

P.I.E. Board Meeting Minutes
When: May 14, 2018 5:00 PM EST
Where: Gasparilla Way Fire House

Present: Lindsay Yates, Sally Johnson, Dave Witters, Monica Errico, Lou Ellen Wilson, Andy Ronald, Jeff Bisgrove, Craig Baresel, Heather Stout, Gary Galloway, and Linda Cotherman (arrived at 5:09)

Present by phone: Dave Mulvaney and Cori Hane Palmere

Meeting called to order: 5:01 PM by Lou Ellen Wilson

1. Roll Call/Establish Quorum
2. Approval Minutes April 9th, 2018 Meeting
 - Minutes: motion to approve. Lindsay motioned to approve the minutes with a correction of “January 8th” to “April 9th”. The motion was approved unanimously.
3. Report of the President (Lou Ellen Wilson)
 - Clarification on Sunshine Act
 - Jeff Bisgrove distributed copies of 617.0832 referencing 617 Conflict of Interest. Not applicable to Sunshine Act
 - On Sunshine, if issues are discussed on MSTU/MSBU’s when more than 1 committee member is in any public setting then only 1 person can speak on an issue.
 - Don Pedro and Knight Islands Street and Drainage Unit - Jeffery Jacobson is the Chair, Lindsay is the Vice Chair of the committee, Jeff Bisgrove, Linda Cotherman and Ralph Artigliere are committee members. Linda was appointed P.I.E. spokesperson for the Road and Bridges MSBU.
 - Barrier Islands Fire Service Unit – Candy Cohen, Wendy Dalzell, Lou Ellen Wilson, James Gordon and Bill Peterson. Committee officers will be elected at the May 24, 2018 MSBU meeting
 - Heather was thanked for her upkeep of the P.I.E. bulletin boards.
 - Craig was thanked for cleaning the landscape island at the barge landing.
4. Treasurer’s Report (Lindsay Yates) (copy attached)
 - April income was \$2000 from member dues, Band in Sand) and \$425 for deputy coverage from local Islanders headed by Barb DeYulio.
 - Lindsay stated that \$425 was received from a group of island citizens that had contributed money last year for additional holiday deputy coverage. This money was given to PIE for security efforts this year. Dave Mulvaney stated the money had been earmarked for additional security for Tropical Nights and that the sheriff's office will be sending PIE an invoice for the coverage - cost \$425.
 - \$300 was used for P.I.E. tax preparation.
 - \$49 was paid to Dave Mulvaney for Weebly (P.I.E. web hosting)

- \$4 was PayPal fees (membership dues)
 - Information only - \$500 is budgeted Sea Turtle T-shirts. This may come in in May. The final expense has not been determined.
 - Monica inquired as to why the check for the turtle conservation was written to BICI. Lindsay explained that the money had been held in trust for the Gopher tortoise Conservation Group because they did not have a bank account at the time the donations were received. The money had always been shown as a liability on PIE's balance sheet. After the PIE board approved Kjell Poltkin's request at the April Board meeting to release the funds, it was at Kjell's request that the check be written to BICI.
 - Motion by Craig to approve the treasurer's report for information. The motion was approved unanimously.
5. Report from the Corresponding Secretary (Dave Mulvaney)
- Website Traffic month over month has increased again from an average of 42 unique individuals per day in March to 60 unique individuals per day in April. Regarding our "Page Views per day" we have seen a month over month increase from an average of 125 pages per day in March to 148 pages per day in April. Top 3 pages viewed besides our main page are: Info Central, Social and then our news page.
 - Member ONLY page - need to finish the security aspect of sending everyone a password, this needs to be done on an individual basis and will take considerable amount of time to complete.
 - We received over a dozen emails that were positive regarding our newsletters, Info Central meetings and various emails that have been sent. We received 1 email suggesting that we list all island activities 1st before all off-island events.
6. Committee Reports
- Info Central (Sally Johnson)
 - See Appendix 1: Recap of Informational Meeting – April 25, 2018 1pm Palm Island Clubhouse
 - CERTS – 3-day program. Will complete as a 3-day course. The presenters feel that 3 days in a row are better for retention. 15-30 people could attend (room capacity) if the training is held at the Resort. Andy motioned to have PIE sponsor but not pay for their lunch. Sally suggested later in November would be better when people are back from the summer. Certification is supplied after the class is complete. Lou Ellen motioned to approve that P.I.E. sponsor the training at the Resort for November. The motion was approved unanimously.
 - AED program – AED is an automated external defibrillator. There is currently a defibrillator at the fire house. It was raised that AED's are life-saving devices. Motion was raised by Sally as to if P.I.E. would like to purchase an AED for other part of the island. No decision was made. Sally will provide additional information in a report to include options for people to purchase as individuals. This topic will be further discussed at the next board meeting.
 - Social Update (Lou Ellen Wilson)
 - No report
 - Island Watch (Linda Cotherman)

- According to WCIND meeting minutes on April 2, and August 18, 2017, WCIND worked with Charlotte County staff, US Coast Guard, and FFWCC, on signage issues for the ferry crossing.
- The new Barrier Islands Fire Service MSBU Advisory Board meeting has been scheduled for May 24, 2018 at 9:30 at the Public Works building off San Casa Drive in Englewood.
- The BCC voted on May 8, 2018 to have the top three bidders for the new fire station do a presentation to the commissioners on May 17, 2018 at 2:30, Room #119, Murdock. Site location appears to be one of the main issues.
- The Charlotte County BCC/Utility quarterly meeting is May 15 at 1:00. Craig Baresel is to attend.
- At the Roads and Bridges meeting on April 30, 2018 numerous items were discussed including: On going progress of bid for contractor to do bridge repairs, traffic monitoring speed sign on N. Gulf will be moved to capture data from resort to barge, request to do R/W mowing on N. Gulf to be done quarterly and budget for FY20 and FY21. The estimated cost to repair the Michael Kosinski Bridge was \$367,750 and repairs to the Anne Merry were estimated at \$10,777 for a total cost of approximately \$378,527.47. The FY18 budgeted amount is \$150,000 however there are sufficient funds in reserve to cover the costs. The bid package is expected to be delivered to the Purchasing Department in May.
- On April 24, 2018 the BCC considered the Beach Renourishment Initial Assessment Resolution. There will be a public hearing held July 19, 2018 at 5:01 at Tringali Center in Englewood. Notices will be mailed to all property owners affected.
- Bocilla Utilities has passed on to customers a water rate increase issued by EWD for bulk water. The next meeting for EWD is June 3 at 8:30 at 201 Selma Ave. in Englewood.
- It has been reported that flood insurance is no longer assumable to new buyers when a house is sold. This may substantially increase flood insurance premiums for the new owner.
- Administration Committee (Candy Cohen and Lindsay Yates)
 - Candy Cohen reported that the committee is working on the By-Law and Article Amendments and will present at the August Meeting.
- Welcoming Committee
 - No report
- Security (Dave Mulvaney)
 - Once again, there have been no Major crimes reported.
 - There was a paid detail for YMCA Tropical Nights which was very effective from a preventative perspective.
- Scholarship Committee (Heather Stout)
 - No applications have been received. Funds at this time are \$10,100.
 - Cori Hane Palmere was appointed to the scholarship committee.
 - Discussion regarding allowing scholarship candidates the option to do their 10 hours of community service with a PIE approved off island organization that PIE

has previously donated funds to. This would allow the PIE board to bridge the gap between those that wanted PIE to continue donating money off island and those who advocated for all monies to stay on the island. Also allowing candidates option to present to the scholarship committee if they have another idea that benefits the island and feel it can be counted towards island community service. The committee wants to make sure that everyone is on the same page regarding what is expected and circumvent any questions of were the hours put in or weren't they. The committee will present concrete details for the board to vote at the June meeting.

- The committee will reach out via PIE email and or newsletter to get a gauge on how many students will be coming up in the next 2 years to ensure they have the funds to support the upcoming candidates.
- The committee is putting together ideas (long range) for the eventual need of a fundraiser to replenish the scholarship program. Any and all ideas are encouraged to be submitted.
- Monica submitted documented hours for John Witters, Erica Mulvaney, and Rylie Pevic on behalf of the Social committee for hours served for beach preparation, setup, manning the welcoming table and clean-up for the Band in the Sand. There was a discussion as to whether all or any of these hours could be accepted as community service. It was advised that the scholarship committee review their current instructions/policies available on the website and to make a recommendation to the board if updates to instructions need to be implemented. Documented hours were left with Lou Ellen for consideration by the committee.

7. Old Business

- Newsletter (Monica Errico, Dave Mulvaney and Lou Ellen Wilson)
 - Update on how the EU's General Data Protection Regulation (GDPR) affects P.I.E. communications:
 - The GDPR goes into effect on May 25, 2018. The regulations were written to benefit European citizens by giving them more control over the data that's collected by any and all online services. These new regulations impact and European and USA-based corporation who handle data of E.U. citizens. P.I.E. is a corporation and because our P.I.E mailing list contains email addresses with European domains we must follow the new guidelines. This includes our website, mailchimp communications and email communications. At the moment we are not concerned with applicable website regulations for the P.I.E. website.
 - Mailchimp - All online communication providers (i.e. Mailchimp) now requires verification of electronic or written "opt-in" authorization 6-months of the effective date. This includes USA-based members of a list if they are merged with European members.
 - a. Our P.I.E. membership application serves as proof of opt-in.
 - b. If a member unsubscribes from a list (P.I.E. newsletter or email through mailchimp) a newly enacted feature in Mailchimp will no

longer allow us to manually over-ride the unsubscribe. The unsubscribed member must re-subscribe through mailchimp.

- More information on the GDPR can be found at <https://www.eugdpr.org>
- Discussion on P.I.E. being careful about sending out too much information throughout the month as it was noted that members have been unsubscribing. It should be noted to that if one “unsubscribes” from the mailchimp mailing list they will no longer receive any communications from P.I.E. via Mailchimp.
- P.I.E. newsletter – The P.I.E. monthly newsletter has an opening rate of ~70% with a 30-40% click rate on links to read more on stories reported. The Info central updates, report on sewers, Social events and on/off-island activities have the highest click rates.
- The newsletter is a fair amount of work to put together because we monitor the county website, several chamber of commerce and community websites to keep members up to date. Monica motioned that the newsletter be moved to a quarterly update as opposed to monthly. Here, the communications committee will prepare an abbreviated update monthly with a high level update on issues that could impact homeowners (i.e. sewer, beach renourishment, Stump Pass dredging, water rates, and etc.). Island activities and important announcements can be sent out monthly as part of the abbreviated newsletter. The motion passed unanimously.
- Water Testing Update (Monica Errico)
 - A review of guidance from the EPA and FL Health Department was presented. P.I.E. currently performs testing of *fecal Coliform* in various places around the island. Two types of tests are acceptable for such testing.
 - Membrane filtration is the method of choice for the analysis of fecal coliforms in water. Samples to be tested are passed through a filter of particular pore size (generally 0.45 micrometer). The microorganisms present in the water remain on the filter surface. The filter is placed in a sterile petri dish with a selective medium, growth of specific colonies can be quantified as parts per 100 mL. This is the test that P.I.E. has been using.
 - For recreational waters (salt water pools, brackish water and beaches), *fecal coliform* was the primary bacteria indicator until relatively 2006, when EPA began recommending *enterococci* as better indicators of health risk from water contact because it is now known that *fecal coliform* cannot survive in salt water. The change occurred because testing for *fecal coliform* requires delicate handling during sample collection and procedures to ensure survival and growth of colonies, so it is not an acceptable indicator of human waste contamination salt water (including brackish waterways).
 - It was noted that the Master Sewer Update Plan for Charlotte County was determined by testing brackish and salt waterways for *enterococci* and Sucralose testing on outgoing tides. (Important because incoming tides are not representative of the secreted factors into the water stream). In areas of high water turnover testing is recommended on a bi-weekly basis.

- Sucralose is a good indicator of septic compromise because it is an inorganic product in many pre-prepared consumer foods that is not found in fertilizers.
 - Charlotte county performs testing for *enterococci* every 2 weeks from our public beach access on North Gulf Blvd. Results can be queried at <http://www.floridahealth.gov/environmental-health/beach-water-quality/index.html>
 - Motion was made by Monica to discontinue testing for fecal coliform as we know it is not an indicator of septic compromise in our salt waterways. The motion was approved unanimously.
 - Issue was raised by Linda if testing for *enterococci* should be started to ensure out canals are swimmable. This topic will be discussed in more detail at the June board meeting.
 - See Appendix II for the report on water testing.
- 8. New Business
 - Trash cans at the ferry landing (Lou Ellen Wilson)
 - Craig and Lou Ellen will coordinate schedule of volunteers to ensure trash is picked up and removed from barge landing.
 - Island cleanup (Lou Ellen Wilson)
 - Pat Gordon stated that cleanup was historically performed closer to an island event. Heather advocated for a date later in the year when it's not as hot. Sally noted areas that need cleanup on certain island areas. If we wait until January we are looking pretty shaggy. Motion was made by Lou Ellen to set the Island Cleanup date for Saturday, November 3rd and hope for cool weather. The motion passed unanimously.
 - Flag Protocol (Ray Smith)
 - Ray spoke thanked P.I.E. for allowing him the privilege to tend to the Flag. Ray also thanked P.I.E. for the lowering of the flag by the ferry landing in honor of the passing of Dan Beckstead.
 - Normally 3 flags are kept in the P.I.E. reserves to use at the flag pole, and replenishment of this reserve is needed. There was a discussion as to whether cloth or polyester flags fair better with our island environment. Mike Szykaly, Sally Johnson, and Andy Ronald will donate flags.
 - Conference Call System for remote P.I.E. board members (Monica Errico)
 - Monica reported that it is very difficult for board members present by phone to hear all communications in the board meeting when joining by the P.I.E. teleconference line. Monica suggested that if P.I.E. were to continue allowance of remote TC into board meetings that P.I.E. should invest in a microphone device to better hear participants. Prices for options ranged from \$179 - \$400. Discussions presumed because such a device would enable recording of Info Central speaker presentations to be recorded so that members who work full-time that cannot attend to be recorded.
 - It was raised by Heather that the lack of incoming funds from social activities, P.I.E. current financial obligations, and an indecision of P.I.E. updated bylaws will

enable remote participation in board meetings should be considered prior to decision by the board.

- 4 validated options will be circulated to the board members for discussion at the next meeting.
 - Go Fund Me page for Ashton Boyer (Lou Ellen)
 - Ashton Boyer, raised on our island and currently servicing many of our residents through his landscaping service has suffered a disabling injury this past month. Ashton was recognized for his many contributions to the island and its residents, including and not limited to, assisting with street cleanup after Hurricane Irma prior to residence return, and removal of foliage debris from the clearing of BICI properties... all done without fee for service and care for the island and its residents.
 - Pat Gordon will reach out to Ashton's parents to discuss their feelings on P.I.E. promoting a Go Fund Me page for Ashton. If they are in agreement, we will send out an email blast with disclosure stating that PIE is not the sponsor.
9. Comments from board members not on the agenda
- Mosquito bags (Andy Ronald)
 - Andy discussed the utility of organic mosquito bug repellent bags. These are small bags that you hang within a 20-Ft perimeter to prevent bites from mosquitos.
 - It was noted that the main ingredient was thyme – an agent that has been implicated with triggering of epileptic seizures if in proximity.
 - Lou Ellen proposed that there be a blast email requesting PIE members interested in purchasing same at a group discount let Andy know. The cost is estimated to be \$15-\$18/bag. Andy will coordinate purchase and delivery.

Meeting Adjourn - 7:06 pm

Respectfully submitted,
Monica Errico, Secretary

APPENDIX 1 – Recap of Informational Meeting – April 25, 2018 1pm Palm Island Clubhouse

Representatives from Three Charlotte County Departments made presentations:

1. Ellen Pinder – Charlotte County Community Emergency Response Teams (CERTS).

- This program offers training to Charlotte County Residents in the following areas:
 - What to expect following a major disaster in terms of immediate service
 - Training in lifesaving skills with emphasis on Disaster Preparedness, proper use of fire extinguishers, rescuer safety, light search and rescue and doing the greatest good for the greatest number as well as first aid including stopping a bleed out, how to recognize stroke and heart attack victims, etc..
 - Training consists of a three day program 9 a.m. to 3 p.m. usually held in Punta Gorda; however, they will provide free of charge training on the island provided we have a minimum of 15 participants.
 - Go to their website: charlottecountycert@gmail.com for full information regarding this program.
- She urged everyone to sign up for Alert Charlotte – which provides accurate information regarding storms/fires, etc. that will affect our island – local TV stations give information for areas that could be several miles away.

Dee Hawkins – Charlotte County Fire & EMS – reviewed programs available to Charlotte County Residents

- Red Dot Medical Information Program – A Red Dot is placed above the exterior of your entry door just above your door knob to alert EMS/Fire responders that medical information is in a “red packet” on the refrigerator door. Also information is recorded in the County 911 database, so it is available to the responders prior to arriving at the scene and also to Emergency Room personnel. The red pouches are available to have in golf carts and/or vehicles. Red Dot sign up packets are available at the island fire station or on line: [www.CharlotteCountyFl.gov /red dot program](http://www.CharlotteCountyFl.gov/red-dot-program) or call (941) 833-5600.
- Knox Home Box: Keys to the home are placed in the Knox Box and EMS/Fire Rescue have the master key – saves valuable time in case nobody can answer the door and/or not at home in case of fire. More information: on line www.KnoxHomeBox.com or call (855)438-5669.
- Save Our Pet Stickers: Place near the front door to alert responding personnel that a pet is in the house. If no one is available to care for your pets, Animal Control will be notified and will provide the proper assistance to the pet’s owner for the care of the animal until the owner is capable to arrange for the pet’s care. “Save Our Pet” stickers cost \$3. 941-86-33-5600
- Reflective Address Markers: Charlotte County Fire & EMS will provide reflective address markers to be placed on your property so they are clearly visible from the street. Cost \$25. Installation is free. 941-833-5600
- Safety Tips: For more information on all programs on line: www.CharlotteCountyFl.gov or call (941)833-5600.

- Flicker of Life –Helps emergency workers find their way to your door. Light is used in a normal way but when you have an emergency you turn the light switch off and then back on, this will cause the light to flash on and off for 20 minutes. 941-833-5600
- Replace all Smoke/Fire alarms in the home after ten years even if they test okay. Local Fire personnel are available to assist you with replacement of alarms/batteries provided it does not require electrical work. To arrange an appointment call headquarters (941)833-5600. (do not contact local fire station personnel)
- Sprinkler Systems – recommend owner review the owner manual or contact manufacturer representative for testing requirements. All systems are different.
- AED – (Automated External Defibrillator) Ensure that locations of AED’s on the island are identified. Also recommended that several be placed on homes throughout the island and locations communicated to all residents.
- Emergency Procedures: Recommended that all homes and rental properties have an emergency procedure information sheet – readily available in the home (inside cabinet door one possibility) with the correct street address, location of fire extinguishers, etc. Also recommended in all home for use by visitors.

Melanie Bailey – Charlotte County 911

- When calling 911 – use landline if available – their system will show the location immediately. If calling from a cell phone be sure to give the correct full STREET address – do not say Palm or Gulf Blvd make sure you identify it as Palm Drive and Gulf Blvd North, etc. within Charlotte County there are several streets with the name Palm or Gulf, etc. Also identify that you are in Palm Island Resort, Don Pedro, or Knights Island -
- Stressed: DO NOT DRIVE TO FIRE STATION OR BARGE – wait for EMS personnel to come to the address given. In NO circumstances GO DIRECTLY TO FIRE STATION. Call 911, fire station personnel could be on another call, doing island inspections, etc. Texting available – however use only when unable to talk – one example: a home invasion and you are hiding in a closet.
- NON EMERGENCY TELEPHONE #: (941) 639-0013
- Regarding 911 calls - For Medical calls Station 14 gets called because they have the ambulance and only the ambulance can transport you to a medical facility. Depending on the nature of the call they may ask for backup. If you go to Fire Station #10, and knock on the door, they still have to call for the ambulance and precious time is wasted. CALL 911. They emphasized that we should NOT WORRY about making a 911 call. When in doubt....CALL. They will take your vitals and if you don’t need to be transported you have the option of having a family member drive you. They are here to help us any way they can, but call 911 first.

APPENDIX II: Water Testing report

PART I - EPA Website:

<https://archive.epa.gov/water/archive/web/html/vms511.html>

5.11 Fecal Bacteria

What are fecal bacteria and why are they important?

Members of two bacteria groups, coliforms and fecal streptococci, are used as indicators of possible sewage contamination because they are commonly found in human and animal feces. Although they are generally not harmful themselves, they indicate the possible presence of pathogenic (disease-causing) bacteria, viruses, and protozoans that also live in human and animal digestive systems. Therefore, their presence in streams suggests that pathogenic microorganisms might also be present and that swimming and eating shellfish might be a health risk. Since it is difficult, time-consuming, and expensive to test directly for the presence of a large variety of pathogens, water is usually tested for coliforms and fecal streptococci instead. Sources of fecal contamination to surface waters include wastewater treatment plants, on-site septic systems, domestic and wild animal manure, and storm runoff.

In addition to the possible health risk associated with the presence of elevated levels of fecal bacteria, they can also cause cloudy water, unpleasant odors, and an increased oxygen demand. (Refer to the section on dissolved oxygen.)

Indicator bacteria types and what they can tell you

The most commonly tested fecal bacteria indicators are total coliforms, fecal coliforms, *Escherichia coli*, fecal streptococci, and enterococci. All but *E. coli* are composed of a number of species of bacteria that share common characteristics such as shape, habitat, or behavior; *E. coli* is a single species in the fecal coliform group.

Total coliforms are a group of bacteria that are widespread in nature. All members of the total coliform group can occur in human feces, but some can also be present in animal manure, soil, and submerged wood and in other places outside the human body. Thus, the usefulness of total coliforms as an indicator of fecal contamination depends on the extent to which the bacteria species found are fecal and human in origin. For recreational waters, total coliforms are no longer recommended as an indicator. For drinking water, total coliforms are still the standard test because their presence indicates contamination of a water supply by an outside source.

Fecal coliforms, a subset of total coliform bacteria, are more fecal-specific in origin. However, even this group contains a genus, *Klebsiella*, with species that are not necessarily fecal in origin. *Klebsiella* are commonly associated with textile and pulp and paper mill wastes. Therefore, if these sources discharge to your stream, you might wish to consider monitoring more fecal and human-specific bacteria. For recreational waters, this group was the primary bacteria indicator until relatively recently, when EPA began recommending *E. coli* and enterococci as better indicators of health risk from water contact. Fecal coliforms are still being used in many states as the indicator bacteria.

E. coli is a species of fecal coliform bacteria that is specific to fecal material from humans and other warm-blooded animals. EPA recommends *E. coli* as the best indicator of health risk from water contact in recreational waters; some states have changed their water quality standards and are monitoring accordingly.

Fecal streptococci generally occur in the digestive systems of humans and other warm-blooded animals. In the past, fecal streptococci were monitored together with fecal coliforms and a ratio of fecal coliforms to streptococci was calculated.

This ratio was used to determine whether the contamination was of human or nonhuman origin. However, this is no longer recommended as a reliable test.

Enterococci are a subgroup within the fecal streptococcus group. Enterococci are distinguished by their ability to survive in salt water, and in this respect they more closely mimic many pathogens than do the other indicators. Enterococci are typically more human-specific than the larger fecal streptococcus group. EPA recommends enterococci as the best indicator of health risk in salt water used for recreation and as a useful indicator in fresh water as well.

Which Bacteria Should You Monitor?

Which bacteria you test for depends on what you want to know. Do you want to know whether swimming in your stream poses a health risk? Do you want to know whether your stream is meeting state water quality standards?

Studies conducted by EPA to determine the correlation between different bacterial indicators and the occurrence of digestive system illness at swimming beaches suggest that the best indicators of health risk from recreational water contact in fresh water are *E. coli* and enterococci. For salt water, enterococci are the best. Interestingly, fecal coliforms as a group were determined to be a poor indicator of the risk of digestive system illness. However, many states continue to use fecal coliforms as their primary health risk indicator.

If your state is still using total or fecal coliforms as the indicator bacteria and you want to know whether the water meets state water quality standards, you should monitor fecal coliforms. However, if you want to know the health risk from recreational water contact, the results of EPA studies suggest that you should consider switching to the *E. coli* or enterococci method for testing fresh water. In any case, it is best to consult with the water quality division of your state's environmental agency, especially if you expect them to use your data.

Sampling and equipment considerations

Bacteria can be difficult to sample and analyze, for many reasons. Natural bacteria levels in streams can vary significantly; bacteria conditions are strongly correlated with rainfall, and thus comparing wet and dry weather bacteria data can be a problem; many analytical methods have a low level of precision yet can be quite complex; and absolutely sterile conditions are required to collect and handle samples.

The primary equipment decision to make when sampling for bacteria is what type and size of sample container you will use. Once you have made that decision, the same, straightforward collection procedure is used regardless of the type of bacteria being monitored. Collection procedures are described under "How to Collect Samples" below.

It is critical when monitoring bacteria that all containers and surfaces with which the sample will come into contact be sterile. Containers made of either some form of plastic or Pyrex glass are acceptable to EPA. However, if the containers are to be reused, they must be sterilized using heat and pressure. The containers can be sterilized by using an autoclave, which is a machine that sterilizes containers with pressurized steam. If using an autoclave, the container material must be able to withstand high temperatures and pressure. Plastic containers either high-density polyethylene or polypropylene might be preferable to glass from a practical standpoint because they will better withstand breakage. In any case, be sure to check the manufacturer's specifications to see whether the container can withstand 15 minutes in an autoclave at a temperature of 121°C without melting. (Extreme caution is advised when working with an autoclave.) Disposable, sterile, plastic Whirl-pak® bags are used by a number of programs. The size of the container will depend on the sample amount needed for the bacteria analysis method you choose and the amount needed for other analyses.

There are two basic methods for analyzing water samples for bacteria:

1. The membrane filtration method involves filtering several different-sized portions of the sample using filters with a standard diameter and pore size, placing each filter on a selective nutrient medium in a petri plate, incubating the plates at a specified temperature for a specified time period, and then counting the colonies that have grown on the filter. This method varies for different bacteria types (variations might include, for example, the nutrient medium type, the number and types of incubations, etc.).
2. The multiple-tube fermentation method involves adding specified quantities of the sample to tubes containing a nutrient broth, incubating the tubes at a specified temperature for a specified time period, and then looking for the development of gas and/or turbidity that the bacteria produce. The presence or absence of gas in each tube is used to calculate an index known as the Most Probable Number (MPN).

Given the complexity of the analysis procedures and the equipment required, field analysis of bacteria is not recommended. Bacteria can either be analyzed by the volunteer at a well-equipped lab or sent to a state-certified lab for analysis. If you send a bacteria sample to a private lab, make sure that it is certified by the state for bacteria analysis. Consider state water quality labs, university and college labs, private labs, wastewater treatment plant labs, and hospitals. You might need to pay these labs for analysis.

This manual does not address laboratory methods because several bacteria types are commonly monitored and the methods are different for each type. For more information on laboratory methods, refer to the [references](#) at the end of this section. If you decide to analyze your samples in your own lab, be sure to carry out a quality assurance/quality control program. Specific procedures are recommended in the section below.

How to Collect Samples

The procedures for collecting and analyzing samples for bacteria consist of the following tasks:

TASK 1 Prepare sample containers

If factory-sealed, presterilized, disposable Whirl-pak® bags are used to sample, no preparation is needed. Any reused sample containers (and all glassware used in this procedure) must be rinsed and sterilized at 121 C for 15 minutes using an autoclave before being used again for sampling.

TASK 2 Prepare before leaving for the sampling site

Refer to [section 2.3 - Safety Considerations](#) for details on confirming sampling data and time, picking up equipment, reviewing safety considerations, and checking weather and directions. In addition, to sample for coliforms you should check your equipment as follows:

- Whirl-pak® bags are factory-sealed and sterilized. Check to be sure that the seal has not been removed.
- Bottles should have tape over the cap or some seal or marking to indicate that they have been sterilized. If any of the sample bottles are not numbered, ask the lab coordinator how to number them. Unless sample containers are to be marked with the site number, do not number them yourself.

TASK 3 Collect the sample

Refer Task 2 in [Chapter 5 - Water Quality Conditions](#) for details on collecting a sample using screw-cap bottles or Whirl-pak® bags. Remember to wash your hands thoroughly after collecting samples suspected of containing fecal contamination. Also, be careful not to touch your eyes, ears, nose, or mouth until you've washed your hands.

Recommended field quality assurance/quality control procedures include:

- **Field Blanks.** These should be collected at 10 percent of your sample sites along with the regular samples. Sterile water in sterilized containers should be sent out with selected samplers. At a predetermined sample site, the sampler fills the usual sample container with this sterile water. This is labeled as a regular sample, but with a special notation (such as a "B") that indicates it is a field blank. It is then analyzed with the regular samples. Lab analysis should result in "0" bacteria counts for all blanks. Blanks are used to identify errors or contamination in sample collection and analysis.
- **Internal Field Duplicates.** These should be collected at 10 percent of your sampling sites along with the regular samples. A field duplicate is a duplicate stream sample collected at the same time and at the same place either by the same sampler or by another sampler. This is labeled as a regular sample, but with a special notation (such as a "D") that indicates it is a duplicate. It is then analyzed with the regular samples. Lab analysis should result in comparable bacteria counts per 100 mL for duplicates and regular samples collected at the same site. Duplicates are used to estimate sampling and laboratory analysis precision.
- **External Field Duplicates.** An external field duplicate is a duplicate stream sample collected and processed by an independent (e.g., professional) sampler or team at the same place at the same time as regular stream samples. It is used to estimate sampling and laboratory analysis precision.

TASK 4 Return the field data sheets and the samples to the lab or drop-off point

Samples for bacteria must be analyzed within 6 hours of collection. Keep the samples on ice and take them to the lab or drop-off point as soon as possible.

TASK 5 Analyze the samples in the lab

This manual does not address laboratory analysis of water samples. Lab methods are described in the references below (APHA, 1992; River Watch Network, 1991; USEPA, 1985). However, the lab you work with should carry out the following recommended laboratory quality assurance/quality control procedures:

- **Negative Plates** result when the buffered rinse water (the water used to rinse down the sides of the filter funnel during filtration) has been filtered the same way as a sample. This is different from a field blank in that it contains reagents used in the rinse water. There should be no bacteria growth on the filter after incubation. It is used to detect laboratory bacteria contamination of the sample.
 - **Positive Plates** result when water known to contain bacteria (such as wastewater treatment plant influent) is filtered the same way as a sample. There should be plenty of bacteria growth on the filter after incubation. Positive plates are used to detect procedural errors or the presence of contaminants in the laboratory analysis that might inhibit bacteria growth.
 - **Lab Replicates.** A lab replicate is a sample that is split into subsamples at the lab. Each subsample is then filtered and analyzed. Lab replicates are used to obtain an optimal number of bacteria colonies on filters for counting purposes. Usually, subsamples of 100, 10, and 1 milliliter (mL) are filtered to obtain bacteria colonies on the filter that can be reliably and accurately counted (usually between 20 and 80 colonies). The plate with the count between 20 and 80 colonies is selected for reporting the results, and the count is converted to colonies per 100 mL.
 - **Knowns.** A predetermined quantity of dehydrated bacteria is added to the reagent water, which should result in a known result, within an acceptable margin of error.
 - **Outside Lab Analysis of Duplicate Samples.** Either internal or external field duplicates can be analyzed at an independent lab. The results should be comparable to those obtained by the project lab.
-

PART II - Charlotte County Beach Testing:

<http://charlotte.floridahealth.gov/programs-and-services/environmental-health/beach-water-quality/index.html>

[Map](#)

Results for Palm Island North:

<http://www.floridahealth.gov/environmental-health/beach-water-quality/beach-detail.html?County=Charlotte&SPLocation=PALM%20ISLAND%20NORTH&SPNo=&SPLat=26.8807827&SPLong=-82.3295937>

In 1998, five of Florida's coastal counties began monitoring for enterococci bacteria under a grant-funded pilot program. By the beginning of 2000, 11 Florida counties were participating in the program, which continued through July 2000. To review the sampling history for those counties who participated in the original program, click here.

In August 2000, the Beach Water Sampling Program was extended to 34 of Florida's coastal counties through state legislation (Senate Bill 1412 and House Bill 2145) and funding. In addition, sampling under the new program now includes fecal coliform as well as enterococci bacteria. The rationale for selecting these two bacteria for analysis and implications of the sampling results are described below. In August 2002, the beach water sampling program began collecting water samples on a weekly basis with additional funding from U.S. EPA. The most recent results from the current program can be reviewed here.

[Florida Healthy Beaches Program](#)

Health Implications

Enterococci are enteric bacteria that normally inhabit the intestinal tract of humans and animals. The presence of enteric bacteria can be an indication of fecal pollution, which may come from stormwater runoff, pets and wildlife, and human sewage. If they are present in high concentrations in recreational waters and are ingested while swimming or enter the skin through a cut or sore, they may cause human disease, infections or rashes.

Enterococci

The statewide testing program tests for enterococci, which the [United States Environmental Protection Agency \(EPA\) opens in new window](#) has recommended states adopt as a saltwater quality indicator. According to studies conducted by the EPA, enterococci have a greater correlation with swimming-associated gastrointestinal illness in both marine and fresh waters than other bacterial indicator organisms, and are less likely to "die off" in saltwater. If an enterococci result were observed to exceed 104 colony forming units per 100 milliliters of beach water sampled and a resampling result also exceeds this value, then an "Advisory" would be issued for the sampling site.

Florida Healthy Beaches Program Categories are:
Good = 0-35 Enterococci per 100 milliliters of marine water
Moderate = 36-104 Enterococci per 100 milliliters of marine water
Poor = 105 or greater Enterococci per 100 milliliters of marine water

PART III - Link to Master Sewer Update (11/2017):

<http://www.charlottefl.com/outreach/agenda/20171121/3.pdf>

Reducing Septic Tank Pollution in Charlotte County: Background Research and Scope of Work

- Brian E. Lapointe, Laura W. Herren, and Sara N. Ouly
- Florida Atlantic University-Harbor Branch Oceanographic Institute Marine Ecosystem Health Program
- Harbor Branch, Florida-Atlantic University
- Date: 3/18/16
- Link:https://www.charlottecountyfl.gov/dept/utilities/Site%20Documents/Lapointe_Ch%20Charlotte%20County%20Red%20Final.pdf

Charlotte County PHASE I: Design of a Long-Term Water Quality Sampling Program

1. Datamine and Synthesize Existing Data
2. Reconnaissance Field Trips, QA/QC sampling across study area
3. Identify Long-Term Monitoring Stations and Outline Sampling Design
4. Identify Laboratories & Volunteer Networks to Collect & Analyze Samples

Phase 1 Report:

<https://www.charlottecountyfl.gov/dept/utilities/Site%20Documents/Charlotte%20County%20HBOI.FAU.PhaseI.Final%20Report.12.12.2016.pdf>

Methods: (Page 18)

3.1.1 Surface water sampling

Surface water was collected at four sites representing the confluence of residential canals and Charlotte Harbor (El Jobean, Ackerman, Spring Lakes, and Yacht Club; **Fig. 14b**). Under the lead of CCUD, the four surface water stations were sampled weekly by Benchmark EnviroAnalytical, Inc. during ebbing tides between the weeks of June 27, 2016 - August 25, 2016 for nutrients, chlorophyll *a*, bacteria (fecal coliforms and *Enterococcus*), total dissolved solids, BOD, and environmental parameters (pH, salinity, conductivity, temperature; **Appendix 2.2**). In addition to the weekly surface water monitoring by Benchmark EnviroAnalytical, Inc., HBOI-FAU collected surface water samples at the cage sites during an outgoing tide on June 24 (dissolved nutrients and aqueous isotopes) and July 25 (sucralose), 2016.

3.1.2 Groundwater sampling

Preliminary nutrient and wastewater tracer analyses were conducted on groundwater (monitoring wells 66, 67, and 68) in the East and West Spring Lake Wastewater Pilot Program area (Fig. 13). The three groundwater stations sampled by HBOI-FAU represented areas where nutrient concentrations, especially nitrogen, were exceptionally high during the East and West Spring Lake Wastewater Pilot Program. Dissolved nutrient analyses were conducted on groundwater samples collected at three wells on June 24 and July 25, 2016 by HBOI-FAU. Groundwater samples were collected using a Masterflex[®] ES portable peristaltic pump with two ft. of silicon tubing connected via silicon junction to 17 feet of Tygon tubing. Monitor well depth was calculated and the well volume was turned over a minimum of three complete times prior to sample collection. All aqueous isotope samples were collected in duplicate and immediately preserved on ice until processing at HBOI-FAU.

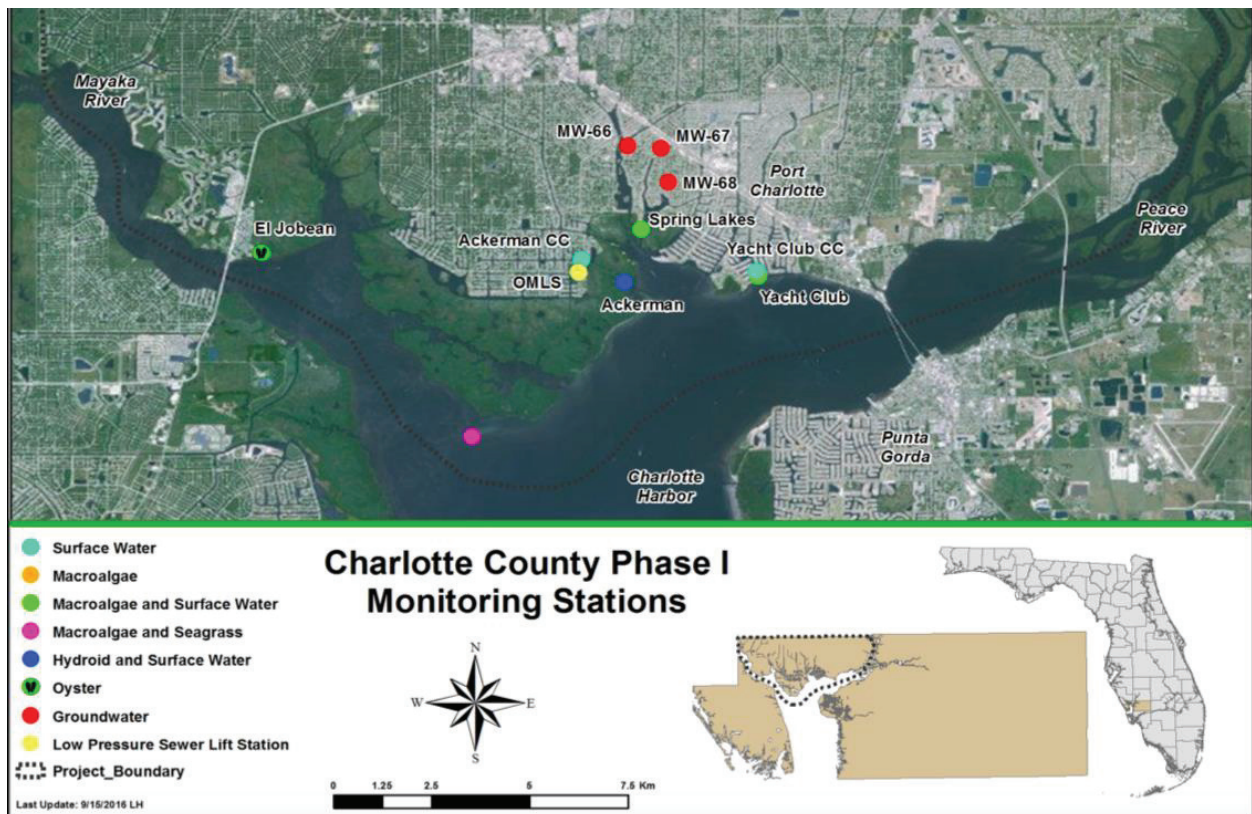


Figure 13. Reconnaissance sampling groundwater monitoring well (MW) and surface water sites and a low-pressure sewer lift station at the intersection of O’Hara and Midway roads (#23; OMLS) incorporated into the Phase I monitoring by Florida Atlantic University- Harbor Branch (HBOI-FAU) and Charlotte County.

3.1.4 Sucralose Sampling

As mentioned in sections 3.1.1 and 3.1.2, sucralose samples were collected on an outgoing tide on July 25, 2016 at the four sites in Charlotte Harbor, three monitoring wells (66, 67, and 68) in the Spring Lakes area, and OMLS. Individual samples were collected in 125 mL HDPE bottles,

placed on ice immediately after collection, frozen when returned to the HBOI-FAU laboratory, and then shipped to FIU-EARL for processing. At FIU-EARL, sucralose was analyzed in accordance with Batchu et al. (2015).

3.2. Results

3.2.1 Surface Water

Overall, average (n=11) surface water nutrient concentrations decreased from west to east in the study area. The El Jobean site had the highest average concentrations of TN and TP, with values decreasing at the more eastern sites (**Fig. 15a,b**). The average TN concentrations at all four monitoring sites were above the NNC for the tidal Myakka and Peace rivers. Additionally, TN concentrations were about 10 % higher than those from the historical East and West Spring Lake Wastewater Pilot Program dataset; this is most likely because the reconnaissance sampling was conducted on an ebbing tide when STE leaching from groundwater to surface water would most discernable. TN:TP ratios indicate that nitrogen is limiting (**Fig. 15c**). In contrast, chlorophyll *a* concentrations generally increased from west to east among the sampling sites, with the highest average value at Yacht Club (**Fig. 15d**). The average chlorophyll *a* at Yacht Club exceeded the NNC and values for Ackerman and Spring Lakes fell within the 95% SD. The concentrations of ammonia, nitrate + nitrite, and the dissolved inorganic nitrogen (DIN):SRP ratio generally decreased from west to east, with no pattern observed for soluble reactive phosphorus (SRP; **Fig. 16a,b,c,d**). Average BOD concentrations at these four sites ranged from 4.4-6.9 mg/L with maximum values as high as 12.1 (**Table 3**); for reference, clean tidal creeks have a BOD of about 2mg/L (Mallin et al., 2006).

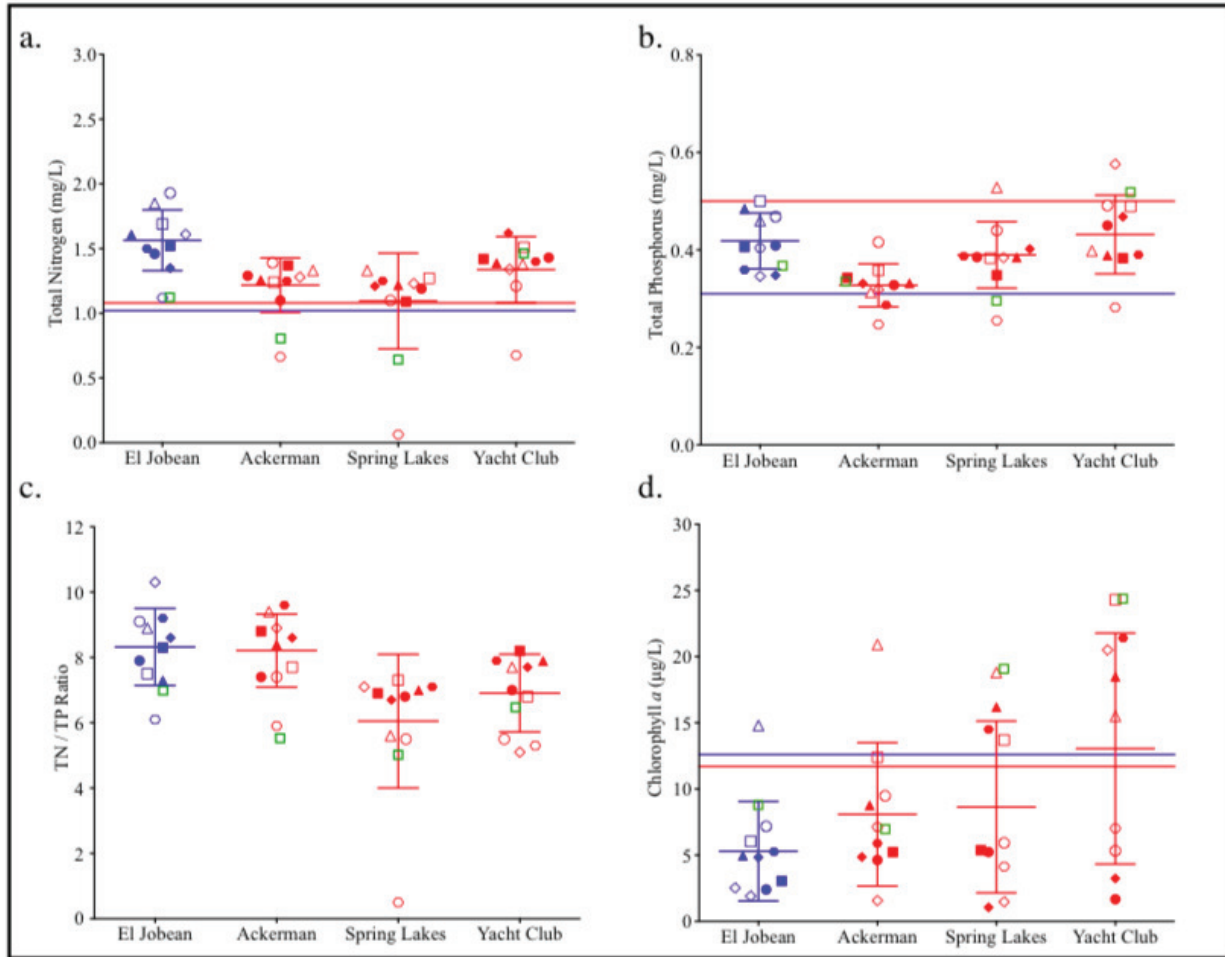


Figure 15. Nutrient concentrations (arithmetic mean \pm SD) observed in weekly reconnaissance sampling of surface waters (canals) within Charlotte County, FL from June 30 to August 31, 2016 showing the respective Numeric Nutrient Criteria (NNC) for surface water, including: a) total nitrogen (TN; NNC= 1.02 mg/L Tidal Myakka River and 1.08 mg/L Tidal Peace River), b) total phosphorus (TP; NNC=0.31 mg/L Tidal Myakka River and 0.50 mg/L Tidal Peace River), c) molar TN:TP ratios, and d) chlorophyll *a* levels (NNC= 12.6 Tidal Myakka River and 11.7 Tidal Peace River). Sampling dates are represented by differently, colored shapes: white triangle = week 1, white square = week 2, white circle = week 3, black triangle = week 4, black square = week 5, black circle = week 6, black diamond = week 7, white diamond = week 8, black hexagon = week 9, and white hexagon = week 10. Any values above the corresponding NNC line are in exceedance of the criteria. Green squares represent data collected by Florida Atlantic University-Harbor Branch Oceanographic Institute during reconnaissance sampling (June 24, 2016). Blue lines denote NNC values for the Tidal Myakka River and red lines denote NNC values for the Tidal Peace River.

3.3 Bacterial Abundance

Bacteria samples (fecal coliforms and *Enterococcus*) were collected during the weekly surface water monitoring events overseen by CCUD. The current standards established by Florida

Department of Health (DOH) Healthy Beaches Program for *Enterococcus* bacteria are: Good (0-35 *Enterococci* / 100 mL seawater), Moderate (36-70 *Enterococci* / 100 mL seawater), and Poor (71+ *Enterococci* / 100 mL seawater). Surface water standards for fecal coliforms are 400 MPN/100 mL (directly relatable to 400 cfu/100 mL) and the presence of fecal coliform bacteria in these samples indicates the presence of waterborne human pathogens and strongly suggests contamination via STE.

The highest average fecal coliform concentrations were observed at El Jobean and decreased from west to east among the sampling sites (**Fig. 17a**). El Jobean and Ackerman had samples that exceeded surface water quality criteria (400 MPN/100mL). A similar spatial trend was observed for *Enterococci* values, which averaged highest at El Jobean and decreased from west to east (**Fig. 17b**). Both the El Jobean and Ackerman sites had average values exceeding the standard for “poor” water quality according to the DOH Healthy Beaches program (>71 *Enterococci*/100 mL seawater).

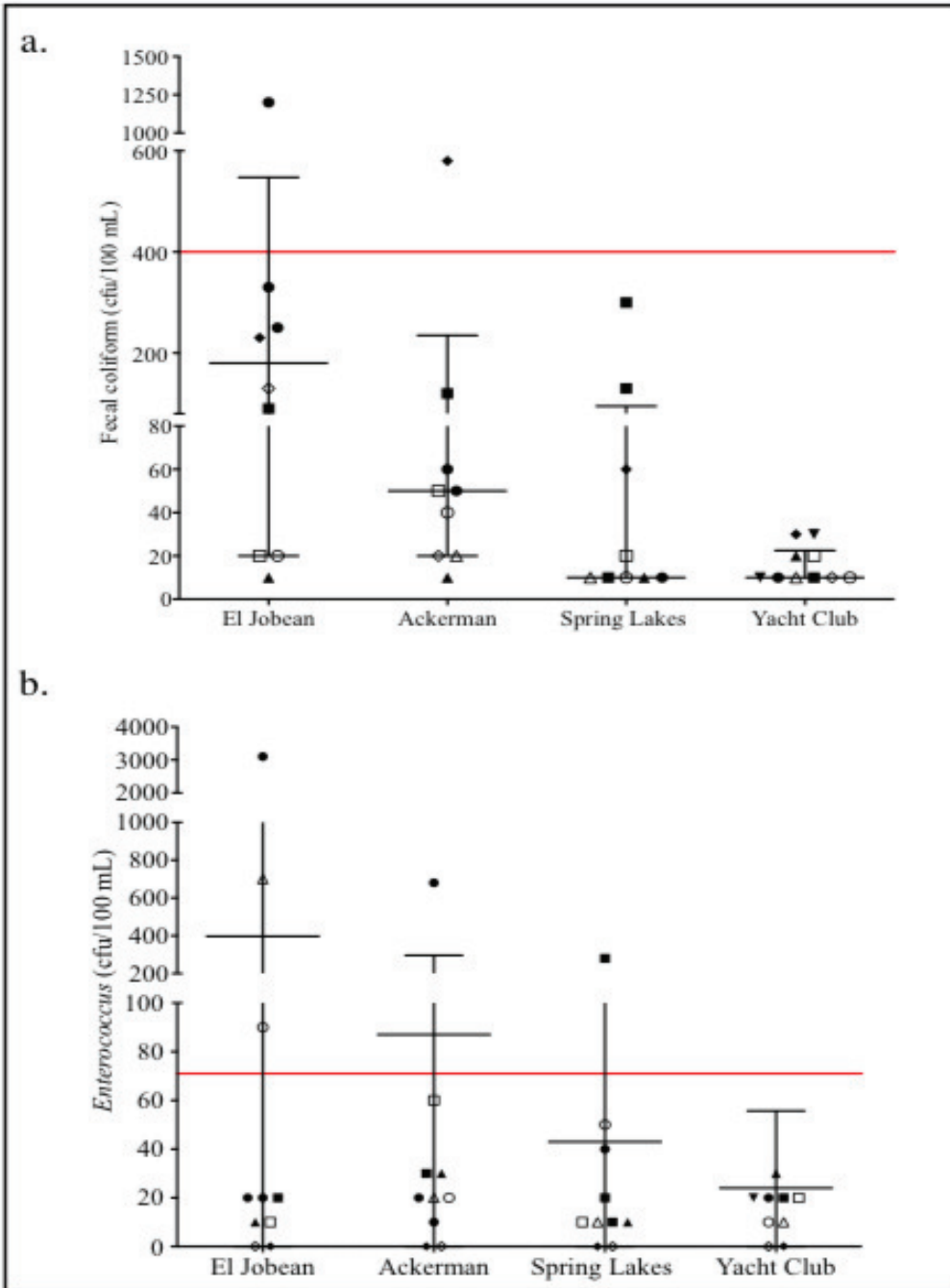


Figure 17. Bacterial abundance in surface waters during reconnaissance sampling in Charlotte County, including: a) fecal coliform, showing surface water quality criteria, and b) *Enterococcus* concentrations from sites within Charlotte County, showing the level at which the Department of Health Healthy Beaches Program considers water quality to be “Bad” (71 cfu/100mL; red line).

Table 3. Water quality parameters from reconnaissance sampling effort (06/30/2016-08/31/2016) at four sites in Charlotte Harbor (El Jobean, Ackerman, Spring Lakes, and Yacht Club) and the O'Hara-Midway low-pressure sewer lift station #23 (OMLS).

Parameter	Site	Ackerman	Spring Lakes	El Jobean	Yacht Club	OMLS
Enterococci (cfu/100 mL)	Mean	157	51	426	27	575000
	SD	271	83	964	30	328131
	Max	680	280	3100	110	960000
	Min	10	10	10	10	190000
	Number	10	10	10	10	6
Fecal Coliform (cfu/100 mL)	Mean	50	10	180	10	245000
	SD	291	98	1236	8	92682
	Max	850	300	4000	30	370000
	Min	10	10	10	10	160000
	Number	10	9	10	10	6
Chlorophyll <i>a</i> (µg/L)	Mean	8.1	8.6	5.3	13.1	na
	SD	5.4	6.5	3.8	8.7	na
	Max	20.9	18.8	14.8	24.3	na
	Min	1.57	1.05	1.91	1.67	na
	Number	10	10	10	9	na
Biological Oxygen Demand (mg/L)	Mean	5.155	4.250	6.891	4.389	39.233
	SD	3.782	4.052	4.761	4.244	53.558
	Max	11.9	10.8	12.1	11	148
	Min	0	0	0	0	12
	Number	10	10	10	10	6
Total Nitrogen (mg/L)	Mean	1.22	1.10	1.56	1.34	71.33
	SD	0.2	0.4	0.2	0.3	16.7
	Max	1.39	1.33	1.93	1.62	88
	Min	0.664	0.063	1.12	0.676	45
	Number	10	10	10	10	6
Nitrate+Nitrite (mg/L)	Mean	0.030	0.014	0.063	0.038	0.014
	SD	0.022	0.012	0.014	0.038	0.018
	Max	0.062	0.038	0.091	0.121	0
	Min	0.004	0.004	0.049	0.006	0
	Number	10	10	10	10	6
Ammonium (mg/L)	Mean	0.047	0.036	0.137	0.047	na
	SD	0.042	0.043	0.075	0.054	na
	Max	0.141	0.132	0.217	0.181	na
	Min	0	0	0	0	na
	Number	10	10	10	10	na
Total Kjeldahl Nitrogen (mg/L)	Mean	1.19	1.08	1.50	1.30	71.32
	SD	0.2	0.4	0.2	0.2	16.7
	Max	1.38	1.33	1.88	1.5	88
	Min	0.639	0.0528	1.03	0.67	45
	Number	10	10	10	10	6
Total Phosphorus (mg/L)	Mean	0.33	0.39	0.42	0.43	6.87
	SD	0.0	0.1	0.1	0.1	1.2
	Max	0.416	0.528	0.5	0.576	8
	Min	0.247	0.255	0.346	0.282	5
	Number	10	10	10	10	6

3.4 Sucralose concentrations

Sucralose concentrations at OMLS were greater than 60 µg/L, with lower, but elevated concentrations of approximately 10 µg/L in the groundwater monitoring wells, and concentrations less than 1 µg/L at the four surface water sites (**Appendix 2.3; Fig. 18a**). These concentrations are similar to those measured in a recent Martin County septic tank study and confirm contamination of surface waters with human-sourced pollutants from STE (**Fig. 18b**).

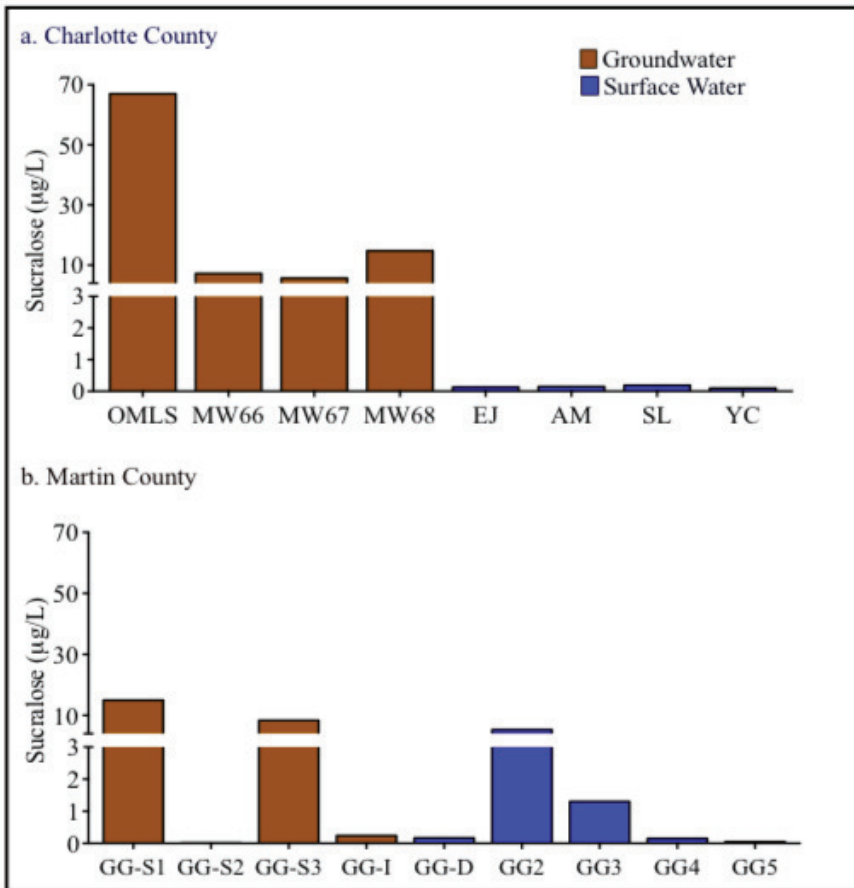


Figure 18. Sucralose concentrations (µg/L) from groundwater and surface water sites within a) Charlotte County, including O'hara-Midway low-pressure sewer lift station (OMLS) compared to sites with b) Martin County.

4.3 Existing Charlotte County Resources (page 31)

Charlotte County, specifically CCUD, contracted several laboratories throughout the course of the East and West Spring Lake Wastewater Pilot Program. It will be important that these and/or similar labs be used as the project progresses so that results are comparable. Contracted labs and their respective responsibilities include:

A) Benchmark EnviroAnalytical, Inc.

Certified by the National Environmental Laboratory Accreditation Program (NELAP) and accredited by Florida's Department of Health. Benchmark EnviroAnalytical, Inc. was contracted to perform analysis on groundwater and surface water throughout the Spring Lakes area. Parameters analyzed in reconnaissance testing all met National Environmental Laboratory Conference Institute (NELAC) standards. Benchmark EnviroAnalytical, Inc. conducts the field sampling as well as the laboratory analysis for reconnaissance sites, including TN, nitrate + nitrite, ortho-phosphorus, TP, chlorophyll *a*, ammonium as NH₄, fecal coliforms, and others. Benchmark EnviroAnalytical, Inc. also completed the BOD analyses for the East and West Spring Lake Wastewater Pilot Program.

B) Charlotte Country East Port Laboratory

NELAP certified with the ability to analyze water samples using methods that are in compliance with NELAC standards. During the East and West Spring Lake Wastewater Pilot Program they have been utilized as an intermediary to hold samples during transfer between Johnson Engineering and Benchmark EnviroAnalytical, Inc. Additionally, they have analyzed samples from the East and West Spring Lake Wastewater Pilot Program for all parameters, excluding BOD. They have state certifications to process groundwater, surface water, and stormwater, and there is potential that they will be called upon for more analyses in future phases.

C) Tetra Tech

Contracted since the onset of East and West Spring Lake Wastewater Pilot Program in 2012. Tetra Tech gathers and analyzes data to make determinations and recommendations for Charlotte County's East and West Spring Lake Wastewater Pilot Program. As per the East and West Spring Lake Quality Assurance Project Plan (QAPP), Task Memo 2, some of the key Phase I tasks included creating a database for surface water and groundwater and evaluating groundwater depths in the monitoring wells to determine flow paths and identify potential monitoring septic plumes (Frick et al., 2015).

D) Johnson Engineering

Subcontracted by Tetra Tech, Johnson Engineering's QAPP was established to quantify the nutrient load reduction achieved by the Revitalize Impaired Waters of Charlotte Harbor- East and West Spring Lakes Project (Johnson Engineering, 2015). Johnson Engineering will conduct pre and post construction sampling at five sampling sites to assess stormwater effects. The QAPP was established to assure QAQC and adherence to appropriate FDEP standard operating procedures throughout the duration of the project. Samples collected by Johnson Engineering are analyzed at the Charlotte Country East Port Laboratory.

PHASE II: Implement Long-Term Sampling to Document Pre-Post Construction Conditions in the Watershed, Myakka and Peace Rivers, and Charlotte Harbor

1. Dissolved Nutrient Concentrations – surface and groundwater
2. Sucralose Concentrations – surface and groundwater
3. Microbial (Enterococcus and Coliform) Counts in surface water
4. Stable N Isotope Signatures and C:N:P in submerged vegetation

PART IV - Fecal Coliform Testing (Wiki): Fecal coliform

https://en.wikipedia.org/wiki/Fecal_coliform

Analysis

Main article: [Bacteriological water analysis](#)

Bacteria reproduce rapidly if conditions are right for growth. Most bacteria grow best in dark, warm, moist environments with food. When grown on solid media, some bacteria form [colonies](#) as they multiply which may grow large enough to be seen. By growing and counting colonies of fecal coliform bacteria from a sample of water, the amount of bacteria originally present can be determined.

[Membrane filtration](#) is the method of choice for the analysis of fecal coliforms in water. Samples to be tested are passed through a filter of particular pore size (generally 0.45 [micrometre](#)). The microorganisms present in the water remain on the filter surface. The filter is placed in a sterile [petri dish](#) with a selective [medium](#), growth of the desired organisms is encouraged, while other non-target organisms is suppressed. Each cell develops into a separate colony, which can be counted directly, and the initial inoculum size can be determined.

Typically sample volumes of 100 ml will be used for water testing and filtered, with the goal of achieving a final desirable colony density range of 20 to 60 colonies per filter. Contaminated sources may require dilution to achieve a "countable" membrane. The filter is placed on a petri dish containing M-FC [agar](#) and incubated for 24 hours at 44.5 °C (112.1 degrees F). This elevated temperature heat shocks non-fecal bacteria and suppresses their growth. As the fecal coliform colonies grow they produce an acid (through fermenting lactose) that reacts with the aniline dye in the agar thus giving the colonies their blue color.

Newer methods for coliform detection are based on specific enzyme substrates as indicators of coliforms. These assays make use of a sugar linked to a dye which, when acted on by the enzyme [beta-galactosidase](#), produces a characteristic color. The enzyme beta-galactosidase is a marker for coliforms generally and may be assayed by hydrolysis of enzyme specific glycosides such as o-nitrophenyl-beta-D-galactose. Assays typically include a second sugar linked to a different dye which, when acted on by the enzyme beta-glucuronidase, produces a fluorescent product. Because E. coli produces both beta-galactosidase and [beta-glucuronidase](#), a

combination of two dyes makes it possible to differentiate and quantify coliforms and *E. coli* in the same pot.

More recently, the chemistry behind enzymatic detection compounds has been updated so that the indicating component is redox active, as opposed to the more usual chromogenic format, allowing fecal indicator bacteria such as *E. coli* and *E. faecalis* to be detected electrochemically without any sample pre-treatment. Since the colour of the detection compound is of no consequence, this allows detection in deeply coloured matrices. [\[5\]](#)