



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

DATE AND TIME: October 18, 2007 at 9:30 am

PLACE: Sylvan Lake Park
845 Lake Markham Road
Sanford, FL 32771

This meeting is open to the public

AGENDA: DRAFT 10/10/2007 Elke Ursin

1. Introductions
2. Review Minutes of Meeting 6/12/2007
3. Wekiva Onsite Nitrogen Contribution Study
4. Brief updates on other projects
 - a. Ongoing projects
 - i. Passive Nitrogen Removal Assessment
 - ii. High Strength Waste Study
 - iii. Manatee Springs, Performance of Onsite Systems Phase II Karst Study
 - iv. Monroe County Performance Based Treatment System Performance Assessment
 - v. Remote Sensing of Optical Brighteners Study: Mote Marine Report
 - vi. Taylor County Source Tracking Study
 - b. Projects coming up
 - i. 319 Project on Performance and Management of Advanced Onsite Systems
 - ii. Coastal Management Program Grant Funding Opportunity
5. Budget Discussion
6. Prioritization of Future Projects
7. Public Comment
8. Closing Comments, Next Meeting, and Adjournment

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

Draft Minutes of the Meeting held at Sylvan Lake Park, Sanford, FL

June 12, 2007

Draft by Elke Ursin 10/03/2007

In attendance:

- **Committee Membership and Alternates:** Sam Averett (alternate, Septic Tank Industry); David C. Carter (Chairman, member, Home Building Industry); John Glenn (member, Environmental Interest Group); Stan Keely (alternate, Professional Engineer); Bill Melton (member, Consumer); Jim Rashley (alternate, DOH-Environmental Health); Patti Sanzone (alternate, Environmental Interest Group); John Schert (member, State University System); Pam Tucker (member, Real Estate Profession); and Ellen Vause (alternate, Septic Tank Industry)
 - **Not represented:** Restaurant Industry
 - **Visitors:** Damann Anderson (Hazen & Sawyer); Rick Baird (Orange County Environmental Protection Department); Quentin Beitel (Markham Woods Association); Alic Berkley (Office of Representative Bryan Nelson); Dominic Buhot (Greens Environmental Services); John Byrd (Aide to Orange County Commissioner Brummer); Bill Carson (Florida Onsite Wastewater Association); John Cochrane (Seminole County Environmental Health Department); Stewart Dawson (Mack Concrete); Kim Dove (Seminole County Environmental Health Department); Frankie Elliott (Orlando Regional Realtor Association); Doug Everson (Plastic Tubing Inc.); Sarah Hardy (Office of Senator Lee Constantine); Roland Harris (Citizen); Henry Hicks (Florida Water Environment Association Utility Council); Justin Hubbard (Infiltrator Systems); Chazz Huston (Citizen, WI Financial); Tony Matthews (Seminole County); Mark Mechling (Ellis & Associates); Steve Meints (Averett Septic); Russ Melling (Lake County Environmental Health Department); Dick Otis (Otis Environmental Consultants, LLC); Harley Pattee (World Wide Water Recycling Inc.); Chris Rowe (Plastic Tubing Inc.); Nicholas Rupnow (Citizen, WI Financial); Gary Smith (Orange County Environmental Health Department); Britt Watson (Averett Septic Tank); Linda Young (University of Florida)
 - **Department of Health (DOH), Bureau of Onsite Sewage Programs:** Paul Booher; Bart Harriss; Mark Hooks; Dr. Eberhard Roeder; and Elke Ursin
1. **Introductions:** Eight out of nine groups were present, representing a quorum. Chairman David Carter calls the meeting to order at 9:40 am.
 2. **Review Minutes of Meeting February 6, 2007:**
 - a. **Motion was made by John Schert and seconded by Bill Melton for the RRAC to approve the May 8, 2007 meeting minutes. No changes were proposed. All are in favor with none opposed, and the motion passed.**
 3. **Wekiva Onsite Nitrogen Contribution Study:**
 - a. Elke Ursin presents a brief overview of the tasks. The department was assigned to look at the nitrogen loading from onsite systems in the Wekiva Study Area. The project was split into four tasks to accomplish this assignment. The first task was to do Wekiva specific field work and to take groundwater samples underneath the drainfield and in the wastewater plume to find the contribution from onsite systems. The second task was to determine the input estimate for onsite systems, and what different

categories are important to determine loading estimates from onsite systems. The third task was to take the input and loading estimates by category and apply Wekiva specific GIS information to determine a total input and loading for onsite systems and then compare that with DEP's estimates for other sources to determine an overall significance. The fourth task was to determine some cost-effective solutions if the overall impact was significant.

- b. Summary of progress as of the last RRAC meeting and decisions made during the current meeting for the DOH study:
 - i. **Task 1** (Field Work, \$200,000): Elke Ursin stated that RRAC reviewed a draft report from Ellis & Associates at the May RRAC meeting. The final report for this task came in on June 1, 2007 and was forwarded to the RRAC committee for review. This final report incorporated comments from DOH and comments from RRAC. One of the main differences between the draft and the final report is that the mass loading calculations are included in the final report. Mark Mechling with Ellis & Associates, Inc. presented the final report on the results of the field work portion of the Wekiva study. Mark Mechling outlined how the mass loading of nitrogen to the surficial aquifer was calculated for each system. His estimates for nitrogen removal by nitrification / denitrification at the three sample sites were between 23% to 52%. Mark Mechling stated that the three sites should not be viewed as average or typical. The total nitrogen from the septic tanks were at the high end of the EPA established range. They developed a table showing estimated total nitrogen loading to the groundwater for a low, moderate, and high effluent load. These estimates were based on three sites, and the Wekiva Study Area has over 55,000 sites, so any total estimates of loading based on these numbers should be viewed cautiously. Pam Tucker states that she does not know whether this field work addresses significance of loading to the groundwater, the aquifer, or the springshed. Mark Mechling states that they were tasked to look at how much nitrogen makes it from a septic tank to the groundwater. They looked at three systems, which does not address all of the 55,000 systems. One of the recommendations he made was to use the study that they performed in conjunction with other studies being done at the same time, and that has been done in the draft final report submitted by DOH. He recommends looking beyond the results of this task and gather further information on the 55,000 systems. He recommends further study to determine whether the EPA baseline is accurate in the Wekiva Study Area. He also recommended that smaller lot subdivisions should be studied to see the potential cumulative impact of onsite systems. Mr. Beitel states that it appears as if there were too few study sites and recommends additional study before significance is determined. Mark Mechling states that this study provides a step beyond anything previously in the Wekiva Study Area. It is expensive to look at numerous sites. One question still remaining is what happens when you have lots of onsite systems together and whether the numbers generated in this task give an adequate estimate of total nitrogen loading down gradient. Denitrification rates calculated in this task are similar to rates previously published in other studies, and are on the high end of the range. Mr. Beitel states that the homeowners in his association are getting excited, in a negative way, about being forced to do something when the reason may or may not have been proven. Mr. Hicks states that the report indicates an estimate of 18 pounds of nitrogen per year per system, which is

lower than previous studies. He asks whether any consideration has been made to the abnormal drought situation that the area is in. Mark Mechling states that more information over more time would be better, but he is confident in the loading estimates that they determined for the three sites because it is based on real data. He does caution again in using the estimates and extrapolating to all the other 55,000 sites. Damann Anderson states that they did a good job identifying the plume, and the calculations show removal of nitrogen, but asks what would happen in the shallow aquifer. Mark Mechling states that it would be beneficial to look downgradient at many sites and does not know if he has enough information to make the determination on what happens in the shallow aquifer. Damann Anderson states that he expects that denitrification will continue in the aquifer. John Byrd states that the report shows nitrogen at background levels in a short distance, and Damann Anderson states that the majority of that is dilution. The mass of nitrogen is important, not dilution. Dilution only hides what is there, the nitrogen is still there. Damann Anderson states that in the late 1980's, early 1990's he looked at subdivisions and there was no evidence of down gradient cumulative plumes from four Florida subdivisions. Mark Mechling states that a follow-up study to see the cumulative impacts could be to install permanent wells at varying depths around a dense subdivision and observe over a year minimum. Stan Keely asks Mark Mechling to highlight the differences between the sites and the EPA results and Mark Mechling states that generally they were in the range, and the nitrogen concentrations of the septic effluent were in the upper range. Damann Anderson states that nitrogen concentration in wastewater has been increasing over the years because of water conserving fixtures. Bill Melton states that it is important to note that none of the three sites were outside the expected parameters. David Carter states that Mark Mechling's recommendation of looking at a subdivision is very similar to what Damann Anderson suggested back in June of last year. Dr. Eberhard Roeder states that the concern is that there is enough mixing underneath the drainfield to find the plumes. Mark Mechling thanks everyone and appreciates the opportunity to work on a project that so many people feel so strongly about.

- ii. **Task 2** (Categorization and quantification of nitrogen loading, \$25,000): Elke Ursin gives a quick update on what has happened since the last meeting. Dr. Richard Otis with Otis Environmental Consultants LLC, presents the final report on the results of this task. The purpose of this task was to estimate the amount of nitrogen coming from onsite sewage treatment and disposal systems in the Wekiva Study Area (WSA) and going to the groundwater. The scope was limited to estimating what makes it to the water table, including the capillary fringe, but not including what is going on in the aquifer. He reviewed literature to get to how much nitrogen is removed. The literature was focused more on different technologies, but not on the soils. The data does not look at characteristics in the soil profile that are providing conditions that are conducive to denitrification. Some of the literature data ranged from 0 – 80%, and his struggle was trying to determine which number to use. Most of the data was on concentrations, with no flow information. To get to mass loading you need concentration and volume. There are a lot of unknowns. He worked with two models for wastewater treatment: the single sludge model, and the two sludge model. He produced a table outlining the percentage of nitrogen reduction in

various soil types found in the Wekiva Study Area. This number was based on the drainage class, the amount of organic content in the soil, where the estimated seasonal high water table was, the soil texture and mineralogy, the fluctuation in the water table, the influent nitrogen species (either total Kjeldahl nitrogen or nitrate), and the type of infiltration system (mounded, in-ground, etc.) Dr. Otis states that the numbers generated in Task 1 are not included in the numbers in Task 2 because the Task 1 numbers included what was going on in the groundwater and the Task 2 numbers only reflect up to the groundwater. He stated that his estimates are conservatively low, and that the fate of nitrogen in the groundwater is not included. Ellen Vause asks what the difference is between gravity systems and dosed systems and Dr. Otis states that there is more nitrogen removal in dosed systems due to the wetting and drying conditions. Dr. Otis states that the trend is moving from public health to a water quality approach. The current rules are written from a public health approach. David Carter asks if there were two systems with the same nitrogen loading and one has a standard drainfield size and the other was spread it over twice the area, would you expect to see nitrogen reduction to be twice as much. Dr. Otis states that that could be, that there is a better chance of getting the organic matter. David Carter states that low pressure dosed systems appear to be a low cost alternative. Dr. Roeder asks whether a well drained soil does much for denitrification and Dr. Otis states that the carbon source is replenished all the time as roots cycle every two days. John Byrd asks whether Task 2 is part of the determination of loading and Dr. Otis states that it is. The percentages he came up with for the different soil types were applied to the actual number of systems in each soil type in Task 3. David Carter states that from a public health perspective the wastewater should go down in the groundwater so limiting soils are removed. Dr. Otis states that the way systems are designed today are not designed to remove nitrogen. There needs to be a balance between public health and water quality. Ellen Vause states that it is a balance. The hydraulics allow for a small footprint on a small lot. Florida has one of the smallest drainfield footprints in the country. Instead, she recommends looking at all options: i.e. if you need a small footprint then you need a PBTS, if you have a larger size lot then put in a larger drainfield. Dr. Otis states that when dealing with water quality, each individual system is different. He stated that removing nitrogen to 12 – 15mg/L is easy, but 10mg/L is much more difficult to achieve. He looked at Linda's report which took the Task 2 information in the MACTEC report and thinks something is wrong with the conversion from input to load. John Byrd asks how can we move forward with this report to the governor. Dr. Otis states that the data needs to be comparing apples to apples and now it is comparing apples to oranges. Dr. Otis states that DOH is working on their own and DEP is working on their own. He would like everyone to get together and describe the entire "creature". The Task 4 report will require cooperation from everyone involved and that is hard. We need to look at the value of what we're doing, how do we put a dollar figure on good clean groundwater. Traditionally it is putting in the cheapest system, ignoring the value of good treatment. John Schert states that the work done in this report is cutting edge. He thinks the department should think about how to educate on putting in better systems. Ellen Vause asks for clarification on how much more benefit there is between 10 mg/L of nitrogen vs. 15 mg/L. Mark

Hooks explains that the 10 mg/L refers to the testing result under controlled conditions. Dr. Roeder states that according to research he did for Task 4 shows that systems that claim to get 10 mg/L are not any less cost effective than those that get 15 mg/L. There will be variations in strength, toxicity, flow volume, etc. in the field that might influence reaching 10 mg/L. Dr. Otis stresses the importance of maintenance on PBTS. Mr. Beitel states the Markham Woods Association supports conservation issues, but he has a problem with there being a lot of science but no facts. Dr. Otis states that it is very difficult to prove a null hypothesis. If nothing is found does it mean that nothing is there? Sam Averett states that every research project has assumptions. Dr. Otis states that if further studies are done then we need to come up with a good hypothesis and test it. Damann Anderson states that one thing that we could all agree on and move forward with are the input numbers. They are easier to collect, they are more finite, there is no questionable nature of what happens in the environment, and we know the sources. He suggests source load reduction goals. He states that scientists can study this groundwater issue for many years and not reach a consensus. He states that he likes the framework established by Dr. Roeder in Task 4. He would like to see a task force between all agencies to come up with a solution.

- iii. **Task 3** (Assessment of the contribution of OWTS relative to other sources, \$25,000): Dr. Linda Young with the University of Florida presented the final report on the results of this task. The report follows the process used in the DEP report but looks at the Wekiva Study Area as opposed to the Wekiva Basin. She put together the Task 2 work and the DEP work to come to some conclusions on loading to the groundwater. There is diversity in the land uses in the Wekiva Study Area. There are over 55,000 septic systems in the area as well as numerous centralized wastewater facilities. There are two wastewater facilities that lie just outside the boundary of the Wekiva Study Area and there was discussion on whether to include them in the calculations or not. One of the facilities, Conserve II, generates more nitrates than all the rest of the facilities put together. The percentage of the contribution from wastewater treatment facilities goes from 6% to 13% if this contribution is fully included. Stan Keely stated that this facility handles wastewater from areas both inside and outside of the WSA and the Wekiva Basin. He cautions not to use the total numbers in the calculations; this is a distribution center which distributes to areas both inside and outside of the WSA. She presented pie charts for both scenarios (100% of two boundary systems included, or 0% of two boundary systems included). The inputs that were considered were fertilizer use, livestock wastes, atmospheric deposition, centralized wastewater facilities, and onsite systems. She took the methodology used by MACTEC and applied it to the Wekiva Study Area. A major part of her effort was scaling it down properly to the study area. Onsite systems were calculated to be 6% of the inputs. There was a discussion on some of the assumptions made in the DEP report and how these assumptions may not be accurate. She explains that the DEP study used nitrate numbers for the majority of the estimates, but used total nitrogen for onsite systems. She made the analogy that the onsite system slice of the pie is an orange in the midst of a basket of apples. David Carter asked how this affects the answer. Dr. Otis stated that if the pie chart for the inputs were to only look at nitrates then the onsite contribution would be zero

because the effluent comes out of the tank as ammonia. He stated that if the wastewater treatment plants are denitrifying they may only be discharging nitrates, but if they are not denitrifying then they are not accounting for all the nitrogen. Damann Anderson stated that most of the wastewater treatment plants are not denitrifying yet, but that they will be required to in the future. Mr. Anderson stated that the wastewater input and loading numbers are grossly underestimated at this point. There are 265,000 people served by sewer in the WSA, and only 160,000 served by onsite systems. If you only look at inputs the sewer should be considerably greater than the onsite but the numbers in the MACTEC report do not show that. This is because they did not look at the total nitrogen numbers from the facilities. Dr. Young stated that although MACTEC stated that nitrate numbers were being considered, if you look carefully through the report they mangle it a lot and may have used total nitrogen numbers and nitrate numbers. Dr. Young stated that she consistently tried to state nitrates throughout her report. Damann Anderson stated that they only used the nitric portion of atmospheric deposition and that's probably less than half the actual amount if the results are similar to Tampa. Dr. Young stated that MACTEC used one monitor for rural and one monitor for urban and used it throughout. Damann Anderson stated that this area is not rural. Dr. Young stated that she is trying to be clear of some of the assumptions that went into the work that she did because the analysis is only as good as the assumptions used. Pam Tucker asked why the MACTEC numbers were used and Dr. Young stated that that is what her task was. Pam Tucker asked if DOH was tasked to use the DEP numbers and Patti Sanzone responded by asking where else these numbers would come from. Dr. Young stated that she was tasked to work with the DEP numbers, that this is the best available data at this point in time. Dr. Young stated that the funding and the timeline were not sufficient enough to do anything other than to use the MACTEC numbers. John Byrd stated that there is still \$200,000 for DEP to use to verify the numbers in the MACTEC report. David Carter stated that Dr. Young was trying to make the RRAC aware of the inconsistencies and limitations of what it was that she had to work with. Pam Tucker stated that Dr. Otis has his limitations and Dr. Young has her limitations, so coming up with any determination of significance is tough at this point. Dr. Young stated that often decisions have to be made on the best available information, and this is the best available information. There can be discussions on how to tweak these numbers, but she does not know of anything that can be used to replace this information. Damann Anderson stated that the pie chart can be corrected fairly easily, because the numbers are there for the inputs. It's the loading that is difficult to estimate. MACTEC used recharge rates to estimate the loading. There was a small portion of the WSA that had no recharge information and most of it was water. For the land portions she took the weighted average of the residential recharge rates and applied it to these areas. Damann Anderson asked whether it is the recharge rate to the surficial or the Floridan aquifer and there was a discussion on this. Ellen Vause asked whether the loadings include the 10-15% that is removed in the septic tank, and Dr. Otis stated that it does. Quentin Beitel asked whether the type of system is taken into consideration, and Dr. Young stated that this information is not available for all 55,000 systems. Dr. Otis stated that the system types are incorporated into his

numbers as it relates to whether it is a subsurface, filled, or mounded system. Damann Anderson stated that there is an agreed amount of what comes out of the tank and then depended on the soil type to determine what other reductions take place. She presented a series of pie-charts showing the low, mid, and high range of loading based on the task 2 estimates. The estimates for the contribution by onsite systems to groundwater loading ranged between 25% and 31%. Damann Anderson stated that the problem with the pie charts is the way the fertilizer loading was calculated versus how the onsite system loading was calculated. The fertilizer loading has gone way down and is inconsistent with how the other numbers were calculated. There was a question from the audience whether the land application from septage was included in the calculations and the county health department representatives stated that there are no land application sites in the WSA. Pam Tucker stated that the MACTEC report would need to be fine-tuned a bit to make this report more accurate. Dr. Roeder stated that there is information available supporting the estimates used by MACTEC for the fertilizer reduction estimates. David Carter asked if Dr. Young is given an updated MACTEC report how difficult would it be to update these numbers, and Dr. Young stated that it is possible but may take some time to do but that better numbers are certainly worth the effort. David Carter stated that he is wrestling with whether there is a number that he can feel confident in at this point. Bill Melton asked how can there be a percentage that they are comfortable with if the measurements are using different parameters. Dr. Young stated that this is a limitation described in her report. Damann Anderson stated that overall input to load reduction ranges between 10% to 23%, but the field work found a starting point reduction at 23% and went up from there. Dr. Roeder stated that what was input into the drainfield was more in the field work, so it actually comes to a wash. Dr. Young stated that she did not incorporate the field work into her numbers unless it impacted Dr. Otis' numbers. There were only three out of 55,000 sites sampled and in two soil types. Bill Melton asked what assumptions were made by MACTEC to justify fertilizer reductions from input to loading and Dr. Young stated that they assumed the nitrogen that is applied is used. Damann Anderson then stated that unless the crop is harvested it does not go away. He stated that if the same methodology is used for onsite systems the loading would be 29 metric tons per year (as opposed to over 350 metric tons per year estimated in Dr. Young's report). Quentin Beitel stated that he would prefer to see fertilizer as one slice of the pie, rather than broken out, because as it is now it visually lessens the impact of its proportion of the pie.

- iv. **Discussion on Draft DOH Final Report:** The RRAC had concerns regarding the final conclusions and recommendations presented in the DOH draft report. Patti Sanzone asked whether anyone sat in on DEP's planning meeting regarding phase II of their task, and John Byrd stated they had one meeting but the scope of work had not been drafted for public review as of yet and that they will meet again in the near future to develop this scope. The SJRWMD presentation on the phase I work was made at the RRAC, TRAP, and Wekiva River Basin Commission meetings. David Carter asked whether DOH staff or Damann Anderson had received any response to Mr. Anderson's letter to DEP regarding the phase I report. The letter is posted on the DOH website. DOH staff and Damann Anderson both indicated that they have not had a response

but that the phase I report will most likely not be rewritten, instead it will be verified in phase II. Mark Hooks stated that some of the issues raised about the MACTEC report will probably not be addressed until the phase II report comes out. Patti Sanzone asked if DEP will take one to three years to do phase II then does DEP expect DOH to wait to act until this has been completed, and Mark Hooks stated that he cannot speak for DEP and does not know. John Byrd stated that there is a DOH draft report that stated that onsite systems are a significant contributor, and he would like to know when RRAC determines whether that is in fact the case. He stated that if DOH is going to meet the June 30th deadline, which DEP is not going to meet, how can significance be determined. David Carter stated that John Byrd's point was whether the committee should come to a decision of significance, and if it does not then should RRAC proceed with discussions on Task 4. Ellen Vause would like to address the executive summary because if RRAC does not agree with the executive summary, and RRAC needs to make a statement independent of the summary, than it would certainly play into how Task 4 is addressed. John Byrd stated that Task 4 doesn't happen if significance is not determined. Dr. Roeder stated that Task 4 is a range of cost-effective strategies if contributions are significant and can be included either way, the question would be on whether they would be implemented or not. John Byrd sees this differently and the statutory language was read. There was a discussion on who determines significance. The DOH draft report stated that the contributions are significant. Dr. Roeder stated that the department can state that it is significant and then it is up to the legislature to agree or disagree with this statement. David Carter stated that in his opinion the legislature wants RRAC to weigh in on whether it is significant or not, the department can have their own separate decision. Pam Tucker stated that at the last RRAC meeting she had requested an outline of the final report. Mark Hooks stated that the department will consider RRAC's comments. Elke Ursin stated that there is an internal review process in DOH that required a draft be routed to the secretary by Friday June 8th. Mark Hooks stated that any policy recommendations as a result of this will require review from TRAP and the variance committee. Ellen Vause stated that in order for her to decide whether onsite systems play a significant part of the impact, she would want some qualifications on the data used to get to the final decision. She cannot state that she is certain of anything right now because there are questions on MACTEC's assumptions. She stated that the information from MACTEC gathered and used as part of the RRAC's task has faults in it and no conclusions can be made at this time. John Byrd stated that these are separate studies, the MACTEC report should not be a part of this process, and that DEP does not consider the MACTEC report as a final determination. Damann Anderson stated that there is no other way to compare onsite contributions to other sources without looking at the MACTEC report. Mr. Anderson stated that if you are going to evaluate significance you cannot base it on the loading. The loadings are not comparative the way they have been calculated. He stated that with some minor adjustments to the input numbers, a determination of significance can be made on the inputs. He also stated that there is still the question of what the definition of significance is. There can be consensus on the inputs, but not on the loadings because there are too many unknown questions and too many discrepancies in the data. Bill

Melton asked whether the inputs were calculated the same, and Mr. Anderson stated that with some minor corrections that can be fixed. The wastewater treatment facilities need to have total nitrogen applied to them, the atmospheric deposition number is only looking at nitrate in a rural setting instead of total nitrogen in an urban setting, and reuse water should be added into the wastewater. Dr. Young stated that the reuse numbers were not included in the MACTEC report as the assumption was made if you have reuse water you do not use fertilizers. John Glenn stated that he had difficulty relating total sales in an area to the input into that same area, and Damann Anderson stated that MACTEC did not use sales information. David Carter stated that the fertilizer input was based on an assumption of an application of a certain amount of fertilizer per acre of residential land. Pam Tucker stated that there is not time to change all the reports and reevaluate all the testing that has been done, but there is time to review the report that is going to the governor. She stated that the assumptions are generally consistent with MACTEC, the methodology is inconsistent, there is mangled information on nitrates vs. nitrogen, rural vs. urban. She stated that the department's report is based on conclusions in ill-matched reports. She stated that MACTEC and Damann Anderson have both stated that the reports are based on assumptions and she understood that studies would not be concluded on assumptions. She stated that she went through the entire report and has several comments that she will not go into at this point, but she does not think that RRAC can endorse this report as it is written. She would like to make a motion that RRAC does not support the report, it needs to be changed, amended, modified, etc. and Sam Averett seconded the motion for consideration and discussion purposes. David Carter asked whether RRAC wants to spend the remainder of the meeting going through the report. Stan Keely stated that RRAC can provide input but that the department will submit the report if they want to submit it. Mark Hooks stated that the report has to be submitted whether it is endorsed by RRAC or not, the department is required to submit. The timelines have not been conducive to get the report boiled down to one final conclusion. David Carter asked if the department could see any circumstance where the department would write a report that stated that the results were inconclusive, and Mark Hooks asked whether the budget language specifically asked for a conclusion. Patti Sanzone pointed out that DEP was also given similar budget language. David Carter stated that one can give a two line report that stated the results are inconclusive. Mark Hooks stated that that is true but is not certain that was one of the options outlined in the budget language. David Carter stated that he does not know how anyone as a scientist and a public health official can tell somebody that something is right, wrong, significant, or insignificant if you have not come to that conclusion. Patti Sanzone was concerned that the first line of the conclusions stated that there is an answer when RRAC is finding out that there is no answer at this time. Mark Hooks stated that there is data and the department recognizes that the data is not ideal and Patti Sanzone stated that she does not read that in the first sentence of the conclusions. Mark Hooks stated that the report does outline where the data came from, that the conclusion was based on this data, and that a conclusion can only be made on the data that is available at the time of the decision. Patti Sanzone stated that RRAC does not know what they need to know in order to make a judgment on

what needs to be implemented. David Carter stated that this is a 50 to 100 million dollar program, and real people are going to have to pay this money. He is okay telling people that they need to spend this money if there is a real problem and this will help solve the problem, but he is not at that point yet. In reading the report he felt that the tone was more conclusive rather than inconclusive. David Carter was under the impression that the department would be handing in a status report. Mark Hooks stated that there is a deadline that needs to be met; a report has to be issued. This does not mean that this is the last say in everything. He stated that there is time between now and the next TRAP meeting in August for RRAC to make comments. Pam Tucker stated that the legislature will most likely not review the report until next March. Mark Hooks stated that there is nothing that prohibits the report from being amended. Damann Anderson does not understand how the department can move forward with a report worded in this way and there is no evidence to support the language in the executive summary. Pam Tucker reads part of the legislative mandate, and points out that the report shall assess whether onsite systems are a significant contributor, and at this point the data is inconclusive. Mark Hooks stated that the department understands the limitations on the data on which the language is based. Bill Melton stated that there are parts of the draft that he agrees with. He stated that if nothing is done, and development continues in this area, that loading is just going to become greater. Some of the recommendations need to be addressed now or the loading will continue to increase. David Carter asked why the department would want to go forward with inconclusive results. Damann Anderson stated that there are a lot of good ideas in the report, but there are many misperceptions that will be maintained once the report comes out. David Carter stated that RRAC and the department got the task, the consultants did the work, and now the results are not gelling. He would like to see RRAC come forward with a solid recommendation that makes sense. The department can put rules forward, but they can be challenged. He stated that you are not really improving the environment unless you come forward with a solid report. Paul Booher stated that he would like to expand on this. There is a new DOH secretary and this is an assignment given to her office and she would appreciate if she did not miss the date. John Byrd pointed out that this task was given to her by Governor Bush. The department has reservations about this and there are three things on the input side that Damann Anderson had suggested that could be correctable within the next two weeks. Then the report could be submitted, with Dr. Roeder's Task 4 report with the first sentence reading: "This appendix of the 2007 Wekiva Study Report suggests a range of strategies that can be employed as a part of a comprehensive onsite sewage treatment and disposal system management program to reduce their particular nitrogen contributions and generally their environmental impact in the Wekiva Study Area, **in the event that onsite sewage treatment and disposal systems are found to be significant relative to other sources.**" Paul Booher continues, stating that the report can say that we did this because we did not have the time, we do not know whether it is significant, and we do not know who is going to determine whether it is significant. John Byrd stated that what he understands Paul Booher is saying is that the department will say they do not know whether it is significant or not, but if it is here is what we propose. Paul Booher stated that if this statement is

added the report, and the introduction is amended to reflect that this report is inconclusive because it is not verified by phase II of the DEP task, then the deadline can be met. When the department receives a copy of the phase II DEP report the department will do the verification and finalize the report. Damann Anderson stated that he thinks this is a good idea if the loadings are left out. John Glenn stated that significance is a range, and the report can state that it is significant to an extent we have not quite determined. David Carter stated that if onsite systems were a big part of the problem then many things would need to be done, but if they are a small part of the problem then a few minor things can be done to make them work better. His reaction to decisions on cost-effective solutions may differ depending on how much of a problem onsite systems are determined to be. He stated that the committee can come to the conclusion that they are satisfied with the inputs, but not satisfied with the loadings which are the key part to determining significance, and not list the numbers as if they are absolute and finite. Quentin Beitel stated that significance is a relative term. As compared to fertilizer, onsite systems are not significant. He recommended to put a definition of significance in the report. He stated that the quality of the report is very good, but he wants to see a quality truthful product. He assured everyone that his association will follow-up on this. Paul Booher suggested that RRAC review the report, make modifications to the input calculations, and withhold the loading part for DEP's phase II. John Byrd stated that the DEP spokesperson stated that they might have the project done in approximately a year and it might be done in time for the next legislative session. Paul Booher stated that this information gathered today goes back to Gerald Briggs and he is the one that makes the final decision. Paul Booher stated that he understands the concerns with the loading pie-chart going out, and suggests withholding that until the DEP phase II information comes in. Stan Keely suggested that RRAC clearly tell the secretary the issues and problems they find regarding the report, RRAC cannot control what the department does. He does not think that the onsite numbers have increased as much as some of the other sources over the last 30-years. David Carter suggested that RRAC develop a list of conclusions. Pam Tucker stated she had an issue with the recommendations, if working with presumptuous conclusions, how do you come up with specific strategies. John Byrd suggested Pam Tucker's earlier motion be amended as Paul Booher stated before moving on to the Task 4 discussion. Pam Tucker restated what Paul Booher stated earlier: Remove the loadings at this time to reevaluate once DEP's phase II is completed, concentrating on the contributions from inputs as updated by Damann Anderson's suggestions. Paul Booher stated also to include Dr. Roeder's report with the modification to the statement he mentioned earlier. John Byrd stated that the determination of significance should be withheld until the DEP phase II information has been received. Dr. Roeder stated that this essentially means that we would not commit to not doing anything for a long time. Pam Tucker withdrew her first motion that RRAC does not endorse the report as it is written now. Pam Tucker makes a new motion in the spirit of what Paul Booher stated. Dr. Young stated that she does not have the information on the total nitrogen numbers from wastewater treatment facilities and there was a discussion that those numbers will be obtained. Dr. Young asked whether she would do anything to the fertilizer

numbers if she adds reuse back to wastewater and the consensus was that she would not need to do anything because reuse was not added to the fertilizer numbers. Dr. Young asked how she was to calculate the atmospheric deposition numbers and Damann Anderson stated that there is much information about the Tampa Bay airshed he could get her. There was a discussion about whether this is an urban or a rural or a mixed airshed, and Damann Anderson stated that it was an urban airshed. There was a discussion about how the total nitrogen numbers would be calculated for the wastewater treatment plants that do not have any information, and it was agreed that for those where there is information the average "blow-up" factor from nitrate to total nitrogen would be applied to those with no information. Stan Keely stated that there is a significant difference in air models in different parts of the state. He recommended that numbers should be obtained for central Florida, and that the coastal numbers will most likely be different than inland numbers. David Carter pointed out that it is better to use urban coastal numbers rather than rural coastal numbers which are the numbers that MACTEC used. Sam Averett pointed out the significant difference in the original pie chart in the DOH report submitted in 2004 for atmospheric deposition: going from 49% to 2%, and Mark Hooks explained that the 2004 report used the Wakulla Springs area which is more rural than the WSA. David Carter stated that the loading numbers should be eliminated from the report because of the inconsistencies in the way the numbers are calculated and portrayed in the MACTEC report. John Byrd stated that the department can submit Task 4 as they see fit, but that implementation of anything in that report shall be contingent on the determination of significance which will come after DEP's phase II. It was made clear that Paul Booher's references to altering the statement regarding the recommendations was in the Task 4 report and not in the 18-page DOH draft report that everyone else was looking at. Paul Booher stated that there were concerns about Task 4 being included in the report if significance has not been decided on and his suggestion is to include it with the caveat that he mentioned earlier. In the case that onsite systems are determined to be significant after the DEP phase II, then the recommendations are there for review. Dr. Roeder asked whether we need to wait for DEP's phase II before continuing or can we use any new information that may develop. Jim Rashley stated that we need to be in agreement with DEP. If new information is uncovered RRAC can review it and make a decision at that time. **Motion by Pam Tucker and seconded by John Glenn to amend the report to use the inputs as presented in Linda Young's report with adjustments for atmospheric deposition to use urban information instead of rural and adding ammonia to make it total nitrogen, to add reclaimed/reuse water to the estimates for wastewater treatment plants, and to use total nitrogen numbers for wastewater treatment plants. Linda Young's report shall be modified to reflect these changes. There shall be no conclusions on loading until the second phase of the DEP report has been completed. The loadings shall be removed from the DOH report and the Appendix.** The motion passed unanimously.

The RRAC and the public request that the final DOH report be available for review by posting the report on the website and emailing the report to the distribution list.

- v. **Task 4** (Cost-effective solutions): Dr. Roeder has drafted a report. There was a discussion on whether this task should be included before there was a conclusion by RRAC that onsite systems are a significant source of loading to the groundwater. Pam Tucker asked whether the Task 4 report will be changed in response to the motion voted on by the RRAC committee to remove the loading numbers from the report. Dr. Roeder stated he can update his report as Paul Booher had suggested. John Byrd stated that if the determination of significance will be withheld until the DEP phase II report is done, then Task 4 can remain in the report but would not be implemented until the determination has been made. David Carter stated that he is uncomfortable with requiring performance based treatment systems (PBTS) on a large scale. It is better to be simple. He asked staff whether it would be better to propose some other strategies that are not as complicated and maintenance intensive. Dr. Roeder stated that if we want to get to nitrogen reduction, adding new systems will increase the contribution. If new systems meet higher requirements, then there is a decrease in the increase of the rate of loading. If sewer could be made available, then there can be a comparison between nitrogen reduction vs. connecting to sewer to make the best decisions on where to install new infrastructure. The question then is how to do the nitrogen reduction. The code has a performance boundary that can be met in several different ways. Dr. Roeder stated that the Seminole County site showed that you cannot only rely on the soil. This is why pretreatment is a strategy. He surveyed installers in the WSA and found that there are two steps in increasing performance levels: an ATU and 10 or 20 mg/L effluent levels with a PBTS. The expensive step is to go to an ATU and then going to a PBTS is not that much more expensive, has similar operation and maintenance, and yields better reductions in nitrogen. David Carter asked whether the costs include maintenance and Sam Averett stated that the more systems there are the cheaper it is to maintain them, and that the costs to install would also go down. Dr. Roeder stated that we could keep the homeowner to maintenance entity structure that currently exists or go to a utility program where there is one utility that oversees everything. A utility would be a cheaper alternative. Paul Booher mentioned Dr. Otis' comment that 12 to 15 mg/L is a passive system and is much more expensive to reach 10 mg/L. Dr. Otis explained that a passive system could include a pump, and that some of the more passive systems are the fixed film systems such as recirculation filters. Paul Booher stated that based on Dr. Otis' comments, 15 mg/L may be more cost effective, but Dr. Roeder's determination based on specific WSA information showed that there was not much of a difference in costs. David Carter asked how different the big picture would look if effluent is brought from 70 mg/L to 15 mg/L vs. 70 mg/L to 10 mg/L. Sam Averett stated that if the restriction is raised to 15 mg/L the market opens up for many more manufacturers. David Carter stated that if there was \$5 million, for example, would it be better to spend that on 500 new PBTS or do 1000 system upgrades to those in the groundwater. Dr. Roeder pointed out that in the Seminole County site there was the separation to the water table but it still was not getting the nitrogen out. Dr. Otis suggested empowering the people and giving them choices. In his experience it is not the same cost to get from 15 mg/L to 10 mg/L. There are many things in the report that it looks like the department would like to do regardless of the results, like

upgrading existing systems. He would suggest that instead of the Task 4 report to have a list of things that DOH would like to implement regardless of the results, and the rest will be on hold until the final determination on significance can be made. Damann Anderson agreed this is a good idea. The report can recommend such things as getting rid of digouts and bringing repair systems up to code, but hold off on the ultimate fix until you know the significance. David Carter stated that the PBTS systems are a riskier expenditure of money. The other things are known to work. Damann Anderson stated that many of these recommendations need to be done anyway. Ellen Vause stated that the department is defining what needs to be done but would like this statement to be put into the report: The department intends to work with the TRAP and RRAC to help develop these recommendations to reduce the inputs. Instead of listing all these recommendations, just state that the department will work with TRAP to develop rule changes to reduce impacts. There was agreement among RRAC and the audience that there are several good ideas presented in the report that would be of benefit throughout the state, and should be considered for implementation. Sam Averett stated that reducing or eliminating digouts will save the homeowner money and will improve the quality of the effluent. Dr. Roeder asked whether there is data to support that this will work and Damann Anderson stated they will work if the drainfield is sized big enough. Sam Averett stated there is nothing in the code that identifies a spodic as a severely limited soil and Mark Hooks stated that it is an organic soil which is defined in the code as a severely limited soil. David Carter asked the RRAC whether they would want to review the recommendations in more detail while waiting for the updated DEP numbers and Pam Tucker stated that she would rather take the entire recommendations section out because the determination of significance has not been made. Paul Booher stated that along with Dr. Otis and Damann Anderson's comments, Task 4 would remain in the report with a statement that these are some things that should be done regardless of the determination of significance and preface some of the other recommendations with if it is determined to be significant we recommend these other things. Ellen Vause asked if the draft is going to be changed, would the recommendations still be listed in the executive summary. Paul Booher explains again that Gerald Briggs is the one who makes the final decision. David Carter stated that everyone needs to understand that all RRAC can do is give a list of recommendations. Ellen Vause asked whether there will be a section of RRAC recommendations if the final report comes out to be significantly different from what was discussed during this meeting; David Carter stated that the motions made as part of this meeting need to be included in the final report. Pam Tucker makes a motion that RRAC does not endorse the conclusions or recommendations of the department report at this time due to outstanding questions that persist over the loading data for sources and the premature nature of the conclusions and recommendations. Ellen Vause stated that she was recommending that the recommendations from RRAC be included in the report to the governor. Pam Tucker modified her original **motion for the department to list the committee recommendations voted on during the meeting in a separate section of the report.** John Glenn seconded the motion. Doug Everson stated that the objective of the study was to determine

whether onsite systems are a significant source and that this was to be determined by the RRAC. He would interject that the motion should encompass whether RRAC has reached a decision as to whether the objective has been met. If the objective has not been met than it should be on record. David Carter thought that was taken care of in the first motion. Pam Tucker stated that they were holding off on significance. Pam Tucker asked whether the department is in agreement with allowing the RRAC comments to be included in the report and Dr. Roeder, Mark Hooks, and Elke Ursin did not think that would be a problem and that Gerald Briggs had indicated that when the TRAP meeting was canceled the TRAP comments would come from Chairman Harper directly to the legislators. There was no further discussion, all were in favor and the motion passed. David Carter stated that there are two options on how to proceed with the recommendations: to include the entire list as is or split the list into two parts: common sense issues to implement now, and more involved recommendations that are only triggered by a finding of significance. Ellen Vause stated there are several good things to address in the report. The report could state that out of these studies things were found that could be corrected and if it is found to be significant then move to the next step. From the executive summary Ellen Vause stated that some of the recommendations she is in favor of are to have all systems inspected and pumped every five years, inspections during real estate transactions, and upgrade repair/modifications to new system standards. Bill Melton stated he has an issue with upgrading repairs to new standards because older homes are built to elevations that make new system standards difficult or impossible to meet. Damann Anderson stated that in that situation a pump would be installed which would increase the nitrogen removal. Bill Melton stated that a three-foot mound in the front yard changes the appearance of the whole piece of property and can reduce the value of the home. Jim Rashley stated that on smaller lots there is also a sacrifice on drainfield size to accommodate shoulders and slopes. Ellen Vause stated that there are many systems being repaired today with the 6-inch separation that have the room to be able to meet a higher separation. Bill Melton stated that this is an issue for the TRAP. Damann Anderson stated that the department should take advantage of this opportunity to say: while we cannot determine significance at this point we realize that onsite systems do have an impact on nitrogen and here are some things we can do immediately to help solve the problem. John Byrd stated that now there is the appearance of being halfway in and halfway out, and feels that until there is a determination of significance to hold off on Task 4. Dr. Roeder stated that significance still has not been defined, but his impression is that there is a cost-effectiveness component to it. If the cost is expensive then it better be really really significant, but if it's cheap it can be a little bit significant. David Carter asked whether RRAC wants to put in a list of strategies now or not. Several members stated that they would rather wait until significance is determined. Pam Tucker stated that she has a problem with the real estate point of sale inspections. Sam Averett makes a motion that nothing be done with Task 4 and Pam Tucker seconded. Bill Melton stated that he is not uncomfortable with adding a caveat stating that Task 4 has been addressed if loading is significant. David Carter paraphrased what Bill Melton stated: the department has evaluated the strategies however a finding of significance is not being made at

this time so no strategies are being put forward at this time. Ellen Vause interpreted what Bill Melton stated as the department recognizes if nitrogen contributions are determined to be significant the following strategies are recommended. This lets the legislature know that Task 4 has been completed. John Byrd stated that the department can come to the legislative session and say that there are some great proposals that came out of this process and here's what the department thinks should be done. John Glenn suggested modifying the motion to state that RRAC has made no determination on strategies at this time. This leaves the opportunity in the future to go back to some things. Sam Averett amended the motion to read: **the RRAC committee recommends no action be taken on Task 4 at this time** and Pam Tucker was in agreement with the change. Damann Anderson asked whether this would mean the Task 4 report would be taken out, and that decision would be up to Gerald Briggs. There was no more discussion, all were in favor and the motion passed.

- vi. **Summary of RRAC Motions:** The committee made the following motions:
 1. Motion by Pam Tucker and seconded by John Glenn to amend the report to use the inputs as presented in Dr. Young's report with adjustments for atmospheric deposition to use urban information instead of rural and adding ammonia to make it total nitrogen, to add reclaimed/reuse water to the estimates for wastewater treatment plants, and to use total nitrogen numbers for wastewater treatment plants. Dr. Young's report shall be modified to reflect these changes. There shall be no conclusions on loading until the second phase of the DEP report has been completed. The loadings shall be removed from the DOH report and the Appendix. The motion passed unanimously.
 2. Motion by Pam Tucker and seconded by John Glenn for the DOH draft report to include the list of RRAC recommendations voted on during this meeting. The motion passed unanimously.
 3. Motion by Sam Averett and seconded by Pam Tucker that the RRAC recommends that no action be taken on Task 4 at this time. Task 4 was to determine cost-effective solutions if contributions of nitrogen are found to be significant. The motion passed unanimously.

4. Public Comment

- a. The public was allowed to comment throughout the meeting and their comments are included throughout the minutes.

5. Closing Comments, Next Meeting, and Adjournment

- a. John Glenn stated that there is nothing stopping the RRAC from taking some of the recommendations made in the report and supporting them. David Carter clarifies that all the motions were made unanimously, and recommended that the minutes reflect that. Ellen Vause stated that the department has worked very hard on this project and does not discount the amount of time and effort that went into doing this. There were some very good things that can be used with this report to upgrade the industry and make sure the environment is protected. John Glenn and David Carter both stated that they were pleased with staffs cooperation and hard work. John Byrd asked whether the final report would be available for RRAC to review before it is sent to the

legislature. David Carter stated that the report can be emailed and/or posted to the website but there is no more time for another meeting. RRAC can submit comments about the report at any time to the department. The RRAC has almost been meeting monthly for this project when they are only required to meet twice a year. The membership can be polled to call a meeting. Sam Averett would like to discuss the Keys study. David Carter would like to have a financial accounting of the department's budget and a list of priorities for the next meeting. John Glenn made a comment about Florida running out of water and there needs to be more support for waterless and self-composting toilets. Ellen Vause stated that Florida needs to stop dumping wastewater into streams and oceans and allowing it to filter down to the aquifer through the soil.

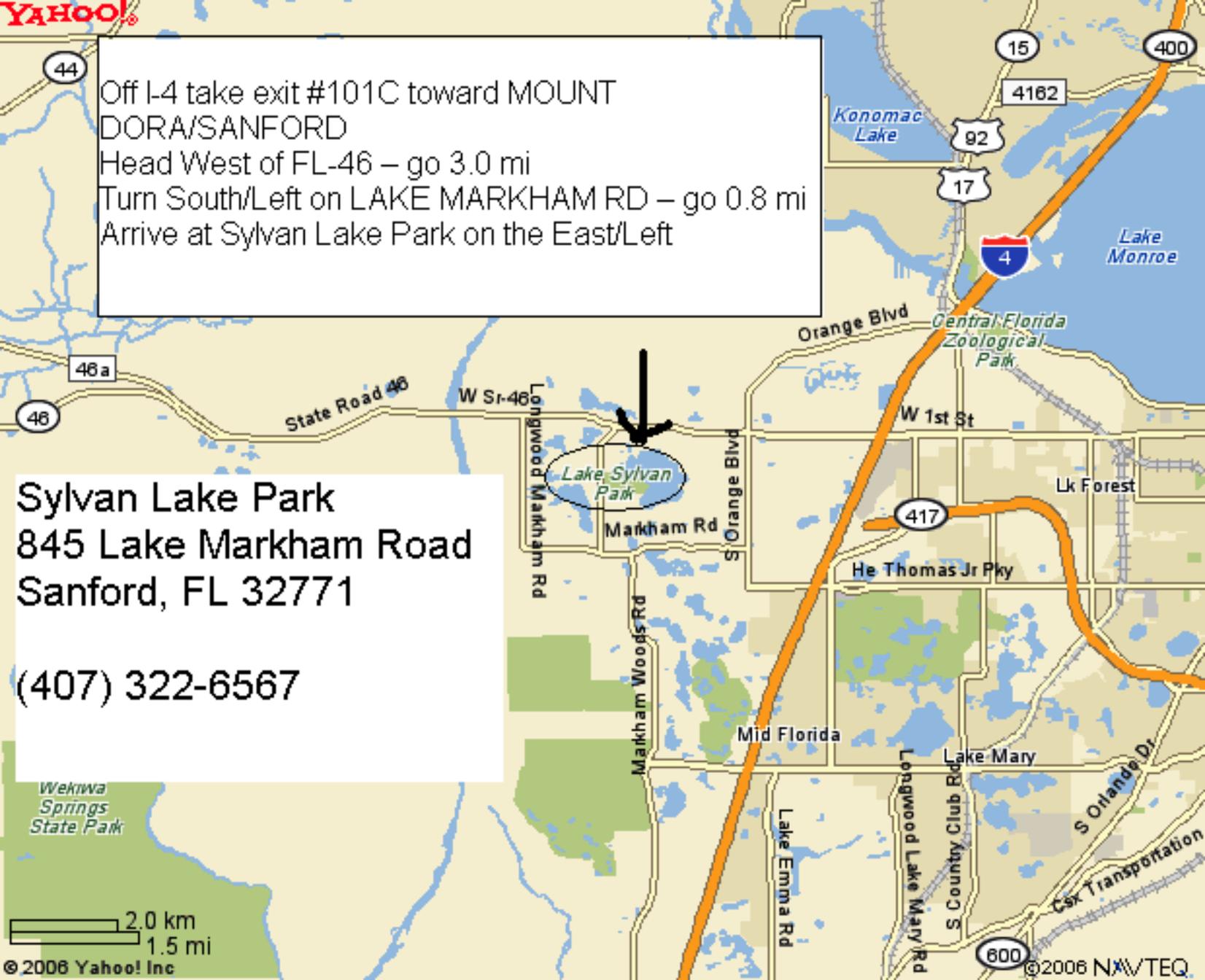
- b. No date was set for the next meeting. Anticipated to be some time in September at a location to be determined. The meeting adjourned at 3:40 pm.

DRAFT

Off I-4 take exit #101C toward MOUNT DORA/SANFORD
Head West of FL-46 – go 3.0 mi
Turn South/Left on LAKE MARKHAM RD – go 0.8 mi
Arrive at Sylvan Lake Park on the East/Left

Sylvan Lake Park
845 Lake Markham Road
Sanford, FL 32771

(407) 322-6567



64E-6.010 SEPTAGE AND FOOD ESTABLISHMENT SLUDGE

(1) through (6) No change

(7) The food establishment sludge and contents from onsite waste disposal systems shall be disposed of at a site approved by the DOH county health department and by an approved disposal method. Untreated domestic septage or food establishment sludges shall not be applied to the land. Criteria for approved stabilization methods and the subsequent land application of domestic septage or other domestic onsite wastewater sludges shall be in accordance with the following criteria for land application and disposal of domestic septage.

(a) through (v) No change.

(w) The land application area shall not be within the Wekiva Study Area as defined in 369.316, F.S.

Specific Authority: 381.0011(4), (13), 381.0065(3)(a), 489.553(3), FS. Law Implemented: 381.0012, 381.0061, 381.0065, 386.041, FS. History: New 12-22-82, Amended 2-5-85, Formerly 10D-6.52, Amended 3-17-92, 1-3-95, 5-14-96, Formerly 10D-6.052, Amended 3-22-00, 05-24-04, 11-26-06,_____.

64E-6.0162-Specific Standards for the Wekiva Study Area

(1) The following standards shall apply to all systems in the Wekiva Study Area as delineated in 369.316, F.S.

(a) Except in areas scheduled by an adopted local wastewater facility plan to be served by a central sewage facility by January 1, 2011, all new systems shall be an performance-based treatment system providing nitrogen reduction. The systems shall provide at discharge from the treatment units before disposal an annual average nitrogen reduction of 70 percent or a limit of 10 milligrams per liter, with a maximum individual sample concentration of 20 mg/L. No increase in authorized flow allowances in 381.0065(4)(a), (b), or (g) or reductions in surface water setbacks in 381.0065(4)(e) or (l) shall be allowed. All systems shall use drip irrigation or low-pressure dosing.

(b) All existing systems requiring repair, modification or re-approval must meet a 24 inch separation from the wet season water table and surface water setbacks in 381.0065(4)(e) or (l), unless a variance has previously been granted by the State Health Office. All treatment receptacles must be within one size of current requirements in Table II and must be tested for water-tightness by a state licensed septic tank contractor or plumber. The bottom of the drainfield shall be no more than 18 inches below finished grade.

(c) All systems shall be pumped out and evaluated by a state licensed septic tank contractor or plumber every five years. Upon completion of the evaluation the contractor shall complete Form DH 4015 page 1 – 4, and submit the application for approval to the department with a \$35 fee. A copy shall also be provided to the owner. The department shall review the application and approve the system for continued use or notify the owner of the requirement for a repair or modification permit. The department shall be responsible for notification and enforcement of the pumpout and evaluation requirement. Initial notifications shall be phased in over a five-year period beginning July 1, 2008.

Specific Authority 369.318, 381.0011(4), (13), 381.0065(3)(a), FS. Law Implemented 369.318, 381.0065, 381.0067, 386.041, FS. History—New _____.

Evaluation of Onsite Sewage Treatment and Disposal Systems in Shallow Karst Terrain

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Submitted to Water Research on 8/21/07

Abstract. Two conventional onsite sewage treatment and disposal systems (OSTDS) at Manatee Springs State Park, Florida USA were studied to assess their impact on groundwater quality in a shallow karst environment. Sulfur hexafluoride and fluorescein were used to establish connections between the drainfields and monitoring wells and to estimate travel velocities. The fluorescein tracer indicated rates of 9.6 ± 10.5 m/day ($n=11$), while SF₆ derived rates were only 0.3 ± 0.2 m/day ($n=11$). Elevated nutrients were found in all wells where significant concentrations of both tracers were observed, with the mean of the highest nitrate concentration observed at each well being 47.8 ± 14.9 ($n=11$) mg/L nitrate-N. The most elevated nutrient concentrations were found directly in the flow path of the effluent as indicated by the tracer experiments. Fecal coliform densities above 10 cfu/100 mL were observed in wells with the most rapid connection to the drainfield. The proximity and connectivity of the surficial soils and the underlying karst aquifer allows rapid contaminant transport and limits the ability of conventional OSTDS to attenuate nutrients.

Keywords: Karst, Groundwater, Sulfur Hexafluoride, Fluorescein, Septic Tank, Onsite Sewage



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“Taylor County Beaches Pathogen and Nutrient Sources Assessment Study Part II Taylor County Source Tracking: Seasonal Variability Study”

-FIELD ACTIVITY REPORT-

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Laboratories for Engineered Environmental Solutions

September 27, 2007

**Taylor County Beaches Pathogen and Nutrient Sources
Assessment Study Part II:
Taylor County Source Tracking:
Seasonal Variability Study**

**Field Activity Report
Seasonal Low Water Table Elevation Event**

May 21 – 25, 2007

Prepared for:

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Approving Signatures and Dates:

Dr. Daniel Meeroff, FAU Principal Investigator

Date

Dr. Fred Bloetscher, FAU Co-Principal Investigator, QA Officer

Date

INTRODUCTION

Florida Atlantic University (FAU) 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 will address 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.

Methodology

The field sampling procedures will be governed according to the previous Quality Assurance Project Plan filed for DOH contract number CO0F7: Taylor County Beaches Pathogen and Nutrient Sources Assessment. According to prior work conducted in Taylor County, additional sampling locations are desired to assist in resolving confounding issues in source tracking hypotheses. 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.

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 1. 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, tidal variability is expected, because some sampling sites are located in shallow (< 6 in.) water.

Table 1. 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 Fenholloway	Developed area without sewer?	No	No	No
HS	Hampton Springs Bridge	Perry	Middle of the Fenholloway	Developed area with 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
Jl	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	3 rd 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

Sampling Sites

The objective of the field study is to distinguish between human sources of pollution and various other types of contamination in coastal waterways within Taylor County, FL. Locations are chosen including coastal canals, inland rivers, and beaches. The sampling locations are 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.

Table 2. Summary of sampling site distribution by category.

Location	Beach	Canal/Creek (Upstream)	Background
Developed without Sewer			
Dekle Beach (ρ = low)	<ul style="list-style-type: none"> • Dekle Beach • Jugg Island Road 	<ul style="list-style-type: none"> • Mexico Road 	<ul style="list-style-type: none"> • Creek at Dekle
Steinhatchee (ρ = high)	<ul style="list-style-type: none"> • Main Street 	<ul style="list-style-type: none"> • Third Avenue Fork • Steinhatchee at Airstrip Drive • Boggy Creek at 51 (tributary) 	<ul style="list-style-type: none"> • Steinhatchee Falls
Developed with Sewer Being Installed			
Keaton Beach (ρ = medium)	<ul style="list-style-type: none"> • Keaton Beach 	<ul style="list-style-type: none"> • Cortez Road Canal (Pump Station) • Cortez Road Canal Upstream (Jet Skis) • Cortez Road Canal Downstream (Marina Rd) 	<ul style="list-style-type: none"> • Blue Creek at Beach Road
Cedar Island (ρ = medium)	<ul style="list-style-type: none"> • Cedar Island Beach house • Seahawk Lane house 	<ul style="list-style-type: none"> • Heron Road Canal 	<ul style="list-style-type: none"> • Blue Creek at Beach Road (same as above)
Other Areas Sampled			
Spring Warrior Beach (ρ = low)	<ul style="list-style-type: none"> • Peterson's Landing 		
Adam's Beach (ρ = low)	<ul style="list-style-type: none"> • Adam's Beach Landing 		
Perry (ρ = high)		<ul style="list-style-type: none"> • Fenholloway Upstream (River @ 19/Alt27) • Fenholloway Downstream (Hampton Springs Bridge) 	

The seven new sites were selected to address several confounding issues that arose during the first year of monitoring. The Fenholloway River set of sites (FR, HS, and PL) attempt 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. During the dry season, this effluent can constitute up to 80% of the river volume (Bortone and Cody 1999). The Fenholloway River is 36 miles long, and its watershed drains approximately 392 square miles of mostly rural area (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 3.

Table 3 - 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	
BOD₅	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 µmhos/cm	--
Color	--	1200 PCU	--

During the December 2006 SLWT event, 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 below 0.15 mg/L as N, the Fenholloway samples were all higher than 3.0 mg/L as N. The additional ammonia could also originate from wastewater treatment facilities or septic tanks. 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 (groundwater recharge). Investigation of the connection between the upstream Fenholloway discharge and its potential impacts along the beaches south of the discharge was beyond the scope of the original study, but in the follow-up sampling it was desired to investigate its effects. The thought process was to follow the effluent from near the original

discharge (FR) to the middle stream (HS) and finally to where the river exits into the Gulf of Mexico (PL). The FR site is located approximately one mile downstream of the industrial discharge of the specialty cellulose mill. The HS site is located about midway from the mill to the ocean along the Fenholloway River. The site is on 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 exits to the ocean, the prevailing north-to-south current 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 see if we can determine a concentration gradient in the flow of bulk transport. Adam's Beach is one of the previously sampling sites. 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. 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 which is also connected to the discharge of the upstream creek (JI).

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 NH₃-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.

Field Sampling Methodology

The field sampling protocol basically replicated the May 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 (The week of May 21 was identified as ideal according to the tidal predictions). Samples were collected and analyzed according to the previous QAPP or similarly effective methods.

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)

Field Log

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.

<p>May 21 – (Monday)</p>	<p>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).</p>
<p>May 22 – (Tuesday)</p>	<p>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.</p>

<p>May 23 – (Wednesday)</p>	<p>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.</p>
<p>May 24 – (Thursday)</p>	<p>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 ($n = 36$).</p>

<p>May 25 – (Friday)</p>	<p>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).</p>
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Additional Experiments

In addition to increasing the site density, additional experimental work was recommended to resolve confounding issues. 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).

Shallow Sediment Re-Growth Studies

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 using sterile gloves. 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 $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 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 $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ 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 or autoclaved (after being wrapped in aluminum foil). 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 168 hours (7 days) later.

Existing Infrastructure Assessment

- Summary of hydraulic regime of the Blue Creek estuary
- Summary of existing sewer network and OSTDS in the study area
- Summary of existing upstream industrial wastewater discharges

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 (NaOCl) disinfection, a holding pond, a spray irrigation field, an office/work-shop, and a back-up power generator. 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-in pipe at the home connecting to 3-in or 4-in mains within the neighborhoods that connect to larger 6-in or 8-in force mains to the plant. Mr. Morgan informed us that the package plant is fed with corn in the winter 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 3 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 MDL).

Unconventional Source Tracking Tools

In previous work, 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. However, given the prior results, molecular techniques and nitrogen isotopic ratios were determined to be more informative as tracers. During the last two sampling events, the molecular techniques proved to be independent of the previous day's microbial density. 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.

Molecular Techniques

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.

For each site, replicate water samples were collected and bacteria and particles were harvested onto membrane filters. Approximately 50-900 mL of sample was filtered onto Whatman 7141-104 cellulose nitrate filters or 0.2 μm Supor-200 filters in sterilized (autoclaved) plastic filter holders. 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. The cellulose nitrate filter membranes were incubated either on mTEC agar (233410 from VWR) for enrichment of *E. coli* and *Salmonella* or M Enterococcus agar (90003-930 from VWR) for enrichment of *Enterococcus* and *Staphylococcus*. Filters were incubated overnight at 35°C in a VWR 1525 signature series general purpose incubator and then delivered to AOML for processing. No positive growth controls were performed. 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.

Supor-200 filters were transferred to an Analyslide[®] Petri dish and frozen immediately, but not at -80°C, without incubation, 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. 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 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.

This work is ongoing, and results will be forthcoming.

Nitrogen Isotope Ratio

Nitrogen isotope ratio experiments will follow the procedures outlined in Heikoop et al. 2000; Sammarco et al. 1999; Risk and Erdmann 2000; Costanzo et al. 2004; among others. Samples for $\delta^{15}\text{N}$ 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 experiment technique for testing source tracking hypotheses in water samples based on nitrogen isotopes in aqueous ammonia and nitrates. The samples will be analyzed after the nutrient analysis is completed. This work is ongoing, and results will be forthcoming.

FLORIDA PASSIVE NITROGEN REMOVAL STUDY

Literature Review and Database

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BACKGROUND

As population growth continues in Florida, so do the potential impacts of on-site wastewater treatment systems to surface and groundwater quality. Nitrogen loading from wastewater treatment systems may be a concern where numerous on-site wastewater treatment systems are located within sensitive environments. Conventional septic tank and soil adsorption systems rely on biological reactions in porous media (setback layer or unsaturated natural soil) to attenuate nitrogen loadings to ground or surface water. Groundwater nitrate concentrations have been shown to exceed drinking water standards by factors of three or greater at distances on the order of several meters from soil adsorption systems (Postma et al., 1992). In a study at Big Pine Key, Florida, the dissolved inorganic nitrogen (DIN) levels in groundwater contiguous to on-site drainfields were greater than DIN levels at a control location (Lapointe et al., 1990). Groundwater $\text{NH}_3\text{-N}$ levels at Big Pine Key reached 2.75 mM, indicating a high fractional breakthrough of ammonia through the on-site treatment system. In another study, conducted on a sandy Florida aquifer system, groundwater levels of both Total Nitrogen and ammonia were elevated above background levels at a distance of 50 meters from a conventional soil adsorption drainfield (Corbett et al., 2002). Available setback distances in Florida locations may often be quite limited, which increases the significance of achieving high nitrogen removal percentages within septic tanks, media filters and other in-tank treatment processes, as well as with in soil treatment units (Siegrist, 2006). A summary review of a wide variety of on-site treatment approaches showed that systems with some degree of “passive” character exhibited Total Nitrogen removal efficiencies of 40 to 75% and produced effluent TN of 10 to 20 mg/L (Anderson and Otis, 2000). FDOH has an interest in exploring the feasibility and practicality of using relatively “passive” on-site treatment systems to accomplish even higher nitrogen reductions in a cost effective manner.

The mission of the Bureau of Onsite Sewage Programs of the Florida Department of Health (FDOH) is “Protecting the public health and environment through a comprehensive onsite sewage program”. FDOH established the Florida Passive Nitrogen Removal Study to identify “passive” treatment systems that can achieve greater nitrogen reductions than exhibited by conventional septic tank/drainfield configurations. The FDOH is specifically interested in approaches that employ filter media, or reactive filter media, which eliminate the need for aeration pumps and minimize the need for liquid pumping. The first step of the Florida Passive Nitrogen Removal Study is to identify treatment configurations, reactive and non-reactive media, performance capabilities of new and demonstrated technologies, and factors influencing performance and longevity. These tasks will be based on a literature review and contacts with experts with knowledge and experience in this field. This report provides the literature review and database for passive nitrogen removal on-site systems.

PASSIVE NITROGEN REMOVAL

The goal of passive nitrogen removal is to provide on-site systems with relatively simple operation and low life cycle costs. Passive nitrogen removal approaches must be cognizant of the speciation of nitrogen (inorganic vs. organic, particulate vs. soluble, oxidized and reduced), the biochemical reaction sequence needed for complete nitrogen removal, and the use of *Total Nitrogen* as the generally accepted metric of system performance:

$$\text{Total Nitrogen (TN)} = \text{Organic N} + \text{Ammonia N} + \text{Nitrate N} + \text{Nitrite N}$$

In septic tank effluent (STE), nitrogen is present in organic and ammonia forms, with virtually no oxidized N. Other nitrogen relationships and delineations are listed below.

$$\text{Total Kjeldahl Nitrogen (TKN)} = \text{Organic N} + \text{Ammonia N}$$

$$\text{Organic Nitrogen} = \text{Filterable Organic N} + \text{Non-filterable Organic N}$$

$$\text{Total Inorganic Nitrogen (TIN)} = \text{Ammonia N} + \text{Nitrate N} + \text{Nitrite N}$$

$$\text{Total Oxidized Nitrogen (TON)} = \text{Nitrate N} + \text{Nitrite N}$$

$$\text{TN} = \text{TKN} + \text{TON}$$

Conventional unmixed septic tanks provide sedimentation and removal of suspended solids and particulate nitrogen. STE contains ammonia, non-filterable organic N, and filterable organic N that has not been removed within the septic tank by sedimentation. The use of strainers to treat effluent from septic tanks (also termed STE “filters”) can enhance removal of filterable organic N. Filterable Organic N in STE would be removed in media filters by the standard physical filtration mechanisms of straining, impaction and sedimentation within the filter bed.

Of great importance to the configuration of passive nitrogen removal systems are biochemical nitrogen transformations. The significant biochemical transformations are listed below in the sequence in which they must generally occur. Hydrolysis converts particulate organic N to soluble organic N, which in turn releases ammonia through ammonification. Both processes can occur in the presence or absence of oxygen.

Hydrolysis



Ammonification



Removal of total nitrogen in on-site systems requires both nitrification (an aerobic process) and denitrification (an anoxic process). Nitrification must occur first and must be followed by denitrification. Passive denitrification filters cannot treat septic tank effluent without pre-treatment with some type of aerobic treatment. Therefore, if septic tank effluent is considered as the starting point for examining nitrogen reduction strategies, a systems view of nitrification and denitrification may be most beneficial.

Nitrification (requires O₂)

Ammonia N → Nitrite N → Nitrate N

Denitrification (requires electron donor)

Nitrate N → Nitrite N → Di-nitrogen (N₂)

Nitrification requires oxygen, while denitrification requires an electron donor. Oxygen for nitrification can be supplied to liquid in septic tanks, pumping tanks, or other treatment tanks using aeration pumps, or by air ingress (assisted or unassisted) into systems containing unsaturated media, such as packed trickling filters, recirculating sand filters, peat filters, textile filters, and the unsaturated zones of drainfields. Here, the unsaturated media are attachment surfaces for nitrifiers and other microorganisms.

To remove nitrogen, both centralized and decentralized wastewater treatment plants must create the conditions necessary to sustain the biochemical reactions required for nitrogen removal. Several different process trains are used in wastewater treatment plants of which two closely mimic the processes that commonly occur in nature. These treatment trains, called the “simultaneous” and “two sludge” systems (Figures 1 & 2) can be used to describe denitrification in soils. “Sludge” in this case refers to the active biomass in the process, which provides the treatment. In the simultaneous process the biomass is a mixture of autotrophs (nitrifiers) and facultative heterotrophs (organic degraders & denitrifiers) while in the two sludge system, the two groups of microorganisms are separated in different reactors.

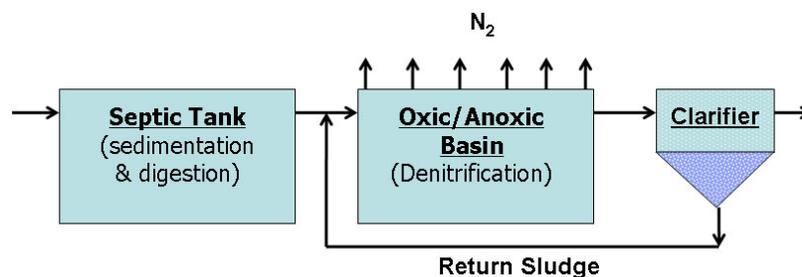


Figure 1. Simultaneous Denitrification System

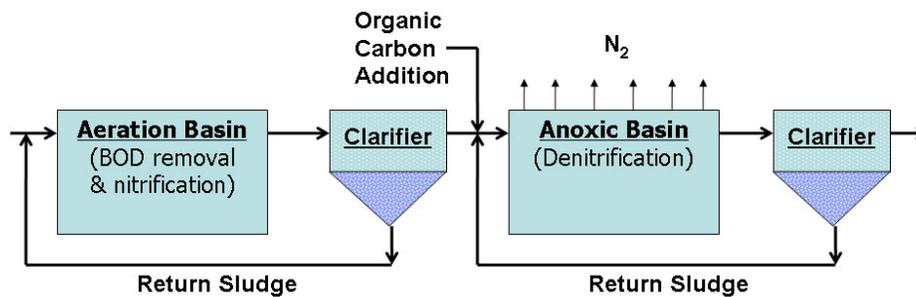


Figure 2. Two Sludge Denitrification System

In the simultaneous system, denitrification is achieved by cycling between oxic and anoxic conditions in a single reactor such that nitrification and denitrification is accomplished “simultaneously” (Figure 1). This process occurs in the soil when wastewater containing ammonium and biodegradable carbon is applied to aerobic soil. In response to the application, facultative heterotrophs quickly degrade the organic carbon and deplete the oxygen in doing so. The ammonium cannot be nitrified under anoxic conditions, so being a positively charged ion; it may be retained in the biomat at the infiltrative surface or adsorbed by clay minerals in the soil. As the soil drains and reaerates, the autotrophic nitrifiers are able to nitrify the ammonium. Without percolating water available to leach the nitrate, the nitrate is held in the soil until the next dose of wastewater. With the addition of new organic carbon, the facultative autotrophs again deplete the oxygen degrading the organic carbon and once the soil is anoxic the heterotrophs turn to the oxygen in the nitrate molecule as a replacement for free oxygen in the electron transfer, which results in denitrifying the nitrate to N_2 and NO_x gases.

This simultaneous process has the advantages of having a reliable supply of organic carbon from the wastewater for the denitrification step, lower oxygen requirements, and it recycles the alkalinity needed for nitrification. However, the amount of denitrification can be limited depending on the frequency and duration of the oxic/anoxic fluctuations with respect to the reaction rates. In a field study, which investigated OWTS design and operation that would maximize denitrification, Degen, et al. (1991) found that this simultaneous process performed best because carbon is the limiting factor for denitrification in soil. However for optimum results, the OWTS must be installed in a surface horizon to ensure an adequate supply of organic matter and dosed on a 48 hour interval to create alternating oxic/anoxic cycles of sufficient duration for the reactions to occur. Also, these requirements imply that the infiltration system must completely drained between applications of wastewater to allow the soil to reaerate.

The two sludge system can achieve nearly complete nitrogen removal because the fluctuating cycle is avoided during which ammonium can by-pass the nitrification step. However, since the nitrification step removes nearly all the organic carbon, a separate source of organic carbon is required (Figure 2). As the wastewater is applied to the soil,

the heterotrophs degrade the organic carbon in the biomat as before. However, if little clay is present to adsorb the ammonium as the wastewater percolates into the soil, the ammonium will move with it. As the ammonium percolates through the biomat into the vadose zone, oxygen is present for the autotrophs to nitrify the ammonium using carbon dioxide as the carbon source. Nitrate is a very soluble compound so it readily moves with the percolating water deeper into the soil profile. If the percolate encounters a shallow saturated zone and sufficient organic carbon is available to deplete the oxygen to create an anoxic environment, the facultative heterotrophs then will use the remaining organic carbon for denitrification of the leached nitrate.

This two sludge process has the advantage that it can achieve more complete nitrogen removal but its disadvantages can prevent full denitrification from occurring. This process is very dependent on an external organic carbon source to be present (Bitton, 1994; Degen, et al., 1991; Oakley, 2005). If the water table is shallow, sufficient organic carbon may be present in the saturated zone from the decay of roots and other soil flora. If the water table is deep, organic matter is more likely to be removed before reaching the saturated zone.

A third process model that has been recognized only recently is an anaerobic, autotrophic bacterial process called Anammox. This process is possible when both nitrate and ammonium occur together under anoxic or anaerobic conditions (Van de Graaf et al., 1995; 1996; 1997). In this process, the autotrophs reduce the nitrate to nitrogen gas while utilizing the oxygen from the nitrate to oxidize the ammonium to nitrate. Because the bacteria are autotrophs, no organic carbon is required to sustain this process. Anoxic or anaerobic conditions are necessary because if not, the heterotrophs would oxidize the ammonium removing the energy source from the autotrophs.

Total nitrogen in the effluent from the treatment sequence will consist of ammonia, nitrate and nitrite, and organic nitrogen. The ammonia nitrogen levels in the effluent from the unit operations preceding the denitrification filter must be consistently at or below target levels for final effluent ammonia nitrogen, since ammonia may behave conservatively as wastewater passes through the anoxic denitrification filter. For passive denitrification filters, solid phase electron donors are employed that provide attachment surfaces for denitrifying microorganisms and electron donor supply through a process of continuous dissolution over extended time periods. While numerous potential solid phase electron donors exist, the most commonly applied have been lignocellulosic materials such as wood chips and sawdust that support heterotrophic denitrification and elemental sulfur (autotrophic denitrification). The total oxidized nitrogen levels in the effluent from the denitrification filter must be consistently at or below target levels for final effluent oxidized nitrogen, which can be established either independently or be apportionment of the target effluent Total Nitrogen among the nitrogen species.

The meaning of the term “passive” for nitrogen removal in on-site wastewater treatment systems can then be addressed within the context of overall STE composition, the forms and speciation of nitrogen, and the mechanisms of nitrogen removal. For the Florida Passive Nitrogen Removal Study, a program specific definition for the term “passive” was provided by FDOH:

Passive *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*

This definition of a “passive” system placed significant restrictions on the types of onsite wastewater treatment systems than can be considered. The definition precludes the use of aeration pumps within any system component: septic tank, dosing tank or other treatment chambers. Oxygen for BOD removal and nitrification must therefore be supplied by unassisted aeration to an unsaturated media filter that operates as a four phase system: solid media, water, gas phase, and attached biofilm. Wastewater is supplied at the top of the media and flows downward by trickle flow or percolation. This very common approach to onsite wastewater systems is applied in sand filters and in other media filters, providing ammonification and nitrification.

Single pass unsaturated media filters can provide some degree of denitrification using wastewater organics. Recirculation of filter effluent to a septic tank chamber or dosing tank can substantially enhance denitrification and produce Total Nitrogen removals of 60% or greater. To achieve higher Total Nitrogen removal percentages and lower effluent TN concentrations, unsaturated filter effluent can be directed to a denitrification filter. Denitrification filters are possibly the only feasible approach to enhancing TN removal in onsite systems beyond that achievable by unsaturated filters. Denitrification filters are saturated with water and are three phase systems: solid media, liquid, and biofilm (possible bubble formation from denitrification is considered insignificant). The solid phase contains a reactive solid media that supplies electron donor for denitrifying organisms. The solid phase electron donors that have been most commonly studied are elemental sulfur and cellulosic materials (sawdust and wood chips).

Another stipulation of the “passive” definition is that only a single effluent dosing pump be used. The dosing pump must provide adequate head to convey wastewater from the septic tank effluent elevation, through filter media, and presumably to a soil treatment unit. Wherever the single pump is positioned within the treatment train, the movement of wastewater before and after the pump must be by gravity. The pump can provide very important treatment features in addition to hydraulic conveyance, including the ability to pressure dose, the ability to time dose, and the ability to spread wastewater uniformly over a filter surface. These features have been exploited in aerobic filters such as intermittent sand filters and are central to successful treatment. An additional feature afforded by a pump is the ability to recirculate a portion of filter effluent, using

various non-powered splitter devices which do not require power or manual operation. Recirculation of the effluent of an aerobic filter effluent (recirculating sand filter for example) increases denitrification using wastewater organics, resulting in substantial increases in TN reduction and decreased in effluent TN.

An additional treatment consideration is alkalinity and the need to maintain appropriate pH conditions for biochemical reactions. Nitrification consumes 7.14 grams of alkalinity as CaCO_3 per gram ammonia N nitrified, and nitrifying microorganisms are inhibited as pH decreases below neutral. For an STE containing 45 mg/L TN, required alkalinity is 321 mg/L. The alkalinity of the starting water supply, as augmented by the increase in alkalinity through domestic water use (perhaps 60 to 120 mg/L), must be sufficient to prevent pH decrease and inhibition of nitrification. Nitrogen removal performance of a total nitrogen removal system could be affected by alkalinity of STE and the effects of pH conditions on biochemical reaction rates. If the pH drops in an aerobic filter due to nitrification, then nitrification might not proceed to completion, leaving a high residual ammonia concentration. Ammonia in the effluent of the first stage aerobic filter could largely pass through a second stage anoxic filter, thereby lowering the overall TN removal efficiency. A benefit of recycle around the aerobic filter is that the partial pre-denitrification would be accompanied by the additional benefit of restoration of alkalinity. Alkalinity restoration may become more important in the future as water conservation trends exacerbate the potential of alkalinity to limit nitrification in non-recycle aerobic systems. The potential advantages of recycle in aerobic systems are increased as TN levels increase in STE.

The first stage filter must achieve a high degree of BOD and ammonia removal because these components may not be degraded in the second stage anoxic filter environment. Additionally, a high quality first stage effluent will limit the amount of solids and BOD added to the second stage filter. Lower loadings to the anoxic filter should reduce the possibility of channeling and enable better long term performance and lower maintenance needs.

Saturated anoxic filters for passive denitrification have far less studied than unsaturated filters. Anoxic filters are usually fully submerged to preclude ingress of oxygen from air. Oxygen in the incoming flow is probably utilized preferentially near the entrance, enabling anoxic conditions to prevail downstream. Denitrifying microorganisms reduce oxidized inorganic nitrogen, predominantly nitrate, to nitrogen gas. The denitrifying microorganisms grow as biofilms on the reactive media, dissolving the reactive media and using it for nitrate reduction. Nitrate is reduced to nitrogen gas, which leaves the reactor dissolved in the liquid effluent or as small bubbles. The principals of porous media biofilm reactors have been well established. Factors that affect performance include the size, specific surface area, tortuosity and porosity of media, average liquid residence time, superficial flow velocity, linear velocity, uniformity of flow (i.e. channeling), mass transfer and biofilm kinetics. A special feature of the passive anoxic filters is the reactive dissolution of the media. The media must supply enough electron

donor for denitrification or nitrate removal may decline. On the other hand, if media dissolution is too rapid, media longevity will be reduced and the reactor effluent will contain excess dissolution product (such as BOD for cellulose based media). A solid phase alkalinity supply, such as limestone or crushed oyster shell, may be required to maintain pH. Over long term continuous operation, flow channeling can result in short circuiting, decreased contact time of with biofilms, and decline in performance.

A “passive” treatment system for nitrogen removal must be seen as an integrated sequence of unit operations/processes that can achieve the treatment goal. If it is assumed that the starting point is septic tank effluent (STE), then the total treatment system must meet the target treatment goal. The treatment goal could be expressed as the Total Nitrogen (TN) concentration “leaving the treatment system,” or “entering the environment.” Suppose the goal is to achieve a TN of 3 mg/L or less before directing the effluent to a soil absorption field. Assuming nitrite levels are negligible, the effluent TN of 3 must be apportioned between 1. organic N, 2. ammonia-N, and 3. nitrate-N:

$$\text{Organic-N} + \text{NH}_3\text{-N} + \text{NO}_3\text{-N} \leq 3.0$$

The biochemical sequence requires ammonification and nitrification before denitrification. If it assumed that the final treatment step will be an anoxic denitrification filter that contains a reactive media, then attention must be focused on the concentrations of organic N and ammonia N in the influent to the denitrification filter. Ammonia levels could increase across the denitrification filter due to ammonification of influent organic N; ammonia levels could decrease across the denitrification filter by nitrification near the inlet using residual dissolved oxygen in the actual denitrification filter influent.

A conceptual approach to process formulation is to estimate the effluent nitrate N achievable in anoxic filters, and allocate the remainder of the target effluent TN to the sum of organic N and ammonia N that are allowed in the anoxic filter effluent. For a target of 3 mg/L TN:

$$\text{TKN}_{\text{allowable}} \leq 3.0 - \text{NO}_3\text{-N}$$

The approach would be conservative from an engineering perspective because, as long as the influent TKN were less than $\text{TKN}_{\text{allowable}}$, the effluent TKN of the anoxic denitrification filter would never exceed $\text{TKN}_{\text{allowable}}$, regardless of ammonification.

This discussion points to the important need to reduce TKN in the treatment that occurs before the anoxic denitrification filter. Producing TKN less than $\text{TKN}_{\text{allowable}}$ should be the first priority of the “first stage” of treatment. For “first stage” systems that accomplish denitrification along with nitrification and ammonification in the same process tank or through recirculation, the critical question is: is the effluent TKN less than $\text{TKN}_{\text{allowable}}$.

From a knowledge of the functioning of aerobic filter systems treating STE, it is hypothesized that optimization of the aerobic treatment process is the most important factor affecting overall nitrogen reduction. This is speculative, because there is limited experience in the coupled operation of aerobic and coupled anoxic filters in passive configurations. If the aerobic process must be optimized, then the single pump that is allowed should be used to supply STE to the aerobic biofilter. The benefits of more frequent doses of lower volume and more uniform flow distribution will accrue to the aerobic filter, and provide a high quality influent to the anoxic biofilter. Using the pump to supply the aerobic biofilter will enable recirculation, which will lessen the nitrate loading to the denitrification biofilter and reduce alkalinity requirements. For low relief Florida environments, the aerobic filter would be placed above grade to enable gravity flow to and through the anoxic filter and then to a soil treatment unit.

The following points summarize the needs that must be satisfied by the passive nitrogen removal technology, and factors that influence the overall approach and configuration:

- the biochemical requirement for initial aerobic reactions (ammonification and nitrification), followed by anoxic denitrification, likely in two separate filters;
- a first stage unsaturated media filter allowing air ingress without aeration pumps;
- first stage filter to achieve target effluent ammonia and organic nitrogen level;
- second stage saturated denitrification filter with reactive solid phase electron donor and possible alkalinity source;
- second stage design to achieve desired effluent oxidized nitrogen level;
- provide adequate head for passive media filtration, enabled by only one effluent dosing pump;
- preferred alternative considered dosing pump to first stage unsaturated (aerobic) filter that enables timed pressure dosing and uniform effluent distribution;
- possible recirculation around first stage (unsaturated) filter.

LITERATURE SEARCH METHODOLOGY

Databases and Search Engines CSA Illumina (<http://www.csa.com/>) and Science Direct (<http://www.science-direct.com/>) search engines were used to access multiple data bases, as shown in Table 2. The American Society of Agricultural and Biological Engineers (ASABE) Technical Library was queried, as ASABE has been sponsoring an on-site wastewater treatment symposium every three years. Search terms listed in Table 2 were combined using *and* operator logic in numerous configurations. In addition, Google (<http://www.google.com/>) and Google Scholar (<http://scholar.google.com/>) searches were conducted on the World Wide Web, using the same search terms listed in Table 3.

Test Centers The on-site centers listed in Table 4 were contacted regarding information on passive nitrogen removal technologies, experience, and theoretical and practical developments. A site visit was made on May 21, 2005 to the Massachusetts Alternative Septic System Test Center on Cape Cod, MA. During this visit, it was determined that nitrogen removal technologies were being evaluated at the test center that were subject to non-disclosure by center staff. As a result of this visit, a memo was prepared and addressed to the test center requesting voluntary information disclosure from technology developers using the test center for evaluation of nitrogen removal technologies. A copy of the memo is included in Appendix A.

Table 1 Search Engines and Databases

CSA Illumina
Biotechnology and Bioengineering Abstracts
Environmental Sciences and Pollution Management
Environmental Engineering Abstracts
Pollution Abstracts
Science Direct (over 2000 peer reviewed journals)
Applied Science and Technology
Civil Engineering Abstracts
American Society of Agricultural and Biological Engineers (ASABE) Technical Library

Table 2 Search Terms

denitrification
wastewater
on site
nitrogen
nitrate
ammonia
nitrification
passive
septic
carbon
wood
sawdust
sulfur
organic
media
filter
filtration
solid
peat filter
recirculating filter
sand filter
coir filter
zeolite filter
soil denitrification

Table 3 On-Site Centers Contacted

Massachusetts Alternative Septic System Test Center
Rhode Island On Site Wastewater Resource Center
Deschutes County Environmental Health Division, Oregon Department of Environmental Quality (La Pine National Demonstration Project)
National Environmental Services Center
Baylor Wastewater Research Program

Personal Contacts Personal contacts were made with individuals who are involved with developing, testing, and evaluating technologies for nitrogen removal in on-site wastewater treatment systems. The individuals contacted are listed in Table 5. Valuable insights were gained through discussions and information transfer, and technical reports and information was obtained that was not otherwise available.

Table 4 Individuals Contacted

Dr. Bruce Tesikar	Texas A & M University
Dr. Robert Siegrist	Colorado School of Mines
George Loomis	University of Rhode Island
George Huefeld	Director, Massachusetts Alternative Septic System Test Center
Damain Anderson	Hazan and Sawyer, Tampa
Barbara Rich	Environmental Health Division, Dechuttes Co, Oregon
Pio Lombardo	Lombardo and Associates
Dr. Sukalyan Sengupta	University of Massachusetts-Dartmouth
Paul Hagerty	Hagerty Environmental
Wesley Brighton	Wastewater Alternatives
Dr. Martin Wanielista	University of Central Florida

LITERATURE SEARCH RESULTS

Database Structure A database was constructed using EndNote software provided by Thomson Research Soft (<http://www.endnote.com/>). EndNote is a contemporary and fully supported standard software tool for publishing and managing bibliographies on the Windows and Macintosh® desktops. Endnote allows internal searches using keywords, and Endnote files can be exported for use in other software. The Passive Nitrogen Removal database contains 224 references, which are listed in Appendix B. The Endnote entries include keywords and abstracts for most citations, and URL addresses are provided for numerous citations. The attached CD includes numerous PDFs for cited articles, and PDFs and Word files containing descriptive and performance data for numerous citations.

Organization of References in Electronic Files References were classified according to the nested tree file framework shown in Figure 1. The files in the attached CD are also organized according to the Figure 1 framework. The numbers in the parenthesis of Table 2 are the numbers of citations or supporting documents in each in each folder.

The overall organization includes general nitrogen removal in on-site systems, nitrification processes, denitrification processes, and drainfield modifications. Denitrification processes are classified into heterotrophic and autotrophic processes. Heterotrophic processes are subdivided into citations for general cellulosic, cellulosic sources and other carbon sources. The cellulosic folder includes several separate folders for processes of for studies for which several citations of supporting files are available. The autotrophic citations are dominated by sulfur based systems, testifying to the extensive research in this area. As an example, an internal Endnote search using the single search term *sulfur* extracted 43 entries in the Florida Passive Nitrogen Removal Study Citation List. The search terms *organic* and *carbon* each extracted a similar number of citations. The search terms *sand filter*, *peat*, and *wetland* extracted 29, 14 and 12 citations, respectively.

The assembled Citation List includes nitrification processes, including recirculation systems. A system using a recirculation pump, such as a recirculating sand filter, would not be “passive” in the sense that a one-pass flow through media filter would be “passive.” In fact, some state regulatory agencies who are considering the certification of passive denitrification filters are requesting that, as part of the certification process, the provider also specify the aerobic treatment system(s) that would be acceptable to the provider as pretreatments for the denitrification filter (Loomis, 2007). If the treatment system under consideration already includes an aerobic treatment process, then addition of a passive denitrification filter could in itself provide substantially increased total nitrogen removal. The term *recirculating* extracted 26 citations from the Florida Passive Nitrogen Removal Study Citation List.

Some references appears in more than one folder in the attached CD for the reason that they cover more than one subject classification or that they have subject common to more than one area. One example is citations in the Drainfield Modification folder. The passive denitrification media applied in in-tank processes are often similar to those that could be applied within drainfields. The organization framework of Table 2 is used in the following section to review the individual citations.

Table 5 Organization of Citations in Electronic Files
() number of files

<p>Onsite Nitrogen Removal (10)</p> <p>Aerobic Unsaturated Filters (Unsaturated)</p> <p>Recirculating Sand Filters (6)</p> <p>Peat Filters (9)</p> <p>Waterloo Biofilter (1)</p> <p>Textile Filters (2)</p> <p>Coir Filters (3)</p> <p>Zeolite Filters (2)</p> <p>Anoxic Filters (Saturated)</p> <p>Heterotrophic Processes</p> <p>Cellulosics (7)</p> <p>Point (4)</p> <p>Nitrex (5)</p> <p>RI Systems (4)</p> <p>La Pine Study (5)</p> <p>Other Carbon Donors (6)</p> <p>Autotrophic Processes</p> <p>Sulfur (38)</p> <p>Sulfide (1)</p> <p>Iron (1)</p> <p>Heterotrophic/Autotrophic Processes (3)</p> <p>Drainfield Modifications (10)</p> <p>Point (4)</p> <p>Black & Gold (1)</p> <p>Multi Soil Layers (5)</p> <p>Soil Denitrification (1)</p>
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REVIEW OF PASSIVE NITROGEN REMOVAL

Technologies with potential for application in passive on-site nitrogen removal systems are discussed here and summarized in the attached spreadsheet *Passive N Technology*. Nitrogen in septic tank effluent occurs in reduced form as organic nitrogen or ammonia. Total nitrogen removal requires aerobic nitrification as a first biochemical reaction followed by denitrification. These must occur within process tanks, in natural systems, or within soil treatment units (drainfields) modified for enhanced nitrogen removal. The complete citation list for the literature review is contained in Appendix B and in the Endnote file that is an integral part of this report. In this section, tables are presented which contain number designations for citations; these refer to the numbered citation list in Appendix B.

Unit Operations

As a biochemical necessity, ammonification and nitrification is required prior to passive denitrification filters. Removal efficiency and effluent concentrations of organic nitrogen and ammonia are of great concern, as well as the quality of the aerobic effluent that could affect operation of the anoxic denitrification filter. Passive denitrification filters operate with lower dissolved oxygen or under completely anoxic conditions, and are limited in their ability to remove reduced nitrogen (i.e. organic and ammonia nitrogen). Initial treatment units that promote nitrification may also denitrify and reduce total nitrogen, and recirculation (as in recirculating sand filters) can increase total nitrogen removal and lower the nitrate loading on the subsequent passive denitrification filters. Recirculation around the aerobic treatment filter also restores alkalinity. In a single pass system, nitrification could result in a decline in pH due to alkalinity consumption. Inhibition of nitrification at lower pH could result in deterioration in ammonia removal performance. The increasing emphasis on domestic water conservation could result in higher total nitrogen (TN) levels in septic tank effluent and increases in the TN/alkalinity ratio. For a given domestic water source, the potential for inhibition of nitrification would increase.

Factors that influence the passive nitrogen removal technology selection include the water quality characteristics of STE, target effluent nitrogen levels of the overall treatment system, and the reliability of providing continuous treatment. It should be realized that there may be limitations on the concept of a completely passive treatment system for removal of Total Nitrogen from onsite wastewater. An inverse relationship may exist, which is not strongly defined, between nitrogen removal effectiveness and treatment system passivity. The literature review was conducted to examine currently employed and possibly new approaches elucidate to passive nitrogen removal, and to identify technologies and combinations of systems that could be used.

Reported nitrogen removal effectiveness of example treatment systems that do not employ aeration pumps but that employed recirculation are listed in Table 1. Table 1 is by no means an inclusive listing of available technologies, and the results in Table 1 are presented as examples only. While non-proprietary recirculating sand filters can provide Total N and ammonia N reductions of 40% and >85%, respectively, proprietary technologies that do not employ aeration pumps can achieve ammonia N reductions of 90% and greater, with or without recirculation. Total N removal can be increased from 20 to 40% without recirculation to 40 to 70% and higher with recirculation. As a general design principal, lower target levels for effluent Total N and greater reliability would require separation of aerobic and anoxic zones, recirculation pumping, and greater levels of process control and maintenance.

Aerobic (Unsaturated) Filters

Prominent nitrification processes include intermittent and recirculating sand filters, peat filters, textile filters, and filters with other media. These systems are summarized in Table 6. All systems contain porous media through which wastewater flows downward by trickle flow. Oxygen is supplied by ingress of air through pore spaces in the media. All systems are capable of substantial reductions of organic nitrogen and ammonia. Another common feature enhancement of total nitrogen reduction by recirculation, which provides pre-denitrification using organic matter in the wastewater as electron donor. Summaries of unsaturated filter technologies have been presented in Jantrania and Gross (2006), Leverenz et al. (2002) and Crites and Tchobanoglous (1998).

Recirculating sand filters (RSF) are capable of achieving ammonia removals of 98% and Total N removals of 40 to over 70% (Kaintz et al., 2004; Loudon et al., 2004; Piluk and Peters, 1994; Richardson et al., 2004;). Effluent ammonia levels of 3 mg/L or less can be achieved (Uryniewicz et al., 2007). Low temperatures have been suggested to adversely affect RSF ammonia removal performance, but the adverse effect of temperature should be of limited significance in the Florida climate. Peat filters can achieve ammonia nitrogen removal efficiencies of 96% or greater from septic tank effluent, with effluent NH₃-N in some cases of 1 mg/L or less (Lacasse, 2001; Lindbo and MacConnell, 2001; Loomis et al., 2004; Patterson, 2004; Rich, 2007). Some studies also suggest that TN can be reduced peat filters. TN reductions of 29 to 41% have been reported in modular recirculating peat filters (Monson Geerts et al., 2001a); 44% in peat filters using pressurized dosing (Patterson et al., 2004); and 15 and 21% in two single pass modular peat filters. Recirculating textile filters achieved 44 to 47% TN reduction (Loomis et al., 2004) from septic tank effluent. In some cases, textile filters treating septic tank effluent have produced effluents with NH₃-N levels of less than 1 mg/L (Rich, 2007). The Waterloo Biofilter is a proprietary treatment system that has been demonstrated to reduce septic tank effluent TN by 62% while also providing over 90% ammonia N removal (132). Textile filters also produce nitrified effluents (McCarthy, et al., 2001; Rich, 2007; Wren et al. 2004) and are often operated at higher hydraulic loading rates (Table 6).

Table 6 Summary of Unsaturated Aerobic Media Filters

System Type	Description	Features	Treatment Performance	Citations (Refer to Appendix B)
Intermittent sand filters	Sand filter Single pass	0.3 to 0.7 mm media 18 to 36 in. depth 0.7 to 1.5 gal/ft ² -day 12 to 48 dose/day	TN Removal: 20 to 50% NH ₃ -N Effluent: 20 to 20 mg/L Effluent: 1.9 to 9 mg/L	10,12,28,41,49, 58,71,87,93,64,110, 133,166
Recirculating sand filters	Sand filter Recirculation	1.5 to 3 mm media 18 to 36 in. depth 3 to 5 gal/ft ² -day 40 to 120 dose/day	TN Removal: 40 to 75% NH ₃ -N Effluent: 15 to 30 mg/L Effluent: 1 to 5 mg/L	20,24,33,40,41,53, 56,83,87,88,93,110, 117,129,130,139, 150,156,163,196, 198,206
Textile filters	Textile filter Recirculation	2 to 3 in. cubes 36 to 72 in. depth 8 to 17 gal/ft ² -day 80 to 140 dose/day	TN Removal: 20 to 60% NH ₃ -N Effluent: 10 to 60 mg/L NO ₃ -N Effluent: 1.7 to 5.9 Effluent: 11 mg/L	47,83,87,110,116, 122,155,215
Peat filters	Peat media filter Single pass or recirculation	246 to 36 in. depth 3 to 6 gal/ft ² -day 12 to 120 dose/day	TN Removal: 10 to 75% TKN Removal: 90 to 95% NH ₃ -N Effluent: 1 mg/L NO ₃ -N Effluent: 20 to 50	20,47,56,83,87,107, 110,116,122,123, 125,126,144-146, 155,160,196,213

Table 6 Summary of Unsaturated Aerobic Media Filters (Continued)

System Type	Description	Features	Treatment Performance	Citations (Refer to Appendix B)
Waterloo biofilter	Open cell foam media, single pass or recirculation	3 to 4 in. cube media 48 in. depth 11 gal/ft ² -day	TN Removal: 62% Effluent: 14 mg/L NH ₃ -N Effluent: 2.4 mg/L NO ₃ -N Effluent: 10 mg/L	134
Zeolite filters	Zeolite media filter	20 to 30 in. depth 6.1 gal/ft ² -day	NH ₃ -N Removal: 98.6% Influent: 70 mg/L Effluent: 1 mg/L NO ₃ -N Effluent: 57 mg/L	148
Coir filters	Coir filter bed, with recirculation	Coconut coir media 30 in. depth 5 to 10 gal/ft ² -day	-	177,178,193

Generally, nitrification processes are reasonably well developed technologies. Synthetic media generally have lower footprints and higher areal hydraulic loading rates. Issues involved include the use and need for recirculation, effluent levels of organic and ammonia N achievable, and reliability of performance. With some media, recirculation may be more important in maintaining ammonia removals, which may be related to oxygen ingress into the site of action of attached nitrifying microorganisms. A rational basis for comparison of aerobic media could potentially be developed using the effective media surface area within the filter bed, as perhaps modified by factors effecting oxygen ingress and by recirculation. The overriding requirement for the aerobic treatment performance is to produce low effluent levels of organic N and ammonia N prior to treatment in anoxic reactive media filters.

Factors affecting performance of unsaturated aerobic media filters are listed in Table 7. The hydraulic loading rate and loading rates of organics and nitrogen are important operating characteristics, particularly as they relate to the functioning of the physical and biological processes within the media. Key factors for successful treatment in an unsaturated media filter are surface area for attachment of microorganisms and for sorption of colloidal constituents in the wastewater, the need for sufficient pore space for assimilation of solids materials and their biodegradation between doses, the water retention capacity of the media, and the pore space that is available for aeration. The characteristics of media that influence performance of unsaturated filters are listed in Table 8. The performance of any unsaturated media filter is determined by the interactions of media characteristics (Table 8) with system parameters (Table 7). A significant interaction between media and system is the water retention capacity of media versus the hydraulic application rate. High water retention capacity is desirable to retain wastewater within the filter and achieve low effluent levels. The water retention capacity of media must exceed the hydraulic application rate per dose to prevent rapid movement of applied wastewater through the filter. More frequent doses (lower volume per dose), coupled with high water retention media, represent the most favorable combination.

Another highly critical factor to optimum functioning of unsaturated media filters is the aeration pore space. Unsaturated media filters are four phase systems: solid media, attached microbial film, percolating wastewater, and gas phase. The total porosity (excluding internal pore spaces within the media) must be shared between attached biofilm, percolating water, and gas phase. A media with a high total porosity will be more likely to be able to allow sufficient oxygen transfer throughout the filter bed, providing more effective utilization of the total media surface area and better treatment. If media size becomes too small, a larger fraction of the pores may become inaccessible to oxygen transfer. Sand may have a total porosity of 38%, but depending on sand size and hydraulic application rate, the aeration porosity may be as low as 2.5% as a percent of the total media volume. Such conditions could decrease

Table 7 Factors Influencing Performance of Unsaturated Aerobic Filters

Feature	Effect
Hydraulic loading rate	Higher rates lower water retention time and treatment
Organic loading rate	Higher loading rates increase rate at which biofilms must process organic matter; nitrification may be inhibited if too high
Nitrogen loading rate	Higher loading rates require higher nitrification rates and higher oxygen utilization rates
Media depth	Deeper beds can give better treatment; upper layers often more reactive
Specific surface area	Higher values give greater attachment surfaces for microorganisms
Superficial velocity	Effects mass transfer between wastewater and biofilms
Average linear velocity	Effects mass transfer between wastewater and biofilms
Hydraulic application rate per dose	Volume per dose should be scaled to field capacity of media
Organic loading rate per dose	Loading per dose must not exceed processing rate
Nitrogen loading rate per dose	Loading per dose must not exceed processing rate
Average water residence time	Longer residence time gives more time for biochemical reactions and better treatment
Uniformity of Dosing	Promotes full utilization of all elements of the filter media
Wastewater	
Suspended solids	Accumulated within pores, may lead to clogging if not biodegraded
BOD	High values require more room for attached growth and metabolism between doses, particularly in upper filter layers
Organic and ammonia nitrogen	Significant component of total oxygen supply requirement
Alkalinity	Consumed by nitrification and restored by heterotrophic denitrification; adequate supply needed to prevent pH decline by nitrification

Table 8 Media Characteristics Influencing Performance of Filters

Feature	Effect
Particle size distribution	Larger particles less subject to clogging Smaller particles have greater surface area per volume for treatment
Uniformity coefficient	Effects flow uniformity
Specific surface area	Higher values give greater attachment surfaces for microorganisms
Air filled porosity	Oxygen supply throughout media depth for BOD oxidation and nitrification in unsaturated filters
Water retention capacity	Higher water retention in unsaturated media filters provides longer time of contact of water with microorganisms and better treatment; affected by intrinsic porosity that favours capillary water retention
Sinuosity and tortuosity	Affect accessibility of pore spaces to exchange of wastewater and air
Specific weight	Effects compression strength required for support in multi media filters
Ion exchange capacity	Ammonia adsorption may improve performance
Compressibility	Effects material resistance to compression when wetted with biofilm and attached solids
Biodegradation	Biodegradation of organic media will limit longevity
Resilience	Prevents compaction under deployment
Hydrophilicity	Attracts water for wetting and rewetting

nitrification effectiveness, and perhaps also increase denitrification within microzones with limited contact with the gas phase. Denitrification within an unsaturated filter would improve total nitrogen removal but could result in less efficient nitrification and higher effluent ammonia concentrations. By contrast, media with high total porosity would be more likely to have a sufficiently high aeration porosity to allow effective utilization of all media surface area and better ammonia removal performance. If the goal is to achieve total nitrogen removal in an overall system containing an unsaturated filter followed by an anoxic, reactive media denitrification filter, then the goal of low effluent ammonia should take precedence over denitrification in the unsaturated filter. An example media with high total porosity and high water retention capability is sphagnum peat moss. The total porosity of sphagnum peat is greater than 85%, and percolating water might occupy two thirds of this available pores. Under these conditions, pore space available for aeration would be over 25% of the total volume of the filter bed. The very low effluent ammonia levels that peat filters appear capable of producing may be related to these factors.

Media with significant ion exchange capacity may offer a method to superior removal of ammonia nitrogen in flowing systems (Philip and Vasel, 2006; Smith, 2006). Zeolite media are excellent surface for biofilm attachment, and have relatively high porosities. Sorption of ammonium ions onto zeolite media can sequester ammonium ions from the water and provide enhanced contact with attached nitrifying organisms under steady flow conditions. Sorption also provides a buffer when loading rates are high, increasing the resiliency of the treatment process. The sorption is reversible, and microorganisms can biologically regenerate the zeolite media in periods of lower loading. A zeolite filter for onsite wastewater treatment removed 98.6% of ammonia and produced an effluent ammonia nitrogen concentration of 1 mg/L when operated at 6.1 gal/ft²-day (Philip and Vasel, 2006). Other bench scale and pilot studies have demonstrated the ability of zeolite filters to maintain high ammonia removal under high non-steady loadings of ammonia nitrogen (Smith, 2006).

Several candidate media can be suggested for the unsaturated media filter which forms the first stage of a passive onsite nitrogen removal system for Florida. Media should possess many of the desirable characteristics that have been discussed above. Coconut coir is a natural, renewable material that is a waste product from coconut production. Coir has many of the same properties of peat that make it a desirable treatment media, including high surface area, high water retention, and high porosity (Talbot, 2006), and has been successfully used as a planting media in greenhouses. While most coir is produced in Asia, Florida contains abundant coconut palm trees that could potentially provide a sustainable material source. A onsite wastewater treatment system using coconut coir has been reported (Sherman, 2006; Sherman, 2007). Synthetic fiber materials could have many of the same advantages as a media as coir. Zeolite filters also have promise for unsaturated flow filters for passive systems. The interaction of cation exchange media with microbial reactions appears to offer potential

for passive treatment with enhanced performance. Other candidate media include expanded clays and shales.

Anoxic (Saturated) Filters

Anoxic saturated media filters form a second stage in the passive nitrogen removal system. The 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 the oxidized nitrogen species is 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 are listed in Table 9. Hydraulic and nitrogen loading rate, surface area of media, pore size, and flow characteristics within the reactor are important considerations. The media is consumed by dissolution, and this process must be sufficiently rapid to supply electron equivalents for nitrate reduction and other possible reactions. On the other hand, rapid dissolution would reduce the longevity of the media. Too rapid a dissolution rate could also lead to the presence of excess dissolution products in the effluent (BOD for wood-based filters; sulfate for sulfur based filters). An aerobic process effluent low in BOD and suspended solids would be less likely to lead to channeling within the anoxic filter. Geometry of the column could affect flow patterns and potential channeling; the later effects could be overcome by use of larger systems. The effects of flow channeling on performance deterioration could require maintenance or media replacement at time scales appreciably shorter than longevities based on theoretical stoichiometric requirements of electron donor for denitrification. A summary of performance of passive anoxic denitrification filters is shown in Table 10.

Heterotrophic Denitrification Passive heterotrophic denitrification systems use solid phase carbon sources including woodchips (Cooke et al., 2001; Greenan et al., 2006; Jaynes et al., 2002; Kim et al., 2003; Robertson et al., 2000; Robertson and Cherry, 1995; Robertson et al., 2005; van Driel et al., 2006), sawdust (Eljamal et al., 2007; Greenan et al., 2006; Jin et al., 2006; Kim et al., 2003; van Driel et al., 2006), cardboard (Greenan et al., 2006), paper (Jin et al., 2006; Kim et al., 2003), and agricultural residues (Cooke et al., 2001; Greenan et al., 2006; Jin et al., 2006; Kim et al., 2003; Ovez, 2006; a, 2006b). In addition, limited studies have been conducted using other carbon sources such as cotton (Della Roca et al., 2005), poly(e-caprolactone) (Horiba et al., 2005), and bacterial polyesters (Mergaert et al., 2001). Cellulosic-based systems using wood are the most developed heterotrophic denitrification filter technology. The Nitrex process uses a proprietary media containing woodchips and other materials (EPA, 2007; NSF, 2003; Lombardo, 2005; Robertson et al., 2000; Robertson and Cherry, 1995; Robertson et al., 2005). Several Nitrex demonstration studies have been conducted, which have followed sand or peat filters, and some have operated for greater than two years (Lombardo, 2005). Combined RSF/Nitrex systems have

Table 9 Factors Influencing Performance of Saturated Anoxic Filters

Feature	Effect
Hydraulic loading rate	Higher rates lower water retention time and treatment
Organic loading rate	Higher loading rates increase rate at which heterotrophic biomass could accumulate
Solids loading rate	Higher loading rates increase rate at which solids could accumulate
Nitrogen loading rate	Higher loading rates require higher denitrification rates and higher rates of electron donor dissolution
Media depth	Deeper beds can give better treatment; uppers layers often more reactive
Specific surface area	Higher values give greater surface area for attachment of microorganisms and dissolution of media
Superficial velocity	Effects mass transfer between wastewater and biofilms
Average linear velocity	Effects mass transfer between wastewater and biofilms
Average water residence time	Longer residence time gives more time for biochemical reactions and better treatment
Wastewater	
Suspended solids	Accumulated within pores, may lead to preferential flow if not biodegraded
BOD	Will create more heterotrophic biomass and may increase potential for preferential flow
Nitrate nitrogen	High loadings require greater surface areas and higher levels of denitrifying activity
Alkalinity	Consumed by autotrophic denitrification; must be balanced by sum of influent alkalinity and alkalinity provided by solid source

Table 10 Summary of Saturated Anoxic Media Filters

System Type	Description	Features	Treatment Performance	Citations (Refer to Appendix B)
Sulfur/oyster shell filter (bench scale)	1 liter bench column synthetic wastewater upflow single pass	Sulphur/oyster shell media (75/25% by volume) Sulphur: 4.7 mm	anoxic only NO ₃ -N Removal: 80% Influent: 50 mg/L Effluent: 10 mg/L	170
Sulfur/oyster shell filter	185 gal. column aerobic effluent upflow single pass	Sulphur/oyster shell media (75/25% by volume) 47 gal/ft ² -day	anoxic only TN Removal: 82% Effluent: 4.2 mg/L NO ₃ -N Removal: 88% Influent: 20 mg/L Effluent: 2.4 mg/L	23
Sulfur/limestone column	237 gal. column groundwater upflow single pass Residence time: 13 hr.	Sulphur/limestone media (67/33% by volume) 63 gal/ft ² -day Sulfur: 2.5 to 3.0 mm Limestone: 2.38 to 4.76 mm	anoxic only NO ₃ -N Removal: 96% Influent: 64 mg/L Effluent: 2.4 mg/L NO ₂ -N Effluent: 0.2 mg/L	46

Table 10 Summary of Saturated Anoxic Media Filters (Continued)

System Type	Description	Features	Treatment Performance	Citations (Refer to Appendix B)
Nitrex™	aerobic effluent gravity flow upflow single pass	Nitrex wood-based media 24 to 30 inch media depth (est.) 4.6 gal/ft ² -day (est.)	<p style="text-align: center;">aerobic+anoxic</p> TN Removal: 79 to 96% Effluent: 3 to 18 mg/L NO ₃ -N Effluent: 0.3 to 8 mg/L	54,62,113,115, 155,157,159,200
Black& Gold™	wood-based media single pass downflow gravity	Influent: STE 280 gal. column Sand/tire crumb/woodchip (85/11/5% by volume) 8.3 gal/ft ² -day	<p style="text-align: center;">aerobic+anoxic</p> TN Removal: 98% Influent: 414 mg/L Effluent: 7.1 mg/L NH ₃ -N Effluent: 4.4 mg/L NO ₃ -N Effluent: 0.05 mg/L	173

produced average TN removals of 88 to 99% from septic tank effluent, with average effluent $\text{NO}_3\text{-N}$ concentrations of 1 to 2 mg/L. In another study, a subsurface leaching chamber was installed beneath an active parking lot for on-site sewage treatment, using sawdust as carbon source (St. Marseille and Anderson, 2002). At a loading of 1.22 gallons/ft²-day; the effluent $\text{NO}_3\text{-N}$ averaged 0.6 mg/L. Other heterotrophic denitrification systems have been successfully tested at laboratory scale.

Factors that affect the long term success of carbon-based denitrification filters include the long term availability of carbon supply for the wastestream being treated and the physical structure of the biodegradable components of the media. As for any packed bed, biologically active media filter which is deployed over extended periods of time, the long term hydraulics of the unit are a possible issue. Accumulation of biological and inorganic solids could lead over time to the development of preferential flow paths within the filter, reducing average residence time and wastewater contact with the media. To the extent that deposition and flow circuiting occur over time, deterioration of performance could result. The practical aspects of media replacement and disposal must be considered, in light of the frequency with which media replacement, maintenance or supplementation are required. Another factor is the release of soluble biodegradable carbon as water passes through the filter, which could increase biochemical oxygen demand (BOD) and chemical oxygen demand (COD). It is possible that this material would be readily consumed within tens of feet of release in a groundwater plume, or within a solid treatment unit receiving the effluent of the carbon-based denitrification filter.

Autotrophic Denitrification The autotrophic denitrification systems that have by far received the most attention are elemental sulfur-based media filters, and they are still under development. Sulfur-based denitrification filters usually employ limestone or oyster shell as a solid phase alkalinity source to buffer the alkalinity consumption of the sulfur-based biochemical denitrification (Brighton, 2007; Darbi et al., 2003a, 2003b; Flere and Zhang, 1998; Kim et al., 2003; Koenig and Liu, 2002; Nugroho et al., 2002; Sengupta and Ergas, 2006; Sengupta et al. 2007; Sengupta et al., 2006; Shan and Zhang, 1998; Zeng and Zhang, 2005; Zhang, 2002; Zhang, 2004).

A pilot scale filter containing elemental sulfur and oyster shell at a 3:1 ratio was operated for 11 months at the Massachusetts Alternative Septic System Test Center (Brighton, 2007). The filter received the effluent from a Clean Solution aerobic treatment system that was treating septic tank effluent. The sulfur/oyster shell filter removed 82% of influent TN, while the aerobic/sulfur treatment train removed 89.5% TN from the septic tank effluent. A pilot scale elemental sulfur/limestone column was operated for 6 months on a well water containing 65 mg/L $\text{NO}_3\text{-N}$; nitrate removal averaged 96% and average effluent $\text{NO}_3\text{-N}$ was 2.4 mg/L (Darbi et al., 2003a). A laboratory sulfur/oyster shell column was operated at an Empty Bed Contact Time of 0.33 to 0.67 days and removed 80% of influent nitrate (Sengupta and Ergas, 2006; Sengupta et al., 2006).

Some factors that affect the long term performance success of autotrophic denitrification filters are similar to those for carbon-based denitrification filters. They include the long term availability of electron donor supply for the wastestream being treated, and the physical structure of the biodegradable components of the media. Versus wood based organics electron donors, elemental sulfur could possibly remain physically intact for longer time periods. As for any packed bed, biologically active media filter deployed over extended periods of time, the long term hydraulics of the unit are a concern. Accumulation of biological and inorganic solids could lead over time to the development of preferential flow paths within the filter, reducing average residence time and wastewater contact with the media. To the extent that these processes occur, deterioration of performance could result. The timescales of media replacement, maintenance and supplementation and the practical aspects of these activities must be considered. Another factor is the release of sulfate as water passes through the filter, and possible odors through hydrogen sulfide generation. The latter could increase chemical oxygen demand (COD).

Several candidate media can be suggested for the saturated media filter which forms the second stage of a passive onsite nitrogen removal system for Florida. Media should possess many of the desirable characteristics that have been discussed above. Both elemental sulfur and wood based treatment systems are readily available, economical candidates. Florida contains abundant softwood materials, for example, and elemental sulfur is also readily available. Crushed oyster shell is another stage product, which could also be used in a single pass unsaturated first stage filter if nitrification would otherwise be inhibited by alkalinity supply. The interaction of cation exchange media with microbial reactions appears to offer potential for passive treatment with enhanced performance. Expanded shales with anion exchange capacity are commercially available and could be used as pre-filters or in mixed media filters to increase the performance second stage denitrification.

Drainfield Modifications

Modifications to drainfields entail the in-situ addition of a permeable media that supports denitrification through the release of carbon or electron donor. Wastewater (septic tank effluent) would initially pass through an unsaturated layer or zone (of sand for example), where nitrification occurs. Following passage through the unsaturated zone, the wastewater would pass through a permeable denitrification layer or zone. Denitrification media could be placed as an underlayment beneath the unsaturated soil, or as a subdivided treatment zone within a drainfield through which effluent from the aerobic zone is directed.

A modified drainfield design using a sulfur/limestone layer beneath a sand layer provided greater than 95% TN removal in laboratory scale columns receiving primary effluent from a municipal wastewater treatment plant (Shan, 1998). Nitrification

occurred in the upper sand layer, and the lower denitrification layer was not maintained in a saturated condition.

A wood based system using a mixture of sand, wood chips, and tire crumb (85/11/4% by mass), was examined in bench scale columns to simulate treatment that would occur in a separate reactive media treatment zone established within a drainfield (Shah, 2007). In this system, septic tank effluent would first pass through an unsaturated sand layer, and then pass through the treatment zone containing the reactive media. Laboratory column experiments with septic tank effluent supplied at a hydraulic residence time of 24 hours resulted in 98% TN removal. Average effluent ammonia and nitrate nitrogen concentrations were 4.4 and 0.05 mg/L, respectively.

Other studies, conducted in the laboratory for the most part, have demonstrated an increase in nitrogen removal using modified drainfield designs with carbon substrates (usually wood chips or sawdust) or inorganic electron donors (elemental sulfur). The general concepts are similar to the drainfield modifications presented above. Questions of concern for modified drainfields include media longevity, replacement intervals, and hydraulic issues related to preferential flow paths. Replacement of in-situ denitrification media would require disturbing the entire drainfield, so the life of the reactive media in the denitrification zone would need to be at least as long as the other drainfield components. The consequences of uncertainty in the life of an in-situ denitrification zone located within a drainfield would be relatively more significant than for an in-tank denitrification filter, whose replacement would not require disruption of other treatment system components. Another issue of possible concern is that in-tank processes could be relatively more easily monitored, although monitoring systems could be possibly installed in some modified drainfield designs

Denitrification in Soil

Biological denitrification is a complex process that requires mineralization and nitrification the nitrogen before denitrification can occur. With the decay of organic matter, nitrogen is released into the environment as organic nitrogen (principally proteins and urea). Bacteria and fungi in the soil quickly “mineralize” the organic nitrogen by converting it to ammonium. The ammonium is nitrified by autotrophic bacteria, which use carbon dioxide for their carbon source instead of organic carbon. These bacteria are obligate aerobes that require an aerobic environment because oxygen is used as the final electron acceptor. Since hydrogen ions are created by this reaction, which can lower the pH to levels that inhibit the biological process, it is essential that sufficient alkalinity be available to buffer the soil solution so that nitrification can be complete. After nitrification, heterotrophic bacteria are able to convert the nitrate to gaseous nitrogen and NO_x as they oxidize available organic matter. However, for this conversion, an anoxic or anaerobic environment is required since the oxygen associated with the nitrate is used as the final electron acceptor in oxidizing the organic matter. If either anoxic conditions or organic carbon are not

available, denitrification does not proceed via this pathway. Other pathways exist, but they are far less prevalent.

The heterotrophic bacterial process models were used to define the mechanisms and the necessary conditions for biological denitrification to occur. By understanding these, the literature could be reviewed for the occurrence of the requisite conditions in soils from which the potential for nitrogen removal could be estimated. The most critical conditions for which data are available were selected to investigate. These included the soil's internal drainage, depth to saturated conditions, and the availability of organic materials. Internal drainage provides a measure of the soil's permeability and the extent of time that it may be unsaturated. Unsaturated conditions are necessary to aerate the soil to allow the autotrophs to nitrify the ammonium nitrogen. The shallower the depth to the water table, the more likelihood organic matter will be leached to where the soil moisture is high enough to restrict soil reaeration to the point that aerobic organic matter decomposition is inhibited preserving the carbon for heterotrophic denitrification. The availability of organic carbon determines the occurrence and extent of denitrification that will occur.

Gable and Fox (2000) and Woods et al. (1999) suspect that the Anammox process could explain why nitrogen removal below large soil aquifer treatment systems (SAT) exceeds what can be attributed to heterotrophic nitrogen removal alone because the organic carbon to nitrogen ratio is typically too low to sustain heterotrophic denitrification. Crites (1985) reports that denitrification below seven large scale SAT systems in the US were observed to achieve total nitrogen removals of 38 to 93% . While Anammox quite likely could contribute substantially to the reduction of nitrogen below OWTS, little is known about the conditions under which it is likely to occur. Until the process requirements are better understood, detection of denitrification via the Anammox process would require actual monitoring data where the nitrogen reduction by the heterotrophic processes can be separated out. Such data were not available so the estimates of nitrogen removal below OWTS reported in this study may underestimate the actual removals.

The extent to which denitrification occurs in soils varies depending on the specific environmental conditions at the particular site, and the design and operation of the OWTS. Numerous investigations into the fate of nitrogen below SWIS have been undertaken. However, the results are quite variable even for sites that appear similar. Gold and Sims (2000) point out the dynamic and open nature of SWIS designs create uncertainties with in-situ studies of the fate of nitrogen in soil. The affects of dispersion, dilution, spatial variability in soil properties, wastewater infiltration rates, inability to identify a plume, uncertainty of whether the upstream and downstream monitoring locations are in the same flow path, and temperature impacts are a few of the problems that challenge the in-situ studies. As a result, even when small differences in concentrations are observed, the spatial and temporal variability can result in large changes in estimates of the mass loss of nitrogen.

Several investigators have performed rather thorough reviews of the fate of nitrogen below SWIS. Siegrist and Jennsen (1989) reviewed national and international literature for both laboratory and field studies of nitrogen removal in SWIS. Laboratory studies using soil columns showed removals of TN from less than 1 to 84 percent. Hydraulic loadings varied from 5 to 215 cm/day and influent TN concentrations from 16 to 74 mg/L. The field studies were performed on systems installed in sands. As in the case of most field studies, influent flows and TN concentrations were not always accurately known. Estimates of TN removal in these studies ranged from 0 to 94 percent. The investigators noted that high TN removals have been observed but that reasonably comparable studies showed limited removals. Based on their review, they provided a table of what they thought were “achievable nitrogen removal efficiencies” below SWIS (Table 11).

Table 11. Estimated Total Nitrogen Removals below SWIS
 (after Siegrist & Jennsen, 1989)

SWIS Type	Achievable N Removals	
	Typical	Range
Traditional In-Ground	20%	10 – 40%
Mound/Fill	25%	15 – 60%
Systems with Cyclic Loading	50%	30 – 80%

Long (1995) reviewed studies of nitrogen transformations in OWTS to develop a methodology for predicting OWTS nitrogen loadings to the environment. Long also found that in-situ studies were confounded with many known and unknown variables that made data interpretation complicated. His review of the data indicated that soil treatment removes between 23 to 100% of the nitrogen. He correlated greater removals with finer grained soils because anoxic conditions would be achieved more frequently, which also would help to preserve available organic carbon for denitrification. Using this correlation, he estimated TN removals as shown in Table 12.

In a study investigating the effects of effluent type, effluent loading rate, dosing interval, and temperature on denitrification under SWIS, Degen, et al. (1991) and Stolt and Reneau, Jr., (1991) reviewed published results of other studies that measured denitrification in OWTS. They found denitrification removals varied substantially depending on the type of pretreatment and SWIS design (Table 13).

In a study investigating the effects of effluent type, effluent loading rate, dosing interval, and temperature on denitrification under SWIS, Degen, et al. (1991) and Stolt and Reneau, Jr., (1991) reviewed published results of other studies that measured

Table 12. Estimates of TN Removal Based on Soil Texture (Long, 1995)

Soil Texture	Estimated TN Removal	Comments
Coarse grained sands	23%	Soils promote rapid carbon and nitrogen oxidation leaving insufficient carbon for denitrification. If anoxic conditions and a source of carbon is available, such as a high or fluctuating water table, TN removal would increase.
Medium grained sands	40%	Soils restrict gas transfer during bulk liquid flow periods to create anoxic conditions.
Fine grained sands	60%	Soils restrict gas transfer for longer periods after bulk flow periods
Silt or clay	70%	Soils further restrict gas transfer and retain nutrients higher in the soil profile.

denitrification in OWTS. They found denitrification removals varied substantially depending on the type of pretreatment and SWIS design (Table 13).

The more significant environmental factors that determine whether nitrogen removal occurs and to what extent include the soil's texture, structure, and mineralogy, soil drainage and wetness, depth to a saturated zone and the degree to which it fluctuates, and amount of available organic carbon present. OWTS design and operation factors include the species of nitrogen discharged to the SWIS, the depth and geometry of the infiltrative surface, the daily hydraulic loading and its method of application, whether it is dosed and, if so its frequency.

Table 13. Total Nitrogen Removal Found in Various Studies of OWTS (Degen, et al., 1991)

System Type	TN Removal	Source
Traditional	0-35%	Ritter & Eastburn (1988)
Sand filter	71-97%	Wert & Paeth (1985)
Low Pressure Dosing Shallow	46%	Brown & Thomas (1978)
Low Pressure Dosing At-Grade	98%	Stewart & Reneau, Jr. (1988)
Mound	44-86%	Harkin, et al. (1979)

Soil Drainage Class Soil drainage class has been found to be a good indicator of a soil's capacity to remove nitrogen (Gold, et al., 1999). The Natural Resources Conservation Service (NRCS) uses seven drainage classes to describe the "quality" of the soil that allows the downward flow of excess water through it (USDA, 1962). The classes reflect the frequency and duration of periods of soil saturation with water, which are determined in part, by the texture, structure, underlying layers, and elevation of the water table in relation to the addition of water to the soil. Table 14 provides a brief description of each of the classes.

Table 14. NRCS Drainage Classes and Descriptions

Drainage Class	Description
Excessively drained	Water is removed from the soil very rapidly. The soils are very porous. These soils tend to be droughty.
Somewhat excessively drained	Water is removed from the soils rapidly. The soils are sandy and very porous. These soils tend to be droughty but can support some agricultural crops without irrigation.
Well drained	Water is removed from the soil readily but not rapidly. The soils are commonly intermediate in texture and retain optimum amounts of moisture for plant growth after rains.
Moderately well drained	Water is removed from the soil somewhat poorly so that the profile is wet for a small but significant period of time. The soils commonly have a slowly permeable layer within or immediately beneath the solum and/or a shallow water table.
Somewhat poorly drained	Water is removed from the soil slowly enough to keep it wet for significant periods of time. These soils commonly have a slowly permeable layer within the profile and/or a shallow water table. The growth of crops is restricted to a marked degree unless artificial drainage is provided.
Poorly drained	Water is removed so slowly that the soil remains wet for a large part of the time. The water table is commonly at or near the soil surface for a considerable part of the year. They tend to be mucky.
Very poorly drained	Water is removed from the soil so slowly that the water table remains at or on the surface the greater part of the year. They commonly have mucky surfaces.

Poorly drained and very poorly drained soils can have a high capacity for nitrogen removal because the saturated zone is shallow, carbon enriched and anoxic while moderately well and well drained soils have a very limited capacity (Groffman et al., 1992; Hansen et al., 1994a, 1994b; Nelson et al., 1995; Parkin and Meisinger, 1989;

Simmons et al., 1992). Groundwater in moderately well drained or well drained typically flows deeper within the subsoil and does not intersect the reduced and organic enriched surface horizons. The groups and their expected impacts on denitrification are given in Table 15.

Organic Matter Heterotrophic bacterial denitrification is often limited by organic matter (Bradley, et al., 1992; Burford and Bremner, 1975; Christensen, et al., 1990; Gambrell, R.P., et al., 1975). The organic carbon is necessary as an energy source for bacterial metabolism. Sources of organic matter in soil are either natural, which is continuously replenished in the soil from the decay of vegetative materials or supplied by the wastewater itself. Studies indicate that denitrification is inhibited where the nitrate to dissolved organic carbon ratio is below 0.73 to 1.3 (Burford & Bremner, 1975).

Table 15. Drainage Class and Expected Impacts on Denitrification

Drainage Class Group	Expected Impact on Heterotrophic Denitrification
Excessively/Somewhat excessively	<ul style="list-style-type: none"> ◆ Well aerated soil capable of achieving complete nitrification of applied TKN ◆ Provides little organic carbon and will likely degrade any added organic matter within the aerobic zone ◆ Short retention time
Well	<ul style="list-style-type: none"> ◆ Sufficiently aerated soil capable of achieving complete nitrification ◆ May allow some organic matter to reach a saturated zone where it would be available for denitrification if a shallow water table is present
Moderately well	<ul style="list-style-type: none"> ◆ Sufficiently aerated soil capable of achieving complete nitrification ◆ Denitrification would be enhanced with a fluctuating water table for a “two sludge” process or with slow drainage for a “single sludge” process
Somewhat poorly/Poorly/Very poorly	<ul style="list-style-type: none"> ◆ Ample organic matter for a carbon source and to create anoxic conditions in saturated zones for significant nitrogen reduction ◆ Insufficiently aerated soil to nitrify TKN requiring nitrification of the wastewater prior to application to the soil

The amount of organic matter in the soil is greatest in the root zone and above (Paul and Zebarth, 1997; Starr and Gillham 1993). Roots regularly exude carbonaceous materials and die and decay. Much of the organic carbon is degraded in the vadose zone through natural degradation within 2-3 ft of the ground surface. Organic matter is typically very low (<1%) below about 3 ft in most soils with a deep vadose zone. However spodic soils, which are common in the WAS, have a horizon that is lower in the soil profile that contains organic matter, iron and aluminum. This organic matter would be available for heterotrophic denitrifiers.

Depth to Water Table Water tables or perched water saturated zones restrict reaeration of the soil. With organic matter present, the saturated zone will become anoxic or anaerobic. This will inhibit nitrification and if nitrate and organic matter are present, will support denitrification. When the air-filled porosity drops below 11 to 14% or the moisture content is greater than 60 to 75% of the soil's water holding capacity, reaeration is sufficiently restricted that anoxic conditions can result (Bremmer and Shaw, 1956; Christensen, et al., 1990; Cogger, et al., 1998; Donahue et al., 1983; Pilot and Patrick, Jr., 1972; Reneau, Jr., 1977; Singer & Munns, 1991; Tucholke et al., 2007). If the water table is deep, little denitrification seems to occur. In soils with thick unsaturated zones, organic matter may not reach the saturated zone because it is oxidized before it can leach to the water table. Where the ground water depths exceed about one meter, denitrification is greatly reduced (Barton et al., 1999; Starr and Gillham, 1993). However, a shallow, fluctuating water table can create the conditions for simultaneous denitrification. This occurs when a seasonally high water table prevents nitrification of the ammonium, which will adsorb to negatively charged clay particles in the soil. The ammonium is held by the soil and after draining and reaerating, the ammonium is nitrified. If organic matter is present and the soil nears saturation again, the nitrate can be denitrified and the newly applied ammonium is adsorbed as before, repeating the process. Cogger, 1988; Reneau, 1977, 1979; Walker et al., 1973a).

Type of Infiltration System The type of infiltration system used can affect the soil's potential for nitrogen removal. Traditional in-ground trench systems are installed with their infiltrative surfaces typically below the A horizon and thus below where organic matter can be expected to be the highest. At-grade and mound systems are typically installed above the O and A horizon thereby gaining the advantage of having a high organic layer available to create anoxic conditions with organic carbon available (Converse et al., 1999; Harkin et al., 1979). However, in Florida, the OWTS rules for mound construction require the removal of the O and A horizons, which removes most of the available organic carbon. Also, "digouts", which are systems on sites where a restrictive horizon in the soil profile is removed, can result in reducing a particular soil's nitrogen removal potential because quite often the restrictive horizon removed is a spodic layer, which can have a sufficiently high organic content and be restrictive enough to create a saturated zone where anoxic conditions may be created for denitrification.

APPROACHES TO PASSIVE NITROGEN REMOVAL

The overall approach to passive nitrogen removal is a two stage filter system. The first stage of an unsaturated media filter for ammonification and nitrification is followed by the second stage of a saturated anoxic filter with reactive media (denitrification). This configuration is mandated by the obligatory biochemical sequence of aerobic nitrification followed by anoxic denitrification. The use of an unsaturated media filter for the initial nitrification is necessary because of the constraint that aeration pumps can not be used in the passive system. The first media filter can be established as a downflow filter, similar to sand filters, and can be hydraulically connected to the second anoxic denitrification filter that operates in the upflow direction. The flow connectivity between the two filter stages would be by gravity and pumping would not be required. In Florida, vertical topography is often small and seasonal high water table elevations near the surface.

The first stage filter must be designed to achieve the targeted final effluent ammonia N levels. Ammonia N may behave conservatively in the anoxic second stage filter, and any additional ammonia N removal in the anoxic filter should be viewed as incidental. The first stage filter will also provide additional processes that will remove biodegradable organics (biochemical oxygen demand) and organic N. Although some denitrification may occur in unsaturated filters that are operated on STE under certain conditions (i.e. simultaneous nitrification/denitrification), the predominant design goal of the first stage filter must be to achieve consistent low levels of ammonia N and organic N. Key factors for first stage media are surface area per volume, ability to supply oxygen (aeration pore volume), ability to retain water, and adequate space within the media to assimilate suspended solids in the wastewater influent and biomass that is synthesized from degradation of influent wastewater constituents. Unsaturated filter performance is governed by the interaction between the filter media and the manner in which septic tank effluent is imposed onto the media surface. Important factors are the average applied hydraulic and organic loading rates, the timing and volume of dosings, and the distribution of wastewater over the entire surface area of the filter. Review of technologies suggests that ammonia nitrogen reductions of 95% and effluent ammonia N levels of 1 mg/L are possible to achieve. Evaluation of specific filter media, hydraulic and organic loading rates, and water quality must be conducted to define the design parameters needed to achieve low effluent ammonia and organic N concentrations. Promising candidate media include peat, coconut coir, zeolites, expanded clays and shales, and synthetic fiber materials. The first stage unsaturated filter should produce an effluent with low TSS and potential for regrowth to minimize potential solids accumulation and channeling in the second stage filter.

The need for recirculation around the first filter must be considered, the point to which recirculation should be directed (i.e. a pumping tank, external recirculation tank, septic tank chamber), and the recirculation ratio (flowrate in relation to the wastewater flowrate). Recirculation can provide pre-denitrification using wastewater organics,

which would lower nitrate loadings to anoxic denitrification filters. Alkalinity recovery would be an accompanying benefit which may be important in the future in preventing nitrification inhibition if water conservation efforts lead to increases the Total Nitrogen of and the TN/Alkalinity ratio in onsite wastewater. Recirculation around the aerobic filter can be accommodated by using a flow splitting device on aerobic filter effluent, while still keeping within the FDOH definition of “passive.”

The second filter must be designed to achieve the targeted final effluent total oxidized N levels, which are expected to be predominantly nitrate N. Considerations for the second stage media involve surface area per volume, propensity for accumulated suspended solids to reduce hydraulic conductivity (clogging), and operating factors such as the applied hydraulic and organic loading rates. The need to provide a continuous supply of electron donor for denitrification, and over extended periods of deployment, is central to the purpose of the reactive media. Review of technologies suggests that effluent nitrate levels of 2 mg/L and less are possible to achieve. Evaluation of specific filter media, hydraulic and nitrate loading rates, and water quality must be conducted to define the design parameters needed to achieve low effluent nitrate concentrations. Candidate media include woodchips, sawdust, and elemental sulfur; other cost effective materials may also be identified. Literature review suggests two additional considerations that must be addressed for deployment of anoxic reactive media. The first is residuals that are added to water by passage through the reactive media. Wood based materials can add biodegradable organics to water, increasing the chemical and biochemical oxygen demand. Elemental sulfur systems can increase sulfate levels. The degree to which residuals are added to the water by the reactive media filters could be reduced to by replacing a fraction of the reactive media with inert filler. However, care must be taken to insure continuous electron donor supply over the target deployment period. Thus, anoxic filter systems must be formulated with sufficient electron donor supply to support denitrification, but with as small an excess release of electron donor as is consistent with achieving the target nitrate removals.

A second factor in anoxic filter design is the hydraulic performance, which may be even more significant to the longevity of anoxic denitrification filters than the duration of electron donor supply. Preferential flow paths can be initiated through deposition of organic and inorganic solids within the filter media, and by methods used to distribute and withdraw flow into and through the reactive media. Preferential flow paths can lead to channelization, reduced contact with reactive media surfaces, and performance deterioration. The ability to predict a priori the propensity for channelization phenomena is limited, particularly in the anoxic filters, which host biochemically reactive systems with complex water chemistries and a significant transition from a predominantly aerobic to an anoxic redox environment. Approaches to overcoming channelization involve manipulation of media, providing a minimum amount of headloss, baffling, using long aspect ratio reactors, using large systems that provide acceptable performance over time even with some degree of channelization, or using smaller systems with lower

retention times that are changes out more frequently. These factors must be addressed through continuous deployment over time periods of months and longer.

The established of the two stage treatment system as an in-tank process is preferred to a subsurface or modified drainfield approach. Achieving acceptably low effluent Total N removals over time periods of many years will require access to filter media for effluent monitoring, media maintenance and change out when required, and verification of desired hydraulic operation. Replacement or maintenance of denitrification media could be accomplished without disturbing the first stage media. The use of the two stage in-tank process, passively connected hydraulically, would avoid the vagaries inherent in verifying the continuing performance of subsurface flow systems.

CONCLUSIONS AND RECOMMENDATIONS

A review was conducted of passive technologies that enhance removal of nitrogen from on-site wastewater treatment systems. The review included searches of peer reviewed literature and conference proceedings, procuring technical reports, searches on the world wide web, discussions with vendors and national experts, and a site visit to the Massachusetts Alternative System Test Center. These efforts provided the basis for a critical assessment of the present state of technology. The following summarize the significant conclusions of this effort.

- To achieve high nitrogen removals from septic tank effluent using “passive” systems as defined by the study goals, a promising and perhaps only feasible approach is a two stage filter system consisting of an unsaturated first stage media filter followed by a directly connected second stage anoxic filter with reactive media for denitrification; pressure and timed dosing to the first stage; with possible recirculation around the first stage.
- Filter media that appear promising for passive nitrogen removal include peat, coir, synthetic fabrics, zeolites, expanded clays and shales (first aerobic stage), and elemental sulfur and cellulosic based materials (sawdust and woodchips) for the second stage.
- As defined by FDOH, a passive system includes only one liquid pump and no aerator pumps. Studies of actual field installations are required to ascertain their ability to perform satisfactorily over extended time periods.
- Passive systems to remove nitrogen from septic tank effluent (STE) must consider the entire nitrogen transformation process, including nitrification (aerobic) and denitrification (anoxic), as well as the integration of these processes for total nitrogen reduction.
- Aerobic, unsaturated filtration technologies have been well studied and in some cases can achieve effluent ammonia nitrogen levels of several milligrams per liter or less. Most prominent technologies include sand, peat and textile media, and often employ recirculation. New media offer exciting possibilities for improved performance.
- Passive denitrification filters employ solid phase electron donors to produce saturated anoxic environmental. Passive technologies are currently under development or in early stages of deployment. Promising filter systems include cellulosic based media (wood, sawdust), other organic media, and elemental sulfur based systems.

- The passive denitrification technologies have not been deployed for sufficiently long periods of time to fully evaluate longer term performance, operation and maintenance requirements, media longevity, and media replacement requirements.
- The ability of passive denitrification media to maintain a long term supply of carbon or electrons for denitrification is a significant factor affecting their longevity. Theoretical stoichiometric calculations provide a starting estimate, but longer term studies are needed to verify these results in practice.
- The longevity of passive denitrification filter systems may be affected by the long term accumulation of organic and inorganic solids within the filter media. This is perhaps more important than the ability of media to provide a long term supply of carbon or electrons. Solids accumulation can result in the development of preferential flow paths, reduced contact of wastewater with solid media, and deterioration of performance. Longer term studies are needed to verify continued performance of denitrification filters in practice, and to determine filter maintenance and media replacement requirements.
- Constituents released by passive denitrification media include biodegradable organic matter (BOD) from carbon-based systems, and sulfate from sulfur-based systems. The environmental acceptability of constituent release must be determined.
- The practicality and life cycle costs of media replacement must be evaluated for all systems, including frequency of replacement, site access issues, replacement volumes, and management of used media.
- Modifications to soil treatment units have been evaluated in limited laboratory systems and some field studies are underway, using denitrification media similar to those used in in-tank treatment processes.
- In-soil denitrification is highly dependent on the specific environmental conditions at a particular site and operation of the onsite wastewater treatment and disposal system.
- It is recommended to perform bench scale evaluations of two-stage filter systems to evaluate various media for nitrogen removal from septic tank effluent.

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APPENDIX A

MEMO TO MASSACHUSETTS ALTERNATIVE SEPTIC SYSTEM TEST CENTER REQUESTING INFORMATION FROM TECHNOLOGY DEVELOPERS



Applied Environmental Technology

TO: George Heufelder, Director
Keith J. Mroczka, Test Center Operator
Massachusetts Alternative Septic System Test Center

FROM: Daniel Smith, PhD, PE
Applied Environmental Technology (AET)
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DATE: 5/29/2007

RE: Florida Passive Nitrogen Removal Study

The Florida Passive Nitrogen Removal Study is a literature review being conducted by AET for the Florida Department of Health (FDOH). The object is to identify and characterize “passive” systems to enhance nitrogen removal in on-site wastewater treatment systems. Florida DOH requests a literature summary and database of available passive nitrogen removal technologies, which can include in-tank systems or modifications to soil treatment units (drainfields). FDOH is interested in technologies that treat influents ranging from septic tank effluent (STE) (i.e. organic and ammonia N, no nitrate) to substantially nitrified effluent. The overall goal is enhanced Total Nitrogen removal: performance, life cycle cost, and permitability, for new or retrofit systems.

During a recent visit to the Massachusetts Alternative Septic System Test Center, it was indicated that systems that are being tested at the center may be of interest to the Florida Passive Nitrogen Removal Study. Vendors with appropriate technologies may be interested in having their technologies included in the Florida DOH study. This memo is to request information from such vendors that describes their technology and characterizes the treatment process, its mode of application within on-site treatment systems, nitrogen removal performance, longevity, operations and maintenance, economics, and any special considerations for deployment.

Vendors are encouraged to contact Dr. Smith directly to discuss this study or to provide the technology information (contact information is listed above). The following list contains some specific information that would be useful to include in the database. It is realized that not all of this information may be available or compiled, or may be included within documents or reports.

Florida Passive Nitrogen Removal Study Technology Description and Characterization

- Name of technology or process
- Name and contact information of provider
- Process description
 - Treatment principal
 - Treatment goals
 - Unit operation sequence; where unit fits in to treatment sequence
 - Operational methods: passive, dosed, other
- Performance evaluation
 - Testing entity
 - Location and duration
 - Operation and monitoring methods
- Provide references for performance evaluations, certifications
 - email reports, documents, papers, citations
 - web links
 - hard copies of reports, documents, papers
- Performance data
 - Physical description of test unit
 - Location within treatment sequence
 - Unit dimensions: plan area, depth, other
 - Operational method: passive, dosed, other
 - Operational history
 - Influent and effluent flowrates
 - Influent and effluent monitoring data
 - Temperature
 - pH
 - Alkalinity
 - BOD, COD, TOC
 - TSS, VSS
 - Nitrogen: Total N, TKN, Organic N, NH₄-N, NO₂-N, NO₃-N
 - Other parameters
- Full scale operations and maintenance
 - regular operation
 - inspection and maintenance requirements
 - media replacement intervals
- Longevity
 - the life of passive media (for denitrification for example) for a typical application based on field data or theoretical calculation; list all assumptions
- Economics
 - installation cost
 - specific breakout of media cost
 - operation
 - maintenance
 - media replacement
 - life cycle cost including all of above
- Special Issues relation to permitting and deployment

APPENDIX B

PASSIVE NITROGEN REMOVAL CITATION LIST

Florida Passive Nitrogen Removal Study

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Florida Passive Nitrogen Removal Study

Laboratory Media Evaluation

Quality Assurance Project Plan

Prepared for:

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Division of Environmental Health
Bureau of Onsite Sewage Programs
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Section 1 Project Organization

The Florida Department of Health has contracted with Applied Environmental Technology to perform a literature review and assemble a database of passive nitrogen removal technologies for onsite wastewater treatment, and to perform experimental evaluations of candidate reactive media to be used in treatment filter systems. Applied Environmental Technology will perform overall project management, will establish and conduct the experimental studies, and will deliver samples to ELAB Inc., a NELAC Certified Analytical laboratory, for water quality analyses. Applied Environmental Technology will review and interpret the resultant data, adjust the experimental program as warranted, and generate a summary report.

Prudent project management will help minimize changes, ensure project continuity, and avoid delays in the project schedule. This type of project is highly specialized, requiring unusual equipment and services. Therefore it is crucial that adequate project management be used to ensure the success of the project.

Section 2 Problem Definition and Background

A. Project Background

The Florida Department of Health (FDOH) has provided funding to evaluate methods that can be used to enhance nitrogen removal in onsite wastewater systems in a passive and cost effective manner. The Florida Passive Nitrogen Removal Study Task 2 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 the study is to perform small scale testing to identify candidate media for subsequent evaluation using full scale onsite wastewater treatment systems.

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.

To perform the media evaluations, it is desired to conduct studies in a manner that closely resembles the functioning of an actual onsite system. The actual candidate media should be used, placed in appropriate depth and distribution. Continuous or dosed filter operation is preferable, where microbial populations will establish their metabolic activities and perform desired biochemical transformations in response to conditions similar to an actual system. The use of actual septic tank effluent (STE) as feed source is deemed preferable to use of a synthetic analog STE. This Quality Assurance Project Plan (QAPP) describes the methods and procedures that will be used to conduct the media evaluations.

B. Candidate Study Sites

Four candidate sites have been identified and approvals are being sought for their use for this study. All sites are acceptable for use in the study. Each site has a source of actual septic tank effluent, can provide a power supply to pump STE to test columns, each site location is isolated from public use and will cause minimal disruption to any activity, and each site has reasonable security. A single site will be used.

1. Flatwoods

Ranger residence, septic tank, county operated park administered by the Southwest Florida Water Management District, 14302 Morris Bridge Road, Thonotosassa FL 33592, Hillsborough County.

2. Morris Bridge

Ranger residence, septic tank, county operated park administered by the Southwest Florida Water Management District, 13330 Morris Bridge Road, Thonotosassa FL 33592, Hillsborough County.

3. Hillsborough River State Park

Visitor center, septic tank, state park, 15402 US 301 North, Thonotosassa FL 33592, Hillsborough County.

4. Branchton

Private residence, septic tank effluent pumping chamber, 11809 Cedar Cove Drive, Thonotosassa, FL 33592, Hillsborough County.

Section 3 Project Description

A. Project Purpose

To evaluate candidate media for use in passive nitrogen removal systems for onsite wastewater treatment.

B. Project Objectives

The objective is to establish small scale experimental systems to evaluate the effectiveness of media in removing total nitrogen from septic tank effluent. The experimental systems will consist of three two-stage filter systems, each consisting of a first stage unsaturated filter followed in series by a second stage filter saturated with wastewater. Septic tank effluent will be applied to the top 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. The significant target reactions are aerobic heterotrophic oxidation (by microorganisms that oxidize organic material and reduce biochemical oxygen demand), hydrolysis and ammonification (releasing ammonia), and nitrification (biochemical conversion of ammonia to nitrate and nitrite). Of particular interest are the organic and ammonia nitrogen concentrations in first stage effluent, as well as nitrate and nitrite.

Effluent from the bottom of the first stage filter will be passed through a saturated anoxic upflow filter that contains a reactive media that supplies electron donor for denitrification (reduction of nitrate and nitrite to N₂ gas). Of particular interest are the oxidized nitrogen concentrations in first stage effluent. 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 interaction of media with applied wastewater governs the treatment process. Key features affecting nitrogen removal performance include:

1. The effects of hydraulic and nitrogen loading rates, on average daily and per dose basis, on first stage effluent nitrogen concentrations.
2. The effects of first stage media on effluent nitrogen levels.
3. Alkalinity consumption in the first stage and its possible effects on nitrification.
4. The effects of hydraulic and nitrogen loading rates, on average daily basis, on second stage effluent nitrogen concentrations.
5. The effects of second stage media on effluent nitrogen levels.
6. Second stage effluent total nitrogen concentrations and speciation into organic, ammonia, and oxidized nitrogen forms.
7. Alkalinity consumption in the second stage and its possible effects on denitrification.
8. Possible use of first stage recycle.

C. Project Tasks

Project tasks are shown in Table 1. The start dates are contingent upon review and approval by FDOH.

Table 1 Scheduled Tasks

Task/Activity	Start	Projected Completion
Task 1 Select study site	Week 1	Week 2
Task 2 Procure media	Week 1	Week 2
Task 3 Construct media filter testing apparatus	Week 1	Week 3
Task 4 Deploy testing apparatus at site	Week 3	Week 4
Task 5 Operate and monitor experiments	Week 5	Week 10
Task 6 Prepare final report (CORY 6 Task 2d)	Week 11	Week 12

Task 1 Select study site

Four study sites have been identified, each of which are acceptable for this research (Section 2B). A final site will be selected based on receiving approval from the agencies with responsibility for the locations and other factors.

Task 2 Procure media

Candidate media for evaluation in Stage 1 (unsaturated) filters and Stage 2 (saturated) filters are listed in Table 2. All media offer high water retention and porosity, and the clinoptilolites additionally provide ion exchange capacity. Media will be procured from vendors for use. For Stage 1 media, four clinoptilolite media are listed with particle sizes of 0.3 to 4.8 mm. These have greater than 45% porosity and high water retention. The clinoptilolites have cation exchange capacities of 1.5 to 1.8 meq./g, which will act to retain ammonia ions for enhanced ammonia removal under non-steady flows and higher loading rates. Livlite is an expanded clay with high water retention characteristics. Coir fiber is produced from coconut husk, has a high lignin fraction, and offers high porosity and water retention.

The Stage 2 electron donor media is elemental sulfur, which will result in an autotrophic denitrification process in the anoxic filter. Crushed oyster shell will be used as an alkalinity source, as sulfur-based autotrophic denitrification will consume alkalinity. Expanded shale is included for its anion exchange capacity, which will bind nitrate and enhance performance under non-steady conditions or higher flowrates.

Table 2 Filter Media

Material	Bulk density, lb/ft³	Particle Size Range	Supplier
ZK406H Clinoptilolite	59	0.8 - 1.7mm	GSA Resources, Tuscon, AZ
AMZ 4/8 Clinoptilolite	55	2.3 - 4.8 mm	Ash Meadows, Armagosa, NV
AMZ 8/20 Clinoptilolite	55	0.8 - 2.3 mm	Ash Meadows, Armagosa, NV
AMZ 16/50 Clinoptilolite	55	0.3 - 1.1 mm	Ash Meadows, Armagosa, NV
Livlite Expanded Clay	41	3 to 5 mm	Big River, Alpharetta, GA
Coir fiber	8.7	0.5 - 9 cm L 0.1 - 0.3 mm D	RoLanka International, Stockbridge, GA
Elemental sulfur	77	2 - 4 mm	Georgia Sulfur, Valdosta, GA
Oyster shell	82	3 - 15 mm	Harold's Farm Supply, Dover, FL
ACT-MX ESF-580 Utelite	54	4 -20 mm	ES Filter, Ogden, UT
ACT-MX ESF-416 Utelite	54	2 - 10 mm	ES Filter, Ogden, UT
ACT-MX ESF-450 Utelite	54	0.4 - 4.5 mm	ES Filter, Ogden, UT

Task 3 Construct media filter testing apparatus

Filter testing apparatus will be fabricated from 3 and 1.5 in. tubing, using a 1/8 inch screening for media support and retention. Six columns will be constructed with the media characteristics shown in Table 3. Three Stage 1 columns will be constructed, two using stratified layers of clinoptilolite and expanded clay, and a third using coir fiber media. Total media depth will be 24 in. in each Stage 1 column. Stratification of media 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 will occur in the upper layer, and the use of larger particle size media will provide greater space for accumulation of solids. Long term operation should be enhanced. The use of an expanded clay in the upper layer of Filter 1B (Table 3) is based on the supposition that the ion exchange is not necessarily critical to the functioning of the aerobic biochemical

Table 3 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	3.0	24.0	Stratified	8 in. expanded clay (3-5 mm) 8 in. clinoptilolite (0.8-2.3 mm) 8 in. clinoptilolite (0.5-1.1 mm)
	1C	3.0	24.0	Nonstratified	100% coir fiber
Stage 2	2A	1.5	24.0	Nonstratified	75% elemental sulfur 25% oyster shell
	2B	1.5	24.0	Nonstratified	60% elemental sulfur 20% oyster shell 20% expanded shale
	2C	1.5	24.0	Nonstratified	45 % elemental sulfur 15% oyster shell 40% expanded shale

processes in the upper media layer. Cation exchange will be most beneficial to ammonia nitrogen retention and nitrification in a lower media depth region. The use of finer particle sized in lower depths will provide better ammonia retention and also 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.

Three Stage 2 columns will be constructed or unstratified media containing elemental sulfur, crushed oyster shell, and expanded shale (Table 3) 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 elemental sulfur fractions. Lower sulfur fractions 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. If the sulfur fraction was too low, denitrification could be starved for electron donor and cause nitrate breakthrough in the effluent. the use of three sulfur fractions will allow this issue to be examined.

Stage 1 filters will be supplied septic tank effluent by a multi-head peristaltic pump, set to operate with a timed dosing of once per hour. A perforated plate will be used to distribute effluent over the surface of the Stage 1 media. Water will percolate downward through the media, through the support plate, and the downward through a tube of about 38 in. The bottom of the 38 in. tube will be at the elevation of the bottom of the upflow Stage 2 column. This arrangement will provide hydraulic head for passive upflow through the Stage 2 filters. Effluent from the Stage 2 filters will be directed to an overflow port, and then routed to a common effluent line. The effluent line will be directed to the outlet pipe of the septic tank, downstream of the point at which STE is withdraw for this experiment.

Initial operation will be commenced at a hydraulic loading to the Stage 1 filters of 2 gal./ft²-day. Operating characteristics of Stage 1 and Stage 2 filters are shown in Tables 4 and 5. At 24 doses per day, a single dose adds a volume that is about 7% of the water retained within the Stage 1 filter bed and the average water residence time is about 13.5 hr. (Table 4). An average water residence time of 18 hr. is provided in the Stage 2 filter (Table 5).

Table 4 Operating Characteristics of Unsaturated Column

Flow, gpd/ft ²	2.00
Diameter, inch	3.00
Media depth, inch	24.0
Flow, gal/day	0.098
Flow, ml/hour	15.5
Time for 250 ml sample, hour	16.2
Doses/day	24
Flow, ml/dose	15.5
Empty bed volume, liter	2.8
Resident water volume, liter ¹	0.21
Single dose volume / resident water volume	0.07
Average water residence time, hour	13.5

¹Assumes 50% pore space, 15% of pore space filled with water

Table 5 Operating Characteristics of Saturated Column

Diameter, inch	1.50
Media depth, inch	24.0
Flow, gal/day	0.098
Flow, gpd/ft ²	8.00
Flow, ml/hour	15.5
Time for 250 ml sample, hour	16.2
Empty bed volume, liter	0.7
Pore volume, liter ¹	0.28
Average residence time, hour	18.0

¹Assumes 40% pore space

Monitoring sample points are septic tank effluent, three Stage 1 effluents, and three Stage 2 effluents (total of seven points). For septic tank effluent, the influent pipes from the pump will be removed from the Stage 1 filters and directed to sample containers, and the pump speed increased during collection (no intermediate collection container). Separate samples will be collected for lab analyses and for field analyses. For Stage 1 effluent, an inline flow through sample reservoir with port and valve will enable withdrawal of adequate size sample volume for lab analysis and another volume for field analysis. For Stage 2 effluent, the upper portion of the filter column extending above the surface of the media will be used as a sample reservoir; a port with a valve will be located about 8 in. below the outfall pipe and used to withdraw Stage 2 effluent directly into a sample container (no intermediate collection container). Sampling order will be influent, Stage 2 effluent, Stage 1 effluent to prevent. At each sample location, a separate sample will be collected for the field analyses and the lab analyses sample will not be used.

Effluent from Stage 1 Filter 1A will be fed to Stage 2 Filter 2A, 2A to 2B, and 3A to 3B. There is no particular rationale for this flow routing scenario. The interpretation of filter performance results could be complicated as a result of this flow routing arrangement. The routing could be modified as the study progresses, to enable examination of different Stage 1 and Stage 2 combinations. The filter configurations (media, diameter, media depth) could also be modified based on ongoing results, although the present duration of the study provides limited time for evaluation of different cases. As study results are compiled, additional filter columns could be constructed or media composition or stratification may be modified. Another potential factor that could be examined is the effects of recirculation around the Stage 1 filters, which would have a host of effects: on Stage 1 loadings, Stage 1 effluent TKN levels, the degree of

denitrification achieved, the effect on alkalinity across Stage 1, and reduction in the size of the Stage 2 filter. The scope of the present investigation provides limited opportunity to address these issues.

Task 4 Deploy testing apparatus at site

The apparatus will be fabricated in the AET laboratory and flow tested with clean water. Pump flowrate calibrations will be performed. Media will be screened if necessary, washed repeatedly to remove fines, and placed to appropriate depths in the columns using funnel and water transport. The apparatus will be disassembled as needed, transported to the test site, and reassembled as needed. The denitrification column will be filled with a clean water source and water will be applied at high flowrate to fill the 38 in. tube below the unsaturated column. A line will be connected to the septic tank effluent source and secured in place in a manner to be determined by the specifics of the site. The pump will be started and flow hydraulics checked. the initial flowrates will be measured and adjusted 24 hr. later.

Task 5 Operate and monitor experiments

Filter system will be operated for 60 days. The analyses template is shown in Table 6. Field parameters include temperature, pH, and dissolved oxygen. Laboratory parameters include the nitrogen series of total kjeldahl nitrogen, ammonia, and oxidized nitrogen, as well as sulfate in the Stage 2 column influent and effluent. Alkalinity will also be measured. Depending on ongoing results, applied hydraulic loading rates could be increased, or filter configurations modified. Flowrate checks will also be performed as needed, and tubing at the peristaltic pump head also changed several times thorough the study.

Task 6 Prepare Final Report

A final report will be prepared describing experimental methods and procedures, results of the research, discussion and conclusions, and all monitoring data.

Table 6 Analyses Template

	Septic tank effluent	Effluent from unsaturated filters	Effluent from saturated anoxic filters	Sampling Days
Temperature	5	5	5	16,27,38,49,60
pH	5	5	5	16,27,38,49,60
DO	5	5	5	16,27,38,49,60
TKN	5	5	5	16,27,38,49,60
NH ₃ -N	5	5	5	16,27,38,49,60
(NO ₃ +NO ₂)-N	5	5	5	16,27,38,49,60
Sulfate	0	5	5	16,27,38,49,60

Section 4 Quality Objectives and Criteria

The objective of this monitoring program is to evaluate media for passive nitrogen removal from septic tank effluent. The following will be performed:

- Three two stage filter system will be constructed and operated on septic tank effluent for 60 days.
- The flowrate to each filter system will initially provide a 2 gal/ft²-day to the first stage.
- Monitoring will be conducted five times of septic tank effluent, effluent from the Stage 1 (unsaturated) filters, and effluent from the Stage 2 (saturated) filters.
- Field parameters will be monitored at the site. Sample will be collected and transported to the laboratory for analysis of nitrogen species and sulfate.
- Operation or configuration of the columns may be modified based on analysis of results and adaptive management.

The monitoring data will be used to calculate:

1. average concentrations and standard deviations of water parameters in septic tank effluent, Stage 1 effluent and Stage 2 effluents;
2. percent removal nitrogen and nitrogen species in Stage 1 filters, Stage 2 filters, and two stage filter systems;
3. changes to dissolved oxygen and pH through treatment stages; and
4. average applied hydraulic loading rate, applied loading rates of total nitrogen and nitrogen species.

A. Precision and Accuracy

Precision describes the reproducibility of results. Accuracy is the degree of agreement between an observed value and an accepted reference value. Accuracy will be evaluated through the analysis of surrogate spikes, Laboratory Control Samples (LCS), Laboratory Control Sample Duplicates (LCSD), matrix spike samples (MS/MSD) and laboratory internal blind audit samples. Precision and accuracy information is tracked by the laboratory, with acceptable ranges updated periodically. In addition, NELAC requirements include the analysis of proficiency test samples to evaluate precision and accuracy. Precision and accuracy requirements for the target analytes and matrices are provided in Table 10.

B. Representativeness

Representativeness refers to the relationship of a sample taken from a site to be analyzed to the remainder of the sample matrix at the site. The samples will be taken directly from the influents and effluent of the filters and will provide representativeness.

C. Comparability

The use of NELAC approved procedures and consistent approved methodologies ensure the comparability of data sets generated by different laboratories.

D. Completeness

Completeness is defined as a measure of the extent to which the data fulfill the data quality objectives of the project. The completeness of the data will be determined during the data validation and verification process.

Section 5 Certifications

ELAB Inc. is located in Tampa, Florida and is FDOH NELAP certified laboratory # E84973. ELAB's Tampa certification documentation is provided in Appendix A. ELAB Inc. also maintains a facility in Ormond Beach, Florida that is FDOH NELAP certified laboratory # E83079. The Ormond Beach certification documentation is also provided in Appendix A

Section 6 Documentation and Records

All documentation archives will be kept for a minimum of 5 years after the date of project completion (Table 7). Reports and deliverables will be submitted in Word or Excel format.

A. Field Documentation

1. Field Notes

Field notes will be documented and maintained by field staff.

2. Field Parameters

Field staff will record specific sample point, date and time of sample collection, parameter name, result and units

3. Sample Collection, Preservation and Transport

Chain of custody forms and sample tags attached to sample bottles will be supplied by the laboratory. A copy of the chain of custody form is provided as Figure 1. Legal or evidentiary chain of custody as defined in the NELAC standards will be executed.

B. Laboratory Documentation and Reporting

Laboratory deliverables will be submitted in Word or Excel format. Laboratory reports will be issued in accordance with NELAC requirements. Certificates from vendors will be retained, whether from a laboratory or commercial vendor. Records of the lot numbers of reagents and other cleaning supplies, with the inclusive dates for use, will be recorded. Pre-cleaned container packing slips, lot numbers of shipments, and certification statements provided by the vendor will be retained by ELAB. All local, state and federal requirements pertaining to waste storage and disposal will be followed.

C. Archival of Electronically Stored Data

Analytical reports generated will be retained by AET and ELAB.

Table 7 Documentation and Records Storage

Document/Record	Location	Retention Time	Format
QAPP and revisions	AET	5 years after project completion	Paper, electronic
Field notes	AET	5 years after project completion	Paper
Chain of custody	AET, ELAB	5 years after project completion	Paper
Laboratory QA manual	ELAB	5 years after project completion	Paper, electronic
Laboratory SOPs	ELAB	5 years after project completion	Paper, electronic
Laboratory data reports	ELAB	5 years after project completion	Paper, electronic
Laboratory equipment maintenance logs	ELAB	5 years after project completion	Paper
Laboratory calibration records	ELAB	5 years after project completion	Paper

Section 7 Sampling Process Methodology

A. Site Location

The project will be conducted at one of the sites listed in Section 2B.

B. Monitoring and Sampling Frequency and Duration

The filter systems will be monitored five times over a duration of 60 days.

C. Number of Samples and Matrices

All sampling will be manually collected aqueous “grab” samples. On each monitoring date, seven samples will be collected: septic tank effluent, the effluents from three Stage 1 columns, and the effluents from three Stage 2 columns. Samples will be collected in sample containers prepared by ELAB, placed in an iced cooler, and transported to ELAB. Samples will arrive at ELAB within one hour after the completion of collection and monitoring activities. Field analysis will be performed at the same time and for the sample locations as aqueous laboratory samples. Samples for field analyses will be collected in separate containers from laboratory samples. Stage 2 effluent parameters will be measured in-situ by placing probes directly into the water column overlying the media surface; this procedure will always be performed after aqueous samples for laboratory water quality analyses have been collected. Shipping coolers will be supplied and decontaminated by the laboratory. Sample preservation and holding times are provided in Table 8. ELAB will follow all local, state and federal requirements pertaining to waste storage and disposal.

Table 8 Aqueous Matrix Containers, Preservation, and Holding Times

Parameter	Container	Preservation	Holding Time
Nitrate + Nitrite	250 ml HDPE	4°C, H ₂ SO ₄ to pH<2	28 days
TKN			28 days
Ammonia			28 Days
Sulfate	125ml HDPE	4°C	28 Days

Section 8 Analytical Methodology

Analytical methods, precision and accuracy, method detection and practical quantification limits are shown in Table 9.

Table 9 ELAB Inc. Aqueous Methodology, Precision and Accuracy, Detection Limits

Parameters	Method	Precision (% Diff. ¹)	Accuracy (% Recovery)	MDL, ppm	PQL, ppm
Nitrate + Nitrite	EPA 353.2	20	90 - 110	0.0050	0.050
Total Kjeldahl Nitrogen	EPA 351.2	20	90 - 110	0.046	0.5
Ammonia	EPA 350.1	20	90 - 110	0.0063	0.05
Sulfate	EPA 300.0	20	90 - 110	0.085	0.5

¹% Diff. = (Result 1-Result 2)/((Result 1+Result 2)/2) x 100

Section 9 Inspection/Acceptance of Supplies and Consumables

A. Sample Containers

To be provided by the laboratory prior to each sampling event.

B. Sample Coolers

To be provided by the laboratory prior to each sampling event.

Section 10 Data Review, Verification and Validation

A. Data Verification

Data verification is the process for evaluating the completeness, correctness, and conformance of the data set against the methodology. This evaluation is integral to the final report.

B. Data Validation

Data validation is an analyte and sample specific process that determines the quality of the data set relative to the end use. Any data deemed to be unusable for the stated objectives will be identified as such in the final report.

Appendix A

ELAB Certification Documentation

Draft



State of Florida
Department of Health, Bureau of Laboratories

This is to certify that

E84973
ELAB, INC. - TAMPA
1211 TECH BLVD, SUITE 106
TAMPA, FL 33619

has complied with Florida Administrative Code 64E-1,
for the examination of Environmental samples in the following categories
DRINKING WATER - MICROBIOLOGY; DRINKING WATER - PRIMARY INORGANIC CONTAMINANTS; DRINKING WATER - SECONDARY INORGANIC
CONTAMINANTS; NON-POTABLE WATER - GENERAL CHEMISTRY; NON-POTABLE WATER - MICROBIOLOGY

Continued certification is contingent upon successful on-going compliance with the NELAP Standards and FAC Rule 64E-1
regulations. Specific methods and analytes certified are cited on the Laboratory Scope of Accreditation for this laboratory and
are on file at the Bureau of Laboratories, P. O. Box 210, Jacksonville, Florida 32231. Clients and customers are urged to verify
with this agency the laboratory's certification status in Florida for particular methods and analytes.

EFFECTIVE August 03, 2007 THROUGH June 30, 2008



Max Saffinger, M.D.
Chief, Bureau of Laboratories
Florida Department of Health
DH Form 1697, 7/04
NON-TRANSFERABLE E84973-04-8/3/2007
Supersedes all previously issued certificates

Charlie Crist
 Governor



Ana M. Viamonte Res. M.D., M.P.H.
 State Surgeon General

Laboratory Scope of Accreditation

Page 1 of 3

Attachment to Certificate #: E84973-04, expiration date June 30, 2008. This listing of accredited analytes should be used only when associated with a valid certificate.

State Laboratory ID: E84973 EPA Lab Code: FL01241 (386) 672-5668

E84973
 ELAB, Inc. - Tampa
 1211 Tech Blvd.
 Suite 106
 Tampa, FL 33619

Matrix: Drinking Water

Analyte	Method/Tech	Category	Certification Type	Effective Date
Alkalinity as CaCO ₃	SM 2320 B	Primary Inorganic Contaminants	NELAP	3/22/2006
Chloride	EPA 325.3	Secondary Inorganic Contaminants	NELAP	7/30/2007
Chloride	SM 4500 Cl- C	Secondary Inorganic Contaminants	NELAP	7/30/2007
Color	EPA 110.2	Secondary Inorganic Contaminants	NELAP	3/22/2006
Conductivity	SM 2510 B	Primary Inorganic Contaminants	NELAP	3/22/2006
Fluoride	EPA 340.2	Primary Inorganic Contaminants	NELAP	7/30/2007
Fluoride	SM 4500 F-C	Primary Inorganic Contaminants	NELAP	7/30/2007
Heterotrophic plate count	SM 9215 B	Microbiology	NELAP	3/22/2006
Nitrite	SM 4500-NO3 E	Primary Inorganic Contaminants	NELAP	3/22/2006
Nitrate as N	EPA 353.2	Primary Inorganic Contaminants	NELAP	7/30/2007
Nitrate-nitrite	SM 4500-NO3 E	Primary Inorganic Contaminants	NELAP	3/22/2006
Nitrite	SM 4500-NO2 B	Primary Inorganic Contaminants	NELAP	3/22/2006
Odor	EPA 140.1	Secondary Inorganic Contaminants	NELAP	3/22/2006
Odor	SM 2150 B	Secondary Inorganic Contaminants	NELAP	3/22/2006
Orthophosphate as P	SM 4500-P E	Primary Inorganic Contaminants	NELAP	3/22/2006
pH	EPA 150.1	Primary Inorganic Contaminants, Secondary Inorganic Contaminants	NELAP	3/22/2006
Residual free chlorine	SM 4500-Cl D	Primary Inorganic Contaminants	NELAP	3/22/2006
Residue-filterable (TDS)	EPA 160.1	Secondary Inorganic Contaminants	NELAP	3/22/2006
Residue-filterable (TDS)	SM 2540 C	Secondary Inorganic Contaminants	NELAP	3/22/2006
Sulfate	EPA 375.4	Secondary Inorganic Contaminants	NELAP	7/30/2007
Sulfate	SM 4500 SO4-E	Secondary Inorganic Contaminants	NELAP	7/30/2007
Total coliforms & E. coli	SM 9223 B	Microbiology	NELAP	3/22/2006
Total nitrate-nitrite	EPA 353.2	Primary Inorganic Contaminants	NELAP	7/30/2007
Total residual chlorine	SM 4500-Cl D	Primary Inorganic Contaminants	NELAP	3/22/2006
Turbidity	EPA 180.1	Secondary Inorganic Contaminants	NELAP	3/22/2006
Turbidity	SM 2130 B	Secondary Inorganic Contaminants	NELAP	3/22/2006

Clients and Customers are urged to verify the laboratory's current certification status with the Environmental Laboratory Certification Program.

Issue Date: 8/3/2007

Expiration Date: 6/30/2008

Charlie Crist
 Governor



Ana M. Viamonte Ros, M.D., M.P.H.
 State Surgeon General

Laboratory Scope of Accreditation

Page 2 of 3

Attachment to Certificate #: E84973-04, expiration date June 30, 2008. This listing of accredited analytes should be used only when associated with a valid certificate.

State Laboratory ID: E84973 EPA Lab Code: FL01241 (386) 672-5668

E84973
 ELAB, Inc. - Tampa
 1211 Tech Blvd.
 Suite 106
 Tampa, FL 33619

Matrix: Non-Potable Water

Analyte	Method/Tech	Category	Certification Type	Effective Date
Alkalinity as CaCO ₃	EPA 310.1	General Chemistry	NELAP	3/22/2006
Alkalinity as CaCO ₃	SM 2120 B	General Chemistry	NELAP	3/22/2006
Ammonia as N	EPA 350.3	General Chemistry	NELAP	7/30/2007
Ammonia as N	SM 4509NH ₃ -D	General Chemistry	NELAP	7/30/2007
Biochemical oxygen demand	EPA 465.1	General Chemistry	NELAP	3/22/2006
Biochemical oxygen demand	SM 5210 B	General Chemistry	NELAP	3/22/2006
Carbonaceous BOD (CBOD)	SM 5210 B	General Chemistry	NELAP	3/22/2006
Chemical oxygen demand	EPA 410.4	General Chemistry	NELAP	7/30/2007
Chloride	EPA 325.3	General Chemistry	NELAP	7/30/2007
Chloride	SM 4500 Cl- C	General Chemistry	NELAP	7/30/2007
Chromium VI	SM 3500-Cr D (180b/190b Ed.)/COLOR	General Chemistry	NELAP	7/30/2007
Color	EPA 110.2	General Chemistry	NELAP	3/22/2006
Color	SM 2120 B	General Chemistry	NELAP	3/22/2006
Conductivity	EPA 120.1	General Chemistry	NELAP	3/22/2006
Conductivity	SM 2510 B	General Chemistry	NELAP	3/22/2006
Fecal coliforms	SM 9222 D	Microbiology	NELAP	3/22/2006
Fluoride	EPA 340.2	General Chemistry	NELAP	7/30/2007
Fluoride	SM 4500 F-C	General Chemistry	NELAP	7/30/2007
Heterotrophic plate count	SM 9215 B	Microbiology	NELAP	7/30/2007
Nitrate	SM 4500-NO ₃ E	General Chemistry	NELAP	3/22/2006
Nitrate as N	EPA 353.2	General Chemistry	NELAP	7/30/2007
Nitrate-nitrite	SM 4500-NO ₃ E	General Chemistry	NELAP	3/22/2006
Nitrate	SM 4500-NO ₂ B	General Chemistry	NELAP	3/22/2006
Orthophosphate as P	EPA 365.2	General Chemistry	NELAP	3/22/2006
Orthophosphate as P	SM 4500-P E	General Chemistry	NELAP	3/22/2006
pH	EPA 150.1	General Chemistry	NELAP	3/22/2006
Phosphorus, total	EPA 365.2	General Chemistry	NELAP	7/30/2007
Phosphorus, total	SM 4500-P E	General Chemistry	NELAP	7/30/2007
Residue-filterable (TDS)	EPA 160.1	General Chemistry	NELAP	3/22/2006
Residue-filterable (TDS)	SM 2540 C	General Chemistry	NELAP	3/22/2006
Residue-nonfilterable (TSS)	EPA 160.2	General Chemistry	NELAP	3/22/2006
Residue-nonfilterable (TSS)	SM 2540 D	General Chemistry	NELAP	3/22/2006
Residue-total	EPA 160.3	General Chemistry	NELAP	3/22/2006
Residue-total	SM 2540 B	General Chemistry	NELAP	3/22/2006
Sulfate	EPA 375.4	General Chemistry	NELAP	7/30/2007

Clients and Customers are urged to verify the laboratory's current certification status with the Environmental Laboratory Certification Program.

Issue Date: 8/3/2007

Expiration Date: 6/30/2008

Charlie Crist
 Governor



Ana M. Viamonte Ros, M.D., M.P.H.
 State Surgeon General

Laboratory Scope of Accreditation

Page 3 of 3

Attachment to Certificate #: E84973-04, expiration date June 30, 2008. This listing of accredited analytes should be used only when associated with a valid certificate.

State Laboratory ID: E84973 EPA Lab Code: FL01241 (386) 672-5668

E84973
 ELAB, Inc. - Tampa
 1211 Tech Blvd.
 Suite 106
 Tampa, FL 33619

Matrix: Non-Potable Water

Analyte	Method/Tech	Category	Certification Type	Effective Date
Sulfide	SM 4500 S04-E	General Chemistry	NELAP	7/30/2007
Total coliforms	SM 9222 B	Microbiology	NELAP	3/22/2006
Total nitrate-nitrite	EPA 353.2	General Chemistry	NELAP	7/30/2007
Turbidity	EPA 180.1	General Chemistry	NELAP	7/30/2007

Clients and Customers are urged to verify the laboratory's current certification status with the Environmental Laboratory Certification Program.

Issue Date: 8/3/2007

Expiration Date: 6/30/2008



State of Florida
Department of Health, Bureau of Laboratories

This is to certify that

E83079
ELAB, INC.
8 EAST TOWER CIRCLE
ORMOND BEACH, FL 32174

has compiled with Florida Administrative Code 64E-1,
for the examination of Environmental samples in the following categories:

- DRINKING WATER - GROUP I UNREGULATED CONTAMINANTS, DRINKING WATER - SYNTHETIC ORGANIC CONTAMINANTS, DRINKING WATER - GROUP II UNREGULATED CONTAMINANTS, DRINKING WATER - OTHER REGULATED CONTAMINANTS, DRINKING WATER - GROUP III UNREGULATED CONTAMINANTS, DRINKING WATER - MICROBIOLOGY, DRINKING WATER - PRIMARY INORGANIC CONTAMINANTS, DRINKING WATER - SECONDARY INORGANIC CONTAMINANTS, NON-POTABLE WATER - EXTRACTABLE ORGANICS, NON-POTABLE WATER - GENERAL CHEMISTRY, NON-POTABLE WATER - METALS, NON-POTABLE WATER - MICROBIOLOGY, NON-POTABLE WATER - EXTRACTABLE ORGANICS, PESTICIDES-HERBICIDES-PCBS, NON-POTABLE WATER - VOLATILE ORGANICS, SOLID AND CHEMICAL MATERIALS - EXTRACTABLE ORGANICS, SOLID AND CHEMICAL MATERIALS - GENERAL CHEMISTRY, SOLID AND CHEMICAL MATERIALS - METALS, SOLID AND CHEMICAL MATERIALS - MICROBIOLOGY, SOLID AND CHEMICAL MATERIALS - PESTICIDES-HERBICIDES-PCBS, SOLID AND CHEMICAL MATERIALS - VOLATILE ORGANICS, BIOLOGICAL TISSUE - METALS, BIOLOGICAL TISSUE - PESTICIDES-HERBICIDES-PCBS

Continued certification is contingent upon successful on-going compliance with the NELAP Standards and FAC Rule 64E-1 regulations. Specific methods and analytes certified are cited on the Laboratory Scope of Accreditation for this laboratory and are on file at the Bureau of Laboratories, P. O. Box 210, Jacksonville, Florida 32231. Clients and customers are urged to verify with this agency the laboratory's certification status in Florida for particular methods and analytes.

EFFECTIVE July 01, 2007 THROUGH June 30, 2008



Max Saifinger, M.D.
Chief, Bureau of Laboratories
Florida Department of Health
DH Form 1697, 7/04

NON-TRANSFERABLE E83079-06-7/1/2007
Supersedes all previously issued certificates

EVALUATION OF EEM METHODOLOGY FOR DETECTION OF OSTDS EFFLUENT IN AMBIENT SURFACE WATERS



SUBMITTED TO:

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BUREAU OF ONSITE SEWAGE PROGRAMS
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Mote Marine Laboratory Technical Report No. 1186

JUNE 22, 2007

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Introduction

Optical brighteners (OB) are organic compound(s) typically added to laundry detergents and paper during manufacture. These water soluble dyes are various compounds which, as a class, absorb in the near-UV (250-400 nm) portion of the spectrum and fluoresce in the visible blue region (between 430-460 nm), making “whites appear whiter.”

Usefulness of OB in the environment is that, unlike nutrients or bacteria, the compounds present a uniquely anthropogenic signature. As OB are rapidly absorbed by soils (Mote Marine Laboratory, unpublished data), detection in the environment typically indicates either the relatively direct input of human wastes (typically consisting of sewage and grey water combined) or the presence of large quantities of wastes from which not all OB have been removed, as in waste water treatment plant (WWTP) effluents. Failing septic tanks (on site treatment and disposal systems - OSTDS) or OSTDS with inadequate filtration are also likely contributors.

Fluorometry is a standard technique with which to identify the presence of OB. Detecting OB in ambient waters, however, present an analytical challenge in that naturally occurring colored dissolved organic matter (CDOM) is also highly fluorescent, particularly in the blue wavelengths where OB have a peak emission. A field technique has been developed by Mote Marine Laboratory (MML) to distinguish between humic and OB fluorescence using a single excitation wavelength and two emission wavelengths that was promising (Dixon, et al., 2005), but variations in the amounts and relative fluorescence of naturally occurring humic compounds can complicate interpretations.

The more detailed fluorescence analyses under this project used excitation-emission matrix fluorescence (EEM) in which samples are successively excited with a wide range of wavelengths and the resulting fluorescence quantified across a similarly large wavelength range, creating a three dimensional matrix of fluorescent data which can act as a “fingerprint” with which to characterize both natural waters and any OB present. Measurement of CDOM absorption for correction of fluorescence data is a necessary part of the EEM analysis. Mathematical techniques are used to separate the components, allowing identification of OB in the presence of CDOM.

EEM analyses were performed on a wide variety source waters, both with and without OB added, to allow confirmation of the dual wavelength technique, to identify limitations in interpretation of EEM, to allow correlations of OB signatures with conventional analyses for wastewater source tracking, and to identify other wavelength pairs that may be suitable for a remote sensing approach to identify OB in the environment. A number of field locations were also sampled for EEM to apply the EEM signatures developed and to quantify OB in the areas of interest.

The overall project was funded through a cooperative agreement between the Environmental Protection Agency (EPA) Gulf of Mexico Program and the Florida Department of Health (FDOH) Bureau of Onsite Sewage Programs, and was conducted by the Florida Department of Health with the Florida Department of Environmental Protection (Southwest District Office) as the prime contractor, principal investigator, and

prime sampling entity. Mote Marine Laboratory, as a subcontractor, acted to provide expertise in sampling for EEM, and to analyze both ambient and laboratory-prepared samples for methods development of EEM interpretation and environmental assessment. Sarasota County was an essential cooperator and contributor in the project, finding accessible OSTDS and WWTP locations and funding the analyses of additional ambient samples for EEM. Sampling and analyses were conducted under a Quality Assurance Project Plan (FDEP, 2006).

Methods

EEM and CDOM SAMPLING

Ambient samples were collected from three regions with suspected wastewater influence and potential OB concentrations, Keaton and Dekle Beaches, Steinhatchee River, Chassahowitzka River, and Phillipi Creek (**Figures 1-5**). EEM samples were collected by Mote Marine Laboratory (MML) initially (September 20-21, 2006) and subsequently by FDEP personnel. A total of 41 ambient samples plus field QC samples (replicates and field blanks) were collected. Additionally, samples were collected from OSTDS (five locations), a tertiary treatment and a secondary treatment wastewater plant in Sarasota County for a total of 48 field samples. Samples for EEM and absorption measurements were collected simultaneously with bacteriological and nutrient parameters, which were analyzed by the prime contractor.

The Phillippi Creek locations had been previously sampled and found to contain OB using a dual wavelength fluorescence method (Dixon and Julian, 2005). The present project resampled some of the same locations in order to confirm presence of OB through EEM analyses. Outside of the project, Sarasota County funded an additional sampling effort (**Figure 6**), collecting EEM samples from a total of eight waterways and tributaries thought to be impacted by OSTDS. These additional samples were collected by Sarasota County personnel under instructions from MML, together with other wastewater parameters. Results for both project and additional samples are included in the following report. MML provided all sample containers, and any necessary intermediate containers to secure EEM samples and field blanks and to maintain these samples on ice. FDEP and other cooperators (Sarasota County) provided any needed well points, well sampling equipment, and any specialized equipment needed to secure samples from the WWTP and the selected OSTDS.

Sample containers for EEM and absorption (125mL amber glass bottles with Teflon-lined caps) were acid washed (10% HCl, deionized water), capped with foil (10% methanol rinsed), and fired (450 °C) for 4 hours. Caps were rinsed with laboratory water, 10% methanol, and a final rinse of laboratory water. Sampling protocol was to obtain ambient water directly in sample bottles from near surface waters after an initial sample rinse that was discarded. Large non-representative particles (algal mats, vegetation) were avoided but moderate amounts of turbidity have been shown not to interfere (MML, unpublished data).



Fixed Sample Locations for Steinhatchee River, Taylor County, FL

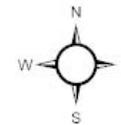
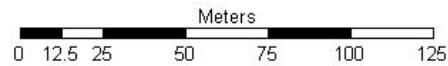


Figure 1. Fixed sample locations for Steinhatchee River, Taylor County, FL. Image courtesy of FDEP-Tampa District Office.



Fixed Sample Locations for Keaton Beach, Taylor County, FL

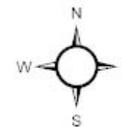
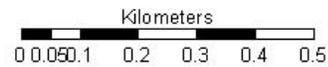
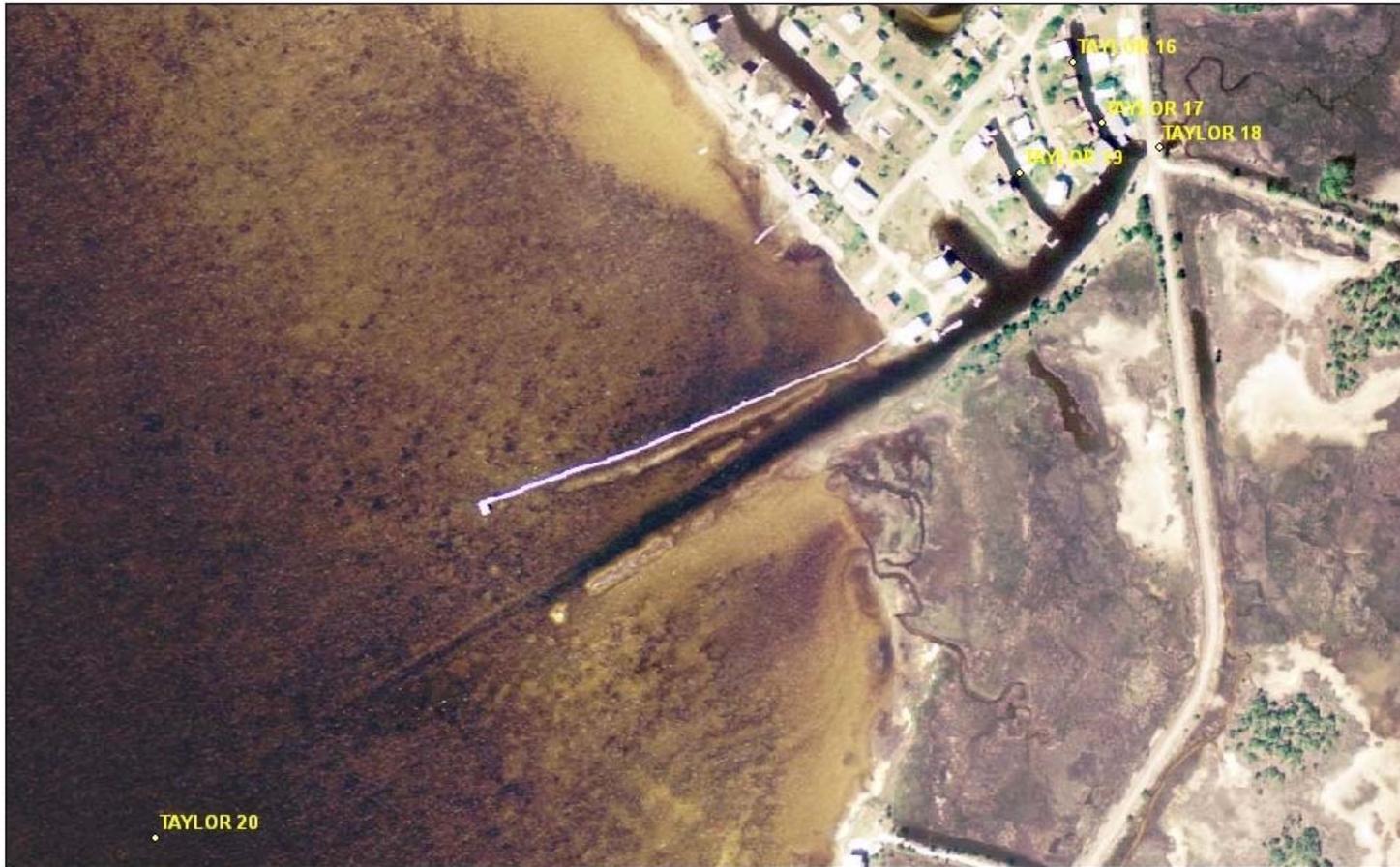


Figure 2.

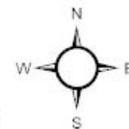
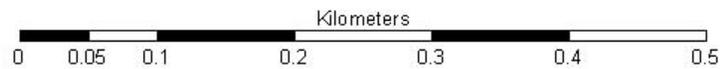
Fixed sample locations for Keaton Beach, Taylor County, FL. Image courtesy of FDEP-Tampa District Office.



Fixed Sample Locations for Dekle Beach, Taylor County, FL



Figure 3.



Fixed sample locations for Dekle Beach, Taylor County, FL. Image courtesy of FDEP-Tampa District Office.



Fixed Sample Locations for Chassahowitzka River, FL

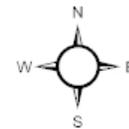
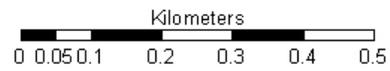
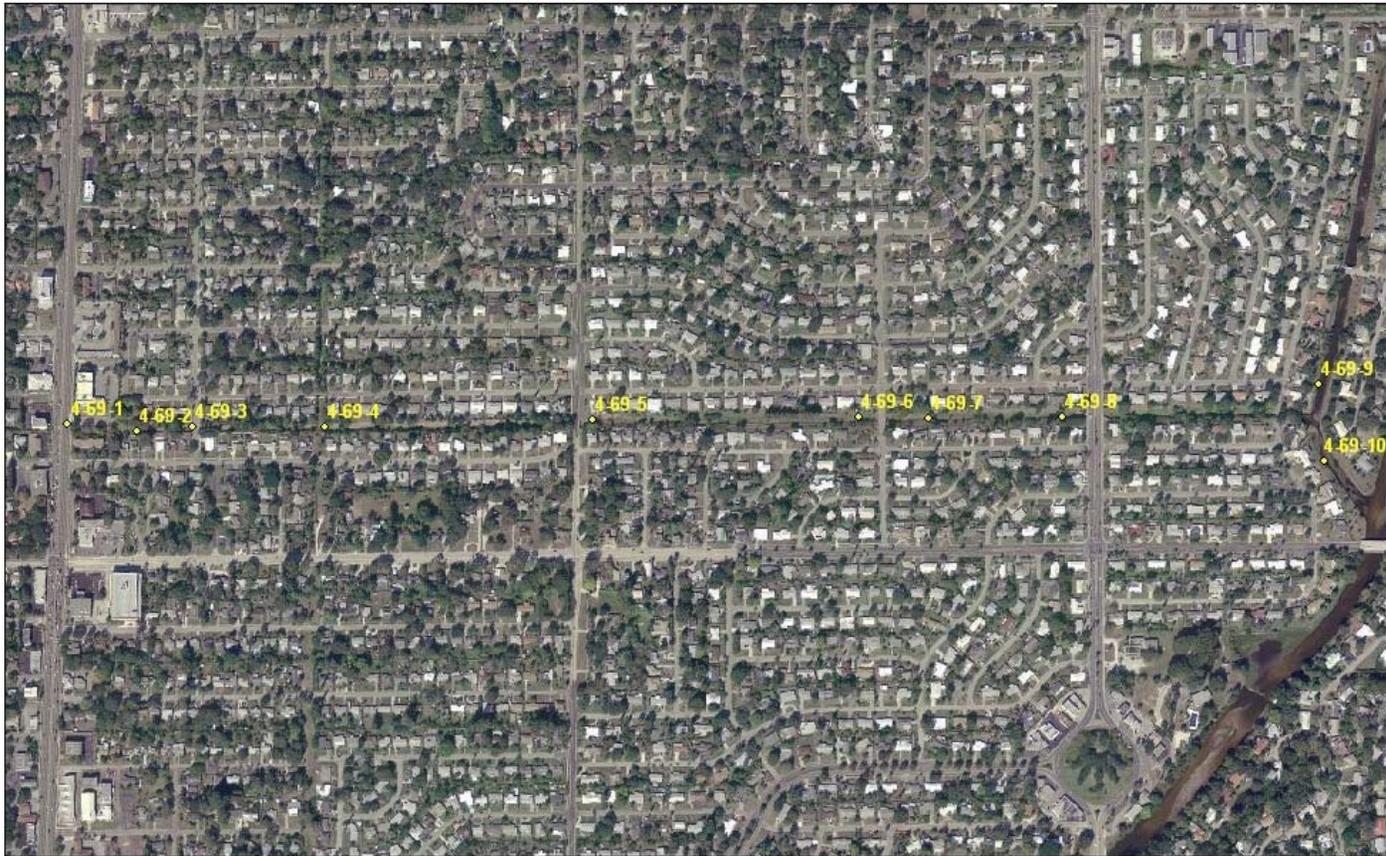


Figure 4.

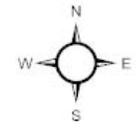
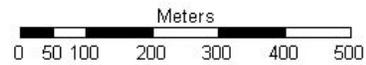
Fixed sample locations for Chassahowitzka River,, FL. Image courtesy of FDEP-Tampa District Office.



Fixed Sample Locations for Canal 4-69 Phillippi Creek, Sarasota County, FL



Figure 5.



Fixed sample locations for Phillippi Creek, Sarasota County, FL. Image courtesy of FDEP-Tampa District Office.

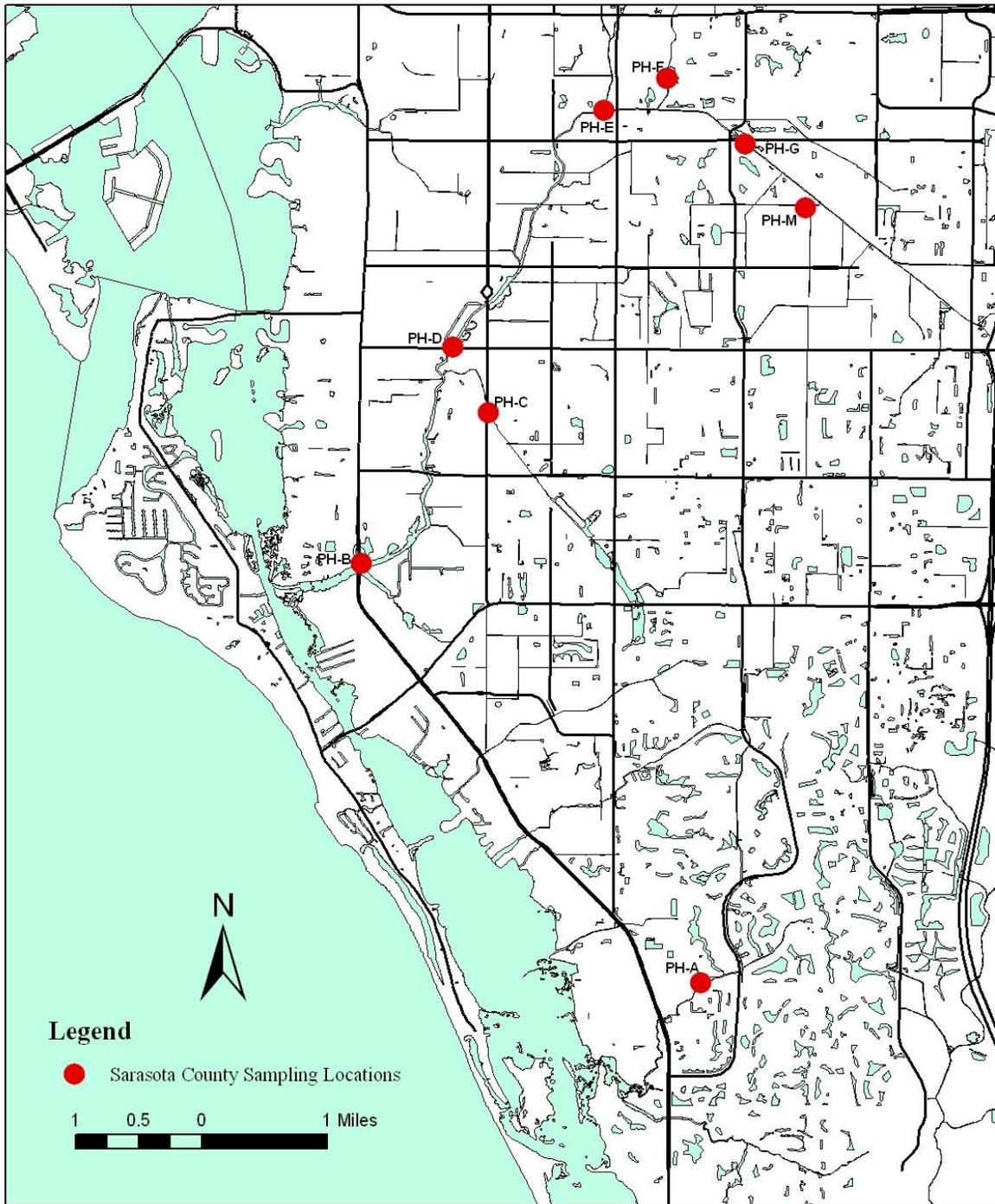


Figure 6. Additional Phillippi Creek fixed stations, Sarasota County, FL. Image courtesy of Sarasota County Integrated Water Resources.

Samples were not filtered. Gloves were worn by sampling crew and contact of sample with plastics was minimized to all extents possible. Samples pumped from OSTDS did come in contact with a short length of silicone peristaltic tubing. All samples were collected in duplicate to ensure no loss of sample from breakage of glass sample bottles. Along with the field samples collected, one replicate per sampling day was collected to enable assessment of system heterogeneity. Daily field equipment blanks were analyzed to test transport, storage, and field handling, while container blanks were to confirm cleanliness of sample containers. Water for field equipment blanks was provided by MML in precleaned glass containers. Temperature blanks were included with sample shipments to confirm appropriate holding temperatures. Samples were iced and maintained in the dark at 4 °C for transport back to MML within 24 hours and were processed for EEM and absorption within one week of collection.

EEM and Absorption Analysis Methods

For EEM analysis, samples were cooled to 18° C, inverted several times to assure homogeneity and directly transferred to a 1 cm quartz cuvette. Those samples that were heavy in particulates were diluted with laboratory deionized water. Samples were not filtered prior to analysis as filtration has been demonstrated to remove OB from solution (MML, unpublished data). All samples were scanned in a PTI QM-4 SE Spectrofluorometer, with excitation wavelengths of 220-455 nm (5nm increments) and emission wavelengths of 250-700nm (2nm increments). The instrument used a scanning fluorescence Xenon arc lamp 75 W and spectral units were based on concave diffraction gratings. Excitation slit width was set at 5nm, emission slit width at 2nm and digital PMT slit width at 5nm.

Analyses for EEM included the daily EEM analysis of reference materials (quinine sulfate) to which fluorescence intensities were normalized to permit intercomparisons of data with other spectrofluorometric systems. Resulting data are presented in Quinine Sulfate Relative Fluorescence units (QSRF). Daily EEM of laboratory water was used to remove matrix effects from subsequent sample data during post processing. Wavelength accuracy was confirmed for at least three locations across the sampling range with Raman emission maxima and agreement with literature values. Initial calibration verifications consisted of another preparation of quinine sulfate from an alternate source and were required to be within 90-110%. Continuing calibrations evaluated quinine sulfate fluorescence at a fixed excitation wavelength to confirm continuing instrumental response (85-115% required) and were repeated as a final calibration check at the completion of an analytical group. Spike recoveries (minimum 1 per 20 samples, 90-110% recovery) were evaluated as fixed excitation scans relative to identical preparations in laboratory deionized water. Duplicate precisions (minimum one per 10 samples, <=15% RSD) were also evaluated at fixed excitation wavelengths.

Linear response of the fluorescence of quinine sulfate was evaluated over 0.02 to 400 ug/l. Linearity for the instrumental conditions in use in this project began to degrade above 10 ug/l. The instrumental thresholds so determined and appearance of fixed

excitation scan spectra when photodetectors were saturated with highly fluorescing samples were used to determine when sample dilution was required prior to analysis.

During the EEM analysis, samples were not filtered in order to retain OB in the sample. Although moderate amounts of turbidity have been demonstrated not to cause either negative or positive interferences, the amount of fine particulates suspended in the OSTDS samples was noteworthy and preliminary dilutions indicated that high turbidity levels resulted in negative interferences. Extremely turbid samples (>100 NTU) were therefore diluted and evaluated by fixed excitation scans until linearity of maximum fluorescence was achieved. The resulting dilution was then analyzed by the complete EEM.

Absorption was determined according to Ocean Optics Protocols for Satellite Ocean Color Sensor Validation (Mueller, *et al.*, 2003). A Perkin Elmer 650 spectrophotometer was used for the determination of full-spectrum absorption profiles. The instrument is a double beam, double monochromator, ratio recording UV/Vis spectrophotometer (tungsten-halogen sources). An all reflecting optical system (SiO₂ coated) used a holographic grating (1440 Lines/mm UV/Vis blazed at 240 nm) for wavelength selection and a R955 Photomultiplier sensitive in the 190 – 900 nm wavelength range for detection. The instrument is linear to 3.0A. Samples were warmed to slightly above room temperature to match temperatures of reference cells in the spectrophotometer. Samples were filtered through 0.2 micron Sterivex cartridges directly into a 10, 5, or 1 cm quartz cuvette, depending on sample color. Dilutions of highly absorbing samples were performed as appropriate to remain within the instrument's range of linearity.

Analyses for absorption included instrumental zero on laboratory water, confirmation of zero stability with re-analysis of laboratory water as a sample, and measurement of solid standards (didymium glass and a 10% T filter) to confirm wavelength accuracy and instrument response within specified limits (90-110% of historical values). Duplicate precision (minimum 1 per 10 samples) was assessed at select wavelengths (<5% RSD at 400 nm, 440 nm). Consistent with protocols, samples were not spiked. While full spectrum data were collected, absorption at 400 nm (a_{400}) was abstracted as a summary indicator of sample absorption.

Expected OSTDS Concentrations

Optical brighteners consist of a variety of compounds. As manufacturer formulations vary and are proprietary, a detergent mixture solution was prepared from different commercially available detergents to mimic the mixture of OB that was expected from a variety of OSTDS and to develop a field method with broad applicability. Ten detergents (**Table 1**) were separately prepared at five times manufacturer's recommended usage for a medium sized load (found to be 16.3 gallons or 61.7 L as an average of four washing machine manufacturers). (Solution was complete for all detergents.) A detergent mixture was prepared using equal volumes of all ten single detergent solutions.

Table 1. Standard detergent preparations. Final concentrations is 5 times manufacturer's recommended concentrations in initial wash water (without rinses), based on a 16.3 gallon average for a medium sized laundry load.

Detergent	Detergent/Load	Detergent /L in wash	Density g/ml	Detergent g/L in 5X Conc.
All Stain Lifter	92.34ml	1.496ml	1.055	7.89
Gain	92.40ml	1.498ml	1.050	7.86
Tide HE	113.5ml	1.839ml	1.066	9.80
Cheer Color Guard	94.40ml	1.530ml	1.058	8.09
Purex	92.20ml	1.494ml	1.053	7.86
Color Bright Clorox 2	81.25ml	1.316ml	1.03	6.78
Tide	92.34ml	1.497ml	1.066	7.98
Oxiclean	113.3g	1.837g		9.19
Arm & Hammer	64.00g	1.037g		5.19
Surf	55.30g	0.896g		4.48

The detergent mixture solution represented a 5X concentration of what would be expected in the wash cycle. The 5X concentration was prepared to minimize dilution of CDOM when spiking high absorption samples. The individual detergents prepared laboratory deionized water and the detergent mixture were also characterized by EEM, as was a single OB reference compound, disodium 4,4'-bis(2-sulfostyryl)biphenyl (DSBP).

The typical amounts of OB in OSTDS effluents was estimated as follows. A typical older model washing machine can generate 30-50 gallons of waste per load of laundry, consisting of a wash cycle with detergent, followed by one or two equivalent volume rinse cycles. EPA (2002) data estimates an average wastewater production of 69.3 gallons per person per day on average, of which 15 gallons is from laundry, or approximately 6 gallons would consist of the soapy water used in the first cycle of the machine.

Breakdown of a typical ODTDS effluent, therefore, was estimated to be:

$$1 \text{ L OSTDS effluent} = 15 \text{ gal} / 69.3 \text{ gal} = \begin{matrix} 216.5 \text{ ml laundry water} \\ 783.5 \text{ ml other wastes} \end{matrix}$$

Using an average of 1.5 rinses:

$$216.5 \text{ ml laundry water} = 216.5 / (1 \text{ wash} + 1.5 \text{ rinse}) = \begin{matrix} 86.6 \text{ ml wash} \\ 129.9 \text{ ml rinse} \end{matrix}$$

OSTDS effluent, therefore, should consist on average of approximately 8-9% wash water. For the detergent mixture used in this investigation (at 5X the concentration as would be in wash water), a volume of 86.6/5 or 17.3 ml of detergent mixture per liter would provide detergent and OB concentrations comparable to OSTDS effluent (termed as 100% OSTDS). Variations due to amount of laundry, detergent type, wash day relative to sampling day, and the like are of course possible, producing fluctuations in both in effluent volume and OB concentration, but the value represents an approximate upper bound for a robust analytical method.

During analyses of ambient samples, spike amounts were selected to be 0.2 ml detergent mix per 25 ml final volume, or 46% of typical OSTDS concentrations, allowing a wider range of samples to be spiked without exceeding the fluorometer's range of linearity. During preparation of laboratory dilution series (see below) to evaluate OB recovery in a variety of sample matrices, spike preparations were 0.875ml and 1.75 ml per 100 ml of sample or 50.5% and 101% the concentrations expected from typical OSTDS effluent.

Dilution Series

The laboratory dilution series of various natural waters was designed to investigate the recovery of a few fixed amounts of OB from a variety of parent waters. Laboratory dilution series were constructed with highly colored, low salinity water (HI CDOM), minimally colored saline water (LO CDOM), OSTDS effluent, and tertiary treatment WWTP effluent, collected in bulk from the same locations as the field samples. The low CDOM sample was collected from saline waters behind MML (33 PSU, $a_{400} = 0.50 \text{ m}^{-1}$), the high CDOM sample was collected from the Myakkahatchee Creek tributary of the Myakka River (Salinity < 2 PSU, $a_{400} = 7.94 \text{ m}^{-1}$). Absorption coefficients (at 400 nm) of the WWTP and OSTDS were 0.90 m^{-1} and 12.5 m^{-1} , respectively.

The high and low CDOM waters were also mixed to prepare intermediate CDOM water (MCDOM). Each of the three CDOM levels were then prepared as a dilution series with both WWTP and OSTDS waters, as 100:0, 50:50, and 0:100 mixtures for a total of 11 unique matrices. Source waters (three CDOM levels, WWTP, and OSTDS) and laboratory deionized water were replicated two or more times to evaluate stability and reproducibility, resulting in a total of 26 evaluations. Absorption analyses of CDOM were performed on each of the mixtures.

Each of the 26 samples for evaluation was spiked with two concentrations of OB, one to represent typical OSTDS concentrations (101% of OSTDS effluent) and another at one half typical concentrations (50.5%). The dilution series was used to evaluate the ability of the planned fluorescence data processing to reliably and quantitatively discriminate OB in the presence of varying quantities of naturally occurring fluorescent materials and in the presence of CDOM and OB from potential sources.

Method Detection Limits

The standard detergent mixture (at 5X manufacturer's recommended application rates) resulted in 7.53 g/L of detergent. Using the calculations and conversion factors described above, the typical OSTDS effluent might be expected to contain 0.130 g/L of mixed detergent. A concentration of 0.005 g/L detergent in laboratory deionized water was prepared and used to determine a single scan MDL, using 350ex/430em peak heights. Obtained MDL was 0.001 g/L of mixed detergent (1600 raw fluorescence counts) or 0.8% of typical OSTDS detergent concentrations. A more or less sensitive technique relative to OSTDS effluent and to detergent weight is of course possible, depending on homeowner laundry volume and detergent selected. Using quinine sulfate in 0.1N H₂SO₄, MDL values obtained were 0.04 ug/L.

Data Processing

Data processing began with data corrected for instrument-specific spectral lamp output, spectral grating efficiencies, and spectral photomultiplier sensitivity with manufacturer correction files, automatically applied. A daily emission wavelength correction was applied based on location of maximum Raman emission in laboratory DI water. Daily normalization for long term lamp and instrument drift was applied by multiplying by a Raman factor (RF), where:

$$RF = 80,000 / F_{275/303} \text{ of deionized water.}$$

It is essential that analyses include full-spectrum absorption profiles for correction of EEM data for inner filter and self-absorption effects. A dilution series of humic substances will experience spurious spectral shifts which may be inappropriately attributed to changes in fluorescent components unless absorption effects are removed (Mobed, et al. 1996). The inner filter effects arise from the absorption of photons by potentially non-fluorescent entities in the sample. In a highly absorbing sample, the energy reaching the sample volume viewed by the detector is much reduced, leading to less fluorescence being emitted. The reduced fluorescence is, in turn, subject to losses by absorption during the path to the detector. The losses are not spectrally neutral as there is a strong exponential shape to CDOM absorption. Failure to correct for self-absorption will result in a false red shift of both excitation and emission maxima that can be interpreted as another substance. The false red-shift is proportional to the amount of CDOM present in a sample and so is particularly important to address when sampling across saline-freshwater gradients.

Correction for absorption was initially applied as proposed and as described in the QAPP. However, although the resulting PARAFAC model was valid, spike recoveries and slope of response strongly covaried with CDOM absorption, leading to the conclusion that the initial absorption correction algorithm was inadequate for these high CDOM samples. From first principles, an alternate absorption correction was derived, using the wavelength CDOM absorptions and resulting %T of the excitation and emission

wavelengths (each across a 0.005 m path for the target volume of a 1 cm X 1 cm cell). Fluorescence data were multiplied by the resulting absorption correction, A, which was:

$$A = 1 / (10^{((a_{em} + a_{ex}) * 0.005 / (-2.303))})$$

The daily EEM of laboratory water, processed as above, was subtracted from each sample EEM data. Data were normalized to quinine sulfate units to permit interlaboratory comparisons of EEM data by dividing by the slope of quinine response for the day;

$$QS \text{ Slope} = F_{QS350/450} / [QS]$$

Lastly, data were smoothed (MATLAB, Ver 6.0 R12) to reduce noise (negative values) at low fluorescence values, and remaining negative data, both primary and secondary Rayleigh and Raman, as well as where emission wavelengths were shorter than excitation wavelengths were set to missing (hard negative weighting; JiJi and Booksh, 2000) prior to analysis. All data were reviewed at selected wavelengths for general efficacy of Rayleigh and Raman removal. Selected samples (generally OSTDS samples and mixtures) with high turbidity revealed inadequate removal of Rayleigh scattering effects and so the width of the Rayleigh mask was increased for those samples only. For initial model development, data were also normalized by dividing all matrix elements by the maximum EEM value within a 290-650 nm / 230:440 nm excitation/emission region to minimize the range between samples.

PARAFAC Analysis

Parallel factor analyses (PARAFAC, PTFools for Matlab, Andersen and Bro, 2003) was then applied to the EEM data from the dilution matrix. This technique of linear unmixing, when used for fluorescence data, generates the spectral shapes and relative components (factors) of excitation and emission needed to produce the observed initiating data, separating fluorescence into that generated by CDOM, OB, and other fluorescing compounds present in the pool of initiating samples. The optimum number of unique factors (unique mixture components) was evaluated (generally from two to six components) via duplicate runs to avoid local minima and the optimum number of factors determined based on the resultant model fits and consistencies. The model was constrained to only present non-negative excitation and emission spectra, but both excitation and emission factors were allowed to be multimodal.

Models factors were initially identified using data normalized to a maximum fluorescence of 1.00 to avoid the effects of having a model skewed by a few extremely high fluorescence samples in the data set. Models determined were confirmed through split-half analysis (Stedmon et al., 2003) where data were randomly assigned to one of two groups; each group analyzed separately, and derived modes and factors compared for similarity. Non-normalized data and EEM results from ambient samples were analyzed lastly, using the identified multimodal factors to arrive at quantitative proportions or

loadings of each factor in each individual sample. Comparisons of loadings with OB added, with CDOM absorption, etc., allowed the linking of identified PARAFAC factors with sample properties.

Results

Quality Assurance

All quality assurance measures were performed and acceptable for both EEM and absorption analyses with the exception of container blanks. Container blanks for the cleaning lot number were not processed as all field blanks from the single cleaning lot number were acceptable. Minor adjustments to wavelengths at which standards were assessed were made in keeping with the standards in use (350ex/450em rather than 300ex/451em). Updated quality assurance criteria appear in Appendix A. Internal audit results as part of MML's Quality Assurance Program appear as Appendix B.

Laboratory Dilution Matrix

Examples of processed data from the dilution matrix appear in Figures 7 and 8. Low CDOM samples had a diffuse, elongated peak at 220-225ex/300-420em. As the feature near 650nm emission did not extend into higher excitation wavelengths, it was unlikely to be chlorophyll and may have been affected by residual tertiary Rayleigh scattering in these unfiltered samples. Higher CDOM samples in the same figure had a more localized fluorescence of 220-230ex/400-500em.

Spiked samples and those in which OB was expected to be present (Figure 8) had a very discrete and characteristic peak (220-230ex/290-300em) that was outside of the visible range. The OSTDS sample, in addition, had some Rayleigh scattering that had not been accounted for in the low wavelength excitation, and also had a number of other smaller features along the 220-230 excitation region. Due to the intensity of the UV peak, the expected fluorescence of OB in the 400-450 range was not clearly evident.

The UV region of the EEM spectra, however, as the result of low lamp output and high gain settings, was among the noisiest portion of the sample and reproducibility may have been problematic. PARAFAC analyses on these complete EEM spectra resulted in models which captured this noise in the factors presented, and which also endeavored to capture any residual Rayleigh emissions. Residuals from these models are distributed and indicated incomplete sample description despite adequate model parameterization. While the UV peak appeared to have little overlap with CDOM, and exhibited high sensitivity to OB, it was also in a region of maximum absorption and maximum absorption correction. The use of the UV peak may be better suited to a screening method for presence/absence of OB rather than a quantitative assessment.

Accordingly, dilution matrix data were truncated to 280-455ex/262-650em and PARAFAC analyses repeated. Example data in Figures 9 and 10 now indicate a much stronger relative response in the 400-450 nm emission range. Characteristic CDOM and OB fluorescence appeared where expected although the OB peak in the OSTDS sample was partially masked by the strength of the UV peak in the region outside of the plot and, to a lesser extent, by fluorescence in the 280ex/650em region which could no longer be attributed to scattering.

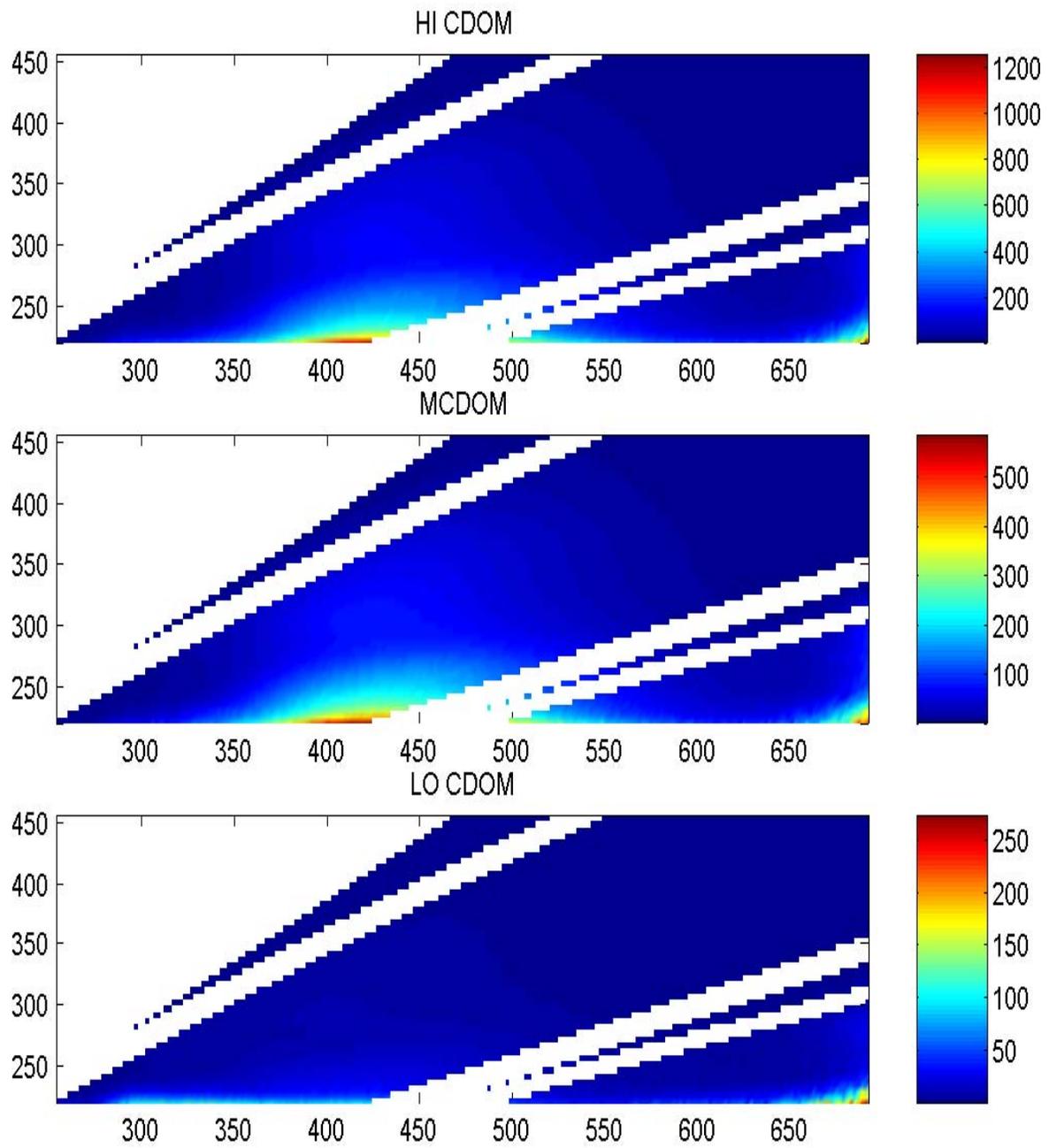


Figure 7. Example EEM data from laboratory dilution matrix.

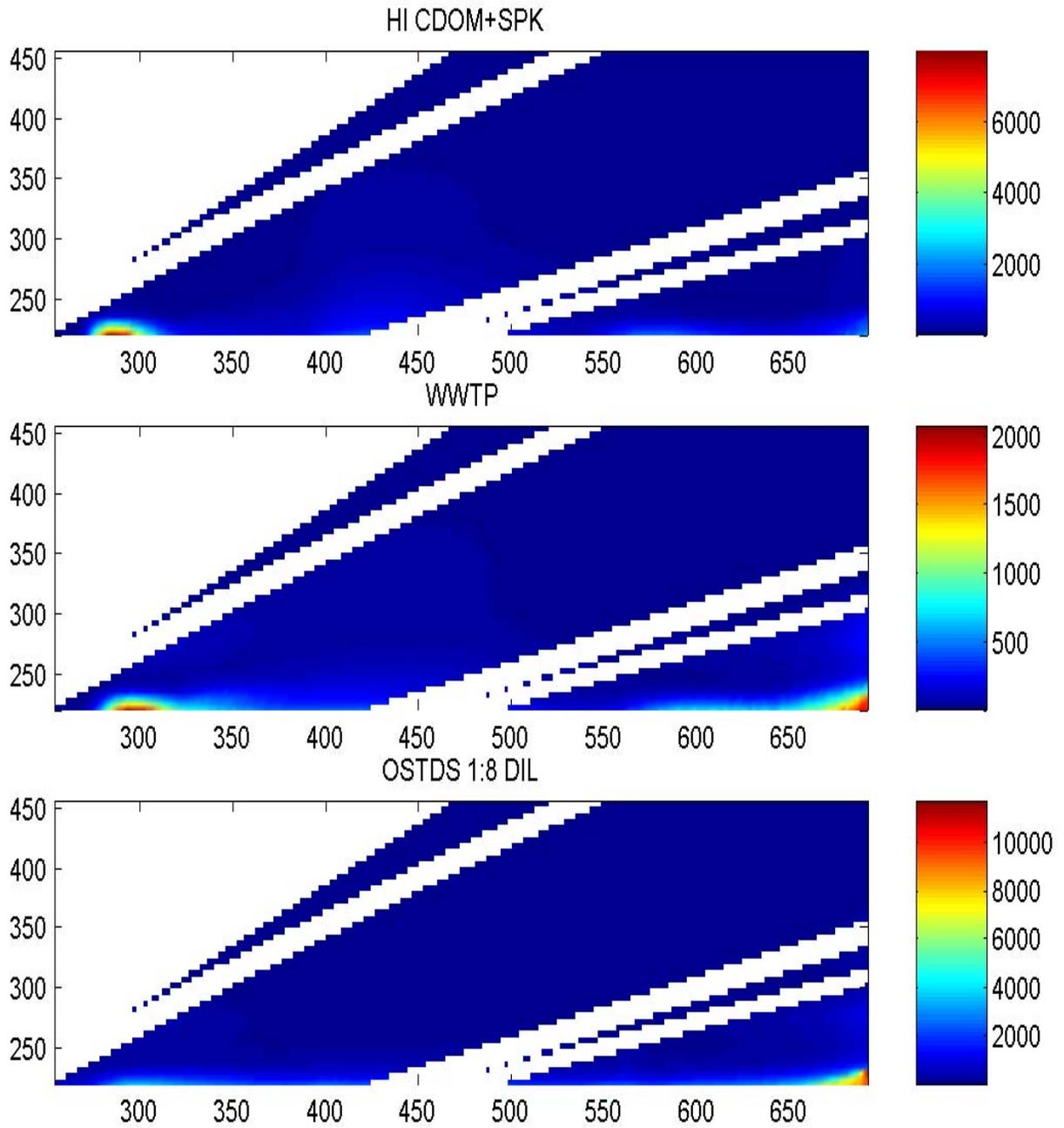


Figure 8. Examples of EEM data from laboratory dilution matrix in which OB was present or expected.

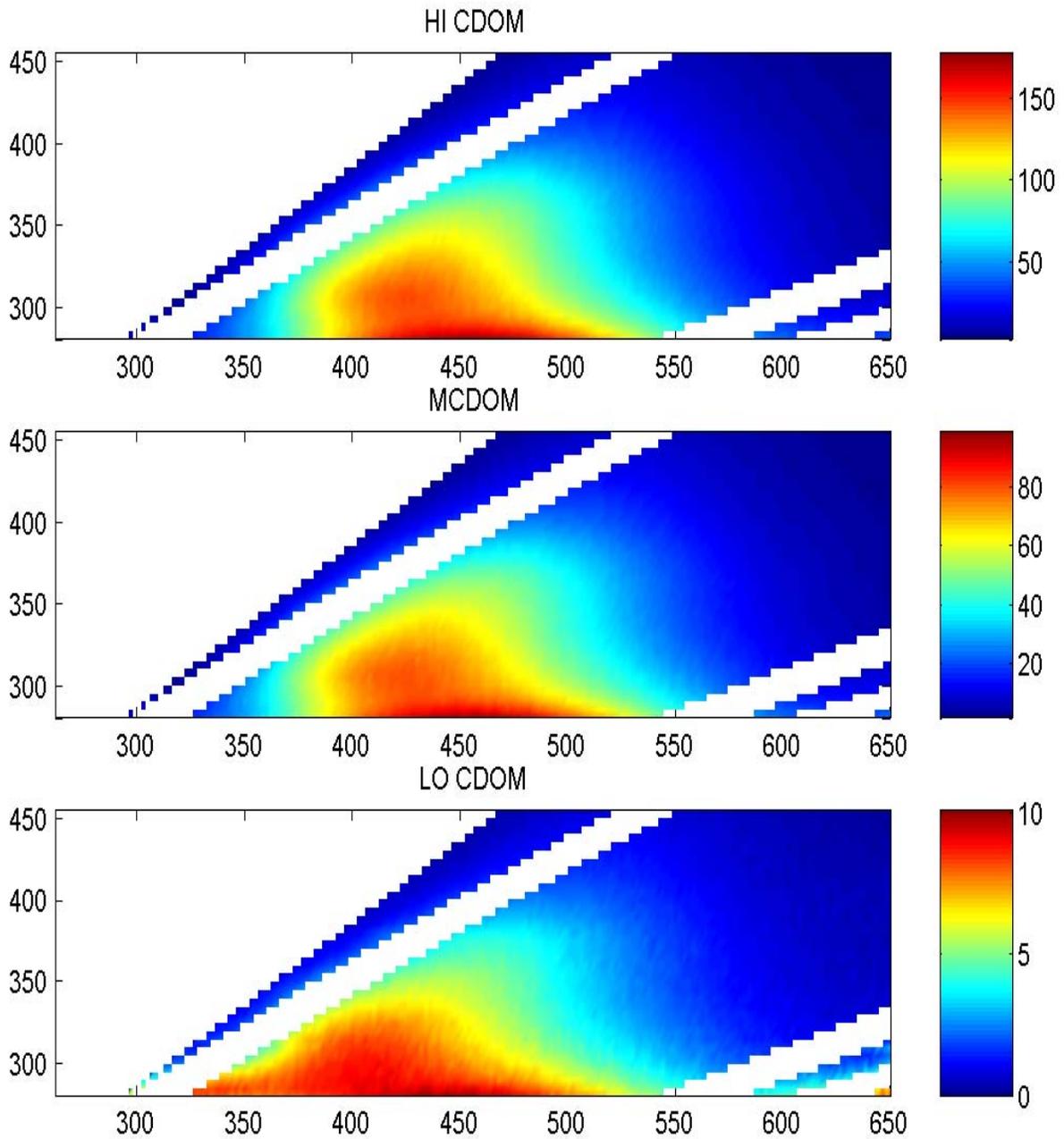


Figure 9. Examples of EEM data from Figure 7 after truncation of low wavelength excitation and emission regions.

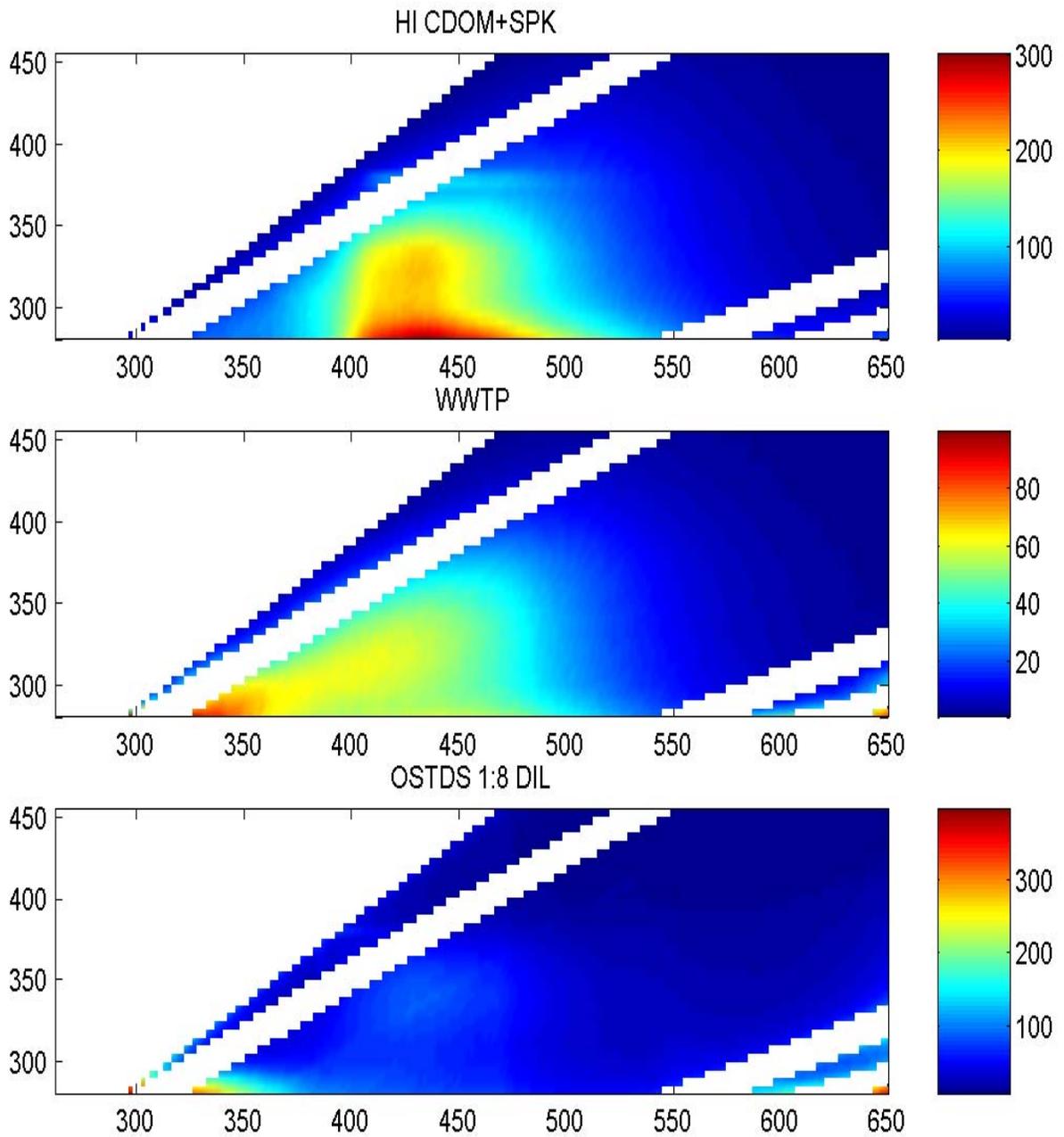


Figure 10. Examples of EEM data from laboratory dilution matrix in which OB was present or expected, after truncation of low wavelength excitation and emission regions.

PARAFAC results on all samples from the truncated dilution matrix determined a best fit of four unique factors (Model 1 - **Figure 11**). Mode#1 was indicative of the per sample loadings of the four factors, and Mode#2 and Mode#3 indicated the spectral properties of emission and excitation of the factors. The order of the factors (which spectral component is assigned as number 1 – PFAC1) was based on the amounts of variance which each factor accounts for in the pool of normalized data used to determine factors.

The individual factors for Model 1 are illustrated as pseudo-3D plots in **Figures 12** and **13** and in **Table 2**. Split-half analysis, where the data are randomly divided and the model recalculated for each half independently (**Figures 14** and **15**), indicate that the model's factors were robust. Computed factor amounts were additive, or consistent with the mixtures of source waters prepared given the computed amounts of factors in the source waters.

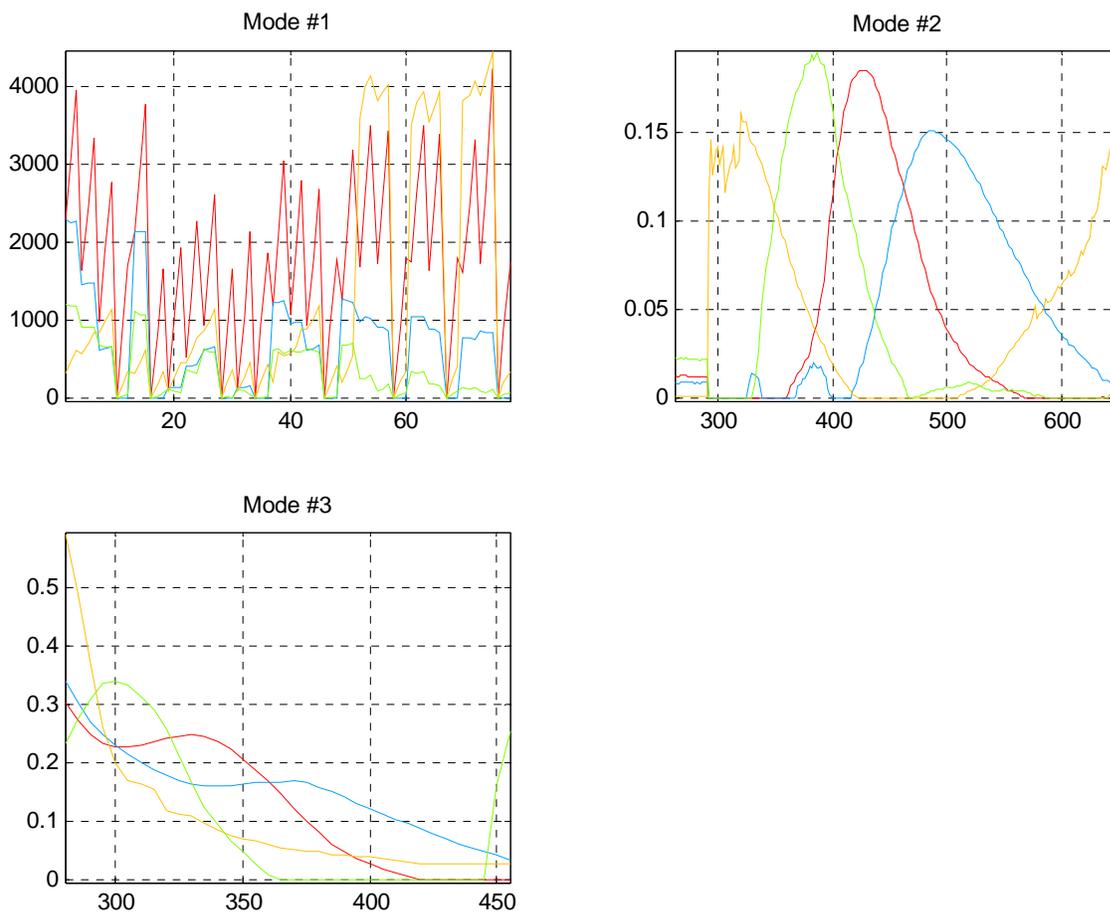


Figure 11. Model 1 - PARAFAC analysis and the four fluorescent factors determined on the laboratory dilution matrix and mixed detergent spikes (1-red, 2-blue, 3-yellow, 4-green). Input data from 280-455ex/262-650em.

PARAFAC Factor 1 (PFAC1) of Model 1 was characterized by an excitation peak near 325-335 nm, an emission maxima of 424-430 nm, and was the most representative of the expected fluorescence of OB. Initial examination of PFAC1 indicated a slight dependence on absorption at 400 nm (a_{400}), indicating a possible incomplete separation of CDOM and OB in the computed factors. It should be kept in mind, however, that the samples as a group, as mixtures of only four source waters, were not truly independent. There was no significant correlation of PFAC1 with a_{400} if only the four source waters were considered. The results are consistent with the presence of OB in the OSTDS and WWTP waters (expected) and the presence of OB in the high CDOM water (likely, see below).

PFAC2 of Model 1 was characteristic of CDOM only, with excitation maxima <290nm and a small shoulder near 370 nm and emission at 484-488 nm. Using the independent samples only ($n=4$), PFAC2 was correlated with a_{400} and displayed little response to added OB. For PFAC3, there were no discrete peaks captured. Maximum excitation for PFAC3 was less than 290 nm, with maximum emission ranges both less than 325 nm and greater than 640 nm. The low wavelength end of PFAC3 captured some residual portion of the OB UV peak discussed above. PFAC3 levels were uncorrelated with a_{400} and were most notable in the ODTDS samples and mixtures (which are also assumed to contain more OB) with a lesser amount present in the WWTP samples. Due to truncation of input data, the high wavelength end of PFAC3 should no longer be affected by the tertiary Rayleigh emissions discussed for the high particulate samples. Due to the noise associated with the near-UV region and the generally lower amplitude of PFAC3, PFAC1 was preferable to quantitatively evaluate OB.

PFAC4 of Model 1 had an excitation shoulder near 295-305 nm and emission near 382-386 nm, was prevalent in the high CDOM waters, and was also present in the WWTP samples. PFAC4 was most closely related to CDOM fluorescence characterized in proteinaceous materials, eutrophic marine waters high in algae, or to Peak “M” designated by a number of authors (Coble, 1999). The few independent samples were not correlated with a_{400} and the peak was not observed in any of the individual detergents or the detergent mixture. The levels found of all factors are plotted in Figure 16 in the same case order as appears in **Table 2**. All samples are followed by the two spikes (~50% and ~100% of typical OSTDS concentrations) to permit visualization of the factors which did and did not respond to OB additions.

Using the PFAC1 and the mean loading of 1746 QSRF for the 101% OSTDS effluent level spike in laboratory deionized water, recovery of all spikes were calculated. Spike recovery ranged between 94% and 110% and averaged 100.9% for all source waters and source water mixtures (**Figure 17**). Agreement among spikes is excellent for all sample matrices. The recoveries of PFAC3 (**Figure 18**), both between and within sample matrices, was less reproducible for the reasons discussed above.

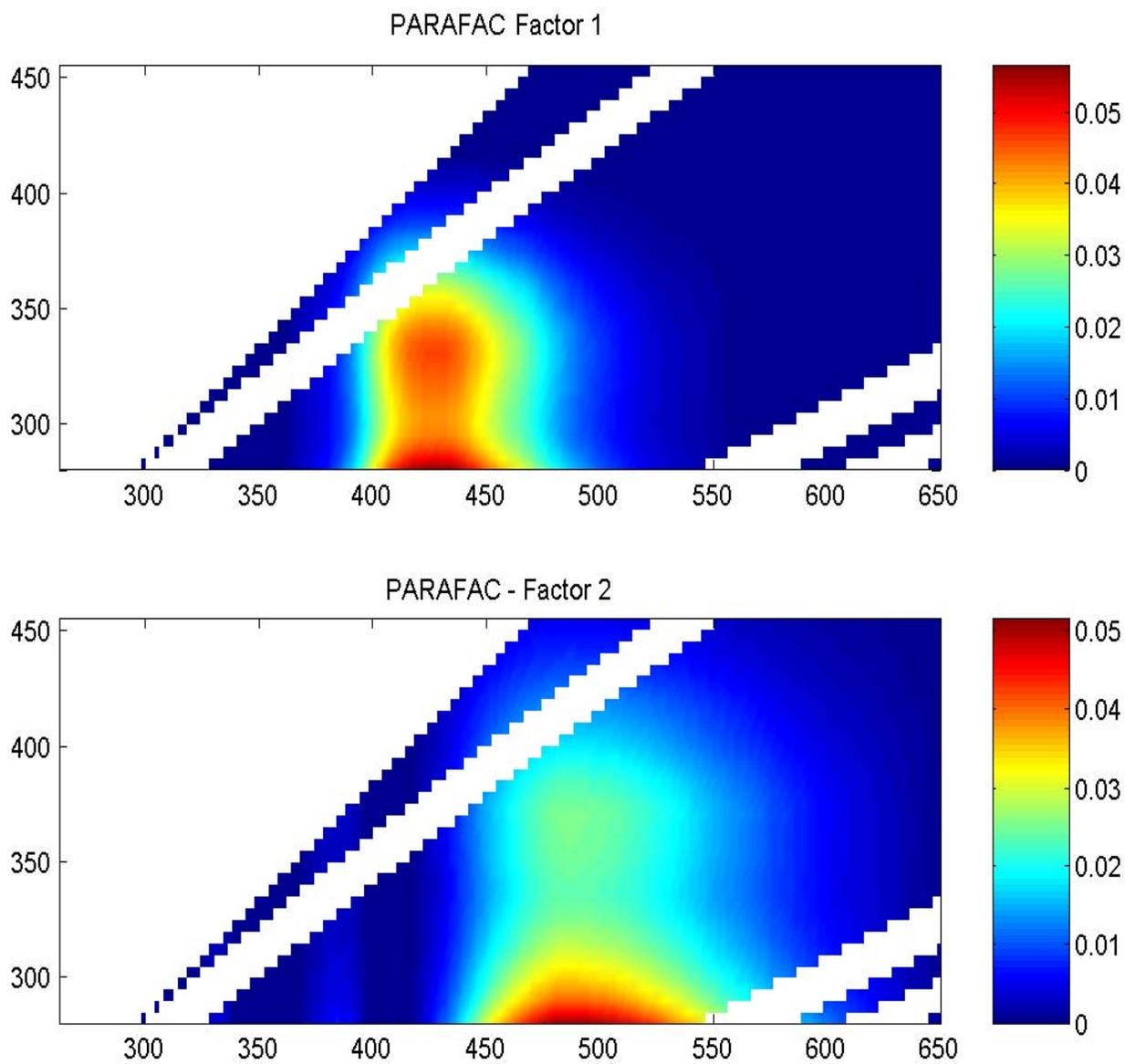


Figure 12. Model 1 - PARAFAC component factors (PFAC1 and PFAC2) determined from the laboratory dilution matrix and mixed detergent spikes.

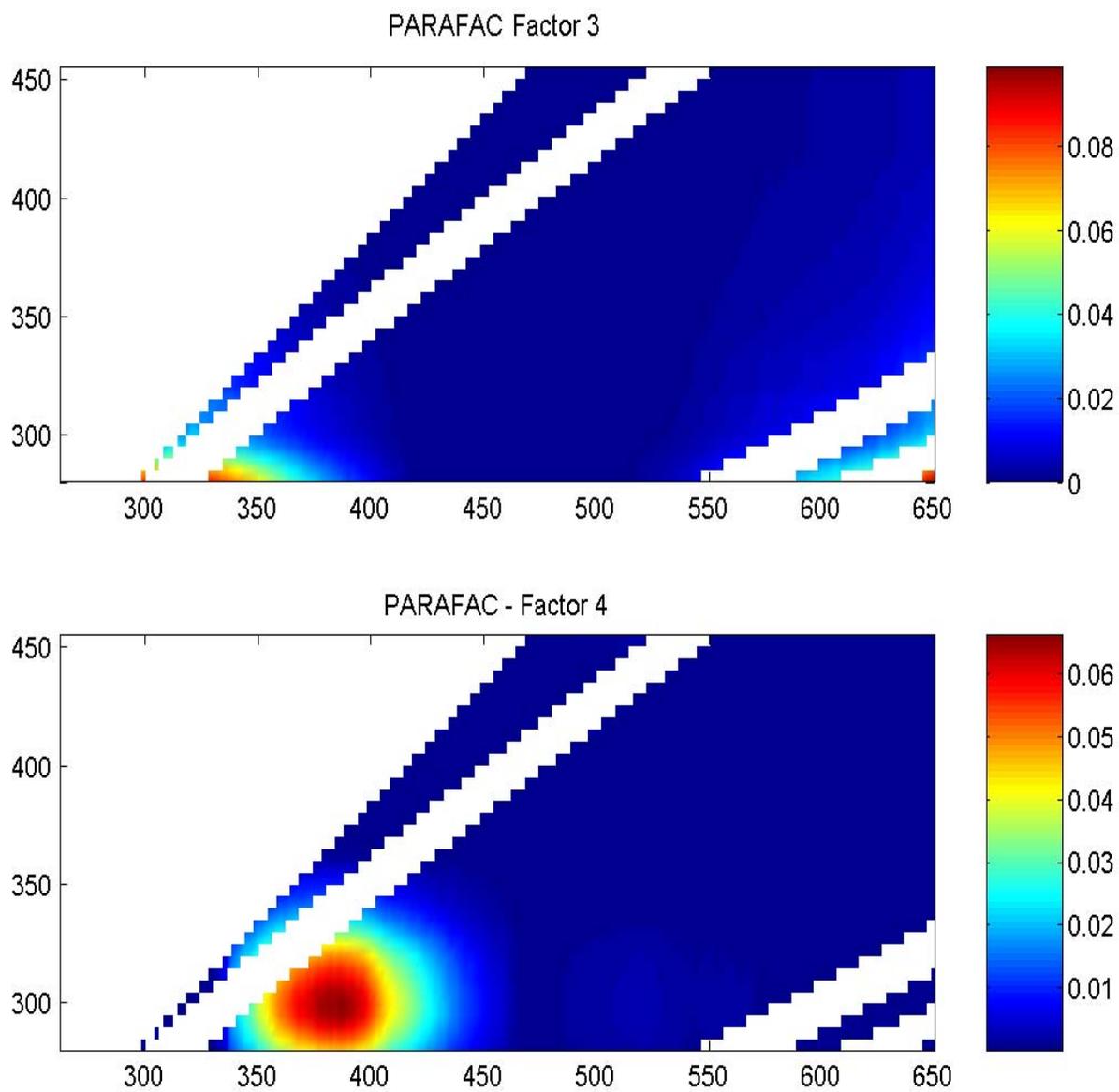


Figure 13. Model 1 - PARAFAC component factors (PFAC3 and PFAC4) determined from the laboratory dilution matrix and mixed detergent spikes.

Table 2. Results of Model 1 PARAFAC analysis on dilution matrix of various source waters. Values are units of factors. Spikes were at 50.5% (1/2SPK) and at 101% (SPK) of expected OSTDS concentrations using mixed detergents (see text). Recovery is the spiked fluorescence less the unspiked sample.

Sample	PFAC1	PFAC2	PFAC3	PFAC4	PFAC1 Recovery	PFAC3 Recovery
HI CDOM	2298	2302	308	1190	0	0
HI CDOM+1/2 SPK	3088	2255	474	1171	790	166
HI CDOM+SPK	3955	2264	603	1166	1657	295
HI CDOM +WWTP	1627	1457	552	908	0	0
HI CDOM+WWTP+1/2SPK	2475	1462	693	904	848	141
HI CDOM+WWTP+SPK	3347	1471	844	894	1721	291
WWTP	976	610	833	663	0	0
WWTP+1/2 SPK	1854	621	978	661	878	145
WWTP+SPK	2761	644	1124	661	1785	291
DI	0	0	0	0	0	0
DI+1/2SPK	885	0	156	17	885	156
DI+SPK	1710	0	338	38	1710	338
HI CDOM	2124	2123	304	1100	0	0
HI CDOM+1/2 SPK	2921	2131	443	1069	797	139
HI CDOM+SPK	3768	2136	607	1064	1644	303
DI	0	0	0	0	0	0
DI+1/2SPK	841	0	181	24	841	181
DI+SPK	1659	0	343	52	1659	343
LO CDOM	124	118	84	96	0	0
LO CDOM+1/2 SPK	976	124	239	73	852	155
LO CDOM+SPK	1916	125	442	64	1791	359
LO CDOM+WWTP	512	395	447	354	0	0
LO CDOM+WWTP+1/2SPK	1307	411	589	338	795	142
LO CDOM+WWTP +SPK	2263	423	755	309	1751	308
WWTP	919	601	862	617	0	0
WWTP+1/2 SPK	1767	625	979	586	848	117
WWTP+SPK	2621	658	1138	580	1702	277
DI	0	0	0	0	0	0
DI+1/2SPK	815	0	191	7	815	191
DI+SPK	1644	0	349	0	1644	349
LO CDOM	136	128	82	105	0	0
LO CDOM+1/2 SPK	1049	134	257	81	913	175
LO CDOM+SPK	2135	150	456	44	1999	374
DI	0	0	0	0	0	0
DI+1/2SPK	927	0	190	0	927	190
DI+SPK	1868	0	419	18	1868	419
MCDOM	1164	1223	203	595	0	0
MCDOM+1/2 SPK	2066	1215	588	622	901	385
MCDOM+SPK	3035	1232	544	549	1870	341

Table 2. Results of PARAFAC analysis on dilution matrix of various source waters. Values are units of factors. Spikes were at 50.5% (1/2SPK) and at 101% (SPK) of expected OSTDS concentrations using mixed detergents (see text). Recovery is the spiked fluorescence less the unspiked sample. (Continued.)

Sample	PFAC1	PFAC2	PFAC3	PFAC4	PFAC1 Recovery	PFAC3 Recovery
MCDOM+WWTP	1063	938	567	614	0	0
MCDOM+WWTP +1/2 SPK	1903	959	653	591	840	86
MCDOM+WWTP +SPK	2788	973	871	589	1725	304
WWTP	930	607	881	627	0	0
WWTP+1/2 SPK	1780	626	1008	601	850	127
WWTP+SPK	2669	674	1168	586	1739	287
DI	0	0	0	0	0	0
DI+1/2SPK	874	0	148	0	874	148
DI+SPK	1768	0	395	11	1768	395
MCDOM	1264	1270	187	680	0	0
MCDOM+1/2 SPK	2175	1247	350	682	911	163
MCDOM+SPK	3182	1213	539	691	1918	352
(MCDOM+OSTDS)1:4DIL	1667	971	3584	250	0	0
(MCDOM+OSTDS)1:4DIL1/2SPK	2620	1030	3993	240	953	409
(MCDOM+OSTDS)1:4DIL+SPK	3498	1016	4134	295	1831	550
OSTDS 1:8 DIL	1714	897	3830	84	0	0
OSTDS 1:8 DIL+1/2SPK	2583	893	3929	97	870	99
OSTDS 1:8 DIL+SPK	3425	860	4015	172	1711	185
DI	0	0	0	0	0	0
DI+1/2SPK	918	0	209	44	918	209
DI+SPK	1793	0	336	62	1793	336
(HICDOM+OSTDS)1:4DIL	1739	1029	3499	337	0	0
(HICDOM+OSTDS)1:4DIL+1/2S	2639	1034	3798	314	900	299
(HICDOM+OSTDS)1:4DIL+SPK	3510	1027	3936	323	1771	437
OSTDS 1:8 DIL	1627	878	3536	170	0	0
OSTDS 1:8 DIL+1/2 SPK	2485	872	3723	177	858	187
OSTDS 1:8 DIL+SPK	3377	838	3941	148	1750	405
DI	0	0	0	0	0	0
DI+1/2SPK	879	0	219	41	879	219
DI+SPK	1791	0	402	89	1791	402
(LOCDOM+OSTDS)1:4DIL	1600	773	3827	116	0	0
(LOCDOM+OSTDS)1:4DIL+1/2S	2474	755	3877	111	873	49
(LOCDOM+OSTDS)1:4DIL+SPK	3324	745	4072	135	1724	245
OSTDS 1:8 DIL	1713	857	3893	87	0	0
OSTDS 1:8 DIL+1/2 SPK	2642	843	4105	63	928	211
OSTDS 1:8DIL+SPK*	4222	836	4424	93	2509	531
DI	0	0	0	0	0	0
DI+1/2SPK	871	0	192	36	871	192
DI+SPK	1737	0	342	67	1737	342

* Spike was at 143% of expected OSTDS concentration

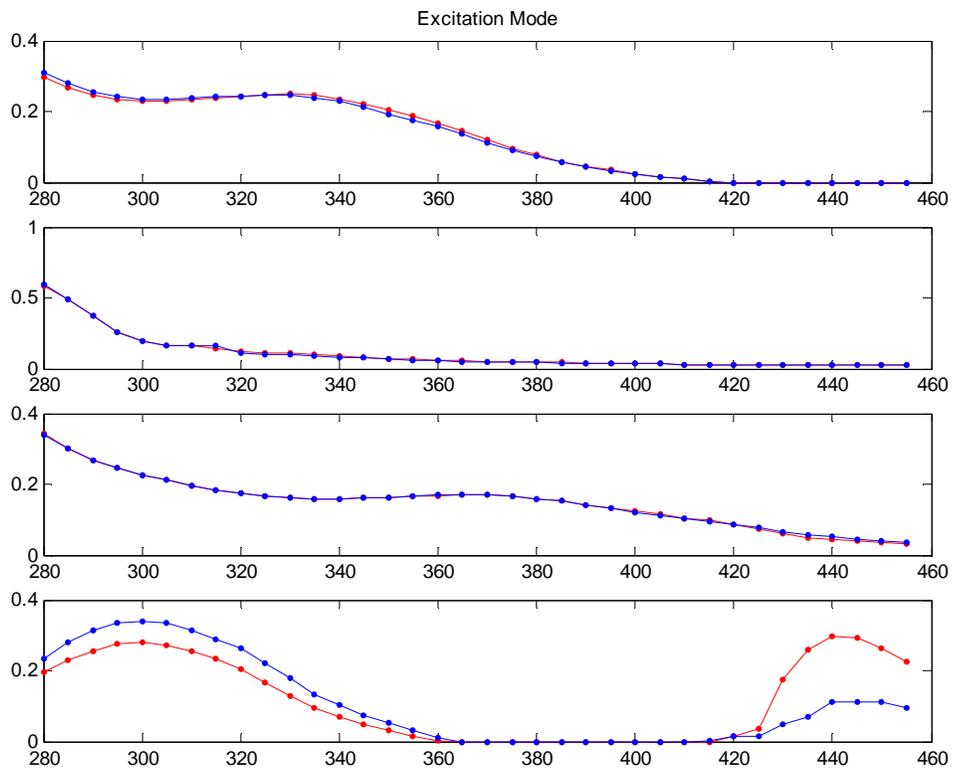


Figure 14. Results of split half analysis for Model 1 showing similarity of independently derived excitation modes.

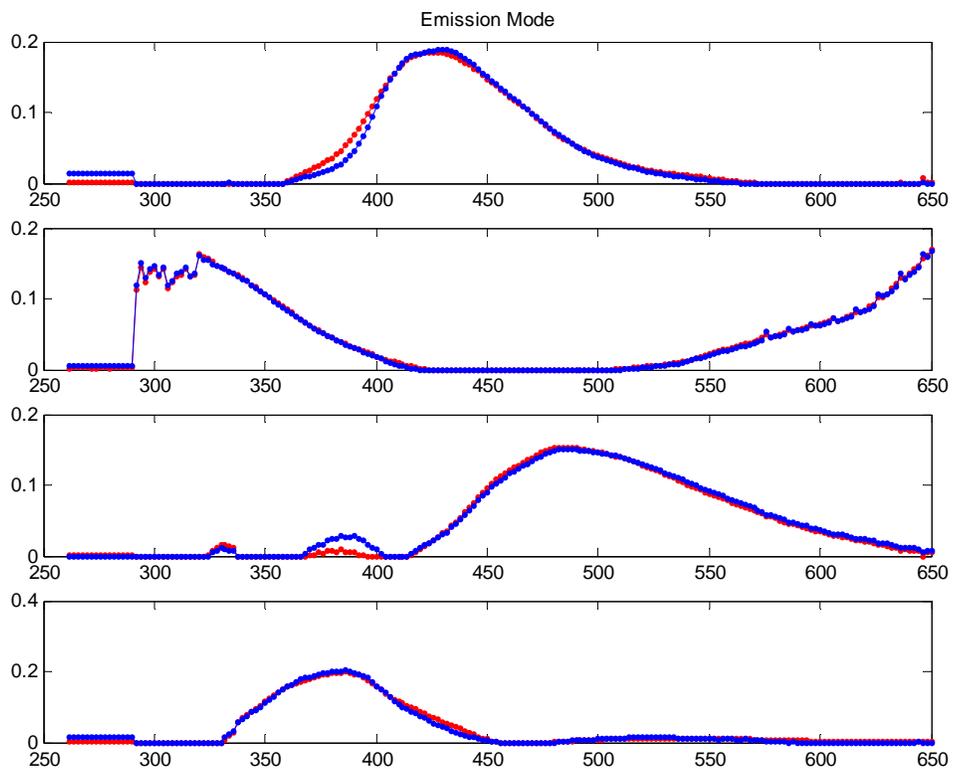


Figure 15. Results of split half analysis for Model 1 showing similarity of independently derived emission modes.

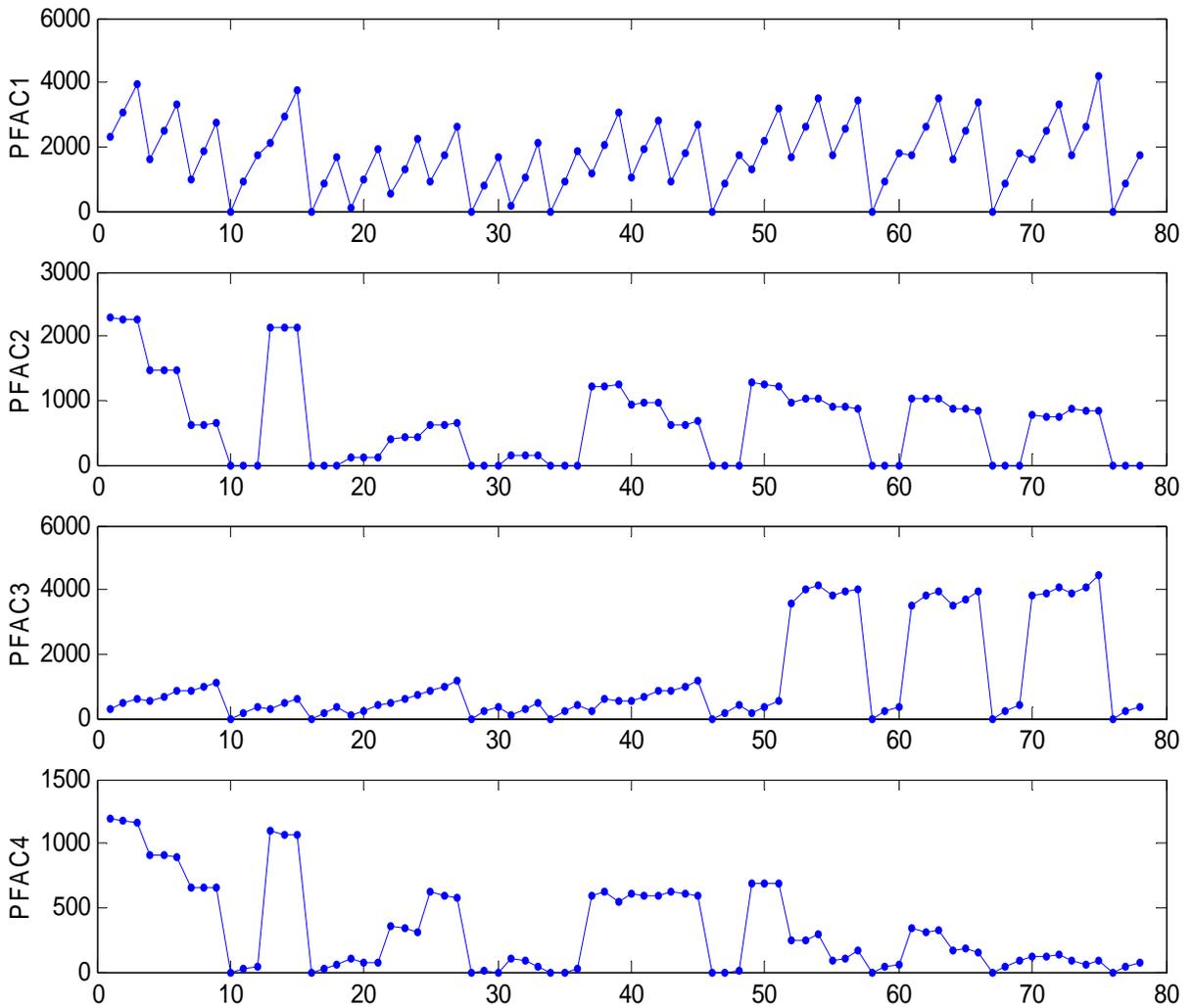


Figure 16. Loading of Model 1 PARAFAC factors from 4-factor model developed on laboratory dilution matrix, from 280-455ex/262-650em. Sample identity is in case order as listed in Table 2. Each sample is followed by a 50% and 100% spike of typical OSTDS concentrations of mixed detergents. PFAC1 and PFAC3 are indicative of OB.

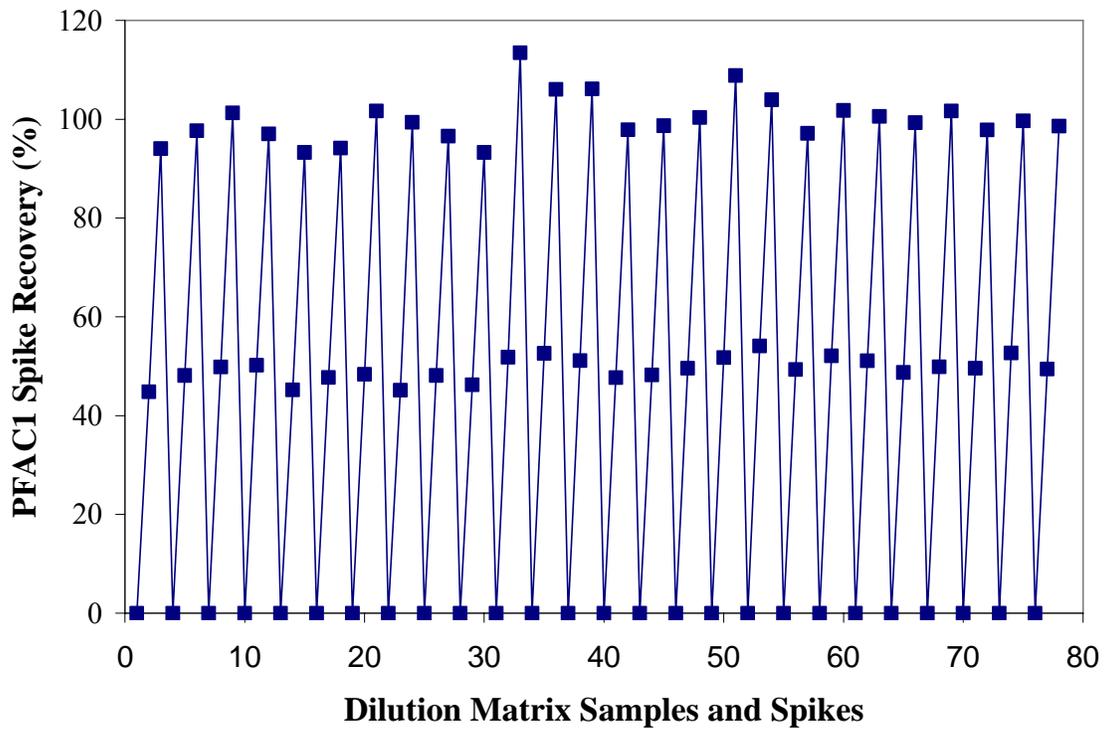


Figure 17. Recoveries of all spikes in all laboratory dilution matrices, calculated from the Model 1 PARAFAC derived amounts of OB-PFAC1 (see text) relative to amounts in deionized water.

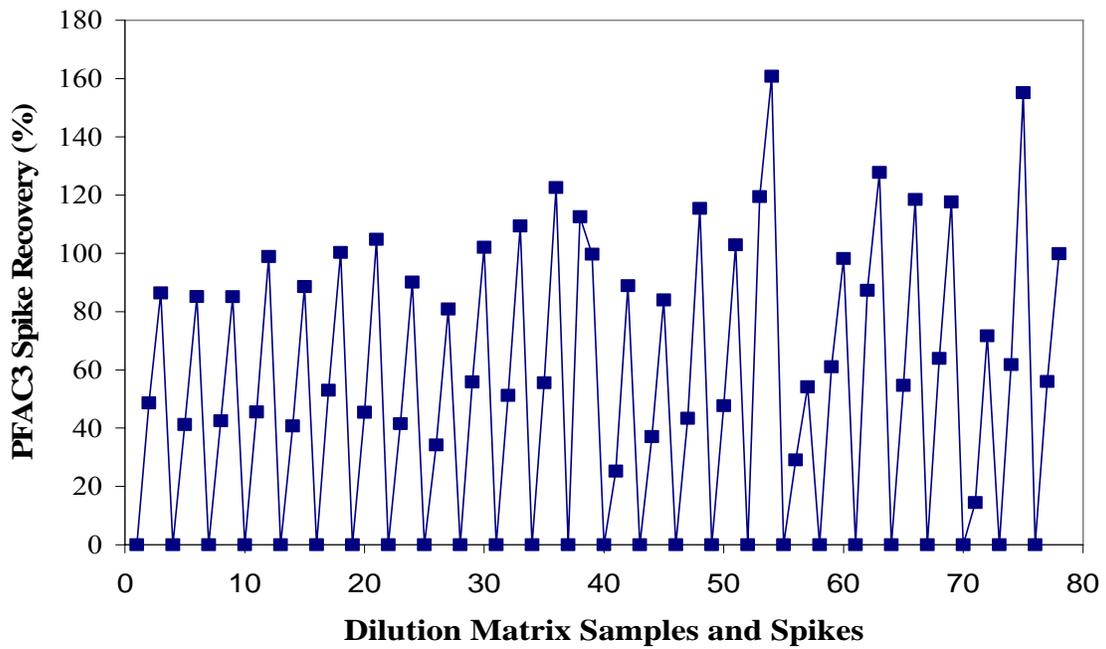


Figure 18. Recoveries of all spikes in all laboratory dilution matrices, calculated from the Model 1 PARAFAC derived amounts of OB-PFAC3 (see text) relative to amounts in deionized water.

Review of the unspiked data from the dilution matrix was interesting as well. The raw values of Model 1 PFAC1 were used to estimate OSTDS influence in the source waters used for the preparation of the matrix (**Table 3**). After accounting for dilution and using 1746 (the mean loadings in laboratory DI water) as indicative of 101% typical OSTDS effluent, OB in the source waters of low CDOM, high CDOM, WWTP, and OSTDS were calculated as percentages of typical OSTDS effluent. These preliminary estimates of OB amounts in high and low CDOM, and the WWTP and the OSTDS samples averaged 128%, 8%, 54% and 773%, respectively, of the typical OSTDS effluent.

The low CDOM water was collected near MML where all nearby residences are served by City/County waste treatment collection system. Salinity was also high indicating minimal freshwater influence at the time although the occasional discharge from the City of Sarasota WWTP occurs to Whitaker Bayou, located across Sarasota Bay and within 3.5 km of the sampling location. Nevertheless, 8% of typical OSTDS levels for this site appears extreme.

The high CDOM water, on the other hand, was collected from a tributary to the Myakka River. The tributary, Myakkahatchee Creek, drains a watershed heavily served by OSTDS (**Figure 19**) and so the presence of OB is not surprising. Again, however, 12% of typical OSDTS effluent appear extreme. The tertiary treatment WWTP sample, with PFAC1-OB at 54% of typical OSTDS effluent, was also not surprising. Collection of WWTP influent would allow an assessment of the amount of OB removed during the various treatment processes. Most surprising were the high levels of OB detected in the OSTDS sample. At over 800% of expected typical OSTDS effluent it was clear that detergent choice and laundry habits could substantially affect the amounts of OB that might reach surface waters even when the volumes of effluent are comparable.

Using Model 1 PFAC3 values to estimate OSTDS influence (**Table 3**), however, generated values many times higher for any samples containing OSTDS and WWTP fractions. Lack of concurrence between these two evaluations urged caution before applying this model to further samples.

Table 3. Comparison of preliminary estimates of OB detected in source waters (grey) and mixtures used for the laboratory dilution matrix, based on Model 1 PFAC1 and PFAC 3 values, corrected for dilution, and referenced to expected OB in typical OSTDS effluent. Wide range in between PFAC results indicates a questionable model.

Sample	PFAC1	PFAC3	%"Typical" OSTDS PFAC1	%"Typical" OSTDS PFAC3
LO CDOM	124	84	7	23
LO CDOM	136	82	8	23
LO CDOM+WWTP	512	447	30	124
MCDOM	1164	203	67	56
MCDOM	1264	187	73	52
MCDOM+WWTP	1063	567	62	157
HI CDOM	2298	308	133	85
HI CDOM	2124	304	123	84
HI CDOM +WWTP	1627	552	94	153
WWTP	976	833	56	231
WWTP	919	862	53	238
WWTP	930	881	54	244
(MCDOM+OSTDS)1:4DIL	1667	3584	386	3967
(HICDOM+OSTDS)1:4DIL	1739	3499	402	3873
(LOCDOM+OSTDS)1:4DIL	1600	3827	370	4236
OSTDS 1:8 DIL	1714	3830	793	8478
OSTDS 1:8 DIL	1627	3536	753	7827
OSTDS 1:8 DIL	1713	3893	793	8618

Individual Detergents

Initial review of matrix results indicated several discrete peaks that were associated with the detergent mixture spikes (**Figure 10**, above). The EEM data from individual detergents (**Figures 20 and 21**) illustrated the range in manufacturer formulations, both in compounds and in relative fluorescence of amounts of OB. Note the scale change and relatively low fluorescence of Detergent D, for example. While all detergents with OB fluoresced in the 400-450 nm region, some had additional peaks below 350 nm emission with <300 nm excitation. Based a PARAFAC analysis of individual detergents alone (results not shown) there were at least three different OB complexes, one of which was similar to the OB standard, DSBP. The individual detergents varied between 0.02 and 4.6 times the amount of DSBP as that contained in the detergent mixture. Most detergents with little DSBP appeared to contain comparably fluorescing amounts of other compounds, while some were mixtures of DSBP and the other factors. At least two detergents tested had little detectable OB.

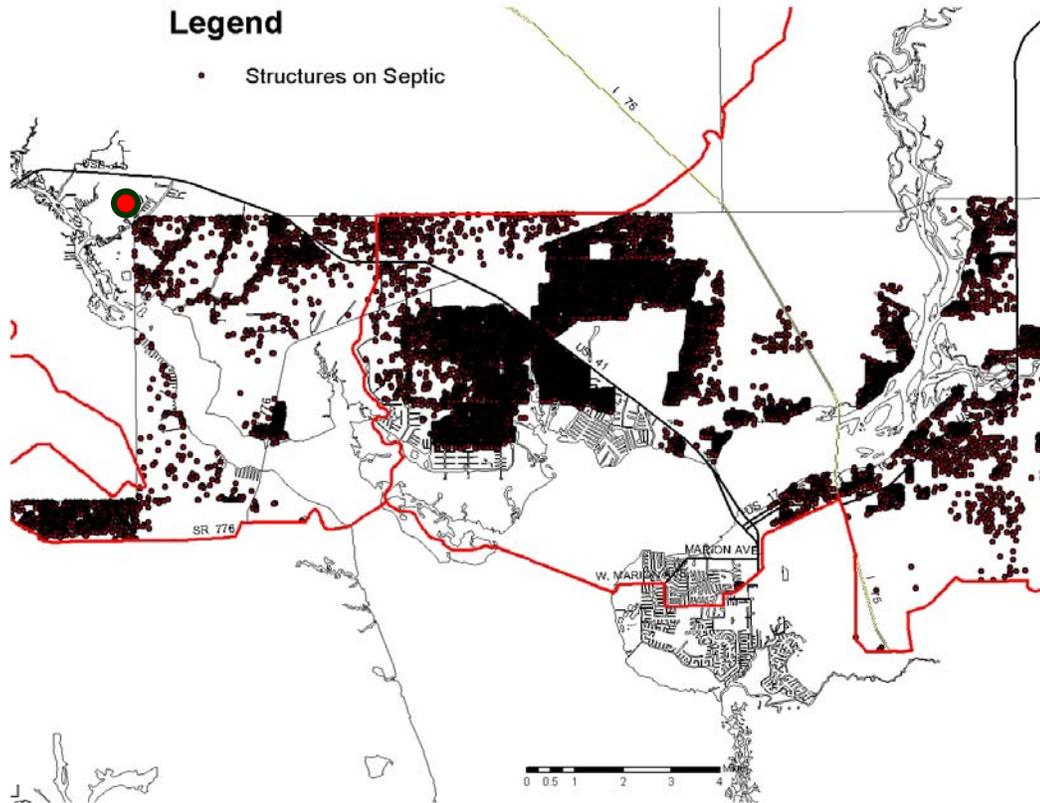


Figure 19. Charlotte County OSTDS locations within the Myakka and lower Peace River basins. Dot in upper left indicates collection location of high CDOM water.

The PARAFAC factor 1 determined during the analysis of the laboratory dilution matrix was the EEM of the standard detergent mixture which resulted from the mixing of equal aliquots of all detergents obtained. The three dimensional shape of the PFAC1 was strictly the product of the relative OB contained in each detergent used and the mixture that was prepared. A detergent-specific or an individual OB-specific PARAFAC analysis would be possible given more samples of individual detergents, however, the utility and application of the information to management actions is questionable. These points highlight the difficulty of determining quantitative environmental impacts when the loadings and the identity of the tracer (the specific OB complex) varies.

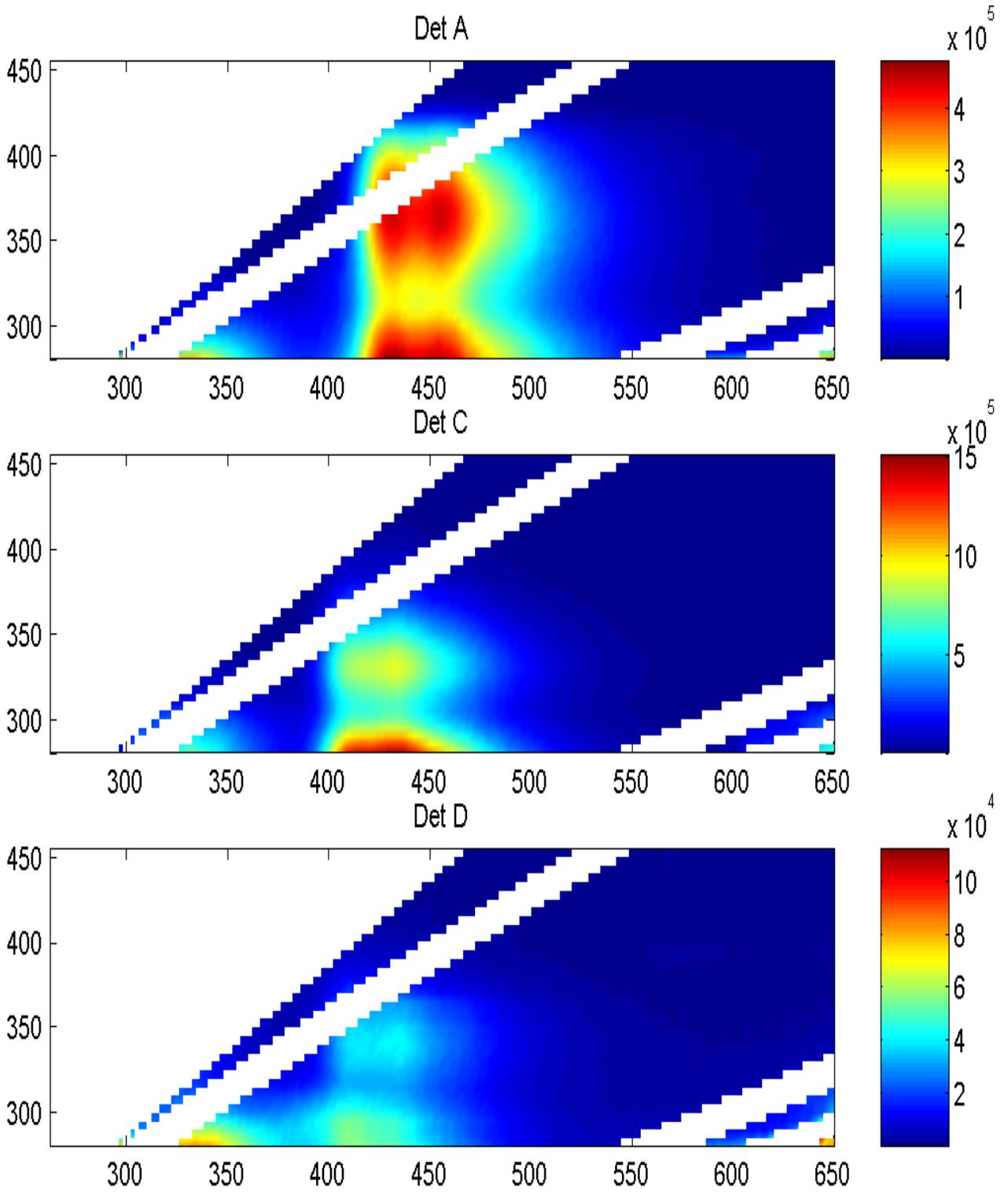


Figure 20. EEM characterization of selected individual detergents. Note scale changes. All are at approximately 50% of expected OSTDS concentrations.

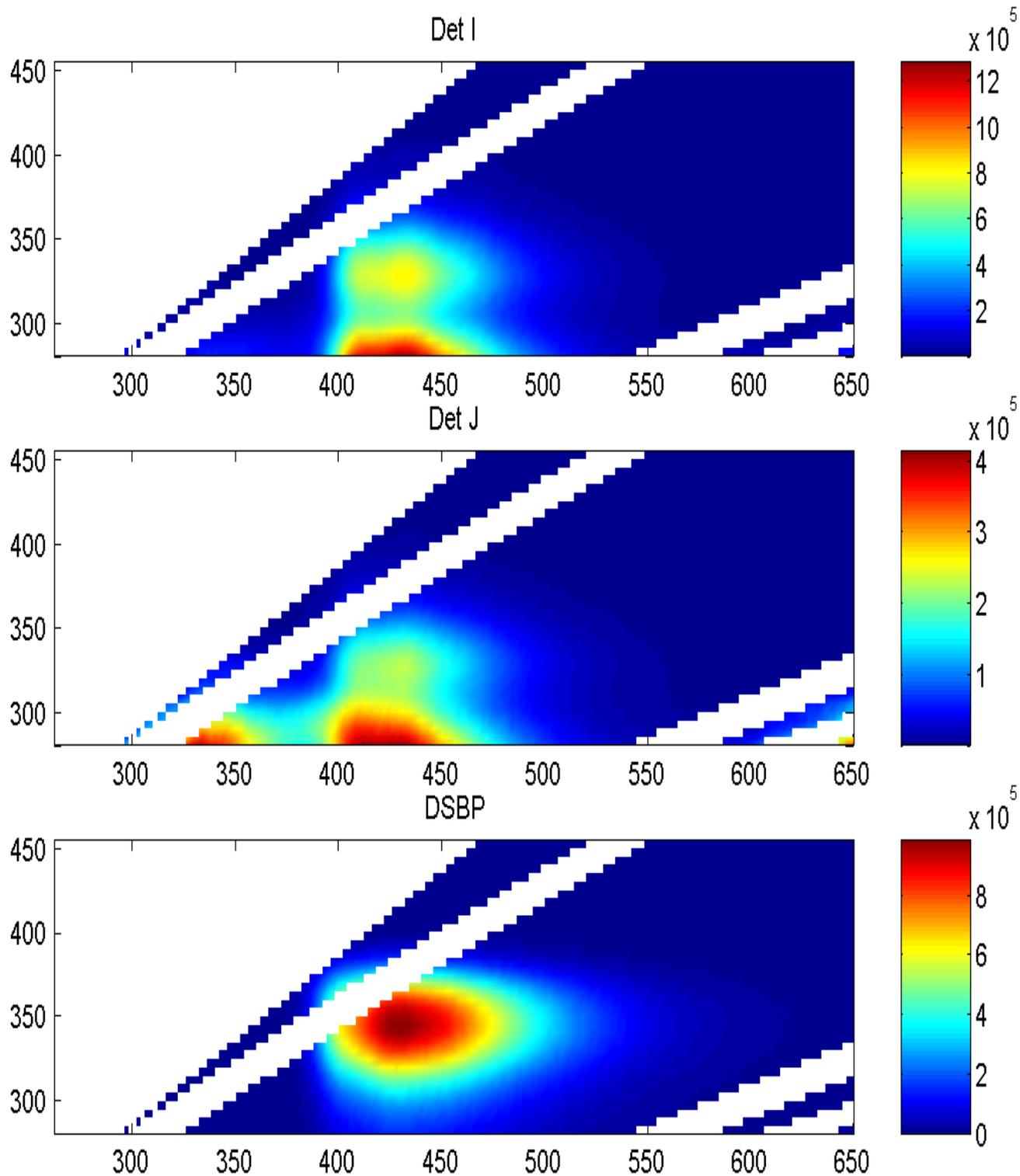


Figure 21. EEM characterization of selected individual detergents. Note scale changes. All are at approximately 50% of expected OSTDS concentrations.

Regions of Interest

PARAFAC modeling was also conducted on the ambient samples collected from the four regions of interest, the OSTDS and WWTP samples, and a few selected samples from the dilution matrix to evaluate model performance. The initial model used EEM factors developed only from the laboratory dilution matrix (Model 1) in which a few ambient samples were combined in a variety of mixtures and enhanced with a standardized detergent mixture. The factors so identified and discussed above (**Figure 12 and 13**) were applied to the ambient samples without further adjustment. A second model (Model 2) was also prepared in which new factors were identified using only the ambient samples plus a 15 selected samples and detergent spikes from the laboratory dilution matrix as input data. As the input data between the models differed, the amounts of variance attributed to individual factors and subsequent factor ranking would be expected to differ, while derived spectral shapes should be similar if EEM factors found in Model 1 represented consistent fluorescent phenomena.

Figures 22 and 23 illustrates the agreement between the two models' factors and indicate that same general peaks appear in both the laboratory mixtures and detergent spikes and in the ambient samples. Excitation and emission maxima developed predominantly from the ambient samples (Model 2 - blue) are generally shifted slightly to longer wavelengths relative to the matrix derived factors (Model 1 – red). Other than for the OSTDS samples, the results of estimated OB concentrations derived between the two models was relatively consistent, although the sample-specific Model 2 factors estimating about 30% higher concentrations of OB than the matrix-specific Model 1 factors.

Unfortunately, however, the loads for the factors most responsive to OB concentration in these two models were generally well correlated with either the factor load representing CDOM or with a_{400} . In a small estuarine system, where freshwater, OB, and CDOM sources all have a common source and where dilution of freshwaters with low CDOM, low OB saline waters is occurring, then a correlation of OB with CDOM as developed from PARAFAC factors might be expected in samples collected across the resulting gradient. In a system with multiple sources of CDOM, or OB, or where the samples do not necessarily represent sampling across a linear gradient, then the correlation of the two factor loadings is less tenable. When data from a number of different regions are combined, then correlation of true OB and CDOM are highly unlikely and levels of computed factors should be viewed with great caution.

The primary factor loadings for OB and CDOM in both models were reviewed and indicated that both overall and by region sampled, nearly all were significantly correlated. The secondary OB factor (PFAC3 in **Figure 11**), with UV excitation and both a long and a short wavelengths emission, was generally uncorrelated with either CDOM factors or with a_{400} . The fact that the maximum UV peak was not entirely captured in the input data and that the lowest excitation was in a region of rapid change for this peak made quantification from the PFAC3 estimates uncertain.

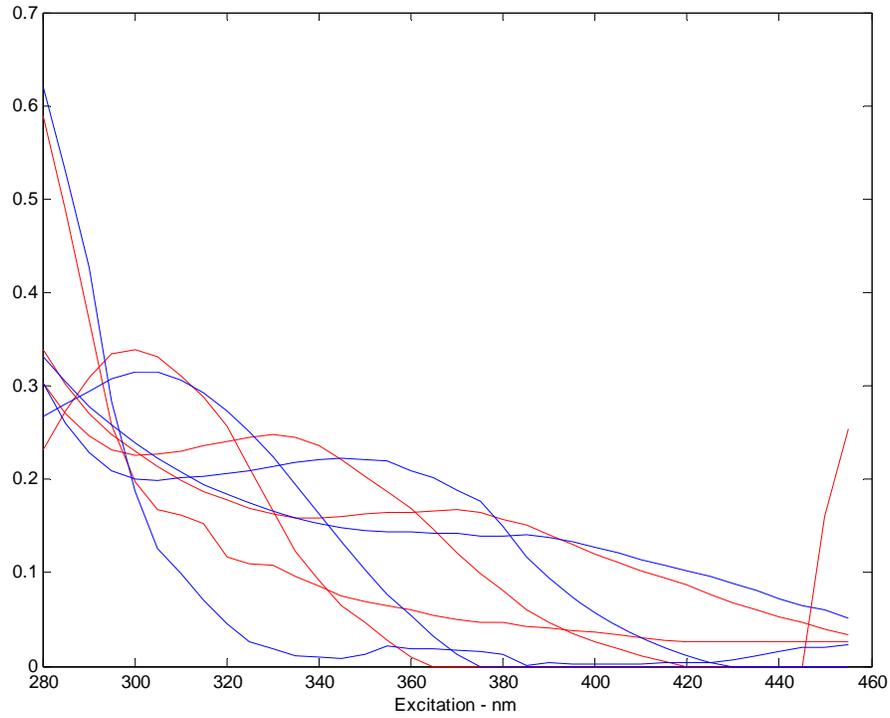


Figure 22. Excitation factors for Model 1 (red) and Model 2 (blue), developed from dilution matrix and ambient samples, respectively.

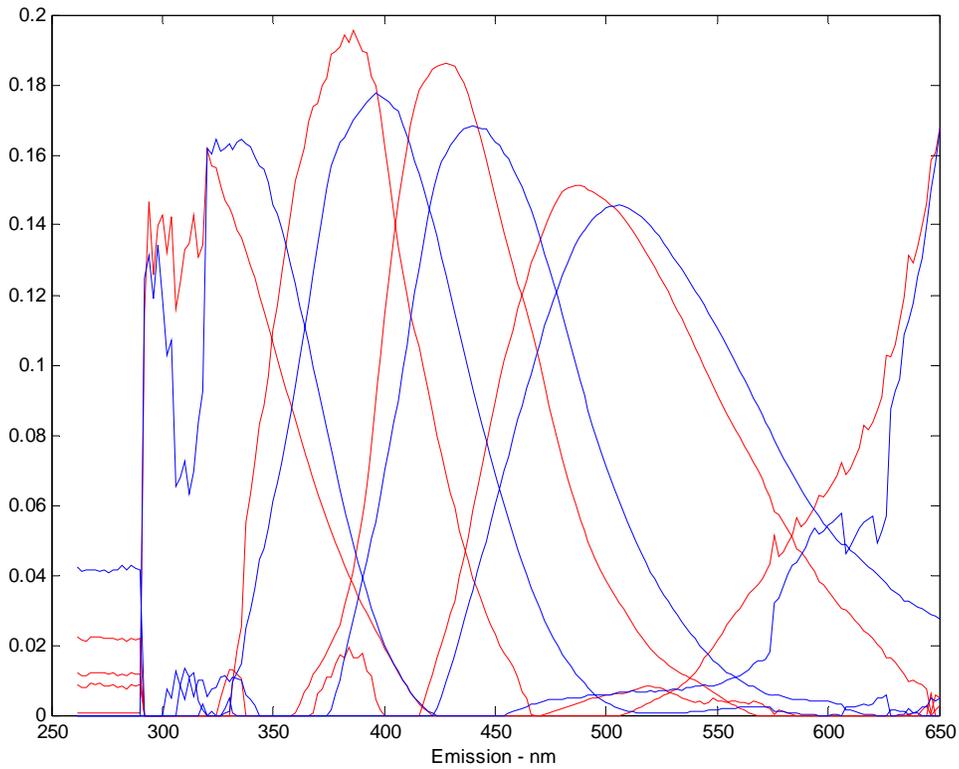


Figure 23. Emission factors for Model 1 (red) and Model 2 (blue), developed from dilution matrix and ambient samples, respectively.

Accordingly a variety of PARAFAC models were prepared that expanded the input data to 230-455ex/256-650em to recapture the UV peak of OB. Models were developed using either combined dilution matrix and ambient samples, or all samples with the exception of the full strength WWTP samples and any sample containing OSTDS waters. (The OSTDS samples were very non-characteristic of all other ambient samples, containing exceptionally high and broad fluorescence levels with multiple discrete peaks superimposed. Eliminating these samples permitted the modeling solution to emphasize the resolution of regional variation in CDOM and known OB regions of fluorescence.)

Valid models of three, four and five factors were examined for each of the two data inputs. Results appear in **Figures 24-28** as Models 3, 4, 5, 6, and 7. (The five factor model of data without OSTDS samples was not significant.) Noteworthy was that the same UV peak of OB was captured as a discrete peak in each of the models, although the peak assignment of importance (whether it was PFAC1 or PFAC2, etc.) varied among models. All UV OB peaks also had a smaller feature at 580 nm emission and a likely noise feature near 450 nm.

The number of factors in the resultant model determined whether separate peaks were indicated for the 400 nm emission of OB and the ~475 nm emission of CDOM, with lower factor models (Models 3, 4, and 6) providing a single blended peak for both elements. When OSTDS samples were included in the input data and when factors were 4 or greater (Models 4 and 5), a characteristic multimodal emission profile for OSTDS was identified. All models identified characteristic WWTP/OSTDS emission peaks near 325 nm and at greater than 620 nm, consistent with high levels of proteinaceous materials. **Figures 29-33** illustrate the factor loads (for the matrix samples only) to demonstrate the relative response of all factors to OB. **Table 4** summarizes factors found in the models and the identity of samples elements (CDOM, OB, etc.) with factors found. **Figure 34** and **35** illustrate the pseudo-3D representation of the factors identified for Model 7 as an example. The factors for identified for OB were evaluated both against a_{400} and against factors for CDOM, by region and for all ambient samples, and found to be generally uncorrelated with CDOM.

Table 4. PARAFAC model parameters, data used to develop models, and sample element associations of factors identified.

Model	Factors	Input Data	PFAC1	PFAC2	PFAC3	PFAC4	PFAC5
3	3	All	CDOM+OB	OSTDS/WWTP	OB(UV)*		
4	4	All	CDOM+OB	OSTDS/WWTP	OB(UV)*	OSTDS	
5	5	All	CDOM(475)+OB	OB(400)+CDOM	OSTDS/WWTP	OB(UV)*	OSTDS
6	3	w/out OSTDS, 100%WWTP	CDOM+OB	OB(UV)	WWTP		
7	4	w/out OSTDS, 100%WWTP	CDOM(475)+OB	OB(400)+CDOM	OB(UV)	WWTP	

* Depressed response in OSTDS matrix

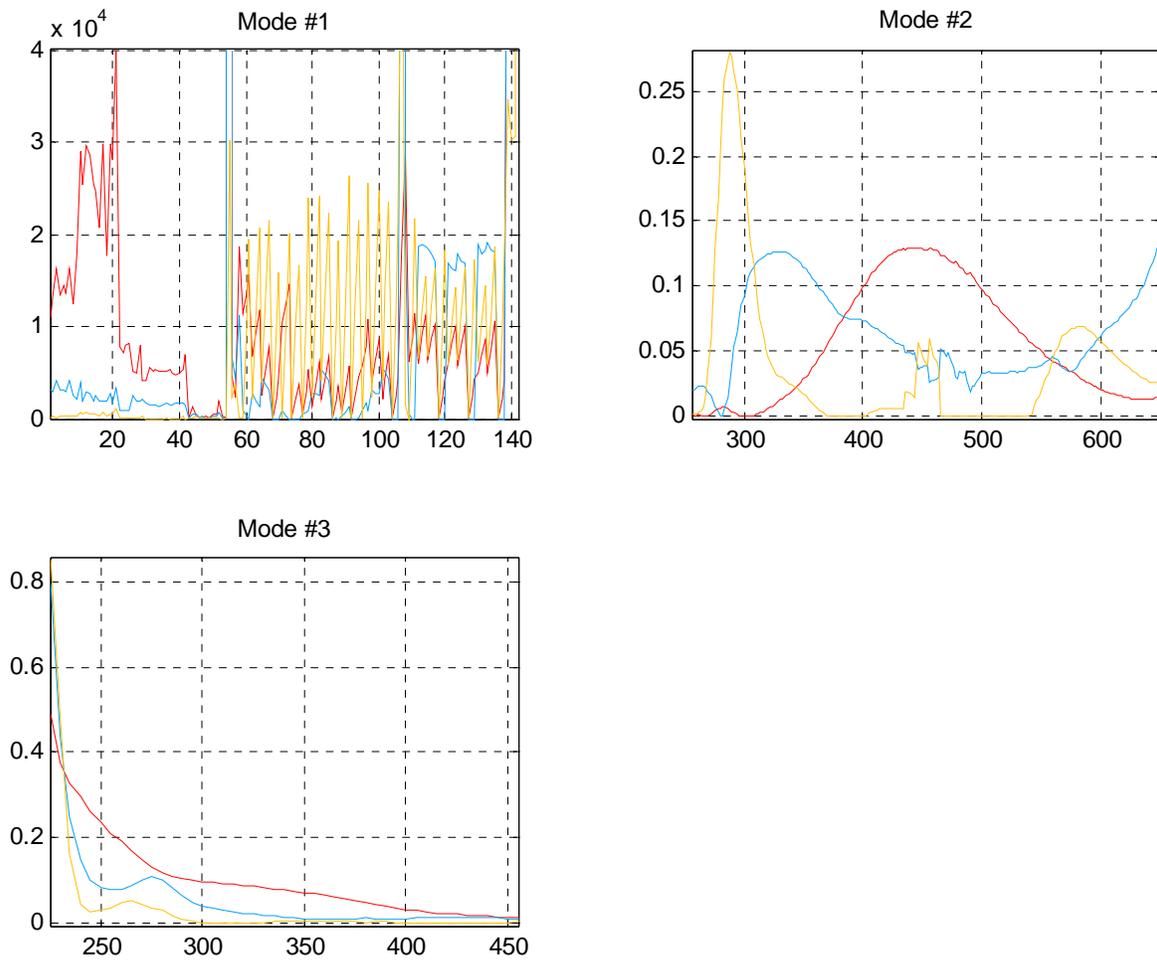


Figure 24. PARAFAC analysis and the three fluorescent factors determined on the combined ambient samples and laboratory dilution matrix and mixed detergent spikes (1-red, 2-light blue, 3-yellow). Input data from 230-455ex/256-650em.

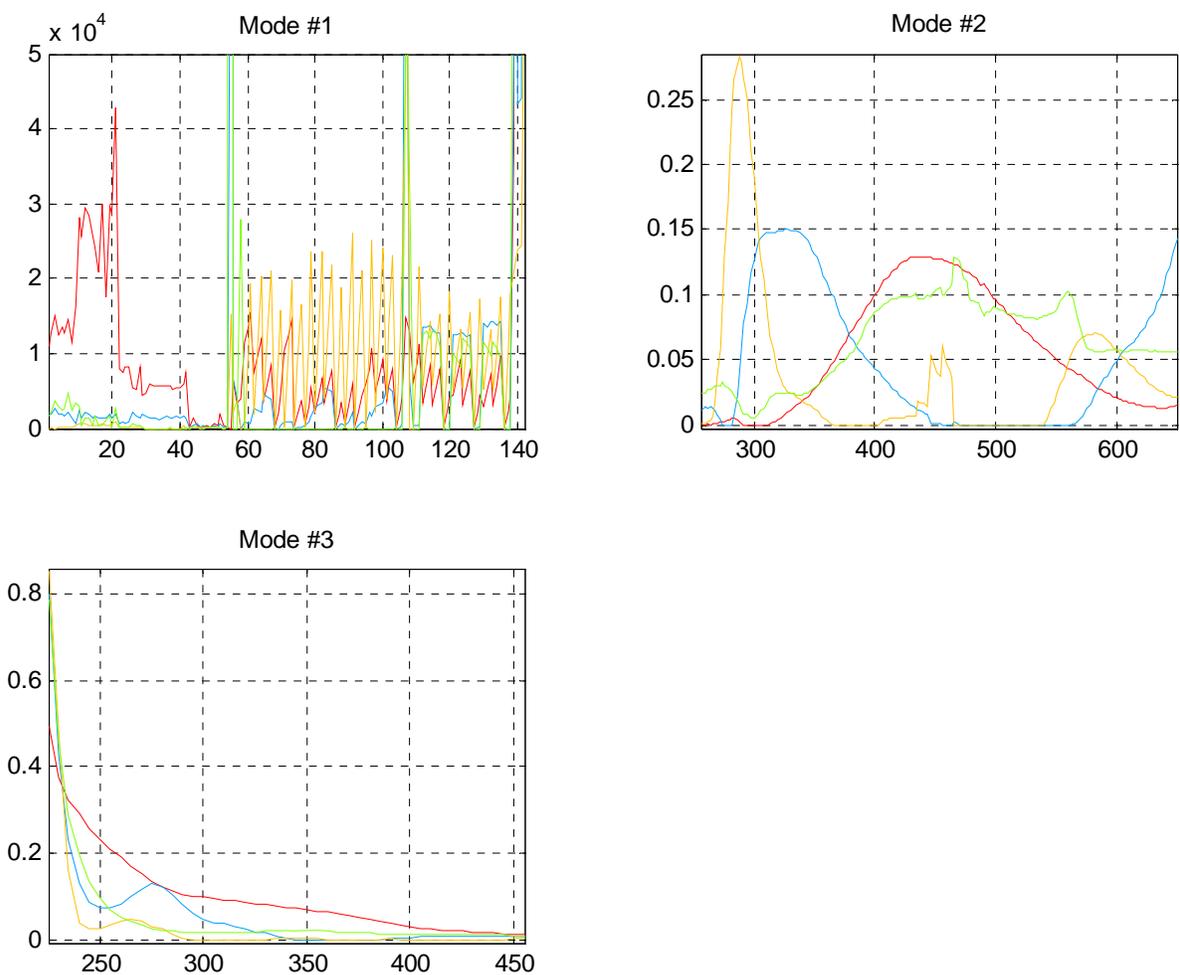


Figure 25. PARAFAC analysis and the four fluorescent factors determined on the combined ambient samples and laboratory dilution matrix and mixed detergent spikes (1-red, 2-light blue, 3-yellow, 4-green). Input data from 230-455ex/256-650em.

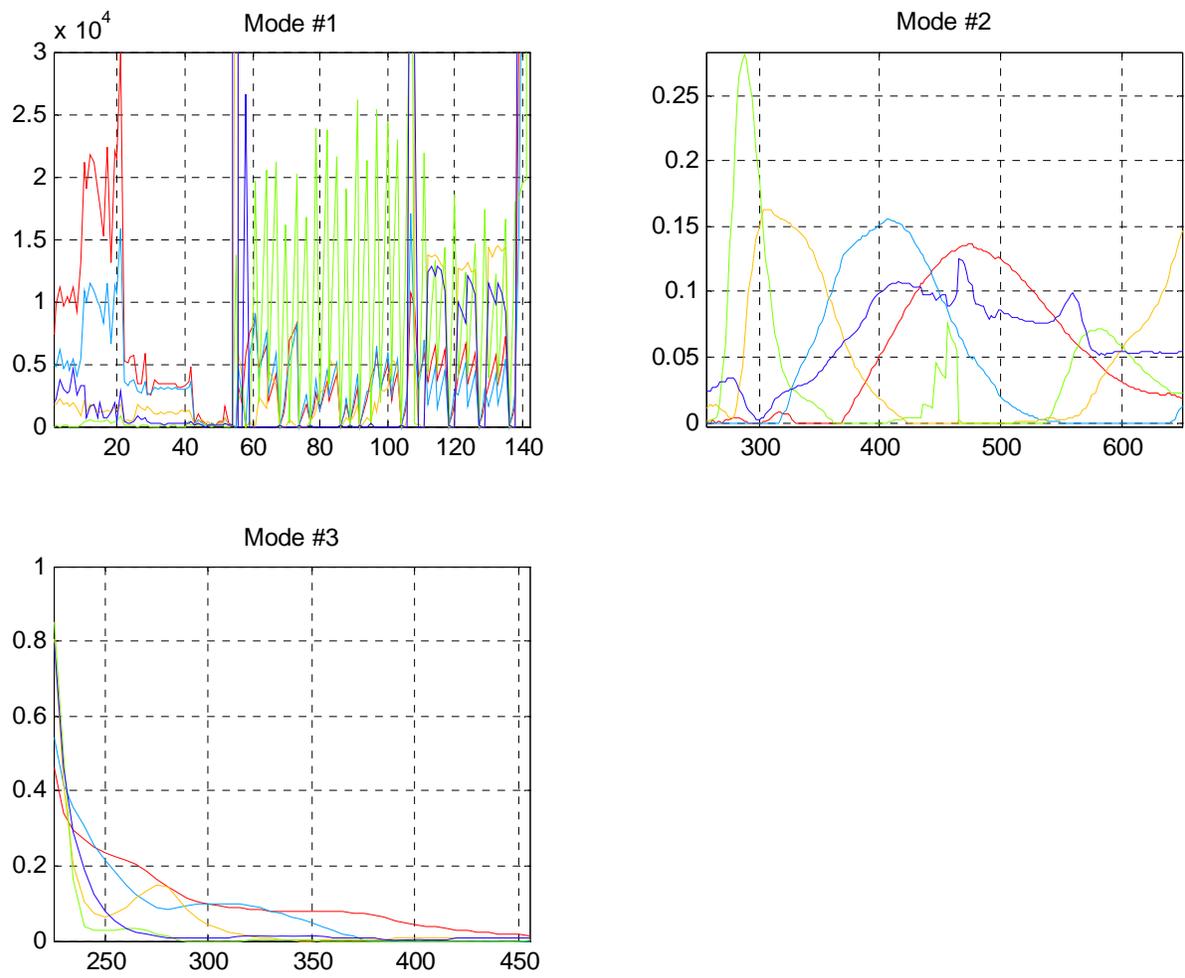


Figure 26. PARAFAC analysis and the five fluorescent factors determined on the combined ambient samples and laboratory dilution matrix and mixed detergent spikes (1-red, 2-light blue, 3-yellow, 4-green, 5-dark blue). Input data from 230-455ex/256-650em.

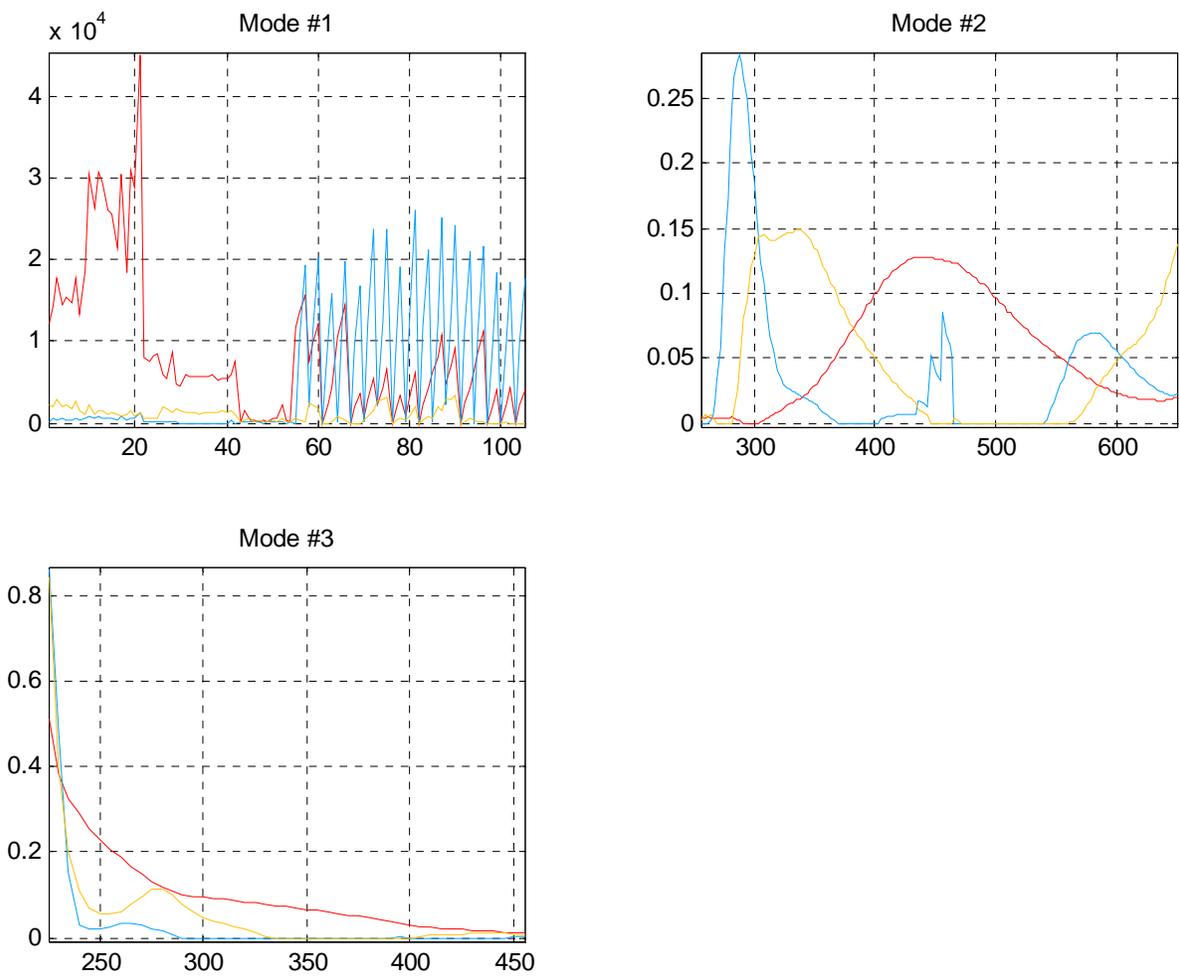


Figure 27. PARAFAC analysis and the five fluorescent factors determined on the combined ambient samples and laboratory dilution matrix and mixed detergent spikes, less full strength WWTP and any mixture with OSTDS (1-red, 2-light blue, 3-yellow). Input data from 230-455ex/256-650em.

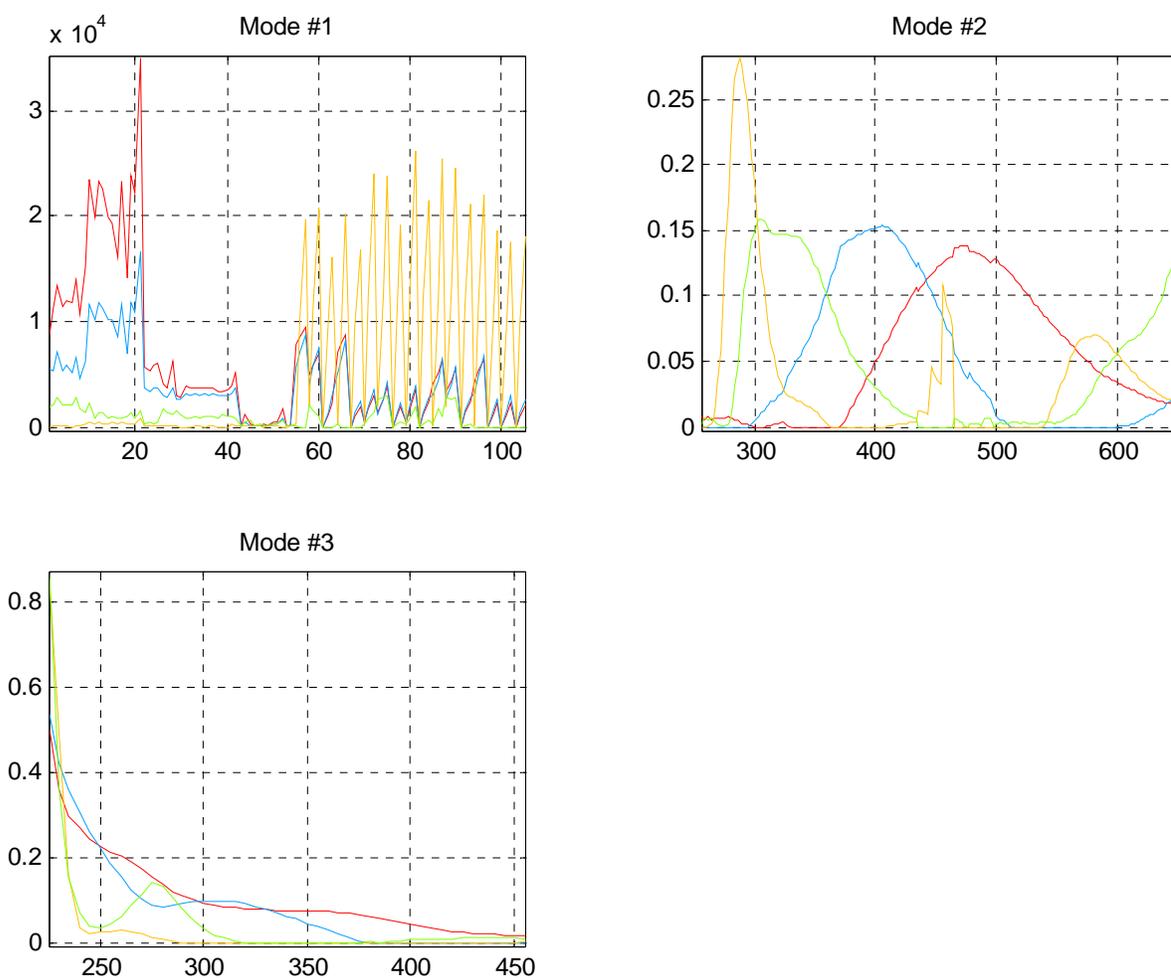


Figure 28. PARAFAC analysis and the five fluorescent factors determined on the combined ambient samples and laboratory dilution matrix and mixed detergent spikes, less full strength WWTP and any mixture with OSTDS (1-red, 2-light blue, 3-yellow, 4-green). Input data from 230-455ex/256-650em.

Based on **Figures 29-31**, even factors which displayed a unique response to OB exhibited a depressed response in the presence of OSTDS waters (Samples 52-69). As a result, ambient samples with a high proportion of direct OSTDS discharge may be expected to be underestimated. This conservative approach was preferable to overestimates. The results of Models 3-7 for the ambient samples appear in **Table 5**. Units in **Table 5** are as a percentage of typical OSTDS effluent, determined by dividing the individual sample PFAC values by mean PFAC loadings determined from all deionized water spikes. (Data are reported without regard to the analytical MDL of OB of ~0.8% OSTDS effluent. The analytical MDL may differ from the MDL determined via EEM and PARAFAC analysis. In all cases the UV peak of OB, at approximately 225ex/290em, was selected as the factor for quantification.

Examination of the results of the model application to the dilution matrix samples revealed an interesting phenomenon (**Table 6**). Recoveries of added OB, relative to the counts obtained in deionized water, were enhanced for medium and low CDOM matrices and were depressed to below 50% of expected in the presence of OSTDS fractions. Reference to the emission modes of the factors associated with OB and OSTDS in the various models indicates an overlap in both the UV and the >600 nm region for these factors. As a result, some portion of OB had the potential to be identified as OSTDS or WWTP component of the sample. As there are no independent measures to identify and eliminate correlations between factors (as with a400 and CDOM-OB interactions discussed above), it is suggested that the estimates of the WWTP/OSTDS components be evaluated to identify samples which should be examined further.

The presence of the factors indicating high levels of algal by-products or OSTDS/WWTP proteinaceous material is not confirmation of OB. The presence of the UV peak, however, could be indicative of high concentrations of OSTDS/WWTP effluents factor which reduce the ability of the model to quantify the OB present. At the high computed concentrations of OSTDS/WWTP, reference to other water quality data (chlorophyll, coliforms) could identify the likely causative factor and determine whether the presumptive OSTDS/WWTP identified should be considered in addition to OB when surveying a region of interest.

Figure 36 summarizes the mean % of typical OSTDS for the ambient samples derived by Models 3-7. Samples are in the case order as in **Table 5**. **Figure 37** illustrates an analogous computation of the fraction of OSTDS present, based on the magnitude of the OSTDS factor loadings and the magnitude in the known OSTDS samples analyzed. The fact that these two computed quantities are the same order of magnitude is reassuring. **Figure 38** illustrates the magnitude of the sample loading relative to WWTP effluent. **Figure 39** illustrates the relative agreement between fractions of OSTDS effluent computed by either the OB specific PARAFAC factors or by the OSTDS specific factors with figure data appearing in Table 7. The sum of the estimated OSTDS effluent percentage is also plotted as the true value of effluent present should be equal to or less than the combination of OB and OSTDS derived data. The sum, therefore, should represent an upper bound and can be used, together with OB derived concentrations alone, to interpret water quality data.

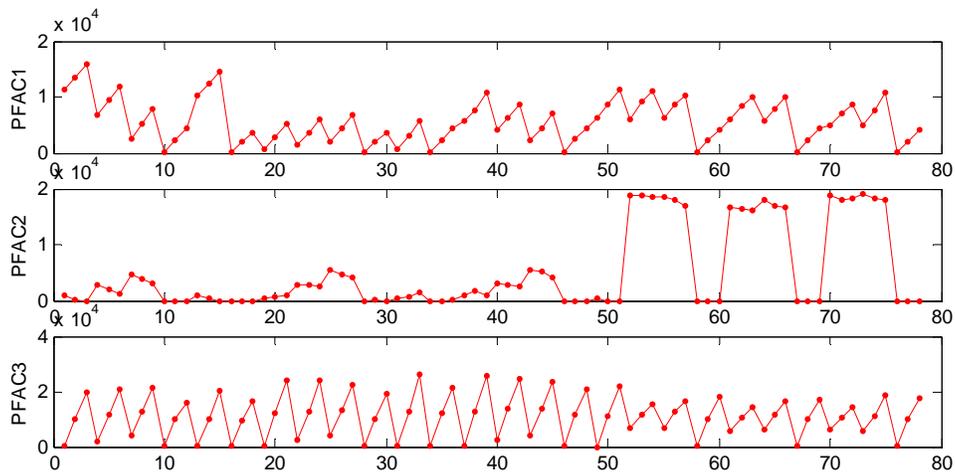


Figure 29. Loading of PARAFAC factors from a 3-factor model developed on combined ambient samples and laboratory dilution matrix (only matrix samples shown), from 230-455ex/256-650em. Sample identity is in case order as listed in Table 2. Each sample is followed by a 50% and 100% spike of typical OSTDS concentrations of mixed detergents. PFAC1 and PFAC3 are indicative of OB.

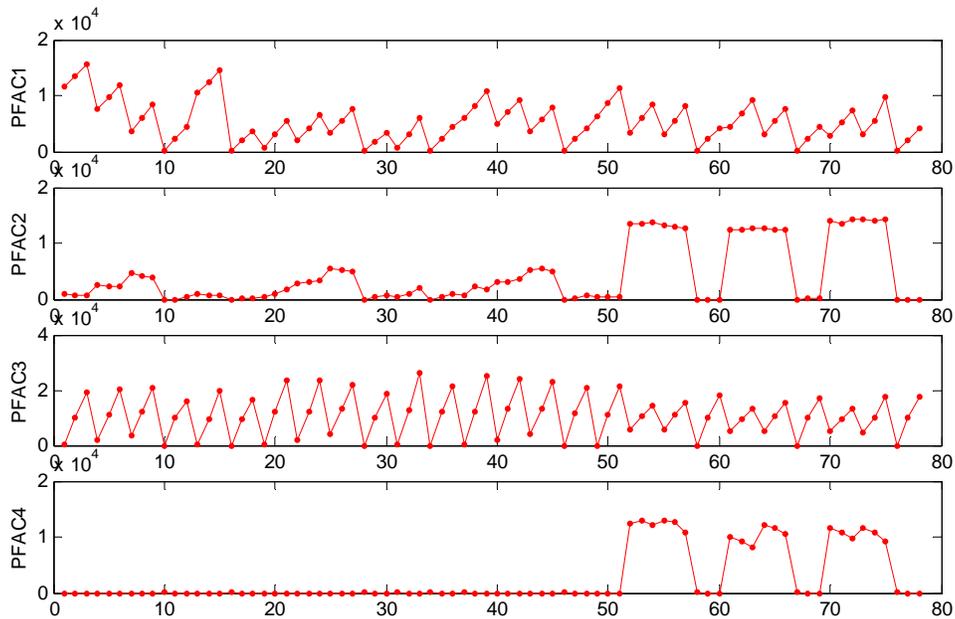


Figure 30. Loading of PARAFAC factors from a 4-factor model developed on combined ambient samples and laboratory dilution matrix (only matrix samples shown), from 230-455ex/256-650em. Sample identity is in case order as listed in Table 2. Each sample is followed by a 50% and 100% spike of typical OSTDS concentrations of mixed detergents. PFAC1 and PFAC3 are indicative of OB.

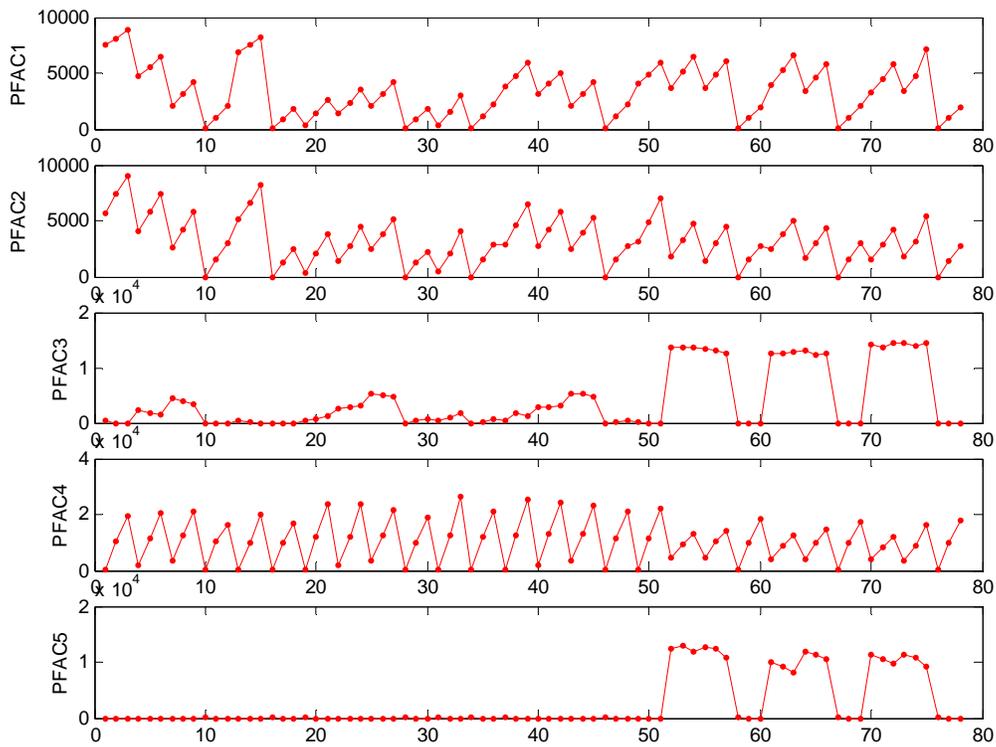


Figure 31. Loading of PARAFAC factors from a 5-factor model developed on combined ambient samples and laboratory dilution matrix (only matrix samples shown), from 230-455ex/256-650em. Sample identity is in case order as listed in Table 2. Each sample is followed by a 50% and 100% spike of typical OSTDS concentrations of mixed detergents. PFAC1, PFAC2, and PFAC4 are indicative of OB.

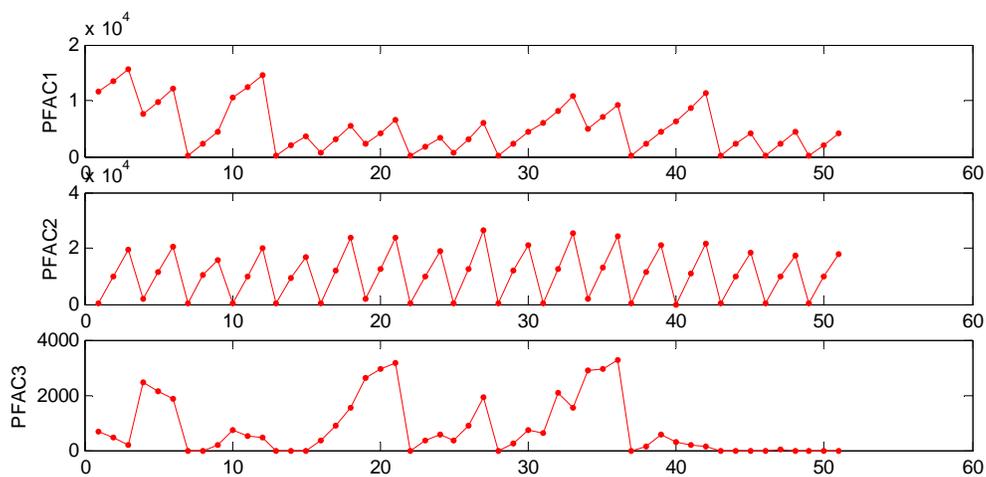


Figure 32. Loading of PARAFAC factors from a 3-factor model developed on combined ambient samples and laboratory dilution matrix, less full strength WWTP and any mixture with OSTDS (only matrix samples shown), from 230-455ex/256-650em. Sample identity is in case order as listed in Table 2. PFAC1 and PFAC2 are indicative of OB.

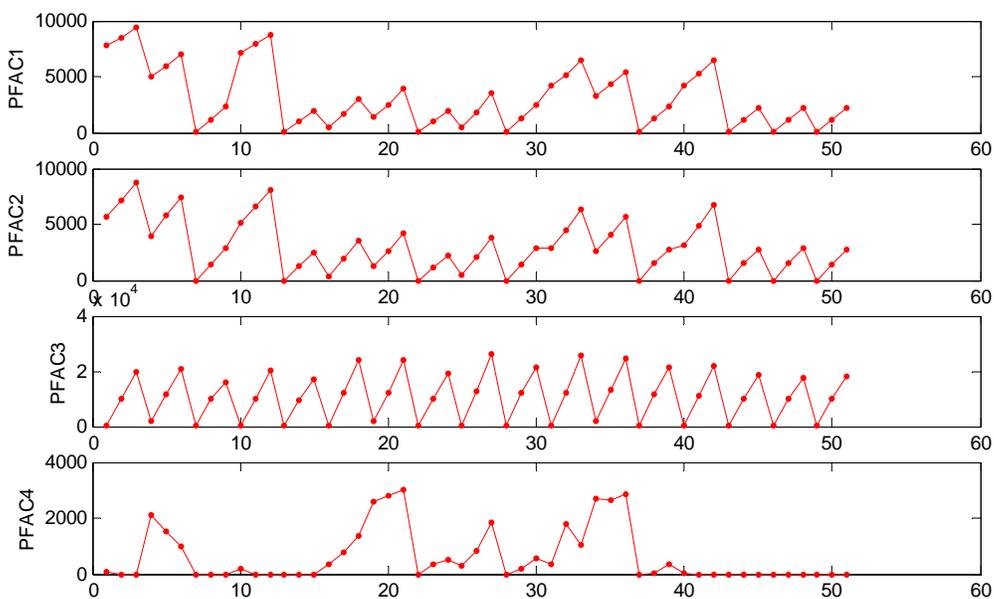


Figure 33. Loading of PARAFAC factors from a 4-factor model developed on combined ambient samples and laboratory dilution matrix, less full strength WWTP and any mixture with OSTDS (only matrix samples shown), from 230-455ex/256-650em. Sample identity is in case order as listed in Table 2. PFAC1, PFAC2, and PFAC3 are indicative of OB.

Table 5. Estimated levels of OB in ambient samples, in units of % of typical OSTDS effluent, generated by PARAFAC Models 3-7, and using the UV OB peak for quantification.

STATION	a_{400} (m ⁻¹)	Model 3	Model 4	Model 5	Model 6	Model 7	Mean
PH-A	10.46	0.1	0.0	0.0	0.6	0.0	0.1
PH-B	15.87	2.0	1.4	0.4	2.7	0.7	1.4
PH-C	16.85	0.8	0.0	0.0	2.0	0.2	0.6
PH-D	15.96	1.9	1.1	0.3	2.8	0.9	1.4
PH-E	17.45	1.7	1.0	0.2	2.5	0.7	1.2
PH-F	14.46	1.1	0.4	0.0	2.1	0.0	0.7
PH-F REP	17.79	1.5	0.3	0.0	3.1	0.6	1.1
PH-G	13.94	1.5	0.9	0.0	2.4	0.4	1.0
PH-M	21.25	2.2	1.5	0.5	3.6	1.1	1.8
D1 (4-69-1)	16.2	3.4	3.3	2.8	4.2	2.5	3.2
D3 (4-69-2)	15.6	3.5	3.0	2.3	4.9	2.6	3.2
D3 REP (4-69-2)	14.63	2.7	2.6	2.1	3.7	1.8	2.6
D5 (4-69-3)	13.99	2.5	2.4	1.9	3.4	1.6	2.3
D9 (4-69-4)	11.3	2.2	2.1	2.1	3.1	2.1	2.3
D10 (4-69-5)	12.45	2.6	2.8	2.5	3.1	1.9	2.6
D11(4-69-6)	12.21	2.4	2.6	2.3	2.9	1.7	2.4
D11 A	24.09	5.7	5.6	4.5	7.3	4.0	5.4
D12 (4-69-7)	13.22	2.8	3.1	2.4	3.3	1.5	2.6
D13 (4-69-8)	14.97	3.5	3.7	3.1	4.1	2.4	3.4
D14 (4-69-9)	19.07	2.1	1.9	1.3	2.6	1.2	1.8
D15(4-69-10)	14.98	2.2	2.2	2.1	2.6	1.7	2.2
TAYLOR 1	5.21	0.0	0.0	0.0	0.0	0.0	0.0
TAYLOR 2	4.63	0.0	0.0	0.0	0.0	0.0	0.0
TAYLOR 2 REP	4.79	0.0	0.0	0.1	0.0	0.2	0.1
TAYLOR 3	4.87	0.0	0.0	0.1	0.0	0.1	0.0
TAYLOR 3 REP	4.85	0.0	0.0	0.1	0.0	0.1	0.0
TAYLOR 4	4.24	0.0	0.0	0.1	0.0	0.1	0.0
TAYLOR 5	4.68	0.0	0.0	0.0	0.0	0.0	0.0
TAYLOR 6	4.31	0.0	0.0	0.0	0.0	0.0	0.0
TAYLOR 7	4.49	0.0	0.0	0.0	0.0	0.0	0.0
TAYLOR 8	4.77	0.0	0.0	0.0	0.0	0.0	0.0
TAYLOR 9	5.33	1.8	1.7	1.9	1.7	1.9	1.8
TAYLOR 10	7.08	0.0	0.0	0.2	0.0	0.2	0.1
TAYLOR 11	4.88	0.1	0.1	0.4	0.2	0.3	0.2
TAYLOR 12	4.71	0.6	0.6	0.8	0.6	0.6	0.6
TAYLOR 13	5.12	0.1	0.1	0.4	0.2	0.3	0.2
TAYLOR 14	5.15	0.4	0.4	0.6	0.4	0.5	0.5
TAYLOR 16	5.07	0.5	0.1	0.0	0.5	0.2	0.3
TAYLOR 17	4.89	0.4	0.2	0.0	0.4	0.2	0.2
TAYLOR 18	8.1	0.4	0.2	0.0	0.6	0.1	0.3
TAYLOR 19	3.85	1.1	0.9	0.8	1.0	0.9	1.0
TAYLOR 20	3.82	0.0	0.0	0.0	0.0	0.0	0.0
CHASS 1	0.09	0.2	0.2	0.1	0.2	0.1	0.1
CHASS 2	0.97	0.5	0.4	0.1	0.5	0.2	0.3

CHASS 3	0.34	1.2	1.1	0.7	1.1	0.7	0.9
CHASS 4	0.16	0.4	0.4	0.3	0.4	0.2	0.3
CHASS 5	0	0.6	0.6	0.4	0.5	0.4	0.5
CHASS 5 REP	0.21	0.2	0.1	0.2	0.1	0.2	0.2
CHASS 6	0.17	0.0	0.0	0.0	0.0	0.0	0.0
CHASS 6 VENT	0.05	0.4	0.4	0.3	0.4	0.3	0.3
CHASS 7	0.62	0.3	0.2	0.1	0.3	0.1	0.2
CHASS 8	0.71	0.7	0.7	0.5	0.7	0.5	0.6
CHASS 9	1.73	0.4	0.3	0.0	0.4	0.0	0.2
CHASS 10	0.62	0.0	0.0	0.0	0.0	0.0	0.0
OSTDS1	15.02	377.3	339.2	283.3			333.2
OSTDS2	11.39	8.4	0.0	1.4			3.3
OSTDS3	5.35	189.2	115.5	98.7			134.5
OSTDS4	83.77	165.4	131.7	105.4			134.2
OSTDS4REP	74.36	167.6	133.8	106.9			136.1
OSTDS5	49.81	687.9	403.4	396.0			495.8
WWTP1	3.17	13.0	11.4	10.3			11.5
WWTP2	1.14	20.8	19.8	18.0			19.5

Table 6. Estimated levels of OB in the laboratory dilution matrix, in units of % of typical OSTDS effluent, generated by PARAFAC Models 3-7, and using the UV OB peak for quantification. Spike recoveries are the sample+SPK value less the sample values and should be 100%.

STATION	a_{400} (m^{-1})	Model 3	Model 4	Model 5	Model 6	Model 7	Mean	Spike Recovery
HI CDOM	7.85	0.1	0.0	1.2	0.1	1.0	0.5	
HI CDOM+1/2 SPK	7.85	55.7	54.9	56.2	55.0	55.7	55.5	
HI CDOM+SPK	7.85	106.7	106.2	106.5	106.5	106.5	106.5	106.0
HI CDOM	7.78	0.5	0.4	1.4	0.4	1.2	0.8	
HI CDOM+1/2 SPK	7.78	53.7	52.8	53.7	52.9	53.7	53.4	
HI CDOM+SPK	7.78	109.8	108.7	109.4	108.9	109.5	109.3	108.5
HI CDOM+WWTP	4.19	11.2	10.5	10.2	10.5	10.1	10.5	
HI CDOM+WWTP+1/2SPK	4.19	62.6	61.6	61.5	61.6	62.2	61.9	
HI CDOM+WWTP+SPK	4.19	113.0	111.4	111.4	111.6	112.8	112.1	101.6
MCDOM	4.34	0.0	0.0	0.2	0.0	0.2	0.1	
MCDOM+1/2 SPK	4.34	60.8	60.4	60.7	60.5	60.7	60.6	
MCDOM+SPK	4.34	119.0	118.9	118.6	119.1	119.1	119.0	118.9
MCDOM	4.26	0.3	0.3	0.6	0.3	0.6	0.4	
MCDOM+1/2 SPK	4.26	68.7	67.6	66.8	67.4	66.9	67.5	
MCDOM+SPK	4.26	139.4	137.9	137.0	137.8	138.1	138.0	137.6
MCDOM+WWTP	2.55	12.3	11.6	10.6	11.6	10.6	11.3	
MCDOM+WWTP +1/2 SPK	2.55	74.3	73.1	71.7	73.0	72.3	72.9	
MCDOM+WWTP +SPK	2.55	135.3	133.5	131.5	133.4	132.7	133.3	122.0
LO CDOM	0.53	0.6	0.6	0.5	0.6	0.5	0.6	
LO CDOM+1/2 SPK	0.53	67.3	66.8	66.1	66.8	66.5	66.7	
LO CDOM+SPK	0.53	131.4	130.6	129.0	130.5	130.0	130.3	129.7
LO CDOM	0.48	0.6	0.6	0.6	0.6	0.6	0.6	
LO CDOM+1/2 SPK	0.48	70.5	70.0	69.1	69.9	69.6	69.8	
LO CDOM+SPK	0.48	144.5	143.6	141.5	143.4	142.4	143.1	142.5
LO CDOM+WWTP	0.86	12.4	11.8	10.5	11.7	10.5	11.4	
LO CDOM+WWTP+1/2SPK	0.86	69.1	68.0	66.1	67.9	66.7	67.6	
LO CDOM+WWTP +SPK	0.86	132.2	130.7	128.2	130.6	129.3	130.2	118.8
BRO	0.00	0.0	0.0	0.0	0.0	0.0	0.0	
BRO+1/2SPK	0.00	55.2	54.7	54.0	54.8	54.6	54.7	
BRO+SPK	0.00	105.2	104.2	102.8	104.3	104.1	104.1	104.1
BRO	0.00	0.0	0.0	0.0	0.0	0.0	0.0	
BRO+1/2SPK	0.00	52.2	52.6	52.0	52.6	52.5	52.4	
BRO+SPK	0.00	91.1	91.5	90.7	91.6	91.6	91.3	91.3
BRO	0.00	0.0	0.0	0.0	0.0	0.0	0.0	
BRO+1/2SPK	0.00	66.0	65.7	65.0	65.7	65.7	65.6	
BRO+SPK	0.00	117.6	116.6	115.3	116.6	116.7	116.6	116.6
BRO	0.00	0.0	0.0	0.0	0.0	0.0	0.0	
BRO+1/2SPK	0.00	63.1	63.0	62.5	63.0	63.2	62.9	
BRO+SPK	0.00	115.3	114.5	113.3	114.5	114.6	114.4	114.4
BRO	0.00	0.0	0.0	0.0	0.0	0.0	0.0	
BRO+1/2SPK	0.00	54.9	55.4	54.8	55.4	55.3	55.2	
BRO+SPK	0.00	100.4	101.2	100.1	101.1	101.0	100.8	100.8
BRO	0.00	0.0	0.0	0.0	0.0	0.0	0.0	
BRO+1/2SPK	0.00	53.9	54.2	53.7	54.2	54.2	54.0	

BRO+SPK	0.00	94.2	94.8	93.9	94.8	94.7	94.5	94.5
BRO	0.00	0.0	0.0	0.0	0.0	0.0	0.0	
BRO+1/2SPK	0.00	54.2	54.6	54.0	54.5	54.5	54.4	
BRO+SPK	0.00	97.1	98.0	96.9	97.8	97.7	97.5	97.5
BRO	0.00	0.0	0.0	0.0	0.0	0.0	0.0	
BRO+1/2SPK	0.00	55.2	55.7	55.1	55.6	55.6	55.4	
BRO+SPK	0.00	87.2	87.1	86.9	87.3	87.6	87.2	87.2
OSTDS1:8DIL	1.61	37.1	31.5	24.2			30.9	
OSTDS1:8DIL+1/2SPK	1.61	68.0	62.5	55.7			62.1	
OSTDS1:8DIL+SPK	1.61	89.2	84.0	77.4			83.5	52.6
OSTDS1:8DIL	1.40	32.1	26.7	19.3			26.0	
OSTDS1:8DIL+1/2SPK	1.40	60.7	55.5	48.2			54.8	
OSTDS1:8DIL+SPK	1.40	102.0	97.1	89.4			96.2	70.1
OSTDS1:8DIL	1.39	34.9	29.5	22.8			29.0	
OSTDS1:8DIL+1/2SPK	1.39	63.7	58.5	52.1			58.1	
OSTDS1:8DIL+SPK	1.39	90.3	85.3	78.8			84.8	55.7
(HICDOM+OSTDS)1:4DIL	2.37	32.0	27.2	20.9			26.7	
(HICDOM+OSTDS)1:4DIL+1/2S	2.37	57.6	53.0	46.7			52.5	
(HICDOM+OSTDS)1:4DIL+SPK	2.37	77.5	73.1	66.6			72.4	45.7
(MCDOM+OSTDS)1:4DIL	1.98	36.3	30.8	23.6			30.2	
(MCDOM+OSTDS)1:4DIL+1/2SPK	1.98	64.6	58.9	51.8			58.5	
(MCDOM+OSTDS)1:4DIL+SPK	1.98	84.6	79.0	72.0			78.5	48.3
(LOCDOM+OSTDS)1:4DIL	1.52	33.9	28.5	20.9			27.8	
(LOCDOM+OSTDS)1:4DIL+1/2S	1.52	58.0	52.9	45.7			52.2	
(LOCDOM+OSTDS)1:4DIL+SPK	1.52	78.8	73.7	66.0			72.8	45.0
WWTP	1.05	23.2	21.9	19.6			21.6	
WWTP+1/2SPK	1.05	75.8	74.1	71.4			73.8	
WWTP+SPK	1.05	129.1	127.0	124.1			126.7	105.2
WWTP	1.10	22.3	21.0	18.6			20.6	
WWTP+1/2SPK	1.10	73.4	71.7	69.1			71.4	
WWTP+SPK	1.10	122.3	120.1	117.2			119.8	99.2
WWTP	1.06	21.9	20.8	19.0			20.6	

Table 7. Mean results from PARAFAC Models 3-7, with estimated OB concentrations as percent of typical OSTDS effluent. Percentage of OSTDS and WWTP estimates are also calculated based on alternate factors.

Sample Number	Station	a ₄₀₀ (m ⁻¹)	Mean OB	Mean OSTDS	Mean WWTP	OB, OSTDS Sum
1	PH-A	10.46	0.1	2.0	43.0	2.1
2	PH-B	15.87	1.4	1.8	39.9	3.3
3	PH-C	16.85	0.6	2.5	55.8	3.2
4	PH-D	15.96	1.4	1.9	41.6	3.3
5	PH-E	17.45	1.2	2.0	42.8	3.2
6	PH-F	14.46	0.7	1.7	37.7	2.4
7	PH-FREP	17.79	1.1	2.3	52.1	3.4
8	PH-G	13.94	1.0	1.5	33.3	2.5
9	PH-M	21.25	1.8	1.0	23.1	2.8
10	D1 (4-69-1)	16.20	3.2	1.9	36.9	5.1
11	D3 (4-69-2)	15.60	3.2	2.4	48.7	5.6
12	D3 REP (4-69-2)	14.63	2.6	1.7	33.2	4.3
13	D5 (4-69-3)	13.99	2.3	1.7	33.6	4.1
14	D9 (4-69-4)	11.30	2.3	1.5	28.6	3.8
15	D10 (4-69-5)	12.45	2.6	1.3	23.9	3.9
16	D11(4-69-6)	12.21	2.4	1.3	24.0	3.7
17	D11A	24.09	5.4	1.9	37.1	7.3
18	D12 (4-69-7)	13.22	2.6	1.3	22.5	3.9
19	D13 (4-69-8)	14.97	3.4	1.3	24.0	4.7
20	D14 (4-69-9)	19.07	1.8	1.3	25.3	3.1
21	D15(4-69-10)	14.98	2.2	1.3	24.0	3.4
22	TAYLOR1	5.21	0.0	1.1	23.1	1.1
23	TAYLOR2	4.63	0.0	1.1	22.2	1.1
24	TAYLOR2REP	4.79	0.1	1.3	25.8	1.3
25	TAYLOR3	4.87	0.0	1.2	23.9	1.2
26	TAYLOR3REP	4.85	0.0	1.2	23.8	1.2
27	TAYLOR4	4.24	0.0	1.0	20.7	1.1
28	TAYLOR5	4.68	0.0	1.2	24.3	1.2
29	TAYLOR6	4.31	0.0	1.1	22.2	1.1
30	TAYLOR7	4.49	0.0	1.3	27.6	1.3
31	TAYLOR8	4.77	0.0	1.2	25.4	1.2
32	TAYLOR9	5.33	1.8	1.3	26.5	3.1
33	TAYLOR10	7.08	0.1	1.1	21.7	1.1
34	TAYLOR11	4.88	0.2	0.6	11.3	0.8
35	TAYLOR12	4.71	0.6	0.6	12.0	1.2
36	TAYLOR13	5.12	0.2	0.6	10.9	0.8
37	TAYLOR14	5.15	0.5	0.6	12.4	1.1
38	TAYLOR16	5.07	0.3	1.8	39.3	2.1
39	TAYLOR17	4.89	0.2	1.5	31.9	1.7
40	TAYLOR18	8.10	0.3	1.3	27.4	1.6
41	TAYLOR19	3.85	1.0	1.6	33.0	2.5
42	TAYLOR20	3.82	0.0	1.3	27.9	1.3
43	CHASS1	0.09	0.1	0.1	2.9	0.3
44	CHASS2	0.97	0.3	0.4	8.3	0.7

45	CHASS3	0.34	0.9	0.5	10.9	1.4
46	CHASS4	0.16	0.3	0.2	3.3	0.5
47	CHASS5	0.00	0.5	0.3	6.4	0.8
48	CHASS5REP	0.21	0.2	0.1	1.1	0.2
49	CHASS6	0.17	0.0	0.1	2.2	0.1
50	CHASS6VENT	0.05	0.3	0.1	1.6	0.4
51	CHASS7	0.62	0.2	0.3	5.7	0.4
52	CHASS8	0.71	0.6	0.2	4.8	0.9
53	CHASS9	1.73	0.2	0.6	12.3	0.8
54	CHASS10	0.62	0.0	0.1	2.5	0.1

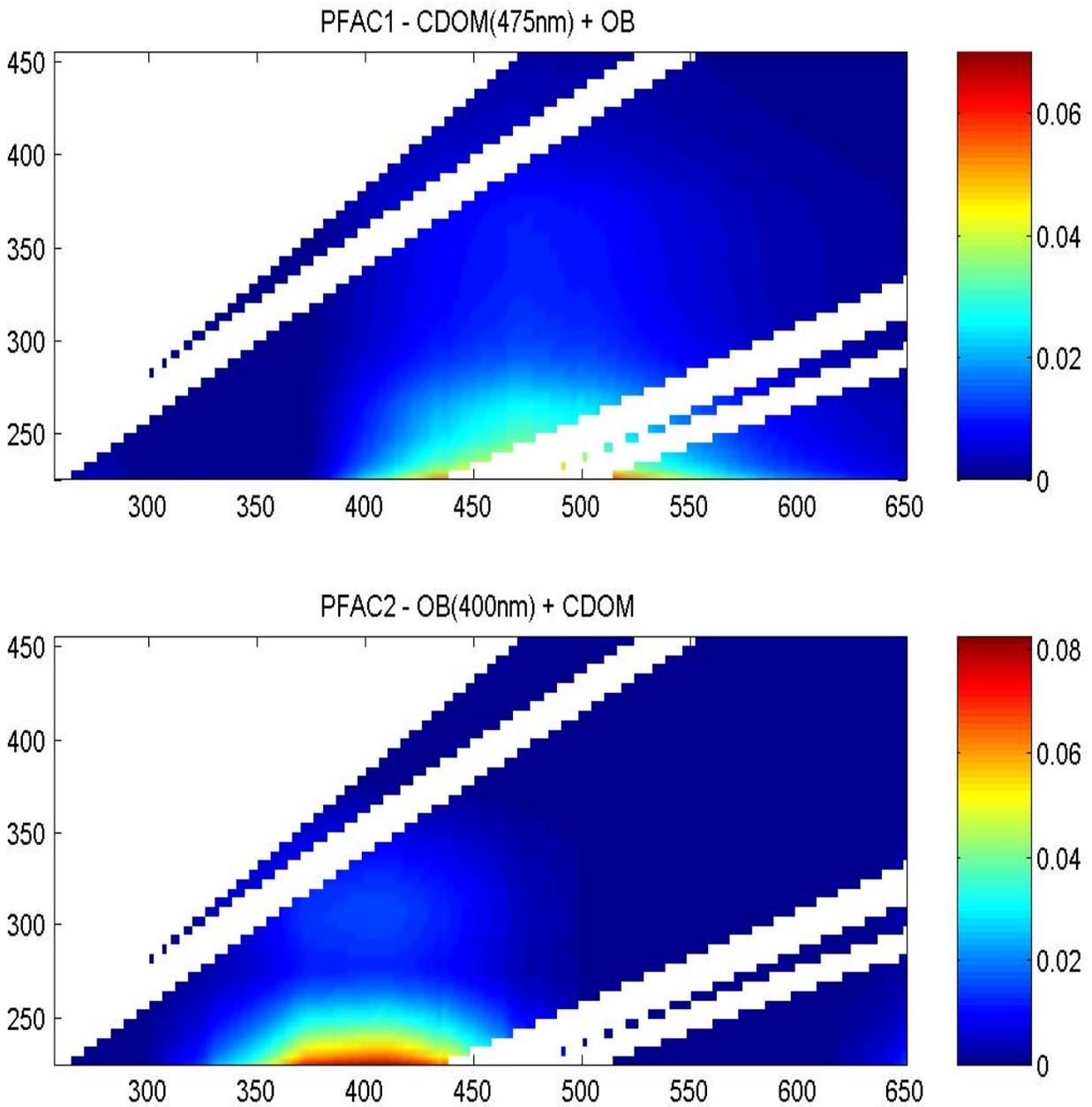


Figure 34. Model 1 - PARAFAC component factors (PFAC1 and PFAC2) determined from Model 7, all samples except WWTP and OSTDS samples.

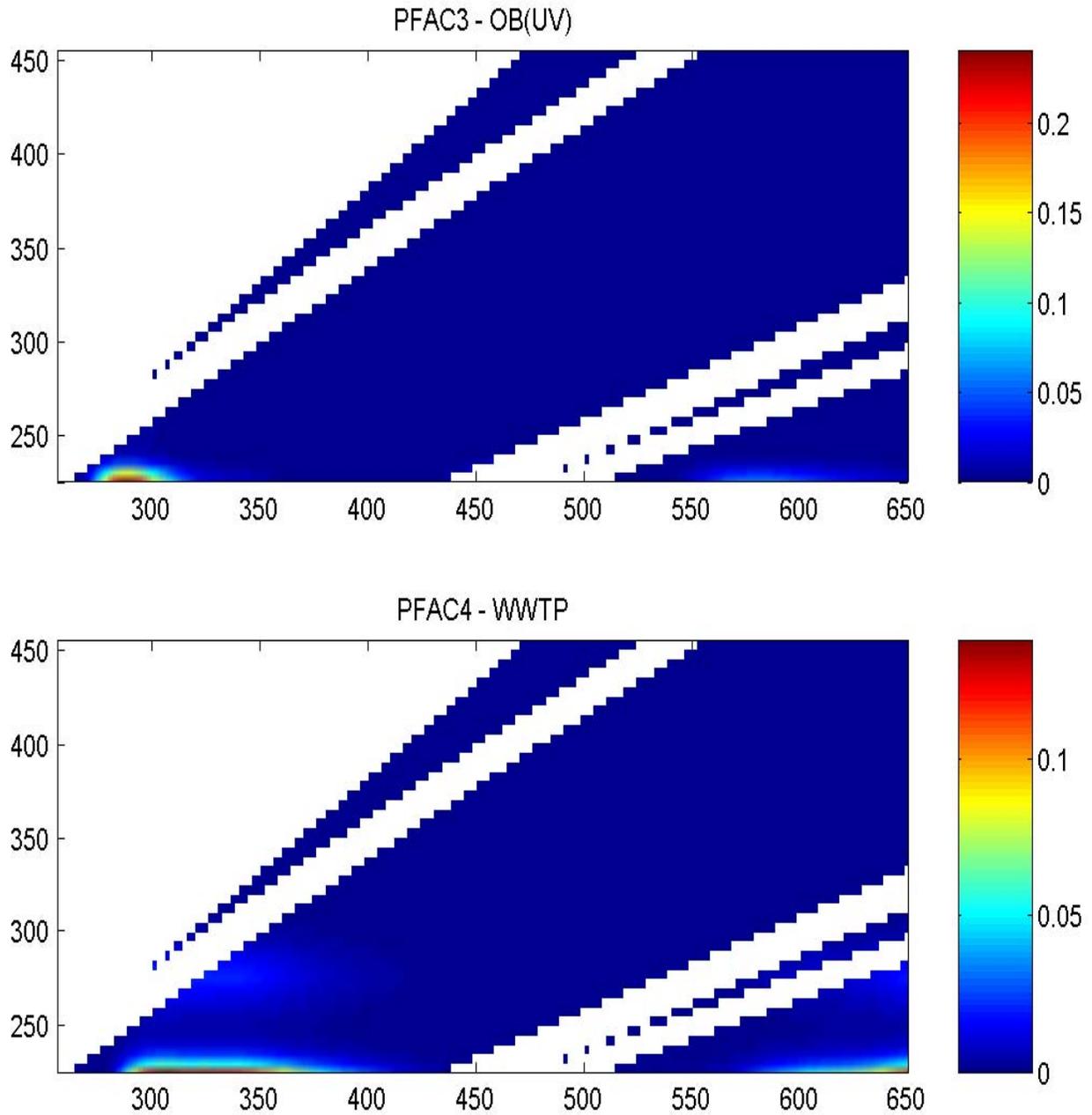


Figure 35. Model 1 - PARAFAC component factors (PFAC3 and PFAC4) determined from Model 7, all samples except WWTP and OSTDS samples.

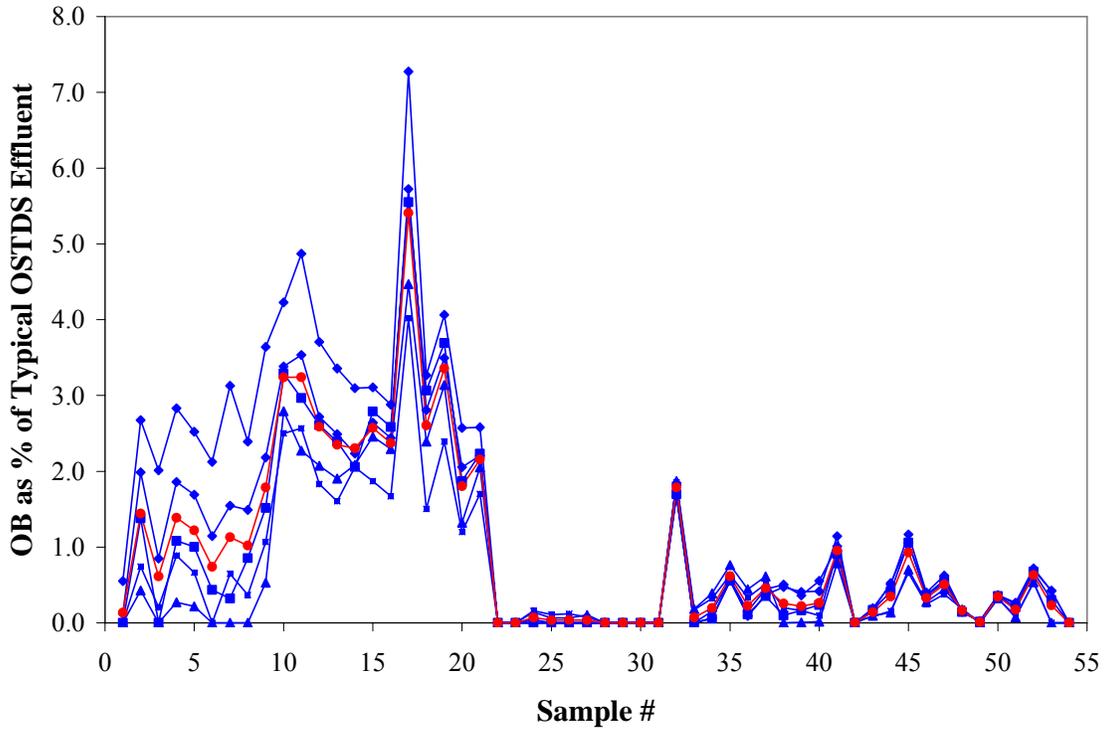


Figure 36. OB determined as % of typical OSTDS effluent via PARAFAC Models 3-7. Mean value in red. Sample order in Table 7.

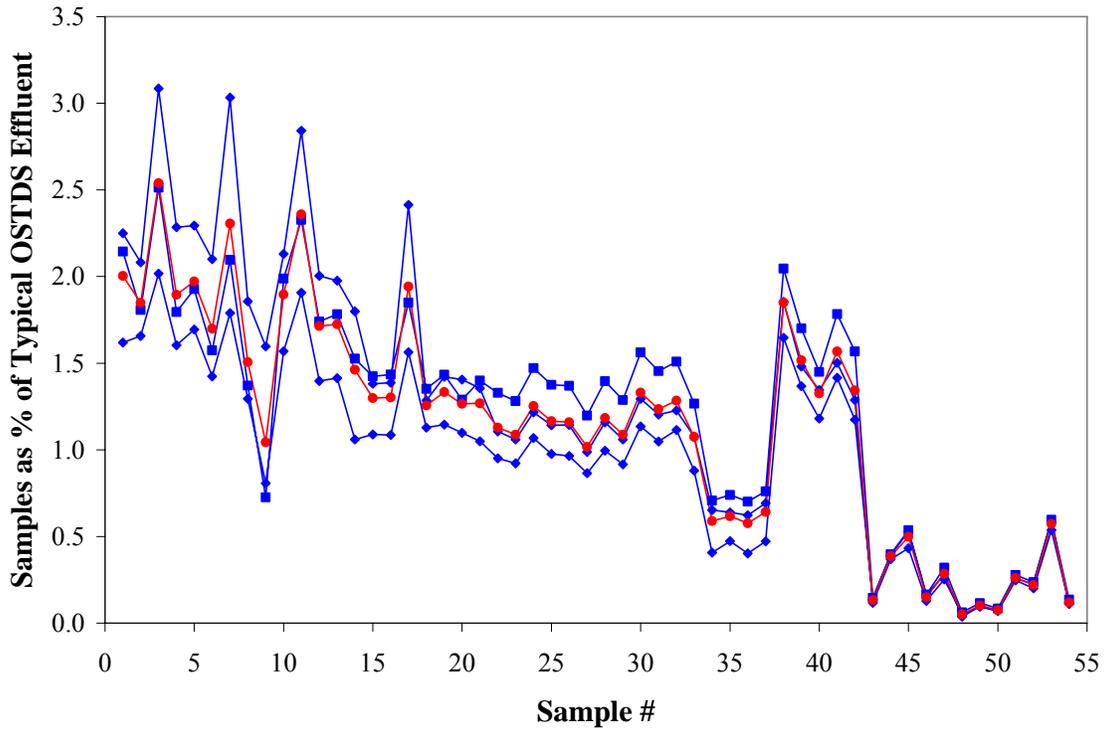


Figure 37. Amount of OSTDS waters present, as % of OSTDS effluent, estimated via PARAFAC Models 3-7. Mean value in red. Sample order in Table 7.

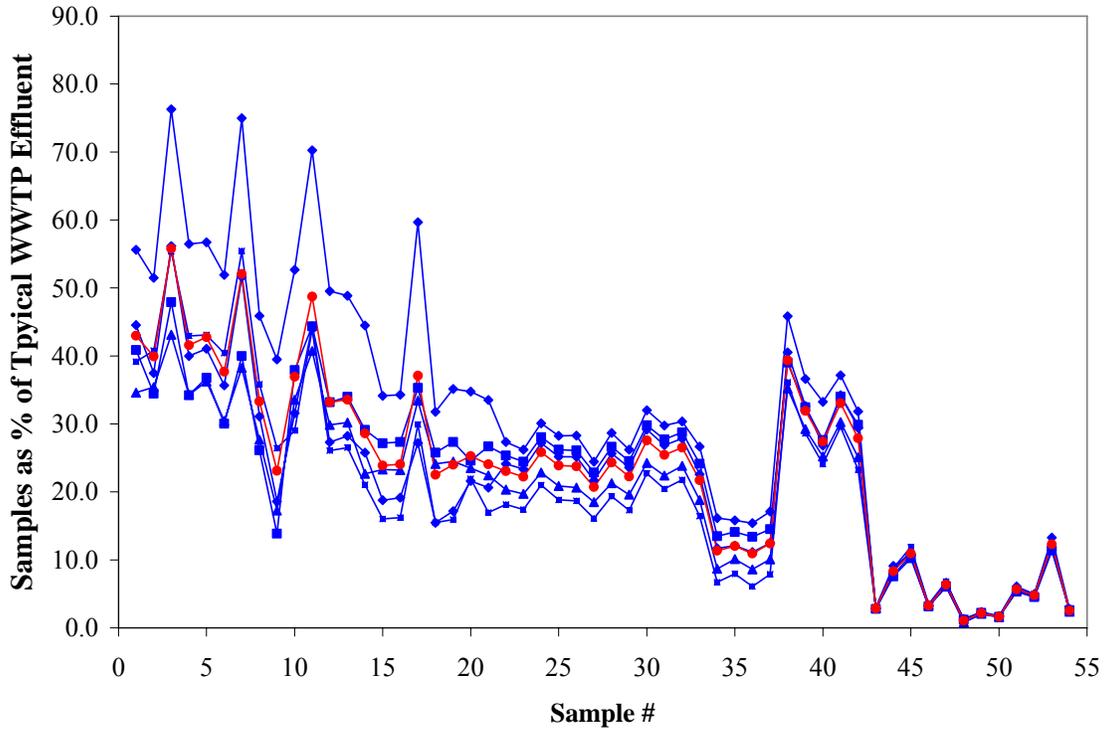


Figure 38. Amount of WWTP waters present, as % of WWTP effluent, estimated via PARAFAC Models 3-7. Mean value in red. Sample order in Table 7.

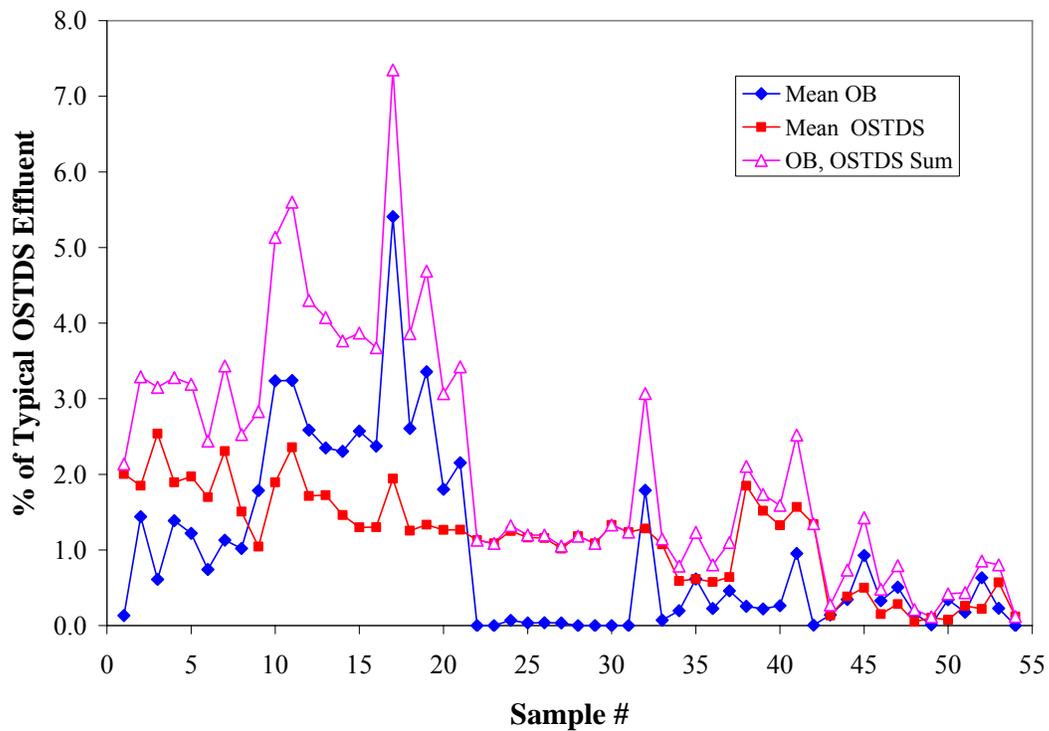


Figure 39. Mean values of OB and OSTDS waters present, and summed, as % of typical OSTDS effluent, estimated via PARAFAC Models 3-7. Sample order in Table 7.

Ambient samples

Using the summed upper bounds of estimated OB concentrations (the sum of OB and OSTDS percentages of typical OSTDS effluent), the values of the ambient samples displayed some interesting results. Levels from the additional Phillippi Creek and tributary stations collected by Sarasota County ranged from about 2-3%. Levels of OB in the Phillipi Creek system sampled by this project were expected to be and were higher than the OB in the Phillippi Creek mainstem samples. In the absence of rainfall, the samples in this very small basin consist solely of baseflow, expected to consist primarily of OSTDS effluent with varying degrees of filtration. Samples at the upper end of the single ditch were higher than downstream, with the exception of a single sample which was collected due to its unusual appearance. This unusual sample proved to contain the highest levels of OB, or over 7% of a typical OSTDS effluent. Values of OB at the downstream end of the ditch where it intersected with Phillippi Creek were comparable to levels found in the Phillippi Creek mainstem samples collected by Sarasota County.

Samples from Keaton Beach (Taylor 1-10) recorded modeled OB levels of 1-1.5% of typical OSTDS effluent except one station with approximately 3% (Taylor 9). Steinhatchee River stations (Taylor 11-14) were approximately 1%. Levels at Dekle Beach (Taylor 16-20) were somewhat higher, 1-2%, with the lowest levels found at the most offshore station. OB modeled at the Chassahowitzka River stations were some of the lowest, generally less than 1% of typical OSTDS effluent levels. Waters from the main spring, sampled at depth were slightly higher than the waters overlying the vent.

In contrast to Table 3, the OSTDS sampled as discrete samples ranged from 3-500% of typical OSTDS, while WWTP samples were 10-20% of typical OSTDS. Source waters used in the laboratory dilution matrix were 0.7%, 0.6%, 21.1%, and 229% of typical OSTDS effluent for high CDOM, low CDOM, WWTP, and OSTDS, respectively.

Optimization of Dual Wavelength Method

The dual wavelength fluorescent field screen method developed in prior work (Dixon, et al., 2005) employed filter fluorimeters configured with 254nm excitation and a 440 nm and a 550 nm emission ranges to separate CDOM and OB fluorescence. A description of the platform and methods follows:

“Filters and lamps were obtained from Turner Designs otherwise specified. For the field, each fluorometer was equipped with a 10-049 G4T5 near-UV lamp, a 300nm reference filter, and a 300-400nm excitation filter. One fluorometer used a 440nm emission filter (Lambda Research Optics, Inc.) and was sensitive to the emission wavelength of OB as well as humic substances. The other fluorometer had a 550nm emission filter (Lambda Research Optics, Inc.) and was therefore predominantly sensitive to the emission wavelength of humic substances only.”

Figure 40 illustrates the EEM scans at 300 nm excitation of the laboratory dilution matrix results for various unspiked and detergent spiked samples. Based on the scans and response to spikes, 440 nm is an excellent choice for capturing the sample fluorescent variations due to OB. A 550 nm emission range, conversely has little response to OB, as is desired. Neither region appear to be subject to Rayleigh and Raman scattering that would make it susceptible to false results from sample turbidity.

A more detailed plot in **Figure 41**, illustrates the high CDOM sample both without and with added OB (Curves A and B). The unspiked high CDOM sample was also multiplied by a factor of three (Curve C) and enhanced by the OB response (Curve B-A) to create curve D. Based on these samples, and also using a 300 nm excitation, optimal response to CDOM with minimal response from OB and detergents is confirmed to be in the 550 nm region. Reference to the PARAFAC models developed indicate that the 550 nm region may have some sensitivity to OB as well as to CDOM although this was not visible in Figures 42 and 43. The wavelength region should respond only minimally to the elements found in mixtures of OSTDS/WWTP (other than OB) as the maximum excitation for these factors are nearer 275 nm. The successful quantitative use of this method would depend on a reproducible relationship between CDOM fluorescence at 440 nm and at 360-380 nm in the absence of OB (either by region or across regions) and on the adequate correction of both fluorescence values for absorption. Using these excitation wavelengths there are no obvious improvements to the dual wavelength method unless an alternate excitation wavelength is explored. (It may be possible to isolate the UV peak of OB that was used in the PARAFAC modeling. The use of filters to isolate excitation wavelengths, however, often make the energy throughput of UV unacceptably low for typical field instrumentation. Highly energetic light sources and grating optics would likely be required to pursue the UV region of OB fluorescence.

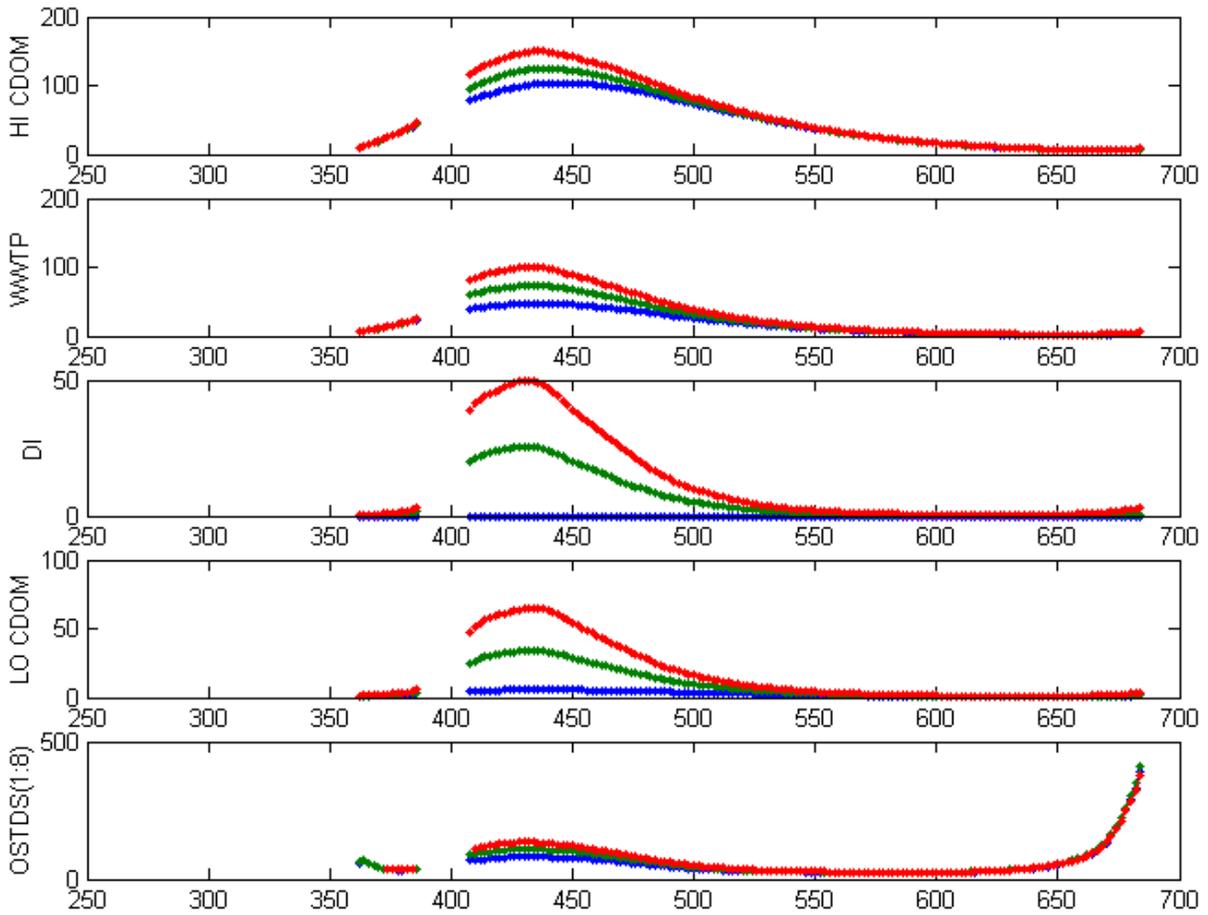


Figure 40. Excitation scans (255nm) of source waters for the laboratory dilution matrix, both raw and with OB at 50% and 100% of typical OSTDS effluent levels added. Rayleigh and Raman regions appear as blank regions of the curve.

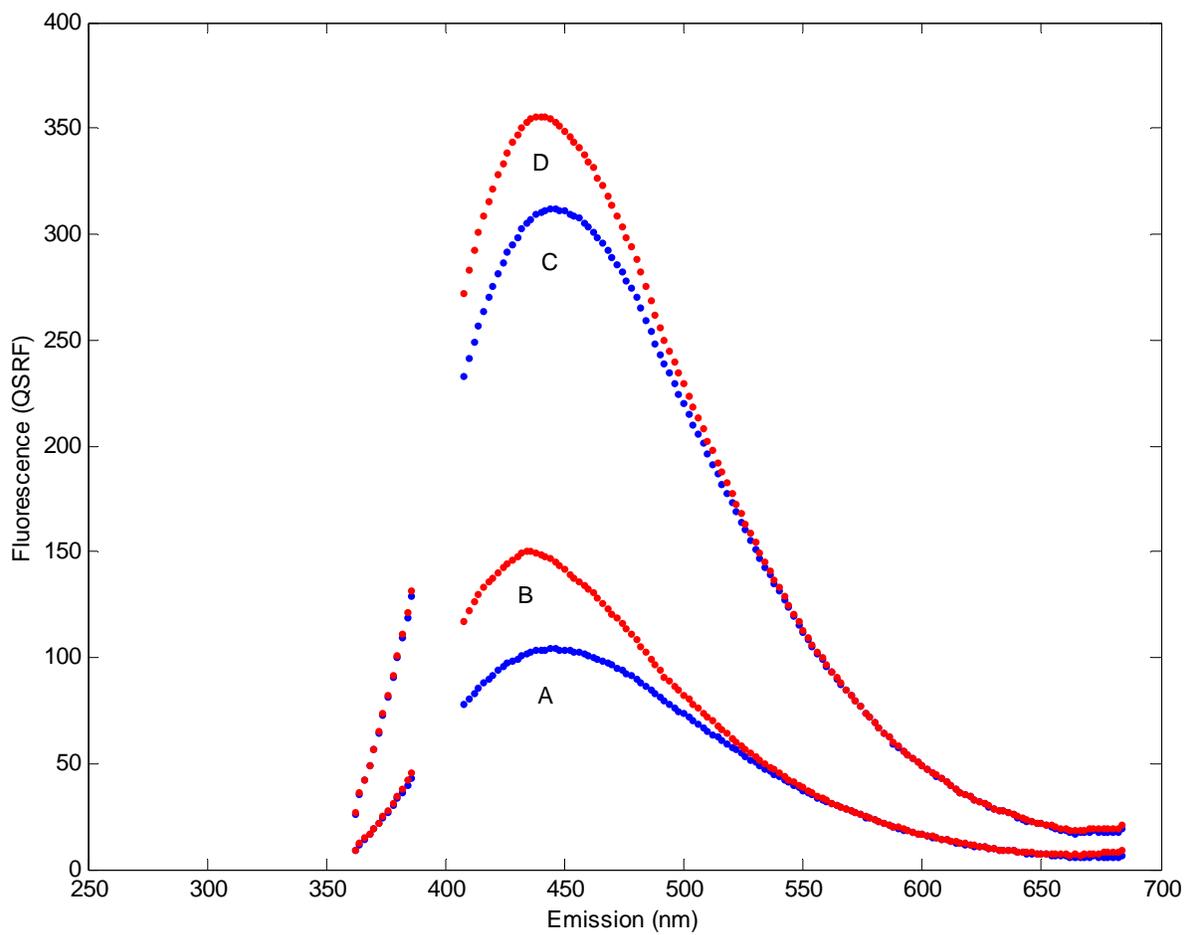


Figure 41. A family of emission curves from 255 nm excitation. Curve C is constructed as three times Curve A. OB has been added to sample from Curve A, with an equivalent response mathematically added to Curve C.

Summary

Accurate correction of sample fluorescence for absorption was critical to recover quantitative amounts of OB from the laboratory dilution matrix samples. Inadequate correction algorithms produced highly spurious results and forced revision of all modeling effort. Adequate correction of absorption in a dual wavelength field survey method may be difficult unless a reproducible correction can be developed and parameterized as a function of sample absorption measured at a single or a few wavelengths. Without adequate correction, field survey methods can still provide relative data for the region under survey, provided background CDOM fluorescence remains relatively constant. Surveying across a saline freshwater gradient would be difficult

Model parameterization was critical to determine reasonable levels of OB present in ambient samples. Derived factor loadings were less sensitive to input data and number of factors than to wavelength range. Removal of full strength WWTP and all OSTDS samples from model initialization did not substantially change factors or loadings, but analytically, care must be taken with highly turbid samples and some turbid samples must have additional masking of Rayleigh scattering before model input. With relatively small numbers of samples, a mathematically significant model can be generated which produces spurious results if identified peaks are too close and factor loadings are correlated. Good performance of fluorescence instruments in the UV range is essential as the most useful peak found to indicate detergent presence was at 225ex/290em.

Although the effect of added detergent to a variety of samples was clearly evident among fixed excitation fluorescent scans, model factors did not uniquely separate the CDOM and OSTDS peaks in the 400-450 region, i.e. a factor responded both to added OB and was dependent on a_{400} . Even when emission peaks were identified at both 400 nm (expected OB emission) and 450 nm (expected CDOM emission), both factor loadings were proportional to a_{400} as well. Using these combined factors to estimate OB concentrations produced extremely inflated results. More significantly, however, the CDOM sampled in the variety of ambient stations did not differ in fluorescent properties sufficiently to be identified as a separate model factor. This presents the possibility that model factors derived under this study can be applied to a broad range of waters with less concern for identifying variations in CDOM as OB. This concern is further satisfied as the region used for OB quantification did not overlap with estimates of CDOM fluorescence. Collection of additional samples with an emphasis on regions which are not expected to have OB influence would be useful to conserve these results.

There were a variety of individual or mixed fluorophores in the detergents examined, not all of which can be termed optical brighteners. The most useful peak for OB quantification emitted in the UV region, where the human eye is not sensitive, and was not contained in the DSBP optical brightener standard, although its presence is referred to as OB throughout the text. Quantitative model results of OB present were necessarily dependent on the detergent or detergent mixture used for standardization. Results of ambient samples should be considered relative as loadings of OB in OSTDS effluent clearly vary widely based on the results obtained in the five OSTDS samples under this

project. Factors leading to OB variation in measured OSTDS levels include timing and amount of laundry activities, as well as consumer choice of detergent. EEM data on ambient samples provided confirmation of samplers' instinct as to unusual sample appearance.

Based on EEM data and matrix scans, the wavelengths selected for the dual wavelength field fluorescence method were confirmed. There appear to be no major wavelength changes needed provided the filter fluorometer in use remains the primary optics platform.

Literature

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APPENDICES

Appendix A Project Quality Assurance Criteria

Appendix B MML Audit Reports the annual systems audit performed by the MML Quality Assurance Officer, Dr. Cathy Walsh

APPENDIX A--Quality objectives and Criteria for EEM analyses

Laboratory QC	Frequency	SOP Acceptance Limits	Corrective Action
Accuracy – wavelength of Raman emission at 300, 450, and 500nm	Daily	+/- 4 nm of theoretical	Reinitialize instrument
Accuracy – ICV Quinine Sulfate	Daily (initially)	90-110%	Rerun standard, remake standard
Accuracy – CCV Quinine Sulfate	Initially and every 10 samples	85-115%	Rerun standard, remake standard, recalibrate , rerun samples since last acceptable CCV
Precision – Duplicates	One every 10 samples	+/- 10nm of maximum λ emission at 350nm excitation; <15% RSD or <3*MDL of 450nm emission at 350nm excitation	Rerun both duplicates, determine cause, if not sample specific (turbidity) rerun since last acceptable duplicate
Instrument Blank	Daily	<5000 counts at 3.0V Gain (350nm ex, 450nm em)	Rerun blank, remake blank
Container Blank	One per cleaning lot number	<3*MDL (350nm ex, 450nm em)	Rerun, determine cause, annotate data sets, revise protocol if necessary
Field Equipment Blank	Daily	<3*MDL (300nm ex, 451nm em)	Rerun, determine cause, annotate data sets, revise protocol if necessary
Representativeness	Daily field replicates or 5%, whichever is greater	<20% RSD or differ by < 5*MDL	Review with sampler, revise sampling protocol if necessary, recognize heterogeneity of sampled system
MDL and linearity	Annually	0.1 – 10 ug/L Quinine Sulfate (350nm ex, 450nm em)	NA
Completeness	By project	>98%	Collect more samples
Comparability - Relative fluorescence in Quinine Sulfate Units	Daily	NA	NA

APPENDIX A--Quality objectives and Criteria for Absorption Analyses

Laboratory QC	Frequency	SOP Acceptance Limits	Corrective Action
Accuracy – absorption (Didymium glass)	Start and end of each analytical batch	90-110% at 444, 578, and 680nm	Rerun standard, recalibrate spectrophotometer
Accuracy – wavelength (Didymium glass)	Start and end of each analytical batch	90-110% at 444 and 680nm	Rerun standard, recalibrate spectrophotometer
Precision – Duplicates	One every 10 samples	<5% RSD or within 3*MDL at 400 and 440nm	Rerun both duplicates, determine cause; if not sample specific, rerun since last acceptable duplicate
Instrument Blank	Daily and every five samples	+/-0.0005A	Remake blank, rerun, rezero,
Container Blank	One per cleaning lot number	<3*MDL	Rerun, determine cause, annotate data sets, revise protocol if necessary
Field Equipment Blank	Daily	<3*MDL	Rerun, determine cause, annotate data sets, revise protocol if necessary
Representativeness	Daily field replicates or 5%, whichever is greater	<20% RSD or differ by < 5*MDL	Review with sampler, revise sampling protocol if necessary, recognize heterogeneity of sampled system
MDL and linearity	Annually	0.010 A at 400nm (10 cm cell)	NA
Completeness	By project	>98%	Collect more samples
Comparability – results reported in absorption coefficients (m ⁻¹)	Daily	NA	NA

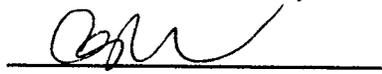
APPENDIX B
MML Audit Reports
the annual systems audit performed by the
MML Quality Assurance Officer, Dr. Cathy Walsh

INTERNAL SYSTEMS AUDIT

Date of audit: 8-10-06

Program: Chemical Ecology

Signatures:
Technical Director: 

QA Officer: 

Brief summary of results:

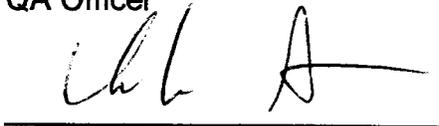
1. A list of approved suppliers should be available according to NELAC 2003 Standard 5.4.6.4 - The laboratory shall evaluate suppliers of critical consumables, supplies and services which affect the quality of environmental testing, and shall maintain records of these evaluations and list those approved.
2. Withdrawn SOPs should have the date withdrawn indicated on them (MML QA Manual Section 4.6 Document Control and Maintenance).

Date for re-check: September 12, 2006

Re-check signatures:


QA Officer

10-12-06
Date


Technical Director

10/13/06
Date

Ari

CHEMICAL ECOLOGY

INTERNAL AUDIT FORM BASED ON NELAC STANDARDS, 2003

PROFICIENCY TESTING

2.5 Are PT samples handles in the same manner as real environmental samples utilizing the same staff & methods as used for routine analysis of that analyte, procedures, equipment, facilities, & frequency of analysis?

2.5.1(a) Does the laboratory not send any PT sample, or portion of a PT sample, to another laboratory for any analysis for which it seeks accreditation, or is accredited?

2.5.1(c) Does the laboratory management & staff not communicate with any individual at another laboratory (including intralaboratory communication) concerning the PT sample?

2.5.2 Does the laboratory maintain copies of all written, printed, & electronic records resulting from the analysis of any PT sample for 5 years or for as long as required by the applicable regulatory program, whichever is greater?

DOCUMENT CONTROL

id 5.4.3.2.1 Is there an established master list or equivalent document control procedure identifying the current revision status & distribution of documents in the quality system? *- location of original copies of SOP's*

5.4.3.2.1 Are the master lists or document control procedures readily available to preclude the use of invalid and/or obsolete documents?

5.4.3.2.2(a) Are authorized editions of appropriate documents available at all locations where operations essential to the effective functioning of the laboratory are performed?

5.4.3.2.2(b) Are documents periodically reviewed and, where necessary, revised to ensure continuing suitability & compliance with applicable requirements

5.4.3.2.2(c) Are invalid or obsolete documents promptly removed from all points of issue or use, or otherwise assured against unintended use *- make withdrawn more clearly*

5.4.3.2.3 Are the quality system documents uniquely identified to include issue date and/or revision identification, page numbering, total number of pages (or marked to signify the end of the document), & the issuing authorities

5.4.3.3.1 Are changes to documents reviewed & approved by the same function that performed the original review, unless specifically designated otherwise?

5.4.3.3.2 Where practicable, is the altered or new text identified in the document or appropriate attachments?

5.4.3.3.3 Are all amendments to documents clearly marked, initialed, & dated, with a revised document formally re-issued as soon as practicable

5.4.3.3.4 Are procedures established to describe how changes in documents maintained in computerized systems are made & controlled? *- convert to pdf - some are - make withdrawn SOP's more clearly*

PURCHASING SERVICES & SUPPLIES

5.4.6.1 Does the laboratory have policies & procedures for selection & purchasing of services & supplies it uses that affect the quality of environmental tests?

5.4.6.1 Do procedures exist for the purchase, reception, & storage of reagents & consumable materials relevant for environmental tests?

8-10-06

Supplemental Questions:

Have there been any changes in staff?

Hillary Crook - left
May Whelan - left
Midge O - going (Sept 1)
two new hires next wk: Melissa Gilbert
Allison Albee
Staff - Dave Julian - red tide subchem work
Have there been procedural changes?
- TSS, VSS, turbidity, BOD, Color
NO

Have there been changes in type of work you are doing?

Analysis for Jim Culter - TCO by Bend
BOD, TSS, VSS, nitrate-nit, nutrients

Have any of your staff participated in training? Is it documented?

- Data Integrity training - certificates
- In-house Data Integrity in October - signed attendance
- ongoing in-house training for new employees - DDC etc.

Evidence of inappropriate actions or vulnerabilities related to data integrity?

NO

✓ 5.4.6.2 Does the laboratory ensure that supplies & services comply with specified requirements and maintain records of actions taken to check compliance with these requirements? - certificates

✓ 5.4.6.3 Do purchasing documents for items affecting the quality of laboratory output contain data describing the services & supplies ordered, and are purchasing documents reviewed & approved for technical content prior to release?

✓ 5.4.6.4 Does the laboratory evaluate suppliers of critical consumables, supplies, & services that affect the quality of environmental testing, and does the laboratory maintain records of these evaluations and list approved suppliers?

- need list of approved suppliers

CONTROL OF RECORDS

✓ 5.4.12 Does the laboratory's record system produce unequivocal, accurate records which document all laboratory activities?

✓ 5.4.12 Does the laboratory retain on record all original observations, calculations & derived data, calibration records, and a copy of test reports for at least 5 years?

✓ 5.4.12 Does the laboratory have a written SOP for carrying out legal chain-of-custody if a client specifies that a sample will be used for evidentiary purposes? - follows QA manual

✓ 5.4.12.1.4 Does the laboratory have procedures to protect & back-up records stored electronically & to prevent unauthorized access to or amendment of these records?

✓ 5.4.12.1.5 Does the record keeping system allow historical reconstruction of all laboratory activities that produced the resultant sample analytical data?

✓ 5.4.12.1.5(a) Do the records include the identity of personnel involved in sampling, sample receipt, preparation, calibration, & testing?

✓ 5.4.12.1.5(b) Has the laboratory documented all information relating to the laboratory facilities equipment, analytical test methods, & related laboratory activities (e.g. sample receipt, sample preparation, & data verification)?

✓ 5.4.12.1.5(d) Are all changes to records signed or initialed by responsible staff, and is the reason clearly indicated in the records (e.g., sampled by, prepared by, or reviewed by)?

✓ 5.4.12.1.5(e) Is all generated data recorded directly, promptly, & legibly in permanent ink?

✓ 5.4.12.1.5(f) Are all corrections to record-keeping errors made by one line marked through the error, with the individual making the correction signing or initialing & dating the correction?

✓ 5.4.12.2.1 Do lab records include the identity of personnel responsible for sampling, performance of tests, & checking results?

✓ 5.4.12.2.3 When corrections are due to reasons other than transcription errors, does the laboratory document the reason for the correction?

✓ 5.4.12.2.4(b) Are all laboratory records retained for a minimum of 5 years from generation of the last entry in the records?

✓ 5.4.12.2.4(d) Has the laboratory established a record management system for control of laboratory notebooks, instrument logbooks, standards logbooks, & records for data reduction, validation, & reporting?

✓ 5.4.12.2.4(e) Does the laboratory have an access log to document access to archived information?

SAMPLE RECORDS

Does the laboratory maintain records of the following procedures & activities to which a sample is subjected while in the lab's possession:

- 5.4.12.2.5.1(a) Sample preservation, appropriateness of sample container, compliance with holding times
- 5.4.12.2.5.1(b) Sample identification, receipt, acceptance or rejection, & log-in
- 5.4.12.2.5.1(c) Sample storage & tracking, including transmittal forms (chain-of-custody form)
- 5.4.12.2.5.1(d) Documented procedures for receipt & retention of test items, including provisions to protect integrity of samples
- 5.4.12.2.5.2(b) Does the laboratory retain all original raw data, whether hard copy or electronic, for calibrations, sample analyses, & quality control measures?
- 5.4.12.2.5.2(h) Does the laboratory retain results of data review, verification, & cross checking procedures?

Does the laboratory's analytical records on strip charts, tabular printouts, computer data files, analytical notebooks, & run logs include the following essential information:

- 5.4.12.2.5.3(a) sample ID code
- 5.4.12.2.5.3(b) Data of analysis, & time of analysis if holding time is 72 h or less or when time critical steps are included in analysis (e.g., extractions & incubations)
- 5.4.12.2.5.3(c) Instrumentation identification & instrument operating conditions/parameters (or reference to such data)
- 5.4.12.2.5.3(d) Analysis type
- 5.4.12.2.5.3(e) All manual calculations
- 5.4.12.2.5.3(f) Analyst or operator initials/signature
- 5.4.12.2.5.3(g) Sample preparation
- 5.4.12.2.5.3(h) sample analysis
- 5.4.12.2.5.3(i) Standard & reagent origin, receipt, preparation, & use
- 5.4.12.2.5.3(j) Calibration criteria, frequency, & acceptance criteria
- 5.4.12.2.5.3(k) Data & statistical calculations, review, confirmation, interpretation, assessment, & reporting conventions
- 5.4.12.2.5.3(n) Method performance criteria including expected quality control requirements

ACCOMMODATION & ENVIRONMENTAL CONDITIONS

- 5.5.3.1 Are the laboratory's facilities for environmental testing, including energy sources, lighting, & environmental conditions, such as to facilitate correct performance of tests?
- 5.5.3.2 Does the laboratory provide for effective monitoring, control, & recording of appropriate environmental conditions (biological sterility, dust, electromagnetic interference, humidity, mains voltage, temperature, and sound & vibration levels)
- 5.5.3.2 Does the laboratory stop environmental tests when environmental conditions jeopardize the results of environmental tests?
- 5.5.3.5 Does the laboratory take adequate measures to ensure good housekeeping in the laboratory & to ensure that any contamination does not adversely affect data quality?

5.5.3.6 Does the laboratory's available work spaces ensure an unencumbered work area (access and entryways to the laboratory, sample receipt areas, sample storage areas, chemical & waste storage areas, data handling & storage areas)?

ENVIRONMENTAL TEST METHODS

5.5.4.1 Does the laboratory use appropriate test methods & procedures for all tests & related activities within its responsibility (sample collection, sample handling, transport & storage, sample preparation, sample analysis, estimations of uncertainty, statistical techniques)?

5.5.4.1 Are all instructions, standards, manuals, & reference data relevant to the work of the laboratory maintained up-to-date and readily available to staff?

5.5.4.1.1(c) Are standard operating procedures accessible to all personnel

5.5.4.1.1(e) Does each standard operating procedure indicate the effective date of the document, revision number, & signature of approving authority?

SELECTION OF TEST METHODS

5.5.4.2.1(a) Does the laboratory ensure that the latest valid edition of a standard is used, unless it is not appropriate or possible to do so

5.5.4.2.1(a) When necessary, is the standard supplemented with additional details to ensure consistent application

5.5.4.2.1(b) When the use of specific test methods for sample analysis are mandated or requested, does the laboratory use only those methods

CONTROL OF DATA

5.5.4.7.1(a) Has the laboratory established standard operating procedures to ensure that reported data is free from transcription & calculation errors

5.5.4.7.1(b) Has the laboratory established standard operating procedures to ensure that all quality control measures are reviewed & evaluated before data are reported

5.5.4.7.2(b) Has the laboratory established & implemented procedures for protecting electronic data - lock #15 after data entered. database - can't overwrite

5.5.4.7.2(d) Has the laboratory established & implemented appropriate procedures for maintenance of electronic data security (prevention of unauthorized access & unauthorized amendment of computer records) network is password protected

EQUIPMENT

5.5.5.1 Is the laboratory furnished with all items of equipment & reference materials required for the correct performance of tests for which accreditation is maintained

5.5.5.1 Does the laboratory ensure that equipment outside its permanent control meet these requirements

5.5.5.2 Does the laboratory calibrate and/or verify all equipment to establish that it meets specified requirements & complies with the relevant standard specifications before being put into service

5.5.5.2.1(b) Is support equipment calibrated or verified annually, using NIST-traceable references when available, over the entire range of use

✓ 5.5.5.2.1(d) Does the laboratory check balances, ovens, refrigerators, freezers, water baths with NIST-traceable references (where commercially available) prior to use on each working day in the expected use range

✓ 5.5.5.2.1(e) Are mechanical volumetric dispensing devices & burettes (except Class A glassware) check for accuracy on a quarterly use basis

✓ 5.5.5.3 Is equipment properly maintained, inspected, & cleaned

✓ 5.5.5.4 Is each item of equipment & software used for environmental testing, & significant to the result, uniquely identified

✓ 5.5.5.5 Does the laboratory maintain records of each major item of equipment and all reference materials significant to the environmental tests performed

-identity

-manufacturer's name

-serial number

-checks that equipment complies with specifications (5.5.5.2)

-current location

-manufacturer's instructions

-dates, results, & copies of reports & certificates of all calibrations, adjustments, acceptance criteria, due date of next calibration

-documentation of routine & non-routine maintenance activities & reference material verifications

-damage, malfunction, modification, or repair to equipment

-date received & placed into service

-condition of equipment when received (new, used, reconditioned)

✓ 5.5.5.7 Does the laboratory clearly identify, isolate, & remove from service any item of equipment that has been subjected to overloading or mishandling, gives suspect results, or has been shown by verification or otherwise to be defective

✓ 5.5.5.8 Is each item of equipment under the control of the laboratory & requiring calibration labeled, marked, or otherwise identified to indicate calibration status, including date when last calibrated and date or calibration criteria when recalibration is due

✓ 5.5.5.9 When equipment goes outside the direct control of the laboratory, does the laboratory ensure that the function & calibration status of equipment are checked & shown to be satisfactory before equipment returned to service

MEASUREMENT TRACEABILITY

✓ 5.5.6.1 Does the laboratory calibrate and/or verify all measurement operations & test equipment having an effect on the accuracy or validity of tests before being put into service and on an on-going basis

✓ 5.5.6.1 Does the laboratory have an established program for calibration & verification of its measuring & test equipment

✓ 5.5.6.2.1 Is the overall program of calibration and/or verification & validation of equipment designed & operated such that laboratory measurements are traceable to national standards of measurement

✓ 5.5.6.2.2 When traceability to International System of Units (SI) is not possible or not relevant, is there traceability to certified reference materials, agreed methods, or consensus standards

REFERENCE STANDARDS & REFERENCE MATERIALS

5.5.6.4(a) Does the laboratory retain records for all standards, reagents, & media including:

- manufacturer/vendor
- manufacturer's certificate of analysis
- date of receipt
- recommended storage conditions
- expiration date after which material shall not be used unless verified

5.5.6.4(b) Does the laboratory label the original containers of standards & reagents (provided by the manufacturer) with an expiration date

5.5.6.4(c) Does the laboratory maintain records on reagent & standard preparation

- traceability to purchased stocks or neat compounds
- reference to method of preparation
- date of preparation
- expiration date
- preparer's initials

5.5.6.4(d) Do all containers of prepared standards & reference materials bear a unique identifier, expiration date, & link to preparation record

5.5.6.5(f) Do containers of prepared reagents bear a preparation date

5.5.6.5(f) Is expiration date for each prepared reagent defined on the container or documented elsewhere as indicated in the laboratory's quality manual or SOP

SAMPLING

5.5.7.1 Where sampling (as in obtaining sample aliquots from a submitted sample) is carried out as part of the test method, does the laboratory use documented procedures & appropriate techniques to obtain representative subsamples

5.5.7.2 Are deviations, additions, or exclusions required by the client from the documented sampling procedure recorded in detail with the appropriate sampling data

5.5.7.3 Do sampling records include sampling procedure, sampler ID, environmental conditions, sampling location

HANDLING OF SAMPLES

5.5.8.1 Does the laboratory have procedures for transportation, receipt, handling, protection, storage, retention, and/or disposal of samples

5.5.8.2(a) Does the laboratory assign a unique ID code to each sample container received in the laboratory

5.5.8.2(b) Does this unique laboratory ID code maintain an unequivocal link with the unique field ID code assigned to each container

5.5.8.2(d) Is the laboratory ID code the link that associates the sample with related laboratory activities, such as sample preparation or calibration

5.5.8.1 Is the sample ID retained throughout the life of the sample

SAMPLE RECEIPT PROTOCOLS

5.5.8.3 Does the laboratory record the condition of the sample, including any abnormalities or departures from standard conditions as prescribed in the relevant test method, upon receipt at the laboratory

- ✓ 5.5.8.3.1(a) Does the laboratory check each sample for all items specified in its sample acceptance policy
- ✓ 5.5.8.3.1(c)(2) Are decisions to proceed with analysis of samples not meeting acceptance criteria fully documented
- ✓ 5.5.8.3.1(c)(2)(i) Does the laboratory note the condition of these samples on the chain-of-custody forms and on laboratory receipt documents
- ✓ 5.5.8.3.1(d) Does the laboratory utilize a permanent chronological record to document receipt of all sample containers
- ✓ 5.5.8.3.1(d)(1) Does the sample receipt log record the following:
 - client or project name
 - date & time of laboratory receipt
 - unique laboratory ID code
 - signature or initials of person making the entries
- ✓ 5.5.8.3.1(d)(2) during the log-in process, is sample collection information unequivocally linked to the log record or included as part of the log
- ✓ 5.5.8.3.1(d)(2)(i) Is the field ID code which identifies each sample container linked to the laboratory ID code in the sample receipt log
- ✓ 5.5.8.3.1(d)(2)(ii) Is the date & time of sample collection linked to the sample container and to the date & time of receipt in the laboratory
- ✓ 5.5.8.3.1(e) Does the laboratory retain all documentation that is transmitted to the laboratory by the sample transmitter
- ✓ 5.5.8.3.1(f) Does the laboratory maintain a complete chain-of-custody record

SAMPLE ACCEPTANCE POLICY

- ✓ 5.5.8.3.2 Does the laboratory have a written sample acceptance policy that clearly outlines the circumstances under which samples will be accepted
- ✓ 5.5.8.3.2.2 Does the sample acceptance policy include:
 - complete documentation (sample ID, location of sample collection, date & time of collection, collector's name, preservation type, sample type, special remarks)
 - Unique ID
 - durable labels and indelible ink
 - use of appropriate sample containers
 - adequate sample volume to perform necessary tests
 - procedures to be used if sample shows signs of damage, contamination, or inadequate preservation
- ✓ 5.5.8.3.2 Are samples that do not meet the laboratory's acceptance policy flagged in an unambiguous manner clearly defining the nature & substance of the variation
- ✓ 5.5.8.4 Does the laboratory maintain, monitor, & record any necessary specific environmental conditions whenever test items have to be stored or conditioned under such conditions
- ✓ 5.5.8.4(a) are samples stored according to the conditions specified by preservation protocols

ASSURING THE QUALITY OF ENVIRONMENTAL TESTS

5.5.9.1 Does the laboratory have quality control procedures to monitor the validity of environmental tests

- internal QC procedures
- participating in PT testing
- use of certified reference materials
- replicate testing using the same or different test methods
- re-testing of retained samples
- correlation of results for different parameters

ESSENTIAL QUALITY CONTROL PROCEDURES

Does the laboratory have detailed written protocols in place to monitor the following quality controls:

5.5.9.2(a)(1) adequate positive & negative controls to monitor tests (e.g., blanks, spikes, reference toxicants)

5.5.9.2(a)(2) Adequate tests to define repeatability and/or variability of laboratory results (e.g. replicates)

5.5.9.2(a)(3) measures to assure test method accuracy that include sufficient calibration and/or continuing calibrations, use of certified reference materials, & proficiency test samples

5.5.9.2(a)(4) Measures to evaluate test method capability (e.g. limit of detection & limit of quantitation) or range of applicability (e.g. linearity)

5.5.9.2(a)(6) Selection & use of reagents & standards of appropriate quality

5.5.9.2(b) Does the laboratory assess & evaluate all quality control measures on an on-going basis

5.5.9.2(b) Does the laboratory use quality control acceptance criteria to determine the usability of data

5.5.9.29(d) Are the quality control protocols specified by the laboratory's method manual followed

List of Suppliers

Consumables/Supplies/Services	Vendors
Chemicals	VWR, Sigma-Aldrich, Fisher, Hach, Spectrum, Turner Designs, Guildline Instruments, Supper Market (Na Hypochlorite 6%)
Reagent Storage Bottles	VWR
Sample Bottles	All American Containers, VWR
General Lab consumables	VWR , Misonix, YSI, Hach, WET Labs, Subchem Systems, Cole Palmer, Upchurch Scientific, Pall Filters, Irma Corp, Seal-Analytical, General Oceanics, Millipore, Westco Scientific instruments, Beckman-Coulter, Li-Cor, Air Gas, Lab Safety Supply, Brooklyn Thermometer Company
RO System Maintenance	SIEMENS (US Filter)
Balance Calibration Check	Quality Assurance Service or Certified Vender
Proficiency testing Samples	APG, RTC or NVLAP Certified vender

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Determination of Limits to Biological Loading Rate in Column Experiments with Florida Sandy Soils

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¹Florida Department of Health, ²University of Florida

Abstract. This study evaluated the effect of wastewater strength and water table separation on failure due to clogging in columns (lysimeters) representing four common Florida drainfield configurations. 107 cm (42") long soil/fill profiles were packed into 20 cm (8") diameter vented columns and covered by 15 cm (6") of gravel. Testing conditions included three different wastewater strengths (average concentrations: low: CBOD₅= 99 mg/L; TSS= 48 mg/L; Oil&Grease =13 mg/L; medium: CBOD₅= 308 mg/L; TSS= 112 mg/L; Oil&Grease =31 mg/L; high: CBOD₅= 640 mg/L; TSS= 164 mg/L; Oil&Grease =50 mg/L), with concentrations based on results of previous field sampling of restaurant wastewaters. Four different hydraulic loading rates, ranging from 2.6 to 4.9 cm/day (0.65 to 1.2 gpd/sqft), were employed, representing code requirements for each drainfield configuration. Two saturation conditions were included, 0.6 m (2ft) and 0.3 m (1 ft) separation from the water table. Each of these conditions was evaluated in triplicate to equal 72 individual columns. Columns were dosed twice daily with synthetic wastewater. Dosing was continued for 250 days or until failure, which was defined as ponding 15 cm (6") above the infiltrative surface at the time of the next morning dose.

Results suggested that wastewater strength was the most important factor determining failure. None of the columns loaded with low strength wastewater failed. 63% of the columns loaded with medium strength wastewater failed over the course of the study, and 83% of the columns loaded with high strength wastewater failed. When wastewater strength (CBOD₅+TSS) and hydraulic loading were combined into a biological rate, the columns showed no failures below 8 g/sqm day (0.0016 lbs/sqft day) and failures beyond 10 g/sqm day (0.002 lbs/sqft day) of CBOD₅ and TSS.

Keywords. Hydraulic loading rates, wastewater, long-term acceptance rate, mass loading rate, clogging, drainfield failure

Introduction

The functioning of drainfield systems requires that the movement of wastewater to the infiltrative surface does not exceed what can be processed long term without failing (Kropf et al., 1977). Failure can occur if the soil limits infiltration or if the drainfield has become clogged. Clogging occurs when the supply of clogging material, from effluent entering the drainfield and from microbiological growth, exceeds what is removed by decay and flow out of the drainfield. The clogging is usually associated with the formation of a thick biofilm or biomat at the interface between the trench and the soil material.

Design of drainfields commonly only considers hydraulic factors. This tradition-based approach may not be suitable if wastewater composition or operating conditions are not comparable to historical averages of residential wastewater (Otis, 1985; Siegrist, 1987). Restaurants produce higher concentrations of pollutants in wastewater (Siegrist et al., 1985; Matejcek et al., 2000; Lesikar et al., 2004).

The objective of this study was to determine the influence of variations in effluent concentrations, water table separation distance, and hydraulic loading rates on occurrence of failures. The study simulated four common Florida drainfield profiles in columns. Three strengths of artificial wastewater were applied under two water table separation regimes. Results presented here are based on work performed at the University of Florida by Brian Matejcek (Matejcek et al., 2000) and Steven Erlsten (Erlsten and Bloomquist, 2001).

Materials and Methods

Soil Selection

The study included three soils and two fill materials. Two well drained sandy soils, Candler and Millhopper, are commonly found on the Central Florida Ridge land resource area. A poorly drained flatwood soil, Pomona, requires usually a mound system due to a seasonal high water table. Two different types of fill material commonly used for mound systems, a Candler loamy sand and an Astatula fine sand, were studied. These were placed above the soil layers normally left intact on a Pomona soil when a mound is constructed.

Soil materials for the three soils were obtained from sites in Alachua County, Florida. Each soil series was verified using a 10 cm (4") diameter soil auger. The Pomona soil series (fine sand) was excavated from the Austin Cary Memorial Forest located north of Gainesville. The Candler soil series (fine sand) was collected from a farm located east of Gainesville. Millhopper (sand to loamy sand) was excavated from the

University of Florida Natural Area Testing Laboratory. The Candler fill (loamy sand) was excavated from a depth greater than 275 cm (109") in a sandpit used by Florida Septic, Inc, near the Town of Interlachen in Putnam County. Astatula fill (fine sand to very fine sand) was obtained in Clearwater in Pinellas County at a residential drainfield replacement by AA Cut Rate Septic Service.

A small vertical trench was excavated at each soil collection site. The trench revealed the soil profile and determined the number of horizons to be collected. Digging then proceeded in a horizontal direction rather than vertical. The top 41 cm (16") of soil was scraped and discarded. The second soil horizon was scraped horizontally and placed in sandbags. The original trench depth was then increased. The next horizon of soil adjacent to the trench was then scraped and bagged. This process continued until 60 sandbags of soil had been collected with a 107 cm (42") profile depth or until the trench was 147 cm (58") deep. Before use, all soil was air-dried and sieved using a screen equivalent to a #15 sieve to remove roots and similar debris. Samples from each horizon and fill layer were sieved to obtain grain size distributions. The sieve analyses showed little difference between Candler fill, Astatula fill, and Candler soil.

Column Construction

The experimental setup included four soil/fill configurations, two water table separation conditions, and three strengths of wastewater. Each of these conditions was repeated in triplicate to equal 72 individual columns. In addition, one control column was constructed for each soil/fill configuration.

Half of the columns modeled absorption trench systems with loading rates of 4.9 cm/day (1.2 gal/sqft) appropriate for the Candler columns and 3.7 cm/day (0.9 gal/sqft) as required by code for Millhopper soils (Florida Administrative Code, Chapter 64E-6.008). The other half simulated mound systems packed with a 66 cm (26") soil profile and 41 cm (16") of fill material. Either fine sand (Astatula fill) or loamy sand (Candler fill) was packed above the Pomona Soil series as separate types of columns. The column loading rates of Pomona with loamy sand fill was 2.6 cm/day (0.65 gal/sqft) and Pomona with fine sand fill was 3.3 cm/day (0.80 gal/sqft) as per Florida Administrative Code, Chapter 64E-6.009.

The column diameter was chosen to be 20 cm (8"). Interior walls were coated with an epoxy and sand mixture to reduce the possibility of water channeling. A 23 cm (9") multi-layered drainage system composed of aggregate and sand with decreasing grain size from drainfield aggregate (20 to 26 mm diameter) to a medium grit sand (0.5 to 1.25 mm diameter) was placed in the bottom of each column. This system retained the soil in the columns but allowed water to flow through the media.

Soil columns represented 107 cm (42") of suitable soil and fill below the infiltrative surface. Soils were dried, sifted, mixed by hand shovels, weighed and poured into each column through a large funnel. Packing took place in increments varying between 10 and 15 cm depending on the actual depth of each soil horizon as measured in the field (Figure 1). Soil densities measured in the field were replicated. The columns were topped off with 15 cm of #5 limestone representing the aggregate located below the drainfield discharge pipe. Construction details are given by Matejcek et al. (2000).

Clear vinyl tubing was used to set the water table height in the columns and for collection of column effluent (Figure 1). A 1.3 cm (1/2") hole was drilled in the bottom of each column for a connector capable of coupling 0.95 cm (3/8") through 1.3 cm (1/2") vinyl tubing. A tee-connector was placed at the required water table level on the column. The tubing extended from the column bottom-drain up to the tee-connector and from there down to a 3.8 L (1 gallon) container. A final length of vinyl tubing extended from the tee-connector to the top of the column and prevented the formation of a siphon.

Air ports were installed to prevent the column walls from creating an anaerobic boundary and to simulate the horizontal flow of oxygen present in actual field conditions. Three symmetrical 4.8 cm (1-7/8") holes were drilled into each pipe to receive 3.8 cm (1 1/2") PVC elbow joints (air ports) located at mid depth of the unsaturated zone (Figure 1). The columns with 0.6 m of unsaturated soil conditions had air ports at approximately 25 cm (10") above the water table. Air ports for the 0.3 m unsaturated conditions were located at approximately 10 cm (4") above the water table.

Two constant head tests were performed on each soil column. Adjusted to a temperature of 20°C, the mean hydraulic conductivity and standard deviation for columns with Candler, Millhopper, Pomona with Candler Fill, and Pomona with Astatula Fill were 1.24, 0.65, 0.59, 0.53 and 0.09, 0.04, 0.06, 0.05 cm/min, respectively.

Synthetic Wastewater

Columns were dosed with synthetic wastewaters in three strengths with concentrations based on results of previous field sampling of restaurant wastewater. The daily dosing requirement of wastewater was 30 L per day for each of the three wastewater strengths or per 24-column set. Consideration of feasibility of obtaining three different wastewater strengths from three different field sites and the variability of wastewater strengths at field sites over time led to the decision to use synthetic wastewater for the column study. The synthetic wastewater mix was composed of Armour SPAM™, Crisco® Vegetable Oil, Purina® Brand Dog Food and dextrose. Each component was individually tested four times for CBOD₅, TSS and

O&G. The results were used to determine a recipe that would result in the desired wastewater composition. Details of the methods used to produce the synthetic wastewaters can be found in Matejcek et al. (2000)

In a subsequent follow-up phase, three wastewater strengths at the lower end of the previous range were dosed on the 24 columns that previously had been dosed with low strength wastewater to investigate the existence of failures in this range. Details of the methods used to produce these synthetic wastewaters can be found in Erlsten and Bloomquist (2001).

Wastewater Dosing

The columns were dosed twice a day with synthetic wastewater, once in the morning and again in the evening. Columns were dosed in numerical order and daily doses each began at different ends of each waste strength category. The purpose of dosing in ascending and descending numerical order was to prevent any column from constantly receiving either a diluted or concentrated dose. Dosing started on March 10, 2000, and continued through June 30, 2000. Dosing continued for another 138 days, but during this time, no synthetic wastewater concentrations, effluent concentrations, or temperatures were measured. Following 250 days of study, dosing was continued for low strength columns only. A follow-up study phase began after 426 days on May 10, 2001 for 90 days with new concentrations dosed on previously low strength dosed columns. Control columns were dosed with tap water.

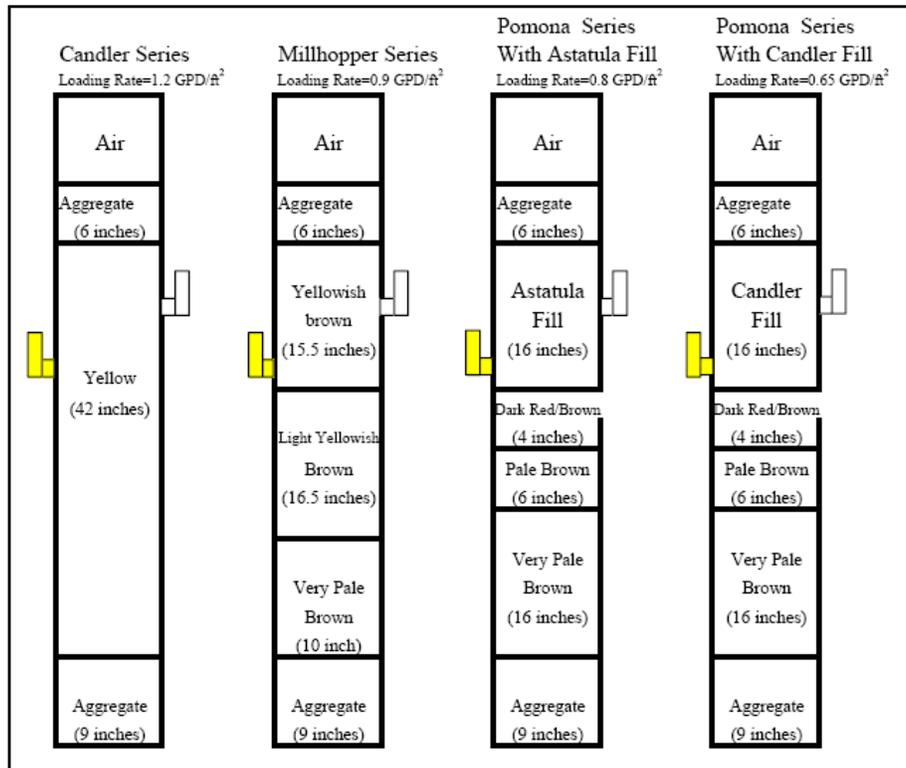


Figure 1. Schematic soil profiles for column study. Left ports indicate air port position in 0.6 m water table separation columns, right ports indicate position in 0.3 m water table separation columns. From Matejcek et al. (2000).

Column Observations

Failures were recorded before the morning dose based on the water level of wastewater being above the drainfield aggregate or 15 cm (6") above the soil surface. The aggregate line represents the location of the simulated discharge pipe. If this condition were to occur in a restaurant situation, it would begin to backup sewage into the septic tank.

Column discharge flowed into 3.8 L (1 gallon) effluent containers. The discharge volume was measured every two days as an estimate of the soil column's ability to process the effluent placed on top of it. Column discharge for 12 columns per week was analyzed for CBOD₅ and TSS between day 23 and day 96. Clean 3.8 L (1 gallon) containers replaced the effluent containers for the 24-hour sample collection period. The daily minimum, maximum and current temperatures were recorded on two opposite sides of the room where the columns stood.

Results and Discussion

Column Construction

A total of 72 columns were built and packed with four soil/fill configurations under two water table separation conditions with triplicate columns. Soil appeared in the effluent of one column (#49) and impeded flow through the tubing, therefore this column was excluded from further analysis. One additional column for each soil/fill configuration was constructed as control and dosed with tap water; these controls did not fail.

Synthetic Wastewater

A total of 64 samples of the synthetic wastewater were analyzed for CBOD₅ and TSS and 35 samples were analyzed for O&G (Table 1). Considerable variability of concentrations occurred even though the recipe was the same. The relatively largest difference between target and measured concentrations was for the high strength of oil and grease. This appeared to be due to particles in the wastewater adhering visibly to the sides of the batch container, the magnetic stir bar and the spinning surface for the stir bar. In addition, scum lines formed on the interior walls of the batch container marking the water line after each dose. Some particles were visible on top of the aggregate after dosing, suggesting that not the entire high strength wastewater concentration contacted the infiltrative surface. The medium strength container had less distinct water lines and particle loss than the high strength. Within each waste strength there was no correlation between CBOD₅ and TSS ($R^2 < 0.1$). The highest but still small correlations existed between TSS and O&G for the high ($R^2 = 0.39$) and medium ($R^2 = 0.29$) waste strength. This suggests independence between analyte measurements. The large variability of measurements resulted overall in low correlations between TSS and O&G ($R^2 = 0.67$), CBOD₅ with O&G ($R^2 = 0.57$), and CBOD₅ with TSS ($R^2 = 0.53$).

Table 1. Target and measured concentrations of synthetic wastewater (n/a =not analyzed).

Parameter	CBOD ₅ (mg/L)			TSS (mg/L)			O&G (mg/L)		
	High	Med	Low	High	Med	Low	High	Med	Low
Target	712	325	112	181	90	39	92	41	14
Mean	640	308	99	164	112	48	50	31	13
Std Dev	144	115	39	62	28	12	17	13	4
Median	628	299	91	168	113	50	49	30	12
N	21	22	21	21	22	21	11	14	10
Follow-Up Study									
Mean	319	245	151	94	53	23	n/a	n/a	n/a
Std Dev	34	21	27	16	26	8	n/a	n/a	n/a

Water table changes during dosing

Water did not enter the air ports in columns with a 0.6 m water table separation and approximately 25 cm (10") of soil between the imposed water table and air port. Water entered the air ports of all columns with 0.3 m water table separation within 15 minutes after every dose. The water level in the air ports rose approximately eight cm (3") before seeping back into the column. This indicates that at the level of the air port, approximately ten cm (4") above the imposed water table, water saturation in the capillary fringe was sufficient to allow the dose to displace water instead of air. This may have lessened the effectiveness of the air port to provide air to the unsaturated zone. The intermittent rise in water table by about 18 cm (7") is consistent with the average dosing rate of 1.7 cm/dose and an effective porosity of 0.10 in the unsaturated zone, the remainder being taken up by water in the capillary fringe and air.

Failures

Indications of incipient failure started one to two weeks before actual column failure. During this period, water at or near the aggregate line was observed after the evening dose but percolated through the soil overnight. Water was never noticeable on top of columns operating without failure. Another indicator of failure was column discharge, which showed a sudden decrease immediately prior to failure.

The cumulative number of failures, in figure 2, shows an initial period of frequent failures starting at day 20, followed by a gradual increase in the number of failures between days 50 and 250. This behavior could be expected from a clogging mechanism where an initial rapid reduction of conductivity is followed by a more gradual decline (Otis, 1985). The observation is confounded by the coincidence that temperature measurements also showed a marked increase during the time of this transition. Temperatures in the experimental building increased from a daily mid-range of about 24°C (75°F) to 28°C (82°F) starting around day 50 or April 29, 2000 and remained at that level through day 112. Temperatures were reported graphically by Matejcek et al. (2000) and are not shown here. Table 2 lists the columns and the time to failure. Even without further statistical analysis, it is striking that no column loaded with the low strength wastewater failed.

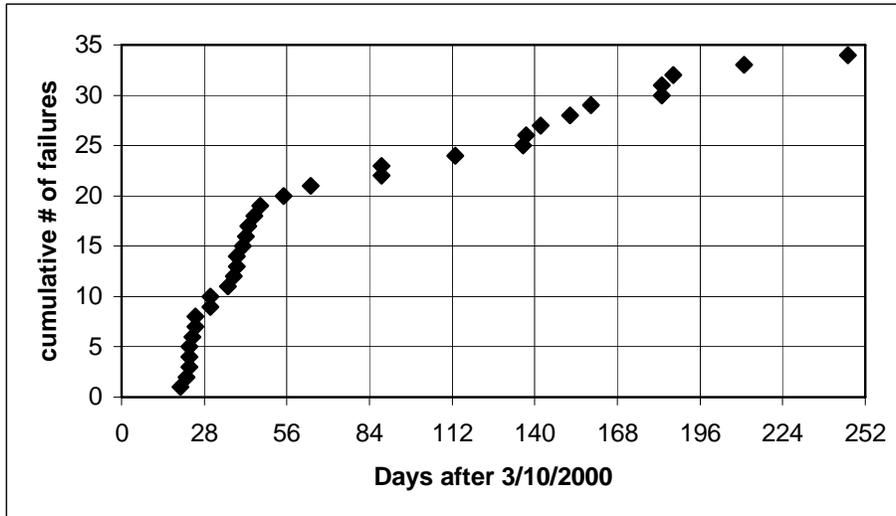


Figure 2. Cumulative number of failures over the course of the study (71 columns).

The cumulative failure curve (figure 2) and the difference in temperatures suggested two time frames for statistical analysis: Early failures through 50 days, and all failures through the end of the experiment. The complete absence of failures at the lowest wastewater strength precluded a statistical analysis of this strength category. Significance of factors that influenced the occurrence of failures for the medium and high wastewater strengths was assessed with multinomial logistic regression (SPSS 12.0, SPSS, Inc. 2003). These regressions on failures of the medium and high strength columns indicated that water table separation was an important factor. After 50 days, 18 columns had failed for the 0.6 m water table separation, compared to 1 failure for 0.3 m water table separation. After 250 days, 21 of 24 columns had failed for the 0.6 m water table separation, but only 13 of 23 columns for the 0.3 m water table separation. No consistent pattern emerged during a more detailed analysis, indicating that other factors did not have a consistent influence for the two water table separation conditions.

For the 0.3 m water table separation alone and medium and high strength wastewater no factor was significant to predict the failure of one medium strength Pomona/Candler column among the 23 columns within the first 50 days. For failures throughout the experiment, wastewater strength was a significant factor, which increased the odds of failure by a factor of 30 between high and medium strength. 10 of 11 high strength columns failed but only 3 of 12 medium strength columns. The remaining high strength column was Pomona with Candler fill, the same drainfield type as two of the three failed medium strength columns.

Table 2. Observed failures and time until failure for columns with 0.3 m and 0.6 m water table separation.

Soil(/fill)	Hydraulic load (cm/d)	Hydraulic load (gpd/sqft)	Waste strength category	CBOD ₅ load (g/m ² d)	TSS load (g/m ² d)	0.3 m days to failure	0.6 m days to failure
Pomona/ Candler	2.6	0.65	Low	2.6	1.3		
Pomona/ Candler	2.6	0.65	Low	2.6	1.3		
Pomona/ Candler	2.6	0.65	Low	2.6	1.3		
Pomona/ Astatula	3.3	0.8	Low	3.2	1.6		
Pomona/ Astatula	3.3	0.8	Low	3.2	1.6		
Pomona/ Astatula	3.3	0.8	Low	3.2	1.6		
Millhopper	3.7	0.9	Low	3.6	1.8		
Millhopper	3.7	0.9	Low	3.6	1.8		
Millhopper	3.7	0.9	Low	3.6	1.8		
Candler	4.9	1.2	Low	4.8	2.3		
Candler	4.9	1.2	Low	4.8	2.3		
Candler	4.9	1.2	Low	4.8	2.3		
Pomona/ Candler	2.6	0.65	Medium	8.2	3.0		38
Pomona/ Candler	2.6	0.65	Medium	8.2	3.0	42	47
Pomona/ Candler	2.6	0.65	Medium	8.2	3.0	55	25
Pomona/ Astatula	3.3	0.8	Medium	10.0	3.6		36
Pomona/ Astatula	3.3	0.8	Medium	10.0	3.6		183
Pomona/ Astatula	3.3	0.8	Medium	10.0	3.6		41
Millhopper	3.7	0.9	Medium	11.3	4.1		30
Millhopper	3.7	0.9	Medium	11.3	4.1		22
Millhopper	3.7	0.9	Medium	11.3	4.1		39
Candler	4.9	1.2	Medium	15.1	5.5	142	113
Candler	4.9	1.2	Medium	15.1	5.5		23
Candler	4.9	1.2	Medium	15.1	5.5		25
Pomona/ Candler	2.6	0.65	High	16.9	4.3	Sand in effluent	23
Pomona/ Candler	2.6	0.65	High	16.9	4.3		23
Pomona/ Candler	2.6	0.65	High	16.9	4.3	211	20
Pomona/ Astatula	3.3	0.8	High	20.9	5.3	159	88
Pomona/ Astatula	3.3	0.8	High	20.9	5.3	183	
Pomona/ Astatula	3.3	0.8	High	20.9	5.3	88	
Millhopper	3.7	0.9	High	23.5	6.0	187	
Millhopper	3.7	0.9	High	23.5	6.0	137	24
Millhopper	3.7	0.9	High	23.5	6.0	246	30
Candler	4.9	1.2	High	31.3	8.0	152	39
Candler	4.9	1.2	High	31.3	8.0	136	43
Candler	4.9	1.2	High	31.3	8.0	64	45

For the 0.6 m water table separation and medium and high strength wastewater, the drainfield profile, but not the hydraulic loading rate, was the strongest predictor for failures up to 50 days, with all six Pomona/Candler columns with the lowest hydraulic loading rates being among the 18 of 24 failed columns. Four of six Pomona/Astatula columns with the second lowest hydraulic loading rate had not failed, this was only significant at the P=0.1 level. After 250 days wastewater strength was the stronger predictor. The only three of 24 medium and high strength columns that had not failed were high strength columns, two with Astatula fill and one with Millhopper sand. The longer survival of high strength wastewater columns was unexpected.

Effluent Concentrations

Between day 23 and day 96, 153 samples were analyzed for TSS and 151 samples were analyzed for CBOD₅. Measurements for CBOD₅ suffered from quantitation problems, many sample results were given as below or above variable quantitation limits. To utilize samples with relatively low and high CBOD₅ concentrations the following numbers were assigned: Samples given as less than a quantitation limit of 8 mg/L or below were assigned the value of that limit. Samples given as above a quantitation limit of 40 mg/L or above were assigned that value. 21 samples with either quantitation limit between 9 and 40 mg/L were not used in the following assessment.

The results for both analytes were not normally distributed. The median values for CBOD₅ and TSS were 6 mg/L and 8 mg/L, respectively, while the 90th-percentiles were 72 mg/L and 48.4 mg/L. The non-parametric Kruskal-Wallis test served to determine significance of differences between relatively high and low effluent results. As had been the case for failure rates, water table separation was a significant influence.

Samples from low strength columns showed very low effluent concentrations. For low strength samples with a water table of 0.6 m, samples before 50 days showed median concentrations of 2 mg/L TSS and 3 mg/L CBOD₅ and 0 mg/L TSS and 3 mg/L CBOD₅ after 50 days. Low strength samples with a water table of 0.3 m were not obtained before 50 days. At later times these columns showed a median TSS of 1 mg/L and 3 mg/L CBOD₅, with Millhopper showing significantly higher TSS values (11.5 mg/L median of four samples).

For column effluent samples from medium and high strength columns, median results categorized by water table separation and drainfield profile are listed in table 3. TSS varied significantly by drainfield profile both for early and later data for the 0.3 m water table separation and for early data for the 0.6 m water table separation. Pomona/Candler had the lowest concentrations while Millhopper had the highest concentrations for either water table separation. TSS values differed less significantly between medium and high wastewater strength.

CBOD₅ for the 0.3 m water table separation varied significantly by drainfield profile (early data only) and by wastewater strength. For early data medium strength effluent had a median of 22 mg/L CBOD₅ (n=4) and high strength a median of 87 mg/L (n=18). For later data the median for medium strength effluent was practically unchanged at 21 mg/L (n=22), and the median for high strength was lower at 45 mg/L CBOD₅ (n=11). CBOD₅ did not vary significantly by either wastewater strength or soil type for the 0.6 m water table separation, with a median in all cases around the lower quantitation limits.

This suggests that a 0.6 m water table separation removes nearly all TSS and CBOD₅, but about 15% of the incoming medium and high strength CBOD₅ and about 10% of incoming TSS remain in the effluent with a 0.3 m water table separation. Both water table separations were similar effective in treating low strength wastewater.

Table 3. Median effluent concentrations (number of samples) for medium and high strength columns

Time Water Analyte	Between t=23 days and t=50 days				Between t=50 days and t=96 days			
	WT= 0.3 m TSS* (mg/L)	CBOD ₅ * (mg/L)	WT= 0.6 m TSS** (mg/L)	CBOD ₅ (mg/L)	WT= 0.3 m TSS* (mg/L)	CBOD ₅ (mg/L)	WT= 0.6 m TSS (mg/L)	CBOD ₅ (mg/L)
Pomona/ Candler	5(5)	31(5)	4(5)	6(4)	8(10)	24(7)	All failed	All failed
Pomona/ Astatula	16(4)	166(4)	5(11)	5(9)	10(18)	28(13)	2(12)	3(12)
Mill- hopper	59(9)	80(5)	33(7)	5(6)	30(14)	31(7)	3(1)	n/s
Candler	23(6)	58(5)	5(10)	7(9)	30(11)	30(6)	3(2)	3(2)
All	35(24)	67(22)	5(33)	6(28)	14(53)	31(33)	2(15)	3(14)

n/s =not sampled * P<0.05 ** P<0.1

Follow-up study

During the 90 days of dosing with three strengths ranging between the former medium and low strength, only one column failed, which was a Candler soil with an intermediate strength, and 0.3 m separation to the water table. This experiment lasted not as long as the main study and the observed failure rate is therefore a low estimate for the number of failures that can be expected to occur over a time period of 250 days.

Limits to the biological loading rate

This study showed that the strength of wastewater was a critical factor to cause failure given the regulatory assigned hydraulic loading rate for the studied drainfield profiles. In order to transfer this information to somewhat different situations, a biological loading rate is suggested that includes the mass loading rate of CBOD₅ and TSS combined (Laak, 1970). A simple addition of CBOD₅ and TSS is suggested here until further studies elucidate a more accurate weighting. This biological loading rate is similar to a contaminant mass loading rate discussed by Otis (1985) or Siegrist and Boyle (1987) but does not consider ultimate CBOD₅ or nitrogenous oxygen demand as they did. One should note that this study included synthetic wastewater with a CBOD₅ to TSS ratio from about three to five.

This study showed that below a biological loading rate of 8-10 g/sqm day (0.0016-0.002 lbs/sqft day) no failures occurred and that beyond this biological loading rate the likelihood of failure increases markedly (table 4). The failure of one Candler column loaded at 14.6 g/sqm day (0.003 lbs/sqft day) during the shorter follow-up study confirms such a threshold.

The threshold identified in this study for the biological loading rate of sandy material under a gravel drainfield generally agrees with results from other studies. Walsh et al (2006) observed continuous ponding in unsaturated columns filled with medium to coarse sand with a biological loading rate of 8.3 g/sqm day at a much higher hydraulic loading rate (20 cm/day). Lowe et al. (2006) found that gravel drainfield test cells in sandy loam loaded with 8 and 15 g/sqm day ponded 20 cm within three years but continued to operate with a loading rate of 4 g/sqm day. Siegrist and Boyle (1987) found that it took more than two years before ponding began and about four before failure (15 cm ponding) occurred with a biological loading rate of 10.9 g/sqm day in lysimeters installed in silty clay loam.

Cumulative mass loading has been suggested as a predictor for failure (Laak, 1970; Siegrist, 1987; Siegrist and Boyle, 1987). If this was the case here, then one would expect the high strength wastewater columns to fail faster than the medium strength wastewater columns. This study did not show a significant difference in time to failure between medium and high wastewater strengths (Kruskal-Wallis H, P=0.05), neither overall, nor distinguished by water table separation. None of the low-strength columns failed, which should have occurred if cumulative loading was an important factor. Cumulative loading may be a more important parameter for a much higher loading rate than that identified here or for longer observation periods.

The wastewater was applied in two doses each day. Dosing allows air into the soil between doses, which has been observed to allow higher loading rates than that found in continuously wetted drainfields (e.g. Otis, 1984; Siegrist, 1987). Due to this effect, the long term acceptance rates found in this study are most applicable to dosed drainfields and could be lower in gravity-fed drainfields.

Table 4. Biological loading rates and failure rates from column testing.

Soil type	Pomona/ Candler	Pomona/ Astatula	Millhopper	Candler
Field-estimated texture	Loamy sand	fine sand	sand	fine sand
Sieve analysis top layer: D10(mm); D60(mm)	0.17; 0.32	0.16; 0.23	0.17; 0.35	0.17;0.33
Hydraulic load (cm/d)	2.6	3.3	3.7	4.9
Hydraulic load (gpd/sqft)	0.65	0.8	0.9	1.2
Low strength biological loading rate CBOD ₅ +TSS (g/sqm d):	3.9	4.8	5.4	7.2
Failure rate (%) within 250 days	0	0	0	0
Medium strength biol. loading rate CBOD ₅ +TSS (g/sqm d):	11.1	13.7	15.4	20.5
Failure rate (%) within 250 days	83	50	50	67
High strength biological loading rate CBOD ₅ +TSS (g/sqm d):	21.3	26.2	29.5	39.3
Failure rate (%) within 250 days	80	67	83	100

This study extended over a period of 250 days and failures occurred throughout the last 230 days of this study. Beyond that, studies have found that reductions in infiltration rates and clogging developed over periods of years (Siegrist and Boyle, 1987; Lowe et al., 2006). In contrast, a shorter study of restaurant and household sewage had shown that biological loading rates above 30 g/sqm day lead to failure within 30 days but biological loading rates of 12 and 21 g/sqm day did not fail within 67 days (Siegrist et al. 1985). This suggests that the threshold suggested by this study should be reduced in design applications to account for more long-term effects. Similarly, Siegrist (1987) recommended a design limit of about 6 g/sqm day for mass loading rates based on total BOD₅, which would equal 2-3 g/sqm day for biological loading rates as defined here.

Conclusions

This study investigated failure due to clogging in column models of four drainfield profiles. 72 columns were constructed that addressed variations in wastewater strength, soil types, and water table separation. Failures began to occur within three weeks and additional failures occurred throughout the study.

We found wastewater strength to be an important factor. No columns failed at the lowest wastewater strength, most columns failed when dosed with medium and high wastewater strength. For similar wastewater types we suggest a biological loading rate that simply adds CBOD₅ and TSS. When this biological loading rate exceeded a value of around 8-10 g/sqm day (0.0016-0.002 lbs/sqft day) risk of failure occurred in the study. This is in agreement with similar previous studies.

Water table separation appeared to be of lesser importance. Fewer columns with 0.3 m water table separation failed, this was due to fewer medium strength columns with 0.3 m water table separation failing compared to columns with 0.6 m water table separation. The narrow range of hydraulic loading rates investigated did not have a consistent effect on failure observations.

At low wastewater strength both the 0.3m and the 0.6m water table separation columns achieved nearly complete removal of CBOD₅ and TSS. At the higher wastewater strengths the 0.6 m water table separation continued to be effective at that level, but the 0.3 m water table columns typically discharged about 10% of loaded TSS and 15% of loaded CBOD₅ concentrations.

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Optical Brightener Detection for Tracking Wastewater Contributions to Coastal, Estuarine, and Freshwater Systems

Task 2 Summary Report

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EXECUTIVE SUMMARY

Accurately quantifying inputs of anthropogenic wastewater to coastal, estuarine, and freshwater systems has been an ongoing challenge for federal, state, and local governments. Recently a field method has been developed that uses fluorescence to detect the presence of optical brighteners in the water column. Optical brighteners, found in most laundry detergents, fluoresce at a specific wavelength and do not occur in nature. Therefore positive identification of optical brighteners in water can provide indisputable evidence of human sources. This project was designed to evaluate the potential of using this technique in-situ and via aircraft remote sensing. Before funds are committed to airborne Light Detection and Ranging (LiDAR), an extensive characterization and reconnaissance of several locations along the Florida Gulf Coast was initiated. This report summarizes the results of this characterization and reconnaissance and provides a go-no-go recommendation for airborne LiDAR. There are several technological challenges in using optical brightener fluorescence for effluent source tracking. For example, much of the original development of this technique was accomplished in parts of the world where dissolved organics make up a relatively small portion of the water column. Along the Gulf Coast, however, dissolved organics are prevalent and are what turn water the color of iced tea. In order to account for this interference, fluorescence of both optical brightener and colored dissolved organic matter, known as CDOM, were measured and the ratio of the two measurements was calculated. To link the optical brightener signal to bacteriological indicators, a weight of evidence approach was employed by collecting bacteriological samples with fluorescence measurements. Sampling took place in coastal Taylor County, the Chassahowitzka River in Citrus County, and in Phillippi Creek in Sarasota County. Samples from onsite sewage treatment disposal systems (OSTDS) and wastewater treatment plants were also collected. Optical brightener fluorescence was measured using a flow-through fluorescence technique as well as by collecting individual grab samples at fixed stations concurrent with bacteriological sampling. Another method of optical brightener detection using cotton pads deployed in the field was used but did not yield any positive results. Bacteriological indicators did not correlate well with optical brightener partly because many of the bacteriological samples were at or below detection limits. Indicators above the laboratory detection limit showed no clear correlation with fluorescence. As a screening tool, the flow-through fluorescence method showed the most promise and revealed some potentially interesting patterns. This was especially true in Phillippi Creek where increases in optical brightener signal corresponded to locations where failed septic systems were known to exist. The canal system above the main spring of the Chassahowitzka River also showed some interesting patterns. Sites in Taylor County were difficult to interpret because of salinity interferences.

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INTRODUCTION

Environmental and public health agencies are interested in new techniques for measuring and mapping the spatial extent of effluent from septic tanks, or onsite sewage treatment and disposal systems (OSTDS), and wastewater treatment plants (WWTP) discharging into natural waters. This goal of this project investigates the potential of using an in-situ technique for detecting optical brighteners using a dual-channel fluorescence approach (Dixon et al. 2005). Optical brighteners (OB) are fluorescent whitening agents used in many commonly used laundry detergents (Wayne 2003). These compounds are unique in that they emit light in the blue range (400 – 440 nm) when excited by wavelengths in the near ultraviolet range (360 – 365 nm) (Hagedorn et al. 2005). Unfortunately, detecting optical brighteners in water is complicated by the presence of colored dissolved organic matter (CDOM) that fluoresces at the same wavelength as OB. On the other hand, peak fluorescence for OB is at 440nm with a relatively narrow band width and is minimal at 550nm (Dixon and Julian 2005). One way to account for the interference in CDOM is to measure fluorescence at two wavelengths and take the ratio of the two (Dixon et al. 2005). This can be done in the laboratory or in the field. We employed a field approach using two field deployable fluorometers and a sample pump. This flow-through fluorescence (FTF) approach provides a cost-effective method of providing very high-resolution data in real-time. When these data are mapped, spatial patterns can be seen and potential hot spots can be identified. This tool may be very useful in guiding confirmatory sampling of bacteriological indicators that are much more labor intense and costly to run.

The intent of this project was to test an optical brightener method of effluent source tracking and determine the feasibility of scaling this technique up to airborne remote sensing using Light Detection and Ranging (LiDAR). To this end, a separate project evaluating the use of a more detailed fluorescence analysis known as Excitation-Emission Matrix (EEM) fluorescence (Dixon and Buehler 2007) was contracted by the Florida Department of Health. Results from that project are forthcoming. The FTF approach would be the primary technique used to ground-truth the airborne survey. This is because FTF allows rapid data collection at spatial resolutions not possible using traditional grab samples.

While airborne remote sensing provides a synoptic picture of large areas, there are some challenges that must be overcome in order to make this technique work. Because of interference, especially from salinity and color dissolved organic matter (CDOM), adequate ground-truth data must be collected, especially in areas where high colored dissolved organic matter (CDOM) and salinity variability exists.

Because of the complex nature of fluorescence in surface waters, especially estuarine and coastal waters, significant “proof-of-concept” testing must take

place before scaling up to an airborne approach. This task and the companion project by Mote Marine Laboratory (Dixon and Buehler 2007) were designed to provide both field and laboratory testing of the optical brightener method. This report summarizes only the work completed under this task. Results from the Mote Marine Laboratory Study is summarized in a separate report (Dixon and Buehler 2007).

METHODOLOGY

Site Locations

Because this was a proof of concept project, we selected sites that would likely have OB fluorescence in the water as well as sites that would not. Three broad areas of interest were chosen along the Florida Gulf Coast (Figure 1). A priori information was used as much as possible in order to conduct testing in a semi-controlled fashion. The experimental design also called for areas where no information was known as to the status of failing septic systems or wastewater distribution lines. The design also called for a range of salinities from estuarine to fresh. The canal systems of Keaton Beach and Dekle Beach, in Taylor County, are located directly on the Gulf of Mexico and therefore represented the most saline systems (18.00 – 30.00psu). The upper Chassahowitzka River, although tidally influenced, was mostly fresh (0.15psu – 3.00psu). Phillippi Creek, in Sarasota County, has a salinity control structure located downstream of the target area and thus was completely fresh.

Flow-Through Fluorescence (FTF)

The technique used for the detection of optical brighteners (OB) was based on a dual fluorometer flow-through approach in which the ratio of optical brightener fluorescence to colored dissolved organic matter (CDOM) was calculated (Dixon, et al. 2005). CDOM concentrations vary considerably in surface waters. This is especially true in estuarine waters where mixing of fresh and salt water occurs. Because CDOM is so variable, it fluoresces across a broad spectrum including the part of the spectrum where peak OB fluorescence occurs (Mobed, et al. 1996). By measuring CDOM fluorescence and taking the ratio of OB to CDOM fluorescence, any interference of the OB signal can be effectively removed.

The project used two field portable fluorometers (Models 10-AU-005-CE and 10-AU-005) from Turner Designs, Inc (Sunnyvale, CA) in a flow-through configuration. The fluorometer used for Optical Brightener (OB) detection was configured with a 300-400 nm excitation filter and a narrow-bandpass emission

filter of 436 nm. The CDOM fluorometer was configured with a 300-400 nm excitation filter and a narrow-bandpass emission filter of 550 nm.

Both fluorometers were blanked with laboratory deionized (DI) water and calibrated against a standard. The standard used for calibration was the fabric brightening agent Tinopal CBS-X. The granular standard was dried for two hours at 120 C and weighed to make a 50 µg/L stock solution. The stock solution was then serially diluted to make a 25 µg/L working solution. All dilutions were made using laboratory deionized water. The 25 µg/L standard was used as the high standard to calibrate both fluorometers.

Cotton Pad Deployment

An additional method using cotton pads as a sentinel for the presence of optical brightener (OB) was used at selected locations in each of the areas of interest (AOI). Sites were selected to cover a broad range of salinities and CDOM. A total of 40 sample locations were selected. Grab samples were collected at these locations and analyzed for water chemistry and bacteriological indicators. The type of cotton pad, deployment chamber, deployment duration, and methodology was based on work performed by Mote Marine Laboratory in Charlotte Harbor, FL (Dixon, et al. 2005) and the Florida Fish and Wildlife Research Institute (Carlson, personal communication). The cotton pads were purchased from a local pharmacy and were made of unbleached cotton cloth. Pads were deployed for three days. Upon retrieving the pads, qualitative analysis using a dual range handheld UV lamp was made. Spectral characterization of each pad was also made using a hand-held spectrometer (HR2000 High-Resolution Fiber Optic Spectrometer, Ocean Optics, Inc., Dunedin, FL) under a 254nm UV lamp.

Grab Samples for Chemistry and Bacteriology

Grab samples were collected from fixed station locations in each of the areas of interest. Several bacteriological indicators were sampled for and included: *E. coli*, Enterococci, Fecal coliform, and *Clostridium perfringens*. Sampling methodology was in accordance with Florida Department of Environmental Protection Standard Operating Procedures and in accordance with the quality assurance project plan (QAPP). In order to maximize the freshwater signal, sampling took place during an outgoing tide or at slack low tide. Samples were collected for a suite of water chemistry samples, including surfactants (MBAS), and were analyzed by the Florida Department of Environmental Protection Central Laboratory. Surfactants were analyzed at an overflow laboratory. The Florida Department of Health Tampa Laboratory analyzed the bacteriological samples. For the purpose of this report, only bacteriological and surfactant (MBAS) data are presented. For bacteriological samples, every effort was made to deliver samples to the laboratory within six hours of collection. However, some locations

were too remote to meet the six-hour requirement. Therefore, some of the bacteriological data are outside the holding time requirement and are flagged with a “Q” value qualifier.

RESULTS AND DISCUSSION

Coastal Taylor County

Coastal Taylor County is located just south of Tallahassee in the Florida Big Bend (Figure 1). The three communities that make up coastal Taylor County include Dekle Beach, Keaton Beach, and Steinhatchee. Most of the County’s land use is rural. The only development along the coast is in the towns of Dekle Beach, Keaton Beach, and Steinhatchee. Most homes in these communities are on septic tanks.

Dekle Beach

Five fixed stations were selected for Dekle Beach (Table 1). Because no a priori information specific to known septic failures existed, these stations were selected to best characterize two canal systems, the main channel, and an offshore site (Figure 4). Samples were collected at low tide to minimize dilution effects from the adjacent Gulf waters. Grab samples were collected on 11 October 2006. All bacteriological counts were either below detection or outside the acceptable range (Table 2). The highest counts occurred at Taylor 17 and Taylor 18 (Table 2). Taylor 17 was located at the end of a short canal while Taylor 18 was located at the mouth of a black rush (*Juncus roemerianus*) marsh. Even though station 17 and 18 represented the highest bacteriological counts for all four indicators, values were still quite low. There does not appear to be any correlation between bacterial counts and OB:CDOM ratio (Table 2).

Flow-through fluorescence data were collected on 10 October 2007. Flow-through was taken during an outgoing tide. Raw fluorescence values for both OB and CDOM were lower offshore relative to the canal systems. However, results were somewhat surprising with respect to the ratio OB:CDOM where higher values were concentrated offshore and not in the canals as was the case with the raw fluorescence data (Figure 2). Salinity plays a major role in quenching the fluorescence signal. Both CDOM and OB fluorescence were strongly influenced by salinity (Figure 3). This relationship between salinity and fluorescence was not linear. Above salinities of approximately 28psu, there was a much sharper decline in fluorescence. Further, the rate of quenching was not the same for CDOM and OB. CDOM fluorescence decreased more rapidly than OB at higher salinities which is why the OB:CDOM ratio increased further offshore (Figure 3). Because the canal system in Dekle Beach is small and directly connected to the Gulf of Mexico, this system is not a good candidate for further testing.

Keaton Beach

Ten fixed stations were selected in the canal system at Keaton Beach (Figure 5). Like Dekle Beach, there was no site-specific information about known septic failures. Therefore stations were based on a field reconnaissance conducted several days prior to station selection. As at Dekle Beach, bacteriological counts were very low (Table 3). All bacteriological counts were either below detection limits or outside the acceptable range. Of all the bacteriological indicators, fecal coliform at station Taylor 10 had the highest count (100cfu/100mL). Coincidentally, Taylor 10 had the highest surfactant value (0.13mg/L) though still representing a very low concentration. Because the bacteriological and surfactant values were so low, it is difficult to draw any conclusions with respect to the fluorescence data.

Flow-through fluorescence data were collected on 10 October 2007. There was considerable spatial variability in OB:CDOM throughout the canal system (Figure 11). In general, maximum OB:CDOM ratios were concentrated in areas closest to the Gulf of Mexico (Figure 8) and was most likely due to salinity (Figure 9). Salinity in Keaton Beach ranged from approximately 16psu to 30psu. Because CDOM fluorescence decreased more rapidly than OB fluorescence with increasing salinity, the OB:CDOM ratio increased with increasing salinity. Because the canal system at Keaton Beach is directly connected to the Gulf of Mexico, it is not recommended that further testing of this method occur in this system.

Steinhatchee River

Five fixed stations were selected along a short canal system that empties directly into the Steinhatchee River just upstream of the town of Steinhatchee (Figure 10). Grab samples were collected on 10 October 2006. Bacteriological counts were very low for all indicators tested (Table 4). With the exception of *Clostridium perfringens*, the highest counts were at Taylor 11 and Taylor 15 (Figure 10). Taylor 11 is the furthest station up the canal and Taylor 15 is located in the river channel. For *Clostridium perfringens*, the highest count was at station Taylor 14 located at the mouth of the canal (Figure 10). The highest counts recorded were for *Enterococci* at 100cfu/100mL. The highest OB:CDOM ratio was at station Taylor 11 while the lowest ratio was at Taylor 14. Although there was a slight decrease in OB:CDOM toward the mouth of the canal, OB:CDOM increased slightly in the river at station Taylor 15. Surfactants were not detected at any of the stations.

Flow-through fluorescence data were collected from the canal where the fixed stations were located downstream to the mouth of the river past the town of Steinhatchee (Figure 11). Several of the canal systems downstream of the canal

where the fixed stations were located were also profiled. There is a sharp increase in OB:CDOM ratio about midway down the transect (Figure 12). Salinity ranged from approximately 4psu to 19psu but this increase was gradual and did not correlate with the step change in OB:CDOM ratio. Toward the mouth of the river at the end of the flow-through transect, OB:CDOM ratio sharply increased (Figure 12). These data were collected in the same vicinity and most probably are an artifact of salinity. Further testing in this system is recommended.

Citrus County

Chassahowitzka River

Ten fixed stations were located throughout the Chassahowitzka canal system upstream of the main headspring of the Chassahowitzka River in Citrus County (Figure 13). Unlike the systems in Taylor County, counts for all bacteriological indicators, except *Clostridium perfringens*, were much higher (Table 5). Only station Chass 6 had *Clostridium perfringens* counts above detection but were still very low (Table 5). The highest bacterial counts occurred at station Chass 9 located at the downstream end of the south canal complex (Figure 13). *E. coli* and *Enterococci* counts were 11,600 cfu/100mL at Chass 9. Unfortunately OB:CDOM ratio at this station did not show a corresponding spike, despite the fact that a laundry facility is located just upstream of station Chass 9. Surfactants were at or below detection for all stations. Additionally, oil and grease samples were collected, all of which were below the practical quantitative limit. Oil and grease results did not co-vary with OB:CDOM ratio suggesting.

Flow-through fluorescence revealed some interesting patterns. Overall, the highest OB:CDOM ratios were toward the dead ends of canals (Figure 14). A potential hotspot was identified at Crab Spring and the associated spring run located downstream of the headsprings away from the canal system (Figure 14). Several homes surround Crab Spring. Unfortunately because the creek is very shallow, especially at low tide, it was not possible to collect bacteriological samples there. Salinity does not appear to be a contributing factor in the pattern of OB:CDOM ratio observed here. Salinity ranged from 0psu – 5psu. Flow-through fluorescence was taken further downstream toward the mouth of the river where salinities were close to 20psu. The OB:CDOM ratios downstream were slightly higher than those in the canal system and were probably an artifact of the increase in salinity.

Sarasota County

Phillippi Creek

Ten fixed stations were selected on canal 4-69 that flows into Phillippi Creek (Figure 15). These stations were chosen based on previous work done by Mote Marine Laboratory (Dixon and Julian 2005). This canal provided a unique opportunity in that the upper portion of the canal was located within the City of

Sarasota where central sewer is in place. The lower portion of the canal is outside of the City limits and the homes along this portion of the canal are still on septic. . Bacteriological counts in this canal were higher than any other system sampled and showed the most promising results. Bacteriological indicators and OB:CDOM ratio responded similarly (Figure 16) increasing from the upper end of the canal to about halfway down the canal and then decreasing toward the mouth of the canal (Figure 16). The highest bacteriological counts occurred in the upper portion of the canal (Table 6). At station 4-69 2, *E. coli* and Fecal Coliform counts were 10,240 cfu/100mL and 12,800 cfu/100mL, respectively. For *Enterococci*, the highest count (3,200 cfu/100mL) occurred further downstream at station 4-69. *Clostridium perfringens* was highest (192 cfu/100mL) at station 4-69 10, the furthest downstream station. Station 4-69 9, located above the mouth of the canal, had a lower OB:CDOM ratio relative to station 4-69 10, downstream of the canal's mouth suggesting the canal is contributing to the OB:CDOM signal. The relatively high bacteriological counts in the canal help to support this.

Because the canal system was very shallow, it was not possible to run flow-through fluorescence directly in the canal. Instead, flow-through fluorescence focused on Phillippi Creek from the weir structure at the upper limit to the salinity control structure at the lower limit. Although differences in OB:CDOM ratios were very small, a distinct pattern emerged (Figure 17). Lower ratios were concentrated in the upper portion of the creek where there are no OSTDS near the creek followed by a steady increase in OB:CDOM further downstream where OSTDS are known to exist. One spike was detected about a third of the way down (Figure 18) but was likely due to air in the pump intake. Because this portion of Phillippi Creek is fresh, salinity did not interfere with the fluorescence signal. However, changes in CDOM composition and concentration may have played a role and further testing should be done to determine whether the OB:CDOM pattern was a true indication of OSTDS inputs or an artifact of some other variable.

OSTDS and WWTP Samples

Five onsite sewage treatment disposal systems (OSTDS) were sampled by installing temporary piezometer wells into each system's drainfield. Two wastewater treatment plants (WWTP) were also sampled. One treatment facility was advanced and the other was secondary. All bacteriological indicators were below detection limit for the WWTP samples (Table 7). Surfactants were also at or near detection. Conversely, OSTDS 1 and OSTDS 2 had very high counts of *E. coli* and Fecal Coliform. For OSTDS 1, the results were too numerous to count. For OSTDS 2, *E. coli* and Fecal Coliform counts were 17,600 and 20,800 cfu/100mL, respectively. For OSTDS 3 – 5, *E. coli* and *Clostridium perfringens* were not analyzed due to laboratory issues. *Enterococci* counts were highest at OSTDS 2 and OSTDS 5 (460 and 270 cfu/100mL, respectively). OSTDS 1 water was essentially raw sewage with an extreme odor and viscous consistency. The other OSTDS samples were not as bad. OSTDS 3 – 5 did not have any odor

associated with them. However, all samples were turbid and had to be filtered prior to fluorescence analysis. Because the CDOM fluorometer was not operational, only OB fluorescence was analyzed and reported as raw fluorescence units. Samples from WWTP 1 and OSTDS 3 had the highest fluorescence values. OSTDS 4 and 5 were not analyzed for fluorescence because of problems with the OB fluorometer.

RECOMMENDATIONS

Two major recommendations came out of this task. The first is that scaling up to airborne LiDAR is not recommended at this time. Although the intent is to eventually transition to an airborne approach, there are still many questions that must be answered with respect to using this technique. The second recommendation is that this technique may not be appropriate in some systems. For example, systems like Dekle and Keaton Beach where there is considerable flushing from adjacent coastal waters presents some major challenges. Salinity variation in these systems is considerable over relatively short periods of time. Furthermore, because of the proximity to the Gulf, dilution of any OB signal that may exist would be significant. Because this is still an experimental method, it is recommended that follow-on work be limited to sites where salinity variation is minimum and flushing is minimal. The Chassahowitzka canal system would be an appropriate site, as would Phillippi Creek. Application of this technique to other systems should be limited to those exhibiting similar characteristics. Further recommendations for the next phase of this project include:

- Limiting further testing to two or three locations close to the testing facility. This will allow for more frequent sampling in response to seasonality, rainfall, reported sewage overflows, and other events.
- Refining flow-through method. Improvements in the flow-through design over the course of this project have made it much easier to deploy the flow-through system. Data processing and turn around time has also been greatly improved.
- Addition of microbial source tracking. Researchers have been working with genetic markers for microbial source tracking in order to better identify human sources of bacteria. Further testing should focus on integrating Dr. Harwood's work with optical brightener detection to further enhance the weight of evidence approach.
- The excitation-emission matrix (EEM) approach being used by Mote Marine Laboratory and others shows continued promise. Follow-on work should incorporate additional EEM sampling.
- A new in-water fluorometer (Turner C-6) is about to go online and will be used as part of this project. The new fluorometer will be equipped with both OB and CDOM sensors. This unit is much smaller than the current fluorometers being used and will allow penetration into much smaller areas.

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Station Name	Latitude	Longitude
<i>Coastal Taylor County – Dekle Beach</i>		
TAYLOR 16	29.849139	-83.616694
TAYLOR 17	29.848722	-83.616472
TAYLOR 18	29.848556	-83.616028
TAYLOR 19	29.848389	-83.617111
TAYLOR 20	29.843900	-83.623861
<i>Coastal Taylor County – Keaton Beach</i>		
TAYLOR 1	29.829500	-83.593722
TAYLOR 2	29.828278	-83.593361
TAYLOR 3	29.827250	-83.593167
TAYLOR 4	29.828806	-83.592306
TAYLOR 5	29.828028	-83.592528
TAYLOR 6	29.827250	-83.592000
TAYLOR 7	29.825694	-83.592806
TAYLOR 8	29.824056	-83.593083
TAYLOR 9	29.822167	-83.593250
TAYLOR 10	29.818806	-83.593278
<i>Coastal Taylor County – Steinhatchee River</i>		
TAYLOR 11	29.683000	-83.361139
TAYLOR 12	29.683056	-83.360778
TAYLOR 13	29.683028	-83.360444
TAYLOR 14	29.683083	-83.360056
TAYLOR 15	29.683028	-83.359278
<i>Citrus County – Chassahowitzka River</i>		
EEM CHASS 1	28.715850	-82.567933
EEM CHASS 2	28.713883	-82.570350
EEM CHASS 3	28.715650	-82.568950
EEM CHASS 4	28.715616	-82.575267
EEM CHASS 5	28.715950	-82.575483
EEM CHASS 6	28.715416	-82.576200
EEM CHASS 7	28.715633	-82.586550
EEM CHASS 8	28.715483	-82.569733
EEM CHASS 9	28.713083	-82.573383
EEM CHASS 10	28.714933	-82.573283
<i>Sarasota County – Phillippi Creek – Canal 4-69</i>		
4-69-1	27.310180	-82.529830
4-69-2	27.310070	-82.528770
4-69-3	27.310130	-82.527930
4-69-4	27.310100	-82.525900
4-69-5	27.310150	-82.521800
4-69-6	27.310150	-82.517720
4-69-7	27.310120	-82.516650
4-69-8	27.310120	-82.514600
4-69-9	27.310520	-82.510680
4-69-10	27.309480	-82.510600

Table 1. Station names and locations for all fixed stations.

Station ID	<i>E. coli</i>	<i>Enterococci</i>	<i>Clostridium perfringens</i>	Fecal Coliform	Surfactants (MBAS)	OB:CDOM
Taylor 16	20 B	60 B	20 B	20 B	0.11 I	0.25554
Taylor 17	100 B	140 B	56 B	100 B	0.1 I	0.25273
Taylor 18	200 B	240 B	44 B	260 B	0.1 I	0.25939
Taylor 19	20 B	120 B	20 B	20 B	0.11 I	0.26521
Taylor 20	20 B	20 U	4 U	20 B	0.11 I	0.29540

Table 2. Fixed station bacteriological and fluorescence results for Dekle Beach in Coastal Taylor County, FL. Bacteriological data units are in colony forming units (CFU) per 100mL Value qualifiers next to bacteriological data indicate the following: (B) Results based on colony counts outside the acceptable range; (I) The reported value is between the laboratory method detection limit and the laboratory practical quantitation limit; (Q) Sample held beyond normal holding time. (U) Not detected. The reported value is the detection limit of the sample analyzed.

Station ID	<i>E. coli</i>	<i>Enterococci</i>	<i>Clostridium perfringens</i>	Fecal Coliform	Surfactants (MBAS)	OB:CDOM
Taylor 1	60 B	60 B	4 B	60 B	0.11 IQ	0.27019
Taylor 2	20 U	20 B	4 B	20 U	0.1 UQ	0.27387
Taylor 3	20 U	20 U	4 U	20 U	0.1 UQ	0.27178
Taylor 4	20 U	20 U	4 U	20 U	0.1 U	0.27704
Taylor 5	20 U	20 U	4 B	20 U	0.1 U	0.27218
Taylor 6	20 U	20 B	4 B	20 U	0.1 I	0.27859
Taylor 7	20 U	20 U	16 B	20 U	0.1 U	0.26997
Taylor 8	20 B	20 B	12 B	40 B	0.1 U	0.27056
Taylor 9	80 B	20 U	8 B	80 B	0.1U	0.26516
Taylor 10	80 B	80 B	12 B	100 B	0.13 I	0.26081

Table 3. Fixed station bacteriological and fluorescence results for Keaton Beach in Coastal Taylor County, FL. Bacteriological data units are in colony forming units (CFU) per 100mL Value qualifiers next to bacteriological data indicate the following: (B) Results based on colony counts outside the acceptable range; (I) The reported value is between the laboratory method detection limit and the laboratory practical quantitation limit; (Q) Sample held beyond normal holding time. (U) Not detected. The reported value is the detection limit of the sample analyzed.

Station ID	<i>E. coli</i>	<i>Enterococci</i>	<i>Clostridium perfringens</i>	Fecal Coliform	Surfactants (MBAS)	OB:CDOM
Taylor 11	100 B	80 B	8 B	100 B	0.1 U	0.28378
Taylor 12	40 B	60 B	8 B	60 B	0.1 U	0.27822
Taylor 13	20 U	20 B	8 B	20 B	0.1 U	0.27927
Taylor 14	40 B	20 B	20 B	60 B	0.1 U	0.27565
Taylor 15	80 B	140 B	16 B	80 B	0.1 U	0.27877

Table 4. Fixed station bacteriological and fluorescence results for Steinhatchee River in Coastal Taylor County, FL. Bacteriological data units are in colony forming units (CFU) per 100mL Value qualifiers next to bacteriological data indicate the following: (B) Results based on colony counts outside the acceptable range; (I) The reported value is between the laboratory method detection limit and the laboratory practical quantitation limit; (Q) Sample held beyond normal holding time. (U) Not detected. The reported value is the detection limit of the sample analyzed.

Station ID	<i>E. coli</i>	<i>Enterococci</i>	<i>Clostridium perfringens</i>	Fecal Coliform	Surfactants (MBAS)	OB:CDOM	Oil and Grease
Chass 1	20 B	20 B	4 U	20 B	0.10 U	0.23486	2.9 I
Chass 2	100 B	160 B	4 U	100 B	0.10 U	0.22262	3.2 I
Chass 3	120 B	140 B	4 U	140 B	0.10 U	0.18967	4.4 I
Chass 4	100 B	120 B	4 B	80 B	0.11 I	0.13114	2.9 I
Chass 5	60 B	40 B	4 U	60 B	0.10 U	0.21587	3.5 I
Chass 6	120 B	60 B	12 B	120 B	0.10 U	0.14510	2.8 I
Chass 7	60 B	40 B	4 U	60 B	0.10 U	0.18070	1.8 I
Chass 8	60 B	80 B	4 U	60 B	0.10 U	0.17143	2.4 I
Chass 9	11600	280 B	4 U	11600	0.10 U	0.17410	4.0 I
Chass 10	260 B	120 B	4 U	260 B	0.10 U	0.14074	4.1 I

Table 5. Fixed station bacteriological and fluorescence results for the Chassahowitzka River canal system upstream of the main spring in Citrus County, FL. Bacteriological data units are in colony forming units (CFU) per 100mL Value qualifiers next to bacteriological data indicate the following: (B) Results based on colony counts outside the acceptable range; (I) The reported value is between the laboratory method detection limit and the laboratory practical quantitation limit; (Q) Sample held beyond normal holding time. (U) Not detected. The reported value is the detection limit of the sample analyzed. Additionally, the parameter oil and grease was also sampled.

Station ID	<i>E. coli</i>	<i>Enterococci</i>	<i>Clostridium perfringens</i>	Fecal Coliform	OB:CDOM
4-69 1	1920 B	6200	16 B	2400 B	0.19305
4-69 2	10240 B	5600	168	12800 B	0.22382
4-69 3	2520	5000	64 B	4200	0.22481
4-69 4	4000	3000 B	148	5000	0.23851
4-69 5	3960 B	3200 B	164	6600	0.23057
4-69 6	200 B	1800 B	56 B	800 B	0.22955
4-69 7	2600 B	2800 B	164	2600 B	0.22195
4-69 8	360 B	1200	52 B	1800 B	0.22209
4-69 9	320 B	1120	32 B	1600 B	0.19895
4-69 10	152	1420 B	192	760	0.21638

Table 6. Fixed station bacteriological and fluorescence results for Phillippi Creek canal 4-69 in Sarasota County, FL. Bacteriological data units are in colony forming units (CFU) per 100mL Value qualifiers next to bacteriological data indicate the following: (B) Results based on colony counts outside the acceptable range; (U) Not detected. The reported value is the detection limit of the sample analyzed. Samples for surfactants were not analyzed due to a shipping problem.

Station ID	<i>E. coli</i>	<i>Enterococci</i>	<i>Clostridium perfringens</i>	Fecal Coliform	Surfactants (MBAS)	OB (raw)
WWTP 1	20 U	20 U	4 B	20 U	0.18 IJ	86
WWTP 2	20 U	20 U	4 U	20 U	0.10 U	54
OSTDS 1	12,000 Z	20 U	4 U	12,000 Z	15 Q	-
OSTDS 2	17,600 B	460	240 B	20,800 B	0.20 U	42
OSTDS 3	-	10 B	-	30 B	0.10 IQY	81
OSTDS 4	-	60 B	-	10 U	1.0 UQY	-
OSTDS 5	-	270	-	70 B	1.0 UQY	-

Table 7. Bacteriological and fluorescence results for two wastewater treatment facilities and five onsite sewage treatment disposal systems (OSTDS). Bacteriological data units are in colony forming units (CFU) per 100mL. OB values are fluorescence values for OB only and are given in raw fluorescence units. Some analyses were not performed due to laboratory problems. Value qualifiers next to bacteriological data indicate the following: (B) Results based on colony counts outside the acceptable range; (I) The reported value is between the laboratory method detection limit and the laboratory practical quantitation limit; (J) Estimated Value; (Q) Sample held beyond normal holding time. (U) Not detected. The reported value is the detection limit of the sample analyzed; (Y) The laboratory analysis was from an unpreserved or improperly preserved sample. The data may not be accurate; (Z) Bacteriological samples too numerous to count at highest dilution performed.

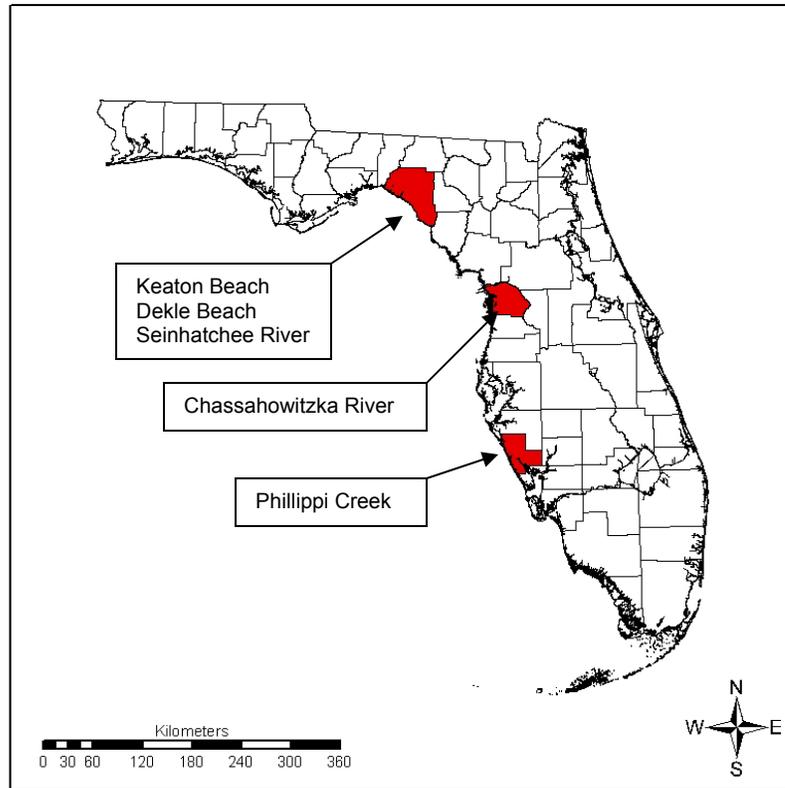


Figure 1. Three focus areas of interest along the Florida Gulf Coast. Taylor County is the northernmost county followed by Citrus County and Sarasota County to the south.



Figure 2. Flow-through fluorescence system being operated off the deck of a small boat. System includes two Turner Designs 10-AU field fluorometers and a variable speed peristaltic pump. Entire payload is being powered by a 12-volt marine battery. Not shown is the YSI 6600 datasonde that is deployed in the water and the GPS receiver. Samples are taken at 2 second intervals.



Figure 3. Cotton pad deployment chambers. Each fixed station location had two deployment chambers per site. The chambers were attached to a concrete cinderblock. A small seine float was attached to each chamber.



Fixed Sample Locations for Dekle Beach, Taylor County, FL

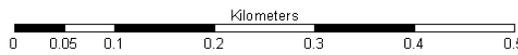


Figure 4. Fixed station locations in Dekle Beach, Taylor County, FL. This is a relatively small canal system with direct connection to the Gulf of Mexico. Anecdotal evidence from local sources suggests that there have been OSTDS failures within this system.



Legend

Dekle Beach FTF for GIS Events

OB:CDOM

- 0.0795 - 0.1602
- 0.1603 - 0.2598
- 0.2599 - 0.2721
- 0.2722 - 0.2880
- 0.2881 - 0.3650

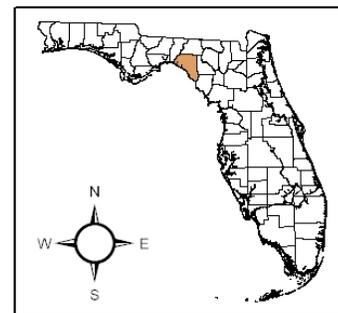
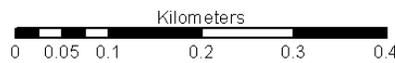


Figure 5. Flow-through fluorescence results for Dekle Beach, Taylor County, FL. Data are presented as the ratio of OB to CDOM.

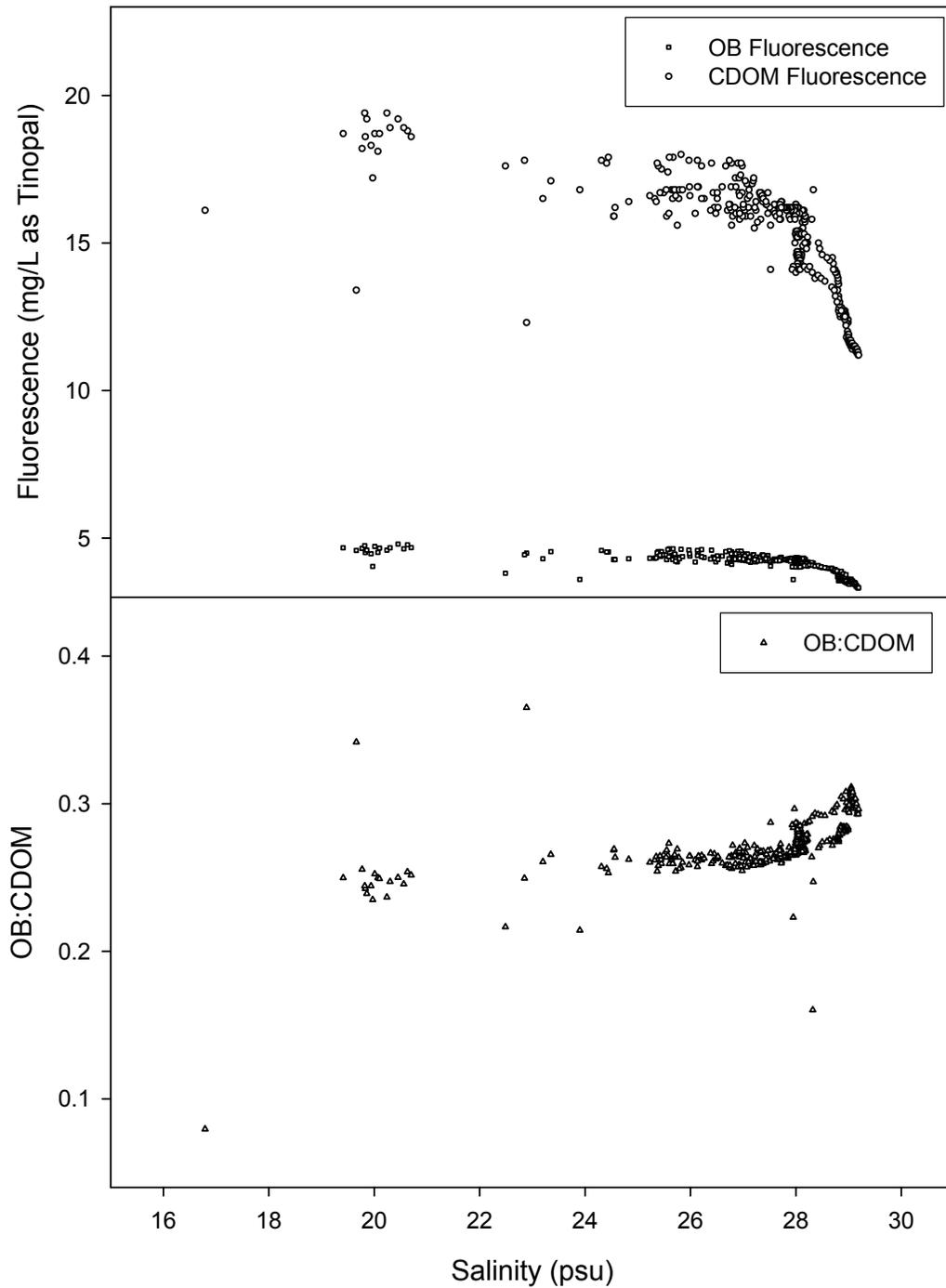


Figure 6. Interference in fluorescence caused by salinity in Dekle Beach, Taylor County, FL. The plot in the top panel shows the response of raw OB and CDOM fluorescence reported as a concentration (mg/L as Tinopal). The lower panel shows the response of the ratio OB:CDOM with changes in salinity.



Fixed Sample Locations for Keaton Beach, Taylor County, FL

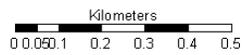


Figure 7. Fixed station locations in Keaton Beach, Taylor County, FL. Homes along this canal system are on septic although a program is currently in place to convert to central sewer.



Legend

Keaton Beach FTF for GIS Events

OB:CDOM

- 0.1860 - 0.2485
- 0.2486 - 0.2645
- 0.2646 - 0.2790
- 0.2791 - 0.2926
- 0.2927 - 0.3196

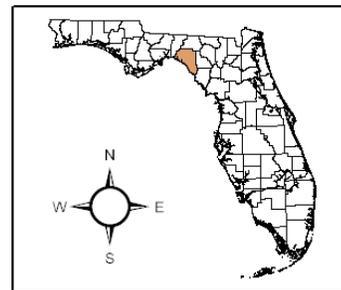


Figure 8. Flow-through fluorescence results for Keaton Beach, Taylor County, FL. Data are presented as the ratio of OB to CDOM.

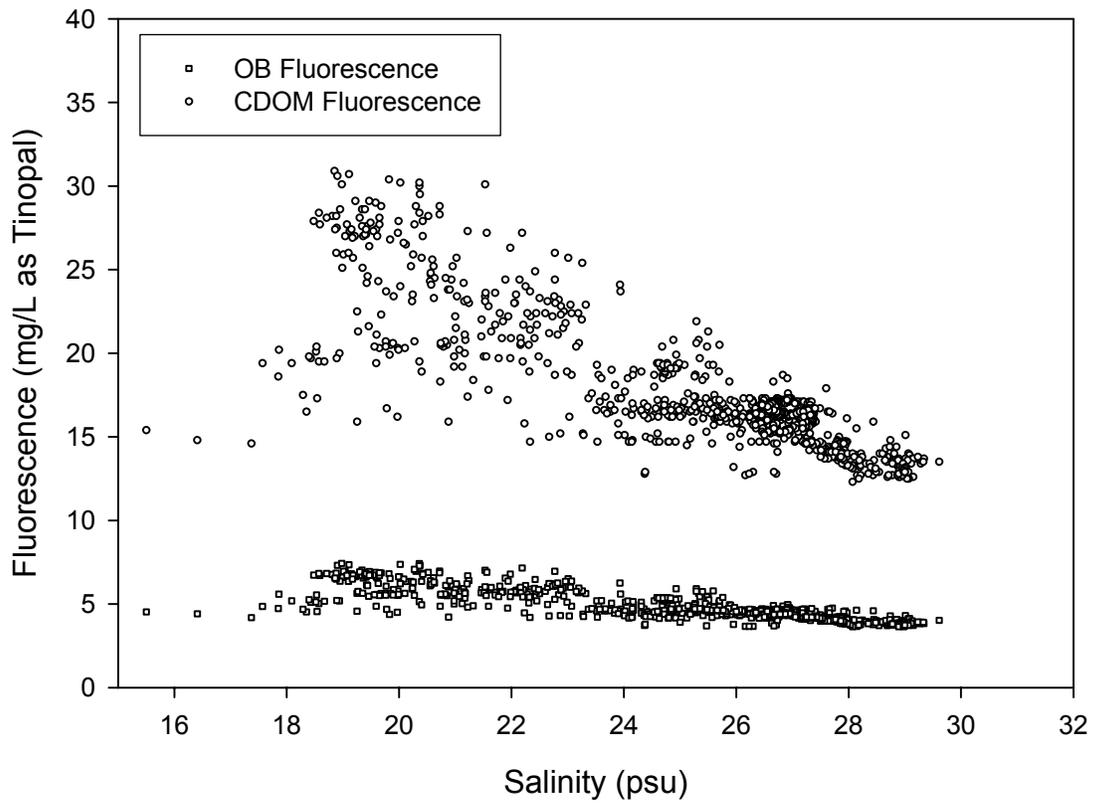


Figure 9. Variation in fluorescence with salinity for Keaton Beach. Fluorescence is reported in mg/L as Tinopal.



Fixed Sample Locations for Steinhatchee River, Taylor County, FL

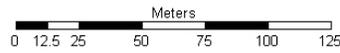
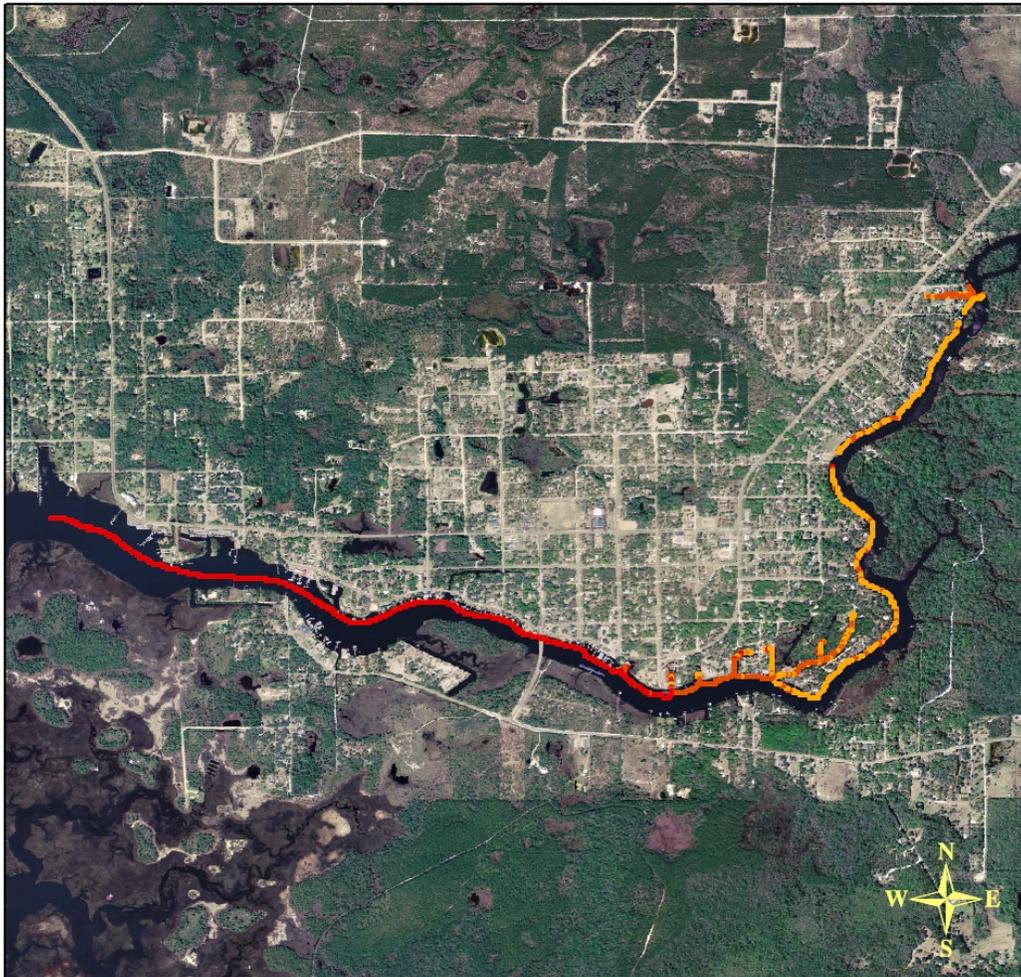


Figure 10. Fixed station locations Steinhatchee River, Taylor County, FL. Station locations are along a small canal upstream of the town of Steinhatchee. All homes on this canal are on septic tanks. Most homes on the canal are at least twenty years old. Age of septic systems is unknown.



Legend

Steinhatchee River FTF for GIS Plots Events

OB_CDOM

- 0.1564 - 0.2111
- 0.2112 - 0.2787
- 0.2788 - 0.2917
- 0.2918 - 0.3811
- 0.3812 - 0.5817

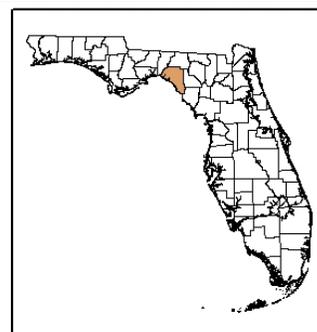
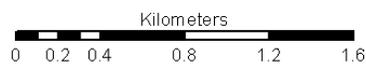


Figure 11. Flow-through fluorescence results for the Steinhatchee River, Taylor County, FL. Data are presented as the ratio of OB to CDOM.

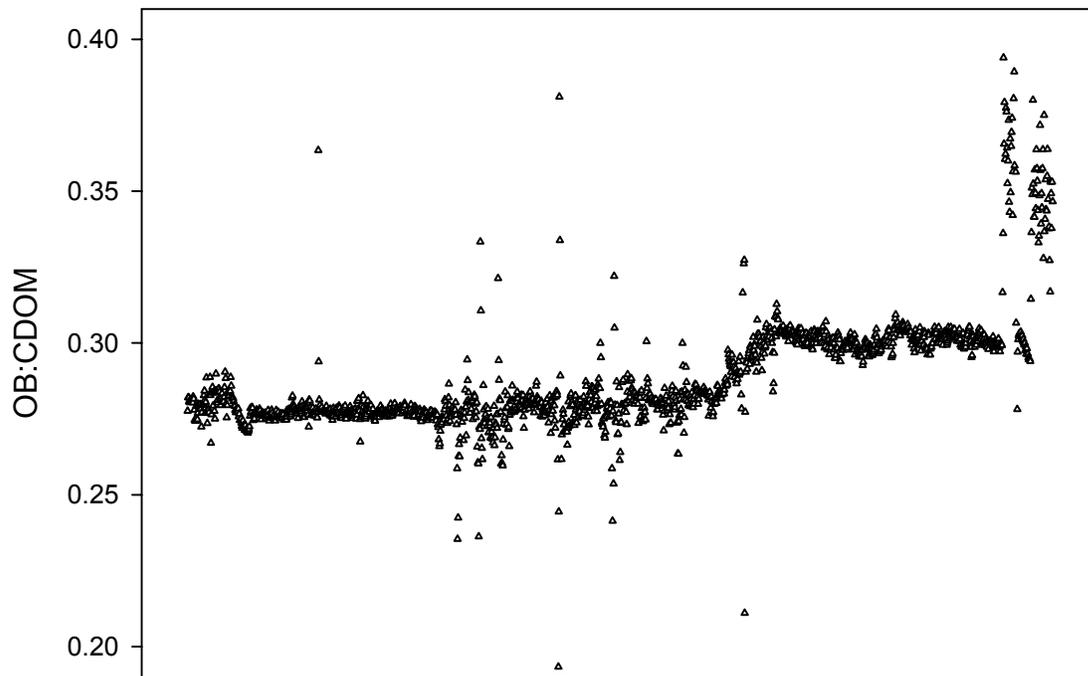
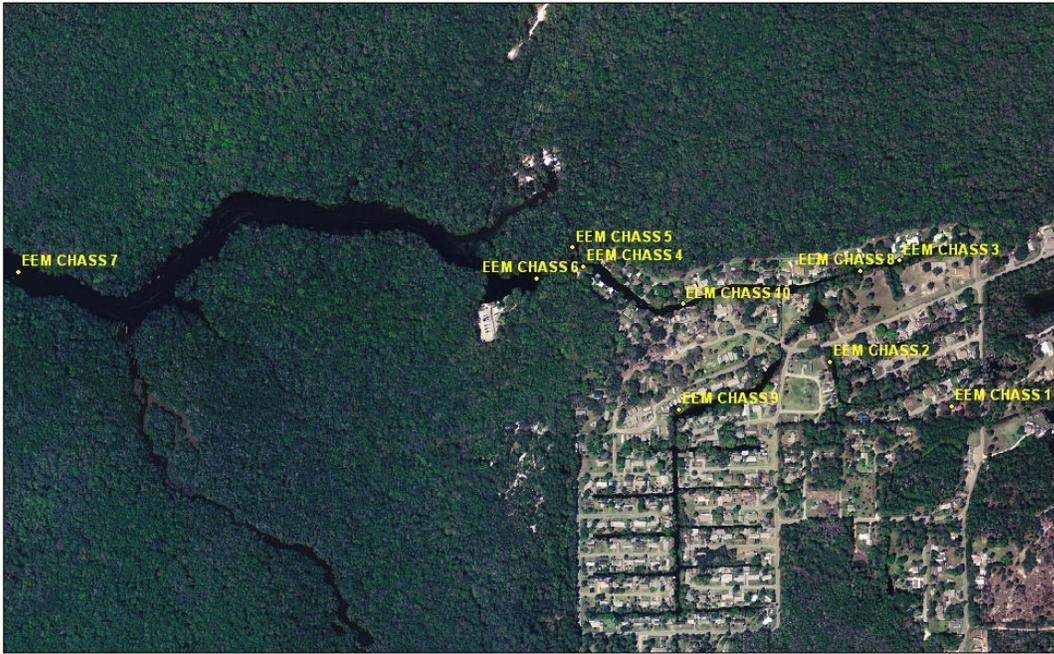


Figure 12. OB:CDOM ratio along a segment of the lower Steinhatchee River. River flows from left (upstream) to right (downstream).



Fixed Sample Locations for Chassahowitzka River, FL

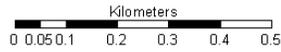
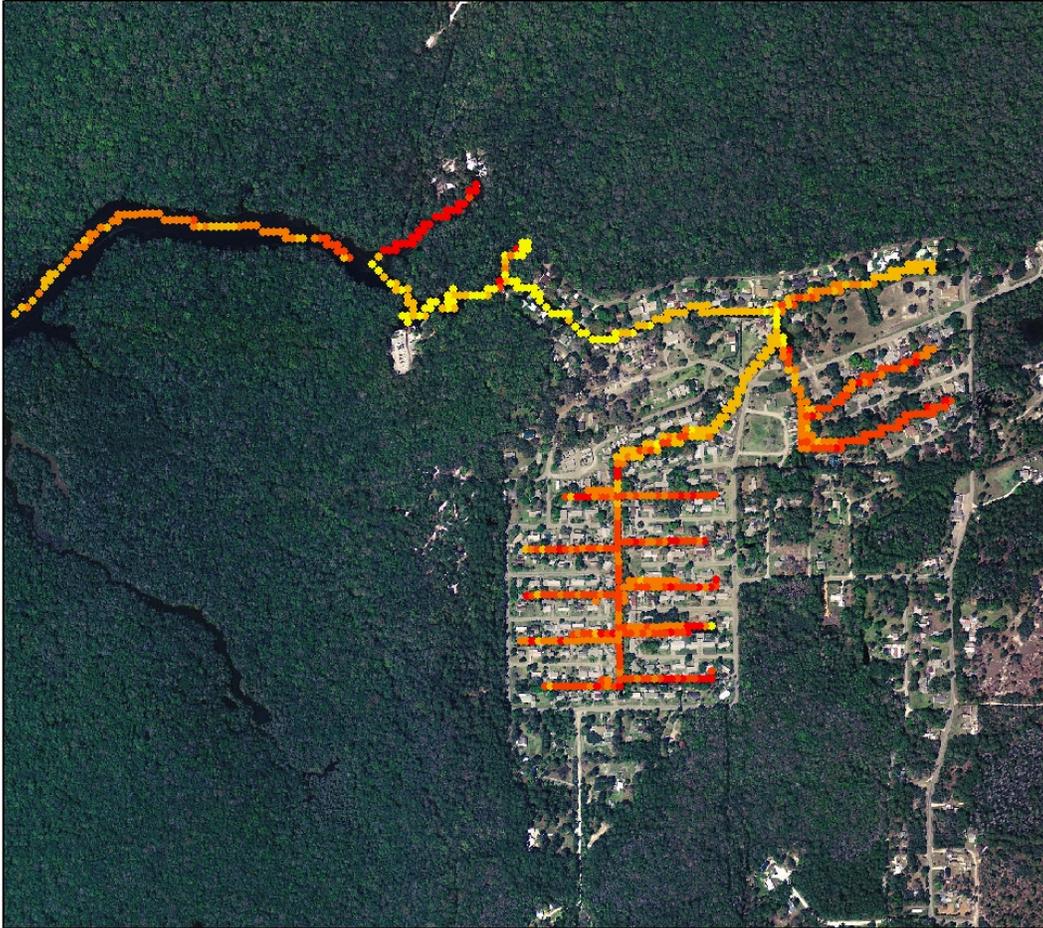


Figure 13. Fixed station locations Chassahowitzka River, Citrus County, FL. Station locations are located in the lower portion of a large canal system upstream of the main spring vent forming the headwaters of the tidally influenced Chassahowitzka River. All homes are on septic tanks, although a project is underway to convert the entire neighborhood to central sewer.



Legend

**Chassahowitzka River FTF
OB:CDOM**

- 0.0264 - 0.1394
- 0.1395 - 0.1806
- 0.1807 - 0.2117
- 0.2118 - 0.2451
- 0.2452 - 0.3951

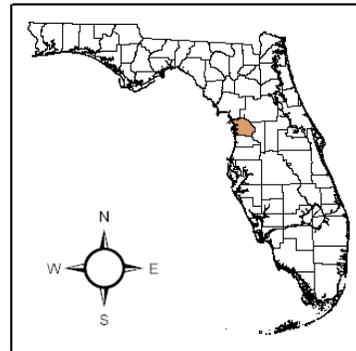
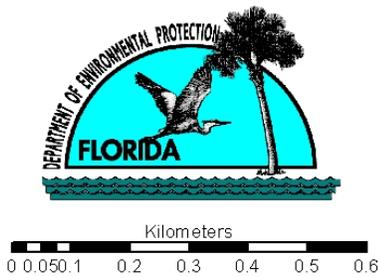
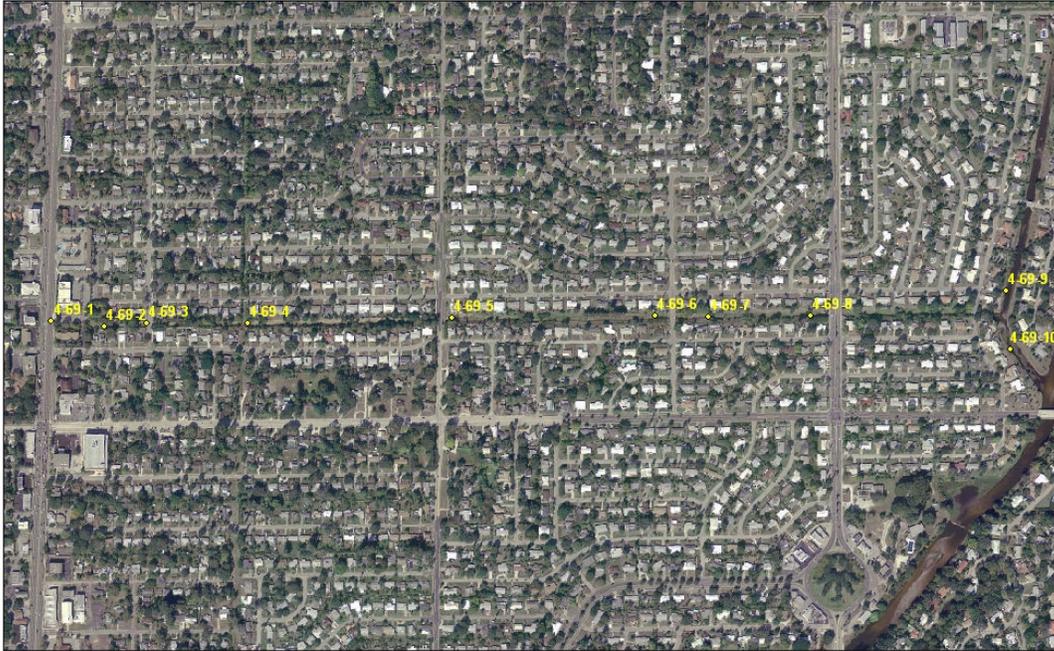


Figure 14. Flow-through fluorescence results for the Chassahowitzka River, Citrus County, FL. Data are presented as the ratio of OB to CDOM.



Fixed Sample Locations for Canal 4-69 Phillippi Creek, Sarasota County, FL

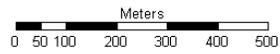


Figure 15. Fixed station locations Canal 4-69 off Phillippi Creek, Sarasota County, FL. This canal drains into Phillippi Creek. This canal was the site of previous optical brightener work performed by Mote Marine Lab (Dixon and Julian 2005). Homes along the eastern portion of the canal are on central sewer. The homes on the west side of the canal are on septic but are slated to be converted to sewer as well.

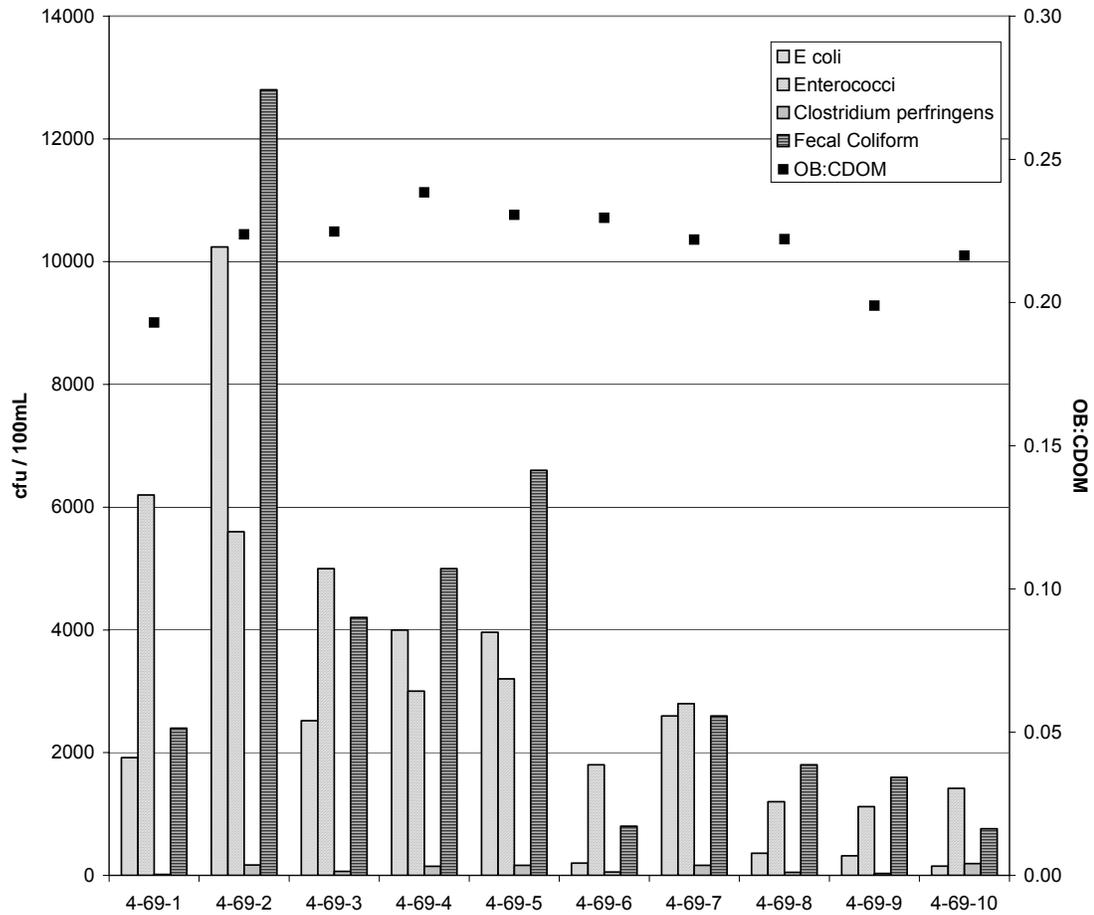
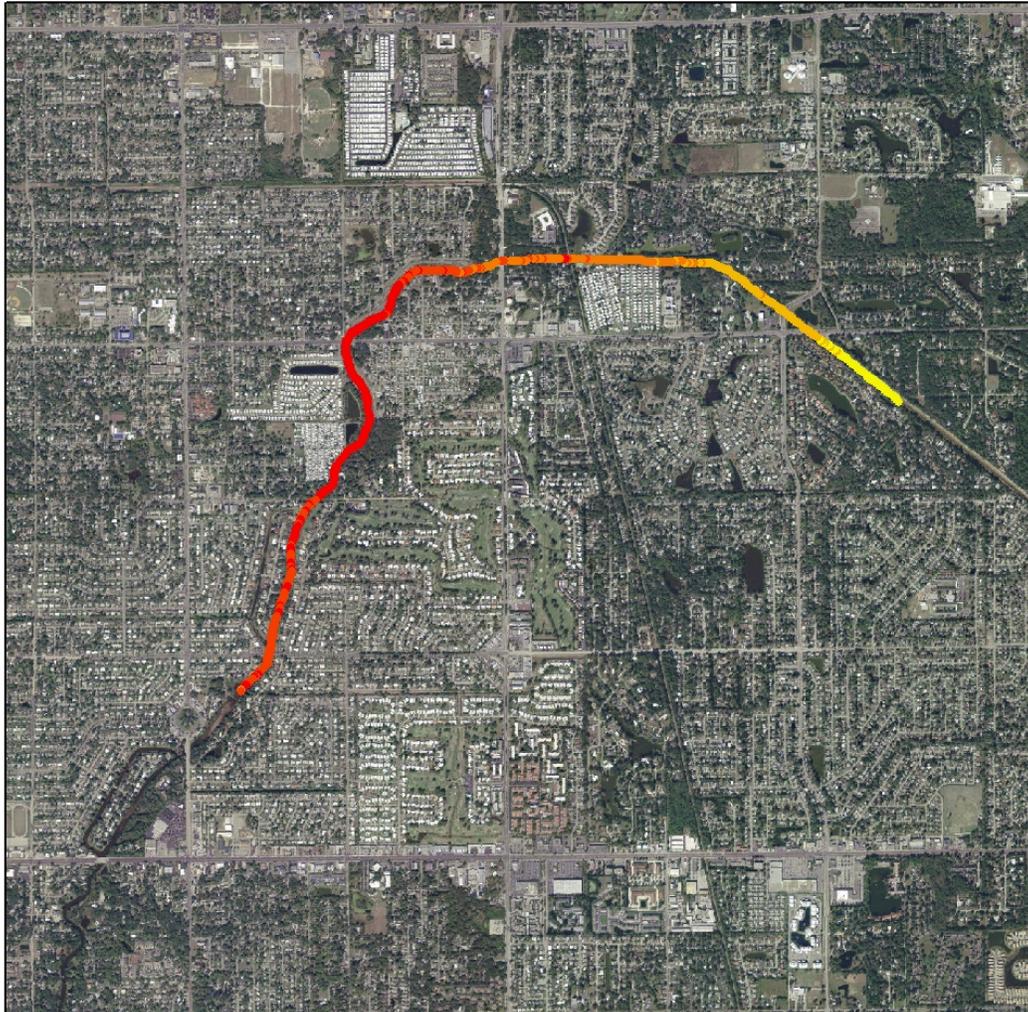


Figure 16. Bacteriological and OB:CDOM results for canal 4-69 on Phillippi Creek. Data were collected on 21 September, 2006.



Legend

Phillippi Creek FTF

OB:CDOM

- 0.2132 - 0.2173
- 0.2174 - 0.2204
- 0.2205 - 0.2237
- 0.2238 - 0.2266
- 0.2267 - 0.2377

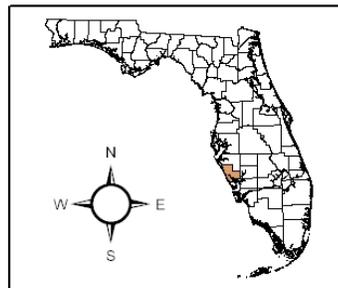
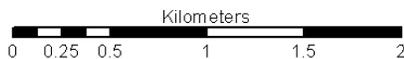


Figure 17. Flow-through fluorescence results for Phillippi Creek, Sarasota County, FL. Data are presented as the ratio of OB to CDOM.

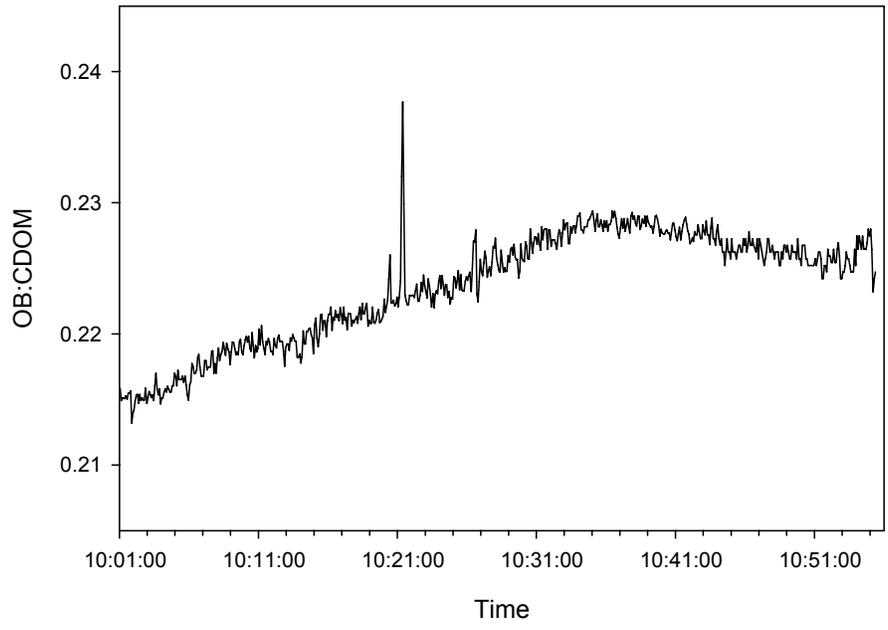


Figure 18. Results of flow-through fluorescence transect for Phillippi Creek, Sarasota County on 22 September 2006.

Florida Department of Health Passive Nitrogen Removal Study

Literature Review and Database

Dr. Daniel P. Smith, P.E.

Dr. Richard J. Otis, P.E.

Quality Assurance Project Plan

Dr. Daniel P. Smith, P.E.

Passive Nitrogen Removal Study

- Goal: Evaluate passive treatment media for on-site wastewater treatment
- Influent: septic tank effluent
- Treatment goal: reduce total N

Nitrogen Removal Study Tasks

- Task 1 Literature review and database
- Task 2 Laboratory experiments
- Task 3 Feasibility
- Task 4 Economic analysis
- Task 5 Final report

Literature review sources

- Peer reviewed: CSA Illumina, Science Direct, Applied Science and Technology,...
- Conference: ASAE, National Onsite Wastewater Recycling Ass.
- Test Centers: Massachusetts Alternative Septic System Test Center, LaPine Oregon national test center
- National experts
- Vendors

Literature review results

- Searchable Endnote database
- 224 entries
- URLs and abstracts provided
- Separate citation file with PDFs
- Summary performance synopsis (Excel)

Citation organization tree (compiled files)

General On-Site Nitrogen Removal (10)

Nitrification Unit Processes

Recirculating Sand Filters (6)

Waterloo Biofilter (1)

Peat Filters (9)

Textile Filters (2)

Coir Filters (3)

Zeolite Filters (2)

Eliminite

Denitrification Unit Processes

Heterotrophic Processes

Cellulosics (7)

Rich (5)

Hagerty (4)

Nitrex (5)

Black Gold (1)

Loomis (4)

Other Carbon Donors (6)

Autotrophic Processes

Sulfur (38)

Sulfide (1)

Iron (1)

Heterotrophic/autotrophic (3)

Drainfield Modifications (10)

Aerobic (unsaturated) filters

System Type	Description	Features	Treatment Performance
Intermittent sand filters	Sand filter Single pass	0.3 to 0.7 mm media 18 to 36 in. depth 0.7 to 1.5 gal/ft ² -day 12 to 48 dose/day	TN Removal: 20 to 50% Effluent: 20 to 20 mg/L NH ₃ -N Effluent: 1.9 to 9 mg/L
Recirculating sand filters	Sand filter Recirculation	1.5 to 3 mm media 18 to 36 in. depth 3 to 5 gal/ft ² -day 40 to 120 dose/day	TN Removal: 40 to 75% Effluent: 15 to 30 mg/L NH ₃ -N Effluent: 1 to 5 mg/L
Textile filters	Textile filter Recirculation	2 to 3 in. cubes 36 to 72 in. depth 8 to 17 gal/ft ² -day 80 to 140 dose/day	TN Removal: 20 to 60% Effluent: 10 to 60 mg/L NH ₃ -N Effluent: 1.7 to 5.9 NO ₃ -N Effluent: 11 mg/L
Peat filters	Peat media filter Single pass or recirculation	24 to 36 in. depth 3 to 6 gal/ft ² -day 12 to 120 dose/day	TN Removal: 10 to 75% Effluent: 10 to 60 mg/L TKN Removal: 90 to 95% NH ₃ -N Effluent: 1 mg/L NO ₃ -N Effluent: 20 to 50

Aerobic (unsaturated) filters

System Type	Description	Features	Treatment Performance
Waterloo biofilter	Open cell foam media, single pass or recirculation	3 to 4 in. cube media 48 in. depth 11 gal/ft ² -day	TN Removal: 62% Effluent: 14 mg/L NH ₃ -N Effluent: 2.4 mg/L NO ₃ -N Effluent: 10 mg/L
Zeolite filters	Zeolite media filter	20 to 30 in. depth 6.1 gal/ft ² -day	NH ₃ -N Removal: 98.6% Influent: 70 mg/L Effluent: 1 mg/L NO ₃ -N Effluent: 57 mg/L
Coir filters	Coir filter bed, with recirculation	Coconut coir media 30 in. depth 5 to 10 gal/ft ² -day	-

Anoxic (saturated) filters

System Type	Description	Features	Treatment Performance
Sulfur/oyster shell filter (bench scale)	1 liter bench column synthetic wastewater upflow single pass	Sulphur/oyster shell media (75/25% by volume) Sulphur: 4.7 mm	anoxic only NO ₃ -N Removal: 80% Influent: 50 mg/L Effluent: 10 mg/L
Sulfur/oyster shell filter	185 gal. column aerobic effluent upflow single pass	Sulphur/oyster shell media (75/25% by volume) 47 gal/ft ² -day	anoxic only TN Removal: 82% Effluent: 4.2 mg/L NO ₃ -N Removal: 88% Influent: 20 mg/L Effluent: 2.4 mg/L
Sulfur/limestone column	237 gal. column groundwater upflow single pass Residence time: 13 hr.	Sulphur/limestone media (67/33% by volume) 63 gal/ft ² -day Sulfur: 2.5 to 3.0 mm Limestone: 2.38 to 4.76 mm	anoxic only NO ₃ -N Removal: 96% Influent: 64 mg/L Effluent: 2.4 mg/L NO ₂ -N Effluent: 0.2 mg/L

Anoxic (saturated) filters

System Type	Description	Features	Treatment Performance
Nitrex™	aerobic effluent gravity flow upflow single pass	Nitrex wood-based media 24 to 30 inch media depth (est.) 4.6 gal/ft ² -day (est.)	aerobic+anoxic TN Removal: 79 to 96% Effluent: 3 to 18 mg/L NO ₃ -N Effluent: 0.3 to 8 mg/L
Black& Gold™	wood-based media single pass downflow gravity	Influent: STE 280 gal. column Sand/tire crumb/woodchip (85/11/5% by volume) 8.3 gal/ft ² -day	aerobic+anoxic TN Removal: 98% Influent: 414 mg/L Effluent: 7.1 mg/L NH ₃ -N Effluent: 4.4 mg/L NO ₃ -N Effluent: 0.05 mg/L

Approach to Passive Treatment

“ 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”

Biochemical Requirement

- Biochemical requirement: require initial aerobic filter followed by anoxic filter
- Aerobic: Organic N \rightarrow NH_4
 $\text{NH}_4 \rightarrow \text{NO}_3$
- Anoxic: $\text{NO}_3 \rightarrow \text{N}_2$

Passive constraints: require two filters in series

- No aerator
- First stage: unsaturated media filter
ammonification, nitrification
- Second stage: saturated media filter
electron donor media
anoxic
denitrification

Treatment Goal: Total N < 3 mg/L

- $TN = \text{Organic N} + \text{NH}_3\text{-N} + \text{NO}_3\text{-N}$

- $TN_{\text{allowable}} < 3.0$

- First stage filter:

$$\text{Organic N} + \text{NH}_3\text{-N} < 1.5$$

- Second stage filter

$$\text{NO}_3\text{-N} < 1.5$$

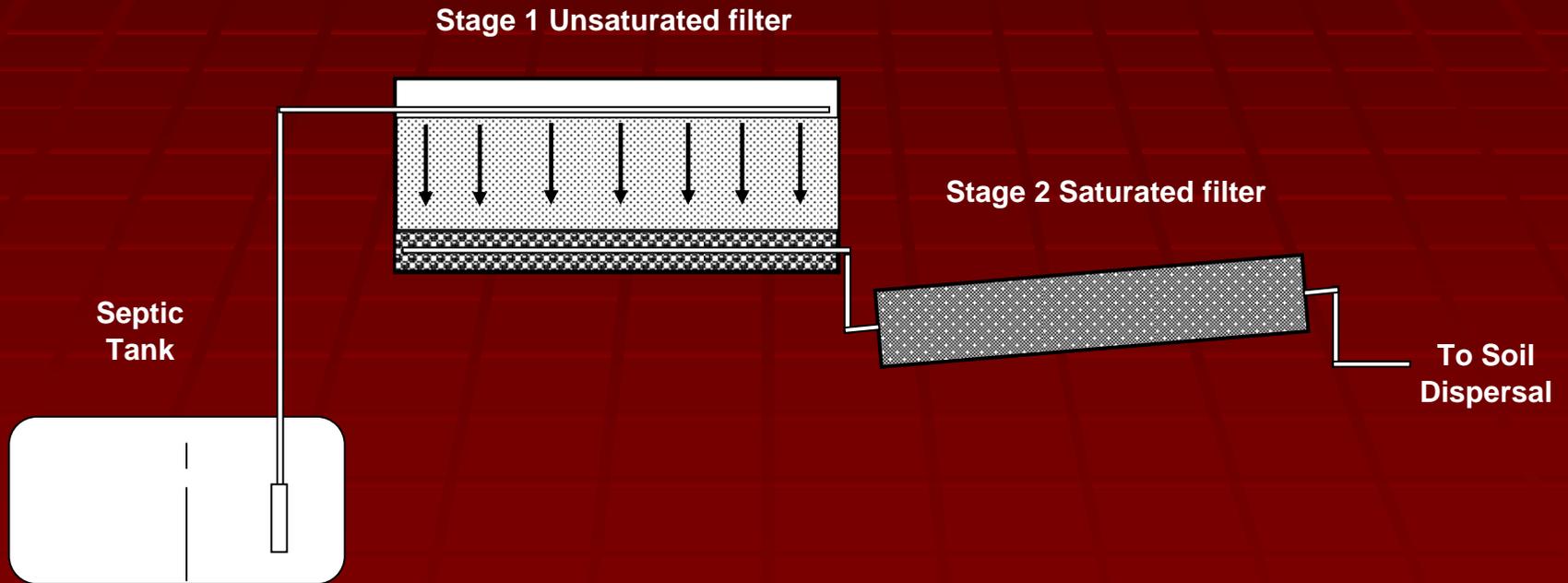
Passive constraints: one pump

- Where to place?
- Hydraulics require gravity flow before and after
- Options:
 - before first stage filter, gravity flow through second stage filter to dispersal field
 - after first stage filter, gravity flow to first stage filter and from second stage filter to dispersal field

Preferred alternative: pump from septic tank or separate chamber

- Allows pressure dosing of first stage filter
- Superior flow distribution over filter surface area
- Timed dosing provides frequent low doses of STE and superior treatment
- 24/day or more
- Potential to recirculate around first stage filter for pre-denitrification

Illustration of One Configuration



Unsaturated Filter Performance Factors

Feature	Effect
Hydraulic loading rate	Higher rates lower water retention time and treatment
Organic loading rate	Higher loading rates increase rate at which biofilms must process organic matter; nitrification may be inhibited if too high
Nitrogen loading rate	Higher loading rates require higher nitrification rates and higher oxygen utilization rates
Media depth	Deeper beds can give better treatment; upper layers often more reactive
Specific surface area	Higher values give greater attachment surfaces for microorganisms
Superficial velocity	Affects mass transfer between wastewater and biofilms
Average linear velocity	Affects mass transfer between wastewater and biofilms
Hydraulic application rate per dose	Volume per dose should be scaled to field capacity of media
Organic loading rate per dose	Loading per dose must not exceed processing rate
Nitrogen loading rate per dose	Loading per dose must not exceed processing rate
Average water residence time	Longer residence time gives more time for biochemical reactions and better treatment
Uniformity of Dosing	Promotes full utilization of all elements of the filter media
Wastewater	
Suspended solids	Accumulated within pores, may lead to clogging if not biodegraded
BOD	High values require more room for attached growth and metabolism between doses, particularly in upper filter layers
Organic and ammonia nitrogen	Significant component of total oxygen supply requirement
Alkalinity	Consumed by nitrification and restored by heterotrophic denitrification; adequate supply needed to prevent pH decline by nitrification

Filter Media Characteristics

Feature	Effect
Particle size distribution	Larger particles less subject to clogging Smaller particles have greater surface area per volume for treatment
Uniformity coefficient	Effects flow uniformity
Specific surface area	Higher values give greater attachment surfaces for microorganisms
Air filled porosity	Oxygen supply throughout media depth for BOD oxidation and nitrification in unsaturated filters
Water retention capacity	Higher water retention in unsaturated media filters provides longer time of contact of water with microorganisms and better treatment; affected by intrinsic porosity that favours capillary water retention
Sinuosity and tortuosity	Affect accessibility of pore spaces to exchange of wastewater and air
Specific weight	Effects compression strength required for support in multi media filters
Ion exchange capacity	Ammonia adsorption may improve performance
Compressibility	Effects material resistance to compression when wetted with biofilm and attached solids
Biodegradation	Biodegradation of organic media will limit longevity
Resilience	Prevents compaction under deployment
Hydrophilicity	Attracts water for wetting and rewetting

Saturated Filter Performance Factors

Feature	Effect
Hydraulic loading rate	Higher rates lower water retention time and treatment
Organic loading rate	Higher loading rates increase rate at which heterotrophic biomass could accumulate
Solids loading rate	Higher loading rates increase rate at which solids could accumulate
Nitrogen loading rate	Higher loading rates require higher denitrification rates and higher rates of electron donor dissolution
Media depth	Deeper beds can give better treatment; uppers layers often more reactive
Specific surface area	Higher values give greater surface area for attachment of microorganisms and dissolution of media
Superficial velocity	Effects mass transfer between wastewater and biofilms
Average linear velocity	Effects mass transfer between wastewater and biofilms
Average water residence time	Longer residence time gives more time for biochemical reactions and better treatment
Wastewater	
Suspended solids	Accumulated within pores, may lead to preferential flow if not biodegraded
BOD	Will create more heterotrophic biomass and may increase potential for preferential flow
Nitrate nitrogen	High loadings require greater surface areas and higher levels of denitrifying activity
Alkalinity	Consumed by autotrophic denitrification; must be balanced by sum of influent alkalinity and alkalinity provided by solid source

Candidate Media

Material	Bulk density, lb/ft ³	Particle Size Range	Supplier
ZK406H Clinoptilolite	59	0.8 - 1.7mm	GSA Resources, Tuscon, AZ
AMZ 4/8 Clinoptilolite	55	2.3 - 4.8 mm	Ash Meadows, Armagosa, NV
AMZ 8/20 Clinoptilolite	55	0.8 - 2.3 mm	Ash Meadows, Armagosa, NV
AMZ 16/50 Clinoptilolite	55	0.3 - 1.1 mm	Ash Meadows, Armagosa, NV
Livlite Expanded Clay	41	3 to 5 mm	Big River, Alpharetta, GA
Coir fiber	8.7	0.5 - 9 cm L 0.1 - 0.3 mm D	RoLanka International, Stockbridge, GA
Elemental sulfur	77	2 - 4 mm	Georgia Sulfur, Valdosta, GA
Oyster shell	82	3 - 15 mm	Harold's Farm Supply, Dover, FL
ACT-MX ESF-580 Utelite	54	4 -20 mm	ES Filter, Ogden, UT
ACT-MX ESF-416 Utelite	54	2 - 10 mm	ES Filter, Ogden, UT
ACT-MX ESF-450 Utelite	54	0.4 - 4.5 mm	ES Filter, Ogden, UT

Granular Zeolite



- Anion exchanger for NH_4^+
- Surface for growth of nitrifiers
- 55% internal porosity
- Retains 18% of its weight as water at 10% relative humidity

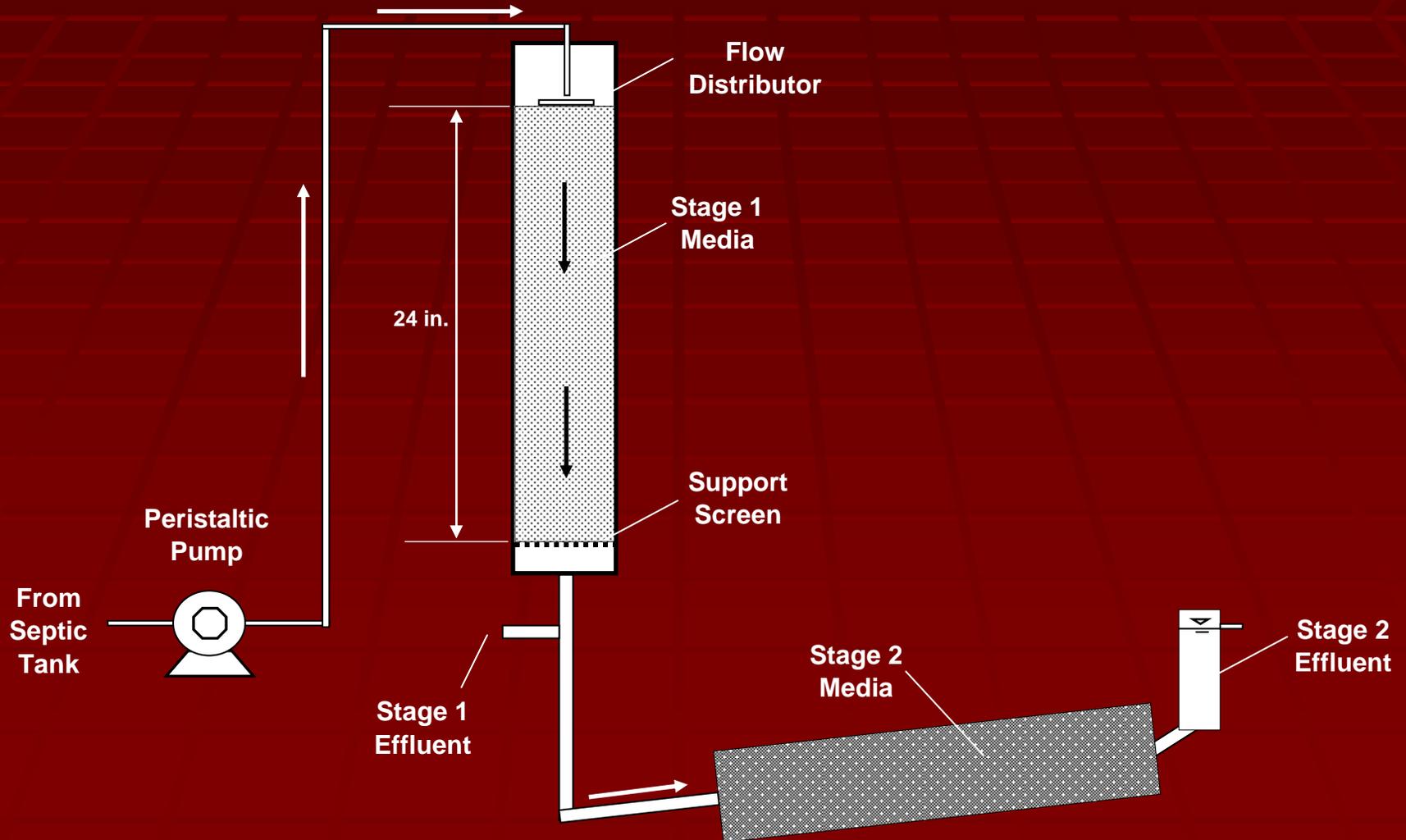
Laboratory Experiments

- Two stage filters systems:
unsaturated/saturated
- Influent: septic tank effluent
- Four sites in Hillsborough County
 - 3 at parks, 1 private
 - 3 single family, 1 visitor center

Media Configuration

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	3.0	24.0	Stratified	8 in. expanded clay (3-5 mm) 8 in. clinoptilolite (0.8-2.3 mm) 8 in. clinoptilolite (0.5-1.1 mm)
	1C	3.0	24.0	Nonstratified	100% coir fiber
Stage 2	2A	1.5	24.0	Nonstratified	75% elemental sulfur 25% oyster shell
	2B	1.5	24.0	Nonstratified	60% elemental sulfur 20% oyster shell 20% expanded shale
	2C	1.5	24.0	Nonstratified	45 % elemental sulfur 15% oyster shell 40% expanded shale

Laboratory Two Stage Filter



Laboratory Experiments

- Stage 1 Aerobic: dose 24 times per day for 2 to 3 min.
- Initial hydraulic loading at 2 gal./ft²-day
- Stage 2 Anoxic: receives gravity flow
- Operate and monitor over 60 days
- Temperature, pH, alkalinity, DO, TKN, (NO₃+NO₂)-N, SO₄ (Stage 2)

Lab Column Configuration

Unsaturated filter

Flow, gpd/ft ²	2.00
Diameter, inch	4.00
Media depth, inch	24.0
Flow, gal/day	0.174
Flow, ml/hour	27.5
Time for 250 ml sample, hour	9.1
Doses/day	24
Flow, ml/dose	27.5
Empty bed volume, liter	4.9
Resident water volume, liter ¹	0.37
Single dose vol. / resident water vol.	0.07
Average water residence time, hour	13.5

¹Assumes 50% pore space, 15% of pore space filled with water

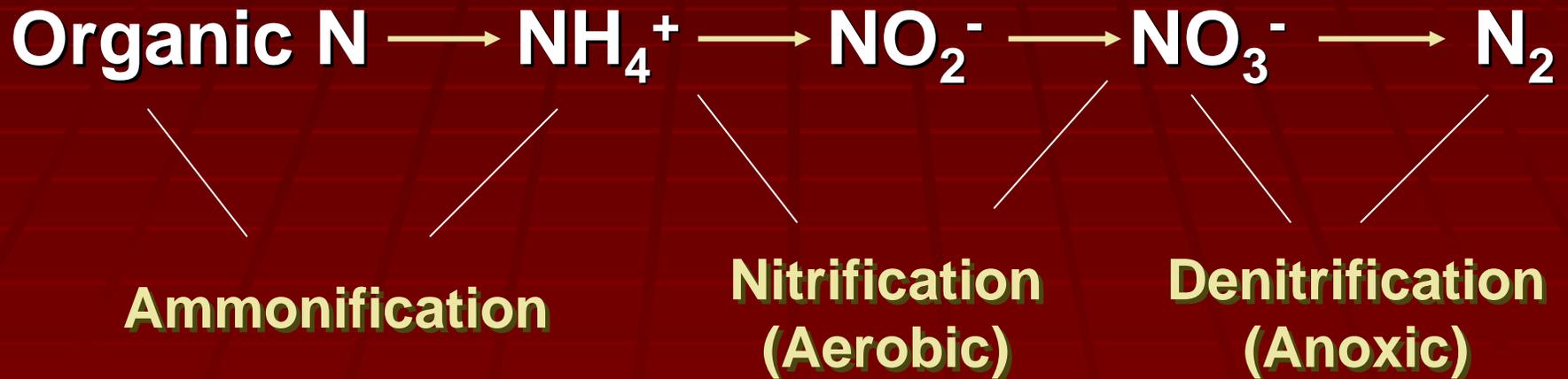
Saturated filter

Diameter, inch	1.50
Media depth, inch	24.0
Flow, gal/day	0.174
Flow, gpd/ft ²	14.22
Flow, ml/hour	27.5
Time for 250 ml sample, hour	9.1
Empty bed volume, liter	0.7
Pore volume, liter ¹	0.28
Average residence time, hour	10.1
¹ Assumes 40% pore space	

Comments/questions

- Literature review and database
- Laboratory studies

Biochemical Transformations



Keys Monitoring Study Update

Onsite Sewage
Research Review and Advisory
Committee
Meeting 10/18/07

Florida Keys Onsite Wastewater Nutrient Reduction Systems Demonstration Project (1996-2000)

- Ayres Associates constructed and operated a testing facility in the Florida Keys under contract with DOH and funded by EPA
- Influent TN: range 19.25-62.55 mg/L; average 38.6 mg/L
- Results:
 - AWT effluent standards can be met for CBOD5, TSS and TP
 - TN reductions of >70% are achievable without supplemental carbon addition
- Recommend county-wide utility to share costs and utilize cluster systems, and other management strategies

Keys Background

- Chapter 99-395 of the Laws of Florida established specific effluent standards for OSTDS in the Florida Keys.
- Keys Standards for onsite wastewater nutrient reduction systems (OWNRS) on a permitted annual average basis:
 - 10 mg/L of carbonaceous biological oxygen demand (CBOD5),
 - 10mg/l of total suspended solids (TSS),
 - 10mg/L of total nitrogen (TN)
 - 1 mg/L of total phosphorous (TP).
- Amended in 2001 to allow for aerobic treatment units without nutrient reduction in areas scheduled to be sewerred by 2010

Keys onsite system sampling

- Through early 2001, operating permit fees could cover yearly sampling of ATUs and engineer-designed systems during annual County Health Department Inspection
- In 2001, legislature reduced fees
- since then, apparently no sampling

Sources of Variability

- Diurnal (not significant in ATU samples, but limited data)
- Daily (significant)
- Monthly (significant)
- Sampling location (significant)
- Operation and Maintenance?
- Technology?
- Design?
- Influent?
- Usage patterns?
- Sampling method?

Keys Monitoring Study

- Objective: characterize performance and develop performance monitoring approach
- Sampling by Monroe County Health Department Staff
- 15 OWNRS systems and 5 interim systems, initially
- criteria:
 - residential system,
 - current maintenance contract,
 - permanent residency in the Keys (homestead exemption).
 - OWNRS system included were those that were volunteered by OWNRS owners that responded to mailings by MCHD sent to all OWNRS systems on record that fulfilled the three criteria (192 out of 326 systems).
 - Interim systems were randomly selected from the total population of interim systems in the Florida Keys using the same inclusion criteria.

Keys Monitoring Study

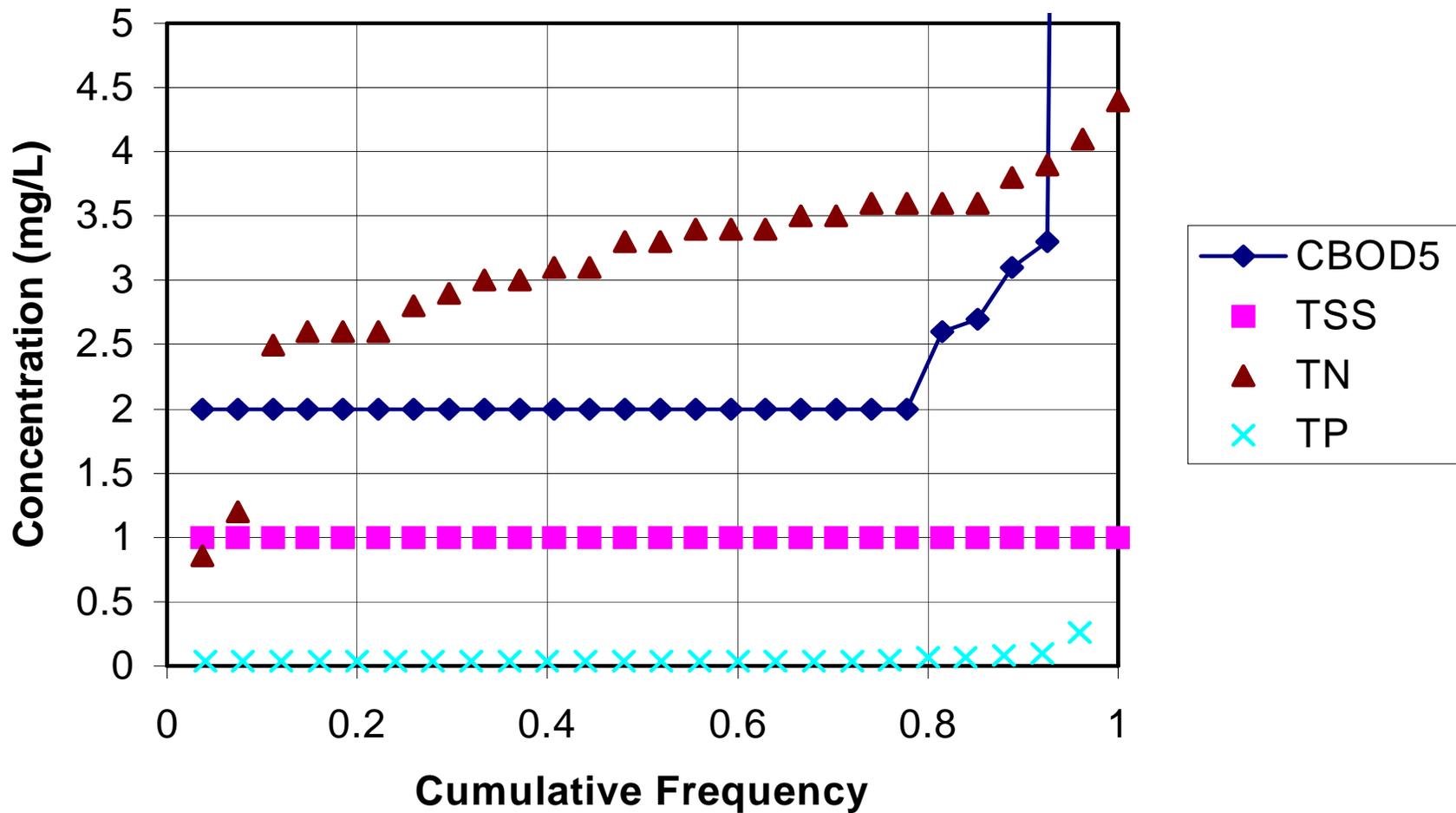
- Sample effluent from P-trap, sample influent where possible from settling tank
- Compare 24-hour time-composite samples to multiple grab samples taken with the same type of equipment
- Each system will be sampled twice during a “peak” season (November through May) and an “off “season (June through October)
- Sampling started 2/18/07, peak season is completed

Status

- 3 interim systems
- 11 OWNRS
- Some staffing and contracting delays

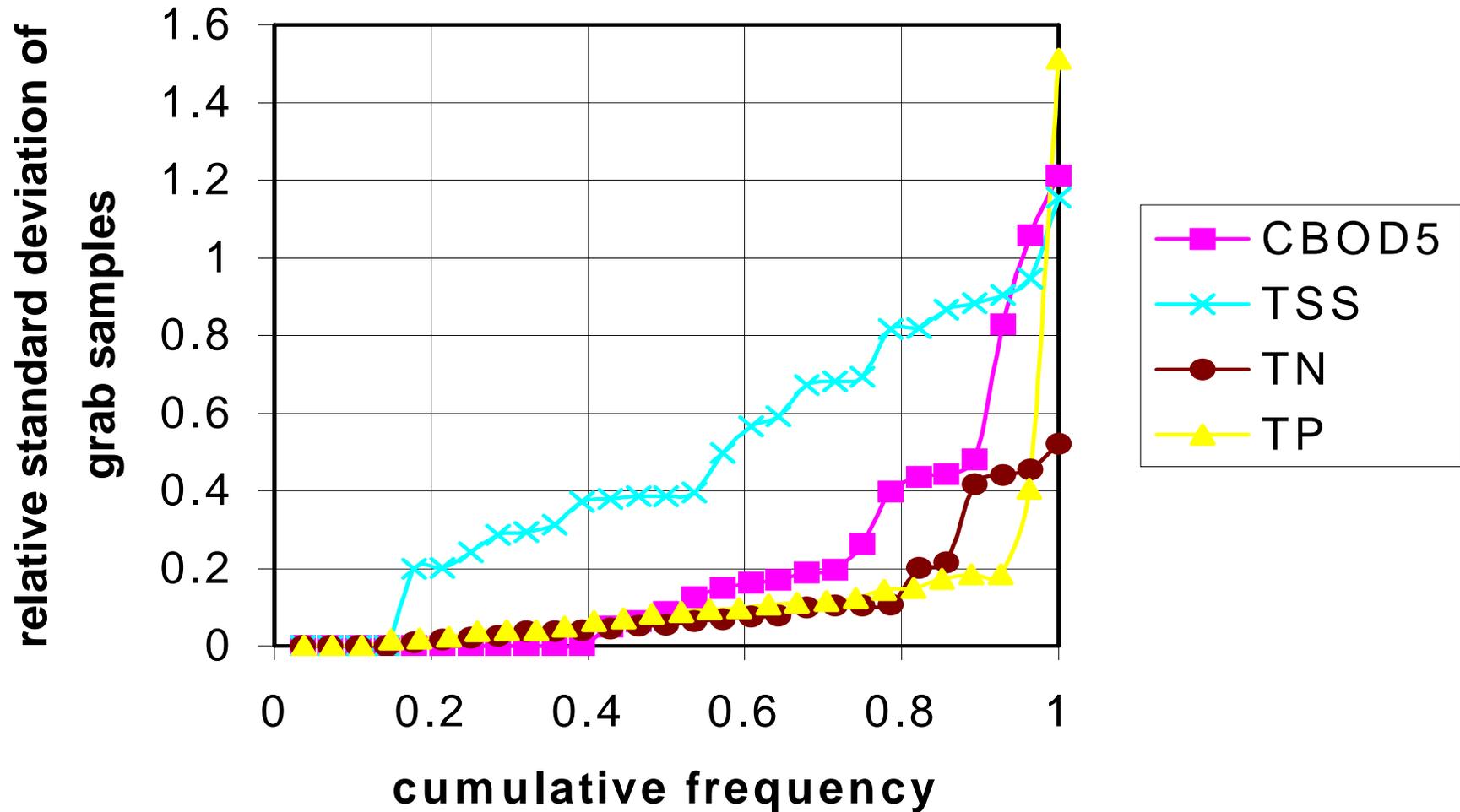
System	Number of Events
Interim 2	2
Interim 4	2
Interim 5	1
OWNRS 1	2
OWNRS 3	2
OWNRS 4	1
OWNRS 5	3
OWNRS 6	2
OWNRS 7	3
OWNRS 8	2
OWNRS 9	2
OWNRS 11	2
OWNRS 14	2
OWNRS 15	2

Tap Water Results

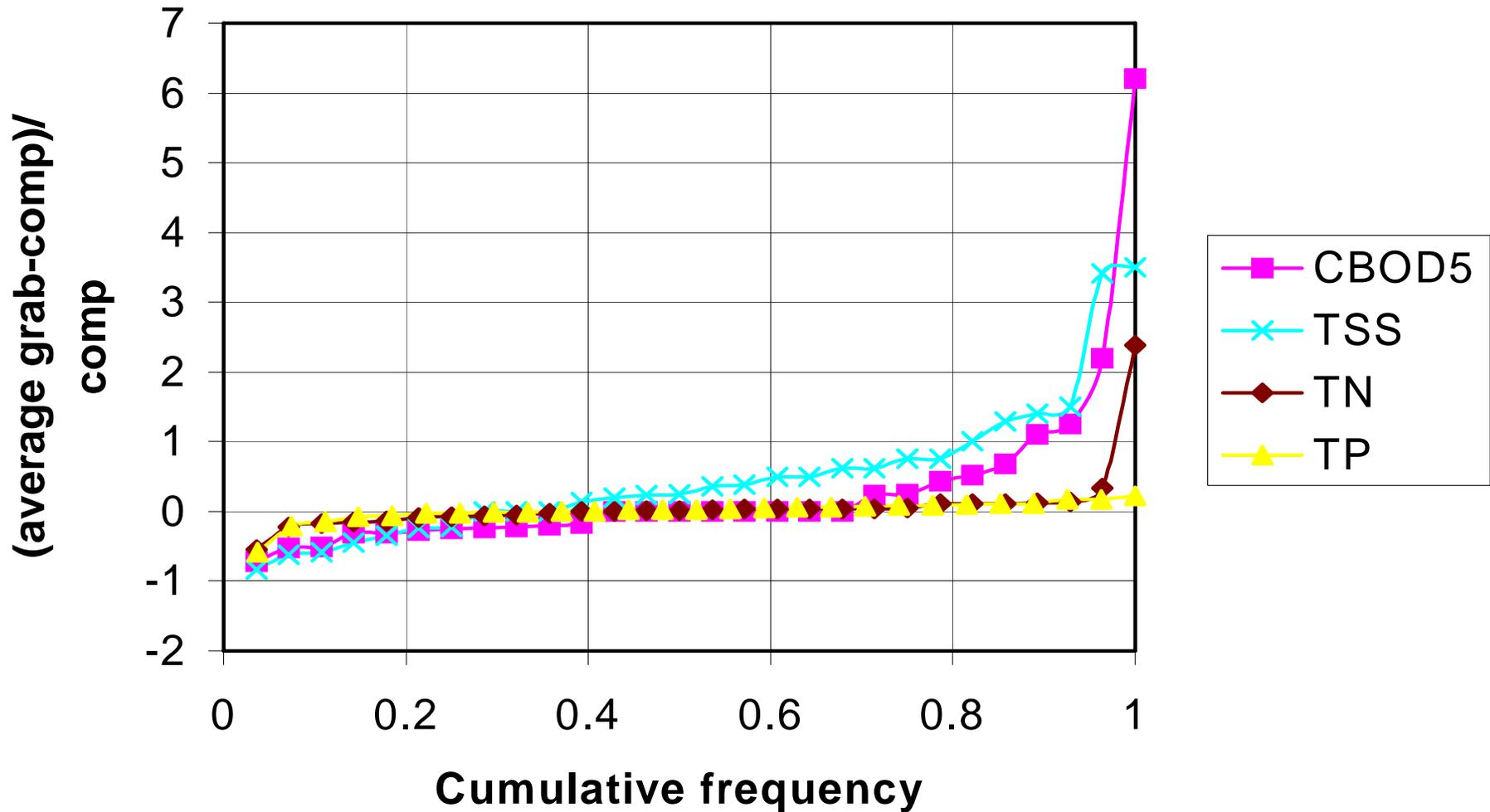


- Note: below detection limit is listed as detection limit value

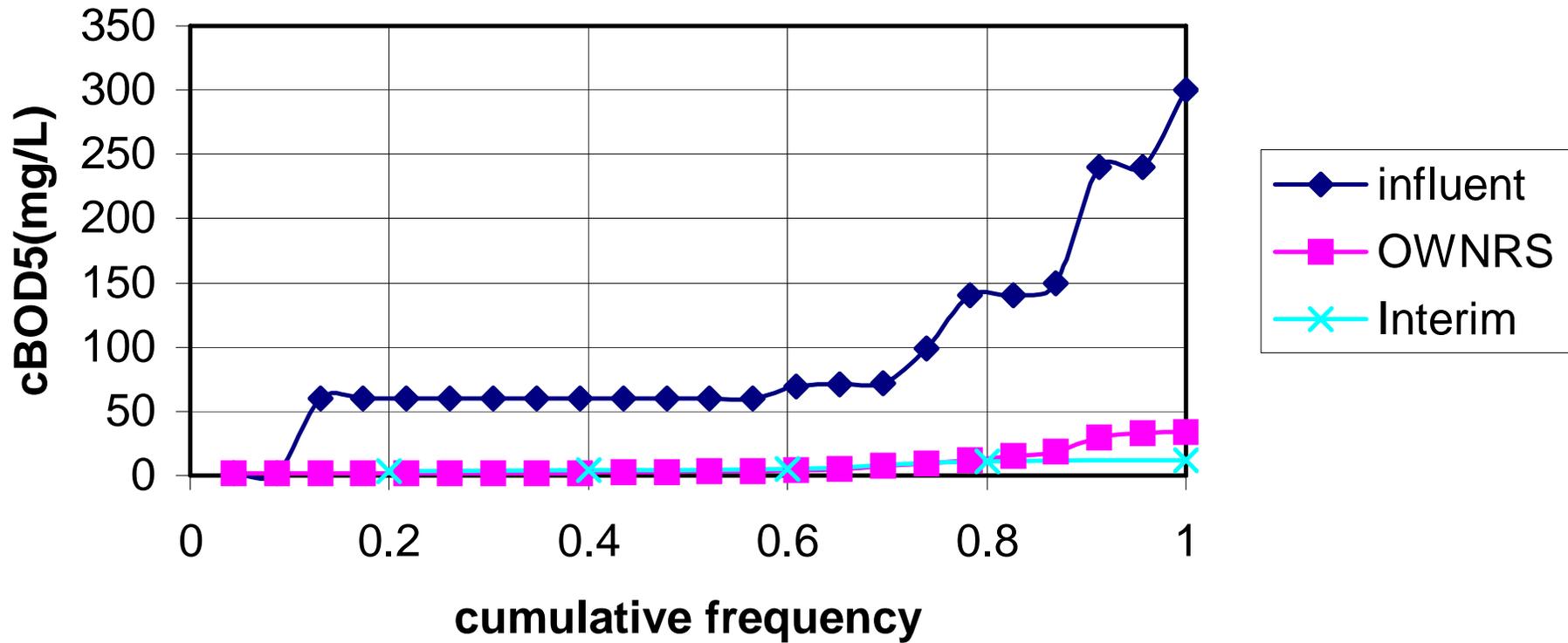
Variability of Grab Samples in a Day



Comparison of Grab and Composite Samples

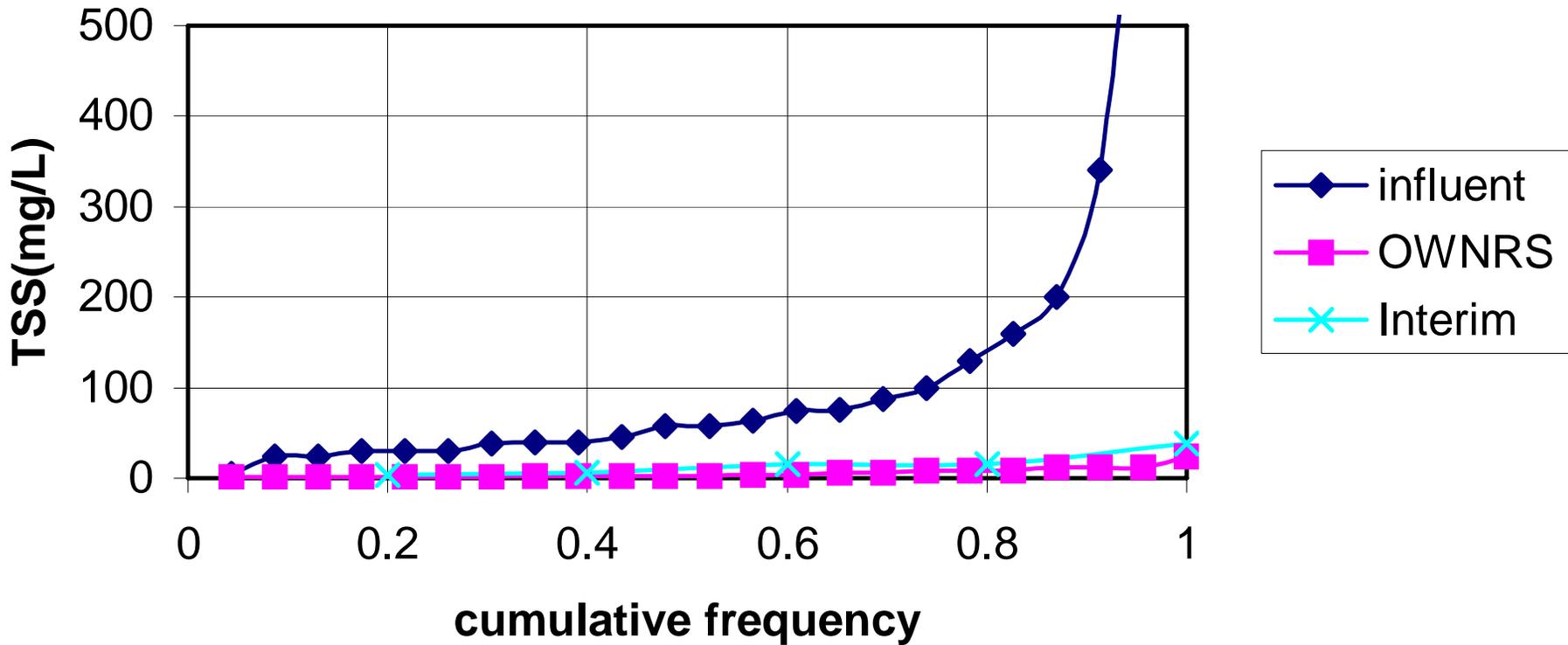


cBOD5 Influent and Effluent Comparison



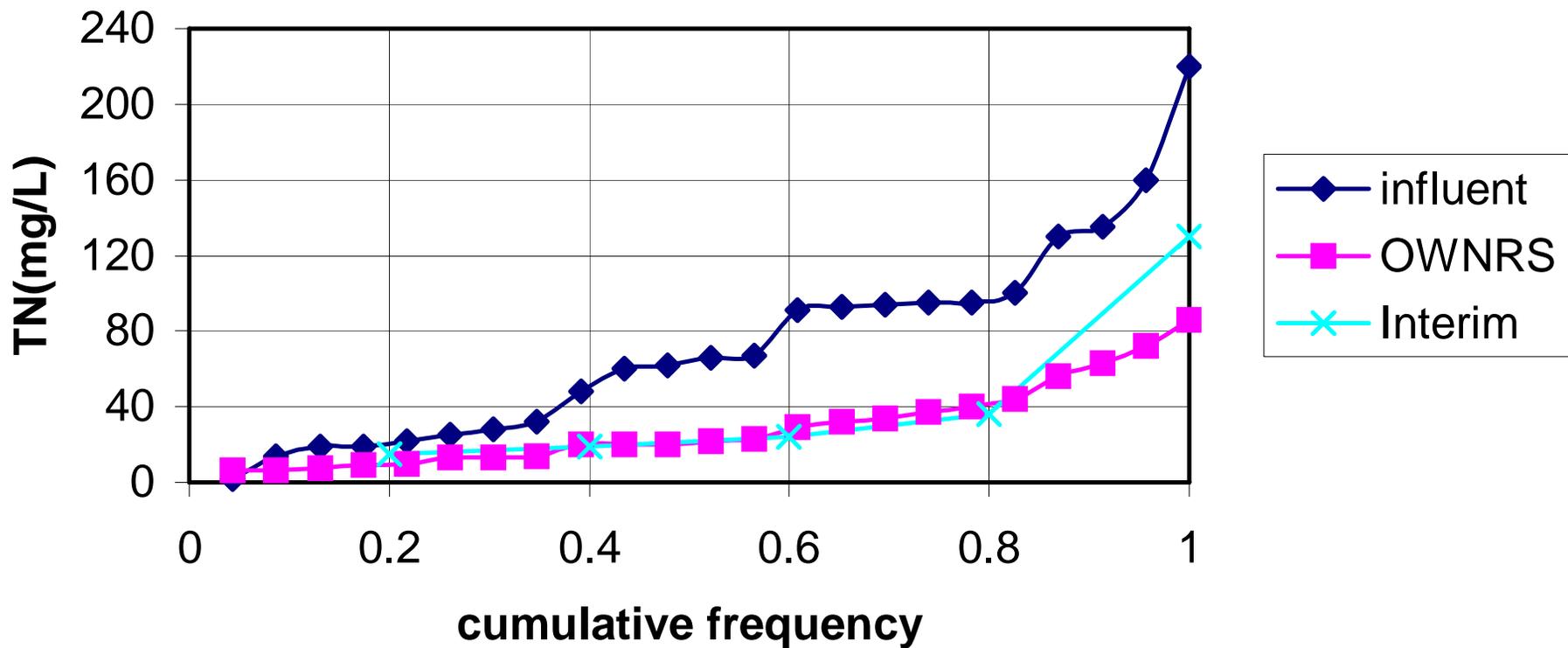
- Note: below detection limit is listed as detection limit value

TSS Influent and Effluent Comparison



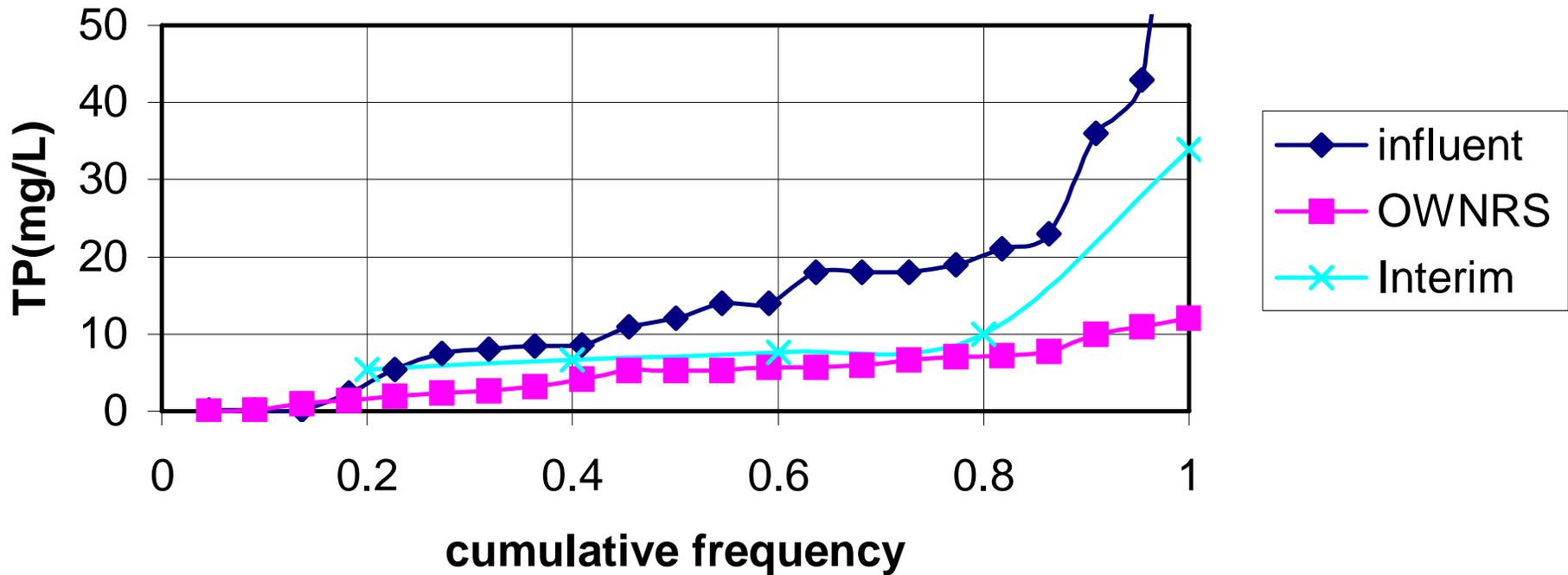
- Note: below detection limit is listed as detection limit value

Total Nitrogen Influent and Effluent Comparison



- Note: below detection limit is listed as detection limit value

Total Phosphorus Influent and Effluent Comparison



- Note: below detection limit is listed as detection limit value

Preliminary Observations

- Only a few odd numbers
- Diurnal variability appears lower for nutrients than for effluent strength
- Nutrient grab samples appear very consistent with time-composite samples, less so for TSS
- Wastewater strength appears to be lower than in Keys OWNRS study
- Nutrient concentrations appear to be higher than in Keys OWNRS study

Off-Peak Season

- Repeat sampling to assess variability for the same system over time
- Added parameters:
 - Fecal coliform
 - Alkalinity and pH

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

Approved Minutes of the Meeting held at Sylvan Lake Park, Sanford, FL
October 18, 2007

Approved by RRAC on January 23, 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); John Glenn (member, Environmental Interest Group); Marc Hawes (alternate, Home Building Industry); Stan Keely (alternate, Professional Engineer); Bill Melton (member, 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:** Phillip Alexander (Superior Septic); George Bartuska (BFA Environmental); Alice Berkley (Office of Representative Bryan Nelson); Dominic Buhot (Greens Environmental Services); John Byrd (Aide to Orange County Commissioner Brummer); David Childs (FWEA Utility Council); Ron Davenport (Infiltrator Systems); Kim Dove (Seminole County Environmental Health Department); Doug Everson (Plastic Tubing Inc.); Chris Ferraro (Florida Department of Environmental Protection); Roxanne Groover (Florida Onsite Wastewater Association); Roland Harris (Complete Ozone Inc.); Jerry Henkins (Seminole County Environmental Health Department); John Higgins (Markham Woods Association); Ken Jones (Markham Woods Association); Tony Matthews (Seminole County); Steve Meints (Averett Septic); Harley Pattee (Complete Ozone Inc.); Daniel Smith (Applied Environmental Technology); Britt Watson (Averett Septic Tank); Walter Wood (Lake County)
 - **Department of Health (DOH), Bureau of Onsite Sewage Programs:** Paul Booher; Dr. 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.
 2. **Review Minutes of Meeting June 12, 2007:**
 - a. **Motion was made by Stan Keely and seconded by Sam Averett for the RRAC to approve the June 12, 2007 meeting minutes. One clarification change was requested on the June 12, 2007 meeting minutes. Under the Closing Comments section, the minutes are to change to: "Ellen Vause stated that Florida needs to stop dumping wastewater into streams and oceans. We need to allow it to filter down to the aquifer through the soil." The minutes were approved as amended. All were in favor with none opposed of approving the amended minutes, and the motion passed.**
 3. **Wekiva Onsite Nitrogen Contribution Study:**

Elke Ursin presented the progress of the study since the June 12th meeting. Linda Young revised the pie charts for Task 3 to eliminate the loading estimates from the other sources and the onsite sewage loading estimates were included. John Byrd asked for clarification

on this. Elke Ursin explained that Task 2 and Task 3 were required to report the estimates on loading from onsite systems. The final report does not include loading estimates for other sources and there is only one pie chart comparing the relative contributions of inputs. John Byrd read from the past meeting minutes that the RRAC made a motion to remove the loading numbers from the report. Pam Tucker stated that the report said that contrary to the RRAC's recommendation it was decided administratively that they were going to put the onsite sewage loadings into the report. The report was sent on June 30th and the deadline was met. There was a TRAP meeting on August 21st. There was a motion made, seconded, and passed to approve rule language prohibiting land application of septage and food establishment sludge within the Wekiva Study Area. There was a motion made, seconded, and passed to table all other proposed rule language specific to the Wekiva Study Area until completion of the DEP phase II study. The vote was 7 in favor, with 2 opposed. The two dissenting votes were Patti Sanzone representing the Florida Environmental Health Association and Russ Melling representing the County Health Departments. Both indicated they wanted the panel to discuss each specific proposal. There was debate over the first issue regarding requiring performance based treatment systems for new systems, but the second issue eliminating grandfathering for separation to wet season water table and surface water setbacks and the third issue requiring all systems to be pumped and evaluated every 5-years were both good recommendations that should not only apply to the Wekiva Study Area, but statewide. There was a discussion on the Wekiva River Basin Commission meeting that was held on October 16, 2007. During the commission meeting Gerald Briggs, Bureau Chief of the Department of Health Bureau of Onsite Sewage Programs, presented the proposed rule language and reported that at that time the department is discussing options with the governor's office but there were no specific plans with moving forward with rule making. Paul Booher reported that after the commission meeting, Mr. Briggs received a call from Dr. Conti, Environmental Health Division Director, advising him that the department would proceed with rule making.

There was a discussion on the process of rule making, filing, and public notification. Paul Booher called Dale Holcomb in the program office and reported that when the office is given the notice to proceed, language will be submitted to the Florida Administrative Weekly (FAW) where it takes 10-days to prepare for advertisement. Then it is advertised for 21-days for public hearings and comments. If there are significant changes, then it would need to be re-advertised. Assuming there are no changes or legal challenges, the rule is filed and becomes effective 20-days after the date filed.

Some of the public education that has been done since the last meeting is that a presentation on Wekiva was made at the Florida Environmental Health Association Annual Education Meeting in August, a poster was also presented at this meeting and received a certificate of excellence, a presentation was made to the Ichetucknee Springs Working Group in October, and an abstract was accepted for presentation at the National Onsite Wastewater Recycling Association 2008 Water Symposium.

Pam Tucker stated that there is no hurry for this rule to be implemented. The Wekiva Parkway can be up to 20-years away. She stated that there is no scientific proof that the nitrogen contribution is significant and the study is not complete on this issue. Bill Melton stated that Pam Tucker should be speaking about relative significance. The data is not

available to make the determination of the relative significance as it relates to other contributors. He does think that a determination can be made that there is a significant amount of nitrogen that is getting to the aquifer from onsite systems in the WSA.

The proposed rule was discussed, with the understanding that this is more in the purview of the Technical Review and Advisory Panel (TRAP), and that the RRAC has not come to a conclusion on relative significance of nitrogen impacts. Some of the discussion points:

- The rule proposal to prohibit land spread of sewage in the Wekiva Study Area (WSA) was approved by TRAP.
- Each of the three counties in the WSA has a comprehensive plan that should illustrate whether sewer is planned to be available to specific areas. It would be helpful to have a GIS map available to better view this information, but it is not clear whether this exists or not.
- The proposed rule does not have any specific requirements for testing. Dr. Roeder stated that the ability for DOH to gather fees that covered testing was taken out of the statute in 2001, but the design engineer can require it. The state code requires an inspection to make sure the system is functioning mechanically as it should. DOH does an inspection annually and the maintenance entity does an inspection a minimum of two times per year. If it does not meet the requirements specified by the engineer, the engineer will be required to redesign the system. Sam Averett stated that one of the biggest issues is who will pay for the sampling. He mentioned a previous TRAP issue regarding the manufacturer to sample a subset of all installed systems and if the subset passes then they are approved. This would require each manufacturer to assure the state that they are performing as stated. It is too expensive to require the homeowner to pay for the sampling. Ellen Vause stated that the homeowners also have a part in whether a system is working or not, because water-use habits determine the strength of the effluent. She stated that if the state feels that this has to be done in the WSA then some assurances need to be set to make sure systems do meet the discharge requirements. In order to verify the 70% nitrogen reduction standard an influent sample would be required which would double the cost. Paul Davis stated that pumping the tank helps bring the system back to normal. The county health departments will not have enough time to sample influent and effluent for each system. Sam Averett stated that it is crucial that DOH develops a maintenance protocol and a testing protocol for each manufacturer and make the manufacturer pay for it. He stated that the performance based language in the code is still vague and that the department could interpret the language to say that monitoring is required on every system at least once or twice a year. This interpretation could be clarified in a memo to allow the existing code to be used to monitor systems.
- The proposed rule does not specifically state **total** nitrogen.
- Requiring a minimum bottom of drainfield elevation of 18-inches below finished grade would wipe out any alternative drainfield product that is more than 12-inches in height. This requirement would also make it difficult to ensure the required fall in the drainlines. An installer commented that only 6-inches of soil cover over the top of the drainfield would make it easy to crush the drainfield when covering.
- There was some confusion over what forms are required, and whether this indicates that a non-certified individual would be allowed to perform a site evaluation. [NOTE: clarification on this issue was received, and a Certified

Environmental Health Professional (CEHP) is required to perform any site evaluation]. Some septic contractors voiced a concern over there being too many forms to fill out and whether there are any other options. They stated that this is time consuming and expensive. Kim Dove stated that if a site evaluation is required there is also an additional fee for the county health departments or other CEHP.

- The proposed rule language as written would prohibit tanks that are larger than within one tank size of current requirements.
- In the existing system language it states that the system would need to meet these requirements if it is in need of repair, modification, or re-approval. Re-approval would include those systems being inspected under part (c) when they are pumped and certified every five years.

Paul Booher stated that there were several good points that would need to be considered, and that staff will report these comments to Gerald Briggs.

David Carter summarized the discussion. At the last RRAC meeting the committee made a motion that no action be taken on Task 4 (to develop recommendations to reduce impact if significance was determined). The department is now prepared to move forward with new rule language. The department staff are taking notes and listening and this is a good group with a lot of expertise and experience. He thinks the department will take into consideration several of the comments made at this meeting. The RRAC is supposed to be a research committee looking at studies and recommending new studies. This is blurring into a TRAP area. Bill Melton agrees that this is not RRAC's purview and making comments is essentially all that can be done. Pam Tucker stated that adopting the rule without DEP's Phase II being completed is wrong. TRAP and RRAC tabled the issue to wait for the scientific data to be completed. She stated that it is important for RRAC to have these inputs and have an agreed upon position. Ellen Vause crafted a motion stating that RRAC stands behind their previous position and that the proposed rules are premature. Bill Melton stated that he is not sure the rules are premature, that onsite systems are contributing nitrogen, but that the data is not there to determine the relative significance. Sam Averett stated that he has no doubt that onsite systems in the Wekiva Area put nitrogen into the Wekiva Springs. Patti Sanzone stated that DOH could propose rules on this issue, even if there was no proposed Wekiva Parkway. Paul Davis stated that most of the discussion so far has been a TRAP committee discussion, not research.

Sam Averett made a motion which was seconded by John Glenn:

RRAC, after review of the Department of Health proposed rule language for Wekiva, still stands behind the previous statement that RRAC is unable to determine relative significance of onsite system impacts of nitrogen to the Wekiva Study Area.

There was a discussion on the relative significance of nitrogen impacts from onsite systems. Several RRAC members were in agreement that onsite systems contribute to the quantity of nitrogen in the Wekiva Study Area, but the relative significance has not yet been agreed upon. After a lengthy discussion, Stan Keely called the motion into question. The members voted and four were in favor: Sam Averett, David Carter, Paul Davis, and

Stan Keely; and three were opposed: John Glenn, Bill Melton, and Pam Tucker and the motion passed. [NOTE: a clarification was made later in the meeting from Pam Tucker stating that she was actually in favor of this motion and would like the minutes to reflect this.]

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

If the proposed rule goes forward, if a pump is required, low pressure dosing should be used due to the increase in system longevity and relatively low additional cost.

There was a discussion that for a minimal additional fee, the life of the system could be extended by years. The members voted and six were in favor with one opposed (Pam Tucker).

It was decided that RRAC would not go through the proposed rule item by item as that is TRAP's area.

After a short break, both the engineer member and alternate left, but there was still a quorum. [NOTE: Clay Tappan returned to the meeting during the updates on other projects].

David Carter stated that DEP has posted the MACTEC report and some additional information on their website www.dep.state.fl.us/water/waterprojectfunding, under Wekiva nitrate sourcing. Chris Ferraro with DEP made an announcement that DEP has been working on the Total Maximum Daily Flows (TMDL's) for the Wekiva Study Area. Tentatively, on November 29th, there will be a public meeting for the TMDL's for the Wekiva Study Area. Bill Melton asked whether DEP will get the MACTEC information refined to help RRAC make a final determination on relative significance. Elke Ursin stated that she had received an email from Bonnie Hall with DEP who stated that they are working on the scope of work right now and the scope is close to being complete. There were no specific dates set at that time, but as soon as there is any additional information she will forward it on.

4. Brief updates on other projects

a. Ongoing projects

- i. Passive Nitrogen Removal Assessment** – Elke Ursin provides a brief overview of the project. The draft literature review report and draft quality assurance project plan were provided in the mailed packets to the RRAC members for review. Dr. Daniel Smith presented on his progress to date. The literature review and database portion was completed with assistance from Dr. Dick Otis. The goal of the study is to evaluate passive treatment media for removal of total nitrogen from onsite wastewater. This project will focus on various filter materials, which are more stable and less subject to variation. The project has five tasks: a literature review and database, laboratory experiments, a feasibility analysis (how the results and recommendations deployed), an economic analysis, and a final report. The literature review task involved searching databases and search engines, looking into test centers,

and personal contacts. Paul Booher recommends that the report include suggestions on how to deal with the material that has been expended and needs to be disposed. Dr. Smith goes over zeolites and coir fiber as aerobic filters. Roxanne Groover stated that Quanics has performed NSF testing on the coir fiber and has information on total nitrogen. Dr. Smith stated that the coir may not need to be tested. He then went over anoxic filters. Next Dr. Smith went over the Quality Assurance Project Plan (QAPP). The Invitation to Negotiate defines passive treatment 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”*. Dr. Smith stated that first the effluent needs to be nitrified and then denitrified, so he is proposing a two stage process. The first stage is an unsaturated media filter that provides ammonification and nitrification. The second stage is a saturated media filter containing an electron donor and is anoxic thus providing denitrification. The next decision is where to put the pump. Dr. Smith decided to place the pump in the front because nitrification will be the trickiest part of this process. This placement of the pump will allow pressure dosing at the first stage filter and will also allow for timed dosing. He has located some potential sites for the laboratory experiments. Septic tank effluent will be used for the experiments. He went over the media configuration, and how the columns will be configured. The experiment will be set up and then monitored to see how well they work. The stage one will be dosed once per hour for 2-3 minutes as needed, at a minimum loading rate of 2 gallons/sq.ft./day. Both stage one and stage two will be operated and monitored over 60 days and will test for temperature, pH, alkalinity, DO, and the entire nitrogen species. In response to a suggestion to change conditions in the experiment, Roxanne Groover asks how the determination will be made to adjust the loading. She stated that she would be more comfortable with a baseline that does not change. Dr. Smith stated that before altering the flow he will gather enough information prior to making a change. He stated that he is planning on running the column for about 3-weeks prior to taking any samples to allow for the microbial population to become established. He stated that the experiments should be run for a longer timeframe, but that the time and budget do not allow for this. The feasibility and economic assessment portions of the project will be based on the best available information in the timeframe allotted for this project. Dr. Eberhard Roeder suggests keeping the parameters the same for the first 6 samples and then an assessment can be done on what to adjust for a potential new project. Paul Davis asks whether the experimental design calls for part of the system to be above the ground, and if so is it possible to do an unsaturated tricking filter for aeration coming directly from the outlet of the septic tank then pumping to the saturated zone to keep the system in the ground. Dr. Smith stated that that the design calls for an unsaturated area and it is possible to configure the system as Mr. Davis suggests but that having the pump at the beginning will be more aggressive at converting to nitrates. Dominic Buhot asks whether lava rock was considered as a media, and Dr. Smith stated that it was not looked at but that it is similar to some of the expanded shale media. Dr. Smith asks if there is anywhere to find that material in a granular form, and Mr. Buhot stated that it can be found at landscaping suppliers. Elke Ursin stated that RRAC and

DOH have to provide comments on the Literature Review report and the Laboratory Experiments report within two weeks of the RRAC meeting. She will send an email to remind the RRAC members.

- ii. **High Strength Waste Study** – Paper submitted to American Society of Agricultural and Biological Engineers. If there are any comments please forward them on.
- iii. **Manatee Springs, Performance of Onsite Systems Phase II Karst Study** – Paper submitted to Water Research on 8/21/07 by Florida State University. Due to contractual and timing issues, this contract has expired and must be re-advertised and re-contract.
- iv. **Monroe County Performance Based Treatment System Performance Assessment** – Dr. Eberhard Roeder presented on the preliminary results of the Monroe County project. Some of the preliminary observations are:
 - 1. Only a few odd numbers
 - 2. Diurnal variability appears lower for nutrients than for CBOD₅ and TSS
 - 3. Nutrient grab samples appear very consistent with time-composite samples, less so for TSS
 - 4. Wastewater strength (CBOD₅ and TSS) appears to be lower than in Keys Onsite Wastewater Nutrient Reduction Study (OWNRS)
 - 5. Nutrient concentrations appear to be higher than in Keys OWNRS study
 - 6. There will be repeat sampling done to assess variability for the same system over time with the added sample parameters of fecal coliform, alkalinity, and pH.

There was some discussion over the strength of the influent being higher than expected. The system may be working properly, but if the influent is too high the effluent may be higher than the 10 mg/L that is required. Sam Averett wanted clarification on whether any of the tested systems are on the list of state approved systems. Dr. Roeder stated that it was primarily one manufacturer with some others that were approved by the county health department. Sam Averett stated that it is difficult to take an influent sample, and the sample may be skewed by fecal matter. Dr. Roeder pointed out that the low number of Total Suspended Solids (TSS) supports that there was low solid fecal matter or other solids that may skew the results, and that the settling tank where the sample was taken from has effectively settled the solids.

- v. **Remote Sensing of Optical Brighteners Study: Mote Marine Report** – Summary report from DEP has been submitted on results of tasks up to the airborne Light Detection and Ranging (LiDAR). The flow-through fluorescence method showed potentially interesting patterns (i.e. one location showed a higher signal corresponding to locations where failed septic systems were known to exist). Contract was amended on Oct. 15th to comply with Contract Administration requirements (end date changed to 12/31/07). New contract will need to be issued using IGA exemption to allow for completion of scope. Phone conference to be held on Oct. 25th to discuss next steps. The Mote Marine portion of this project looked into the optical properties of water and optical brighteners in great detail. They also took some wastewater from onsite systems and characterized it. They discovered two inputs that could be an indicator of wastewater. The results were very promising and now DEP will look into how to incorporate these results in what they have to do.

vi. **Taylor County Source Tracking Study** – RRAC made motion on May 8, 2007 meeting for staff to look into a follow-up sampling event to capture the May seasonal low water table event. FDEP was contacted to see if funds were available, and they were not available for a May sampling event, FDOH utilized research \$ to fund the project (just under \$14,000). Request for proposal was sent to various interested parties and FAU was selected to conduct the study. The sample site locations were determined to be the same as the original list with the exception of one site, which the previous study did not find a marked difference between another site in close proximity, which could be replaced with a new one. An interim progress report was submitted at the end of June 2007 outlining the May 2007 seasonal low water table sampling event, and is included in the packets sent to the RRAC. FDEP's 319 program has funded a September 2007 sampling event. Analysis is ongoing, and a final project report compiling all sampling events will be submitted in January 2008.

b. Projects coming up

i. **319 Project on Performance and Management of Advanced Onsite Systems** – \$300,000 grant through the EPA 319 program administered by FDEP. FDOH will provide \$200,000 in matching funds through the Monroe County project. Tasks:

1. Monroe County detailed study of variability of performance of advanced systems (Keys study)
2. Statewide database of advanced systems based on permit records
3. Survey of the perceived strengths and weaknesses of the current management of advanced onsite systems. County health department employees, septic contractors, homeowners will be polled and each set will have different questions.
4. Statewide assessment of operating condition and performance of advanced systems (random sample of 600 systems)
5. Quarterly influent and effluent sampling for a sample of systems (approximately 70 systems) to see seasonal variability
6. Booklet with case studies outlining both strengths and weaknesses of the current program and best practices in advanced onsite management

Elke Ursin stated that she needs RRAC to vote on whether this project scope is acceptable to move forward, so that she can present it to the TRAP. Sam Averett made a motion that was seconded by Paul Davis:

RRAC recommends moving forward with the 319 project.

Bill Melton stated that he thinks it is not a good idea to mix ATU's, PBTS, and interim systems with the sampling. Dr. Roeder stated that these are all in the category of advanced. Bill Melton thinks this category is too broad. Dr. Roeder stated that including all these classifications would allow for a distinction between the types of systems to see if there is a difference in treatment effectiveness between the different types. Patti Sanzone stated that she views this project as a program check. Sam Averett stated that it is critical to make sure the data is collected effectively. The specifics will be discussed at future meetings. There was a discussion on making sure what is sampled will be statistically significant. The database will give an indication of the population of

systems, how many there are and of what type. Then a number can be determined on what systems to sample. If a system has too few units installed to be statistically significant, they may be removed from the sampling scheme and the extra numbers reallocated to other systems. The members voted and all were in favor with none opposed.

- ii. **Coastal Management Program Grant Funding Opportunity** – FDEP has sent out a notification for a grant funding opportunity due November 15, 2007. One idea is to utilize this funding to sample in the Town of Suwannee, Cedar Key, and areas of Taylor County where areas have converted from onsite systems to sewer and where there is previous sample data from when the areas were still on onsite systems.

Sam Averett made a motion that was seconded by Clay Tappan:

RRAC recommends FDOH apply for the FDEP Coastal Management Program grant funding opportunity.

The members voted and all were in favor with none opposed.

5. **Budget Discussion** – This item is to be discussed at the next meeting
6. **Prioritization of Future Projects** – This item is to be discussed at the next meeting, RRAC members are encouraged to develop a list of potential future project ideas to assist in the discussion.
7. **Public Comment** - The public was allowed to comment throughout the meeting and their comments are included throughout the minutes.
8. **Closing Comments, Next Meeting, and Adjournment**
 - a. David Carter requested that staff work on filling some of the vacant RRAC positions. The Real Estate Industry and State University System have vacancies for the alternate category, both member and alternate of the Restaurant Industry have been absent for many meetings, and the regular member of the Septic Industry has been absent for many meetings as well. David Carter requested that letters be sent to those four groups requesting that they find someone who will attend the meetings. The next meeting will also have an election for the chairperson and vice chairperson and it will be important to get someone in the position so that continuity is maintained.
 - b. Pam Tucker asked for clarification on whether the passive nitrogen systems being studied in Dr. Smith's project would work with performance based treatment systems, and asked for clarification on what is passive about them. It was explained that passive systems just sit there and work with minimal outside influence. David Carter stated that we are always looking for ways to improve how systems are working, and this project is not necessarily tied into Wekiva. The results of the Passive Nitrogen Removal project could show, for example, that for \$2,000 you can put this gizmo on a system and achieve an 80% reduction. Sam Averett stated that no private company wants to study this because you can't patent oyster shells, for example. Paul Davis stated that no one is going to study this except for entities like the State of Florida.
 - c. No date was set for the next meeting. Next meeting anticipated to be some time in January 2008 at a location to be determined. Pam Tucker motioned to adjourn and Clay Tappan seconded. The meeting adjourned at 2:25 pm.



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

Thursday October 18, 2007
9:30 am - 3 pm

Sylvan Lake Park
845 Lake Markham Road
Sanford, FL 32771



Agenda:

- Introductions
- Review minutes of meeting 06/12/07
- Wekiva Onsite Nitrogen Contribution Study
- Updates on other projects
- Budget discussion
- Future projects
- Public comment
- Closing comments, next meeting, and adjournment



Introductions & Housekeeping

-
- Travel reimbursement forms



Review Minutes of Meeting 06/12/2007

- See draft minutes



Wekiva Onsite Nitrogen Contribution Study

Since last meeting:

1. Report was completed and sent to the governor by June 30, 2007
2. TRAP meeting on August 21, 2007
3. Wekiva River Basin Commission Meeting October 16, 2007
4. Currently discussing options with the governor's office



Wekiva Onsite Nitrogen Contribution Study and Public Education

- Presentation given at the Florida Environmental Health Association (FEHA) Annual Education Meeting August 2007
- Poster presented at FEHA AEM, received Certificate of Excellence August 2007
- Presentation to the Ichetucknee Springs Working Group October 2007
- Abstract selected to present at National Onsite Wastewater Recycling Association (NOWRA) 2008 conference in Memphis, TN



Wekiva Onsite Nitrogen Contribution Study

June 30, 2007 - final report submitted to
Governor Crist & Legislature



Proposed language (see handout)



Land application

64E-6.010 SEPTAGE AND FOOD ESTABLISHMENT SLUDGE

(1) through (6) No change

(7) The food establishment sludge and contents from onsite waste disposal systems shall be

disposed of at a site approved by the DOH county health department and by an approved disposal method. Untreated domestic septage or food establishment sludges shall not be applied to the land. Criteria for approved stabilization methods and the subsequent land application of domestic septage or other domestic onsite wastewater sludges shall be in accordance with the following criteria for land application and disposal of domestic septage.

(a) through (v) No change.

(w) The land application area shall not be within the Wekiva Study Area as defined in 369.316, F.S.

Specific Authority: 381.0011(4), (13), 381.0065(3)(a), 489.553(3), FS. Law Implemented: 381.0012, 381.0061, 381.0065, 386.041, FS. History: New 12-22-82, Amended 2-5-85, Formerly 10D-6.52, Amended 3-17-92, 1-3-95, 5-14-96, Formerly 10D-6.052, Amended 3-22-00, 05-24-04, 11-26-06, .



New systems

64E-6.0162-Specific Standards for the Wekiva Study Area

- (1) The following standards shall apply to all systems in the Wekiva Study Area as delineated in 369.316, F.S.
 - (a) Except in areas scheduled by an adopted local wastewater facility plan to be served by a central sewage facility by January 1, 2011, all new systems shall be an performance-based treatment system providing nitrogen reduction. The systems shall provide at discharge from the treatment units before disposal an annual average nitrogen reduction of 70 percent or a limit of 10 milligrams per liter, with a maximum individual sample concentration of 20 mg/L. No increase in authorized flow allowances in 381.0065(4)(a), (b), or (g) or reductions in surface water setbacks in 381.0065(4)(e) or (l) shall be allowed. All systems shall use drip irrigation or low-pressure dosing.



Existing systems

(b) All existing systems requiring repair, modification or re-approval must meet a 24 inch separation from the wet season water table and surface water setbacks in 381.0065(4)(e) or (l), unless a variance has previously been granted by the State Health Office. All treatment receptacles must be within one size of current requirements in Table II and must be tested for watertightness by a state licensed septic tank contractor or plumber. The bottom of the drainfield shall be no more than 18 inches below finished grade.



Pump and certify every 5 years

- (c) All systems shall be pumped out and evaluated by a state licensed septic tank contractor or plumber every five years. Upon completion of the evaluation the contractor shall complete Form DH 4015 page 1 - 4, and submit the application for approval to the department with a \$35 fee. A copy shall also be provided to the owner. The department shall review the application and approve the system for continued use or notify the owner of the requirement for a repair or modification permit. The department shall be responsible for notification and enforcement of the pumpout and evaluation requirement. Initial notifications shall be phased in over a five-year period beginning July 1, 2008.

Specific Authority 369.318, 381.0011(4), (13), 381.0065(3)(a), FS. Law Implemented 369.318, 381.0065, 381.0067, 386.041, FS. History—New .



8/21/07 TRAP meeting

- There was a motion made, seconded, and passed to approve rule language prohibiting land application of septage and food establishment sludge within the Wekiva Study Area.
- There was a motion made, seconded, and passed to table all other proposed rule language specific to the Wekiva Study Area until completion of the DEP phase II study. The vote was 7 in favor, with 2 opposed. The two dissenting votes were Patti Sanzone representing the Florida Environmental Health Association and Russ Melling representing the County Health Departments. Both indicated they wanted the panel to discuss each specific proposal. There was debate over the first issue regarding requiring performance based treatment systems for new systems, but the second issue eliminating grandfathering for separation to wet season water table and surface water setbacks and the third issue requiring all systems to be pumped and evaluated every 5-years were both good recommendations that should not only apply to the Wekiva Study Area, but statewide.



RRAC discussion on final DOH report and recommendations



Ongoing projects



Passive Nitrogen Removal Project

- Received draft literature review report and database
- Received draft Quality Assurance Project Plan (QAPP)
- Presentation from Dr. Daniel Smith on project



High Strength Waste Study

- Paper submitted to American Society of Agricultural and Biological Engineers



Manatee Springs, Performance of Onsite Systems Phase II Karst Study

- Paper submitted to Water Research on 8/21/07
- Due to contractual and timing issues, this contract has expired and must be re-advertised



Monroe County Performance Based Treatment System Performance Assessment

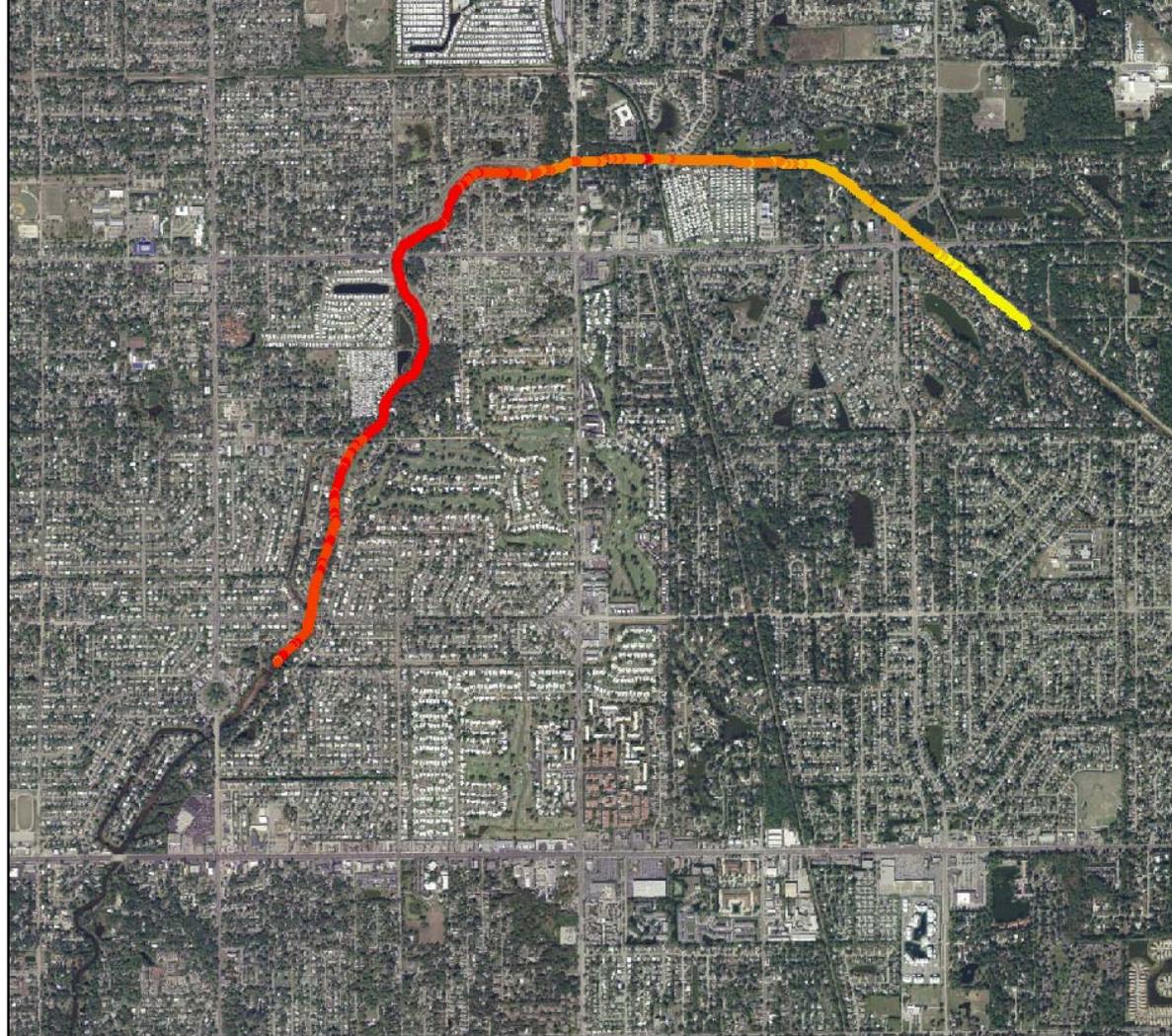


Remote Sensing of Optical Brighteners Study & Mote Marine Report

- Summary report from DEP on results of tasks up to the airborne Light Detection and Ranging (LiDAR)
- The flow-through fluorescence method showed potentially interesting patterns (i.e. one location showed a higher signal corresponding to locations where failed septic systems were known to exist)
- Contract was amended on Oct. 15th to comply with Contract Administration requirements (end date changed to 12/31/07). New contract will need to be issued using IGA exemption to allow for completion of scope.
- Phone conference to be held on Oct. 25th to discuss next steps



Flow-through fluorescence results for Phillippi Creek, Sarasota County, FL. Data are presented as the ratio of OB (optical brightener) to CDOM (colored dissolved organic matter).

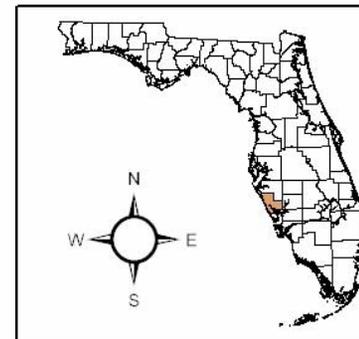


Legend

Phillippi Creek FTF

OB:CDOM

- 0.2132 - 0.2173
- 0.2174 - 0.2204
- 0.2205 - 0.2237
- 0.2238 - 0.2266
- 0.2267 - 0.2377





Mote Marine Report

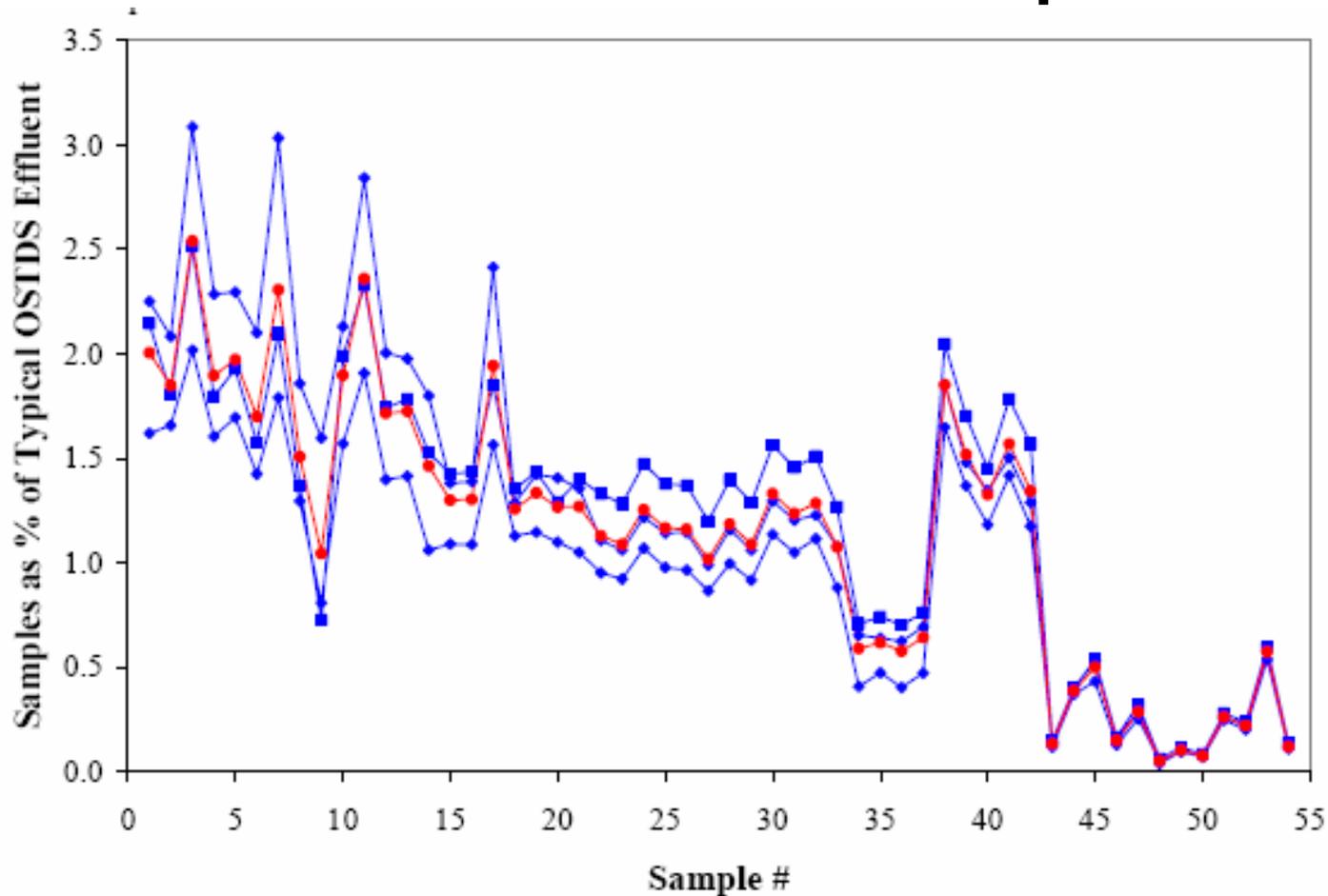


Figure 37. (from report) Amount of OSTDS waters present, as % of OSTDS effluent, estimated via PARAFAC Models 3-7. Mean value in red. Sample order in Table 5.



Taylor County Source Tracking

- RRAC made motion on May 8, 2007 meeting for staff to look into a follow-up sampling event to capture the May seasonal low water table event
- FDEP was contacted to see if funds were available, and they were not available for a May sampling event, FDOH utilized research \$ to fund the project (just under \$14,000)
- Request for proposal was sent to various interested parties and FAU was selected to conduct the study
- The sample site locations were determined to be the same as the original list with the exception of one site, which the previous study did not find a marked difference between another site in close proximity, which could be replaced with a new one
- An interim progress report was submitted at the end of June 2007 outlining the May 2007 seasonal low water table sampling event, and is included in the packets sent to the RRAC
- FDEP's 319 program has funded a September 2007 sampling event
- Analysis is ongoing, and a final project report compiling all sampling events will be submitted in January 2008



Projects coming up



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

Tasks:

1. Monroe County detailed study of variability of performance of advanced systems (Keys study)
2. Statewide database of advanced systems based on permit records
3. Survey of the perceived strengths and weaknesses of the current management of advanced onsite systems
4. Statewide assessment of operating condition and performance of advanced systems (random sample of 600 systems)
5. Quarterly influent and effluent sampling for a sample of systems (approximately 70 systems)
6. Booklet with case studies outlining both strengths and weaknesses of the current program and best practices in advanced onsite management



319 Project on Performance and Management of Advanced Onsite Systems

- RRAC to discuss moving forward with this project



Coastal Management Program Grant Funding Opportunity

- Deadline November 14, 2007
- Funding range: \$15,000 - \$150,000, no match required
- Funds available July, 2008
- Project should contribute to the protection, management, and enhancement of Florida's ocean and coastal resources
- Project idea: re-sampling Suwanee, Cedar Key, etc.



Research Budget

For fiscal year 2006 - 2007:

*Wekiva funding is not included in this amount as the funding source was not from research

For fiscal year 2007 - 2008:



Prioritization of Future Projects



Ideas for potential projects:

- See priority list handout



What does RRAC want to study?

Studies related to:

- Human health
- Performance of systems
- Environmental impacts from onsite systems



Rank Projects



Public Comment



Closing Comments, Next Meeting, and Adjournment

Important dates:

TRAP meeting: **November 8, 2007**
 Time: 9:00 am
 Place: Orlando Airport Marriott

Wekiva Commission Meeting: To be determined

Florida Department of Health

Research Review and Advisory Committee Meeting Summary

Meeting on October 18, 2007 at Sylvan Lake Park, Sanford

- **RRAC Members/Alternates Present:** Sam Averett, David Carter, Paul Davis, John Glenn, Marc Hawes, Stan Keely, Bill Melton, Jim Rashley, Patti Sanzone, Clay Tappan, Pam Tucker, and Ellen Vause. Seven out of nine groups were present, representing a quorum.
- **Review of Previous Meeting Minutes:** One clarification change requested on the June 12, 2007 meeting minutes. The minutes were approved as amended.
- **Wekiva Onsite Nitrogen Contribution Study:** There was a discussion on the Wekiva River Basin Commission meeting that was held on October 16, 2007. During the commission meeting Gerald Briggs, Bureau Chief of the Department of Health Onsite Sewage Program, presented the proposed rule language and reported that at that time the department is discussing options with the governor's office but there were no specific plans with moving forward with rule making. Paul Booher reported that after the commission meeting, Mr. Briggs received a call from Dr. Conti, Environmental Health Division Director, advising him that the department would proceed with rule making. There was a discussion on the process of rule making, filing, and public notification. Paul Booher called Dale Holcomb in the program office and reported that when the office is given the notice to proceed, language will be submitted to the Florida Administrative Weekly (FAW) where it takes 10-days to prepare for advertisement. Then it is advertised for 21-days for public hearings and comments. If there are significant changes, then it would need to be re-advertised. Assuming there are no changes or legal challenges, the rule is filed and becomes effective 20 days after the date filed. The proposed rule was discussed, with the understanding that this is more in the purview of the Technical Review and Advisory Panel (TRAP), and that the RRAC has not come to a conclusion on relative significance of nitrogen impacts. Some of the main discussion points:
 - The proposed rule does not have any specific requirements for monitoring.
 - The proposed rule does not specifically state **total** nitrogen.
 - Requiring a minimum bottom of drainfield elevation of 18-inches below finished grade would wipe out any alternative drainfield product that is more than 12-inches in height. This requirement would also make it difficult to ensure the required fall in the drainlines. An installer commented that only 6-inches of soil cover over the top of the drainfield would make it easy to crush the drainfield when covering.
 - The proposed rule language as written would prohibit tanks that are larger than within one tank size of current requirements.
 - In the existing system language it states that the system would need to meet these requirements if it is in need of repair, modification, or re-approval. Re-approval would include those systems being inspected under part (c) when they are pumped and certified every five years.
 - There was some confusion over what forms are required, and whether this indicates that a non-certified individual would be allowed to perform a site evaluation. [NOTE: clarification on this issue was received, and a Certified Environmental Health Professional is required to perform any site evaluation]

Some septic contractors voiced a concern over there being too many forms to fill out and whether there are any other options. They stated that this is time consuming and expensive.

Paul Booher stated that there were several good points that would need to be considered, and that staff will report these comments to Gerald Briggs.

Sam Averett made a motion which was seconded by John Glenn:

RRAC, after review of the Department of Health proposed rule language for Wekiva, still stands behind the previous statement that RRAC is unable to determine relative significance of onsite system impacts of nitrogen to the Wekiva Study Area.

There was a discussion on the relative significance of nitrogen impacts from onsite systems. Several RRAC members were in agreement that onsite systems contribute to the quantity of nitrogen in the Wekiva Study Area, but the relative significance has not yet been agreed upon. The members voted and four were in favor and three were opposed.

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

If the proposed rule goes forward, if a pump is required, low pressure dosing should be used due to the increase in system longevity and relatively low additional cost.

The members voted and all were in favor with none opposed.

- **Brief updates on other projects**

- Ongoing projects

- **Passive Nitrogen Removal Assessment** – Dr. Daniel Smith presented the status of the project at this point in time. There is a draft final report on the literature review task and a draft Quality Assurance Project Plan for the laboratory experiments task. RRAC members and DOH staff are to have any comments/ideas/corrections sent within two weeks of the meeting. Elke Ursin instructed RRAC members to submit anything directly to her and she will compile and send to Dr. Smith.
- **High Strength Waste Study** – Paper submitted to American Society of Agricultural and Biological Engineers
- **Manatee Springs, Performance of Onsite Systems Phase II Karst Study** – Paper submitted to Water Research on 8/21/07. Due to contractual and timing issues, this contract has expired and must be re-advertised.
- **Monroe County Performance Based Treatment System Performance Assessment** – Dr. Eberhard Roeder presented on the preliminary results of the Monroe County project. Some of the preliminary observations are:
 - Only a few odd numbers
 - Diurnal variability appears lower for nutrients than for effluent strength
 - Nutrient grab samples appear very consistent with time-composite samples, less so for TSS
 - Wastewater strength appears to be lower than in Keys Onsite Wastewater Nutrient Reduction Study (OWNRS)
 - Nutrient concentrations appear to be higher than in Keys OWNRS study

There will be repeat sampling done to assess variability for the same system over time with the added sample parameters of fecal coliform, alkalinity, and pH.

- **Remote Sensing of Optical Brighteners Study: Mote Marine Report** – Summary report from DEP has been submitted on results of tasks up to the airborne Light Detection and Ranging (LiDAR). The flow-through fluorescence method showed potentially interesting patterns (i.e. one location showed a higher signal corresponding to locations where failed septic systems were known to exist). Contract was amended on Oct. 15th to comply with Contract Administration requirements (end date changed to 12/31/07). New contract will need to be issued using IGA exemption to allow for completion of scope. Phone conference to be held on Oct. 25th to discuss next steps.
 - **Taylor County Source Tracking Study** – RRAC made motion on May 8, 2007 meeting for staff to look into a follow-up sampling event to capture the May seasonal low water table event. FDEP was contacted to see if funds were available, and they were not available for a May sampling event, FDOH utilized research \$ to fund the project (just under \$14,000). Request for proposal was sent to various interested parties and FAU was selected to conduct the study. The sample site locations were determined to be the same as the original list with the exception of one site, which the previous study did not find a marked difference between another site in close proximity, which could be replaced with a new one. An interim progress report was submitted at the end of June 2007 outlining the May 2007 seasonal low water table sampling event, and is included in the packets sent to the RRAC. FDEP's 319 program has funded a September 2007 sampling event. Analysis is ongoing, and a final project report compiling all sampling events will be submitted in January 2008.
- Projects coming up
- **319 Project on Performance and Management of Advanced Onsite Systems** – \$300,000 grant through the EPA 319 program administered by FDEP. FDOH will provide \$200,000 in matching funds through the Monroe County project. Tasks:
 1. Monroe County detailed study of variability of performance of advanced systems (Keys study)
 2. Statewide database of advanced systems based on permit records
 3. Survey of the perceived strengths and weaknesses of the current management of advanced onsite systems
 4. Statewide assessment of operating condition and performance of advanced systems (random sample of 600 systems)
 5. Quarterly influent and effluent sampling for a sample of systems (approximately 70 systems)
 6. Booklet with case studies outlining both strengths and weaknesses of the current program and best practices in advanced onsite management

Sam Averett made a motion that was seconded by Paul Davis:

RRAC recommends moving forward with the 319 project.

There was a discussion on making sure what is sampled will be statistically significant. The members voted and all were in favor with none opposed.

- **Coastal Management Program Grant Funding Opportunity** – FDEP has sent out a notification for a grant funding opportunity due November 15, 2007. One idea is to utilize this funding to sample in the Town of Suwannee, Cedar Key, and areas of Taylor County where areas have converted from onsite systems to sewer and where there is previous sample data from when the areas were still on onsite systems. Sam Averett made a motion that was seconded by Clay Tappan:

RRAC recommends FDOH apply for the FDEP Coastal Management Program grant funding opportunity.

The members voted and all were in favor with none opposed.

- **Budget Discussion** – This item is to be discussed at the next meeting
- **Prioritization of Future Projects** – This item is to be discussed at the next meeting, RRAC members are encouraged to develop a list of potential future project ideas to assist in the discussion.
- **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 January 2008 at a location to be determined.