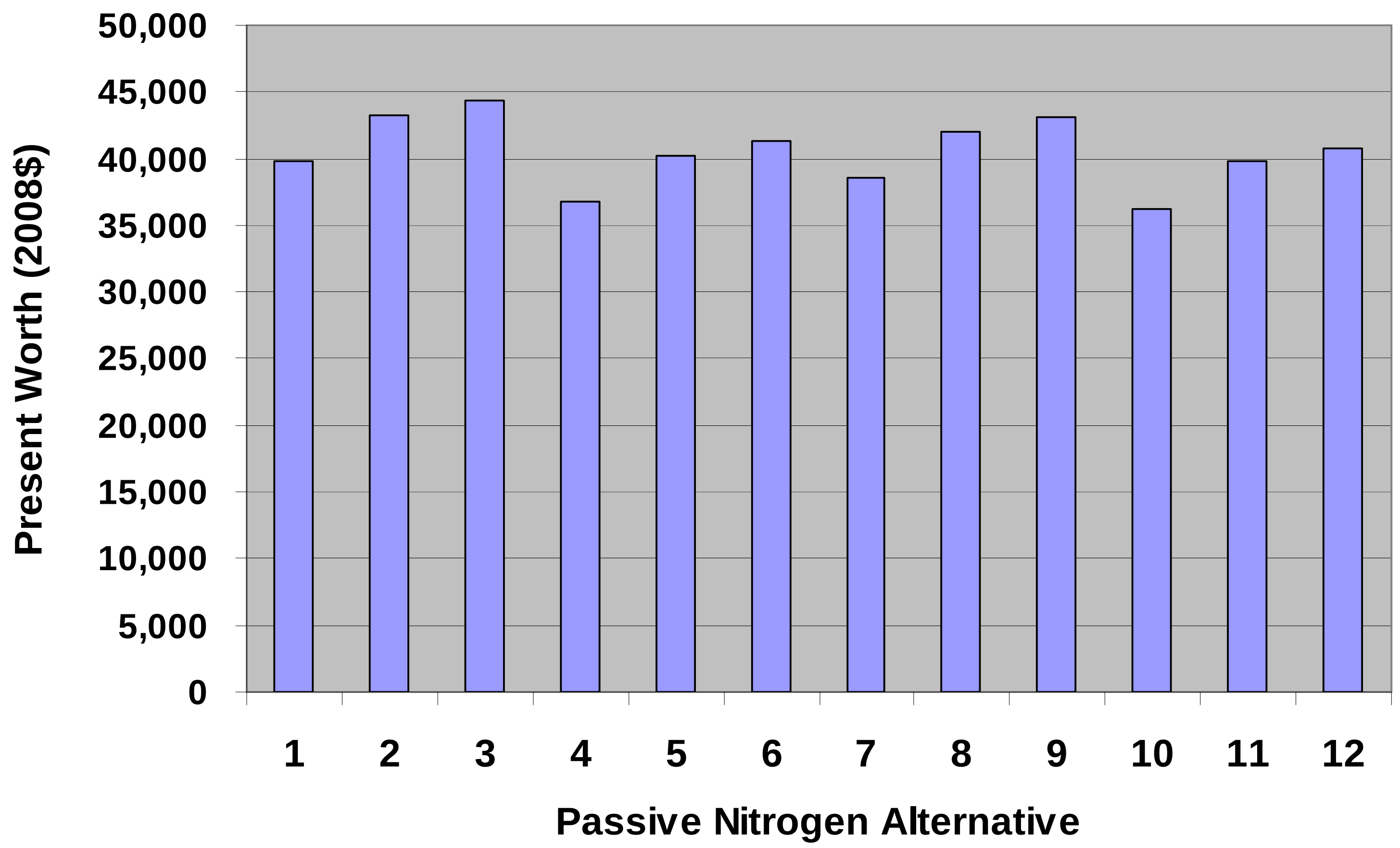
SYSTEM ID	STAGE 1		STAGE 2		PASSIVE NITROGEN REMOVAL LIFE CYCLE COST 30 year, i=4%		TOTAL SYSTEM LIFE CYCLE COST 30 year, i=4%	
	Media	Plan Area	Media Volume	Replace	Uniform Annual Cost	Present Worth	Uniform Annual Cost	Total Life Cycle Cost: Present Worth
	Type	ft^2	ft^3	Years	2008 ($)	2008 ($)	2008 ($)	2008 ($)
1	Clino	100	100	15	1,844	31,908	2,297	39,742
2	Clino	100	50	5	2,047	35,413	2,500	43,247
3	Clino	100	11	1	2,109	36,483	2,562	44,317
4	Clay	100	100	15	1,669	28,878	2,122	36,712
5	Clay	100	50	5	1,872	32,383	2,325	40,217
6	Clay	100	11	1	1,934	33,453	2,386	41,287
7	Clino	75	100	15	1,774	30,695	2,227	38,529
8	Clino	75	50	5	1,977	34,201	2,430	42,035
9	Clino	75	11	1	2,039	35,271	2,491	43,105
10	Clay	75	100	15	1,643	28,423	2,096	36,257
11	Clay	75	50	5	1,845	31,928	2,298	39,762
12	Clay	75	11	1	1,907	32,998	2,360	40,832

Notes: Stage 1 Media: Clino: Clinoptilolite AMZ Clay: Livlite Expanded Clay
Stage 2 Media: 3:1 Elemental Sulfur & Oyster Shell
Total System Costs includes base septic tank and drainfield installation

Uniform Annual Cost 2008$ (with primary treatment & drainfield)



Present Worth 2008$
(with primary treatment & drainfield)



Life Cycle Cost Analysis

- Present Worth of Total Treatment System with PNRS: 36,200 to 44,300
- PNRS component about 80% of total
- O & M typically about 35% of total
- Total treatment system with PNRS:

  $ 0.19 to 0.23 per gallon treated

  $ 22 to 29 per pound nitrogen removed

Cost Distribution: Total System w/ PNRS

(Stage 1: 100 ft^2 Exp. Clay, Stage 2: 375 gal., 5 year)

	2008$	% of Total
Primary Treatment / Pumping		
Installation	2,800	7.0
O&M	1,284	3.2
Total	4,084	10.2
PNRS Stage 1		
Installation	3,770	9.4
O&M	6,801	16.9
Total	10,571	26.3
PNRS Stage 2		
Installation	3,417	8.5
O&M	6,801	16.9
Media replacement	10,844	27.0
Total	21,062	52.4
Total PNRS Component	31,633	78.7
Drainfield	4,500	11.2
Total Installation	14,487	36.0
Total O&M	14,886	37.0
Total System Life Cycle Cost	40,217	100.0

PNRS and RSF Comparison

System	Uniform Annual Cost (2008 $)[1]	Present Worth (2008 $)[1]
Two Stage Filter with Clinoptilolite[2]	2,418	41,829
Two Stage Filter with Expanded Clay[2]	2,264	39,178
Recirculating Sand Filter, One Stage	1,631	28,221

[1]30 year life, i=4%

[1]Average of 6 alternatives

Recommendations: Design

- Lift pump/flow equalization
- Screened STE
- Daily flow measurement
- Pressure dosing to Stage 1
- Stage 1: 100 ft^2 expanded clay @ 24 in.

  Stratified media @ 24 in.

  Underdrain/ sample port
- Stage 2: 375 gal. sulfur media/sample port

Recommendations: Installation

- Installation

  Develop complete hydraulic profile

  Attention to media preparation and placement

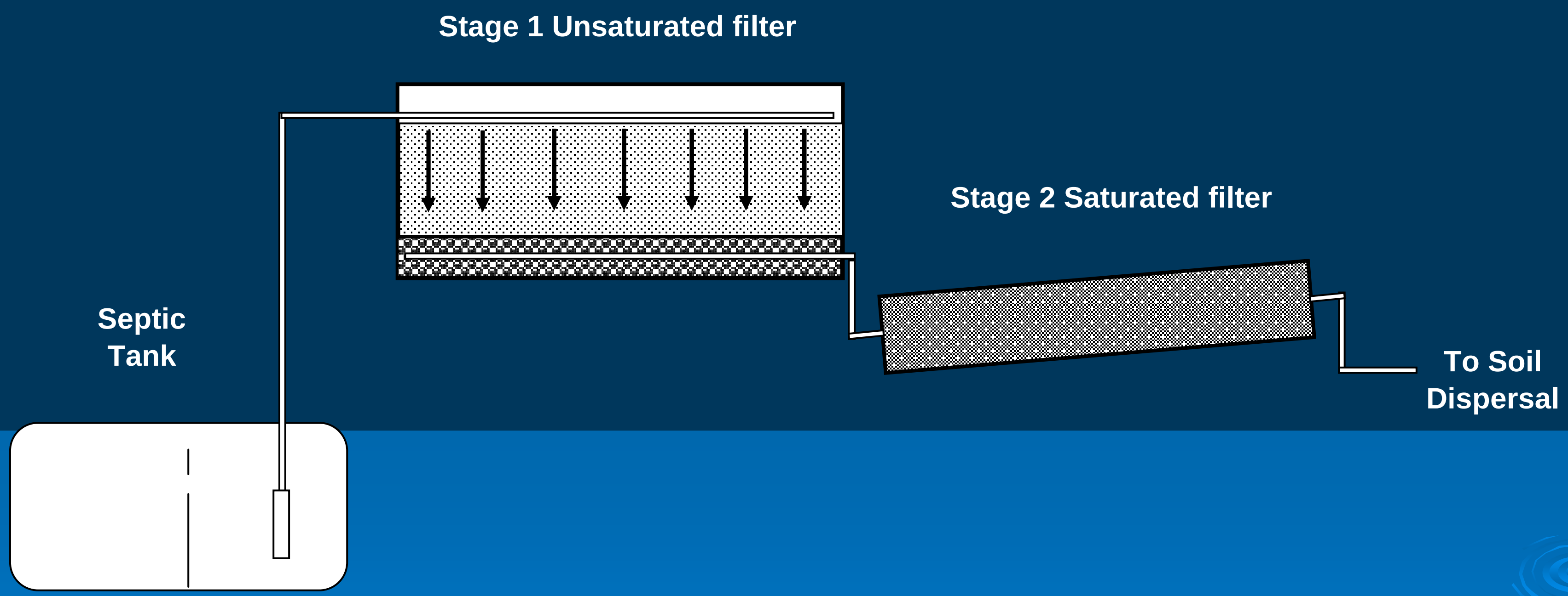
  Watertightness testing

- Elevations

  375 gal. Stage 2 filter: 18 inch diameter

  top of pipe near ground surface

Example On-Site Configuration



Recommendations: Permitting

- F.A.C. 64E-6
- No apparent prohibitions or special rules required for Passive Nitrogen Removal Systems
- Full scale evaluations needed
- NSF Environmental Technology Verification
- Possible drainfield size reductions
- Additives rule

Recommendations: Maintenance and Monitoring

- Maintenance

  6 month: check counters and meters

  12 month: inspect electrical/mechanical parts

  flush/test flow distribution system

- Monitoring

  12 month: Stage 1 and Stage 2 effluent

  TKN, NO_x, $CBOD_5$, DO

Recommendations: Media Replacement

Stage 2 Design Option	Filter volume, gallon	Replacement interval, year
High filter volume, infrequent media replacement	750	15
Intermediate volume and media replacement	375	5
Low filter volume, frequent media replacement	75	1

Residuals: regeneration and reuse in process

land application as crop soil amendment

landfill

Recommendations: Next steps

- Continue operation of small scale columns to verify continued performance
- Full scale demonstration testing of various process configurations, media
- Longevity of denitrification process
- Refine design and cost analysis
- Examine other water quality parameters

Biochemical Transformations

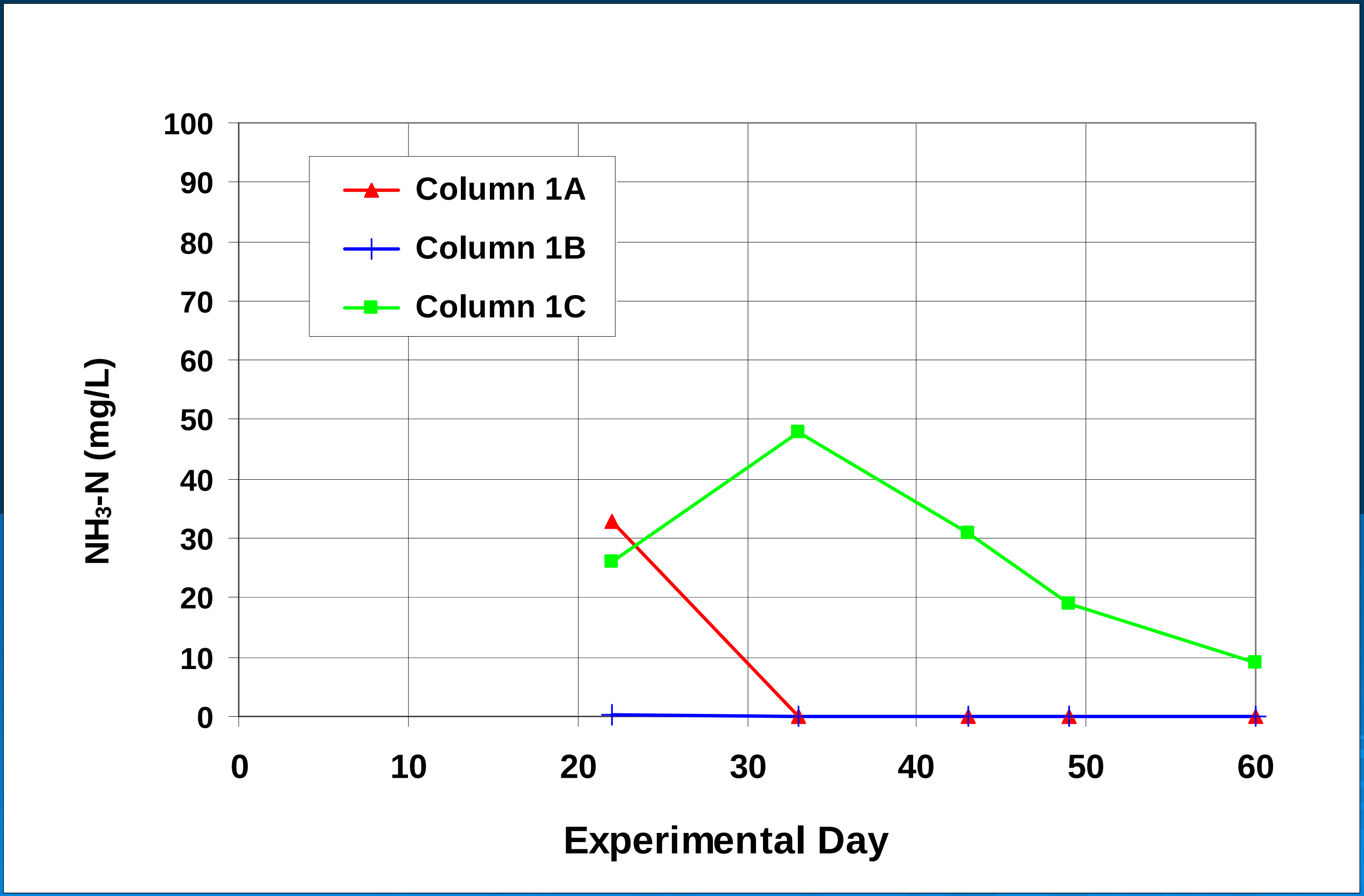
$$\text{Organic N} \longrightarrow NH_4^+ \longrightarrow NO_2^- \longrightarrow NO_3^- \longrightarrow N_2$$

Ammonification

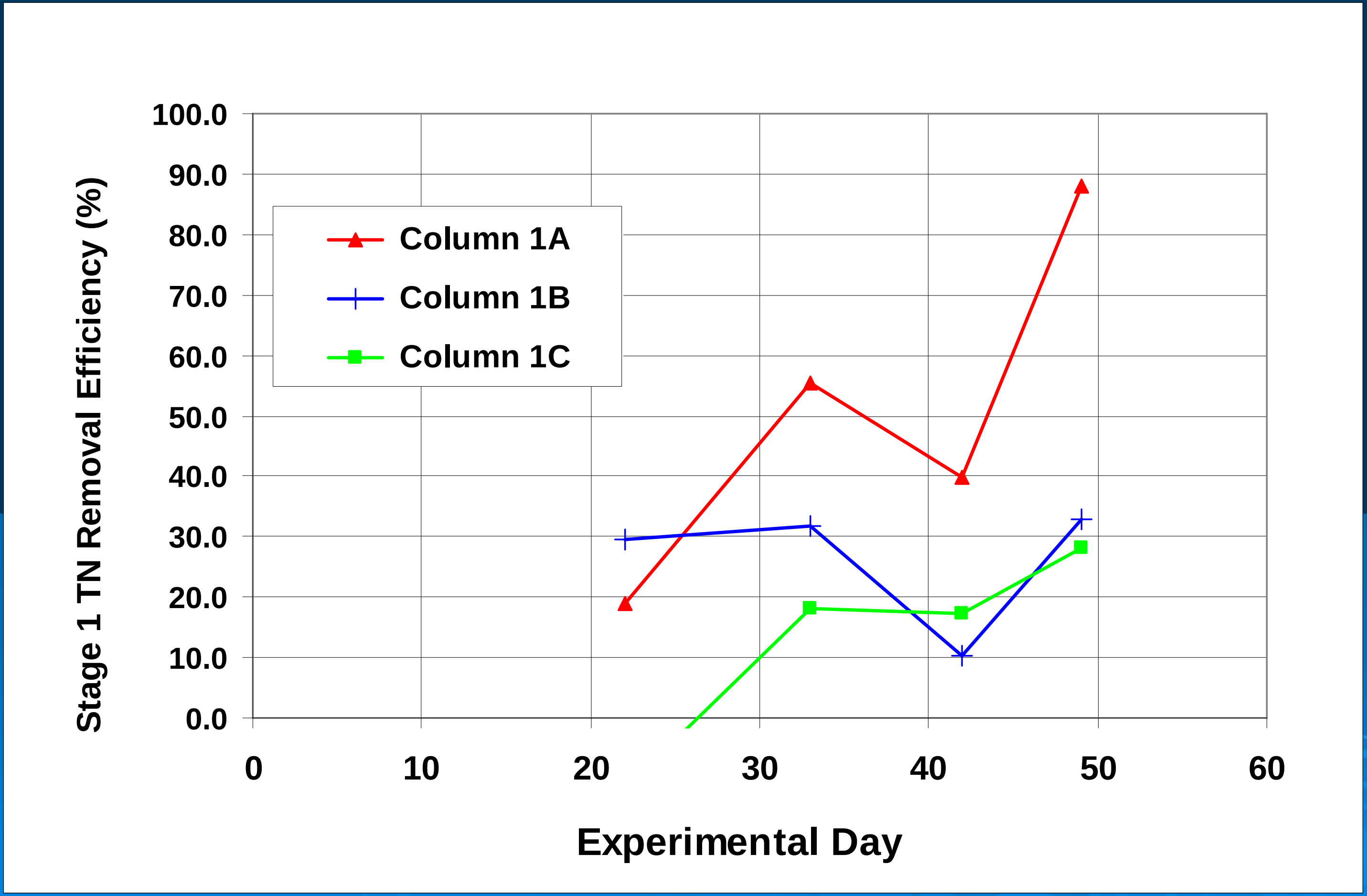
Nitrification
(Aerobic)

Denitrification
(Anoxic)

Results: Stage 1 Effluent NH_4^+



Stage 1 TN Removal Efficiency



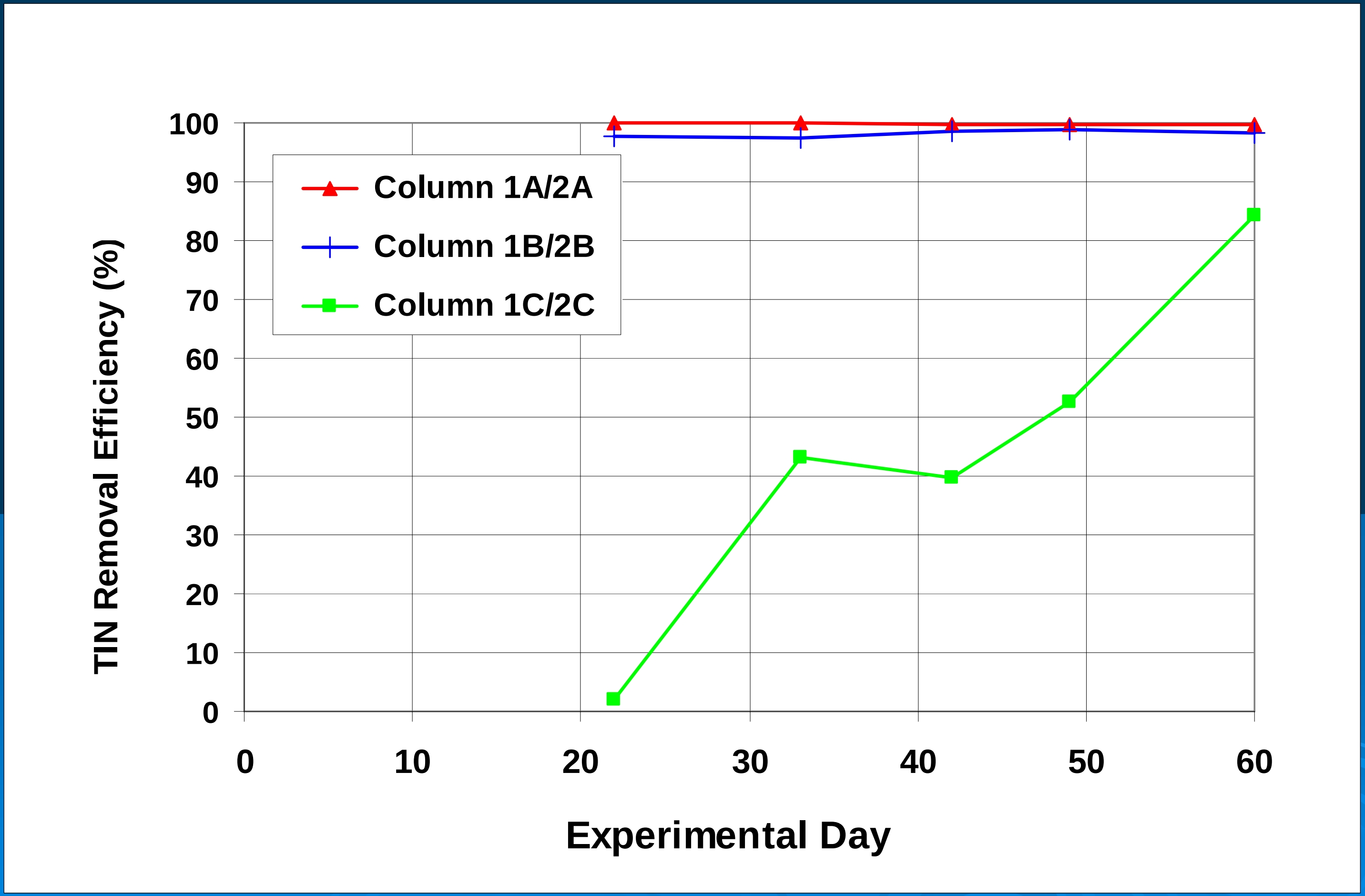
Obligatory Biochemical Sequence for TN Reduction

- Aerobic: Organic N → NH_4
  NH_4 → NO_3
- Anoxic: NO_3 → N_2

Media Selection Factors
Stage 1   Aerobic Unsaturated

- Particle size
- Specific surface area
- External porosity
- Air filled porosity
- Water retention capacity
- Cation Exchange Capacity (CEC)

Total Inorganic Nitrogen Removal Efficiency



Department of Health
Bureau of Onsite Sewage Programs
Research Review and Advisory Committee

Thursday May 29, 2008
9:30 am – 3 pm

Orange County Health Department
Administrative Services
6101 Lake Ellenor Dr.
Orlando FL 32809

Agenda:

- Introductions: New RRAC members/alternates
- Review of minutes from 01/23/2008
- Discussion on Legislative Session
- Brief update on Wekiva
- Updates on ongoing projects
- Updates on future projects
- Other business
- Public comment
- Closing comments, next meeting, and adjournment

Introductions
&
Housekeeping

New RRAC members/alternates:

- Professional Engineer Alternate: James H. Peters

Procedural question:

- Discussion on meeting packets and CD-ROM's

Review Minutes of Meeting 01/23/08

- See draft minutes

2008 Legislative Session

SB 1318:

- Appoints a local government representative to RRAC and TRAP
- Eliminates certification requirement for individuals working under an engineer

Special Appropriation 1682:

- $1,000,000 study on passive nitrogen
- $150,000 inventory of onsite systems
- Report by October 1, 2008 identifying costs for 5 year inspection program

Wekiva Update

Since last meeting:

1. Under review by Governor's Office. No new information available
2. DOH revised loading projections and revised estimates, report discussed at last RRAC meeting is now final
3. Paper presented at National Onsite Wastewater Recycling Association (NOWRA) Nitrogen Symposium in April
4. DEP Total Maximum Daily Load Report Finalized
5. DEP Phase II proposal developed

TMDL Update

- Reports released in April. DEP did not receive objections. Going forward to final adoption.
- Reports available here: http://www.dep.state.fl.us/water/tmdl/final_tmdl.htm
- Includes TMDLs for Wekiwa Spring, Rock Spring, Wekiva River, Rock Springs Run, Little Wekiva Canal & River, & 7 lakes
- Surface loading and springs contribute majority of nutrient load into Wekiva River/Rock Springs Run (Section 4.2.2.1)
- Septic tanks are one source of nutrient loading in surface runoff and springs (Section 4.2.2.1)
- Implementation of TMDLs will require a close look at septic tank loadings along with all other watershed sources
- TMDL implementation plan (BMAP) development will begin this summer

TMDL Questions:

Jennifer Gihring
Watershed Planning & Coordination Section
Florida Department of Environmental Protection
2600 Blair Stone Road, MS 3565
Tallahassee, FL 32399
Ph: (850) 245-8418
Fax: (850) 245-8434
Jennifer.Gihring@dep.state.fl.us

DEP Phase II

- Study looking at what impacts fertilizer use has on water quality in residential areas in the Wekiva Basin
- DEP has contracted with SJRWMD
- Anticipated start date for sampling sometime in June

Ongoing projects

University of Central Florida Research Facility

- Update on UCF Research Facility on passive nitrogen removal

Passive Nitrogen Removal Project

- Received final laboratory experiment report
- Received draft economic analysis of passive systems report and recommendations for passive nitrogen systems report
- Dr. Smith presented a paper at National Onsite Wastewater Recycling Association (NOWRA) Nitrogen Symposium in April
- Laboratory experiment sampling has been extended (5 additional sampling events over 5 months and evaluation of treatment media quality)

Presentation by Dr. Smith

Discussion on Draft Final Report

Optical Wastewater Tracers Study

(old Remote Sensing of Optical Brighteners Study)

- Contract executed between DOH and DEP in May
- Grant extension received from EPA to extend to end December
- Purpose: Test the feasibility of detecting wastewater inputs to Florida surface waters using optical characteristics such as optical brighteners from laundry detergents as tracers
- Project budget: $66,680

Optical Wastewater Tracers Study

(Project Tasks)

- Select lab (contract with Mote Marine in progress)
- QAPP update
- Field sampling

LOCATION	Sampling Events	Number of Sites	Total
Wastewater Treatment Plant	2	2	4
Onsite Sewage Treatment and Disposal System	2	5	10
Laundromat	2	2	4
CDOM-Rich Waters	1	15	15
De-ionized Water	1	1	1
		TOTAL:	**34**
PROPOSED FIELD SAMPLE MATRIX			

- Lab EEM and Parallel Factor Analysis
- Final report and presentation of results

Manatee Springs, Performance of Onsite Systems Phase II Karst Study

- Complete upgrades of systems to include nitrogen reduction
  - New research collecting permit received from DEP
  - Tanks pumped and certified and site evaluations performed
  - Working on repair permit applications
  - Budget for upgrades and some sampling: $34,000
- Need new agreement to perform sampling
  - FSU expressed interest in being the provider
  - Purpose of agreement: perform environmental sampling of monitoring wells around nutrient-removing systems to determine effectiveness
  - Budget for agreement: $24,000

Manatee Springs Phase II Karst Study Discussion on scope of work

- Task 1: Revise QAPP from Phase I
- Task 2: Performance assessment sampling
  - Assess diurnal and daily variability of performance
  - Sample influent and effluent over a 1-week period
- Task 3: Environmental sampling
  - Once every 2 months over 1 year (will need to be reduced to meet grant completion date)
- Task 4: Report development

Taylor County Source Tracking

- Draft report on Phase I submitted for comments from RRAC
- Final report from Florida Atlantic University submitted and distributed to RRAC members
- Grant ends 6/30/2008

Taylor County Source Tracking, Cont.

Remaining grant tasks:

- Task 6, Report Development: A tri-fold brochure targeted towards the residents of the coastal communities. The brochure will present the findings of the main report in layman’s terms in a manner that will educate them about the impact of OSTDS on water quality.
- Task 7, Community education: Community education will be done through the following: extension service outreach, local news media, public meetings, and mailings to coastal residents.

Taylor County Source Tracking, Cont.

Progress:

- FAMU MPH intern currently working on designing tri-fold, creating a mailing list, and developing any policy change recommendations

Main points in tri-fold:

- Beaches were all about the same regardless of development
- Going upstream (creeks and canals) generally worse water quality than beaches
- Environmental factors like rainfall and temperature effect the numbers
- No big difference between OSTDS and sewer, use whatever is appropriate for the desired density and existing OSTDS conditions

Monroe County Performance Based Treatment System Performance Assessment

- Discussion on sampling protocol (in packets) and whether any changes need to be made for the statewide assessment quality assurance project plan

Projects coming up

2008 Research Priorities

- Top 5 ranked projects presented to TRAP
- All were approved by TRAP at 1/24/2008 meeting

2008 Research Priorities

1. **Restoration of the University of South Florida (USF) Lysimeter Station**
   - $20,000 - $50,000 approximate cost
   - If restored, several projects that RRAC wanted to pursue could potentially be conducted at the station
   - USF faculty has expressed interest in partnering on this, could be linked with Kiran C. Patel Center for Global Solutions which has ranked potable water and sanitation as a top priority in their research agenda
   - Dependant on updating the memorandum of understanding between USF and FDOH (draft Memorandum of Agreement in packet)

2008 Research Priorities

2. Phase II of the Florida Passive Nitrogen Removal Project

- $200,000 approximate cost
- Build on the results of the Phase I study to go from a lab scale project to a prototype scale project
- Could be accomplished under special appropriation 1682

2008 Research Priorities

3. **Wekiva Onsite Sewage Treatment and Disposal System (OSTDS) Seasonal Variability Assessment**
   - $200,000 approximate cost
   - Investigate if there is a seasonal variability of nitrogen concentrations from OSTDS in the Wekiva Study Area of Central Florida
   - Could be accomplished under special appropriation 1682

2008 Research Priorities

4. **Alternative Drainfield Product Assessment**
   - $200,000 - $300,000 approximate cost
   - Compare the functioning of alternative drainfield materials to standard aggregate
   - This project may need to wait until the next budget cycle

2008 Research Priorities

5. Long-term deformation of tanks of different materials

- $20,000 approximate cost
- Draft request for proposal in packets, proposing two phases:
  - Phase I: literature review on plastic tanks with assessment protocol to include different tank materials (fiberglass, concrete)
  - Phase II: field sampling numerous tanks of different materials base on the Phase I protocol

319 Project on Performance and Management of Advanced Onsite Systems

- Grant amount: $300,000
- Matching: $200,000 (Keys Study)
- Assess water quality protection by advanced onsite sewage treatment and disposal systems

319 Project on Performance and Management of Advanced Onsite Systems

Focus on Task 2:

- Statewide database of advanced systems based on permit records

Draft Request for Proposal for vendor to provide:

- Electronic database of all advanced OSTDS in Florida with permit information
- Cost $6,000

Coastal Management Program Grant Funding Opportunity

- Grant to resample the Town of Suwannee to see what effects sewering has had on water quality
- DEP has included the project with the state's application to NOAA in the amount of $68,000
- Working on grant agreement
- NOAA will decide around July 1, 2008, if they approve we will begin solicitation for a contract provider
- Draft Invitation to Negotiate scope of work submitted with packet

Coastal Management Program Grant Funding Opportunity

Proposed Tasks

1. Develop a QAPP
2. Gather supplemental data from other agencies
3. Perform sampling (8 weekly samples during November and December at same/similar locations as previously sampled (2 upstream of town, 5 in town, 1 monitoring well, 2 downstream)
4. Data analysis of sampling results
5. Final report development

Research Budget FY 08/09

Requested budget:

• Operating Budget	$90,000
• Phase II Manatee Springs	$34,000
• Columbia County River Front Survey	$5,000
• Lysimeter Station Restoration	$50,000
• Passive Nitrogen Phase II	$100,000
• Deformation of Tanks Study	$20,000
• Wekiva Seasonal Variability Study	$100,000
• Alternative Drainfield Product Assessment	$100,000
• Other	$30,000
Total	**$529,000**

FY 07-08 Budget was $242,475

Other Business

Public Comment

Closing Comments, Next Meeting, and Adjournment

Important dates:

TRAP meeting: **June 5, 2008**
Time: 9:00 am
Place: Orlando Airport Marriott

Next RRAC issues:

- Select provider for Suwannee Study
- Respond to Special Appropriation 1682 if/when signed by governor

Possible next RRAC meeting: July 2008

Florida Department of Health (FDOH)

Research Review and Advisory Committee (RRAC) Meeting Summary

Meeting on May 29, 2008, Orange County Health Department, Orlando, FL

- **RRAC Members/Alternates Present**: David Carter, Paul Davis, Anthony Gaudio, Marc Hawes, Bill Melton, Eanix Poole, Jim Rashley, Patti Sanzone, Clay Tappan, Pam Tucker, and Ellen Vause. Six out of nine groups were present, representing a quorum.

- **Review of Previous Meeting Minutes**: The minutes were approved as submitted with a modification on meeting location.

- **Discussion on Legislative Session**:
  - o Senate Bill 1318: Appoints a local government representative to RRAC and TRAP and eliminates certification requirement for individuals working under an engineer (still would require soil certification). The local government representative would be knowledgeable about domestic wastewater treatment and is to be recommended by the Florida Association of Counties and the Florida league of Cities.
  - o Special Appropriation 1682: $1,000,000 study on passive nitrogen, $150,000 inventory of onsite systems, and a report by October 1, 2008 identifying costs for 5 year inspection program. This item was introduced to the RRAC for consideration and will be discussed at future meetings if/when the appropriation has been signed into law. There was a question from the audience whether RRAC is in favor of this and the general consensus is that RRAC carries forward with whatever charge is given to them. This appropriation is much broader than just the Wekiva issue, the focus is more statewide.
  - o Both are waiting to be signed into law.

- **Wekiva Onsite Nitrogen Contribution Study**: There is no new information on rulemaking; options are still being discussed with the governor's office. At the last RRAC meeting Dr. Eberhard Roeder presented revised the input and loading estimates. The RRAC was given an opportunity to provide comments, the report has been finalized, and is available on the DOH website:

  http://www.doh.state.fl.us/environment/ostds/wekiva/WekivaEstimateFinal.pdf

  There was a discussion on how this report could be final without RRAC having approved the report. Any report generated by or for the department is presented to the RRAC for review and comment, per the statute requirement. The RRAC was allowed to comment at the last meeting. The RRAC does not generally vote on a report. The RRAC is an evolving study group where if new information comes to light it can be considered at that time.

  A brief overview of the Total Maximum Daily Loads (TMDL) process was given by Dr. Eberhard Roeder. Elke Ursin provided a brief update of the TMDL for the Wekiwa Spring, Rock Spring, Wekiva River, Rock Springs Run, Little Wekiva Canal & River, and seven lakes. The reports were released in April. DEP did not receive objections and is going forward to final adoption. Surface loading and springs contribute the majority of nutrient load into Wekiva River/Rock Springs Run. Septic tanks are one source of nutrient loading in surface runoff and springs. Implementation of TMDLs will require a close look at septic tank loadings along with all other watershed sources. TMDL implementation plan (Basin Management Action Plan, or BMAP) development will begin this summer. The reports are available here:

http://www.dep.state.fl.us/water/tmdl/final_tmdl.htm

An update was presented on the FDEP Phase II study looking at what impacts fertilizer use has on water quality in residential areas in the Wekiva Basin. DEP has contracted with SJRWMD and there is an anticipated start date for sampling sometime in June.

- **Brief updates on other projects**
    - Ongoing projects
        - **University of Central Florida Research Facility** – UCF has a grant with FDEP to look at nutrient reducing onsite systems and to develop a research facility with test beds. Dr. Ni-Bin Chang presented to the RRAC the current status of the project.
        - **Passive Nitrogen Removal Assessment** – The final laboratory experiment report, draft economic analysis of passive systems report, and recommendations for passive nitrogen systems report, and the draft final project report have been submitted for review. Dr. Daniel Smith presented a paper at National Onsite Wastewater Recycling Association (NOWRA) Nitrogen Symposium in April. The laboratory experiment sampling has been extended for 5 additional sampling events over 5 months and a final evaluation of the quality of the treatment media. Dr. Smith presented on the project with an extensive question and answer period occurring throughout the presentation.
        - **Optical Wastewater Tracers Study (old Remote Sensing of Optical Brighteners Study)** – Contract executed between DOH and DEP in May. Grant extension received from EPA to extend to end December. The purpose of this study is to test the feasibility of detecting wastewater inputs to Florida surface waters using optical characteristics such as optical brighteners from laundry detergents as tracers. Project budget: $66,680.
        - **Manatee Springs, Performance of Onsite Systems Phase II Karst Study** – The immediate task is to complete the upgrades of the systems to include nitrogen reduction. A new research collecting permit has been received from DEP. The tanks were pumped and certified and site evaluations were performed. DOH is currently working on repair permit applications. The budget for upgrades and some sampling is $34,000. A new agreement will need to be prepared to perform the sampling once the systems have been installed. FSU expressed interest in being the provider with an anticipated budget of $24,000.
        - **Taylor County Source Tracking Study** – Draft report on Phase I submitted for comments from RRAC. Final report from Florida Atlantic University submitted and distributed to RRAC members. Grant ends 6/30/2008. The tasks that are remaining are developing a tri-fold

brochure and write the final grant report. FAMU MPH intern currently working on designing tri-fold, creating a mailing list, and developing any policy change recommendations. Some of the main points in the tri-fold:

- Beaches were all about the same regardless of development
- Going upstream (creeks and canals) generally worse water quality than beaches
- Environmental factors like rainfall and temperature effect the numbers
- No big difference between OSTDS and sewer, use whatever is appropriate for the desired density and existing OSTDS conditions

▪ **Monroe County Performance Based Treatment System Performance Assessment** – The revised sampling protocol is to be reviewed by the RRAC for the remaining work.

o Projects coming up

▪ **2008 Research Priorities** - Top 5 ranked projects presented to TRAP. All were approved by TRAP at 1/24/2008 meeting.

- **Restoration of the University of South Florida (USF) Lysimeter Station** - $20,000 - $50,000 approximate cost. If restored, several projects that RRAC wanted to pursue could potentially be conducted at the station. USF faculty has expressed interest in partnering on this, could be linked with Kiran C. Patel Center for Global Solutions which has ranked potable water and sanitation as a top priority in their research agenda. The restoration is dependant on updating the memorandum of understanding between USF and FDOH which is currently being routed internally.
- **Phase II of the Florida Passive Nitrogen Removal Project** - $200,000 approximate cost. Build on the results of the Phase I study to go from a lab scale project to a prototype scale project. Could be accomplished under special appropriation 1682
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- **Long-term deformation of tanks of different materials** - $20,000 approximate cost. Draft request for proposal submitted for RRAC review. Current draft proposal emphasizes less on other tank materials, but the intent is to include these. Proposing two phases:
    - Phase I: literature review on plastic tanks with assessment protocol to include different tank materials (fiberglass, concrete)

    - Phase II: field sampling numerous tanks of different materials based on the Phase I protocol
  - **319 Project on Performance and Management of Advanced Onsite Systems** – Grant is still in review process with DEP. Task 1 is the Monroe County project discussed previously. Task 2 is the statewide database of advanced systems based on permit records. Draft Request for Proposal submitted to RRAC for review for a vendor to provide an electronic database of all advanced OSTDS in Florida with permit information. Cost $6,000.
  - **Coastal Management Program Grant Funding Opportunity** – Grant to resample the Town of Suwannee to see what effects sewering has had on water quality. DEP has included the project with the state's application to NOAA in the amount of $68,000. DEP is currently working on the grant agreement. NOAA will decide around July 1, 2008, if they approve we will begin solicitation for a contract provider. Draft Invitation to Negotiate scope of work submitted for RRAC's consideration.
- **Research Budget** – A draft budget for the 2008 – 2009 fiscal year has been suggested:

Operating Budget	$90,000
Phase II Manatee Springs	$34,000
Columbia County River Front Survey	$5,000
Lysimeter Station Restoration	$50,000
Passive Nitrogen Phase II	$100,000
Deformation of Tanks Study	$20,000
Wekiva Seasonal Variability Study	$100,000
Alternative Drainfield Product Assessment	$100,000
Other	$30,000
Total	**$529,000**

FY 07-08 Budget was $242,475

- **Other Business** – There was no other business discussed.
- **Public Comment** – The public was allowed to comment throughout the meeting.
- **Next Meeting**: No date was set for the next meeting. Next meeting anticipated to be some time in July 2008 at a location to be determined with a preference for the Gainesville area. Some of the items for the next meeting are to select a provider for Suwannee Study, and to respond to Special Appropriation 1682 if/when signed by the Governor.

PERFORMANCE-BASED PASSIVE ON-SITE WASTEWATER TREATMENT SYSTEMS AT UNIVERSITY OF CENTRAL FLORIDA

FUNDED BY FLORIDA DEPARTMENT OF ENVIRONMENTAL PROTECTION

Dr. NI-BIN CHANG, UCF
Dr. MARTIN WANIELISTA,UCF

University of Central Florida
Stormwater Management
ACADEMY
"Managed Stormwater is Good Water"

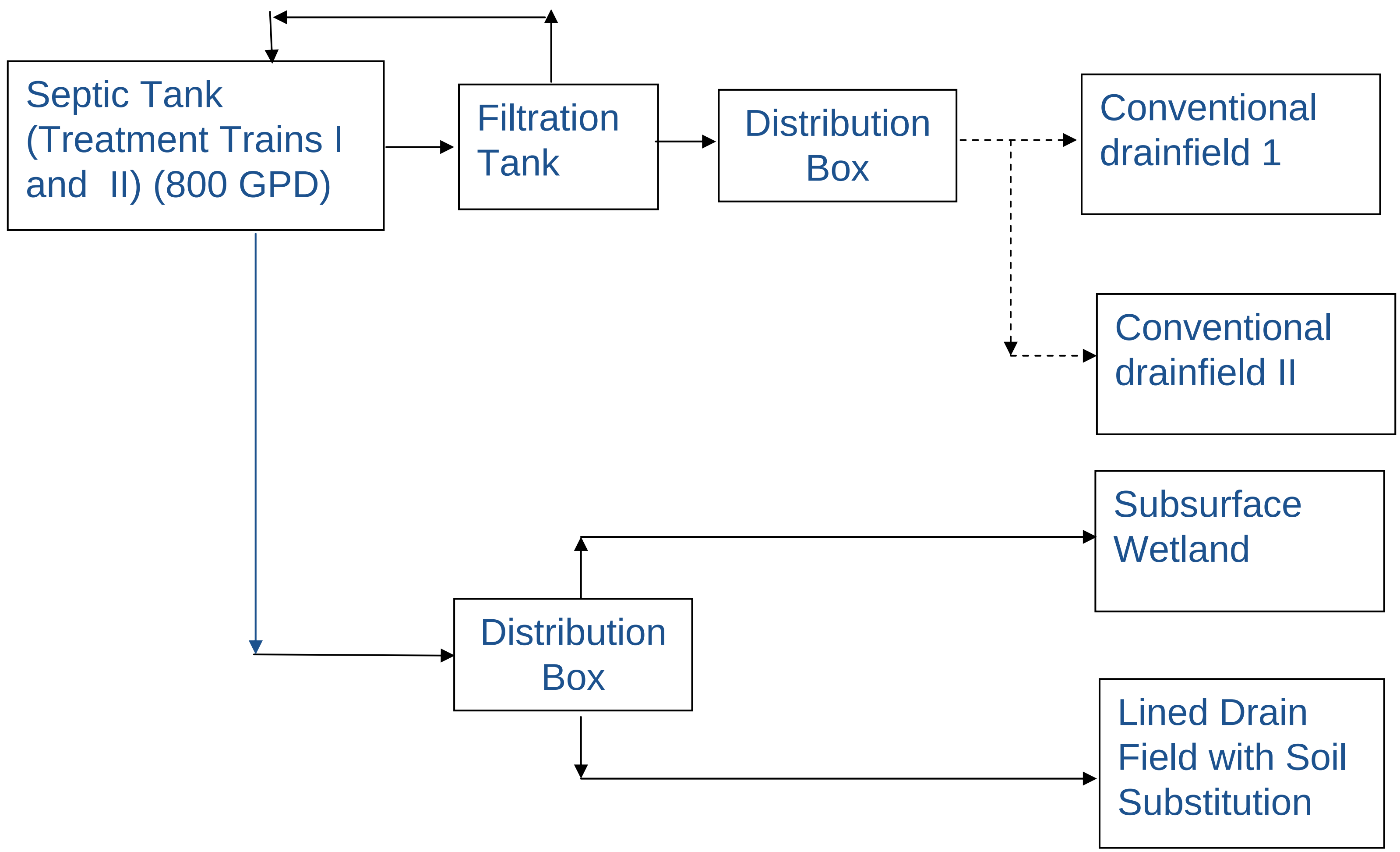
BACKGROUND

- UCF research project on alternative drainfield media for reducing nutrients generated by septic systems
- Waste Water source – BPW Scholarship House, UC

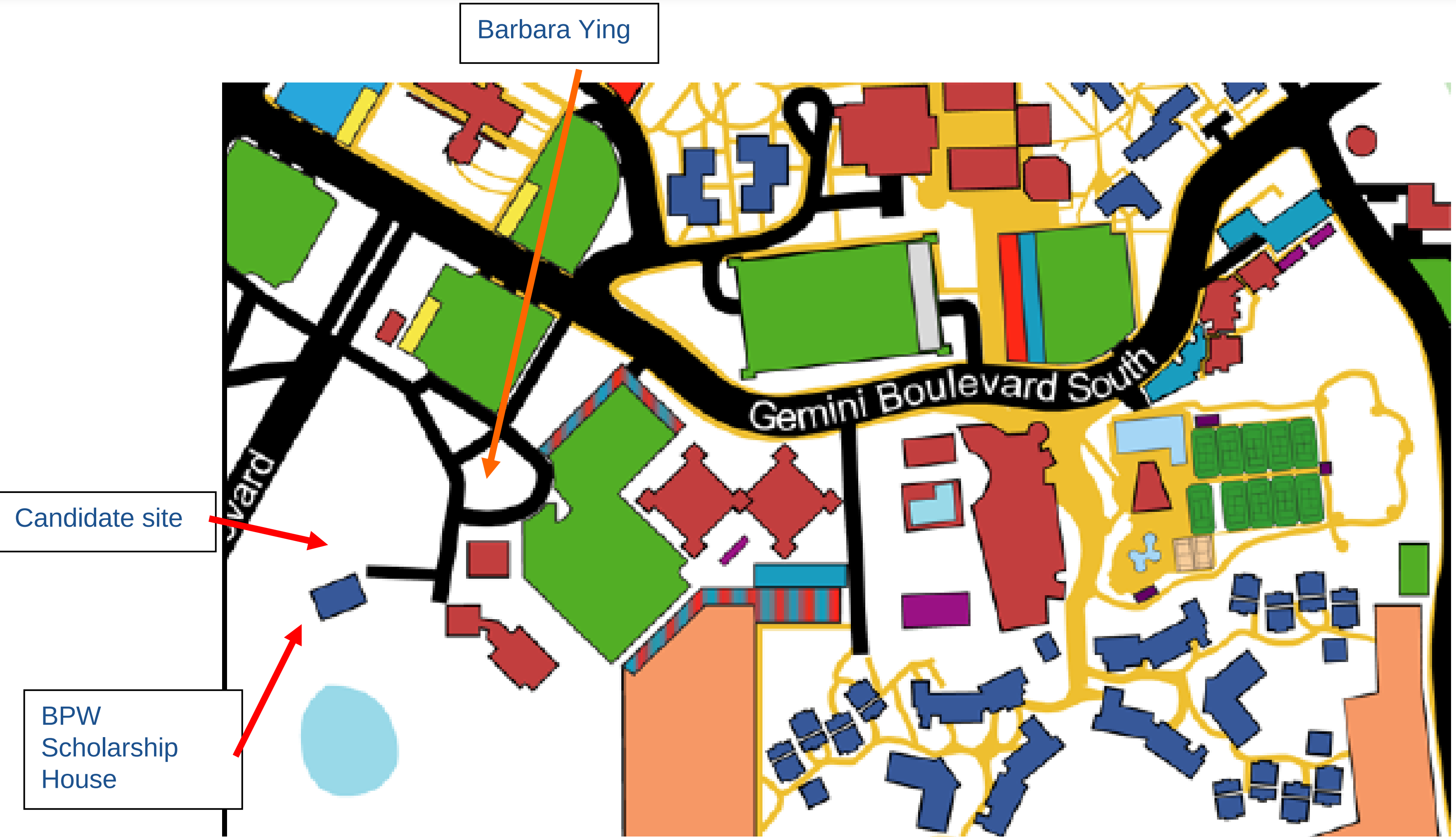
BACKGROUND

- This project include four treatment trains:
  - a septic tank followed by a recirculating sand filter (RSF) with effluent discharged to an unlined gravel-filled gravity field,
  - a septic tank followed by a recirculating sand filter (RSF) with effluent discharged to a low pressure mound drain field,
  - a septic tank followed by a subsurface flow (SSF) wetland system, and
  - a septic tank with effluent discharged to a lined underground drainfield with soil substitution, to ensure the effective removal of nutrient flux from septic tanks.

TREATMENT TRAINS



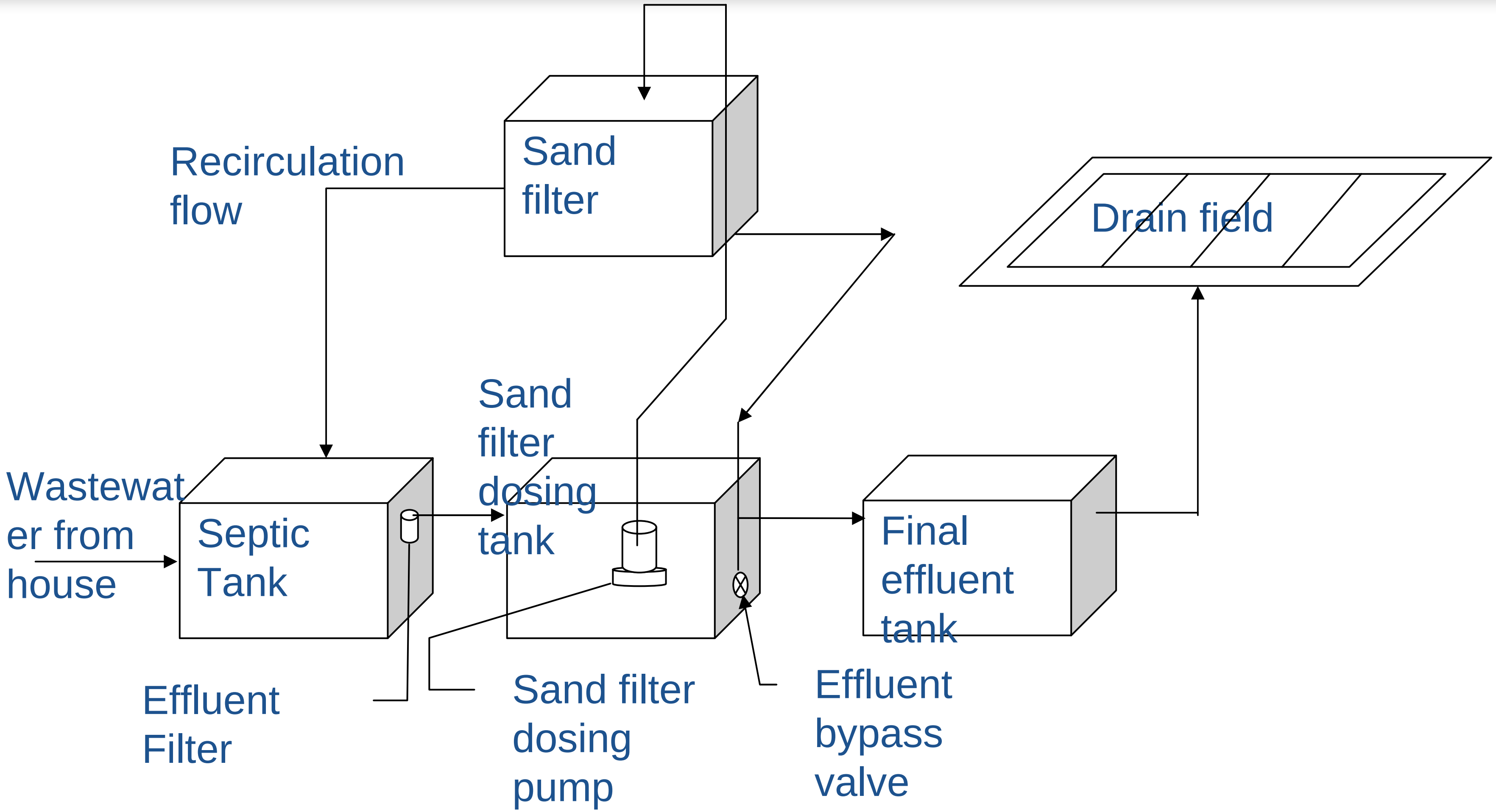
LOCATION



Barbara Ying
Candidate site
BPW Scholarship House
Gemini Boulevard South
vard

SITE VIEW

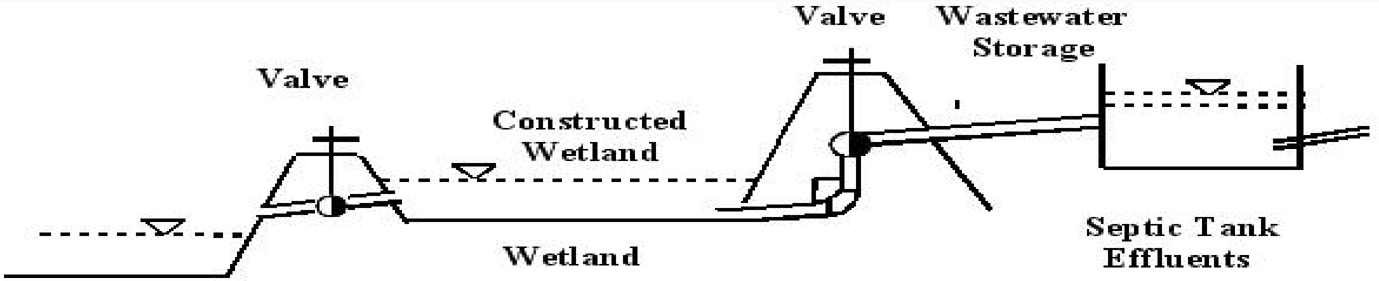
SYSTEM #1

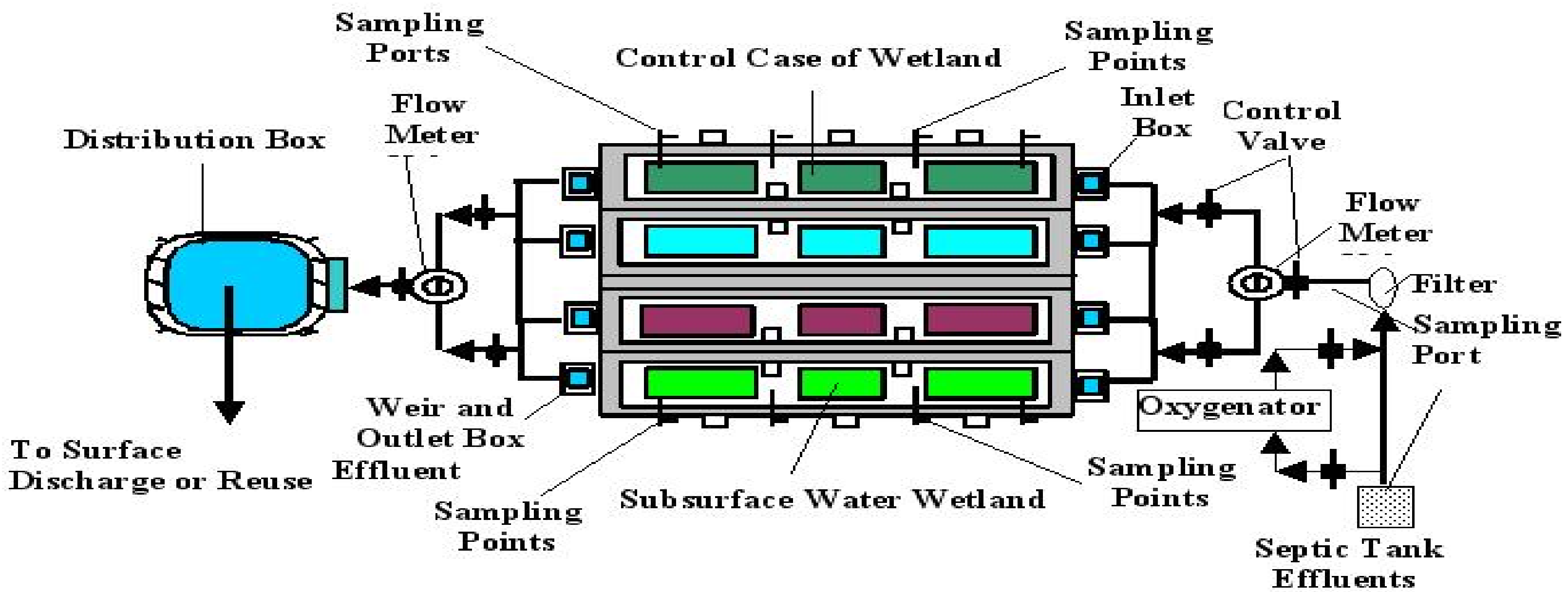


a septic tank followed by a recirculating sand filter (RSF) with effluent discharged to an unlined gravel-filled gravity field,

SYSTEM #3
CONSTRUCTED WETLAND

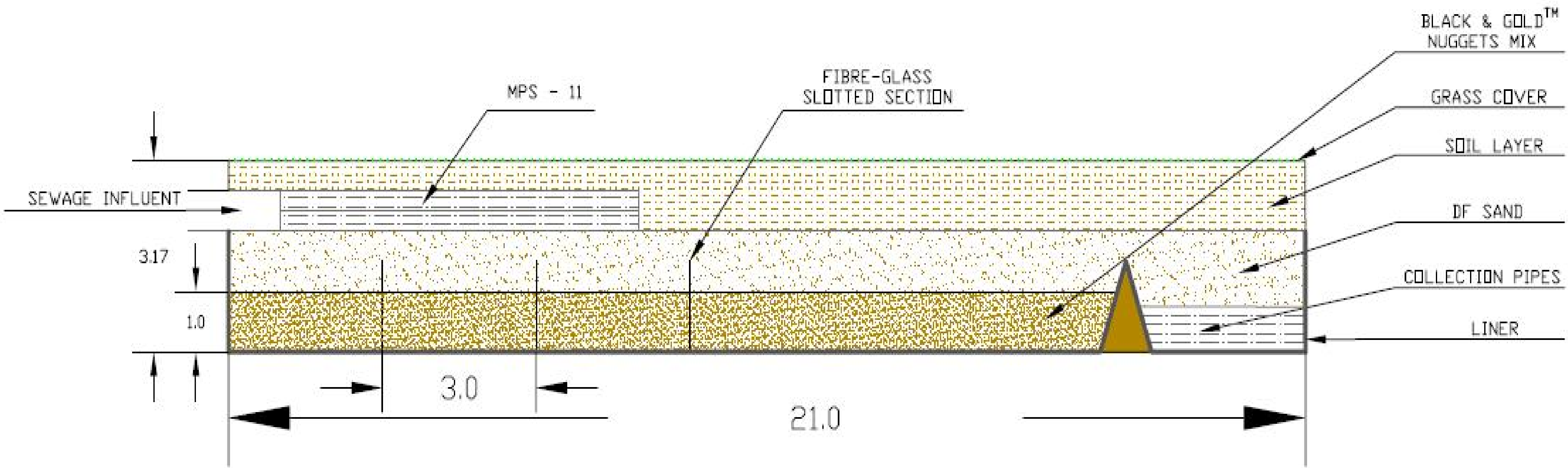
University of Central Florida
Stormwater Management
ACADEMY
"Managed Stormwater is Good Water"

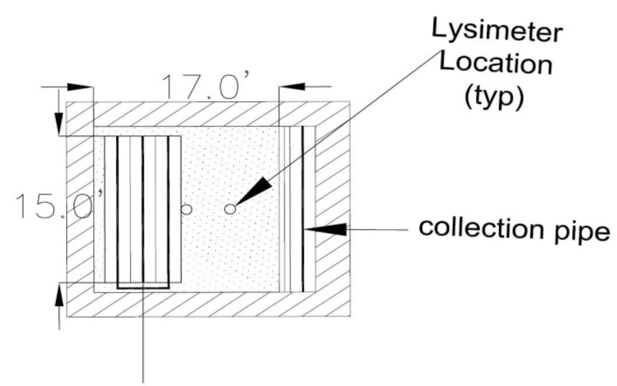




SYSTEM #4
BLACK & GOLD™ DRAINFILED

University of Central Florida Stormwater Management Academy
"Managed Stormwater is Good Water"





Construction

May 2008

Layout

Lift Station

Return to Layout

Lift Station and pipeline

Return to Layout

Lift Station and Bypass

Return to Layout

Septic 1 and 2 Systems

Return to Layout

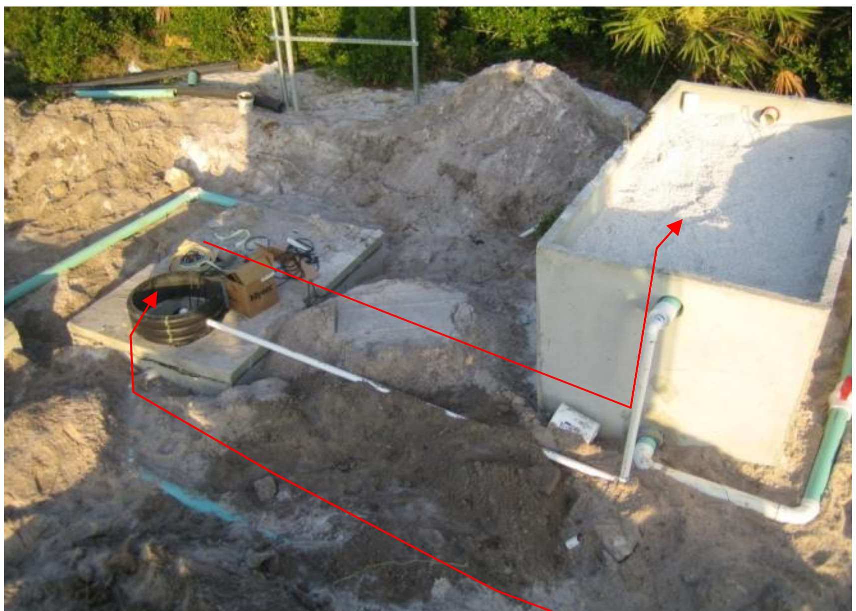
Septic Tank 1

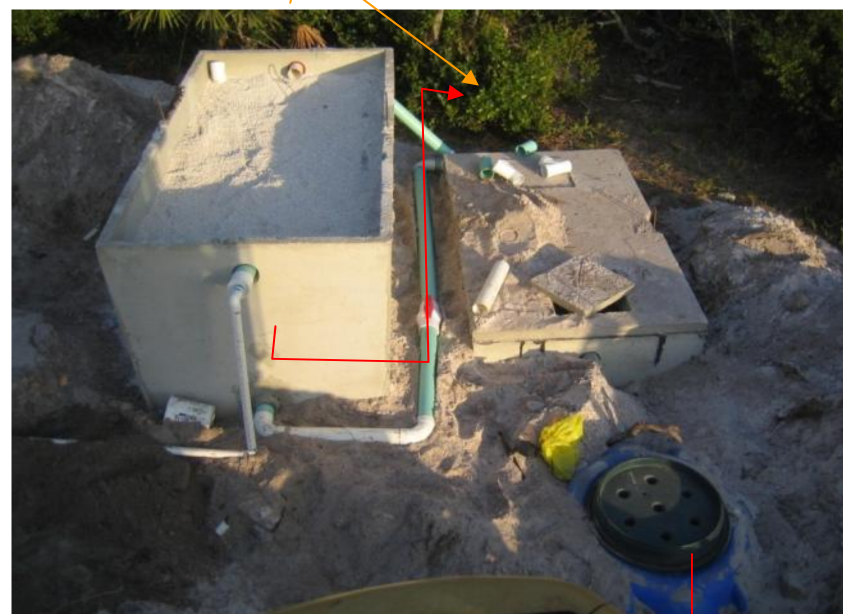
Return to Layout

Septic Tank #2

Return to Layout

Dosing and Sand Filter Tanks





Return to Layout

Distributing Tank

Return to Layout

Making the Sand Filter

Return to Layout

Overflow Pipe of the Sand Filter

Return to Layout

Drainfield 1 and 2

Wash Builder's Sand

Citrus Grove Sand

Return to Layout

Measuring Infiltration Rate

Return to Layout

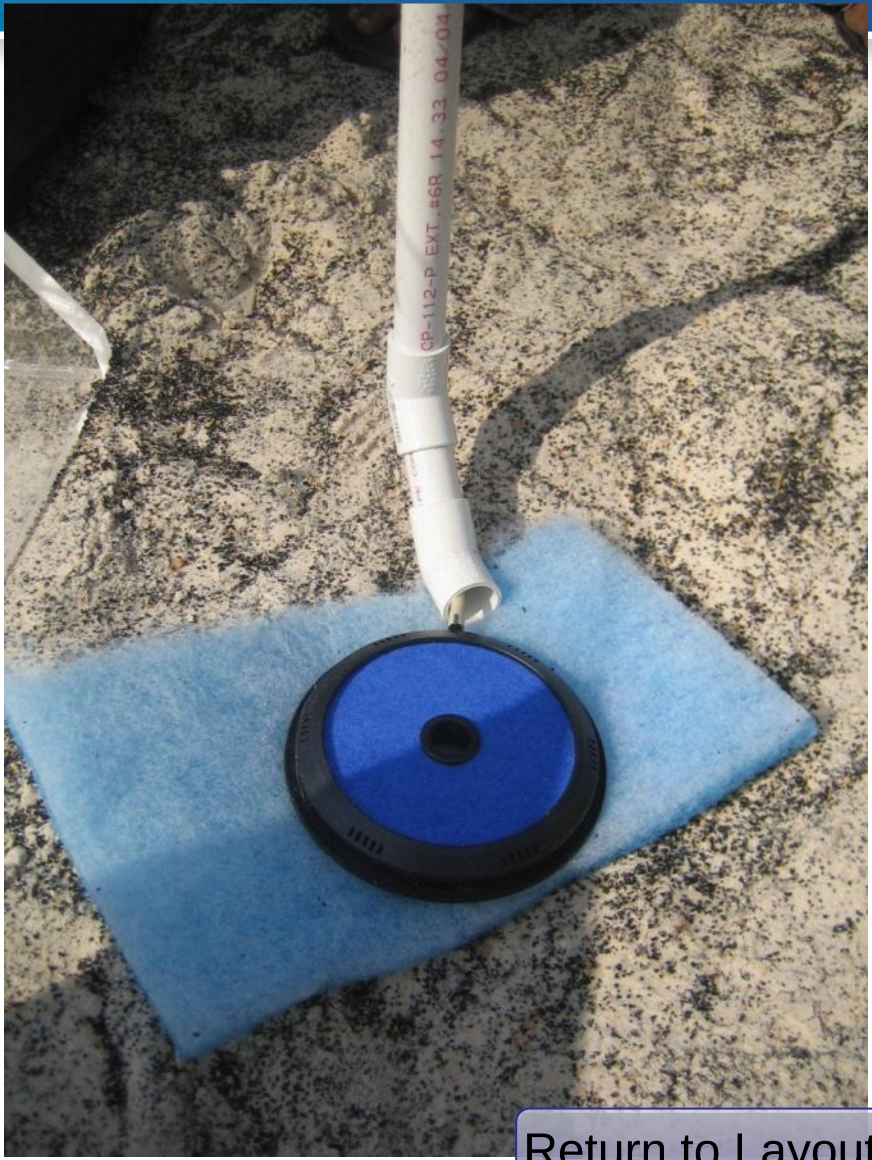
B&G DF liner

Return to Layout

B&G DF Buffers

Return to Layout

Water Sampler



Return to Layout

PTI 11 in B&G DF

Return to Layout

Completed B&G Drainfield

Return to Layout

Wetland's liners

Return to Layout

Wetland Input Header

Return to Layout

Water Samplers in the Wetlands

Return to Layout

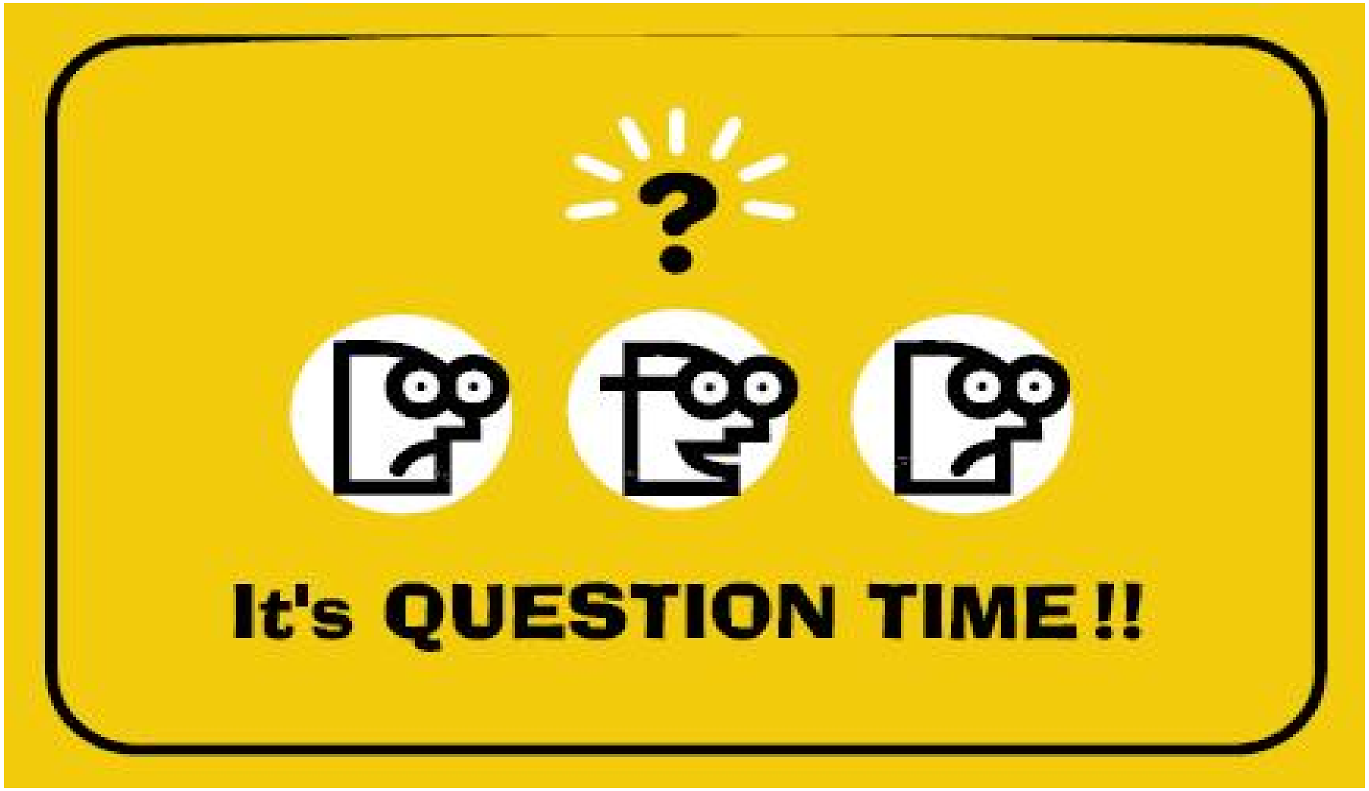
Media-filled Wetlands

Return to Layout

Return Line to the Manhole

Return to Layout

Questions ?



Thank you for your time!