The Importance of OSTDS Contaminant Loading to the IRL

Final Project Report
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IRL National Estuary Program
St. John's River Water Management District

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PROJECT OBJECTIVES AND APPROACH:

This study addresses the importance of OSTDS as a source of nutrients and fecal coliform bacteria to the lagoon---a key issue for future management because of the sensitivity of the lagoon to nutrient and bacterial contamination. To help answer this question, this study was divided into two parts. Part I involved the identification and seasonal sampling of three residential OSTDS test sites in Brevard and St. Lucie Counties. The collected can be added to data obtained by the Principal Investigator from residential sites previously investigated on the St. Lucie River and Estuary in Martin and St Lucie Counties, as well as data obtained from his current Mosquito Lagoon study funded by the National Park Service. The combined data, under a variety of site conditions, will increase the database and enable more informed conclusions to be made. We believe this site-specific residential approach avoids many of the errors common to other approaches, such as modeling, and provides definitive data for each particular site. With the addition of the three proposed sites (sampled three times), at least 14 nearly randomly chosen sites, covering a wide range of site conditions, have been evaluated in various lagoon watershed areas by the P.I. Collectively, these data give a reasonable indication of the importance of OSTDS nutrient and bacterial loading to the IRL System. Included in this evaluation is an estimate of contaminant plume travel rates (ft/yr) for the various test parameters (NH_4-N, NO_3-N, SRP, FC bacteria) and the identification of the most critical (worst case) site factors. The site-specific residential approach, employing

piezometers, pushpoint samplers, peristaltic pumps and seepage meters, is explained in detail in this report.

Part II involved the thorough testing of chemical indicators to determine their effectiveness in detecting sources of human fecal contamination. We chose caffeine (CAF), Triclosan® (TCS) and fluorescent whitening agents (FWA) as our methods of choice. These methods were used near our IRL NEP residential sites, as well as at other selected sites, such as sewage treatment plants and control areas. Reliable methods for fecal contamination source tracking are needed by management personnel because typical nutrient and bacterial sampling and analysis is expensive, time consuming and usually not conclusive. Usually answers are needed in a timely manner, and the procedures employed in Part I often require too much time, effort and manpower for routine use. Although bacterial source tracking (BST) comprises a diverse suite of effective microbiological methods for detecting pollution, it has not performed well at identifying specific sites or points of origin, either. BST is also expensive and time consuming, and samples must be collected and transported to a lab where often generations of bacteria must be incubated sequentially. With the molecular biological methods of BST, highly specialized and expensive equipment is required. In view of the above, a need exists for cost-effective, rapidly performed method of identifying anthropogenic pollution while clearly indicating a point of origin. The chemical indicators, CAF, TCS and FWA were believed to be good candidates to serve that need, and their effectiveness was investigated in Part II of this study.

BACKGROUND AND GENERAL SITE DESCRIPTION:

The Indian River Lagoon (IRL) system is an Estuary of National Significance (EPA, 2007) spanning approximately 250 km of Florida's central east coast from Ponce Inlet in Volusia County to Jupiter Inlet in Palm Beach County (Steward et al, 2003). It is comprised of three main bodies of water (Indian River Lagoon, Banana River, Mosquito Lagoon), has five open seawater inlets (Ponce Inlet, Sebastian Inlet, Ft. Pierce Inlet, St. Lucie Inlet, Jupiter Inlet), and many freshwater tributaries of various size.

The IRL System is a vital biological and economic resource to eastern Florida, representing a biogeographic transition zone rich in habitat and fish and wildlife species. Because of this diversity, and the fact that it is home to approximately nine federally protected threatened and endangered species, it is recognized as an important commercial and recreational area. It appears the water quality of the lagoon is declining, however, and there are a growing number of biological impacts that seem to be caused by this water quality decline. For example, disease and tumors in fish, and increases in algal blooms, fish kills and invasive species have hinted at a system under stress. Many biological experts suspect poor water quality is responsible for these impacts, and septic tanks may be contributing greatly to the water quality degradation through nutrient and bacterial loading in the lagoon system. Nutrient enrichment also represents a very real threat to seagrasses, since the lagoon system is a seagrass based ecosystem that depends on the existence of thriving seagrasses. The nutrient levels required for optimum seagrass growth are extremely low, and elevated nutrient levels may cause more algae and harmful algal blooms (HAB's) to proliferate with a concurrent reduction in seagrasses. A change to and an algal-based system with continued nutrient loading would have disastrous ecological consequences to the system.

In 1995 there were more than 120,000 septic tanks along the IRL and at least 90,000 of the total are located in areas with poor conditions, such as a high water table, poor soil conditions, close proximity to the lagoon, etc.(Kearney and Roesen, 1995). These high 1995 numbers are undoubtedly greater today, indicating the very real potential that OSTDS may serve as significant contributors of nutrients and bacteria to the IRL. This project investigates that question through detailed field studies at selected OSTDS sites.

GENERAL APPROACH AND RATIONALE:

At each residence, control site locations were established in close proximity to the test sampling locations (near the drainfield), but away from OSTDS influence. Although two general neighborhoods were targeted for testing, the exact residence locations in each area were dependent on the willingness of the individual homeowners to allow us to study their OSTDS installations. The sites in this study represent properly functioning OSTDS and, although not selected in a statistically random fashion, they represent a range of site conditions (e.g. soil type, location of drainfield relative to river, high vs. low vertical and horizontal hydraulic gradient, bulkhead vs. natural slope, low vs. high tank loading, etc.).

The three FY 09 study sites represent natural gradient (no bulkheads) areas and are located at 3001 and 2601 Indian River Drive, Ft. Pierce (Lounibos and Grimes residences, respectively) and 511 E. Melbourne Avenue, Melbourne (Huy residence). The general locations of the three residential test sites are shown in Figure 1. All of these homes are older, with the Huy house being built in 1951, and the Grimes house/apartment building and Lounibos home being built in 1901 and 1936, respectively. The Huy house was inhabited by five people until approximately 1975, at which time the three children moved away. The system was rarely pumped out and the drainfield is 115 feet from Crane Creek---a tributary to the IRL. The Grimes home has eight bedrooms and was turned into an apartment house several decades ago. This home/apartment building converted to a new raised aerobic septic system in 1990. The system has a 2,000 gallon tank and two 30 by 21 ft. drainfields. The two drainfields are approximately 105 feet from the lagoon. The system is pumped out each year for maintenance. The Lounibos home, located less than 0.5 mi from the Grimes residence, has three bedrooms and was occupied by four people until about 25 years ago, at which time it reverted to two inhabitants. The septic tank was occasionally pumped and the drainfield is located 160 feet from the IRL. Soil type, hydraulic gradient and other site characteristics are discussed later. Sampling dates in were on 1/2/09, 3/23/09, 6/23/09, and 8/6/09.

This project involved the collection of water quality and hydrologic data three times from each site during 2009. Groundwater samples were collected at each site from piezometers and PushPoint samplers located adjacent to and down gradient from the septic tank drainfield, to the edge of the water body. Water level measurements in piezometers allowed head differences and hydraulic gradients (vertical and horizontal) to be calculated during each sampling trip. The OSTDS layout at each site was mapped and the sediment and hydrologic characteristics measured and documented. The general sampling scheme used at each site is shown in Figure 2, and individual sampling site locations are shown in Figures 3 through 5. Samples for conductivity, ammonia, soluble reactive phosphate, nitrate, dissolved oxygen, temperature, turbidity, and bacteria analyses were collected from the groundwater and surface water sampling locations at each site. Site characterization (soil characteristics, hydraulic gradients etc,) provided hydrogeologic data for use in data analysis.

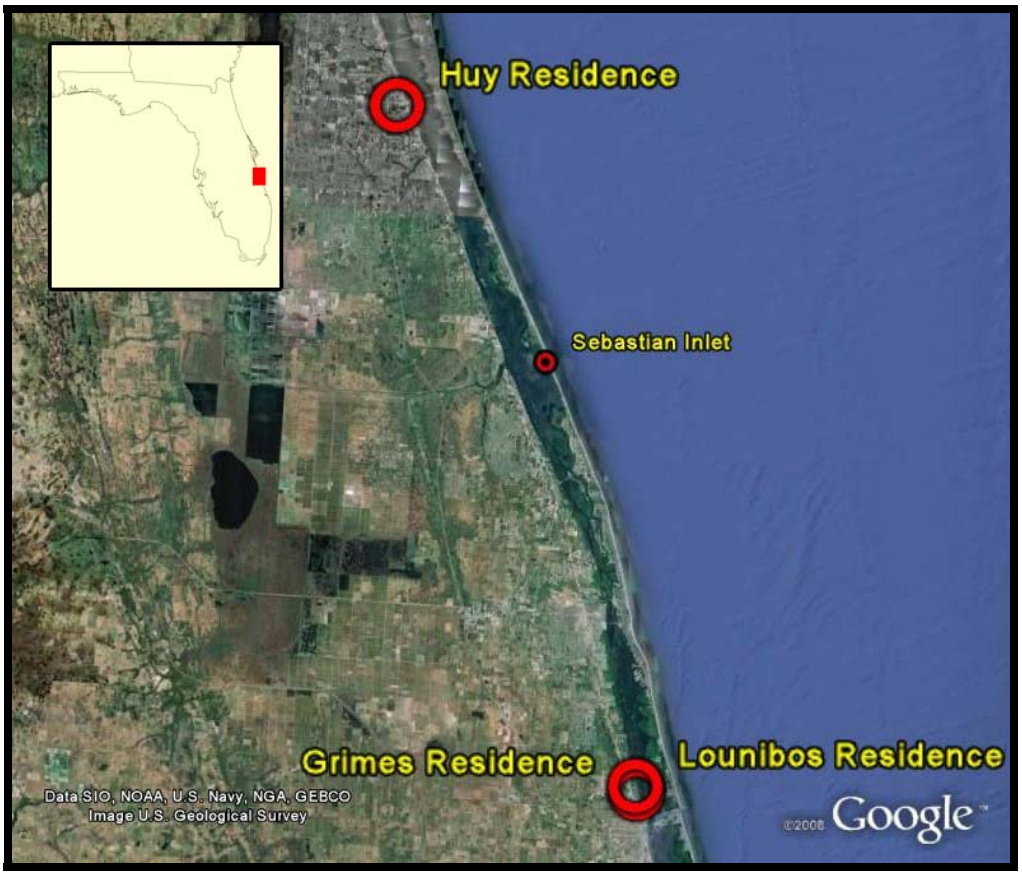


Figure 1. General Locations of the Three Residential Test Sites

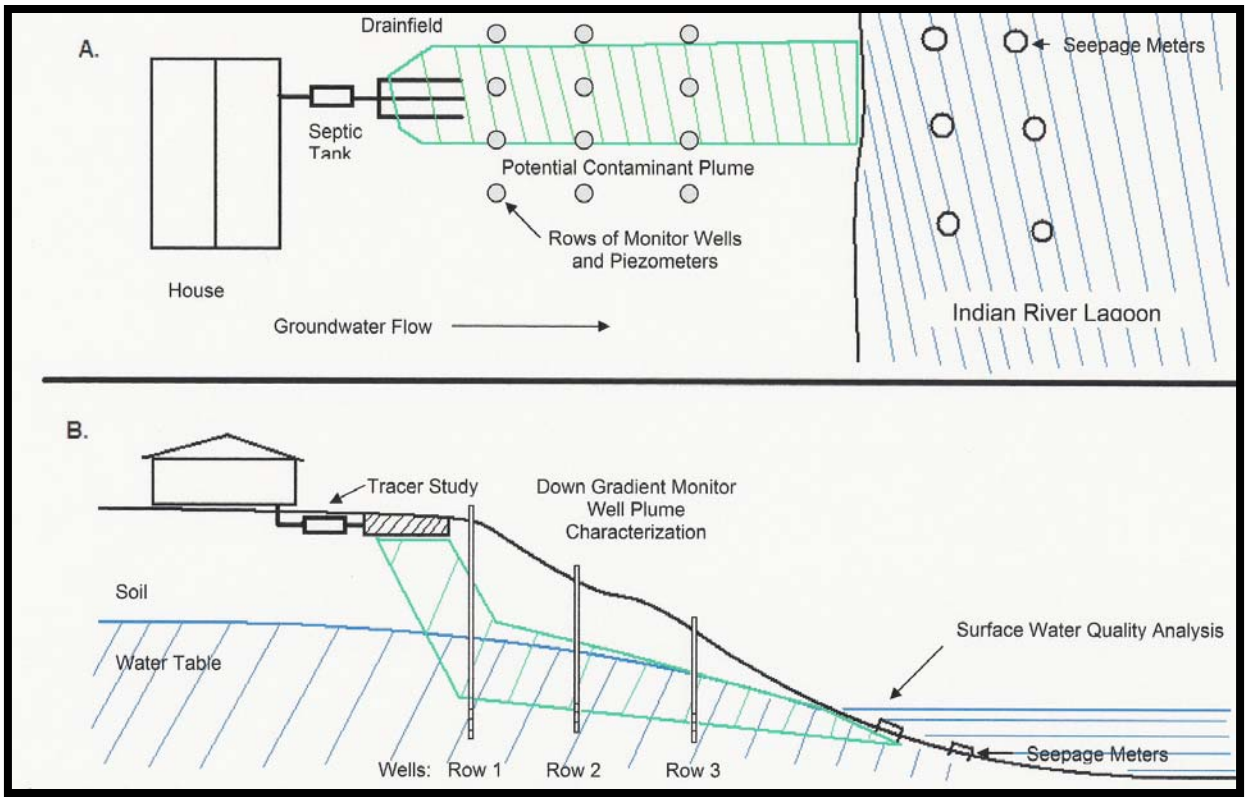


Figure 2. General Sampling Scheme for Study

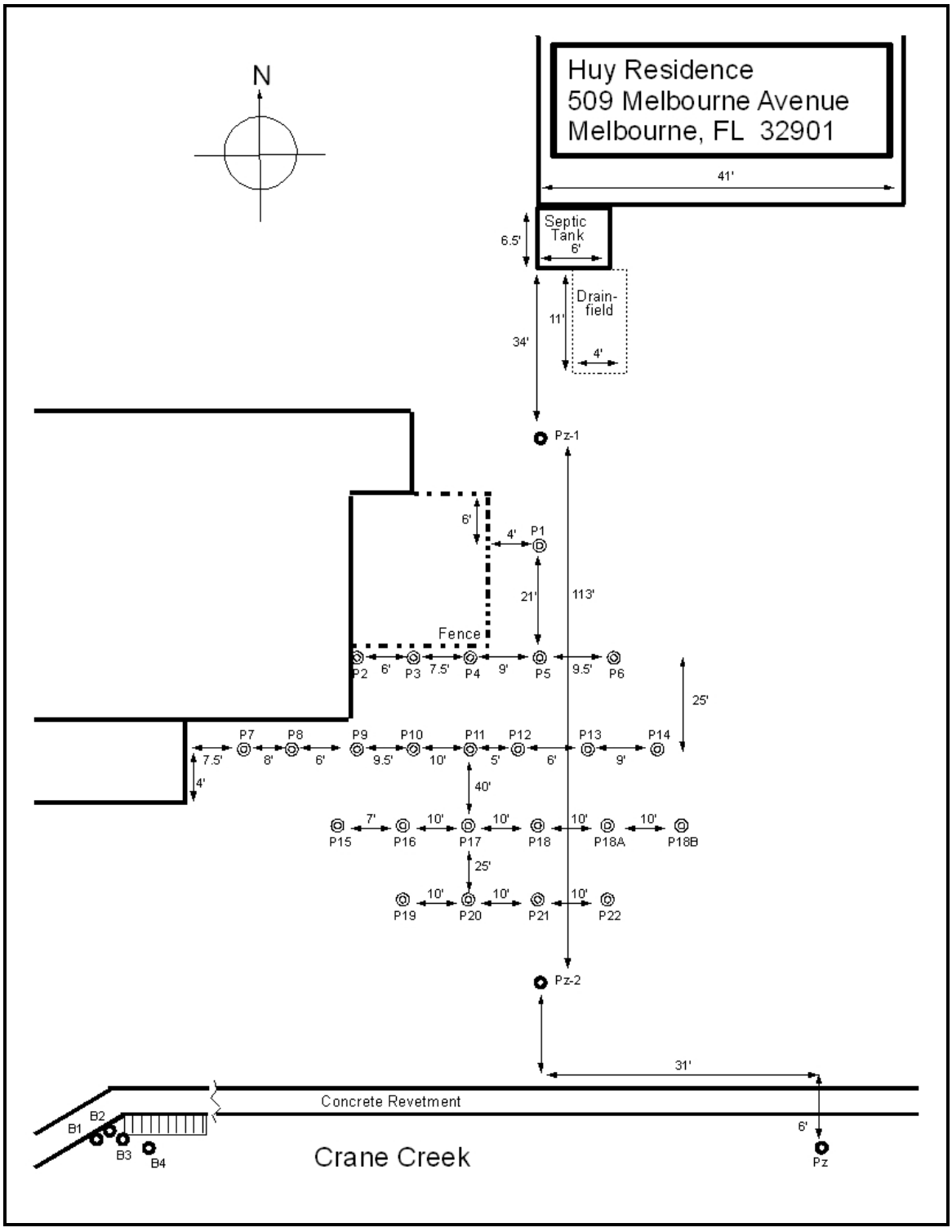


Figure 3. The Huy Residence (Drawing not to scale).

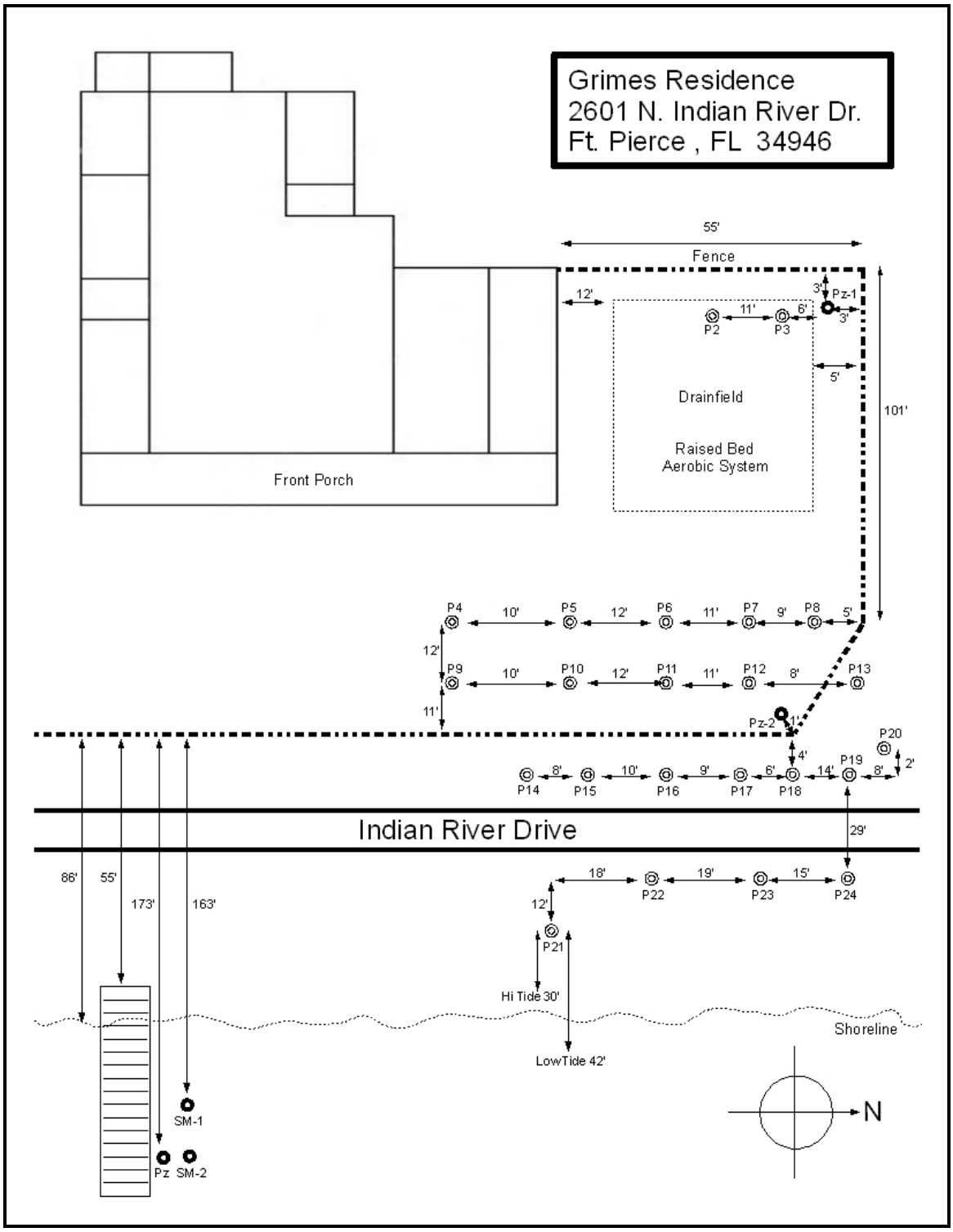


Figure 4. The Grimes Apartment Building (Drawing not to scale).

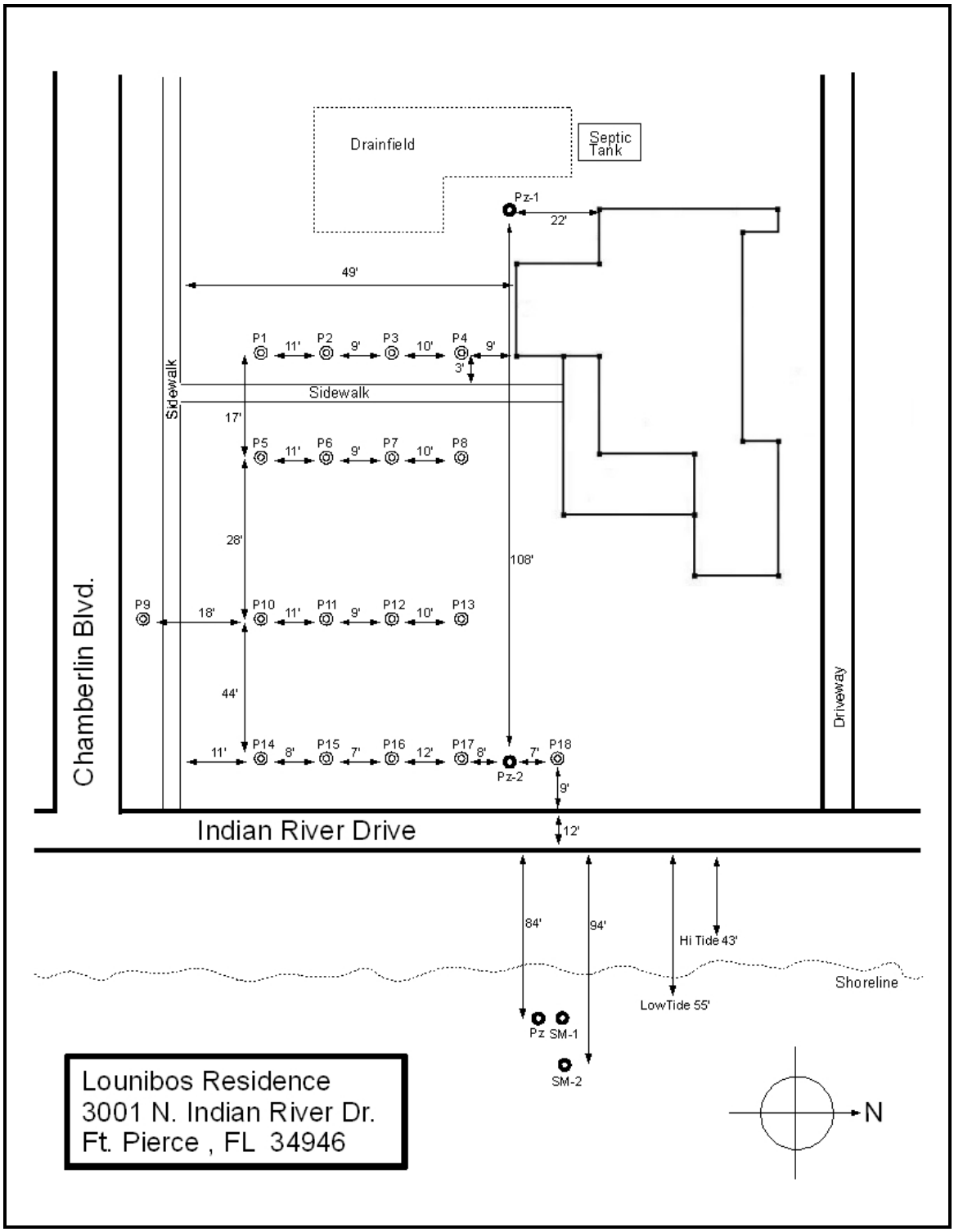


Figure 5 The Lounibos Residence (Drawing not to scale).

GENERAL METHODS:

With the exception of the Huy residence, two seepage meters were positioned near the shore at each site. Two two inch piezometers were installed at each site--one up gradient from the septic tank drainfield and one down gradient near the edge of the river. These piezometers were used to establish site groundwater levels for use in calculating hydraulic gradients. One in situ ¾ inch piezometer was positioned near the shoreline at each site and collected water level data were used to calculate vertical hydraulic gradients. Groundwater samples were obtained from 2 inch piezometers using M.H.E. PushPoint sediment pore water samplers using a peristaltic pump. Groundwater samples were usually collected 0.5 ft below the water table and at additional depths at selected sites. A generalized schematic of the sampling plan is shown in Figure 2. Surface water samples were obtained from grab samples one to two feet below the surface. Sampling protocols described in the South Florida Water Management District's Field Sampling Manual (12/01/02) were followed. Samples were analyzed for soluble reactive phosphate (PO_4), ammonia (NH_4-N), nitrate plus nitrite (NO_X-N), color and turbidity in the lab using EPA approved methods, while specific conductance, temperature and dissolved oxygen were measured in the field using a Myron Ultrameter. Nutrients were analyzed using the CHEMetrics VVR water analysis system. All lab samples were kept on ice in the field and analyzed as soon as possible, or were frozen for later analysis. The methodologies for the chemical indicators caffeine, Triclosan®, and fluorescent whitening agents were investigated and analyzed using what we believe to be the best and most accurate techniques. This is discussed under Specific Methods, below. Bacteria were collected in sterile jars provided by Harbor Branch, kept on ice and delivered to the Harbor Branch Oceanographic Institution lab for total and fecal coliform analysis with three to four hours of sampling. At Harbor Branch they were immediately analyzed using the EPA approved MPN technique (APHA, 1985).

The major objective of this research was to investigate the feasibility of three distinctly anthropogenic chemical compounds as indicators of human fecal contamination and eutrophication of ground and surface waters originating from improperly installed or poorly functioning on-site treatment and detention systems (OSTDS). Specifically at

issue are residential septic systems in close proximity (<200') to the Indian River Lagoon (IRL) and their contribution to nutrient loading and pollution.

The criteria employed in selecting which indicator chemicals to use were based largely on the summary of Young, et al (2008) as described below:

> "Ideally, chemical indicators used for monitoring of microbial risks from sewage spills to surface waters should originate exclusively in raw wastewater, occur therein at elevated and relatively constant levels, adsorb to microorganisms, be removed along with suspended microorganisms during sewage treatment, be insensitive to environmental pH changes, and attenuate in natural waters at rates comparable to those of pathogenic microorganisms" (Young, et al, 2008).

Given the diverse environmental and systemic variables encountered when engaging in the task of microbial source tracking, it is expected that "no single indicator or method is capable of identifying specific sources of fecal pollution in the environment with absolute certainty" (Scott et al, 2002). Therefore several compounds have been chosen for use in this study and will be employed together: caffeine (CAF), Triclosan® (TCS), and a class of organic stilbenes commonly referred to as either optical brighteners or fluorescent whitening agents (FWA). All these compounds recently have been shown to meet the criteria of Young et al (Young et al., 2008; Peeler et al., 2006; Sankararamakrishnan and Guo, 2005; Boving et al., 2004).

Though the three chemical indicators selected for this study meet the established criteria for reliable indicators, and though they have all have had some demonstrated success in predicting anthropogenic impacts, the original impetus for undertaking the study of these three particular compounds was a desire to move away from lengthy, laboratory-based procedures requiring specialized and expensive equipment and toward a more rapid and in situ methodology. As explained in the following sections, preliminary investigations into selected new methodologies for the rapid and in situ determinations of TCS and CAF have revealed such problems as incompatibility for use in a field environment and a lack of necessary sensitivity. This has necessitated the selection of alternate detection methodologies for these two indicators. Fortunately, new methods for

both CAF and TCS, utilizing an Enzyme-Linked Immunosorbent Assay (ELISA), have become available in recent months (Shelver et al, 2007).

SPECIFIC METHODS:

Seepage Meters

Water fluxes through the sediment interface were measured directly using a seepage meter positioned near the shore. The seepage meter technique is cited by EPA as one of the best methods for this purpose (USEPA, 1988). Seepage meters followed the design of Lee (1977), with slight modifications (Figure 6). Each meter consisted of a 55 gallon steel drum cut to produce a hollow cylinder, open at one end, with a surface area of 0.29 m^2. A hole in the top of the meter was connected to a plastic collection (reservoir) bag by a polyethylene tube fitted through a rubber stopper

Meters were installed without a reservoir bag and left undisturbed for a minimum of one day prior to measurement, allowing time for the initial flow disturbance to subside and the meter to settle into a fixed position. When the meter was ready, a reservoir bag with one L of water was attached and the change in volume in the bag was determined over a defined time period. If a meter was not located during sampling a new one was inserted and measured the next day, if possible. Similarly, when bags were lost, measurements were sometimes repeated, if time permitted. The seepage inflow or outflow was measured in change in volume per square meter per hour (mL/m^2-hr). These units are dimensionally equivalent to units of millimeters per hour (mm/hr). Correction factors were applied to the data to correct for flow field disturbance and friction losses within the meter (Erickson, 1981; Cherkauer and McBride, 1988; Belanger and Montgomery, 1992).

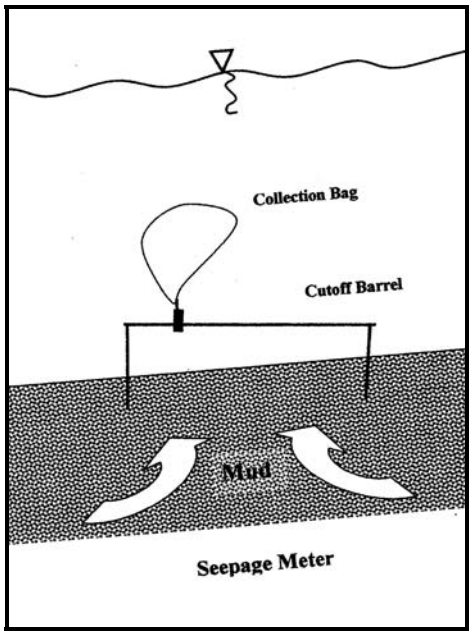


Figure 6. Diagram of a Seepage Meter.

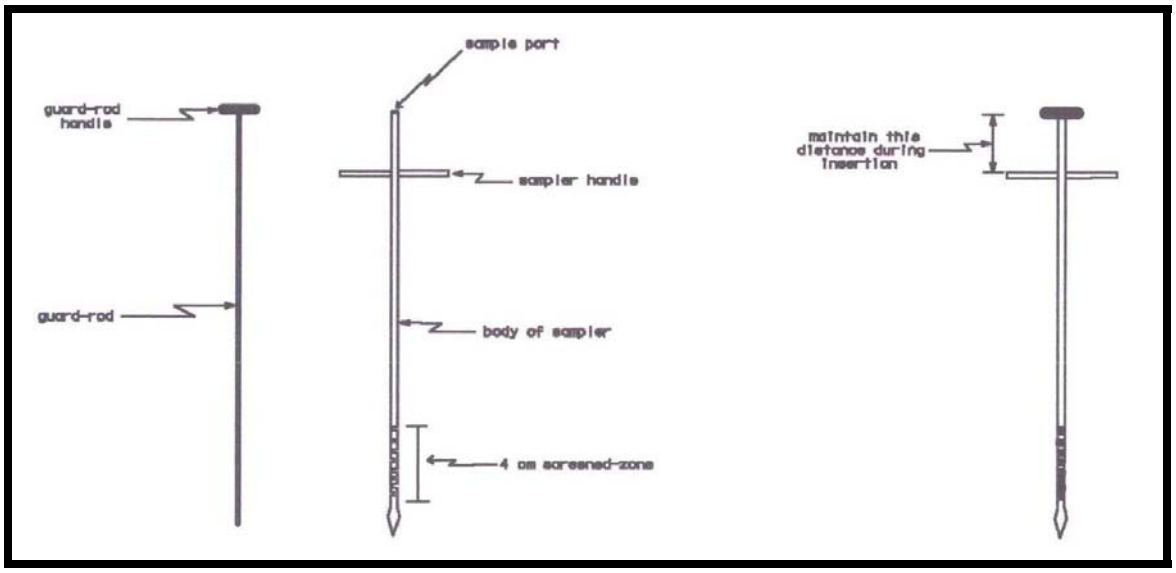
In Situ Piezometers

Shallow (2-5 ft) ¾ inch in situ piezometers were installed in the benthic sediment at nearshore sites (one per site) The piezometers had 1 ft screened intervals with 0.010 inch slot screen. The in situ river piezometers were installed by jetting in a 1 ¼ inch temporary casing outside the piezometer pipe with a 1½ h.p. Honda water pump connected to a 1 ¼ inch hose line. After the piezometers were allowed to settle and equilibrate for several days, the head difference between the surface water level (outside piezometer water level) and the groundwater (inside piezometer water level) was routinely measured (ΔH). The vertical hydraulic gradient can be obtained by dividing the ΔH by the depth of the screen below the sediment surface. The horizontal gradients were calculated by dividing the vertical difference in water level between two points (up-gradient and down-gradient piezometer) by the horizontal distance between the two piezometers or between a piezometer and the river water level. Both vertical and horizontal hydraulic gradient units are dimensionless (ft/ft).

PushPoint Samplers

The M.H.E. PushPoint sampling tool allowed us to rapidly and accurately locate and sample groundwater: in essence to map and track contaminated groundwater movement in the area down gradient from OSTDS. The PushPoint device is a very simple, precisely machined tool consisting of a tubular body fashioned with a screened zone at one end and a sampling port at the other (Figure 7). The bore of the PushPoint body is fitted with a guard rod that gives structural support to the PushPoint and prevents plugging and deformation of the screened zone during insertion into sediments. The screened-zone consists of a series of interlaced machined slots which form a short screened-zone with approximately 20% open area. The PushPoint is made of 316 stainless steel and comes in various lengths. In this study we primarily used 36 inch length and ¼ inch diameter PushPoints.

A.



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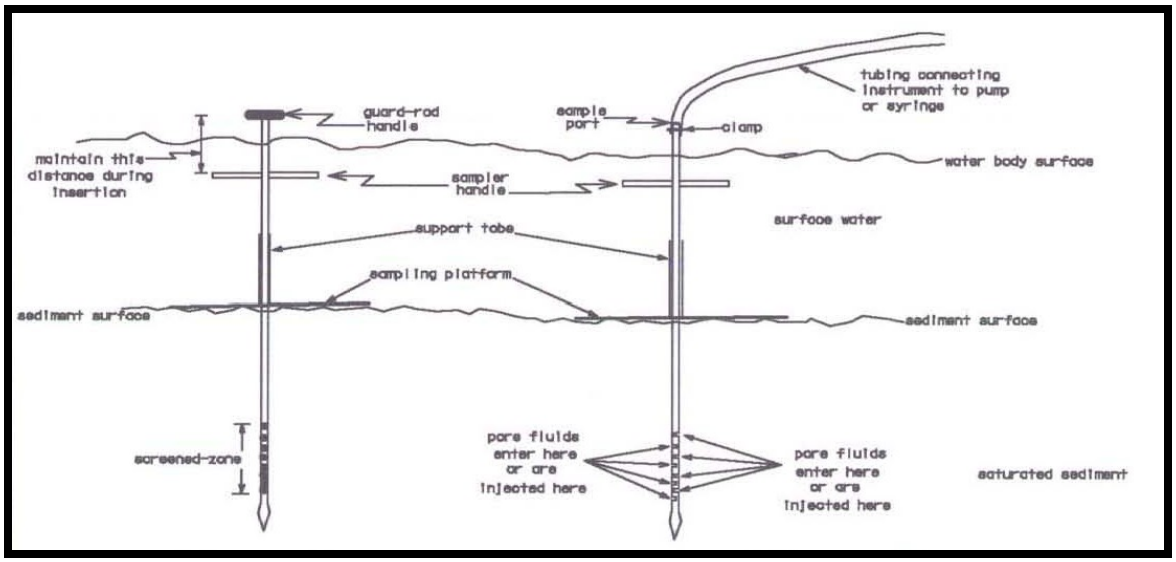

Figure 7. PushPoint Sampler Design (A) and Sampling Configuration (B).

The device is held in a manner that squeezes the two handles towards each other to maintain the guard-rod fully inserted in the PushPoint body during the insertion process. With the device held in this manner, the PushPoint was pushed into the sediment to the desired depth using a gentle twisting motion. When the desired depth was reached the guard-rod from the PushPoint body was removed without disturbing the position of the deployed sampler. A GeoPump peristaltic pump was attached to the PushPoint sample port via Tygon tubing and water was withdrawn at a low-flow sampling rate (50-200 ml/min.). The first 20-50 ml of groundwater is generally turbid and this "development" water is discarded. Once non-turbid aliquots have been withdrawn, representative samples are collected for on-site and off-site analysis. Several depths from the same location can be sampled by pulling the PushPoint up to a successively shallower sediment depth.

Water Quality Parameter and Chemical Indicator Sampling and Analysis

Sampling

Since the monitoring wells allow for an easy determination of the distance to the water table, groundwater samples were obtained by sinking the appropriate length (either 36", 48", or 72" long) ¼" inside diameter PushPoint samplers (Field Investigation Samplers – M.H.E. Products, Inc.) so as to extract water from the top one foot of the water table. Samples are then extracted with a peristaltic pump (GeoPump II – GeoPump, Inc.). At selected locations, samples from 0.5 and 1.5 ft. were obtained.

All samples were collected in 250 mL, polyethylene, Nalgene® bottles. Prior to use in the field, the bottles, sampling line, and PushPoint samplers were first washed with soap in hot tap water. After rinsing with hot tap water, the sample bottles were then triple rinsed in distilled, de-ionized water. Next, all sample bottles and PushPoint samplers were acid-washed in a solution of 10% HCl and allowed to dry thoroughly. In the field, the sampling line and individual PushPoints were cleaned between uses by flushing with approximately 50 mL each of 70% Isopropanol followed by 10% HCl and then DI water. Once pumping began and prior to the actual sample collection, several hundred milliliters were allowed to flow through the PushPoint and collection line to ensure any remaining Isopropanol and HCl were removed.

Surface water samples for nutrient analysis were taken as grab samples from one to two feet below the water's surface and directly down gradient from the septic drainfield. For surface water samples, it is important that the bottles are opened under water to avoid any potential contamination by surface films that may be present (Andresen et al, 2007). Samples for fecal coliform bacterial analysis were collected in sealed, sterile HDPE bottles containing sodium thiosulfate to neutralize any residual chlorine. All sample containers were immediately placed in a cooler on ice. The bacterial samples were delivered to the Harbor Branch Oceanographic Institute within three to four hours of collection for processing in accordance with current EPA standards. Bacterial concentrations were reported as the colony forming units (cfu) per 100 mL.

Nutrient Analysis

Analyses of Nitrogen as Ammonia (NH_4^+) were conducted in the Florida Tech Environmental Lab within 24 hours of collection. Analyses of soluble reactive phosphate (PO_4) and nitrogen as nitrite and nitrate (NO_x) were also conducted in the laboratory. If analyses could not be completed within 24 hours of collection the samples were frozen until such time the analyses could be completed.

All the aforementioned nutrient analyses were performed in accordance with current EPA standards and carried out using a CHEMetrics V-2000 Multi-Analyte Photometer (CHEMetrics, Inc.). Turbidity was also quantified in the laboratory using a Hach Turbidimeter. Specific Conductance, pH, and temperature were measured in the field at the time of collection using a multifunction meter (Myron Ultrameter II – Myron L. Company, Inc.)

FWA Analysis

Fluorescent whitening agent (FWA) detection was accomplished using a Turner Designs Model 10-AU Field Fluorometer configured with a daylight lamp, a 310-390 nm excitation filter, and a 436 nm (+/- 10 nm) emission filter . This particular emission filter has been specifically developed by Turner Designs to discriminate FWA fluorescence from the potentially interfering background fluorescence arising from the presence of Colored Dissolved Organic Matter (CDOM).

The blank used to calibrate the fluorometer was distilled, de-ionized (DI) water. DI water was also used to prepare the standard/reference solution. To prepare the standard solution, six common FWA-containing laundry detergents (Cheer®, Arm & Hammer®, Purex®, Tide®, Sunburst®, All®) were purchased and was combined in equal parts so as to represent an average FWA-containing detergent. Although exact formulas for these laundry detergents are proprietary information and the exact type and amount of FWA in each is unknown, FWA are estimated to comprise about 0.15% of laundry detergents (Sankararamakrishnan and Guo, 2005). Preliminary evaluations of this method indicate good results in both DI water and environmental waters to concentrations as low as 100 ppb. (See Figures 8 – 13 below).

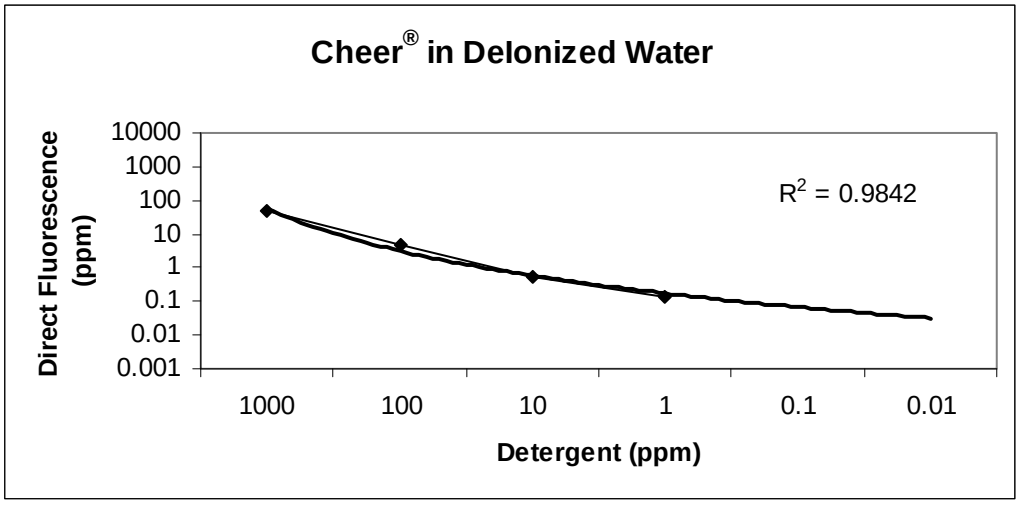

Figure 8. Fluorescence of a serial dilution of Cheer® in DI water

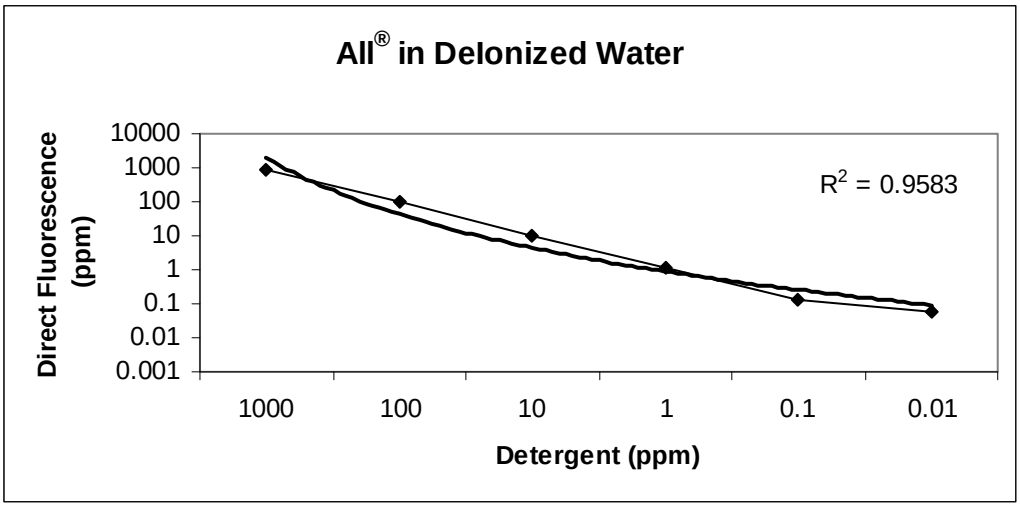

Figure 9. Fluorescence of a serial dilution of All® in DI water

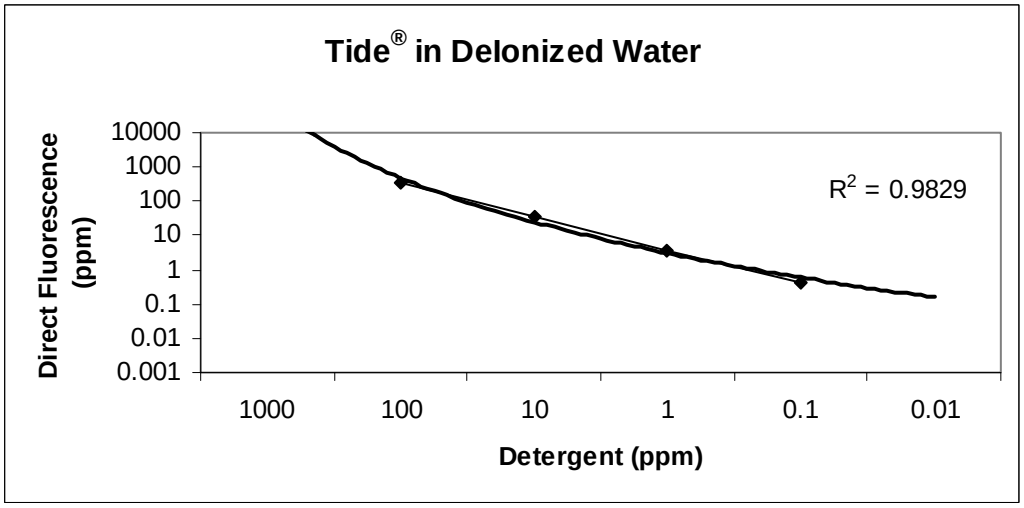

Figure 10. Fluorescence of a serial dilution of Tide® in DI water

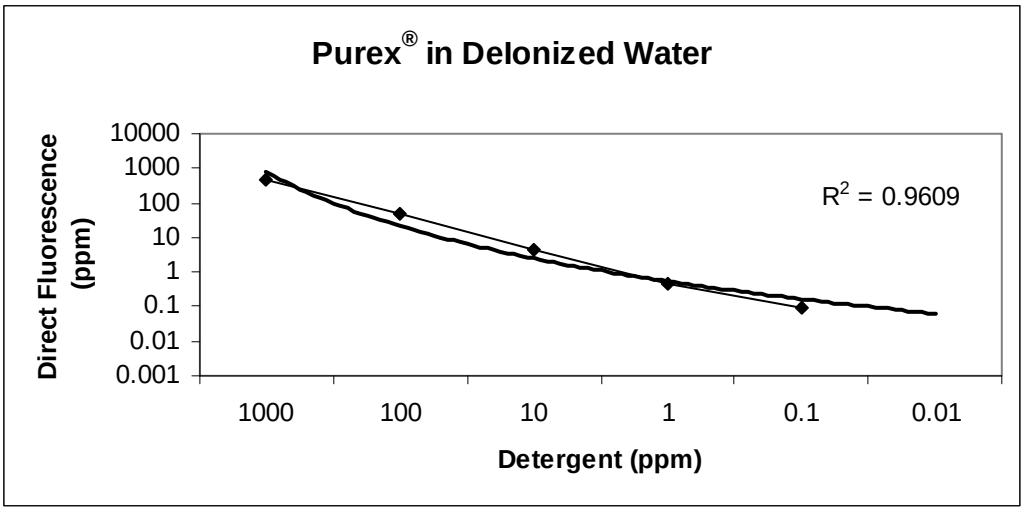

Figure 11. Fluorescence of a serial dilution of Purex® in DI water

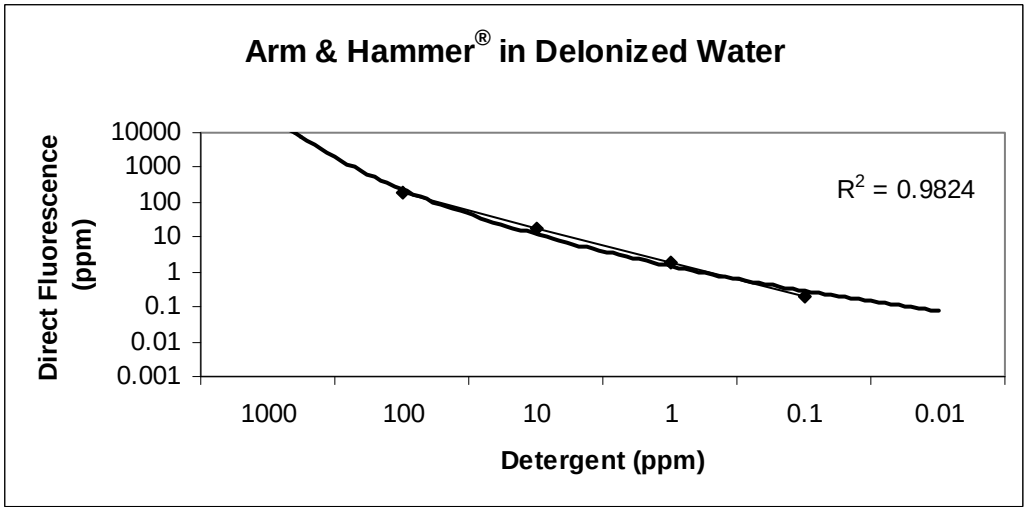

Figure 12. Fluorescence of a serial dilution of Arm&Hammer® in DI water

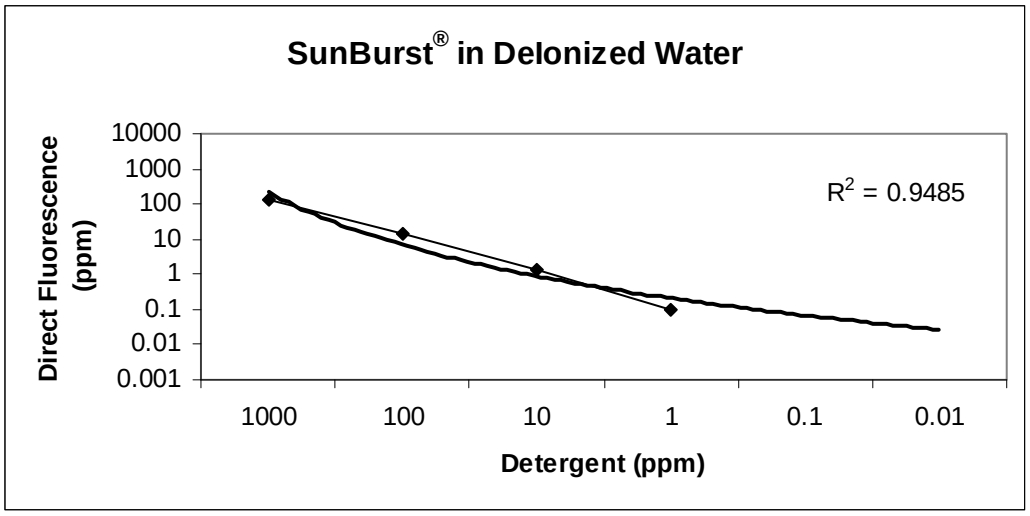

Figure 13. Fluorescence of a serial dilution of Sunburst® in DI water

CAF Analysis

Only 65 plant species worldwide produce caffeine (CAF) and of those only one, *Ilex vomitoria* Ait., is endemic to the southeastern United States and native to Florida (USDA-NRCS, 2009). This natural source of CAF does not appear to contribute significantly to the concentrations of CAF detected in the environment. Peeler et al (2006) determined that in Florida watersheds containing *Ilex vomitoria*, natural background CAF levels in streams and rivers were consistently below their detection limit of 5 ng•L^{-1}.

Human activity, however, is responsible for the addition of much CAF to the environment. The average cup of home-brewed coffee, for example, contains between 83 and 741 mg•L^{-1} CAF (Stavric et al, 1988). One study reported that CAF is among the most frequently detected contaminants in environmental waters with a frequency of occurrence of 70.6% (Kolpin et al, 2002). Though CAF is extensively metabolized with only approximately 3% of ingested CAF entering OSTDS via human urine (Tang-Liu et al, 1983), CAF containing beverages are often disposed of "down the drain" as waste and directly introduced to OSTDS. Therefore in environmental waters CAF only occurs at detectable levels if introduced by humans. Although there exist in the literature examples of the inadequacy of CAF as a predictor of human fecal contamination (Young et al, 2008), the presence of CAF in environmental waters has been shown to be a promising

predictor of human contamination (Peeler et al, 2006; Buerge et al, 2003; Ferreira et al, 2005; Chen et al, 2002; Seiler et al, 1999). In fact, when CAF occurs in groundwater coincident with pharmaceuticals and elevated nitrite concentrations, it is regarded as "clear unambiguous evidence" of domestic wastewater contamination (Seiler et al, 1999).

Determination of CAF most often relies on sophisticated laboratory techniques such as cartridge-based solid phase extractions and high performance liquid chromatography (Seiler et al, 1999; Hawthorne and Miller, 1992) and as such has not been conducive to field determinations. In the interest of moving toward a rapid and in situ assay for the presence of CAF, the procedure of Yang et al (1997) using Solid Phase Microextraction (SPME) followed by GC/MS analysis was modified for use with environmental samples and a preliminary investigation conducted as to its suitability for inclusion in this study.

SPME equipment was purchased from Supelco, Inc. and included sampling stand and puck, fiber holder for manual use, and 24 gauge StableFlex® polydimethylsiloxane/divinylbenzene fibers with a 65 μm core. These fibers were recommended by Supelco for extracting CAF as they are specifically designed for the extraction of non-volatile compounds of similar molecular weight in aqueous solutions.

The extraction begins by immersing a fiber in 4 mL of the sample for 5 minutes while stirring vigorously with a magnetic stir bar. Use of the sampling stand and puck is just one of many ways in which identical extraction conditions are ensured from sample to sample. Use of the Supelco fiber holder also ensures that the surface area of the exposed fiber remains the same from sample to sample.

An HP 5890 GC/MS equipped with a new, unpacked, splitless injection port liner and a 25m x 0.32 mm I.D. column was fitted with a Merlin MicroSeal® septum to allow the direct introduction of the fibers to the injection port. Thermal desorption occurs for one minute at 250° C. The temperature of the GC oven is then raised from 200° C to 275° C at a rate of 30° C•min^{-1} while the injector and transfer line remain at 250° C and 220° C respectively. The carrier gas is Helium flowing at a rate of 1.0 mL•min^{-1}.

Attempts were made at incrementally increasing the sample size (up to 100 mL) and the exposure time (as long as 1 hour) and still the lowest concentration successfully detected by this method was only 200 μg•L^{-1}. The purchase of additional fibers for

further experimentation is cost-prohibitive given the budgetary constraints of this study. Therefore this methodology was found to be not suitable in providing detection of CAF in environmental samples where concentrations are expected to be in the range of 80 - 100 ng•L^{-1}. For this reason an alternative methodology, ELISA, has been chosen to detect CAF in this study.

TCS Analysis

Like CAF, triclosan (TCS) is one of the most commonly detected organic wastewater contaminants (Kolpin et al, 2002). It has been found in environmental waters with a frequency of 57.6%, median concentrations of 0.14 *u*g•L^{-1} (~4.8 x 10^{-10} M) and maximum reported concentrations of 2.3 *u*g•L^{-1} (~7.9 x 10^{-9} M) (Kolpin et al, 2002).

TCS is produced under license from the Swiss-based chemical company Ciba. It has been used since the 1960s as a general antibacterial and antifungal (Levy et al., 1999. It is one of the most common micro-biocides in use today and is found in diverse applications and formulations (Adolfsson-Erici et al., 2002). Glaser (2004) has compiled a list of common consumer products containing TCS and these are listed in Appendix 1.

TCS is bacteriostatic for gram positive and gram-negative bacteria as well as molds, fungi, and yeast (McAvoy et al., 2002). Historically, it has been considered a "non-specific biocide" (McMurry et al., 1998). In the 1990s TCS's mechanism of action on microbial cells, specifically the site-specific binding mechanisms, was described and confirmed (McMurry et al., 1998; Levy et al., 1999). TCS inhibits lipid synthesis in the bacterial cell by blocking the ENR enzyme, enoyl-acyl reductase (McMurry, et al., 1998). Therefore TCS is a highly specific bacteriostatic and not a non-specific biocide. It prevents the bacterial cell from growing and dividing by preventing the construction of new cell membranes (Levy et al., 1999).

The high degree of efficacy for TCS as a biocide can be seen by a comparison. A TCS concentration of 0.24 μg/ml caused a 92% blockage of lipid synthesis while the commonly prescribed Ciprofloxacin® (a DNA synthesis inhibitor) caused 2% at a concentration of 0.045 μg/ml (McMurry, et al., 1998).

While some bacterial strains undergo cell lysis at significantly higher concentrations of TCS than that needed for inhibition of lipid synthesis, other strains of

bacteria can be inherently resistant to the effects of TCS due to a single amino acid substitution in the ENR enzyme (McMurry et al., 1998; Levy et al., 1999).

TCS traditionally has been determined in environmental samples by procedures utilizing a variety of extraction techniques coupled with gas or high performance liquid chromatography and mass spectrometry (McAvoy et al, 2002; Kolpin et al, 2002; Latch et al, 2003). Pemberton and Hart (1999) developed a detection method based on the electrochemical oxidation of TCS at a screen-printed carbon electrode using cyclic voltammetry. Though potentially useful in an industrial setting as a mechanism to gauge quality control of products containing high concentrations of TCS, this method lacks sufficient sensitivity for environmental sampling since the detection limit was determined to be 1.2×10^{-6} M, well below the median concentrations one would expect to find in the environment (Pemberton and Hart, 1999).

Martin et al (2001) developed a slightly less cumbersome method of TCS detection based on the differential bioluminescence of *Vibrio fischeri* pre- and post-exposure to TCS. However, this method also lacks sufficient sensitivity for environmental sampling since the reported detection limit is approximately 0.2% w/v, or approximately 7×10^{-3} M (Martin et al, 2001).

Song and Song (2007) developed a fluorescence method of detection that relies on the phototransformation of TCS to a light-emitting precursor in the presence of Fluorescein in alkaline medium whereby the chemiluminescence of TCS is triggered by N-Bromosuccinimide (NBS) and detected by a photomultiplier tube. The phototransformation to and activation of the chemiluminescent compound were carried out using micromolar quantities of reagent in a flow injection manifold. The manifold carries the reactants at a constant and controllable rate and delivers the reactants to a flow cell where they mix directly in front of the photomultiplier tube of a MP1-A Capillary Electrophoresis Luminescence Instrument (MP1-A) manufactured by X'ian Remax Electro-Science and Technology Co., Ltd. A schematic diagram of Song and Song's (2007) phototransformation and detection apparatus is presented in Figure 14.

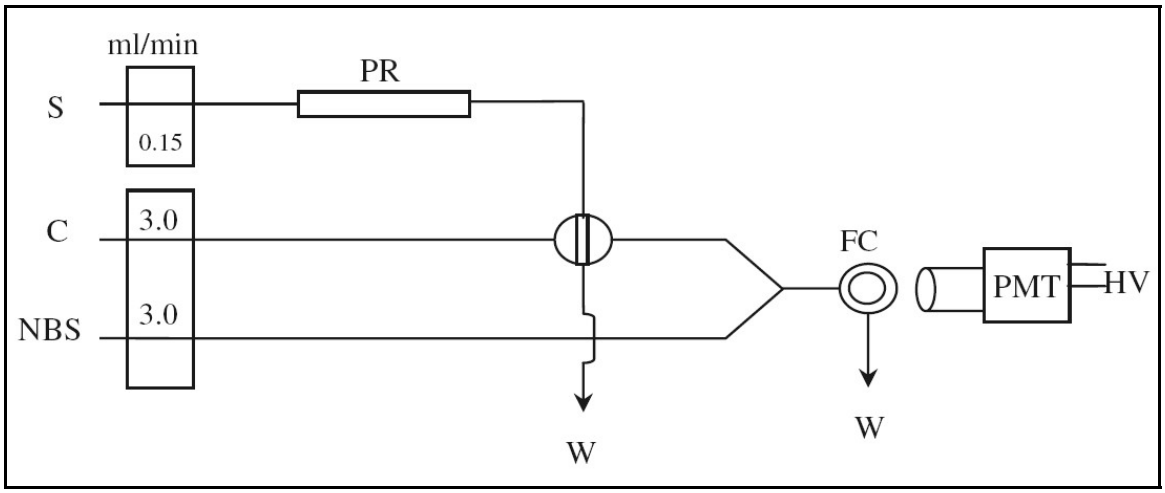

Figure 14. Diagram of an online phototransformation-flow injection chemiluminescent manifold. S=Sample, C=Carrier, NBS=n-Bromosuccinimide, PR=Photoreactor, W= waste, FC=Flow cell, PMT=Photomultiplier Tube, and HV=high voltage. (Song et al, 2007).

Song et al (2007) report that the entire process of phototransformation and detection could be accomplished in six minutes with a detection limit of 5.0×10^{-8} M. This detection limit is still not sufficient for the downstream concentrations documented by Kolpin et al (2002) but is close enough to be promising in detecting the higher concentrations of TCS that would be expected adjacent to a failed or failing OSTDS.

In the interest of moving toward a rapid and in situ assay for the presence of TCS, the method of Song et al (2007) was evaluated for adaptation to the field environment. Since the instrumentation used by Song et al (2007) is cost-prohibitive for this study (approximately $50,000) and is not suitable for use in a field environment, the applicability of the Turner Designs Model 10-AU Field Fluorometer was investigated since it is designed for field work and relies on the use of a photomultiplier tube in apparently the same way as the MP1-A instrument used by Song et al (2007).

Identical reagents were purchased and conventional laboratory equipment chosen to approximate as closely as possible the automated analytical procedures of phototransformation and detection. The photo-reactor was the only component of the apparatus used by Song et al (2007) that could be exactly reconstructed given budgetary constraints.

Reagents were mixed by hand using conventional laboratory glassware and equipment and then introduced to a clean glass cuvette in the fluorometer's sample compartment in the following manner: 0.15 mL of the standard or sample solution is passed through the photoreactor and added over the course of one minute to a glass cuvette while simultaneously adding 3.0 mL NBS over the same time period. The cap protecting the fluorometer's sample chamber was modified to allow the controlled introduction of the reagents without admitting any light to the sample compartment. The fluorometer was run with the light source switched off and no emission filter so as to allow any produced luminescence to reach the photomultiplier tube unimpeded.

To validate the new method, the fluorometer first was calibrated using DI water as a blank and a prepared standard solution of reagent grade TCS dissolved in DI water. Since TCS is poorly soluble in acidic water the pH was adjusted as necessary by dropwise addition of 0.1M NaOH until all TCS has dissolved. An empty cuvette functioned as the flow cell and was placed in the fluorometer's sample compartment. The reagents were simultaneously introduced at a constant rate of 0.15 $mL \bullet min^{-1}$ for the TCS standard and 3.0 $mL \bullet min^{-1}$ for NBS. A broad range of TCS concentrations were prepared from the serial dilution of the TCS standard and processed in hopes of producing a standard curve.

To our dismay, this methodology yielded no results and a curve could not be constructed. The most likely explanation for the negative result is that the reaction was proceeding so quickly as to exceed the fluorometer's ability to register and record a signal (J.C. Baum, Ph.D., Personal Communication, December 1, 2008). It is for this reason that an alternative methodology, ELISA, has been chosen to detect TCS in this study. This methodology study took considerable time and although dependable and accurate methods were eventually found, complete analyses (FWA, CAF, TCS) were completed at all NEP sites on only two dates.

CAF and TCS ELISA Analysis

Photolytic degradation of TCS (Aranami and Readman, 2007; Latch et al, 2003; Mezcua et al, 2004, Morrall et al, 2004; Lindstrom et al, 2002; Tixier et al, 2002) is a

serious concern and therefore great care is taken during collection and analysis to minimize exposure of the samples to direct light of any kind.

Preliminary investigation into the previously discussed detection methodologies for CAF and TCS found them to be either incompatible for use in a field environment or not sufficiently sensitive. Therefore an approach (Shelver, et al. 2007) that is less focused on rapidity and location and more focused on reliable and accurate detection was chosen.

Recently, the Biosciences Research Laboratory within the U.S. Department of Agriculture's Agricultural Research Service, in conjunction with a private company, Abraxis, LLC, brought to market a new Enzyme Linked Immunosorbent Assays (ELISA) for TCS (USDA-ARS, 2009). The CAF ELISA has been available from Abraxis for several years. Both ELISAs are available from Abraxis, LLC in the form of a kit complete with all necessary standards, controls, reagents, and an industry-standard 96-well microtiter plate.

Immunoassays generally function on a well-established principle of immunology, that is, the interaction and complexation of a particular antibody that has a known specificity for a particular antigen, in this case CAF and TCS are the antigens. ELISAs are distinguished from other immunoassays by a competitive reaction that occurs between the antigen, that may or may not be in the sample, and the enzyme-labeled antigen analog. Both antigen and enzyme-labeled analog compete for the antibody binding sites.

The CAF and TCS ELISAs both utilize a colorimetric/absorbance method to detect the presence of the antigen. After the competitive reaction occurred and the antigen complex was formed, the sample wells are washed and a color solution (an enzyme substrate of hydrogen peroxide and a chromogen of 3,3',5,5' tetramethylbenzidine) was added. The enzyme-labeled antigen-antibody complex catalyzes the conversion of the substrate/chromogen mixture to a colored product. After a 20 minute incubation period, the reaction was stopped by the addition of a dilute solution of sulfuric acid. Since the enzyme-labeled antigen conjugate was in competition for the antibody sites with the unlabeled antigens potentially in the samples, the intensity

of the color developed is inversely proportional to the concentration of antigen present in the sample.

The absorbance at 450 nm of the final solution in each well is measured using a Model 6+ Miniphotometer manufactured by Metertech, Inc. A dose-response curve was then generated by plotting the percent of relative absorbance of each sample (relative absorbance is calculated by dividing the sample's absorbance by that of the control), vs. concentration. In the TCS ELISA, the concentration of TCS was plotted on the x-axis (3-cycle log scale) and percent relative absorbance was plotted on the y-axis (logit scale). In the CAF ELISA, the concentration of CAF was plotted on the x-axis (3-cycle log scale) and percent relative absorbance was plotted on the y-axis (linear scale). By plotting the percent relative absorbances vs. the known concentrations of the standards, a curve was generated and the concentrations of antigen in the respective samples were interpolated.

Sediment Analyses

Percent sediment particle size analyses were completed on mixed auger sediment sample sat up gradient (near the drainfield) and down gradient (near the river) samples using standard sieving techniques, and silt/clay, very fine sand, fine sand, medium sand, coarse sand and granule or larger fractions were recorded. Percent organic matter (O.M.) was also determined by combusting the sample at 550 degrees C in a muffle furnace. Procedures are outlined by Belanger et al. (1993).

Field Measurements

Field water level measurements were made in the piezometers with a Herron "Little Dipper" water level measurement pressure transducer and used for horizontal and vertical gradient calculations. Specific conductance, temperature and pH were measured with a Myron Ultrameter that was frequently calibrated.

RESULTS AND DISCUSSION:

Hydraulic Gradients, Groundwater Seepage, Sediment, Rainfall, Field Data

Field and lab data collected from the various OSTDS sites on four dates between 1/3/09 and 6/23/09, sites are shown in Tables 1 through 4. Groundwater temperatures ranged from 20.7 to 31.4 degrees C during the study. The surface water specific conductance values were related to the proximity of the individual sites to inlets, averaging 3,966, 50,346 and 50,106 uS, respectively, for the Huy, Grimes and Lounibos sites. The higher conductance values at Grimes and Lounibos sites are a result of their close proximity to the Ft. Pierce inlet (higher salinity) and lack any fresh surface water inputs. Groundwater specific conductance levels at the Ft. Pierce sites reflected these facts, also.

Table 1: Field and Lab Data for Ground and Surface Water - January 2, 2009											
Sample ID	Physical Parameters				Nutrient Analyses			Chemical Indicators			Bacteria
	pH	Conductivity	Turbidity	Temp	Nox-N	NH4-N	SRP(as PO4)	Triclosan	Caffeine	Fluorescence	Coliform
	---	(uS)	(ntu)	(deg C)	(mg/L)	(mg/L)	(mg/L)	(ppb)	(ppb)	(ug/L)	(cfu/100mL)
Huy Residence											
P1	7.37	331.1	39	---	0.32	0.45	3.71	---	---	---	---
P2	6.54	2700.0	15	---	0.47	0.79	2.25	---	---	---	---
P3	6.37	3387.0	23	---	0.48	1.10	2.32	---	---	---	---
P4	6.13	2614.0	16	---	0.60	0.55	1.64	---	---	---	---
P5	7.42	468.2	5	---	0.22	0.17	1.65	---	---	---	---
P6	6.83	606.7	20	---	>9.00	0.93	14.94	---	---	---	---
P7	6.58	2219.0	40	---	0.25	0.93	1.46	---	---	---	---
P8	6.72	2614.0	33	---	0.34	1.11	2.22	---	---	---	---
P9	7.23	1054.0	16	---	0.14	0.57	2.19	---	---	---	---
P10	7.26	444.5	17	---	0.22	0.50	3.92	---	---	---	---
P11	7.38	494.9	41	---	1.87	0.46	7.72	---	---	---	---
P12	7.20	291.6	41	---	0.15	0.44	7.82	---	---	---	---
P13	6.93	2525.0	>100	---	0.13	0.57	2.89	---	---	---	---
P14	7.09	475.1	85	---	0.10	0.26	5.11	---	---	---	---
P15	7.03	1020.0	57	---	0.12	0.54	3.08	---	---	---	---
P16	7.17	540.6	7	---	0.17	0.53	3.42	---	---	---	---
P17	7.06	567.4	37	---	0.17	0.81	4.62	---	---	---	---
P18	6.74	---	25	---	0.13	0.24	5.93	---	---	---	---
P19	7.08	942.7	5	---	0.16	0.90	4.03	---	---	---	---
P20	7.25	507.8	11	---	0.17	1.02	4.14	---	---	---	---
P21	7.06	591.8	9	---	0.11	0.57	5.21	---	---	---	---
SW	8.41	4201.0	17	---	0.37	1.30	0.53	---	---	---	---

Sample ID	Physical Parameters				Nutrient Analyses			Chemical Indicators			Bacteria
	pH	Conductivity	Turbidity	Temp	Nox-N	NH4-N	SRP(as PO4)	Triclosan	Caffeine	Fluorescence	Coliform
	---	(uS)	(ntu)	(deg C)	(mg/L)	(mg/L)	(mg/L)	(ppb)	(ppb)	(ug/L)	(cfu/100mL)
Grimes Residence											
P1	---	---	---	---	---	---	---	---	---	---	---
P2	7.62	1446.0	8	---	0.35	2.23	2.30	---	---	---	---
P3	7.58	587.6	15	---	0.24	0.00	0.76	---	---	---	---
P4	7.31	2342.0	11	---	0.30	1.01	0.74	---	---	---	---
P5	7.38	2324.0	8	---	9.60	0.69	2.97	---	---	---	---
P6	7.56	1878.0	9	---	5.49	0.63	6.57	---	---	---	---
P7	7.54	1709.0	9	---	8.43	0.65	3.74	---	---	---	---
P8	7.80	1751.0	10	---	2.02	0.00	5.43	---	---	---	---
P9	6.87	1899.0	7	---	0.25	1.05	1.50	---	---	---	---
P10	7.06	1540.0	15	---	0.51	1.53	6.36	---	---	---	---
P11	7.23	1679.0	7	---	0.41	0.25	6.98	---	---	---	---
P12	7.44	1596.0	5	---	0.41	0.95	5.35	---	---	---	---
P13	7.24	1323.0	4	---	0.19	0.94	5.25	---	---	---	---
P14	6.83	2726.0	7	---	0.37	1.97	0.84	---	---	---	---
P15	7.10	2285.0	8	---	0.12	2.06	4.28	---	---	---	---
P16	7.14	1526.0	10	---	0.11	1.57	5.99	---	---	---	---
P17	7.37	1288.0	6	---	0.11	1.04	7.05	---	---	---	---
P18	7.14	2082.0	11	---	0.19	1.94	3.49	---	---	---	---
P19	7.48	1555.0	15	---	0.20	1.07	4.56	---	---	---	---
P20	7.24	1365.0	14	---	0.15	1.08	5.27	---	---	---	---
P21	7.21	2641.0	28	---	0.21	1.56	1.40	---	---	---	---
P22	7.12	2588.0	17	---	0.16	2.24	2.11	---	---	---	---
P23	7.44	4463.0	---	---	1.30	4.23	0.02	---	---	---	---
P24	6.91	3986.0	13	---	1.04	3.72	2.65	---	---	---	---
SW	7.80	48820.0	5	---	0.92	0.00	0.01	---	---	---	---

Sample ID	Physical Parameters				Nutrient Analyses			Chemical Indicators			Bacteria
	pH	Conductivity	Turbidity	Temp	Nox-N	NH4-N	SRP(as PO4)	Triclosan	Caffeine	Fluorescence	Coliform
	---	(uS)	(ntu)	(deg C)	(mg/L)	(mg/L)	(mg/L)	(ppb)	(ppb)	(ug/L)	(cfu/100mL)
Lounibos Residence											
P1	6.90	2472.0	25	---	0.52	3.03	0.87	---	---	---	---
P2	7.04	4628.0	5	---	0.93	3.89	2.09	---	---	---	---
P3	6.95	5391.0	4	---	0.71	3.99	2240.00	---	---	---	---
P4	7.18	5051.0	5	---	1.19	3.40	3.52	---	---	---	---
P5	6.94	2518.0	16	---	0.56	2.27	0.99	---	---	---	---
P6	7.32	3414.0	15	---	0.44	2.63	1.80	---	---	---	---
P7	7.47	3576.0	9	---	1.80	1.70	6.10	---	---	---	---
P8	7.06	5766.0	6	---	1.45	4.90	3.52	---	---	---	---
P9	7.25	711.3	15	---	0.17	1.32	1.46	---	---	---	---
P10	6.77	2851.0	10	---	0.58	2.59	0.89	---	---	---	---
P11	6.76	2848.0	45	---	0.64	2.48	1.18	---	---	---	---
P12	7.20	2043.0	14	---	0.30	1.26	5.77	---	---	---	---
P13	7.04	1012.0	10	---	0.15	1.58	3.55	---	---	---	---
P14	6.77	4428.0	10	---	0.80	3.61	0.75	---	---	---	---
P15	6.81	3463.0	9	---	0.53	2.57	1.25	---	---	---	---
P16	6.67	3628.0	6	---	0.72	3.23	1.71	---	---	---	---
P17	6.79	4860.0	9	---	0.58	3.59	4.05	---	---	---	---
P18	6.83	6346.0	5	---	0.98	4.12	4.61	---	---	---	---
SW	8.03	49010.0	6	---	0.37	0.21	0.12	---	---	---	---

Table 2: Field and Lab Data for Ground and Surface Water - March 23, 2009											
Sample ID	Physical Parameters				Nutrient Analyses			Chemical Indicators			Bacteria
	pH	Conductivity	Turbidity	Temp	Nox-N	NH4-N	SRP(as PO4)	Triclosan	Caffeine	Fluorescence	Coliform
	---	(uS)	(ntu)	(deg C)	(mg/L)	(mg/L)	(mg/L)	(ppb)	(ppb)	(ug/L)	(cfu/100mL)
Huy Residence											
P1	---	---	---	---	---	---	---	---	---	---	---
P2	---	---	---	---	---	---	---	---	---	---	---
P3	---	---	---	---	---	---	---	---	---	---	---
P4	---	---	---	---	---	---	---	---	---	---	---
P5	---	---	---	---	---	---	---	---	---	---	---
P6	---	---	---	---	---	---	---	---	---	---	---
P7	---	---	---	---	---	---	---	---	---	---	---
P8	---	---	---	---	---	---	---	---	---	---	---
P9	6.61	4408.0	3	20.6	0.28	0.95	1.68	---	---	---	1
P10	7.18	1900.0	36	20.4	0.13	0.25	4.57	---	---	---	1
P11	7.02	968.8	14	20.2	8.54	0.25	6.93	---	---	---	1
P12	6.66	950.1	30	21.5	8.49	0.44	5.02	---	---	---	1
P13	6.95	1085.0	39	21.3	2.17	0.31	3.09	---	---	---	1
P14	7.44	496.0	26	21.4	0.20	0.17	1.51	---	---	---	1
P15	6.50	4307.0	6	20.8	0.91	0.65	0.40	---	---	---	1
P16 Deep	6.73	2730.0	14	21.2	0.59	0.65	2.81	---	---	---	1
P16 Shallow	6.51	3850.0	37	---	0.94	0.56	1.11	---	---	---	1
P17 Deep	6.71	542.0	55	21.3	0.54	0.21	0.11	---	---	---	1
P17 Shallow	6.95	398.7	14	---	0.21	0.09	3.28	---	---	---	1
P18	6.45		18	20.2	0.23	0.58	14.40	---	---	---	1
P18A	6.75	593.9	23	21.5	0.42	0.27	3.41	---	---	---	1
P18B	6.57	1745.0	16	21.2	0.21	0.35	0.82	---	---	---	1
P19	6.89	2170.0	3	21.0	0.30	0.34	1.98	---	---	---	1
P20	6.78	574.0	6	21.2	0.34	0.55	2.30	---	---	---	1
P21	6.93	571.0	7	21.0	0.19	0.29	2.46	---	---	---	10
P22	7.15	770.0	14	20.7	0.17	0.44	4.11	---	---	---	1
SW	7.05	3966.0	3	20.4	0.29	1.07	0.29	---	---	---	1300

Sample ID	Physical Parameters				Nutrient Analyses			Chemical Indicators			Bacteria
	pH	Conductivity	Turbidity	Temp	Nox-N	NH4-N	SRP(as PO4)	Triclosan	Caffeine	Fluorescence	Coliform
	---	(uS)	(ntu)	(deg C)	(mg/L)	(mg/L)	(mg/L)	(ppb)	(ppb)	(ug/L)	(cfu/100mL)
Grimes Residence											
P1	---	---	---	---	---	---	---	---	---	---	---
P2	---	---	---	---	---	---	---	---	---	---	---
P3	---	---	---	---	---	---	---	---	---	---	---
P4	7.24	2578.0	9	22.5	9.24	0.22	3.86	---	---	---	1
P5	7.02	2594.0	9	22.0	13.91	0.30	5.94	---	---	---	1
P6	7.29	2570.0	10	24.0	>15.00	0.28	1.55	---	---	---	1
P7	7.22	2313.0	10	25.6	8.07	0.38	1.27	---	---	---	1
P8	7.29	1350.0	9	24.0	0.21	0.21	4.23	---	---	---	1
P9	7.05	2542.0	5	22.3	2.39	0.32	0.78	---	---	---	1
P10	7.15	2031.0	6	21.4	0.15	0.54	6.12	---	---	---	1
P11	7.14	2360.0	4	24.6	>6.00	0.21	6.57	---	---	---	1
P12	7.25	1840.0	14	24.8	0.68	0.75	6.96	---	---	---	1
P13	7.11	1277.0	5	22.2	0.54	0.47	6.66	---	---	---	1
P14	7.14	1781.0	4	23.5	0.42	0.93	1.22	---	---	---	1
P15	6.75	1721.0	3	22.5	0.46	1.10	2.06	---	---	---	1
P16	7.11	1702.0	14	22.8	0.23	0.83	6.74	---	---	---	1
P17	7.04	1606.0	10	22.8	0.31	0.70	8.25	---	---	---	1
P18	7.12	1820.0	4	23.5	0.19	1.59	5.36	---	---	---	1
P19	7.05	1434.0	5	23.0	0.29	0.78	6.53	---	---	---	1
P20	7.03	1393.0	20	23.2	0.20	1.30	3.39	---	---	---	1
P21	7.04	3850.0	5	22.7	0.40	2.11	1.62	---	---	---	1
P22	7.22	5784.0	11	22.3	0.67	1.58	2.92	---	---	---	1
P23	7.08	3540.0	17	22.6	0.33	2.90	3.86	---	---	---	10
P24	6.94	5125.0	4	22.4	0.53	2.39	2.10	---	---	---	3400
SW	8.08	51020.0	2	22.9	0.85	0.01	0.25	---	---	---	100

Sample ID	Physical Parameters				Nutrient Analyses			Chemical Indicators			Bacteria
	pH	Conductivity	Turbidity	Temp	Nox-N	NH4-N	SRP(as PO4)	Triclosan	Caffeine	Fluorescence	Coliform
	---	(uS)	(ntu)	(deg C)	(mg/L)	(mg/L)	(mg/L)	(ppb)	(ppb)	(ug/L)	(cfu/100mL)
Lounibos Residence											
P1	6.66	3658.0	15	24.0	1.58	4.47	0.48	---	---	---	1
P2	6.96	2518.0	6	24.4	1.33	3.37	1.74	---	---	---	1
P3	7.01	4732.0	9	24.2	1.05	4.36	2.15	---	---	---	1
P4	7.08	5161.0	5	24.4	2.39	3.38	2.98	---	---	---	100
P5	6.66	3411.0	3	23.5	0.95	2.87	1.12	---	---	---	1
P6	6.92	1586.0	6	23.5	0.19	1.24	0.95	---	---	---	1
P7	7.24	2299.0	25	23.3	0.22	1.68	2.94	---	---	---	1
P8	7.02	5108.0	2	23.7	1.82	3.49	0.15	---	---	---	1
P9	7.18	604.1	4	22.6	0.83	0.60	0.68	---	---	---	1
P10	6.89	4973.0	5	22.1	1.82	3.51	1.11	---	---	---	1
P11	6.80	3205.0	7	21.8	2.79	2.58	3.40	---	---	---	1
P12	6.76	2333.0	5	21.6	1.39	2.19	2.00	---	---	---	1
P13	6.92	5130.0	3	21.9	1.09	3.43	3.14	---	---	---	1
P14	6.74	6395.0	7	22.0	2.56	4.57	1.02	---	---	---	1
P15	6.75	6674.0	5	22.3	0.93	5.49	0.89	---	---	---	1
P16	6.68	5503.0	7	21.7	1.57	5.61	0.09	---	---	---	1
P17	6.80	4394.0	3	21.0	3.27	3.31	1.14	---	---	---	1
P18	6.92	4794.0	5	20.7	1.28	3.47	2.63	---	---	---	1
SW	8.30	49860.0	4	23.5	0.98	0.00	3.66	---	---	---	10

Table 3: Field and Lab Data for Ground and Surface Water - June 23, 2009											
Sample ID	Physical Parameters				Nutrient Analyses			Chemical Indicators			Bacteria
	pH	Conductivity	Turbidity	Temp	Nox-N	NH4-N	SRP(as PO4)	Triclosan	Caffeine	Fluorescence	Coliform
	---	(uS)	(ntu)	(deg C)	(mg/L)	(mg/L)	(mg/L)	(ppb)	(ppb)	(ug/L)	(cfu/100mL)
Huy Residence											
P1	---	---	---	---	---	---	---	---	---	---	---
P2	---	---	---	---	---	---	---	---	---	---	---
P3	---	---	---	---	---	---	---	---	---	---	---
P4	---	---	---	---	---	---	---	---	---	---	---
P5	---	---	---	---	---	---	---	---	---	---	---
P6	---	---	---	---	---	---	---	---	---	---	---
P7	---	---	---	---	---	---	---	---	---	---	---
P8	---	---	---	---	---	---	---	---	---	---	---
P9	6.98	2233.0	10	26.9	0.07	0.72	2.70	---	---	---	1
P10	7.01	1820.0	10	26.9	0.09	0.50	1.67	---	---	---	1
P11	6.97	1469.0	14	26.6	0.06	0.29	2.65	---	---	---	1
P12	7.39	639.9	9	27.0	0.73	0.37	4.81	< 0.02	3.00	10.50	1
P13	6.99	775.9	27	26.6	0.63	0.30	3.12	---	---	---	1
P14	6.76	1094.0	15	26.3	2.30	0.15	1.75	---	---	---	1
P14A	7.22	884.1	8	26.4	0.14	0.07	0.53	---	---	---	1
P15	---	---	---	---	---	---	---	---	---	---	---
P16 Deep	6.48	3854.0	15	27.0	0.08	0.98	5.75	---	---	---	1
P16 Shallow	---	---	---	---	---	---	---	---	---	---	---
P17 Deep	6.62	1757.0	5	27.4	0.13	0.81	0.57	---	---	---	1
P17 Shallow	6.77	1374.0	17	27.6	0.08	0.22	3.55	---	---	---	1
P18 Deep	---	---	19	---	0.06	0.16	0.08	< 0.02	1.30	9.45	1
P18 Shallow	---	---	12	---	0.62	0.48	2.09	---	---	---	1
P18A	6.59	1048.0	9	28.5	0.06	0.50	4.34	---	---	---	1
P18B	---	---	---	---	---	---	---	---	---	---	---
P19	6.68	1591.0	5	27.5	0.05	0.31	1.17	---	---	---	1
P20	6.57	2234.0	2	27.2	0.10	0.56	4.66		---	---	1
P21	---	---	20	---	0.08	0.65	5.69	< 0.02	< 0.175	17.40	1
P22	---	---	5	---	0.07	0.10	2.70	---	---	---	1
SW	---	---	6	---	0.08	0.68	0.09	< 0.02	< 0.175	30.00	100

Sample ID	Physical Parameters				Nutrient Analyses			Chemical Indicators			Bacteria
	pH	Conductivity	Turbidity	Temp	Nox-N	NH4-N	SRP(as PO4)	Triclosan	Caffeine	Fluorescence	Coliform
	---	(uS)	(ntu)	(deg C)	(mg/L)	(mg/L)	(mg/L)	(ppb)	(ppb)	(ug/L)	(cfu/100mL)
Grimes Residence											
P1	---	---	---	---	---	---	---	---	---	---	---
P2	7.82	642.5	40	31.4	0.10	1.09	0.83	---	---	---	1
P3	---	---	---	---	---	---	---	---	---	---	---
P4	7.16	2402.0	5	30.3	1.55	0.38	4.18	---	---	---	5
P5	7.07	3236.0	3	30.0	14.18	0.42	5.55	< 0.02	< 0.175	35.25	1
P6 Deep	7.14	3050.0	3	29.7	16.48	0.33	5.32	---		---	1
P6 Shallow	7.42	4171.0	5	30.1	9.08	0.60	5.78	---	---	---	1
P7	7.38	3197.0	3	30.4	>9	1.12	0.74	---	---	---	1
P8	7.20	3337.0	4	30.1	17.40	0.63	4.16	< 0.02	< 0.175	31.50	1
P9	7.06	2776.0	5	29.9	3.57	0.42	1.29	---	---	---	1
P10	7.14	2570.0	4	31.0	9.81	0.46	4.26	---	---	---	1
P11	7.19	2441.0	5	29.7	6.47	0.41	6.24	---	---	---	1
P12	7.38	2554.0	2	30.3	>9	0.40	4.49	---	---	---	3
P13	7.32	2068.0	7	29.8	0.10	1.08	3.09	---	---	---	1
P14	7.07	2953.0	6	29.4	0.08	1.32	0.70	---	---	---	1
P15	6.85	4181.0	5	29.9	0.08	1.09	1.89	---	---	---	1
P16	6.93	3824.0	5	29.7	0.17	1.11	3.40	---	---	---	1
P17	7.20	2393.0	15	29.7	0.10	0.73	4.55	---	---	---	1
P18 Deep	7.01	2146.0	5	29.8	0.07	1.30	5.34	---	---	---	3
P18 Shallow	7.16	2544.0	15	29.6	0.08	0.83	5.06	< 0.02	2.00	34.65	1
P19	7.24	2785.0	3	30.2	0.09	1.45	3.17	---	---	---	1
P20	7.25	1530.0	5	30.1	0.07	1.79	3.94	---	---	---	1
P21	---	---	---	---	---	---	---	---	---	---	---
P22	7.05	2076.0	25	29.4	0.08	0.00	0.76	---	---	---	1
P22A	7.00	6060.0	4	30.3	0.09	1.68	1.14	---	---	---	1
P23	6.85	2710.0	9	28.9	0.14	1.49	1.22	---	---	---	1
P24	7.01	2830.0	2	30.3	0.11	1.75	2.36	---	---	---	1
SW	7.89	51200.0	6	30.1	0.13	0.03	0.06	< 0.02	< 0.175	1.50	1

Sample ID	Physical Parameters				Nutrient Analyses			Chemical Indicators			Bacteria
	pH	Conductivity	Turbidity	Temp	Nox-N	NH4-N	SRP(as PO4)	Triclosan	Caffeine	Fluorescence	Coliform
	---	(uS)	(ntu)	(deg C)	(mg/L)	(mg/L)	(mg/L)	(ppb)	(ppb)	(ug/L)	(cfu/100mL)
Lounibos Residence											
P1	6.75	3425.0	1	29.7	0.11	2.48	0.39	---	---	---	1
P2	---	---	---	---	---	---	---	---	---	---	---
P3	7.30	3006.0	2	30.2	0.11	1.55	21.69	---	---	---	1
P4	7.05	1134.0	5	30.0	0.12	1.49	6.94	< 0.02	2.40	54.60	1
P5	---	---	---	---	---	---	---	---	---	---	---
P6	---	---	---	---	---	---	---	---	---	---	---
P7	---	---	---	---	---	---	---	---	---	---	---
P8	7.30	619.7	4	29.0	0.15	1.06	7.63	0.03	1.54	49.65	1
P9	---	---	---	---	---	---	---	---	---	---	---
P10	6.95	3528.0	2	28.2	0.12	1.14	0.62	---	---	---	1
P11	---	---	---	---	---	---	---	---	---	---	---
P12	7.29	3033.0	2	28.2	0.21	1.53	1.30	---	---	---	1
P13	7.05	837.0	3	28.1	0.10	0.83	1.69	---	---	---	1
P14	---	---	---	---	---	---	---	---	---	---	---
P15	---	---	---	---	---	---	---	---	---	---	---
P16	7.06	3269.0	5	28.3	0.10	1.23	3.29	---	---	---	1
P17	7.16	4980.0	2	29.2	0.10	1.88	0.63	---	---	---	100
P18	7.24	5780.0	5	29.6	0.02	2.53	5.31	0.03	> 5.00	79.50	1
SW	8.22	51450.0	3	30.9	0.00	0.08	0.03	0.07	< 0.175	0.60	33

Table 4: Field and Lab Data for Ground and Surface Water - August 6, 2009											
Sample ID	Physical Parameters				Nutrient Analyses			Chemical Indicators			Bacteria
	pH	Conductivity	Turbidity	Temp	Nox-N	NH4-N	SRP(as PO4)	Triclosan	Caffeine	Fluorescence	Coliform
	---	(uS)	(ntu)	(deg C)	(mg/L)	(mg/L)	(mg/L)	(ppb)	(ppb)	(ug/L)	(cfu/100mL)
Huy Residence											
Pz1	---	---	---	---	---	---	---	0.19	0.19	15.50	1
Pz2	---	---	---	---	---	---	---	0.16	< 0.175	19.30	100
P1	---	---	---	---	---	---	---	---	---	---	---
P2	---	---	---	---	---	---	---	---	---	---	---
P3	---	---	---	---	---	---	---	---	---	---	---
P4	---	---	---	---	---	---	---	---	---	---	---
P5	---	---	---	---	---	---	---	---	---	---	---
P6	---	---	---	---	---	---	---	---	---	---	---
P7	---	---	---	---	---	---	---	---	---	---	---
P8	---	---	---	---	---	---	---	---	---	---	---
P9	---	---	---	---	---	---	---	---	---	---	---
P10	---	---	---	---	---	---	---	---	---	---	---
P11	---	---	---	---	---	---	---	0.04	0.21	29.80	1
P12	---	---	---	---	---	---	---	---	---	---	---
P13	---	---	---	---	---	---	---	0.09	0.24	20.00	1
P14	---	---	---	---	---	---	---	---	---	---	---
P14A	---	---	---	---	---	---	---	---	---	---	---
P15	---	---	---	---	---	---	---	0.11	< 0.175	12.60	1
P16 Deep	---	---	---	---	---	---	---	---	---	---	---
P16 Shallow	---	---	---	---	---	---	---	---	---	---	---
P17 Deep	---	---	---	---	---	---	---	---	---	---	---
P17 Shallow	---	---	---	---	---	---	---	---	---	---	---
P18 Deep	---	---	---	---	---	---	---	0.11	< 0.175	11.30	1
P18 Shallow	---	---	---	---	---	---	---	---	---	---	---
P18A	---	---	---	---	---	---	---	0.15	< 0.175	9.49	1
P18B	---	---	---	---	---	---	---	---	---	---	---
P19	---	---	---	---	---	---	---	---	---	---	---
P20	---	---	---	---	---	---	---	---	---	---	---
P21	---	---	---	---	---	---	---	---	---	---	---
P22	---	---	---	---	---	---	---	---	---	---	---
SW	---	---	---	---	---	---	---	0.13	0.18	27.30	100

Sample ID	Physical Parameters				Nutrient Analyses			Chemical Indicators			Bacteria
	pH	Conductivity	Turbidity	Temp	Nox-N	NH4-N	SRP(as PO4)	Triclosan	Caffeine	Fluorescence	Coliform
	---	(uS)	(ntu)	(deg C)	(mg/L)	(mg/L)	(mg/L)	(ppb)	(ppb)	(ug/L)	(cfu/100mL)
Grimes Residence											
P1	---	---	---	---	---	---	---	---	---	---	---
P2	---	---	---	---	---	---	---	---	---	---	---
P3	---	---	---	---	---	---	---	---	---	---	---
P4	---	---	---	---	---	---	---	0.04	< 0.175	38.10	1
P5	---	---	---	---	---	---	---	0.06	1.20	34.90	1
P6 Deep	---	---	---	---	---	---	---	0.03	0.45	38.20	1
P6 Shallow	---	---	---	---	---	---	---	---	---	---	---
P7	---	---	---	---	---	---	---	0.11	1.25	39.00	1
P8	---	---	---	---	---	---	---	---	---	---	---
P9	---	---	---	---	---	---	---	---	---	---	---
P10	---	---	---	---	---	---	---	---	---	---	---
P11	---	---	---	---	---	---	---	---	---	---	---
P12	---	---	---	---	---	---	---	---	---	---	---
P13	---	---	---	---	---	---	---	---	---	---	---
P14	---	---	---	---	---	---	---	---	---	---	---
P15	---	---	---	---	---	---	---	---	---	---	---
P16	---	---	---	---	---	---	---	---	---	---	---
P17	---	---	---	---	---	---	---	---	---	---	---
P18 Deep	---	---	---	---	---	---	---	---	---	---	---
P18 Shallow	---	---	---	---	---	---	---	---	---	---	---
P19	---	---	---	---	---	---	---	---	---	---	---
P20	---	---	---	---	---	---	---	---	---	---	---
P21	---	---	---	---	---	---	---	---	---	---	---
P22	---	---	---	---	---	---	---	0.05	0.70	52.70	1
P22A	---	---	---	---	---	---	---	---	---	---	---
P23	---	---	---	---	---	---	---	---	---	---	---
P24	---	---	---	---	---	---	---	0.27	1.25	76.10	100
SW	---	---	---	---	---	---	---	0.02	< 0.175	6.78	100

Sample ID	Physical Parameters				Nutrient Analyses			Chemical Indicators			Bacteria
	pH	Conductivity	Turbidity	Temp	Nox-N	NH4-N	SRP(as PO4)	Triclosan	Caffeine	Fluorescence	Coliform
	---	(uS)	(ntu)	(deg C)	(mg/L)	(mg/L)	(mg/L)	(ppb)	(ppb)	(ug/L)	(cfu/100mL)
Lounibos Residence											
P1	---	---	---	---	---	---	---	0.20	0.36	101.00	1
P2	---	---	---	---	---	---	---	---	---	---	---
P3	---	---	---	---	---	---	---	0.04	0.23	81.30	1
P4	---	---	---	---	---	---	---	0.15	0.25	94.20	1
P5	---	---	---	---	---	---	---	---	---	---	---
P6	---	---	---	---	---	---	---	---	---	---	---
P7	---	---	---	---	---	---	---	0.13	0.22	93.40	1
P8	---	---	---	---	---	---	---	0.22	< 0.175	81.00	1
P9	---	---	---	---	---	---	---	---	---	---	---
P10	---	---	---	---	---	---	---	---	---	---	---
P11	---	---	---	---	---	---	---	---	---	---	---
P12	---	---	---	---	---	---	---	---	---	---	---
P13	---	---	---	---	---	---	---	---	---	---	---
P14	---	---	---	---	---	---	---	---	---	---	---
P15	---	---	---	---	---	---	---	---	---	---	---
P16	---	---	---	---	---	---	---	---	---	---	---
P17	---	---	---	---	---	---	---	0.04	< 0.175	75.10	1
P18	---	---	---	---	---	---	---	0.09	0.20	95.80	1
SW	---	---	---	---	---	---	---	0.19	< 0.175	9.84	100
IRL - SW	---	---	---	---	---	---	---	0.18	0.31	8.46	---

Table 5 presents tide and precipitation data for the various sites, while Table 6 provides additional groundwater seepage and horizontal and vertical hydraulic gradient data. Although data are limited, vertical gradients were highest at the Huy site and low at the Ft. Pierce sites (Grimes and Lounibos). Mean values ranged from >0.32 at the Huy site to 0.01 at the Grimes site. Even though the ground elevation drop was much greater at the Huy site than the other locations, horizontal hydraulic gradients were low at all sites, with means of 0.004, 0.005 0.002 at the Huy, Grimes, and Lounibos sites, respectively (Table 6). In our other studies, higher horizontal hydraulic gradients were always found at sites with bulkheads, while lower horizontal hydraulic gradient sites occurred at natural gradient locations with little fill material, such as is the case in this study. Groundwater seepage was not measured at site H due difficulty in seating meters due to the soupy, organic nature of the sediment, but was found to be fairly high at the other sites, averaging 1.30 and 1.37 L/m2-hr at Grimes and Lounibos, respectively. Highest seepage rates were found during falling tidal cycles, as expected. Typically, at most locations, an inverse relationship between tide height and seepage rate should be observed. The tidal cycle, hydraulic conductivity and depth of the sediment influences groundwater seepage rates, and these parameters can change greatly from site to site. Because of this, we feel nutrient loading estimates using the measured seepage rates from only a few measurements would represent very crude and perhaps misleading estimates. Ideally, the seepage rates should be measured at numerous shoreline locations over many tidal cycles. Because of the above, and the fact at no sites were elevated nutrient or bacterial levels reaching the IRL, loading estimates were not calculated and only concentration data near the shoreline and plume migration rate data are presented. However, these data are still very instructive.

Table 5: Tide and Precipitation Data												
Date	**Study Site**	**Time on Site**	**Reporting Station**	**Distance to Site**	**Tides (Relative to MLLW) High Tide**	**Height (ft)**	**Low Tide**	**Height (ft)**	**Condition**	**Precipitation (inches) 1 Day Total**	**2 Days Prior**	**2 Day Total**
2-Jan	Huy	08:00 - 10:00	Patrick AFB	10 miles	11:05	+ 3.2	04:52	+ 0.3	Rising			
			MLB Airport	3 miles						0.0	0.0	0.0
	Lounibos	11:00 - 14:00	Ft Pierce Inlet	3 miles	11:55	+ 1.7	17:59	+ 0.1	Falling			
	Grimes		St Lucie Airport	2 miles						0.0	0.0	0.0
23-Mar	Huy	08:00 - 10:00	Patrick AFB	10 miles	06:09	+ 3.2	12:25	+ 0.3	Falling			
			MLB Airport	3 miles						0.13	Trace	0.13
	Lounibos	11:00 - 14:00	Ft Pierce Inlet	3 miles	06:59	+ 1.7	13:02	+ 0.3	Falling			
	Grimes		St Lucie Airport	2 miles						0.33	0.4	0.37
23-Jun	Huy	08:00 - 10:00	Patrick AFB	10 miles	08:30	+ 3.9	14:47	- 1.0	Falling			
			MLB Airport	3 miles						0.0	0.0	0.08
	Lounibos	11:00 - 14:00	Ft Pierce Inlet	3 miles	09:20	+ 2.1	15:24	- 0.9	Falling			
	Grimes		St Lucie Airport	2 miles						0.08	0.0	0.08
6-Aug	Huy	08:00 - 10:00	Patrick AFB	10 miles	08:29	+ 3.6	14:43	+ 0.1	Falling			
			MLB Airport	3 miles						0.04	0.08	0.12
	Lounibos	11:00 - 14:00	Ft Pierce Inlet	3 miles	09:19	+ 2.0	15:20	+ 0.1	Falling			
	Grimes		St Lucie Airport	2 miles						0.28	Trace	0.28

Table 6: Groundwater Seepage, Tide, and Hydraulic Gradient Data											
Date	**Study Site**	**Tide Height**	**Condition**	**Groundwater Seepage ($L/m^2 \cdot hr$)**			**Depth to Groundwater**		**Horizontal Gradient**	**Vertical**	**Vertical Gradient**
				SM1	**SM2**	**Mean**	**Pz-1**	**Pz-2**	**Pz-1 to Pz-2**	**Height**	**(in situ piezometer)**
1/2/2009	Huy	+0.3	Rising	---	---	---	7.65	1.20	0.007	+0.88	+0.44
	Grimes	+0.1	Falling	0.944	0.27	0.61	2.90	2.11	0.005	0.00	0.00
	Lounibos	+0.1	Falling	1.28	0.69	0.99	3.48	2.18	0.003	+0.19	+0.10
3/23/2009	Huy	+0.3	Falling	---	---	---	8.65	1.00	0.003	-0.70	>0.35
	Grimes	+0.3	Falling	0.84	2.54	1.69	2.60	1.70	0.004	+0.10	+0.05
	Lounibos	+0.3	Falling	2.08	0.61	1.35	3.45	1.85	0.001	-0.04	-0.02
6/23/2009	Huy	-1.0	Falling	---	---	---	8.21	1.06	0.001	>0.34	>0.17
	Grimes	-0.9	Falling	1.4	1.79	1.59	2.12	1.33	0.005	-0.04	-0.02
	Lounibos	-0.9	Falling	1.78	---	1.78	2.55	1.25	0.003	=	-

In terms of sediment particle size, there were distinct differences between the Huy site in Melbourne and the Indian River Drive sites in Ft. Pierce (Grimes and Lounibos), shown in Table 7. Subsamples from three ft. cores were composited at up-gradient and down-gradient two inch piezometer locations at each residential site (Figures3-5). Although each site is sandy, the Huy site soils are comprised of fine sand (mean = 49.3%), with lesser percentages of medium (mean =20.3%) and very fine sand (mean = 18.0%). The Lounibos and Grimes residences in Ft. Pierce were dominated by medium sand, with percentages greater than 60%.

Table 7: Particle Size (%) and Organic Matter (%) for Upgradient and Downgradient Residential Site Locations

Sample	Gravel	Sand					Silt/Clay	Moisture	Organic Matter
		Very Coarse	Coarse	Medium	Fine	Very Fine			
H-1	0.160	0.233	3.173	20.172	53.595	21.954	0.713	20.838	0.859
H-2	3.628	5.304	8.093	20.423	44.913	14.079	3.561	49.392	11.832
L-1	0.320	0.214	4.610	63.005	28.685	2.896	0.270	18.881	0.844
L-2	0.688	0.396	7.208	60.861	28.138	2.438	0.270	20.483	0.894
G-1	0.108	0.357	5.641	59.258	29.398	4.677	0.561	20.071	1.183
G-2	0.503	1.083	8.450	64.167	23.529	1.795	0.473	22.519	2.545

Fecal Coliform Bacteria

In general, the soil at the three sites proved to be an effective filter for fecal coliform bacteria, as levels were low at groundwater sampling sites. Most sites exhibited levels of < 1 cfu/100 ml. An anomaly occurred at the Grimes site P24 on 3/23/09 when 3400 cfu/100 ml were found. This is puzzling and must have been due to a contamination problem of unknown origin, such as dumped material at that location, as the site was located in uncut vegetation near Indian River Drive. Surface water fecal coliform levels averaged 500, 67, 48 cfu/100 ml for the Huy, Lounibos and Grimes sites, respectively. A very high concentration occurred in Crane Creek adjacent to the Huy site on 3/23/09 (1300 cfu/100 ml) and may have been due to the numerous manatees observed at that location on that date.

Nutrients

Nutrient data collected at the three sites are presented in Tables 1 through 3. A nutrient plume (NOx-N, SRP) on 1/2/09 and 3/23/09 appears to have migrated approximately 82 ft. from the Huy drainfield to P6, while 3/23/09 SRP data show an impact at P18 109 ft. from the drainfield. 6/23/09 data were difficult to interpret and no pattern was evident. At the Grimes residence there was a distinct OSTDS influence on 1/2/09 at P5, 6 and 7, 20 ft. down-gradient from the drainfield. During wetter conditions on 6/23/09, much higher levels of nutrients were seen at sites 5-12, also indicating a plume movement of 32 ft. In general, our data indicate that the mounded aerobic system at the Grimes residence, installed in 1990, is very effective at treating waste and reducing the plume migration distance from this highly loaded system. At the Lounibos site, nutrient (NOx-N, SRP) concentrations were generally lower, as the system is lightly loaded (two adults). However, elevated nutrient levels reached P7 and P8 on 1/2/09 and 6/23/09, 30 ft. away from the drainfield. No clear impact was observed on 3/23/09.

Chemical Indicators of Human Fecal Contamination

Levels of chemical indicators of human fecal contamination were determined on NEP site samples taken on 6/23/09 and 8/6/09 and compared to sewage and probable control sample levels. Considerable time and effort was spent in the lab testing and choosing methods prior to field sampling. Chemical indicator data are presented in Tables 8, 9 and 10, for FWA, CAF and TCS, respectively. All chemical indicator concentrations were high in sewage treatment plant influent, but were greatly reduced in the effluent by the various treatment processes. Crane Creek (FIT Jungle) and IRL (center) samples, while intended to represent controls, were moderately high in all indicators and were similar to treatment plant effluent levels, indicating human sources to the water bodies are widespread and significant.

Table 8: Relative Fluorescence (ug/L) for Selected Sewage Treatment Plants, Water Bodies, and NEP Residential Sites					
Sample	**5/22/2009**	**6/23/2009**	**7/20/2009**	**8/3/2009**	**8/6/2009**
MELBOURNE IN	48.0	---	---	41.0	---
MELBOURNE OUT	27.0	---	---	35.1	---
COCOA IN	48.0	---	---	60.7	---
COCOA OUT	19.5	---	---	15.5	---
VIERA IN	36.0	---	---	39.9	---
VIERA OUT	10.5	---	---	17.0	---
VIERA CELL #2	---	---	---	29.3	---
VIERA CELL #4	---	---	---	15.0	---
CRANE CREEK	27.0	---	---	21.5	---
IRL	4.5	---	---	8.4	---
HUY - Pz1	---	---	---	---	15.5
HUY - Pz2	---	---	---	---	19.3
HUY - 11	---	---	---	---	29.8
HUY - 12	---	10.5	---	---	---
HUY - 13	---	---	---	---	20.0
HUY - 15	---	---	---	---	12.6
HUY - 18	---	9.5	---	---	11.3
HUY - 18A	---	---	---	---	9.5
HUY - 21	---	17.4	---	---	---
HUY - SW	---	30.0	---	---	27.3
GRIMES - 4	---	---	---	---	38.1
GRIMES - 5	---	35.3	---	---	34.9
GRIMES - 6	---	---	---	---	38.2
GRIMES - 7	---	---	---	---	39.0
GRIMES - 8	---	31.5	---	---	---
GRIMES - 18s	---	34.7	---	---	---
GRIMES - 22	---	---	---	---	52.7
GRIMES - 24	---	---	---	---	76.1
GRIMES - SW	---	1.5	---	---	6.8
LOUNIBOS - 1	---	---	---	---	101.0
LOUNIBOS - 3	---	---	---	---	81.3
LOUNIBOS - 4	---	54.6	---	---	94.2
LOUNIBOS - 7	---	---	---	---	93.4
LOUNIBOS - 8	---	49.7	---	---	81.0
LOUNIBOS - 17	---	---	---	---	75.1
LOUNIBOS - 18	---	79.5	---	---	95.8
LOUNIBOS - SW	---	0.6	---	---	9.8
IRL-SW	---	---	---	---	8.5

Table 9: Caffeine (ppb) Levels for Selected Sewage Treatment Plants, Water Bodies, and NEP Residential Sites					
Sample	**5/22/2009**	**6/23/2009**	**7/20/2009**	**8/3/2009**	**8/6/2009**
MELBOURNE IN	---	---	---	AD	---
MELBOURNE OUT	---	---	---	0.38	---
COCOA IN	---	---	---	AD	---
COCOA OUT	---	---	---	0.50	---
VIERA IN	---	---	---	AD	---
VIERA OUT	---	---	---	0.40	---
VIERA CELL #2	---	---	---	0.45	---
VIERA CELL #4	---	---	---	0.40	---
CRANE CREEK	---	---	---	0.20	---
IRL	---	---	---	0.25	---
HUY - Pz1	---	---	---	---	0.19
HUY - Pz2	---	---	---	---	BD
HUY - 11	---	---	---	---	0.21
HUY - 12	---	3.00	---	---	---
HUY - 13	---	---	---	---	0.24
HUY - 15	---	---	---	---	BD
HUY - 18	---	1.30	---	---	BD
HUY - 18A	---	---	---	---	BD
HUY - 21	---	BD	---	---	---
HUY - SW	---	BD	---	---	0.18
GRIMES - 4	---	---	---	---	BD
GRIMES - 5	---	BD	---	---	1.20
GRIMES - 6	---	---	---	---	0.05
GRIMES - 7	---	---	---	---	1.25
GRIMES - 8	---	BD	---	---	---
GRIMES - 18s	---	2.00	---	---	---
GRIMES - 22	---	---	---	---	0.70
GRIMES - 24	---	---	---	---	1.25
GRIMES - SW	---	BD	---	---	BD
LOUNIBOS - 1	---	---	---	---	0.36
LOUNIBOS - 3	---	---	---	---	0.23
LOUNIBOS - 4	---	2.40	---	---	0.25
LOUNIBOS - 7	---	---	---	---	0.22
LOUNIBOS - 8	---	1.54	---	---	BD
LOUNIBOS - 17	---	---	---	---	BD
LOUNIBOS - 18	---	AD	---	---	0.20
LOUNIBOS - SW	---	BD	---	---	BD
IRL-SW	---	---	---	---	0.31

Table 10: Triclosan (ppb) Levels for Selected Sewage Treatment Plants, Water Bodies, and NEP Residential Sites					
Sample	**5/22/2009**	**6/23/2009**	**7/20/2009**	**8/3/2009**	**8/6/2009**
MELBOURNE IN	---	---	---	AD	---
MELBOURNE OUT	---	---	---	1.00	---
COCOA IN	---	---	---	1.40	---
COCOA OUT	---	---	---	0.25	---
VIERA IN	---	---	---	AD	---
VIERA OUT	---	---	---	0.22	---
VIERA CELL #2	---	---	---	0.22	---
VIERA CELL #4	---	---	---	0.10	---
CRANE CREEK	---	---	---	0.09	---
IRL	---	---	---	0.13	---
HUY - Pz1	---	---	---	---	0.19
HUY - Pz2	---	---	---	---	0.16
HUY - 11	---	---	---	---	0.04
HUY - 12	---	BD	---	---	---
HUY - 13	---	---	---	---	0.09
HUY - 15	---	---	---	---	0.11
HUY - 18	---	BD	---	---	0.11
HUY - 18A	---	---	---	---	0.15
HUY - 21	---	BD	---	---	---
HUY - SW	---	BD	---	---	0.13
GRIMES - 4	---	---	---	---	0.04
GRIMES - 5	---	BD	---	---	0.06
GRIMES - 6	---	---	---	---	0.03
GRIMES - 7	---	---	---	---	0.11
GRIMES - 8	---	BD	---	---	---
GRIMES - 18s	---	BD	---	---	---
GRIMES - 22	---	---	---	---	0.05
GRIMES - 24	---	---	---	---	0.27
GRIMES - SW	---	BD	---	---	0.02
LOUNIBOS - 1	---	---	---	---	0.20
LOUNIBOS - 3	---	---	---	---	0.04
LOUNIBOS - 4	---	BD	---	---	0.15
LOUNIBOS - 7	---	---	---	---	0.13
LOUNIBOS - 8	---	0.03	---	---	0.22
LOUNIBOS - 17	---	---	---	---	0.04
LOUNIBOS - 18	---	0.03	---	---	0.09
LOUNIBOS - SW	---	0.07	---	---	0.19
IRL-SW	---	---	---	---	0.18

Relative fluorescence (FWA) was moderately high in selected groundwater samples at each residential site, indicating human impact (Table 8). Although levels were high, no clear impact from the OSTDS drainfield was observed via defined plume migration. Since the water table was so close to the surface (Ft. Pierce sites), with very low hydraulic gradients (all sites) and significant tidal influence (Ft. Pierce sites), FWA may have spread out in all directions rather than in defined and distinct plumes. The observed values, however, were similar to preliminary FWA data collected by the P.I. behind septic tank drainfields on the Lower St. Johns River (LSJR) in Jacksonville, Fl (Table 11), and indicate probable impact. Because there are so many natural sources of fluorescence, and it's difficult to find any natural area that can serve as a "control", we feel the FWA data are the least reliable of the indicators tested, and results should be viewed with caution.

Table 11: Chemical Indicator Concentrations at 12 Residential Sites In Jacksonville, FL July 20, 2009

Residential Site	Sample	Triclosan (ppb)	Fluorescence (ug/L)
Ben Quick	SW1	BD	23.0
	GW1	BD	1.50
Catherine Seay	SW1	BD	16.5
	GW1	BD	31.2
Darren Hurst	SW1	BD	18.0
	GW1	BD	20.4
	GW2	BD	35.5
Don Ellis	SW1	BD	14.6
	GW1	BD	25.5
Jim Behrens	SW1	BD	15.6
	GW1	BD	2.09
Larry Palmer	SW1	BD	18.5
	GW1	BD	29.8
Maryanne Gruber	SW1	BD	20.0
	GW3	BD	13.4
Michael Miller	SW1	BD	17.6
	GW1	BD	23.8
Mario Rivera	SW1	BD	7.75
	GW1	BD	1.52
Randy Thompson	SW1	BD	16.3
	GW1	BD	20.4
	GW2	BD	19.1
Winton Hinson	GW1	BD	2.15
Neil Garrison	DITCH	BD	18.5

At the Huy residence on 6/23/09, groundwater levels of caffeine (CAF) were high at P12 (3.0 ppb) and P18 (1.3 ppb), indicating impact 107 ft from the drainfield. Groundwater levels of CAF were elevated throughout the Lounibos site, and CAF was also measurable in the IRL just north of the Lounibos residence. CAF appears to have definitely reached P8 on 6/23/09, 30 ft. away, and P18 (on 8/6/09), 102 ft. down-gradient from the Lounibos OSTDS drainfield (Table 9). The Grimes site showed elevated CAF levels 42 ft. from the OSTDS drainfield (2.00 ppb at P18) on 6/23/09 when conditions were fairly dry. Levels were below detection at that time, however, in the adjacent surface water. High CAF concentrations were also found at several Grimes sites on 8/6/09 when rainfall levels were higher (Table 9), indicating a drainfield leachate migration of approximately 70 ft. However, the surface water was below detection on that date, as well.

Triclosan (TCS) was below detection in nearly all the NEP site groundwater samples on 6/23/09, but was observed in low levels in the groundwater and surface water at all three residential sites on 8/3/09. Although no distinct plumes from the OSTDS drainfields were discernible, the wetter conditions in August evidently allowed TCS to spread throughout the sampling locations at each site, and the measurable levels indicate probable OSTDS impact. This is a significant finding because the OSTDS must be the source of the TCS, indicating these sites likely have the potential for various types of pollutional loading (nutrients, bacteria) under favorable hydrologic scenarios. In a current study for the City of Jacksonville, funded by DEP and using the same exact approach as this study, TCS was below detection in surface water and in groundwater samples taken very close to twelve OSTDS drainfields located in the LSJR watershed (Table 11). This indicates the significance of the measurable levels at the NEP sites.

Chemical indicator results were mixed, as sometimes nutrient levels compared well with CAF and TCS levels, but at other times they did not. On 6/23/09, the only date that nutrients and chemical indicators were analyzed together at the same sites, high CAF at Huy (P12), Lounibos (P8, P18), and Grimes (P18) correlated well with high nutrient

levels, but at other locations on that date they did not (e.g. Grimes: P5). High CAF and TCS levels were found at the Lounibos sites P4 and P8, when very high SRP (>6.0 mg/L) was present there. CAF and TCS concentrations were high at a number of sites on 8/1/099 (e.g. Huy: Pz1, Pz2, P11; Grimes: P5, P7, P24; Lounibos: P1, P3, P4, P7, P8), but nutrient data were not completed on that date for comparison.

When TCS and CAF were high, no corresponding increase in bacteria was observed. There was no significant correlation between fecal coliform bacteria and chemical indicator concentrations because fecal coliform bacteria were low or absent at nearly every groundwater sampling site.

SUMMARY AND CONCLUSIONS:

This FY O9 NEP OSTDS study has provided additional information on the nutrient and bacterial impact of selected OSTDS sites in the IRL watershed. Data were obtained that determined estimates of contaminant (nutrient, bacteria) plume migration distances at the various sites (Part I), and the effectiveness of selected chemical indicators of human fecal contamination was evaluated (Part II). The OSTDS sites are representative of properly functioning systems, not failing systems, and are therefore typical of most OSTDS in the watershed. Although these sites were not discharging high nutrient loads to the surface water (NO_X-N, SRP), whether or not loading to the IRL occurs at any site depends on the actual distance of the OSTDS drainfield from the lagoon or tributary as well as a variety of other site factors. Data are presented that show estimated nutrient travel distances at the various sites, each with different sediment, hydraulic gradient, age and OSTDS loading characteristics (Table 12).

Table 12: OSTDS SITE CHARACTERISTICS												
Parameter		**SFWMD (FY 2005)**				**SFWMD (FY 2006)**				**NEP (FY 2009)**		
		Site A1	**Site B1**	**Site C1**	**Site D1**	**Site B2**	**Site C2**	**Site D2**	**Site E2**	**Huy**	**Grimes**	**Lounibos**
Year Built		1950's	mid 1970's	1984	2003	1985	1986	1975	1970	1951	1901	1936
Current # Residents		2	2	2	4-6	4	2	4	4	2	8	2
Maximum # Residents		4 (until '96)	unknown	3 (until '91)	4-6	4	2	4	5	5	10	4
Mean Vertical H		0.11	-0.01	0.14	0.29	0.07	---	0.06	-0.02	>0.32	0.01	0.02
Mean Horizontal H		0.022	0.018	0.055	0.024	0.002	0.033	0.080	0.007	0.004	0.005	0.002
Groundwater Seepage		-172	9	122	---	---	162	32	0	---	113	1137
Mean % Medium Sand		70.52	55.24	20.07	---	54.84	20.20	38.42	61.03	20.30	61.70	61.90
Mean % Fine Sand		29.47	44.76	79.43	---	45.16	79.80	61.58	38.97	49.30	26.50	28.40
Mean % Organic Matter		0.47	2.80	0.41	---	2.43	0.35	2.48	1.19	5.80	1.90	0.86
Estimated Nutrient Migration (ft/yr)	**NH4-N**	---	55	65	---	---	---	44	---	---	---	---
	NOx-N	>76	55-63	65	---	---	>15	30	23	1.80	0.30	0.40
	SRP	52	55-85	65	---	---	>15	>76	45	1.80	0.30	0.40

All of the residential sites investigated in this study represent older OSTDS systems with low hydraulic gradients (except for a high vertical hydraulic gradient at the Huy site), and are characterized by very sandy soils. At each of the three NEP sites, nutrients and FC bacteria were not reaching the adjacent surface water, and contaminant plumes were, in many cases, not well defined. Because of this, the chemical indicator evaluation was not entirely conclusive. High nutrient levels were often found where elevated chemical indicators occurred, but not in every case, as anomalies occurred. In addition, fecal coliform bacteria were seldom found at high chemical indicator concentration locations, as the these bacteria were effectively filtered and reduced to low levels at most sites by the soil. Our methodology and sampling data indicate that FWA is the least reliable chemical indicator, as there are many natural sources of fluorescence and the measurement method appears imprecise. Measurable concentrations of FWA were found at all sites, including our intended control sites. TCS and CAF, whose primary source at these sites is OSTDS, have the greatest potential as chemical indicators.

Plume migration distances, based on NOx-N and SRP, were not great for the older OSTDS investigated in this NEP study, and were estimated to be 107, 32, and 30 ft. respectively for the Huy, Grimes and Lounibos sites. However, data interpretation at the Ft. Pierce sites (Grimes, Lounibos) is undoubtedly complicated by significant up-gradient tidal influence through the sandy calcareous soils that exist there. The estimated plume migration distances stated above equate to plume migration rates of 1.8, 0.3 and 0.4 ft/yr for the Huy, Grimes and Lounibos sites, respectively, but the Grimes and Lounibos rates may be underestimates. At these sites, nutrient and bacterial movement may occur and increase in pulses with rainfall and tidal events. Our sampling schedule was fixed in advance and did not necessarily occur after individual rainfall event, after weeks of heavy rain or during low or outgoing tides, and therefore our data do not represent worst-case scenarios. Although we did sample under a range of rainfall and other conditions, we would expect nutrient and bacteria levels to travel farther with higher rainfall and soil saturation conditions, as well as during low or falling tidal cycles.

Nutrient and bacterial travel distances for three completed OSTDS studies by the P.I. (this study and two SFWMD studies) are summarized in Table 12 along with other

pertinent site data. Although only three of the eleven sites were shown to be contributing nutrients to surface water, the nutrient migration distance data are more instructive since the OSTDS installations were located various distances from the IRL and its tributaries. In the two combined SFWMD studies, NO_X-N traveled > 55 ft. at three sites (1.6 – 3.0 ft/yr), NH_4-N traveled > 55 ft. at two sites (1.1 – 3.0 ft/yr), while SRP migrated > 45 ft. at five of the eight sites (0.95 – 2.7 ft/yr). The NEP site migration rates were generally slower than SFWMD study rates, due to the slow plume migration rates found at the low gradient Ft. Pierce sites (0.3 and 0.4 ft/yr for the Grimes and Lounibos sites, respectively). It is clear these low gradient, tidally impacted sites are difficult to sample and interpret. It may be that nutrient and bacterial loads are delivered in outgoing tidal pulses that we may have missed. At those times groundwater movement and nutrient loading to the IRL is likely great. From our overall data, however, it appears that residence age and horizontal hydraulic gradient are two very important factors in determining plume migration distance at any site (Table 12). If the Ft Pierce sites are not included, as we believe they are unique and tidally impacted, our data (from the SFWMD studies and this study) indicate nutrient travel distances (SRP, NO_X-N) are generally in the 1-3 ft/yr range at all sites. Aerobic conditions in and near the drainfield at most sites prevented high NH_4-N concentrations from occurring.

Sediment type can be a very important factor, but our sites exhibited similar sediment and therefore cause/effect sediment impacts were difficult to determine. The sandy (primarily medium/coarse grain) calcareous soils characteristic of the area are not particularly effective in adsorbing phosphate, as significant SRP travel distances were measured at the many of the OSTDS residential sites. Although slightly elevated fecal coliform bacteria levels were measured 28 to 55 ft. down-gradient at two SFWMD sites, the soil generally appears to be an effective bacterial filter at most sites

Based on results from our FWA methodology studies and data collection efforts, we would discourage the use of FWA as a chemical indicator of human fecal contamination, due to its widespread occurrence and imprecise measurement method. Although some apparent data discrepancies occurred, the sewage treatment plant and NEP site data generally indicated that TCS and CAF can be effective indicators that would indicate further site investigation. Although the NEP study is a very good

beginning, more concentration data for these chemical indicators are needed so that results can be correctly interpreted without doubt. Triclosan and caffeine were found in low levels in Crane Creek (FIT Jungle) and the center of the IRL, at sites that we intended to be control sites. Whether the levels found at the control sites are significant, and what would be critical "cutoff" levels, are questions that are still largely unanswered. Answers to the above questions are critical for chemical indicator data interpretation and can only be answered by additional sampling and analysis, as well as further literature review.

Eleven specific OSTDS sites were investigated in the combined FY O5 and FY O6 SFWMD Issues Team Studies and this FY 09 NEP study, and estimated nutrient migration data are presented in Table 12. In addition to the above three studies, data from an on-going OSTDS study funded by the National Park Service for sites surrounding the Mosquito Lagoon will be available in the Fall of 2010. Also, data from another current study of residential OSTDS sites in the LSJR watershed (in Jacksonville, Florida), funded by DEP, will be available in January, 2011. In total, 27 residential sites will have been investigated under different seasonal and hydrologic scenarios by 2011. This NEP study was a key contributor to this database. Collectively, the considerable nutrient, bacteria and chemical indicator data that will be generated from the above five studies should give regulatory agencies the critical information they need to make informed decisions. To date, data from this NEP study and from the two completed SFWMD Issues Team studies indicate that OSTDS is not the "smoking gun", especially in terms of bacterial loading, that many have predicted.

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