EVALUATION OF WATER QUALITY AROUND THE TOWN OF SUWANNEE, FLORIDA, AND COMPARISON TO HISTORIC DATA

CONTRACT COQOT—EXTENSION

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EXECUTIVE SUMMARY

In 1989 to 1990 *Salmonella* contamination was detected in commercially harvested oysters from an area around the town of Suwannee. The contamination was suspected to be caused and/or contributed to by onsite sewage treatment and disposal systems (OSTDS) in the town. To alleviate the contamination source, plans were approved to abandon OSTDS and route all sewage to a central wastewater treatment plant (WWTP). All OSTDS were closed by March 1998, and the WWTP became operational in October 1997.

The Florida Department of Health and Rehabilitative Services (FDHRS) contracted with Environmental Consulting & Technology, Inc. (ECT), to conduct sampling in 1996 and 1997 in and around the town to evaluate potential differences in water quality immediately before and after construction and operation of the WWTP. A report of this study was issued in 1998. The results of the study suggested the town was not the sole source of *Salmonella*, as this organism was routinely found upstream of the town indicating a potential regional issue.

Unfortunately, the pre- and postconstruction comparisons were somewhat compromised, because in 1997, an El Niño episode persisted during the time of postconstruction sampling, which produced high river flows and potentially introduced other bacterial contamination sources. As such, this weather anomaly affected the postconstruction results and limited the ability to compare with preconstruction data.

In September 2008, the Florida Department of Health (FDOH) received funding to conduct a follow-up study. The intent of the study was to provide an updated evaluation of the environmental impacts of abandoning the OSTDS and sewering the town to a central WWTP. Because of scheduling limitations on the funding, the study was conducted during the summer of 2009, which was not ideal for comparison with the 1996 data that were collected in the winter.

The 1996 and summer 2009 study designs were intended to have common study components to facilitate data comparison. However, in 2009 other analytical parameters were added to provide additional information: total phosphorus, enterococci, and deoxyribonucleic acid (DNA) source (human versus animal) tracking.

The results of the summer 2009 study provided valuable comparisons between pre- and postconstruction water quality data. Statistical techniques were used to separate the changes observed at the river stations (used as controls) and the canal stations. The most notable result was a statistically significant reduction in fecal coliform values observed in the canals between 1996 and the summer of 2009. However, the results of the comparison were somewhat in question, because the studies were conducted during different seasons, which might have biased some of the microbial data.

Fortunately, FDOH provided additional funding to repeat the sampling events during the winter of 2009. The sampling was conducted in November and December and aligned with the eight weekly events conducted in 1996. Four river stations, five canal stations, and one monitoring well were sampled. The parameter list for both 1996 and 2009 included fecal coliform, total coliforms, *Salmonella* (presence/absence only), nitrate + nitrite (NO_x) , and total Kjeldahl nitrogen (TKN) . In addition, enterococci and human gene biomarker (HGB) analyses were added in 2009.

The goal of this study was to evaluate the long-term effects of closing approximately 850 OSTDS in the town of Suwannee and installing a central WWTP. The approach was to sample water quality in the Suwannee River and the canals within the town of Suwannee and compare the results with data collected in 1996 prior to OSTDS closures. The two previous attempts to provide postconstruction data for comparison provided valuable information but were not ideal because of extreme river discharge conditions and seasonality concerns. The most recent study was conducted during the same season and during comparable river discharge conditions as the 1996 baseline survey conducted prior to septic tank removal. Therefore, this study provides a more defensible data set to evaluate potential improvements in the area 13 years after septic tank closure.

The results did not suggest that there was large improvement in water quality in the canals between 1996 and 2009 that could be attributed to closing the OSTDS. However, several specific observations and some improvements were noted:

- Salmonella occurrences were equal to or higher in the river than in the canals in both 2009 and 1996, indicating the canals were not the primary source of *Salmonella*. The percent occurrence of *Salmonella* in the canals was greater in 2009 than in 1996, indicating septic tank closure did not reduce *Salmonella* in the canals. While in 1996, upstream river stations had higher percentages of *Salmonella* occurrences than downstream river stations. This pattern was reversed in 2009.
- The source tracking results (HGB) during the winter 2009 event indicated human material was present approximately 82 percent of the time in the canals (average of two stations) and only 50 percent of the time in the river (Station 10). It appears that, despite septic tank closure, the canals remain a possible source of HGB. Source tracking was not conducted in 1996.
- During the summer 2009 sampling event, HGB was present 38 percent of the time in canals as compared to 50 percent presence in the river. During the winter 2009 event, HGB was present 81 percent of the time in the two canal stations sampled compared to 50 percent in the upstream river station.
- The total and fecal coliform values were higher in the canals than in the river in both 1996 and 2009. Fecal coliform decreased from 1996 to 2009 in both the canals and the river stations, whereas total coliforms increased from 1996 to 2009. The higher values in the canals as compared to the river could be from domestic animals or wildlife concentrated near the canals and, in 1996, could have also been from onsite sewage systems.
- Comparison of the 2009 results with the 1996 results indicated there were three statistically significant changes in the measured parameters between 1996 and 2009:
	- o There was a 59-percent decrease in fecal coliform in the canals.
	- o There was a 230-percent increase in total coliforms in the river.
	- o There was a 6-percent decrease in total nitrogen in the river.

All other observed changes in the surface water samples were not statistically significant.

- NO_x exhibited a strong correlation with river flow and decreased with increasing river flow. TKN increased with increasing river flow, and total nitrogen remained relatively constant. There was consistently more NO_x in the river samples than in the canals.
- Additional statistical tests were conducted that evaluated the differences between average canal and river station results for each event. The results indicated the magnitude of reduction in fecal coliform concentrations from 1996 to 2009 was greater in the canal stations and could be a possible benefit of closing the OSTDS.
- The monitoring well data indicated dramatic improvement from 1996 to 2009 in most of the parameters. The fecal coliform counts dropped from an average of 232 colonies per 100 milliliters (col/100 mL) to nondetectable. The nitrogen parameters all dropped in excess of 82 percent. Since the well was located downgradient of the septic tank drain field, closing the septic tank resulted in marked improvement in the groundwater at this location. However, total coliforms and the percent occurrence of *Salmonella* increased in 2009.
- Comparison of the winter 2009 data with the summer 2009 data indicated that total coliform counts were higher in the summer, but fecal coliform, enterococci, and *Salmonella* occurrences were higher in the winter for both the river and canal stations.

In summary, the results indicated that there was a statistically significant 59-percent reduction of fecal coliform in the canals between 1996 and 2009 that could not be attributed to changes observed in the river stations. There was also an improvement in groundwater measured near a septic tank drain field. No other significant improvements in the water quality of the canals was identified that could be attributed to OSTDS closures.

The winter 2009 study provided a unique opportunity to examine the water quality in the canals and the river around the town of Suwannee 13 years after closure of 850 OSTDS in the area. Analysis of the data indicated there was a significant reduction of fecal coliform in the canals, but there was not a significant reduction of nitrogen or total coliforms, which might have been anticipated. There was no clear reduction in the occurrence of *Salmonella*, which was expected given that the 1996 study had pointed to a river-based source of this parameter. There was a marked improvement of the groundwater near a septic tank drain field.

It is unlikely that additional studies of these parameters would identify further improvements attributable to septic tank removal, since additional improvements were not apparent after 13 years. Although the study plan attempted to isolate the removal of the OSTDS as the only variable for testing between the pre- and postconstruction sampling, it was not possible to control all environmental factors, such as rainfall, river flow, and water temperature. It is recommended that future studies follow a similar protocol and establish a series of test and control stations that lend themselves to rigorous statistical analysis. Future studies at other sites should again be designed to conduct the pre- and postconstruction sampling during comparable seasonal (temperature) and river discharge conditions. It is also recommended that the additional source tracking techniques such as HGB be used more extensively to help separate human impacts from natural sources.

1.0 INTRODUCTION

1.1 PROJECT HISTORY

A cooperative study by the Florida Department of Natural Resources (FDNR, now the Florida Department of Environmental Protection [FDEP]), the Florida Department of Agriculture and Consumer Services (FDACS), and the U.S. Food and Drug Administration (FDA) in 1990 (Glatzer, 1990), investigated an incident of gastroenteritis in Florida during the fall and winter of 1989 to 1990. At least two of the cases were indicative of salmonellosis. Samples of oysters from Louisiana and Florida were analyzed for *Salmonella*. Approximately 39 percent of the oysters tested positive for *Salmonella*; approximately 90 percent of these oysters were from Suwannee Sound and adjacent areas to the north and south—Horseshoe Beach and Cedar Key, respectively. In addition, *Salmonella* were detected in water samples taken upstream and downstream of the town of Suwannee. Possible sources identified by Glatzer (1990) were the waterfowl and wildlife in the area. In May 1990, FDNR reclassified the oyster areas of Suwannee Sound. This reclassification included changes in closure areas and a new management plan based on rainfall amount.

According to the Florida Department of Health and Rehabilitative Services (FDHRS, 1991), now the Florida Department of Health (FDOH), the town of Suwannee had a total of 717 onsite sewage and treatment disposal systems (OSTDS). Of these, based on agency assessment criteria, seven (i.e., less than 1 percent) systems were considered adequate. The remaining 710 inadequate OSTDS were identified as one of the possible sources for *Salmonella* contamination of the oysters in Suwannee Sound and adjacent areas. Because of the number of inadequate OSTDS, plans were approved to construct a central wastewater treatment plant (WWTP). The facility became operational in October 1997, and connections to the system began immediately. The WWTP is located approximately 2.5 miles northeast of the town and uses primary clarification and aeration basins for treatment of the wastewater. The OSTDS were pumped out and abandoned (filled with sand) at the same time each household was connected to the WWTP system. By the end of November or mid-December 1997, all but approximately 50 of the OSTDS were closed. The remaining 50 OSTDS were closed by March 1998. Instead of the

717 OSTDS initially reported by FDHRS (1991), 850 OSTDS were found; all were properly abandoned.

To investigate the impacts the OSTDS closures and use of a central WWTP would have on surface water around the town of Suwannee, FDHRS contracted Environmental Consulting $\&$ Technology, Inc. (ECT), to conduct a water quality study. Sampling was conducted in 1996 prior to the OSTDS closure and again from November 1997 through January 1998 following the OSTDS closure, and a report was issued in 1998 (ECT, 1998). The study included analyses for nutrients, *Salmonella*, and coliforms. Other fecal contaminant indicators were considered for analyses including coprostanol, epicoprostanol, and linear alkylbenzenes (detergent whitener). Of these indicators, coprostanal was selected but provided inconclusive results.

In September 2008, FDOH issued an invitation to negotiate (ITN) titled Evaluation of Water Quality around the Town of Suwannee. The intent of the proposed study was to provide an updated evaluation of the environmental impacts of abandoning the OSTDS and sewering the town to a central WWTP. ECT responded to the ITN and was selected to conduct the study. Sampling for this project was conducted in June and July 2009, and a final report was issued in September 2009.

Because of delays in getting the quality assurance project plan (QAPP) approved, the study was conducted in the summer of 2009, which was not ideal for comparison with the preconstruction study that was conducted in the winter (November and December) of 1996. Subsequently, FDOH funded a fourth survey that was completed in November and December 2009. This provided a data set obtained 13 years after the preconstruction survey was completed in 1996 that was completed during the same season. This report provides the results of the winter 2009 study and comparison with the 1996 preconstruction baseline data.

1.2 PROJECT GOALS

The goal of the initial program, as well as the summer 2009 survey, was to evaluate the potential for restoration of commercially viable oyster harvesting in Suwannee Sound following the connection of the town of Suwannee to a WWTP. The specific objectives included:

- Conduct a preliminary online literature search to identify and evaluate various methods for detecting domestic sewage in receiving waters.
- Prepare a plan of study (POS) and QAPP that would lay out a sampling strategy to meet the goals of the project.
- Conduct preconstruction (of the WWTP) field sampling that would: (1) determine the optimum day of the week to sample, if any; (2) confirm that low tide was the ideal worst-case time to sample; (3) evaluate the various methods selected for detection of domestic sewage; and (4) quantify water quality conditions in the Suwannee River in the vicinity of the town of Suwannee prior to the construction of the WWTP and subsequent abandonment of the OSTDS.
- Conduct postconstruction sampling to determine what changes, if any, resulted from the town of Suwannee converting from OSTDS to the WWTP with land disposal.
- Evaluate the field data and data from other sources in light of the information obtained from the ongoing online literature search and determine if there has been any change in water quality and if the change is statistically significant.

The primary goal of the current project is to generate a comparative water quality database by duplicating the previous study's (1996) weekly sampling effort. The specific sampling approach designed to achieve this goal include:

- Collect samples at the same ten stations (nine surface water and one groundwater) as used in the 1996 to 1998 studies.
- Collect samples over the same duration (eight consecutive weekly events).
- Collect surface water samples during the same tidal cycle (low slack).
- Analyze samples for the same microbiological and nutrient parameters plus the addition of enterococci and deoxyribonucleic acid (DNA) source tracking.
- Use the same surface water sampling and *in situ* data collection protocols. The groundwater sampling technique was revised from using a bailer to use of a peristaltic pump and tubing as required by FDEP.
- Sampling on the same day each week (Monday) as was done during the earlier study.
- Collect data during the same season (November and December) so that water temperature, air temperature, sunlight, and, hopefully, river discharge would be comparable to the preconstruction conditions.

2.0 STUDY COMPONENTS

2.1 SAMPLING EVENTS

2.1.1 SAMPLING SCHEDULE

A total of eight consecutive weekly sampling events were conducted to collect water quality samples and *in situ* data. Prior to the June 2009 sampling, a reconnaissance field trip was conducted jointly by ECT and FDOH project management personnel to inspect current conditions at the proposed sampling locations and confirm station locations. The same stations were used during this study, so an additional reconnaissance trip was not needed.

Sampling was performed on Monday of each week and began on November 9, 2009, and was completed on December 28, 2009. Each weekly sampling event was scheduled so the surface water sampling duration would bracket the projected time of a low slack tide. Tide projections were obtained from an internet Web site (www.saltwatertides.com), which provided daily semi-diurnal tide time projections for the tide at the mouth of the Suwannee River. Based on experienced gained during previous sampling efforts, the total duration for sampling the surface water stations by boat was approximately 2 hours. Therefore, this part of the sampling began 1 hour before the projected time of the low slack tide. Sampling was conducted at low tide to assure samples in the canals collected water issuing from the canals and not water entering from the river.

2.1.2 SAMPLING LOCATIONS

Ten water quality sampling locations consisting of nine surface water stations and one groundwater station were monitored for this project. Figure 2-1 displays the locations of all ten stations. The groundwater station (Station 1) was a shallow well (6 feet below land surface [ft bls]) located on Leon Drive and was the same property as the previous studies. The well was positioned downgradient from an abandoned residential OSTDS site drainfield and installed with a hand auger.

The surface water stations included four stations in the river, including Station 10, located approximately 2 miles upstream of the town, and three other stations located in

major passes of the Suwannee River delta, specifically East Pass (Station 9), Alligator Pass (Station 8), and Wadley Pass (Station 7). Stations 2, 3, 4, 5, and 6 were located in the canals. To ensure the same station locations were occupied on each sampling event, the station's latitude and longitude coordinates were programmed during the reconnaissance trip and stored in a global positioning system (GPS) receiver for future navigation to stations. Table 2-1 provides the position coordinates for all stations.

2.1.3 SAMPLING PARAMETERS

Water temperature, specific conductance, pH, and dissolved oxygen (DO) were measured *in situ* at all stations during each survey. The measurements at Station 1 (monitoring well) were done as required by the FDEP standard operating procedure (SOP) for well sampling to demonstrate adequate purging of the well prior to sample collection. Measurements at the surface water stations were made at three depths (surface, mid-depth, and bottom) to document the physical characteristics in the river/canals water column at the time of sampling and assess any stratification. The surface and bottom reading were done 1 foot (ft) below the surface and 1 ft above the bottom, respectively.

Water quality samples were collected from within the first 1 ft of the water column and analyzed for several nutrients and microbiological parameters. Table 2-2 presents a list of the parameters analyzed as well as ancillary information pertaining to the samples.

2.2 SAMPLING AND ANALYTICAL METHODS

2.2.1 FIELD PROTOCOLS

In situ measurements of water temperature, specific conductance, pH, and DO were made at three depths in the water column at surface water stations using a Yellow Springs Instrument® (YSI) Model 556 multiparameter system. During monitoring well purging, turbidity was also measured with a Hach Model 2100P turbidimeter.

In situ measurements at surface water stations were recorded at 1 ft below the surface, mid-depth, and 1 ft above the bottom on standardized forms developed by ECT. Data collection time and depths were also recorded along with the total depth at each station. The total water depth and measurement depths were determined by graduations on the YSI

Station	Latitude	Longitude
$\mathbf{1}$	29° 18′ 55.40"	83° 08' 21.16"
$\overline{2}$	29° 19' 15.80"	83° 08' 43.64"
3	29° 19' 16.18"	83° 08' 48.74"
4	29° 19' 57.32"	83° 08' 20.76"
5	29° 19' 23.97"	83° 08' 37.12"
6	29° 19' 30.91"	83° 08' 20.35"
τ	29° 18' 28.16"	83° 09' 49.57"
8	29° 18' 11.02"	83° 09' 25.43"
9	29° 18' 55.55"	83° 07' 09.68"
10	29° 19' 29.18"	83° 06' 42.70"

Table 2-1. Town of Suwannee Water Quality Station Coordinates

Source: ECT, 2010.

Table 2-2. Town of Suwannee Water Quality Sample Information

Note: \degree C = degree Celsius. EPA = U.S. Environmental Protection Agency. H_2SO_4 = sulfuric acid. SM = Standard Method (APHA, 1998). TKN = total Kjeldahl nitrogen.

Source: ECT, 2010.

meter cable, which was attached to a weighted polypropylene line. *In situ* measurements during the monitoring well purging prior to sample collection were done per the requirements in the FDEP groundwater sampling SOP FS 2200 and were recorded along with other SOP required ancillary data/information on FDEP form FD 9000-24.

The *in situ* measurement instruments were calibrated at the beginning and end of each sampling day, and the calibration results were documented on FDEP-generated forms. Per a request from FDOH, all field records included in weekly field data/information packets have been transmitted as a separate electronic data submittal to FDOH, prior to submission of this report.

Per the previous studies of 1996 and summer 2009, surface water samples were collected as surface grab samples from within the top 1 ft of the water column. The sample was collected using an extra precleaned 1-liter sample container provided by the laboratory. This technique is consistent with the surface water sampling FDEP SOP FS 2100, specifically FS 2110(1.1.1). A new sample container was used at each station precluding the need to decontaminate the sampling device between stations and avoiding the potential for station cross-contamination.

Samples were collected using the following steps:

- Samples were collected from the bow of the boat and away from the outboard motor.
- The sampler wore a powder-free shoulder-length glove to submerge the sample container and a standard length powder-free latex glove when handling the sample containers. New gloves were used at each station.
- The 1-liter sampling container cap was removed, and the container was slowly submerged with the opening first into the water.
- The bottle was held with the opening pointed upstream, and water was allowed to fill the container.
- The container was retrieved, and aliquots were dispensed to the individual sample containers for preservation, storage, and shipment to the laboratory.

Please note one modification from the FDEP SOP (FS 2100[1.1.2]) sampling process: the extra sample container used to collect samples was not rinsed prior to sample collection to avoid residuals from surface water sheens and surface floating vegetation that could be caused by multiple container immersions.

Each station's sample kit had one prepreserved container with sulfuric acid (H_2SO_4) for nutrient analyses. Acid preservation is done to maintain sample integrity and requires lowering the sample pH to 2 standard units (s.u.) or below. Adequate preservation was checked during the first two sampling events using color-coded pH sticks. All checks yielded results below 2 s.u. and ranged from 1.0 to 1.6 s.u.

The monitoring well sample was collected with a variable-speed peristaltic pump and tubing. Well purging and sampling was done per FDEP SOP 2200, referencing specific sections of the SOP pertaining to use of a peristaltic pump and other aspects of the SOP addressing the overall purging and sampling process. Per the SOP, general procedures followed included:

- Wearing powder-free latex gloves when handling tubing and sample containers.
- Use of new tubing during each sampling event.
- Controlled pump rate to maintain constant water level in the well and minimize entrainment of solids.
- Use of rolled plastic around the well to prevent pump tubing from contacting surrounding soils when deploying.
- Stabilization of *in situ* parameters within SOP criteria before collecting samples.

Each station's sample container kit was stored in a sealable (e.g., Zip-Loc®) bag prior to and following sampling to prevent station cross-contamination. Samples were placed in ice immediately following collection and until delivery to the laboratory. Samples were delivered to the laboratory within the 6-hour holding time required for the microbiological parameters and accompanied by the laboratory chain of custody form that included the following information:

- Laboratory client name and contact information.
- Project name, number, and location.
- Sample identifications.
- Sample type.
- Date and time of sample collection.
- Number of containers per sample.
- Sample preservation method.
- Parameters to be analyzed.
- Types of samples containers used.
- Name and affiliation of sampler.

2.2.2 LABORATORY METHODS

Table 2-2 summarized the analytical methods used for water quality samples. The sample analyses were conducted by Advanced Environmental Laboratories, Inc. (AEL), Gainesville, Florida, with the exception of DNA source tracking, which was done by Source Molecular Laboratory, Inc., Miami, Florida.

2.3 QUALITY ASSURANCE/QUALITY CONTROL

Prior to initiation of field activities and per Task 1 of the contract, the Quality Assurance Project Plan (QAPP) was updated by ECT and approved by FDOH (ECT, 2010). The document provides methodologies used for water quality sampling, data collection, sample analyses, data review and verification, and reporting.

2.3.1 SAMPLING ACTIVITY

For each of the eight weekly sampling events, a field data/information packet was assembled and completed to provide guidance/details to the sampling personnel to ensure that required activities and necessary documentation were completed per the FDEP SOP employed for project execution. The packet, consisting of reference material and ECT and FDEP standardized forms to document information and data, contained the following:

- A form listing itemization of the various records and logs to be completed during sampling and data collection.
- Identification of the *in situ* parameters to be monitored and procedures to be followed.
- Identification of field personnel, sampling date and time period, and project and site name.
- Equipment checklist.
- Identification of laboratory parameters, analytical method numbers, sample preservation requirements, and sample holding times.
- A daily field activity log.
- A project sampling schedule with sample start times based on predicted time of low slack tide and identification of quality assurance/quality control (QA/QC) samples types (i.e., duplicates, field and equipment blanks) to be collected per trip.
- List of project team member phone numbers.
- List of sampling station coordinates.
- Site map.
- Surface water sampling/in situ data collection form.
- Groundwater sampling form.
- Instrument calibration forms.

As previously discussed, sampling activities followed the applicable sections of the FDEP SOPs. Those sections included SOP FS 2100 for surface water sampling and FS 2200 for groundwater sampling. The instruments used to collect *in situ* data were calibrated at the beginning and completion of each sampling day and documented on FDEPdeveloped forms. The parameters calibrated on each survey were specific conductance, pH, DO, and turbidity. Step one of calibration consisted of measuring and adjusting meter responses to vendor-supplied standards for specific conductance (two standards), pH (three buffer solutions), and turbidity (four primary formazin standards). DO was calibrated following the air calibration procedure in a water-vapor saturated chamber. The DO reading was adjusted to read the correct concentration based on ambient temperature in the calibration chamber and referencing Table FT 1500-1, Solubility of Oxygen in Water at Atmospheric Pressure, in the FDEP SOP FT 1500 for measuring DO. The temperature thermistor on the YSI meter was checked periodically against a National Institute of Standards and Technology-traceable thermometer.

Immediately following calibration and to confirm meter accuracy, an initial calibration verification (ICV) was conducted consisting of remeasuring a calibration standard for specific conductance, pH, and DO in the water vapor saturated calibration chamber. Calibration adequacy and meter accuracy were deemed acceptable if the ICV meter responses were within FDEP-stipulated acceptance criteria. For DO, the acceptance criteria is $+0.3$ milligrams per liter (mg/L) of the solubility table concentration for the ambient temperature in the calibration chamber during the ICV; specific conductance is within \pm 5 percent of the standard concentration; for pH within \pm 0.2 s.u. of the buffer value; and for turbidity, the acceptance criteria ranges from 5 to 10 percent, dependent on the concentration of the standard. At the end of the sampling day, a post- or continuing calibration verification (CCV) was conducted to check on meter reading stability over the course of the sampling day. The CCV responses were deemed acceptable based on the same criteria for the ICV.

The ICV and CCV meter responses were within acceptance criteria for the eight sampling events, with the exception of December 7, 2009 (Event 5), when the 100-nephelometric turbidity unit (NTU) turbidimeter standard read 109 NTU (9 percent), which was marginally outside the acceptance criteria of +6.5 percent. The reason for the offset is unknown, and the other turbidity calibration responses were within criteria. Also on December 14 (Event 6), the DO CCV reading was 8.30 mg/L and should have been 8.89 mg/L. This response was outside the +0.3-mg/L criteria but only marginally and was not considered a justification to censure the DO measurements for that event and data were included in the project database.

Per the contract and routine FDEP sampling program requirements, 10 percent of the laboratory samples were QA/QC samples consisting of either a field blank, equipment blank, or field duplicates. Based on ten samples per 8 weeks of sampling, which equates

to a total of 80 samples, a minimum of eight QA/QC samples were required for the project. This requirement was met as a total of eight QA/QC samples were collected. Table 2-3 presents a listing, by sampling event, of the types of QA/QC samples generated to satisfy the project requirements.

The field blank sample was generated by pouring laboratory-provided analyte-free water directly into a set of sample containers to assess the potential for sample contamination from the sampling environment and during handling/transport from the field to the laboratory. The equipment blank was generated by processing analyte-free water through the sampling apparatus (pump/tubing, sample container, and dipper used to collect surface water samples) to simulate sample collection and assess whether the sampling apparatus could contaminate the samples. Duplicate samples were generated by filling two sets of sample containers consecutively at the assigned station using the identical sampling procedure.

Table 2-4 presents the results of the field and equipment blank samples collected on sampling Events 1, 2, 3, and 7. Sampling Event 1 equipment blank was generated using the polyethylene sampling bottle used at Station 10. The equipment blank on Event 2 was generated with the polyethylene dipper used to collect the sample at Station 4. The equipment blank on Event 7 was generated using the pump tubing for Station 1. The data for the blank samples were below the analytical method detection limits (MDLs) with the following exceptions: the equipment blank from Event 1 and the field blank for Event 3 for nitrate + nitrite (NO_x) at 0.009 and 0.027 mg/L, respectively, and the equipment blanks from Events 2 and 7 for total Kjeldahl nitrogen (TKN) at 0.18 and 0.14 mg/L, respectively.

Please note the Event 1 equipment blank NO_x value of 0.009 mg/L, which was "I" flagged as being between the MDL and the practical quantitation limit, was less than 1 percent of the associated project sample from Station 10 at 1.15 mg/L. Therefore, any bias from the sampling device was considered inconsequential. Similarly, the NO_x value from the field blank on Event 3 of 0.027 mg/L was only 3 percent of the average NO_x concentration of the samples for Event 3 of 0.812 mg/L. Thus, no significant bias on

Table 2-3. Project Mandated Quality Assurance/Quality Control (QA/QC) Samples

Source: ECT, 2010.

Table 2-4. Town of Suwannee QA/QC Blank Sample Results

Note: $col/100$ mL = colonies per 100 milliliters of sample.

 $U =$ analyzed but not detected.

 $I =$ value between MDL and practical quantitation limit.

Sources: AEL, 2009. ECT, 2010.

sample results was attributable to the sampling environment or sample handling and transport.

The TKN concentration for the equipment blank on Event 2 was 0.18 mg/L, which is high relative to the associated project sample from Station 4 at 0.32 mg/L. However, as the Station 4 concentration on Event 2 was the lowest of the eight sampling events, it was not deemed significantly positively biased by the sampling device and was, therefore, not excluded from the project database. The Event 7 TKN value of 0.14 mg/L for the equipment blank sample is approximately 13 percent of the associated project sample from Station 1 at 1.03 mg/L. As the value of 1.03 mg/L was deemed representative of the overall station database for the eight sampling events with an 8-week average of 1.35 mg/L, it was included in the data analyses.

Table 2-5 presents the results of the field-generated duplicate samples collected on four of eight sampling events. Duplicate sample analysis is a means to evaluate analytical data precision or reproducibility as it relates to sample collection and laboratory analysis. Duplicate samples were collected by consecutively filling two sets of sample containers with the same sampling device and using common procedures to handle, store, and transport the samples.

To evaluate the results of the field duplicate samples and per the QAPP, ECT used the laboratory acceptance criteria for the nutrient parameters TKN and NO_x for duplicate analyses of matrix spike and matrix spike duplicate samples. Duplicate sample acceptance criteria is the relative percent difference (RPD) between the two samples and is calculated by dividing the concentration difference of the two samples by the average concentration of the samples and converting the result to a percentage value.

Reviewing Table 2-5 indicates only a single instance where duplicate field sample results did not fall within the acceptance criteria. That was from Event 5 analyses for TKN with an RPD of 18 percent. The reason for the difference in the duplicate sample results is unknown, but the overall project dataset for this parameter is considered valid as the other two field duplicate TKN results are well within acceptance criteria.

Table 2-5. Town of Suwannee QA/QC Field Duplicate Sample Results

Note: RPD = relative percent difference.

 $col/100$ mL = colonies per 100 milliliters of sample.

The RPD is calculated as a percentage by dividing the difference of the two concentrations by the average concentration of the sample and duplicate.

Source: ECT, 2010.

Microbiological analyses methods do not require development of acceptance criteria for duplicate samples. The method includes analyses of duplicates only as a general guide to evaluate consistency in method protocol based on data reproducibility or precision. According to communication with the project contract laboratory, agreement in microbiological duplicate samples values within the same order of magnitude is generally considered adequate. As such, no RPD criteria for microbiological parameters are included in Table 2-6. Based on general acceptance for microbiological duplicates agreeing within the same order of magnitude, the data displayed on Table 2-6 are for the most part good. The total coliform results have a couple of instances of numerical values having considerable differences, specifically Events 5 and 8. However based on the acceptability of duplicate microbiological data agreeing within the same order of magnitude the results were deemed acceptable and included in the data analyses.

One duplicate sample each for source tracking analyses was collected from Stations 2, 5, and 10 during sampling. The Station 2 duplicates both were negative for human DNA in enterococci cultures. The Station 5 duplicates were both positive for human DNA presence, and the Station 10 duplicates were both negative for human DNA presence.

2.3.2 LABORATORY ANALYSES

Microbiology QA/QC procedures used in the laboratory for coliforms, enterococcus, and *Salmonella* included the following:

- Blanks—Pre-, post-, and mid-sample analyses (after every ten samples). The source of positive results in a blank sample are investigated to include reagent water, media, instruments, and general housekeeping adequacy.
- Duplicates—Duplicate analyses are performed weekly, and the precision is calculated per method procedures to assess the overall ongoing laboratory QA/QC program and do not apply to an individual batch of sample results.
- Positive and Negative Controls:
	- o Coliforms—Ten positive colonies plus atypical colonies verified by incubation in lauryl tryptose broth/brilliant green lactose bile broth/escherichia coli (LTB/BGB/EC) medias.
- o Enterococcus—Ten typical and atypical colonies verified on brainheart infusion broth (BHIB) + 6.5-percent sodium chloride (NaCl), BHIB at 44.5 degrees Celsius (°C), bile esculin azide (BEA) agar, biochemically with calalase and gram stain.
- o *Salmonella*—For positive controls, *Salmonella* organisms are inoculated with urea reagent and incubated. The *Salmonella* colonies should urease negative and remain orange in color. Negative controls are done with *S. aureus*. The *S. aureus* culture should urease positive and turn pink in color.

Additional QC measures included temperature monitoring of incubators at the beginning and completion of an incubation period, chlorine residual check of all samples, and a monthly double-count check by a second analyst.

Laboratory QA/QC procedures for DNA source tracking included initial performance recovery (IPR), ongoing performance recovery (OPR), matrix spikes (MS), negative and positive control analysis, method blanks, and media sterility checks. OPR analysis occurs after every 20 field and matrix spike samples or one per week that samples are analyzed. IPR and OPR analyses require preparation of a 100-milliliter (mL) sample of water and seeding it with approximately 20 colony-forming units (cfu) of enterococcal surface protein (ESP) gene-containing *Enteroccus faecium* (C68) and then processing the samples as outlined in the procedure. IPR is performed with four samples. The method performance is based on a positive polymerace chain reaction (PCR) signal for all *Enteroccus faecium* (C68) seeded samples. Negative controls are run using sterile reagent water, non-ESP *Enteroccus faecium*, or autoclaved field samples. All negative control samples should result in a negative PCR signal. Analysis of positive and negative controls is conducted whenever new media or reagent is used. Method blanks are tested to see the sterility of equipment used, and a media sterility check is incubated at 36.5 degrees Celsius (\degree C) + 1.0 \degree C for $24 + 2$ hours and analyzed for growth.

Laboratory chemical analyses QA/QC included daily instrumentation calibration and use of several precision and accuracy evaluation samples to determine the acceptability of each batch of sample analyzed. The types of samples used include method blanks, matrix spike, matrix spike duplicates, and secondary source calibration check standards. The results of these QA/QC samples must meet the laboratory's established acceptance criteria in order for project sample results to be deemed reportable. Table 2-6 provides acceptance criteria for calibration standards, method blanks, matrix spike, and matrix spike duplicates samples as well as other ancillary information on the analytical methods employed for this project.

Another item regarding laboratory QA/QC samples is that the project contract-required matrix spike and matrix spike duplicate samples be designated for this project at a set frequency during the sampling period as follows:

- The first time a sample is collected (Event 1).
- One in each additional 20 samples after the first 20 samples (Events 4 and 6).
- The last time a sample is collected (Event 8).

Matrix spike and matrix spike duplicate samples are included in each batch of samples analyzed during a laboratory work shift. A sample batch may consist of up to 20 samples and may be comprised of samples from a number of different projects and therefore potentially different matrix characteristics. The spiked samples are a means to assess the possibility of positive/negative bias in parameters of interest for this project, TKN, and NO_x , caused by the chemical and/or physical composition of a sample. Typically, samples selected for spiking are arbitrarily selected by the laboratory, unless a client requests their sample(s) be used.

As mentioned, this project required samples from three events be used for the matrix spike and matrix spike duplicates. The laboratory was notified verbally and on the chainof-custody forms on each event that this project's samples were to be spiked, which were Events 1, 4, 6, and 8.

Table 2-7 presents a listing of chemistry analyses QA/QC sample results that did not meet acceptance criteria and the laboratory's assessment of sample data usability.

Table 2-6. Chemistry Analyses QA/QC Operations Information and Data Acceptance Criteria

Typical matrix spike concentrations range from 1 to 2 mg/L for TKN and 0.4 to 1 for nitrate + nitrite.

Sources: AEL, 2010. ECT, 2010.

Table 2-7. Laboratory QA/QC Sample Result Excursion Information

2-20

Sources: AEL, 2009. ECT, 2010. Three laboratory values were excluded from the analytical results database as outliers. In all three cases, the values were an order of magnitude either below or above the other values for the particular station and parameter. These values were TKN of 3.83 mg/L at Station 4 on Event 1, NO_x of 0.021 also from Station 4 on Event 1, and enterococci at 1,300 colonies per 100 milliliters of sample (col/100 mL) from Station 8 on Event 1.
3.0 SUPPLEMENTAL DATA

ECT conducted an online search of possible data sources in the project area including state organizations such as FDEP, FDACS, Suwannee River Water Management District (SRWMD), and individual research professors at the University of Florida, who have conducted research work in Suwannee Sound. These professors included Dr. Tom Frazer, Dr. Ed Philips, and Dr. Shirley Baker at the Institute of Food and Agricultural Sciences (IFAS). Water quality data were not available from IFAS but were available from the other three state agencies. Additionally, river flow data have been obtained from the U.S. Geological Survey (USGS), which has maintained temporary and ongoing monitoring stations in the lower Suwannee River basin. Precipitation data have also been obtained for the SRWMD station closest to the project area. Section 4.0 of this report summarizes the river flow and rainfall data.

FDACS collects and manages water quality data in and bordering the project area for their Shellfish and Environmental Assessment Section (SEAS) program. Also, FDEP's Storage and Retrieval (STORET) database compiles biological, chemical, and physical data for ground- and surface waters of Florida. Within STORET are 27 monitoring stations in the vicinity of the project area, of which only nine had water quality data. Five of these nine stations are operated by FDACS; the remaining four stations are maintained by SRWMD. Table 3-1 presents information on the nine STORET-listed stations, and Figure 3-1 presents these station locations as well as the ECT stations to illustrate the proximity of the STORET and ECT stations.

Water quality data from FDACS and SRWMD were screened to retain the parameters that are common to this project, including total and fecal coliform, NO_x , and TKN. These data were updated through December 2009. It should be noted that the FDACS fecal coliform data were updated through November 2009 as the agency sampled only from the estuarine portion of the area in December 2009. Enterococci and *Salmonella* were not available from either source. Table 3-2 presents a data inventory for individual parameters for each station and a statistical summary of the updated data record. The table also lists the project stations closest to the STORET stations.

Note: CM = channel marker.

File: M:\acad\081081\Sampling_Locations.mxd

Table 3-2. Supplemental Water Quality Data

Fecal coliform is monitored at nine stations by SRWMD and FDACS in the project area. As shown in Figure 3-1, some of the SRWMD and FDACS stations are in close proximity to the project stations, and a few are farther afield. For the nine stations, average fecal coliform values range from 77 to 523 col/100 mL. Minimum values are 1 col/100 mL for all except SRE080C1. Maximum counts range from 540 to 5,500 col/100 mL. SRWMD also monitors total coliform in the project area at four locations. Average total coliform counts at these locations range from 496 to 766 col/100 mL. Minimum and maximum values range from 1 to 9 and 3,700 to 12,000 col/100 mL, respectively.

SRWMD also monitors TKN and NO_x at four stations. TKN, a combination of organic nitrogen and ammonia/ammonium nitrogen, has average values from 0.56 to 0.72 mg/L. Minimum values at the four stations are nondetectable concentrations at the detection limit of 0.05 mg/L. Maximum concentrations are from 1.56 to 5.90 mg/L. Concentrations of NO_x , on average, range from 0.33 to 0.68 mg/L. Minimum values are generally below MDLs.

Appendix A provides the complete supplemental water quality data set.

4.0 RESULTS

This section presents the results of the November through December 2009 study. Comparison of these results with the previous studies and assessment of the benefits of closing the OSTDS are presented in Section 5.0.

4.1 RAINFALL AND RIVER FLOW DATA

Approximately 25 miles upstream of the project area, USGS maintains a long-term river stage and flow gauging station near Wilcox, Florida (Station 02323500). Figure 4-1 presents the daily flow hydrograph at this station from October 1941 through December 2009. The highest daily flow observed at Wilcox was 84,700 cubic feet per second (cfs) in 1948. Table 4-1 presents the annual mean discharge values at Wilcox from 1942 to 2009. The annual mean discharge from 1942 through 2009 ranged from 3,275 cfs in 2002 to 24,560 cfs in 1948 (USGS, 2010).

SRWMD has maintained a rainfall gauging station in the vicinity of Wilcox and Fanning Springs (Station 2323500) from 1998 to present. Table 4-2 presents the monthly rainfall total for this period. This project's sampling was conducted between November 9 and December 28, 2009. River daily discharge and rainfall data are presented on Figure 4-2 for the sampling period. 1996 precipitation data are not available at this station. However, for comparison purposes, data collected at a nearby station, Usher Tower in Levy County (southwest of Wilcox and Fanning Springs), are also provided in Table 4-2, which covers from January 1996 through April 1998. The dates of each sampling event are also displayed on this figure. During the sampling period, the highest daily rainfall was 1.0 inch on December 2. Additionally, the cumulative rainfall for November and December 2009 was 5.78 inches. In November and December 1996, total rainfall was 8.79 inches. Peak river flow during sampling occurred on the last sampling event on December 28 at 9,540 cfs and gradually increased for the remainder of the month.

4.2 WATER QUALITY DATA

Weekly water quality samples and *in situ* data were collected from November 9 to December 28, 2009. *In situ* measurements included temperature, specific conductance, pH,

Water Year	Discharge (cfs)	Water Year	Discharge (cfs)		
1942	12,340	1976	9,546		
1943	6,229	1977	12,060		
1944	9,954	1978	10,870		
1945	11,230	1979	8,657		
1946	12,500	1980	10,760		
1947	9,856	1981	5,612		
1948	24,560	1982	8,234		
1949	12,980	1983	13,660		
1950	7,600	1984	17,140		
1951	6,704	1985	6,887		
1952	9,179	1986	12,520		
1953	7,496	1987	14,310		
1954	9,290	1988	9,732		
1955	4,291	1989	6,776		
1956	4,640	1990	6,875		
1957	6,201	1991	14,920		
1958	13,210	1992	9,122		
1959	13,990	1993	10,330		
1960	12,930	1994	10,440		
1961	10,590	1995	10,890		
1962	7,142	1996	5,970		
1963	7,172	1997	8,746		
1964	15,050	1998	15,480		
1965	19,270	1999	6,415		
1966	15,040	2000	3,406		
1967	9,549	2001	5,339		
1968	5,301	2002	3,275		
1969	6,335	2003	10,090		
1970	13,300	2004	6,442		
1971	9,080	2005	16,310		
1972	11,920	2006	6,523		
1973	15,560	2007	3,563		
1974	8,554	2008	4,678		
1975	12,760	2009	7,605		

Table 4-1. Annual Mean Discharge of Suwannee River near Wilcox at USGS Station 02323500

Note: Average annual river flow for period of record = 9,926 cfs.

Sources: USGS, 2010. ECT, 2010.

Month and Year	Rainfall (inches)	Month and Year	Rainfall (inches)	Month and Rainfall Year (inches)		Month and Year	Rainfall (inches)	
Jan 96	4.33	Jul 99		Jan 03	0.13	Jul 06	6.44	
Feb 96	0.98	Aug 99		Feb 03	6.96	Aug 06	5.67	
Mar 96	12.96	Sep 99		Mar ₀₃	6.87	Sep 06	2.32	
Apr 96	3.01	Oct 99		Apr ₀₃	2.11	Oct 06	1.36	
May 96	2.36	Nov 99		May 03	1.46	Nov 06	1.35	
Jun 96	7.14	Dec 99		Jun 03	7.3	Dec 06	4.05	
Jul 96	9.26	Jan 00		Jul 03	5.93	Jan 07	2.72	
Aug 96	12.06	Feb 00		Aug 03	5.3	Feb 07	1.63	
	4.55	Mar 00		Sep 03	2.52	Mar ₀₇	1.01	
Sep 96 Oct 96	4.87	Apr 00	$\overline{}$	Oct 03	2.01	Apr 07	1.07	
					1.5		0.46	
Nov 96	1.02	May 00	0.25	Nov 03		May 07	6.69	
Dec 96	7.77	Jun 00	6.66	Dec 03	1.18	Jun 07		
Jan 97	2.64	Jul 00	7.27	Jan 04	2.05	Jul 07	5.38	
Feb 97	0.82	Aug 00	1.45	Feb 04	7.52	Aug 07	6.73	
Mar 97	2.11	Sep 00	8.5	Mar 04	1.41	Sep 07	5.08	
Apr 97	8.46	Oct 00	0.3	Apr ₀₄	2.06	Oct 07	2.51	
May 97	2.62	Nov 00	1.24	May 04	1.83	Nov 07	1.29	
Jun 97	7.05	Dec 00	0.85	Jun 04	0.82	Dec 07	2.83	
Jul 97	8.21	Jan 01	1.23	Jul 04	0.04	Jan 08	4.1	
Aug 97	8.7	Feb 01	0.38	Aug 04	$\boldsymbol{0}$	Feb 08	2.78	
Sep 97	1.74	Mar ₀₁	3.22	Sep 04	$\mathbf{0}$	Mar ₀₈	4.85	
Oct 97	8.17	Apr 01	1.38	Oct 04	3.73	Apr 08	1.51	
Nov 97	6.47	May 01	0.07	Nov ₀₄	2.98	May 08	0.9	
Dec 97	9.68	Jun 01	6.08	Dec 04	1.89	Jun 08	5.79	
Jan 98	5.67	Jul $01\,$	12.14	Jan 05	1.13	Jul 08	11.42	
Feb 98	15.2	Aug 01	1.76	Feb 05	1.82	Aug 08	16.1	
Mar 98	4.05	Sep 01	7.03	Mar 05	3.78	Sep 08	1.79	
Apr 98	0.88	Oct 01	0.04	Apr 05	5.78	Oct 08	2.61	
May 98	0.87	Nov 01	0.43	May 05	4.45	Nov 08	2.12	
Jun 98	1.73	Dec 01	1.48	Jun 05	4.34	Dec 08	0.92	
Jul 98	1.85	Jan 02	4.07	Jul 05	8.59	Jan 09	3.64	
Aug 98	$\overline{}$	Feb 02	0.87	Aug 05	5.39	Feb 09	1.61	
Sep 98		Mar ₀₂	2.9	Sep 05	1.4	Mar 09	4.82	
Oct 98	$\overline{}$	Apr 02	1.83	Oct 05	1.59	Apr 09	3.17	
Nov 98		May 02	1.5	Nov ₀₅	3.07	May 09	5.22	
Dec 98		Jun 02	4.45	Dec 05	7.06	Jun 09	12.27	
Jan 99	$\overline{}$	Jul 02	6.05	Jan 06	2.32	Jul 09	7.74	
Feb 99	$\overline{}$	Aug 02	5.98	Feb 06	5.11	Aug 09	4.33	
Mar 99		Sep 02	5.63	Mar 06	0.11	Sep 09	3.61	
Apr 99		Oct 02	5.78	Apr 06	0.95	Oct 09	3.03	
May 99		Nov 02	5.47	May 06	1.89	Nov 09	2.51	
Jun 99		Dec 02	8.31	Jun 06	8.27	Dec 09	3.27	

Table 4-2. Monthly Total Rainfall at SRWMD Station 02323500 near Wilcox/Fanning Springs

Note: $_\$ = no data.

 Precipitation data from January 1996 through April 1998 was collected in a nearby station, Usher Tower (NDCC COOP ID 089120) in Levy County, which is located approximately 15 miles to the southeast from Wilcox/Fanning Springs.

Sources: SRWMD, 2010. ECT, 2010.

4-5

and DO. Water samples were analyzed for TKN, NO_x, total and fecal coliform, *Salmonella*, enterococci, and DNA human source tracking. The *Salmonella* and the DNA human source tracking analyses were qualitative (presence/absence), not quantitative.

The initial presentation of data is provided as statistical summaries and grouped into two categories: canal stations and river stations. The rationale for this grouping is based on the canal stations being near-field relative to the previous locations of the OSTDS and river stations are far-field and include upstream Stations 9 and 10. Canal stations are Stations 2 through 6, and river stations are 7 through 10. Additionally, data assessment used this grouping scheme in the earlier study, and this facilitated comparative analyses of the two databases. Station 1 is the monitoring well and has not been included in the station grouping analyses.

Table 4-3 presents *in situ* parameters by station group. Chemical and microbiological water quality sample parameters have been statistically summarized by individual canal and river stations in Tables 4-4 and 4-5, respectively. As shown in Table 4-5, the average values of the upstream stations (9 and 10) were comparable to the values of the downstream stations (7 and 8). Consequently, to aid in statistical comparisons, these four river stations were grouped for comparison with the canal stations (2, 3, 4, 5, and 6). Table 4-6 provides the water quality sample statistical summary for the grouped canal and river stations, as well as the monitoring well station. Tables of the complete raw data set for individual stations are contained in Appendix B. Given the proximity of the well to canal Station 4, water quality results for the well are included in Table 4-4.

4.2.1 *IN SITU* **PARAMETERS**

In situ measurements of pH, temperature, DO, and specific conductance were conducted at three depths in the water column: 1 ft below the surface (surface), mid-depth, and 1 ft above the bottom (bottom) at each surface water sampling station. Table 4-3 provides the summary statistics for each of the parameters at the three depths at the river and canal stations.

Viewing the surface water *in situ* data both vertically in the water column and spatially within the study area indicates there is no large variation in the measurements. Spatially,

Table 4-4. Summary of Water Quality Parameters for Canal Stations

*Value excluded an outlier of 3.59 from canal Station 4. †Value excluded an outlier of 0.021 from canal Station 4.

	Average at Station				Minimum at Station				Maximum at Station			
Parameters	7	8	9	10	7	8	9	10	7	8	9	10
Enterococci $\left(\frac{\text{col}}{100mL}\right)$	64	$37*$	32	29	6	$4*$	6	3	137	94*	52	67
Total coliform $\left(\frac{\text{col}}{100} \text{mL}\right)$	468	732	366	694	154	154	154	154	1,230	2,310	770	2,160
Fecal Coliform $\left(\frac{\text{col}}{100} \text{mL}\right)$	168	133	96	84	28	23	24	22	310	320	200	136
TKN (mg/L)	0.34	0.38	0.40	0.36	0.13	0.15	0.04	0.09	0.72	0.63	0.73	0.85
NOx (mg/L)	0.74	0.80	0.85	0.87	0.32	0.38	0.41	0.41	0.95	1.03	1.09	1.15
Total nitrogen (mg/L)	1.08	1.18	1.25	1.23	0.45	0.53	0.45	0.50	1.67	1.66	1.82	2.00

Table 4-5. Summary of Water Quality Parameters for River Stations

*Value excluded an outlier of 1,300 from river Station 8.

Table 4-6. Statistics for Water Quality Parameters at Canal, River, and Monitoring Well Stations

*Statistics exclude a suspected outlier value of 3.83 from a canal station. †Statistics exclude a suspected outlier value of 0.021 from a canal station. ‡Statistics exclude a suspected outlier value of 1,300 from a river station.

the greatest difference when comparing the vertical averages of the canal and river stations is for specific conductance with canal stations at 2,814 microSiemens per centimeter (μ S/cm) and the river stations at 1,492 μ S/cm. River stations show approximately 47 percent less conductivity as compared to the canal stations. This is due to the large fresh water flow of the river as well as the residual effects from the more saline flood tides coupled with incomplete flushing of canals during ebb tides. The vertical averages of the other three parameters are comparable. Evaluating differences vertically in the water column by comparing the average surface and average bottom measurements for conductance values indicates that the canal stations varied by 53 percent top to bottom (1,964 versus $4,221 \mu S/cm$), and the conductance varied by 57 percent $(1,013 \text{ versus } 1)$ 2,376 μ S/cm) at the river stations. Additionally, pH, temperature, and DO data have only relatively minor differences in the vertical with the largest difference being the DO in the canal station that varied 4 percent, but pH and temperature vertical differences are approximately 1 percent. This consistency in data indicates waters are vertically uniform with regard to temperature, pH, and DO and show only slight stratification. This supports using surface grab samples as a good representation of water quality through the water column.

4.2.2 NUTRIENT PARAMETERS

Nutrient parameters include TKN and NO_x . Total nitrogen was derived by summing TKN and NO_x . Each of these is briefly described in the following subsections and is presented in Table 4-6. The discussion includes comparison with the supplemental data presented in Section 3.0 and specifically on Table 3-2.

4.2.2.1 Total Kjeldahl Nitrogen

The monitoring well TKN concentrations were approximately three times the canal and river average, minimum, and maximum values. The average TKN concentration in the well was 1.35 mg/L compared to canal and river averages of 0.47 and 0.37 mg/L, respectively. The canal and river basic statistics were similar, indicating spatial uniformity throughout the surface water monitoring stations. Project surface water TKN data were slightly lower than the averages of those in the supplemental database for river stations, which range from 0.56 to 0.64 mg/L.

4.2.2.2 Nitrate + Nitrite

The average NO_x was approximately 17 percent higher in the river stations at 0.82 mg/L than the canal stations at 0.68 mg/L. The monitoring well had the lowest NO_x , which averaged only 0.01 mg/L over the sampling period. The maximum river and canal NO_x concentrations were 1.15 and 1.00 mg/L, respectively. The monitoring well maximum concentration was 0.02 mg/L.

The average river NO_x concentration of 0.82 mg/L was slightly higher than the range of average supplemental data river station values of 0.56 to 0.68 mg/L.

4.2.2.3 Total Nitrogen

Total nitrogen was derived by adding TKN and NO_x . Average total nitrogen was similar in both the canal and river stations at 1.15 and 1.19 mg/L, respectively. The monitoring well's total nitrogen average concentration was slightly higher at 1.36 mg/L. The maximum total nitrogen concentration was 2.24 mg/L at the well, compared to 1.52 and 1.54 mg/L at canal and river stations, respectively.

4.2.3 NUTRIENT-DISCHARGE RELATIONSHIP

To explore the relationship between river discharge and nutrient parameters, the average values for TKN, NO_x , and total nitrogen in surface water were determined. These averages were calculated including all of the river stations but excluding the canal stations and monitoring well station. Statistical analysis between the overall average values of the selected water quality parameters and average river discharge for the sampling day revealed weak but nonsignificant relationships. For example, a positive correlation $(R² = 0.29, P = 0.12)$ existed between river discharge and TKN (i.e., TKN increased with the increase in the discharge). Also, a nonsignificant and negative correlation ($R^2 = 0.56$, $P = 0.07$) existed between river discharge and NO_x. In addition, a negative correlation $(R² = 0.30, P = 0.17)$ existed between total nitrogen and discharge. The variations of TKN, NO_x , and total nitrogen with the river flow are presented in Figure 4-3. It is apparent from the figure that the correlations are influenced by the single high-flow event on December 28 at the end of the monitoring period.

Figure 4-4 illustrates the correlation coefficients (R^2) , which measure the degree of association between the data values.

4.2.4 MICROBIOLOGICAL PARAMETERS

Microbiological parameters measured in November through December of 2009 included total coliform, fecal coliform, enterococci, and *Salmonella*. These parameters were expressed in terms of col/100 mL except *Salmonella*, which was reported qualitatively as present or absent. Table 4-6 presents the summary results of the microbiological data.

4.2.4.1 Total Coliform

Total coliform in the monitoring well varied substantially over the sampling duration. Concentrations ranged from a minimum of below detection to 2,310 col/100 mL. The average well total coliform count was 845 col/100 mL. River stations total coliform average was 565 col/100 mL. Canal stations average counts of 654 col/100 mL were less than those found in the well. River and canal stations also had wide variations in counts over the 8 weeks, ranging from 154 to 2,310 col/100 mL. The average river stations value of 565 col/100 mL is similar to supplemental data averages for river stations ranging from 496 to 766 col/100 mL.

4.2.4.2 Fecal Coliform

The average fecal coliform count was highest in the canal stations at 218 col/100 mL, compared to the river and well stations at 120 and 1 (below detection) col/100 mL, respectively. The canal stations also exhibited the highest maximum fecal coliform count, at 666 col/100 mL compared to the river and well maximums of 320 and 1 (below detection) col/100 mL, respectively. The groundwater from the well was below detection for fecal coliform. However, these bacteria were detected in all river and canal station samples over the 8-week sampling period.

The supplemental data average fecal coliform counts was 111 col/100 mL, which is 49 percent less than the project's canal average of 218 col/100 mL. Similarly, the

supplemental data average fecal coliform counts range of 69 to 119 col/100 mL compares well with the project's river average fecal coliform count of 120 col/100 mL.

4.2.4.3 Enterococci

The enterococci bacteria were below detection in the monitoring well. The average well count was 1 col/100 mL (below detection), compared to the canal and river averages at 116 and 40 col/100 mL, respectively. The maximum enterococci count in the canal and river stations were 340 and 137 col/100 mL, respectively.

Figure 4-5 illustrates that the enterococci values were consistently higher in the canal stations than in the river stations. The canal and river stations enterococci values were relatively constant for the first four sampling events and then decreased, while discharge first decreased, recovered, and then increased. This resulted in low concentrations both for the highest and lowest discharge of the monitoring period.

The U.S. Environmental Protection Agency (EPA) has four criteria levels for body contact for enterococci levels. The most stringent is for beach areas at 61 counts per 100 milliliters (#/100 mL), and the most tolerant is for infrequent full body contact at a level of 151 #/100 mL. Figure 4-5 presents these values for comparison with the results. As illustrated, the average enterococci values in the river generally comply with the most stringent criteria, but the canal values frequently exceed the least protective criteria.

4.2.4.4 *Salmonella*

Salmonella were analyzed qualitatively as presence or absence in the samples. *Salmonella* were present in the monitoring well 50 percent (four out of eight) of the time. The percentage of presence of *Salmonella* in the river and canal stations was the same at 62.5 percent. Table 4-7 presents the detection of *Salmonella* at each sampling location during the sampling period. The comparable *Salmonella* occurrences in the river suggest that the canals are not the source of *Salmonella*. Also, data in Table 4-7 indicate that the high occurrence of *Salmonella* at Stations 7 and 8 (75 percent) suggests there may be a downstream source of *Salmonella* not associated with the town and canal stations, which in general have a lower percentage of occurrence than Stations 7 and 8. The lowest

Table 4-7. *Salmonella* Results

Sources: AEL, 2009-2010. ECT, 2010.

occurrence of *Salmonella* occurred during Weeks 1 and 7, when discharge was approximately the same as during the weeks with the highest occurrence (Weeks 3, 4, and 6).

4.2.5 MICROBIOLOGICAL PARAMETERS—DISCHARGE RELATIONSHIP

Weak negative correlations were found between total and fecal coliform with the river discharge. As these correlations were not significant, graphical representation are not included in this report.

4.2.6 SOURCE TRACKING

Water samples from three locations (Stations 2, 5, and 10) were analyzed for *Enterococcus faecium*, esp human gene biomarker (HGB), to track the presence of human fecal contamination as opposed to other animal sources. The stations were selected in consultation with FDOH staff. Samples from Stations 2, 5, and 10 were collected for eight sampling weeks in November and December of 2009. Table 4-8 summarizes the DNA source tracking results. The results showed no consistent patterns within and among the sampling locations.

For example, the first and the third week's samples were all positive for human DNA presence. The second, fourth, and eighth week's sampling had identical results with Stations 2 and 5 results positive for human DNA and Station 10 negative. However, on the fifth week's samples, Stations 2 and 5 were negative. The same stations on the eighth week were positive for human DNA. For the seventh week, Station 2 was negative, whereas Stations 5 and 10 were positive for human DNA. Out of 16 samples collected from two canal stations, 13 samples (81 percent) tested positive for human DNA. Whereas, out of eight samples from the river station, four samples (50 percent) were positive for human DNA. The three samples from the canals that tested negative all occurred when the enterococci values were 31 col/100 mL or less, suggesting higher enterococci values enhance the chances of detecting HGB. However, HGB was detected in the river stations with the enterococci value as low as 3 col/100 mL. Overall, HGB was detected 71 percent of the time at all three stations with the canals showing appreciably higher occurrences. At least one station tested positive for HGB during each week.

Sampling		Percent Positive							
Station		$\overline{2}$	3	$\overline{4}$	5	6		8	(within stations)
2	✔	V	V	V		V		V	75
5	V	V	V	V		V	✔	V	88
10	✔		V		✓		✔		50
Percent positive (among stations)	100	67	100	67	33	67	67	67	

Table 4-8. DNA Human Source Tracking Analyses Result

Sources: Molecular, 2009 and 2010. ECT, 2010.

5.0 ANALYSIS AND DISCUSSION

The primary goal of the water quality sampling program near the town of Suwannee was to document the water quality effects of installing a central wastewater treatment facility and closing approximately 850 septic tanks. More specifically, the study was to evaluate if closing the septic tanks would reduce pollution and enhance the viability of oyster harvesting in Suwannee Sound. The baseline study for the program was completed in 1996 prior to closure of the septic tanks. The intent was to sample 1 year later to evaluate potential improvements. Unfortunately, the value of the postconstruction sampling was compromised by two factors: (1) the septic tank closure was delayed, and not all tanks were closed prior to the 1997 sampling; and (2) 1997 was an El Niño year, and the river flows were two to three times greater, which affected the results and limited the ability to compare with preconstruction values.

In a continued attempt to evaluate the affects of septic tank closure, FDOH has funded this study to investigate if positive effects are measurable 12 years after the septic tanks were closed. This overall program consisted of two monitoring episodes: May through July 2009 and November through December 2009 (which is discussed in this report). The results of the summer 2009 study were presented in a previous report (ECT, 2009). The results of the 2009 winter monitoring effort were presented in Section 4.0; this section provides a comparison of these results with the 1996 preconstruction data.

5.1 ANCILLARY DATA

For a controlled study it is desirable to keep all variables constant except the study parameter. In this case, the study parameter was the effect of closing septic tanks on water quality. One of the key parameters that could affect or bias the study is river flow. The Suwannee River discharge flow for the sampling periods from 1996 (November through December), 1997 (November through December), 2009 (May through July), and 2009 (November through December) are presented in Figure 5-1 for comparison.

For the baseline or preconstruction year 1996 (November through December), the river flow remained relatively constant. However, in 1997, because of El Niño, the river flow

increased sharply, which made it difficult to interpret preconstruction and postconstruction results. The river flow in 2009 was quite variable, but no large flow increases as observed in 1997 occurred. Consequently, the effects of river flow in adding bias to the data were probably small or certainly less than observed in the 1997 results.

The comparison of the 1996 (November through December) and 1997 (November through December) results (ECT, 1998) and the comparison of the 1996 (November through December) and 2009 (May through July) results (ECT, 2009) were provided in the previous reports and will not be repeated in this report. In this section, 1996 (November through December) data (hereafter referred to as 1996 data) are compared with 2009 (November through December) data (hereafter referred to as 2009 data). Comparison of 1996 and 2009 requires evaluation of other parameters that might influence the data comparison. During both sampling events, specific conductivity, DO, pH, and temperature were routinely measured. The results are presented in Figures 5-2 (specific conductivity) and 5-3 (DO, pH, and temperature) and give the vertical average values from measurements made at three different depths for all stations for the entire sampling periods.

The results indicate the average specific conductivity in Canal Stations $(1.406 \mu S/cm)$, equivalent to a salinity of approximately 0.8 parts per thousand [ppt]) in 1996 was less than in 2009 $(2,814 \mu S/cm)$, equivalent to a salinity of approximately 1.6 ppt), whereas specific conductivity in the river stations was higher at $1,653 \mu S/cm$ in 1996 as compared to 1,492 µS/cm in 2009. A t-test for paired two samples for means showed that this difference in conductivity was significant at $P = 0.01$ level. Canal stations' DO in 1996 (6.0 mg/L) was less than that in river stations (7.1 mg/L) in 2009, which also showed significant difference at $P = 0.03$ level. River stations' DO was also lower at 6.8 mg/L in 1996 as compared to 7.5 mg/L in 2009 (significant difference at $P = 0.02$). An increase in pH was observed in both canal and river stations in 2009 as compared to those in 1996 (both significantly different at P less than 0.01). Temperatures, on the other hand, were slightly lower in 2009 in both the canal and river stations as compared to 1996. These changes, although relatively small, could have an influence on some of the parameters measured, but are probably minor. The variability in these ancillary data is presented to

describe and illustrate other parameters that could influence the interpretation of the preand postconstruction results.

5.2 NUTRIENTS

Preliminary examination of the NO_x data for the river stations indicated there was a correlation between NO_x and river flow. The weekly average values for the river stations are plotted against river flow and are shown in Figure 5-4. The figure illustrates the strong correlation ($R^2 = 0.78$) between river flow and NO_x for 1996 data, whereas the correlation is moderate ($R^2 = 0.56$) for 2009 data. This is partly because the river flow was relatively constant at approximately 5,000 cfs in 2009 sampling events except for one high flow value. This did not provide a good range of values to examine possible correlations.

To further illustrate the relationship between river discharge and NO_x , the data from the four surveys were plotted and are presented in Figure 5-5. This provided NO_x for a greater range of river discharges and illustrates the strong negative correlation ($R^2 = 0.84$) of NO_x with river discharge. It is uncertain if this is simply dilution caused by the higher flows or a chemical process.

To further examine the 1996 data and the 2009 NO_x postconstruction data, the average values for the river stations and the canal stations were calculated and presented in Table 5-1. The data for TKN, total nitrogen, and the coliform data are also summarized on this table. Even though there was an overall reduction in NO_x in 2009 as compared to 1996 in both canal and river stations, these differences were 7 percent. Combining all stations, the NO_x reduction from 1996 to 2009 was approximately 8 percent.

In 1996, NO_x and total nitrogen in the canal stations were lower, but TKN was higher than those in the river stations. Table 5-2 shows these results. Similar results were observed in 2009. However, the TKN concentration in the canal stations in 2009 was 27 percent higher than in the river stations as compared to that in 1996 at only 8 percent. Differences in NO_x concentrations in 1996 and 2009 were similar.

5-8

Table 5-1. Changes in Average Concentrations between the 1996 and 2009 Sampling Events

*Average excludes a suspected outlier value of 3.83 from a canal station.

†Average excludes a suspected outlier value of 0.021 from a canal station.

‡Average excludes a suspected outlier value of 1,300 from a river station.

Note: Negative percentage is decrease from 1996 values. Positive percentage is increase from 1996 values. Data are presented for river stations, canal stations, monitoring well, and combined river and canal stations (all stations except monitoring well).

Table 5-2. Difference in Average Concentrations between River and Canal Stations in the Years 1996 and 2009

Note: Negative percentage is decrease from river station values. Positive percentage is increase from river stations values.

The TKN in the canal stations increased slightly from 1996 to 2009, but the values observed in the river decreased slightly in 2009 as compared to 1996. The increase in canal stations' TKN was offset by the decrease in NO_x such that the total nitrogen remained nearly unchanged between 1996 and 2009 as shown in Figure 5-6. However, both NO_x and TKN in river stations decreased slightly in 2009 as compared to 1996. Station average NO_x concentrations were higher in 2009. TKN and total nitrogen concentrations were lower for 2009. There is no clear indication from these results to attribute improvement of water quality resulting from removal of the septic tanks.

5.3 MICROBIOLOGY

The following section compares the results of the microbiology from 1996 with the recent samples. Source tracking and enterococci analyses were not completed in 1996 and, consequently, are not presented here, but were discussed in Section 4.0.

5.3.1 *SALMONELLA*

Salmonella samples were analyzed for presence/absence only, and the 2009 results are presented in Section 4.0. In 2009 *Salmonella* were present in 63 percent of both the river and canal samples. This indicates that *Salmonella* issuing from the canals may not be the primary source of *Salmonella* in the river.

In 1996 *Salmonella* were present in the river stations 75 percent of the time with 100-percent occurrence at Stations 8, 9, and 10 and no occurrence at Station 7. *Salmonella* were present in the five canal stations only 15 percent of the time in 1996. Consequently, the occurrence of *Salmonella* in the river decreased from 75 to 63 percent from 1996 to 2009; however, in the canal stations, the occurrence increased from 15 percent in 1996 to 63 percent in 2009. The results indicate that during both studies the occurrence of *Salmonella* was equal or higher in the river than in the canals. Further, since the occurrences in the canals were higher in 2009 as compared to 1996, there was no observed reduction resulting from septic tank closure.

5.3.2 COLIFORMS

Table 5-1 presents the average observed coliform values for both fecal and total coliforms for 1996 and 2009 and for all river and canal stations. Figure 5-7 illustrates these results. Several key items are apparent in the data. The fecal coliforms are much higher in the canals than in the river in both 1996 and 2009 suggesting that the canals are a source of fecal coliforms to the river. This is not surprising given the concentration of wildlife in canal areas.

The data also indicate that there was a reduction in fecal coliforms in 2009 as compared to 1996 in both the canals (59-percent reduction) and the river station (29-percent reduction). Fecal coliform reduction in the canals might be attributed to closing of the septic tanks because the reduction is significantly greater than observed in the river.

Fecal coliforms were higher in canal stations in both 1996 (216-percent higher) and 2009 (82-percent higher) as compared to the river stations (refer to Table 5-2). Similar to the fecal coliform data, the total coliform values were higher in the canals in both 1996 (214-percent higher) and 2009 (16-percent higher). Comparison of canal and river stations' total and fecal coliform data indicates that the canals are a source of coliforms to the river. However, contrary to the fecal coliform results, the total coliform counts increased in 2009 at both the river and canal stations. Consequently, closing the septic tanks did not reduce the total coliforms. Closer examination of the 1996 data indicated the total coliform data were low and are somewhat suspect, so the observed increase from 1996 to 2009 might not be as great.

5.4 STATISTICAL TREATMENT

The primary goal of the 2009 study was to evaluate and document any potential improvements in water quality from closing 850 septic tanks in the town of Suwannee and establishing a central wastewater treatment system. The results indicated the differences between the concentrations of canal and river stations for a few of the indicator parameters were reduced in 2009 as compared to 1996 (refer to Table 5-1). To help determine if these differences were attributable to septic tank closure, water quality data from the 1996 and 2009 sampling events were further analyzed using statistical techniques for five

indicator parameters including total and fecal coliforms, NO_x , TKN, and total nitrogen. For each sampling week, each indicator parameter was grouped as a canal station or river station. Averages of canal stations and river stations were calculated for each of the parameters and are presented in Table 5-3.

A two-tailed t-test was performed to compare the weekly mean values of water quality parameters for both the canal stations and river stations between 1996 and 2009. Although not all of the parameters passed the normality test, the t-test was still applied to compare the mean values. In addition, a nonparametric test (Mann-Whitney rank sum test) was used to compare the median values, and both tests agreed on which parameters had significant differences. Only the results of the t-test are presented for comparison of the mean values. Figures 5-6 and 5-7 (presented previously) illustrate the data used for this analysis. Table 5-4 provides the results of the tests.

Of the five parameters tested for the canals, only fecal coliforms were significantly different between the 1996 and 2009 replicate samples (Table 5-4) and showed a 59-percent reduction.

For the river stations, the results indicated that there was a significant increase in total coliform in 2009 as compared to 1996 and statistically significant decrease in total nitrogen.

Even though there was a significant reduction in fecal coliforms in the canals from 1996 to 2009, it was not certain if this was the result of septic tank closure or other reasons. To examine this further the changes in the river stations (control stations) were compared to the changes observed in the canals.

In an attempt to assess the septic tank closure contribution to changes in canal water with respect to river water background, the differences between the average concentrations of indicator parameters between the canal and river stations were determined for each of the eight weekly replicate sampling periods. Table 5-3 presents these differences.

Table 5-3. Weekly Average Value of River and Canal Stations and Their Differences for the 1996 and 2009 Results

Source: ECT, 2010.

Table 5-4. Before and After Comparison for Canal Stations and River Stations Mean Values between 1996 and 2009

*Indicates significant difference.

Note: $P =$ value is the probability of being wrong in concluding that there is a true difference between the groups.

Source: ECT, 2010.

Eight replicate weekly means of differences for each of the indicator parameters were also tested for the differences. This analysis was completed to test if there was a significant difference in the observed difference in canal versus river stations between 1996 and 2009. Of the five parameters tested, fecal coliform and total coliforms were significantly different between the 1996 and 2009 replicate samples (Table 5-5). For example, the fecal coliform values in 1996 averaged 367 col/100 mL higher in the canals than the river. But in 2009, the fecal coliform values averaged only 98 col/100 mL higher in the canals than in the river. This reduction in the difference was significant ($P = 0.023$) and was the result of a reduction in the fecal coliform values in the canals from 1996 to 2009.

The significant difference observed in the total coliform data is the result of a large increase in total coliforms in the river from 1996 to 2009 as compared to a relatively small change in the canals. The difference is not the result of large changes in the canals and probably cannot be attributed to septic tank closure.

However, the significant difference in the fecal coliform is the result of the observed significant decrease in fecal coliform in the canals from 1996 to 2009. The fecal coliform counts in the river stations (controls) changed very little; consequently, the reduction in fecal coliform was unique to the canals and could possibly be attributed to septic tank closure.

5.5 COMPARISON OF THE WINTER AND SUMMER 2009 RESULTS

Although not an integral component of the study, the results provide a unique opportunity to compare winter and summer data for the study area. Table 5-6 presents the data results (except HGB) for both the summer and winter 2009 surveys, and Figures 5-8 through 5-10 illustrate these results.

A few of the key comparisons include:

- The average water temperature was 17.85°C in the winter and 27.01°C in the summer.
- Total nitrogen concentration is relatively constant, with the NO_x concentration being higher in the winter and TKN being lower in the winter for both the canal and river stations.

Table 5-5. Before and After Comparison for River and Canal Stations Average Differences between 1996 and 2009

*Indicates significant difference.

Note: $P =$ value is the probability of being wrong in concluding that there is a true difference between the groups.

Source: ECT, 2010.

Table 5-6. Changes in Average Concentrations between the 2009 Summer and Winter Sampling Events

*Summer 2009 average value excludes suspected outliers of 1,150 and 2,100 from a canal station and monitoring well, respectively.

Note: Data are presented for river stations, canal stations, monitoring well, and combined river and canal stations (all stations except monitoring well). Negative percentage is a decrease from summer 2009 values. Positive percentage is an increase from summer 2009 values.

Source: ECT, 2010.

- The total coliforms concentration is higher in the summer, and the fecal coliform concentration is higher in the winter for both the canal stations and river stations.
- Enterococci values were slightly higher in the winter.
- The occurrences of *Salmonella* were substantially higher in the winter, with the winter occurrences nearly twice as high as the summer (33 versus 63 percent, excluding the monitoring well data).
- The occurrence of HGB was the same at the river station (Station 10) for winter and summer but substantially higher in the winter at the two canal stations (average of 82 versus 38 percent).
- At the monitoring well, there were substantial reductions in enterococci, total coliforms, fecal coliform, and NO_x values in the winter as compared to the summer.

In general, the key microbiological parameter concentrations, including enterococci, fecal coliform, and occurrences of *Salmonella*, were higher in the winter.

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

The goal of this study was to evaluate the long-term effects of closing approximately 850 OSTDS in the town of Suwannee and installing a central WWTP. The approach was to sample water quality in the Suwannee River and the canals within the town of Suwannee and compare the results with data collected in 1996 prior to OSTDS closures. The two previous attempts to provide postconstruction data for comparison provided valuable information but were not ideal because of extreme river discharge conditions and seasonality concerns. The most recent study was conducted during the same season and during comparable river discharge conditions as the 1996 baseline survey conducted prior to septic tank removal. Therefore, this study provides a more defensible data set to evaluate potential improvements in the area 13 years after septic tank closure.

The results did not suggest that there was large improvement in water quality in the canals between 1996 and 2009 that could be attributed to closing the OSTDS. However, several specific observations and some improvements were noted:

- *Salmonella* occurrences were equal to or higher in the river than in the canals in both 2009 and 1996, indicating the canals were not the primary source of *Salmonella*. The percent occurrence of *Salmonella* in the canals was greater in 2009 than in 1996, indicating septic tank closure did not reduce *Salmonella* in the canals.
- NO_x exhibited a strong correlation with river flow and decreased with increasing river flow. TKN increased with increasing river flow, and total nitrogen remained relatively constant. There was consistently more NO_x in the river samples than in the canals. There was a slight but significant decrease of 6 percent in total nitrogen in the river stations.
- The source tracking results (HGB) indicated human material was present approximately 82 percent of the time in the canals (average of two stations) and only 50 percent of the time in the river (Station 10). It appears that, despite septic tank closure, the canals remain a possible source of HGB. Source tracking was not conducted in 1996.
- During the summer 2009 sampling event, HGB was present 38 percent of the time in canals as compared to 50 percent presence in the river.
- The total and fecal coliform values were higher in the canals than in the river in both 1996 and 2009. Fecal coliform decreased from 1996 to 2009 in both the canals and the river stations, whereas total coliforms increased from 1996 to 2009. The higher values of coliforms in 2009 in the canals as compared to the river could be from domestic animals or wildlife concentrated near the canals, whereas in 1996, higher coliforms count could have been from OSTDS.
- A simple before and after comparison of the 2009 results with the 1996 results indicated there were three statistically significant changes in the measured parameters between 1996 and 2009:
	- o There was a 59-percent decrease in fecal coliform in the canals.
	- o There was a 230-percent increase in total coliforms in the river.
	- o There was a 6-percent decrease in total nitrogen in the river.

All other observed changes in the surface water samples were not statistically significant.

- Additional statistical tests were conducted that evaluated the differences between average canal and river station results for each event. The results indicated the magnitude of reduction in fecal coliform concentrations from 1996 to 2009 was greater in the canal stations and could be a possible benefit of closing the OSTDS.
- The monitoring well data indicated dramatic improvement from 1996 to 2009 in most of the parameters. The fecal coliform counts dropped from an average of 232 col/100 mL to nondetectable. The nitrogen parameters all dropped in excess of 82 percent. Since the well was located downgradient of the septic tank drain field, closing the septic tank resulted in marked improvement in the groundwater at this location. However, total coliforms and the percent occurrence of *Salmonella* increased in 2009.

• Comparison of the winter 2009 data with the summer 2009 data indicated that total coliform counts were higher in the summer, but fecal coliform, enterococci, and *Salmonella* occurrences were higher in the winter for both the river and canal stations.

In summary, the results indicated that there was a statistically significant 59-percent reduction of fecal coliform in the canals between 1996 and 2009 that could not be attributed to changes observed in the river stations. There was also an increase in total coliforms in the canals, but it was less than the increase in the river. There was an improvement in groundwater measured near a septic tank drain field. No other significant improvements in the water quality of the canals was identified that could be attributed to OSTDS closures.

6.2 RECOMMENDATIONS

The winter 2009 study provided a unique opportunity to examine the water quality in the canals and the river around the town of Suwannee 13 years after closure of 850 OSTDS in the area. A before and after comparison of sampling results for canal and river stations indicated a significant decrease in canal fecal coliform, increase in river total coliforms, and decrease in river total nitrogen. A comparison of differences between canal and river stations in 1996 and 2009 indicated a reduction in fecal and total coliform evaluations in canals, but there was not a significant reduction of nitrogen, total coliforms, or occurrences of *Salmonella*, which might have been anticipated. There was a marked improvement of the groundwater near a septic tank drain field.

It is unlikely that additional studies of these parameters would identify further improvements attributable to septic tank removal, since additional improvements were not apparent after 13 years. Although the study plan attempted to isolate the removal of the OSTDS as the only variable for testing between the pre- and postconstruction sampling, it was not possible to control all environmental factors, such as rainfall, river flow, and temperature. It is recommended that future studies follow a similar protocol and establish a series of test and control stations that lend themselves to rigorous statistical analysis. Future studies at other sites should again be designed to conduct the pre- and postconstruction sampling during comparable seasonal (temperature) and river discharge conditions. It is also recommended that the additional source tracking techniques such as HGB be used more extensively to help separate human impacts from natural sources.

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APPENDIX A

SUPPLEMENTAL WATER QUALITY DATA

Appendix A data may be found on the CD enclosed in the hard copy of this report.

APPENDIX B

IN SITU **AND ANALYTICAL DATA (NOVEMBER AND DECEMBER 2009)**

Town of Suwannee Water Quality Sampling

Water Quality Parameters: *In-situ* measurement

				Parameters			
Station	Date	Sampling Location	Sampling Depth (f ^t)	Specific Conductance $(\mu$ mhos/cm $)$	Temperature $({}^{\circ}C)$	pH (s.u.)	D _O (mg/L)
	11/09/09						
10 10	11/09/09	Bottom	23 12	353 349	20.58 20.57	7.91 7.91	8.31 8.33
		Middle					
10	11/09/09	Top	$\mathbf{1}$	348	20.58	7.93	8.43
10	11/16/09	Bottom	23.0	425	19.81	7.80	7.16
10	11/16/09	Middle	12.0	421	19.88	7.84	7.04
10	11/16/09	Top	1.0	421	19.88	7.97	7.04
10	11/23/09	Bottom	23.0	388	20.63	7.93	7.65
10	11/23/09	Middle	12.0	388	20.58	7.98	7.63
10	11/23/09	Top	1.0	387	20.59	8.00	7.64
10	11/30/09	Bottom	23	422	17.48	7.59	7.56
10	11/30/09	Middle	12	413	17.57	7.75	7.66
10	11/30/09	Top	$\mathbf{1}$	410	17.56	7.97	7.68
10	12/07/09	Bottom	23.0	402	16.79	7.94	8.58
10	12/07/09	Middle	12.0	400	16.79	7.88	8.47
10	12/07/09	Top	1.0	398	16.82	7.96	8.47
10	12/14/09	Bottom	22.0	272	17.21	6.97	7.15
10	12/14/09	Middle	11.5	270	17.18	6.88	7.15
10	12/14/09	Top	1.0	269	17.16	6.98	7.21
10	12/21/09	Bottom	23.0	216	15.21	7.39	7.28
10	12/21/09	Middle	12.0	215	15.20	7.52	7.24
10	12/21/09	Top	1.0	214	15.24	7.72	7.31
10	12/28/09	Bottom	23.0	168	13.73	7.76	7.78
10	12/28/09	Middle	12.0	167	13.74	7.79	7.78
10	12/28/09	Top	1.0	166	13.75	7.88	7.79

Table B-2. *In Situ* Data—General Format (Page 5 of 5)

Table B-3. Analytical Data—Tabular Format (Page 1 of 3)

Town of Suwannee Water Quality Analysis Results

Station 1

Station 2

Water Quality Parameters Units 11/09/09 11/16/09 11/23/09 11/30/09 12/07/09 12/14/09 12/21/09 12/28/09 Enterococci colonies/100ml 200 93 230 111 14 120 12 28 Fecal Coliforms colonies/100ml 420 125 162 450 67 370 39 103
Nitrate-Nitrite mg/L 0.968 0.927 0.946 1.00 0.921 0.569 0.480 0.340 Nitrate-Nitrite mg/L 0.968 0.927 0.946 1.00 0.921 0.569 0.480 0.340 Salmonella Absent Present Present Present Present Present Absent Absent Total Coliforms colonies/100ml 1390 1080 462 770 462 616 154 462 Total Kjeldahl Nitrogen mg/L 0.38 0.59 0.19 0.44 0.40 0.46 0.61 0.68 Sampling Dates

Station 3

Station 5

Table B-3. Analytical Data—Tabular Format (Page 2 of 3)

Station 6

Station 7

Station 8

Station 9

Station 10

 $\mathbf{U} =$ analyte not detected at or above the method detection limit

I = value is between the laboratory method detection liomit and the practical quantitation limit.

Town of Suwannee Water Quality Analysis Results Enterococcus faecium esp Human Gene Biomarker (HGB) for Human Fecal Contamination*

*Detection Method - Polymerase Chain Reaction (PCR) DNA Analytical Technology

HGB Negative

X HGB Positive

Table B-4. Analytical Data—Generalized Format (Page 1 of 10)

Table B-4. Analytical Data—Generalized Format (Page 2 of 10)

Table B-4. Analytical Data—Generalized Format (Page 3 of 10)

Table B-4. Analytical Data—Generalized Format (Page 5 of 10)

Table B-4. Analytical Data—Generalized Format (Page 6 of 10)

Table B-4. Analytical Data—Generalized Format (Page 7 of 10)

Table B-4. Analytical Data—Generalized Format (Page 9 of 10)

Notes: $U =$ analyte not detected at or above the method detection limit

 $I =$ value is between the laboratory method detection liomit and the practical quantitation limit.

MDL = method detection limit

PQL = practical quantitation limit

 $VQ =$ value qualifier