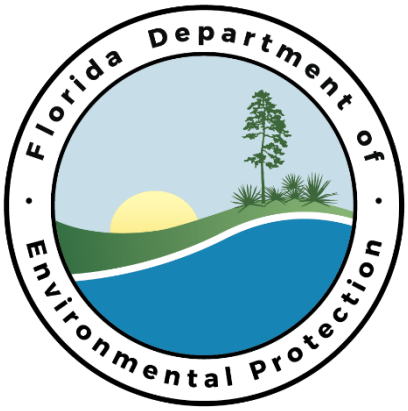
Follow-up Study of Clam Bayou, Pinellas County, Florida



Water Quality Standards Program
Florida Department of Environmental Protection

March 2017

Introduction

This document presents the results of a study of Clam Bayou conducted in 2016. The study was requested by the City of Gulfport following discharges of untreated sewage into the stormwater system draining into Clam Bayou following heavy rainfall in July and August 2015. This study replicated most of the sampling conducted during a previous 2011 study (water quality, sediments, and selected biological communities) and additionally collected bacteria samples and microbial source tracking (MST) analyses designed to evaluate the potential impacts of the stormwater discharges on bacterial levels in the bayou (**Table 1**). This document also compares the results of the current study with those of the 2011 study to evaluate the effectiveness of restoration efforts in the Clam Bayou watershed.

Clam Bayou is a 170-acre estuary in Pinellas County, Florida, that is surrounded by the cities of Gulfport to the west, St. Petersburg to the north and east, and Boca Ciega Bay to the south. Beginning in the 1920s, the Clam Bayou watershed was intensively urbanized, which substantially altered the hydrology of the system. In 1995, land was acquired and construction projects were undertaken to restore native habitats and treat stormwater. The project was funded in three phases over a 17-year period. The stormwater treatment and habitat restoration project was completed in early 2012.

In 2011, in response to a legal complaint (Case No. 8:09-cv-1070-T-17-TBM) that asserted that urban stormwater was responsible for increased sediment loads and the introduction of chemical contaminants that reduced the health of the biological communities throughout the bayou, the Florida Department of Environmental Protection (DEP) conducted a study (DEP 2012) to ascertain the effects of stormwater runoff on the biological, water, and sediment quality in Clam Bayou. The study found elevated levels of several chemicals in fish tissue samples of three fish species from three different trophic levels (common snook, striped mullet, and sheepshead). The target fish concentration (TFC) was exceeded for cis and trans-chlordane in the tissue of both the common snook and the striped mullet, and the TFC for dieldrin was exceeded in all three species. Both chemicals are pesticides that have been banned for use in the U.S. for over twenty years. The TFC for total Polycyclic Aromatic Hydrocarbons (PAHs) was also exceeded in all three species tested.

The study also found elevated levels of contaminants in the sediments of Clam Bayou. Sediment results indicated that the mean concentration for 10 of the 81 compounds analyzed were above the Probable Effect Level (PEL). Eight of those compounds were PAHs, and the other two compounds were cis and trans-chlordane and DDT, both of which are banned pesticides.

Objectives

1. Collect water, sediment, fish tissue, and benthic macroinvertebrate samples from Clam Bayou and compare the results to those from 2011 to assess the effectiveness of the stormwater treatment and habitat restoration enhancements in the Clam Bayou watershed.
2. Monitor several locations in and around Clam Bayou to evaluate potential impacts to the bayou from the diversion of untreated sewage into the stormwater treatment ponds in 2015 and 2016 due to heavy rains.

Methods

Where applicable, all field and laboratory methods followed DEP Standard Operating Procedures (DEP SOPs, see http://www.dep.state.fl.us/water/sas/sop/sops.htm for details). The DEP Quality Assurance Rule (Chapter 62-160, FAC) applied to all sampling, analysis, and data usability evaluations (see http://www.dep.state.fl.us/water/sas/qa/index.htm). All water, sediment, and fish tissue samples were analyzed by the DEP Laboratory in Tallahassee. Benthic macroinvertebrate samples were collected by the Pinellas County Watershed Management Department staff and processed and analyzed by the Environmental Protection Division of Hillsborough County (EPCHC).

Microbial Source Tracking and Bacterial Indicators

DEP has developed several sophisticated microbial source tracking (MST) tools since 2011 to provide a more complete picture of restoration needs related to potential pathogens. Tracking efforts to date have focused on identifying waters affected by untreated human fecal waste because it represents the greatest potential human

health risk. The DEP lab uses two chemical tracers, the artificial sweetener sucralose (Splenda®) and the pain reliever acetaminophen (Tylenol®), and two molecular markers, the human associated Bacteroides HF183 marker to distinguish human from non-human sources of fecal pollution, and the Gull2 marker for coastal bird fecal sources (*e.g.,* wading birds, shorebirds, and coastal pigeons).

Sucralose and acetaminophen enter wastewater treatment plants at similar high concentrations compared to other pharmaceuticals (Du *et al*. 2014). Sucralose passes through our bodies and wastewater treatment plants with very little degradation. A 2012 FDEP survey of 58 Florida domestic wastewater facility effluents found sucralose concentrations ranging from <1.0 µg/L to 41.0 µg/L, with a mean of 19.0 µg/L. Sucralose is also quite stable in the environment, making it an excellent tracer of human wastewater. Due to its stability through the wastewater treatment process, sucralose alone cannot discriminate untreated from treated wastewater sources. Acetaminophen, on the other hand, is very efficiently removed by common wastewater treatment processes (*e.g.*, three log reduction) and is rarely observed in treated wastewater. When similar levels of sucralose and acetaminophen are observed in surface waters (within the same order of magnitude), the human wastewater source is most likely untreated.

The Bacteroides HF183 and Gull2 molecular markers are analyzed using Quantitative Polymerase Chain Reaction (q-PCR), that quantifies portions of DNA sequences specific to the organisms of interest, including bacterial markers strongly or uniquely associated with human fecal waste. Identification of the specific sources of fecal indicators allows for more accurate risk assessments, since human fecal contamination (*e.g.*, sewage) is much more likely to contain human pathogens than fecal material from other sources.

In 2016, DEP adopted new Class III criteria for bacteria for fresh and marine waters (Rule 62-302.530(11) (c, d))[1] that are based on recommended values by the Environmental Protection Agency (EPA). *Escherichia coli* (*E. coli*) replaced fecal coliforms in freshwater, and enterococci became the criterion for marine waters. Fecal coliforms criteria now only apply to Class II (shellfish harvesting) waters, and fecal coliform results provided in this study are presented for informational purposes. The new criteria include a monthly geometric mean (MGM) value (126 for *E. coli* and 35 for enterococci) and an upper value not to be exceeded in more than 10% of the samples in a 30-day period (Ten Percent Threshold Value or TPTV; 410 for *E. coli* and 130 for enterococci). The MGM requires a minimum of 10 samples in a 30-day period in Class III waters, which was not achieved in this study, and thus only the TPTV applies.

On August 4, 2015, in response to the diversion of untreated and partially treated sewage into the stormwater treatment ponds, the City of Gulfport began daily monitoring for fecal coliforms and enterococci at four sites in the Clam Bayou stormwater ponds and at two sites as the water exits into Boca Ciega Bay, and weekly sampling of MST analytes (**Figure 1**). The City suspended the sampling in Clam Bayou on October 27, 2015, after levels consistently met the criteria for the Florida Department of Health's (FDOH) Healthy Beaches Program for nearly three weeks. FDOH Healthy Beach Program guidelines can be found on the FDOH website: http://www.floridahealth.gov/environmental-health/beach-water-quality/index.html. The City then began collecting daily enterococci samples at one location in Clam Bayou (Gulfport Shores/Clam Bayou seawall) and two sites in Boca Ciega Bay (Gulfport Marina and Gulfport Beach, **Figure 2**). From mid-February 2016 through mid-March 2016, the City of Gulfport collected weekly MST samples along with their daily routine bacteria sampling. The source tracking samples were analyzed at the DEP laboratory, and the routine bacteria samples were processed at the St. Petersburg Environmental Compliance Laboratory. Sampling results and other information related to water quality in the Gulfport area can be found on the city's website: http://mygulfport.us/waterquality/.

In March 2016, MST sampling was expanded as part of the current study to cover sites labeled as "MST" in **Figure 3** (also **Table 1**). These sites were intended to capture the inputs into the three stormwater ponds, the levels as they exit each of the ponds, and the concentrations throughout Clam Bayou. Surface water samples were collected by DEP following DEP SOP FS 2100 on four occasions (March 9, March 22-23, May 5, and July 27, 2016) to capture different hydrologic conditions. Sampling was designed to collect samples during a drier period, immediately after a heavy rain event, and during the wet season (**Figure 4**). Samples for bacteriological indicators were not collected on March 23 due to a scheduling error. Wet season samples were scheduled once there was a clear increase in surficial groundwater levels near Clam Bayou (**Figure 5**; Source: Southwest Florida Water Management District Information System, 21134 ROMP TR 13-1X).

[1] Proposed rules were approved by the Environmental Regulation Commission on December 9, 2015 and went into effect for State purposes in February 2016. However, they must be approved by the EPA before going into effect for Clean Water Act purposes.

Figure 1. City of Gulfport post-release monitoring locations. (source: http://mygulfport.us/waterquality/.)

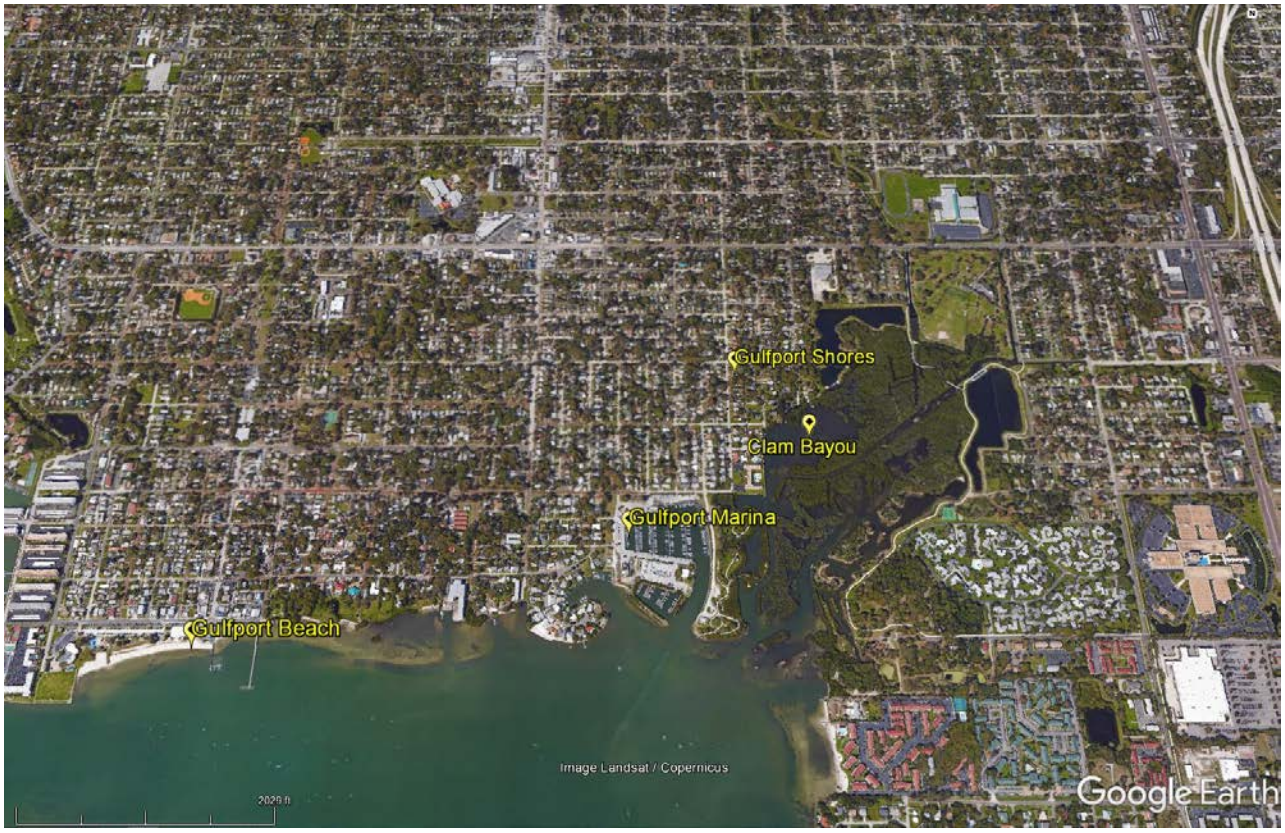


Figure 2. City of Gulfport routine monitoring locations.

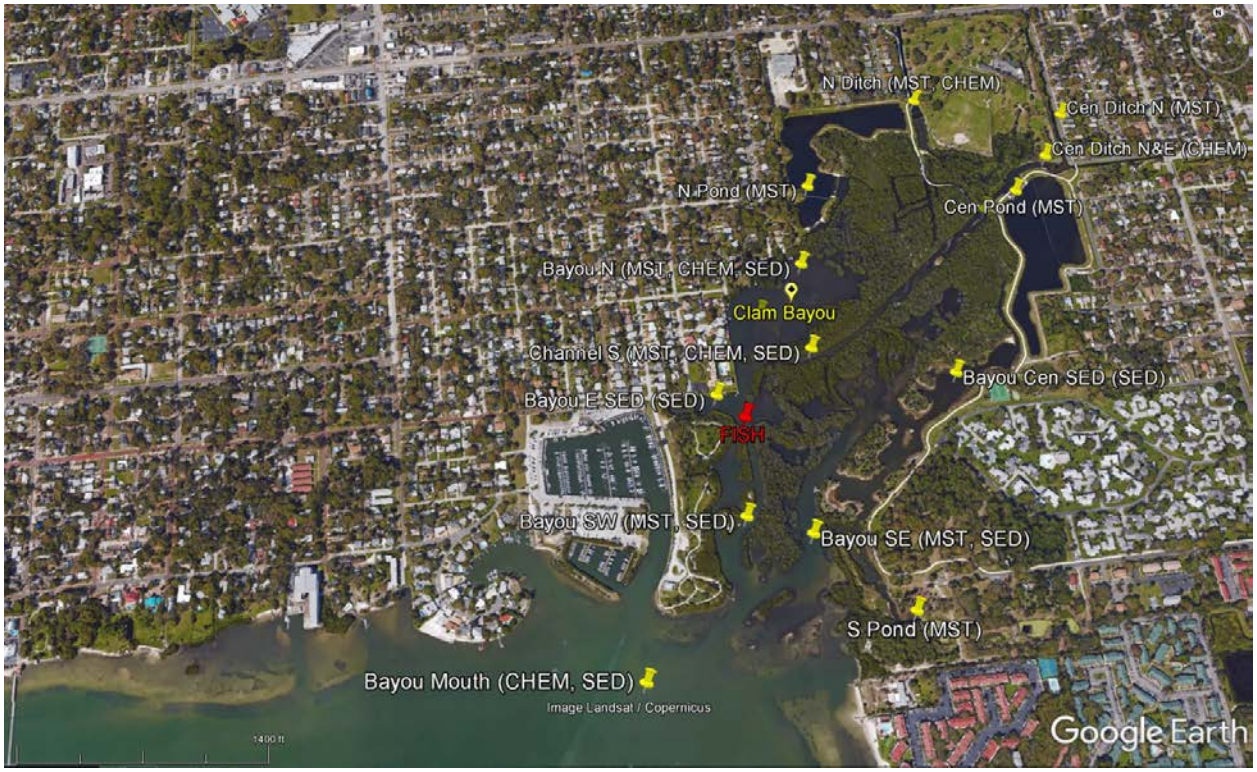


Figure 3. Sampling locations and analyte groups collected at each location in Clam Bayou and the surrounding area.

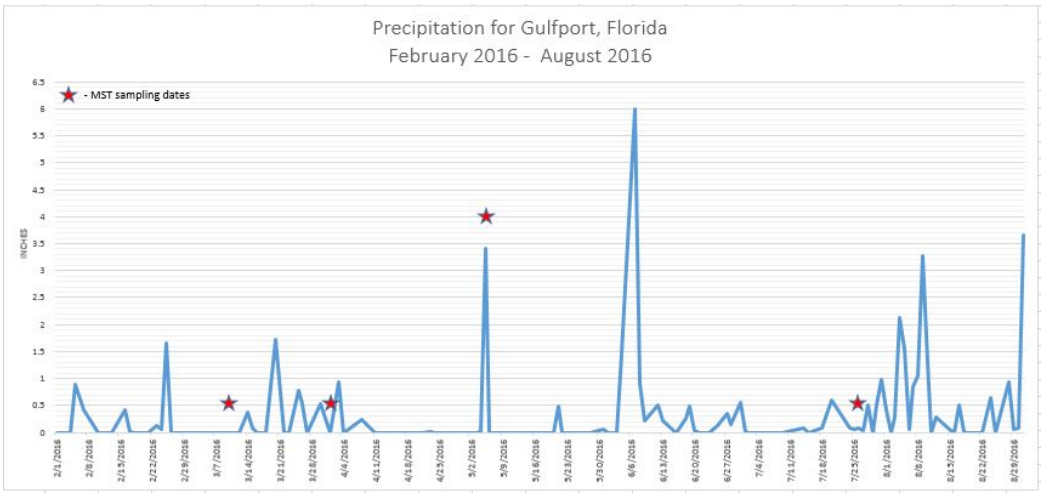


Figure 4. Rainfall totals in inches for Gulfport, Florida. February - August 2016 (Source: Climod Gulfport 0.9 NNW, FL).

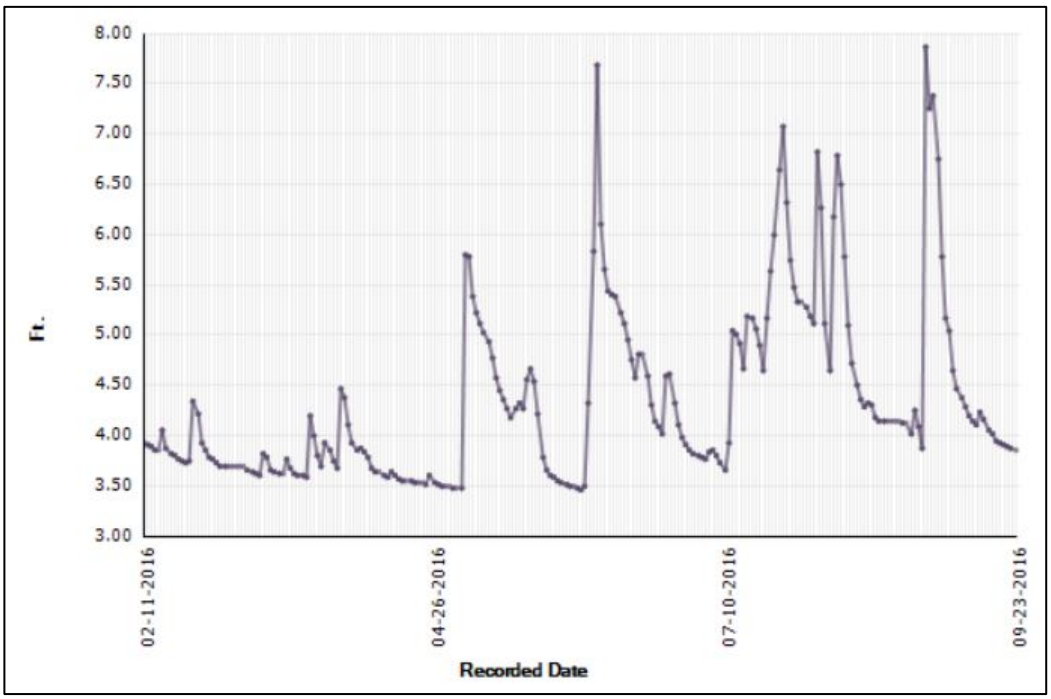


Figure 5. Surficial aquifer levels in the Clam Bayou area, February 2016 – September 2016.

Water Chemistry

Nutrients, turbidity, color, alkalinity, metals, and organic contaminants (pesticides and base-neutral/acid extractable compounds, and PAHs) were collected by DEP following DEP SOP FS 2100 on March 22-23, May 5, and July 27 at sites labeled as "CHEM" in **Figure 3** (also see **Table 1**). The nutrient suite included analysis of total Kjeldahl nitrogen (TKN), ammonia, nitrate+nitrite, and total phosphorus. Metals included arsenic, cadmium, calcium, chromium, copper, iron, lead, magnesium, manganese, mercury, potassium, sodium, strontium, and zinc. Two samples were collected in ditches prior to entering Clam Bayou: one in the North Ditch (N Ditch) and a second from the confluence of the North Central and East Central ditches (Cen Ditch N&E). Three sites were sampled after the water passed through the treatment ponds and into Clam Bayou: one from the terminus of the central channel that runs roughly through the center of Clam Bayou (Channel S), one in the north lobe of the bayou (Bayou N), and one just prior to mixing with Boca Ciega Bay (Bayou Mouth, **Figure 3**).

Metered parameters (dissolved oxygen, specific conductance, pH, and temperature) were also measured at each water chemistry station. Measurements followed DEP SOPs FT 1000, FT 1100, FT 1200, FT 1400, and FT 1500.

For water chemistry, results were evaluated against applicable Class III water quality criteria from Chapter 62-302, F.A.C. Sites were categorized as predominantly marine or predominantly fresh using a conductivity threshold of 4,850

µmhos/cm (**Table 1**). During the current study, the North and Central Ditch sites were predominantly fresh, and the Channel and Bayou sites were predominantly marine. Class III water quality criteria do not apply within the stormwater ponds. The numeric nutrient criteria do not apply to ditches, canals and other man-made conveyances that are predominantly used for water management purposes as is the case for the ditches sampled in this study. In lieu of numeric standards, the narrative nutrient criteria apply; however, information on the biological communities must be considered to interpret the narrative nutrient criterion, and that information was not collected during this study.

Sediment

Sediments were collected by DEP following DEP SOP FS 4000 (sediment sampling) from nine sites in Clam Bayou with a petite ponar on March 23 and April 28, 2016, and are identified as "SED" in **Figure 3**. The sediment collection locations were intended to represent various areas throughout Clam Bayou and to evaluate changes after the completion of the restoration project. All samples were analyzed for 81 priority pollutants and compared to sediment PEL (MacDonald 1994) to determine potential environmental risk. Samples from both dates were analyzed for total phosphorus, organic contaminants (pesticides, total PAHs[2], and base-neutral/acid extractable compounds), and metals (arsenic, cadmium, calcium, chromium, copper, iron, lead, magnesium, mercury, potassium, sodium, strontium, and zinc). A particle size analysis was performed on the samples collected on April 28, 2016.

In 2011, DEP evaluated the Total PAH results by replacing data that were detected below the minimum detection limit (MDL = "U") with ½ of the MDL for each of the thirteen (13) individual compounds and all data qualified with an "I" were replaced with the MDL. One unintended consequence of this approach was that total PAH values could exceed the threshold even if individual PAHs were undetected in all the samples. DEP recognized this problem, and in the current study analyzed the sediment total PAH data in a way that is consistent with requirements in subsection 62-303.320(12), F.A.C. Specifically, data qualified as less than the detection limit ("U") were replaced with 1/26th of the MDL to account for 13 component PAHs in the total. All data qualified with an "I" were once again replaced with the MDL.

Fish Tissue

As in the 2011 DEP study, Florida Fish and Wildlife Conservation Commission (FWC) biologists collected 12 individuals of Common Snook (*Centropomus undecimalis*), Sheepshead (*Archosargus probatocephalus*), and Flathead Mullet (*Mugil cephalus*) from a popular fishing area of Clam Bayou (labeled as "FISH" in **Figure 3**). These fish species were chosen to represent different trophic levels and feeding strategies among common game fish in the area. Fish were collected by hook and line fishing techniques in a single collection effort on March 30, 2016, and filet samples were prepared by FWC per DEP SOP FS 6200 (finfish tissue sampling) and shipped to the DEP Central Lab for analysis. Filets were analyzed for 63 chemicals, including pesticides, metals, and PAHs.

For contaminants that exceeded detection limits, DEP calculated a TFC level in fish tissue that would estimate the associated water column water quality criterion. In 2011, DEP evaluated the Total PAH results by replacing data that were detected below the minimum detection limit (MDL = "U") with ½ of the MDL for each of the ten (10) individual compounds. As was mentioned in relation to sediment total PAHs, a potential result of this approach is to end up with a value that exceeds the threshold even though individual PAHs were undetected in all the samples. In the current study, DEP replaced data qualified as less than the detection limit ("U") with 1/20th of the MDL to account for 10 component PAHs in the total. All data qualified with an "I" were once again replaced with the MDL. For chlordane (cis- and trans-chlordane), a banned pesticide, data qualified as less than the detection limit were replaced with 1/4 of the TFC or 1/4 of the MDL, whichever was lower. Data for DDE-p,p', a banned pesticide, reported as less than the detection limit were replaced with 1/2 of the TFC or 1/2 of the MDL, whichever was lower. Data for dieldrin, a banned pesticide, reported as less than the detection limit were replaced with 1/2 of the TFC or 1/2 of the MDL, whichever was lower.

Biological Health

Benthic Macroinvertebrates

Benthic macroinvertebrates were collected on August 25, 29, and 30, 2016, by Pinellas County Watershed Management Department staff and processed and analyzed by the Environmental Protection Division of Hillsborough County (EPCHC) as part of the annual Tampa Bay Estuary Program's Special Study Sites (Karlen *et al*. 2017). Samples for benthic macroinvertebrate community analysis were taken at ten sites within Clam Bayou (**Figure 6**) using a Young-Modified Van Veen grab sampler. The macroinvertebrate sampling locations were

[2] Total PAHs is a combination of seven low molecular weight PAHs (acenaphthene, acenaphthylene, anthracene, fluorene, 2-methylnaphthalene, naphthalene, and phenanthrene) and six high molecular weight PAHs (benzo(a)anthracene, benzo(a)pyrene, chrysene, dibenzo(a,h)anthracene, fluoranthene, pyrene).

intended to represent the various areas throughout Clam Bayou and to duplicate work done in previous studies to be able to analyze changes over time and since the implementation of restoration efforts (Karlen *et al.* 2009). Benthic sorting and identification work was conducted by EPCHC staff. Benthic sediment samples were rough sorted under a dissecting microscope into general taxonomic categories (Annelids, Molluscs, Crustaceans, and Miscellaneous Taxa). Resorting was done on 10% of the samples completed by each technician for QA/QC. The sorted animals were identified to the lowest practical taxonomic level (species level when possible, per DEP SOP LT 7900) and counted. Taxonomic identifications were conducted using available identification keys and scientific literature. All identification and count data were recorded on laboratory bench sheets and entered into a Microsoft Access® database maintained by the EPCHC.

PRIMER v7 software (PRIMER-E, Ltd. 2015; Clarke and Gorley 2015) was used for all multivariate statistical analysis and for calculating univariate biological metrics (species richness, abundance, Shannon diversity index and Evenness) (Karlen *et al.* 2017). Species richness (*S*) was defined as the total number of taxa. Abundance (*N*) was expressed as the number of individuals per m^2 (calculated as the raw count x 25) except for colonial organisms, which were counted as present/absent. The Shannon diversity index (*H'*) calculations employed the natural logarithm opposed to log base 2 (Clarke and Warwick 2001). The zero-adjusted Bray-Curtis similarity (Clarke *et al.* 2006) was calculated on square root transformed abundance data and the resulting similarity matrix was used for running Cluster Analysis, Non-metric Multi-Dimensional Scaling (MDS), Similarity Percentage (SIMPER), and Analysis of Similarity (ANOSIM). The BIO-ENV procedure (Clarke and Ainsworth 1993) was used to find correlations between the environmental parameters and benthic community structure. All environmental parameters were normalized and log transformed prior to analysis.

Bird Observations

Information on birds in the Clam Bayou study area from 1988-2010 were compiled and provided by Ron Smith of the National Audubon Society as part of their annual Christmas Bird Counts (CBC) for the St. Petersburg, Florida Circle (FLSP). The CBC is an established program of the National Audubon Society during which thousands of citizens across the US and Canada count birds over a 24-hour period between December 14 and January 5 each year. Volunteers count every bird encountered during the day in designated areas within a 15-mile diameter circle. All birds are counted, indicating the total number of birds in the circle that day. The list of birds presented for the Clam Bayou area are those that are aquatic or shoreline-dependent.

Table 1. STORET station names, analyte groups collected, and applicable water quality standards for Clam Bayou sampling sites.

STORET Station	Site	MST Collected	Water Chemistry Collected	Sediments Collected	Applicable Class III Standards
PIN16CLB1	N Ditch	X	X		Fresh
PIN16CLB2	N Pond	X			N/A
PIN16CLB3	Cen Ditch N	X			Fresh
PIN16CLB4	Cen Ditch E	X			Fresh
PIN16CLB5	Cen Pond	X			N/A
PIN16CLB6	S Pond	X			N/A
PIN16CLB7	Channel N	X		X	Marine
PIN16CLB8	Channel S	X	X	X	Marine
PIN16CLB9	Bayou N	X	X	X	Marine
PIN16CLB10	Bayou SW	X		X	Marine
PIN16CLB11	Bayou SE	X		X	Marine
PIN16CLB12	Cen Ditch N&E		X		Fresh
PIN16CLB13	Bayou N SED			X	Marine
PIN16CLB14	Bayou Cen SED			X	Marine
PIN16CLB15	Bayou E SED			X	Marine
PIN16CLB16	Bayou Mouth		X	X	Marine

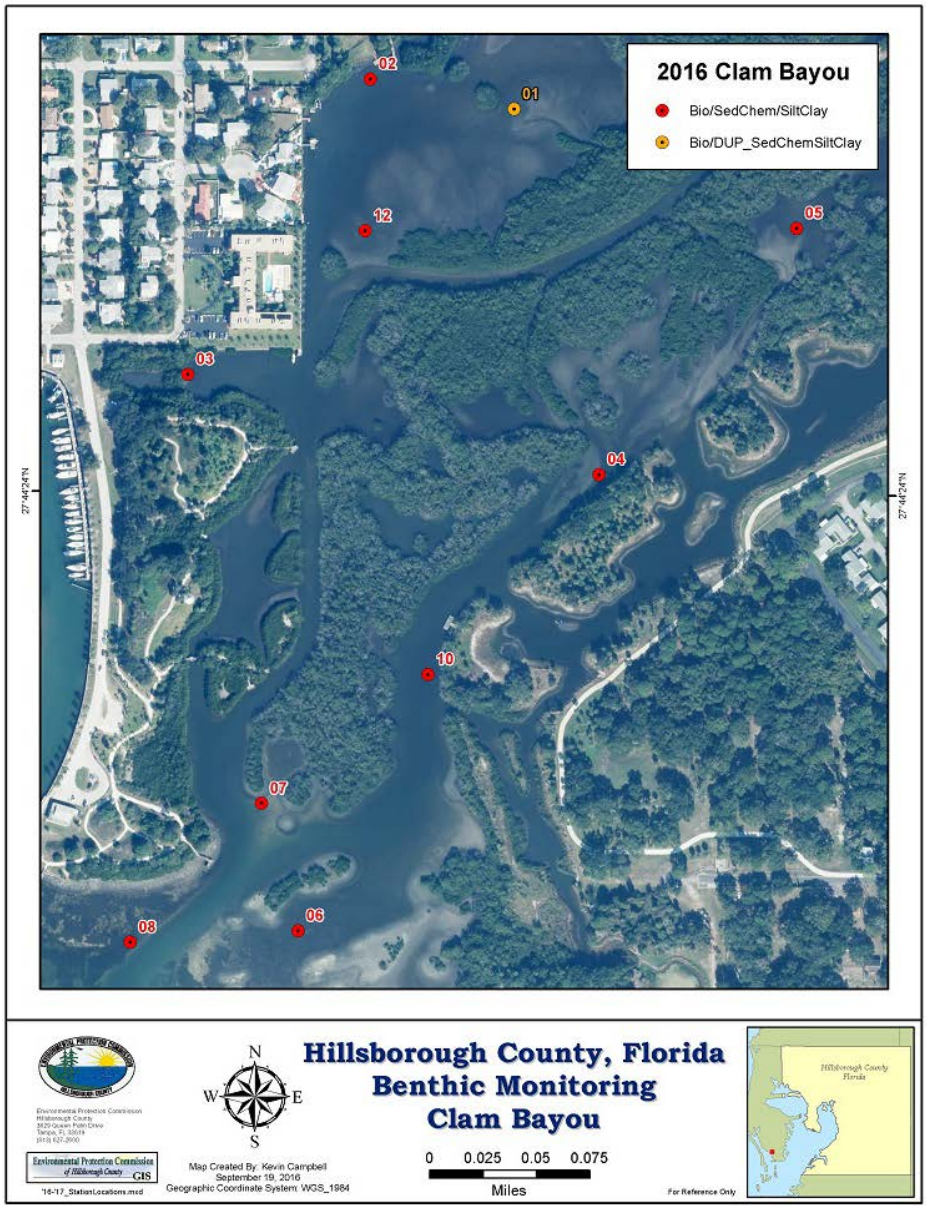


Figure 6. Invertebrate sampling locations within Clam Bayou (from Karlen *et al.* 2017).

Results

Microbial Source Tracking

City of Gulfport MST Sampling: February 16, 2016 – March 14, 2016

Acetaminophen was detected in samples collected by the City of Gulfport at the Gulfport Marina on February 16 (0.0064 µg/L) and February 29, 2016 (0.0043 µg/L). Sucralose was detected at similar levels at all locations and dates (0.14 µg/L – 0.26 µg/L). The human fecal source marker, HF183, was undetected at all locations and dates. The gull source marker, Gull2, was detected during the February 16, 2016, sampling at Gulfport Beach. Detailed results of samples processed at the City of Gulfport Laboratory can be found on the city's website: http://mygulfport.us/waterquality/.

DEP MST Sampling March 9, 2016 – July 27, 2016

A wide range in concentrations of bacteriological indicators and microbial tracers were detected at MST sites during the study, with the highest indicator concentrations on May 5, when *E. Coli* and enterococci exceeded the Class III Fresh and Marine water quality criteria, respectively (**Table 2**; Note that Class III Water Criteria do not apply to the treatment ponds). It should be noted that *E. coli* and enterococci samples collected on May 5 were analyzed outside the acceptable holding time. Sucralose was detected at low levels at all sites on all sampling dates except

for South Pond during the May 5 and August 27 sampling events where the sucralose had higher results of 3.7 and 3.6, respectively. Acetaminophen was detected at low levels in two of the stormwater ponds in the March 22/23 sampling event, and was detected at moderate levels at all sites on May 5 and several locations on July 27 (**Table 2**). The human fecal source marker, HF183, was detected at low levels in the North Ditch on March 22/23 and in the Central Pond on May 5 sampling events. There were no other detections of the HF183 marker. High levels of the Gull2, Gull and shore birds fecal source marker, were detected at most sites including ditches, stormwater ponds, and in the main channels of Clam Bayou on May 5. There were also two detections in the channel and Bayou on the March 9 sampling event. Samples for bacteriological indicators were not collected on March 22-23, 2016, due to a scheduling error.

Water Chemistry

Water chemistry results that were at or above the practical quantitation level (PQL) are presented for March 22-23 (**Table 3**), May 5 (**Table 4**), and July 27, 2016 (**Table 5**). **Table 6** shows the mean concentrations for all sampling events. Although several significant rain events and an increasing trend in groundwater levels occurred in the Clam Bayou area from the onset of the study to the rainy season sampling on July 27, individual analytes did not vary greatly among the sampling events and no additional constituents exceeded the Class III Fresh or Marine Criteria (**Tables 3**, **4** and **5**).

There was insufficient data to calculate annual geometric means for numeric nutrient determination purposes. The single sample total phosphorus values exceeded the thresholds for the Peninsula region (0.12 mg/L) in the North Ditch in March, May, and July, 2016, and in the combined Central North and East Ditch in May. The mean concentration of total phosphorus (0.19 mg/L) also exceeded the threshold in the North Ditch. Numeric nutrient criteria were not adopted until December 2011, but the mean concentration of total phosphorus in the Central North and East Ditch (0.29 mg/L at CLB 4 in the 2012 report) was above the freshwater threshold at that time (FDEP 2012). The numeric nutrient criteria do not apply to ditches, canals and other man-made conveyances that are predominantly used for water management purposes as is the case for the ditches in this study. Further, numeric thresholds must be evaluated as annual geometric means and in conjunction with floral and faunal information.

Three organic contaminants (atrazine, fluoranthene, and metribuzin) were detected at levels that were above the practical quantitation limit, but below the Class III criteria, where applicable. Two organic compounds (fluoranthene and norflurazon) were detected in 2011. Concentrations of total ammonia and nitrate+nitrite were generally higher in the freshwater ditches than in the marine bayou, which is expected for these forms of nitrogen. TKN concentrations were mostly consistent among sites for all sampling dates, though concentrations were higher in the ditch sites than in the bayou sites on March 22-23. Turbidity was higher in the bayou sites than in the ditch sites, but levels were low for all sites and dates. The metals that were elevated at the marine Clam Bayou stations (*e.g*. sodium, potassium, chloride, strontium, magnesium, calcium) are typical constituents of brackish and marine waters (Wilson 1975).

The only exceedance of Class III water quality standards in Clam Bayou in 2011 was an iron exceedance (CLB 4); there were no iron exceedances in the current study.

Sediments

Table 7 shows the site-specific results for compounds or mixtures that were detected in sediments above the PELs (DDT, DDE, cis and trans-chlordane, lead, dibenzo(a,h)anthracene, benzo(a)anthracene, and phenanthrene). Parameters with average concentrations that exceeded the PEL included two PAHs (dibenzo(a,h)anthracene at 176 µg/kg and benzo(a)anthracene at 858 µg/kg) and two banned pesticides (DDE at 9.37 µg/kg and cis and trans-chlordane at 4.85 µg/kg**).** The total PAH concentration was 8,666 µg/kg, which did not exceed the PEL (16,800 µg/kg).

Figure 7 shows the mean area-wide sediment concentrations of compounds that exceeded the PELs for 2011 and 2016, using the MDL replacement technique discussed in the Methods section above for both surveys. Eight of the nine compounds or mixtures that exceeded the PELs in 2011 also exceeded in the PELs in 2016 (benzo(a)anthracene, benzo(a)pyrene, cis and trans-chlordane, chrysene, DDE-p'p', dibenzo(a,h)anthracene, fluoranthene, and pyrene). Phenanthrene exceeded the PEL in 2011, but not in 2016. With the exception of dibenzo(a,h)anthracene, all concentrations were lower in 2016 compared to 2011. The average concentration of Total PAHs in 2011 (21,540 ug/kg) exceeded the PEL (16,770 ug/kg), while levels in 2016 (7,136 ug/kg) were below the PEL (**Figure 7**). This difference represents an overall decline of 67%.

Table 2. Results of the microbial source tracking (MST) analyses in Clam Bayou.

		N Ditch	Cen Ditch N	Cen Ditch E	N Pond	Cen Pond	S Pond	Channel N	Channel S	Bayou N	Bayou SW	Bayou SE
3/9/2016	*Escherichia Coli* (MPN/100mL)	74.5 Q	96.9 Q	63.2 Q	465.4 Q	175.9 AQ	553.9 Q	305.1 Q	237.8 Q	52.1 Q	10 Q	10 UQ
	Enterococci (MPN/100mL)	40.9 Q	74.5 Q	30.6 Q	189 Q	57.4 AQ	108.9 Q	86 Q	121.1 Q	30.6 Q	10 UQ	10 UQ
	Fecal Coliforms (CFU/100mL)	46 AQ	58 Q	58 Q	200 Q	54 Q	490 Q	64 Q	110 Q	13 BQ	7 BQ	2 UQ
	GULL2-qPCR (TSC/100mL)	14000 U	14000 UJ	14000 UJ	14000 UJ	21000 U	14000 U	3400 TJ	10000 UJ	10000 UJ	10000 UJ	2880 TJ
	HF183-qPCR (GEU/100mL)	6400 U	6400 U	6400 U	6400 U	9500 U	6400 U	6400 U	4800 UJ	4800 U	4800 U	4800 U
	Sucralose (µg/L)	0.48	0.27	0.32	0.17	0.28	0.23	0.21	0.17	0.18	0.18	0.18
	Acetaminophen (µg/L)	0.004 U	0.004 U	0.004 U	0.004 U	0.004 U	0.004 U	0.004 U	0.004 U	0.004 U	0.004 U	0.004 U
3/22 and 3/23/2016	GULL2-qPCR	10000 U	14000 UJ	21000 U	21000 UJ	10000 UJ	14000 UJ	14000 U	14000 UJ	14000 UJ	14000 UJ	14000 UJ
	HF183-qPCR	850 T	1400 U	2000 U	2000 U	1000 U	1400 UJ	1400 U	1400 U	1400 UJ	1400 UJ	1400 U
	Sucralose	0.5	0.37	0.29	0.098	0.24	0.48	0.19	0.16	0.23	0.17	0.22
	Acetaminophen	0.004 UJ	0.004 UJ	0.004 UJ	0.0078 IJ	0.0064 IJ	0.004 UJ	0.004 UJ	0.004 UJ	0.004 UJ	0.004 UJ	0.004 UJ
5/5/2016	*Escherichia Coli*	ǂ6131.4 Q	ǂ1860 Q	ǂ6488.2 Q	24196 JQ	24196 JQ	8164.1 Q	24196 JQ	24196 JQ	24196 JQ	24196 JQ	24196 JQ
	Enterococci	4105.8 Q	2281.8 Q	4105.8 Q	24196 JQ	12996.5 Q	5794.3 Q	ǂ24196 JQ	ǂ24195.7 Q	ǂ7167.8 AQ	ǂ8664.4 Q	ǂ7269.9 Q
	Fecal Coliforms	6000 ZQ	24000 BQ	6000 ZQ	12000 ZQ	6000 ZQ	15000 BQ	6000 ZQ	6000 ZQ	6000 ZQ	20000 BQ	6000 ZQ
	GULL2-qPCR	21000 U	32000 T	16000 T	64000	18000 T	21000 U	5710 T	17000	41000 T	21000 U	42000 U
	HF183-qPCR	2000 U	4100 U	2000 U	2000 U	757 T	2000 U	4100 U	2000 U	4100 U	2000 U	4100 U
	Sucralose	0.39	0.29	0.29	0.17	0.11	3.7	0.16	0.34	0.37	0.3	0.33
	Acetaminophen	0.023	0.012 I	0.015 I	0.023	0.057	0.014 I	0.069	0.025	0.029	0.016 I	0.029
7/27/2016	*Escherichia Coli*	126.4	82.6	104.2	1008.6	1067.4	427	4374.4	2142.4	4961.8	1578.8	2827.4
	Enterococci	82.6	147.6	264.6	402.2	20	168.8	104.2	104.2	ǂ192	20	20 U
	Fecal Coliforms	2400	4900	2800	10000	940 B	440 B	2200	1100 B	940 B	250 B	190 B
	GULL2-qPCR	69000 U	100000 U	69000 U	100000 U	69000 U	69000 U	69000 U	69000 U	69000 U	69000 U	69000 U
	HF183-qPCR	2700 U	4100 U	2700 U	4100 U	2700 U	2700 U	2700 U	2700 U	2700 U	2700 U	2700 U
	Sucralose	0.57	0.13	0.37	0.056	0.19	3.6	0.21	0.16	0.26	0.13	0.16
	Acetaminophen	0.004 U	0.004 U	0.0084 I	0.004 U	0.012 I	0.004 U	0.0079 I	0.0052 I	0.0099 I	0.004 U	0.0054 I

Applicable Class III Criteria: Fresh Waters: *E. Coli*: TPTV ≤410 CFU/100mL. Predominantly Marine Waters: *Enterococci*: TPTV ≤130 CFU/100mL.
ǂ - Exceeds the applicable Class III Criteria.
A – Value represents the average of two or more determinations.
B – Results based on colony counts outside the acceptable range.
I – Value is between the laboratory method detection limit and the laboratory practical quantitation limit.
J - Reported value failed to meet the established quality control criteria for either precision or accuracy.
Q – Sample held beyond the accepted holding time.
T - Value reported is less than the laboratory method detection limit. For DNA analyses during this study period, these values were detections, but not quantifiable. Represents a positive DNA detection.
U - Compound was analyzed for but not detected. Value reported is the method detection limit.
Z - Too many colonies were present (TNTC); the numeric value represents the filtration volume.

Table 3. Water chemistry results from March 22 and 23, 2016, in and around Clam Bayou.

March 2016 - Dry Season	Criteria	Class III Fresh		Class III Marine		
		N Ditch	Cen Ditch N&E	Channel S	Bayou N	Bayou Mouth
Organic Constituents (µg/L)						
None detected	-	-	-	-	-	-
Nutrients (mg/L)						
Total Ammonia	ǂ≤1.65	0.078	0.12	0.011	0.013	0.004 I
Nitrate+Nitrite	-	0.16	0.46	0.005 I	0.004 U	0.004 U
Total Kjeldahl Nitrogen	-	0.83	0.7	0.42	0.45	0.58
Total Nitrogen	*1.54	0.24	0.58	0.016	0.017	0.008
Total Phosphorus	*0.12	***0.18	0.057	0.046	0.06	0.044
Chlorophyll-a, Corrected (MC ≤6.3 µg/L)	-	6.1	0.67	2.2	1.6	4.2
Metals (µg/L unless otherwise noted)						
Arsenic	≤50	2.98	0.92	1.93	2.33	2.12
Cadmium	-	0.05 U	0.02 U	0.06 U	0.06 U	0.06 U
Calcium (mg/L)	-	95.1	64.9	366	358	381
Chromium	**≤189	0.46	0.58	0.32	0.35	0.48
Copper (MC ≤3.7)	**≤21.2	1.04	0.67	2.5	1.5	1.3
Iron (MC ≤300)	≤1000	120	340	120 U	120 U	120 U
Lead (MC ≤8.5)	**≤10.8	0.24 I	0.32	0.52	1.01	0.45
Magnesium (mg/L)	-	119	24	1170	1130	1220
Manganese	-	15.3	9.5	4 U	4.3 I	4 U
Potassium (mg/L)	-	35	8.7	332	324	347
Sodium (mg/L)	-	913	176	9150	8860	9450
Strontium	-	803	303	6390	6330	6740
Zinc	**≤270	11 I	5.8 I	20 U	20 U	20 U
General Physical Parameters						
Color (PCU)	-	34	42 A	5.2 I	8.1	2.5 U
Turbidity (NTU)	≤29 above background	2.3	1.2	4.5	3	9.2

I - Value is between the laboratory method detection limit and the laboratory practical quantitation limit.
U - Compound was analyzed for but not detected. Value reported is the method detection limit.
ǂ - Single Sample Criteria. When sites have different pH and temperatures, the most stringent criteria are presented.
*Peninsular Numeric Nutrient Class III Freshwater Criteria.
**Criteria based on the hardness at the time of sampling. When sites have a different hardness, the more stringent criteria are presented.
***Indicates a single value that exceeds the threshold, but that must be evaluated as an annual geometric mean.
MC - Class III Marine Criteria are presented when different from the Fresh Water Criteria.

Table 4. Water Chemistry results from May 5, 2016, in and around Clam Bayou.

May 2016 – Rain Event	Criteria	Class III Fresh		Class III Marine		
		N Ditch	Cen Ditch N&E	Channel S	Bayou N	Bayou Mouth
Organic Constituents (µg/L)						
Atrazine	-	0.29	0.71	0.25	0.26	0.021 I
Fluoranthene	≤0.370	0.026 I	0.14	0.038 I	0.04 I	0.024 U
Nutrients (mg/L)						
Total Ammonia	ǂ≤1.48	0.051	0.12	0.13	0.14	0.008
Nitrate+Nitrite	-	0.094	0.19	0.079	0.096	0.004 U
Total Kjeldahl Nitrogen	-	0.66	0.75	0.75	0.78	0.42
Total Nitrogen	*1.54	0.145	0.31	0.209	0.236	0.012
Total Phosphorus	*0.12	***0.26	***0.18	0.22	0.26	0.069
Chlorophyll-a, Corrected (MC ≤6.3 µg/L)	-	3.9	1.9 I	4.4	3.3 I	7.8
Metals (µg/L unless otherwise noted)						
Arsenic	≤50	2.55	1.25	2.46	2.4	2.34
Calcium	-	38.8	27.6	112	128	339
Chromium	**≤103.3	0.52	0.83	0.53 I	0.58 I	0.22 I
Copper (MC ≤3.7)	**≤11.3	2.59	3.16	2.5	2.3	1.1 I
Iron (MC ≤300)	≤1000	120	140	170 I	150 I	120 U
Lead (MC ≤8.5)	**≤4.2	0.84	1.42	1.54	1.53	0.35 I
Magnesium (mg/L)	-	6.78	15.7	298	350	1120
Manganese	-	13.4	13.4	14 I	16	4.9 I
Potassium (mg/L)	-	4.6	5.8	102	117	329
Sodium (mg/L)	-	55.8	123	2660	3100	9190
Strontium	-	133	154	1740	2040	6100
General Physical Parameters						
Alkalinity (mg /L)	-	83	55	79	80	118
Color (PCU)	-	65	65	56	40	7.5 U
Turbidity (NTU)	≤29 above background	2.2	3.4	4.7	4.9	5.3

I - Value is between the laboratory method detection limit and the laboratory practical quantitation limit.
U - Compound was analyzed for but not detected. Value reported is the method detection limit.
ǂ - Single Sample Criteria. When sites have different pH and temperatures, the most stringent criteria are presented.
*Peninsular Numeric Nutrient Class III Freshwater Criteria.
**Criteria based on the hardness at the time of sampling. When sites have a different hardness, the more stringent criteria are presented.
***Indicates a single value that exceeds the threshold, but that must be evaluated as an annual geometric mean.
MC - Class III Marine Criteria are presented when different from the Fresh Water Criteria.

Table 5. Water Chemistry results from July 27, 2016, in and around Clam Bayou.

July 2016 - Wet Season	Criteria	Class III Fresh		Class III Marine		
		N Ditch	Cen Ditch N&E	Channel S	Bayou N	Bayou Mouth
Organic Constituents (µg/L)						
Metribuzin	-	0.15 U	0.02 U	0.031 U	0.097	0.019 U
Nutrients (mg/L)						
Total Ammonia	ǂ≤1.48	0.065	0.2	0.048	0.004 I	0.008
Nitrate+Nitrite	-	0.097	0.13	0.032	0.005 I	0.005 I
Total Kjeldahl Nitrogen	-	0.64	0.68	0.62	0.65	0.67
Total Nitrogen	*1.54	0.162	0.33	0.80	0.009	0.0013
Total Phosphorus	*0.12	***0.19	0.11	0.09	0.1	0.065
Chlorophyll-a, Corrected (MC ≤6.3 µg/L)	-	6.2	7	6.4	9.2	6.9
Metals (µg/L unless otherwise noted)						
Arsenic	≤50	5.2	0.99	2.37	3.29	3.26
Calcium	-	61.3	46.6	187	276	330
Chromium	**≤200	0.5	0.44	0.23 I	0.2 I	0.17 I
Copper (MC ≤3.7)	**≤23.6	2.43	1.39	1.2 I	1.1 I	1 I
Iron (MC ≤300)	≤1000	110 I	190	120 I	90 U	90 U
Lead (MC ≤8.5)	**≤11.9	0.26	0.9	0.67	0.75	0.49 I
Magnesium (mg/L)	-	31.1	47.6	539	858	1050
Manganese	-	21.3	12.7	14	13	8.8 I
Potassium (mg/L)	-	11.5	15.5	156	250	306
Sodium (mg/L)	-	276	400	4440	7050	8710
Strontium	-	309	360	2930	4570	5640
Zinc	**≤288	4 I	6.5	3.5 I	3 U	3 U
General Physical Parameters						
Alkalinity (mg /L)	-	118	77	95	109	109
Color (PCU)	-	46	49	35	42	12
Turbidity (NTU)	≤29 above background	1.4	2	2.2	4.2	3.2

I - Value is between the laboratory method detection limit and the laboratory practical quantitation limit.
U - Compound was analyzed for but not detected. Value reported is the method detection limit.
ǂ - Single Sample Criteria. When sites have different pH and temperatures, the most stringent criteria are presented.
MC - Class III Marine Criteria are presented when different from the Fresh Water Criteria.
* Peninsular Numeric Nutrient Class III Freshwater Criteria.
**Criteria based on the hardness at the time of sampling. When sites have a different hardness, the more stringent criteria are presented.
***Indicates a single value that exceeds the threshold, but that must be evaluated as an annual geometric mean.

Table 6. Mean water chemistry results for all sampling events in and around Clam Bayou.

Mean Concentrations - All Sampling Events	Criteria	Class III Fresh		Class III Marine		
		N Ditch	Cen Ditch N&E	Channel S	Bayou N	Bayou Mouth
Organic Constituents (µg/L)						
Atrazine	-	0.290	0.375	0.250	0.260	-
Metribuzin	-	-	-	-	0.097	-
Fluoranthene	≤0.370	-	0.140	-	-	-
Nutrients (mg/L)						
Total Ammonia	ǂ≤1.48	0.06	0.15	0.06	0.08	0.01
Nitrate+Nitrite	-	0.12	0.26	0.06	0.10	-
Total Kjeldahl Nitrogen	-	0.71	0.71	0.60	0.63	0.56
Total Nitrogen	*1.54	0.83	0.97	0.65	0.72	0.56
Total Phosphorus	*≤0.12	***0.21	0.12	0.12	0.14	0.06
Chlorophyll-a, Corrected (MC ≤6.3 µg/L)	-	5.4	7.0	4.3	9.2	6.3
Metals (µg/L unless otherwise noted)						
Arsenic	≤50	3.58	1.05	2.25	2.67	2.57
Calcium (mg/L)	-	65.07	46.37	221.67	254.00	350.00
Chromium	**≤103.3	0.49	0.62	-	-	-
Copper (MC ≤3.7)	**≤11.3	2.02	1.74	2.50	1.90	1.30
Iron (MC ≤300)	≤1000	120	223	-	-	-
Lead (MC ≤8.5)	**≤4.2	0.55	0.88	1.11	1.10	-
Magnesium	-	52.3	29.1	669.0	779.3	1130.0
Manganese (mg/L)	-	16.7	11.9	14.0	14.5	-
Potassium (mg/L)	-	17.0	10.0	196.7	230.3	327.3
Sodium (mg/L)	-	415	233	5417	6337	9117
Strontium	-	415	272	3687	4313	6160
Zinc	**≤270	-	6.50	-	-	-
General Physical Parameters						
Alkalinity (mg/L)	-	100.5	66.0	87.0	94.5	113.5
Color (PCU)	-	48.3	52.0	45.5	30.0	12.0
Turbidity (NTU)	≤29 above background	2.0	2.2	3.8	4.0	5.9

ǂ - Single Sample Criteria. When sites have different pH and temperatures, the most stringent criteria are presented.
MC - Class III Marine Criteria are presented when different from the Fresh Water Criteria.
* Peninsular Numeric Nutrient Class III Freshwater Criteria.
**Criteria based on the hardness at the time of sampling. When sites have a different hardness, the more stringent criteria are presented.
***Indicates a mean value (n=3) that exceeds the threshold, but that must be evaluated as an annual geometric mean (with n=4).

Table 7. Site specific sediment chemistry results of PEL exceedances.

Date	Site	Component	Probable Effects Level (PEL)	Result	MDL	PQL
Pesticides (µg/Kg)						
3/23/16	Bayou N SED	DDT-p,p'	4.77	9.6 J	0.22	0.87
		DDE-p,p'	3.74	44	0.12	0.48
		Cis and trans-chlordane	4.79	17 J	0.091	0.36
3/23/16	Bayou E SED	DDE-p,p'	3.74	26 J	0.081	0.32
		Cis and trans-chlordane	4.79	13 J	0.12	0.49
3/23/16	Bayou SE SED	DDE-p,p'	3.74	3.7 J	0.039	0.16
3/23/16	Bayou Cen SED	DDE-p,p'	3.74	5.3 J	0.041	0.16
4/28/16	Bayou E	DDE-p,p'	3.74	30	0.088	0.35
		Cis and trans-chlordane	4.79	15	0.13	0.54
4/28/16	Bayou SW	DDE-p,p'	3.74	11	0.05	0.2
		Cis and trans-chlordane	4.79	9.2	0.076	0.3
Metals (mg/Kg)						
3/23/16	Bayou N SED	Lead	112	119	0.63	2.5
3/23/16	Bayou E SED	Lead	112	122	0.68	2.7
PAHs (µg/Kg)						
3/23/16	Bayou N	Dibenzo(a,h)anthracene	135	380	18	74
		Benzo(a)anthracene	693	1900	18	74
		Phenanthrene	544	680	18	74
3/23/16	Bayou E	Dibenzo(a,h)anthracene	135	390	24	98
		Benzo(a)anthracene	693	1500	24	98
		Phenanthrene	544	600	24	98
4/28/16	Bayou E	Dibenzo(a,h)anthracene	135	620	27	110
		Benzo(a)anthracene	693	2400	27	110
		Phenanthrene	544	760	27	110
3/23/16	Bayou SE	Benzo(a)anthracene	693	920	12	48
3/23/16	Bayou Cen SED	Dibenzo(a,h)anthracene	135	300	13	50
		Benzo(a)anthracene	693	1700	13	50
		Phenanthrene	544	910	13	50
4/28/16	Bayou SW	Dibenzo(a,h)anthracene	135	350	15	61
		Benzo(a)anthracene	693	1900	15	61
		Phenanthrene	544	620	15	61

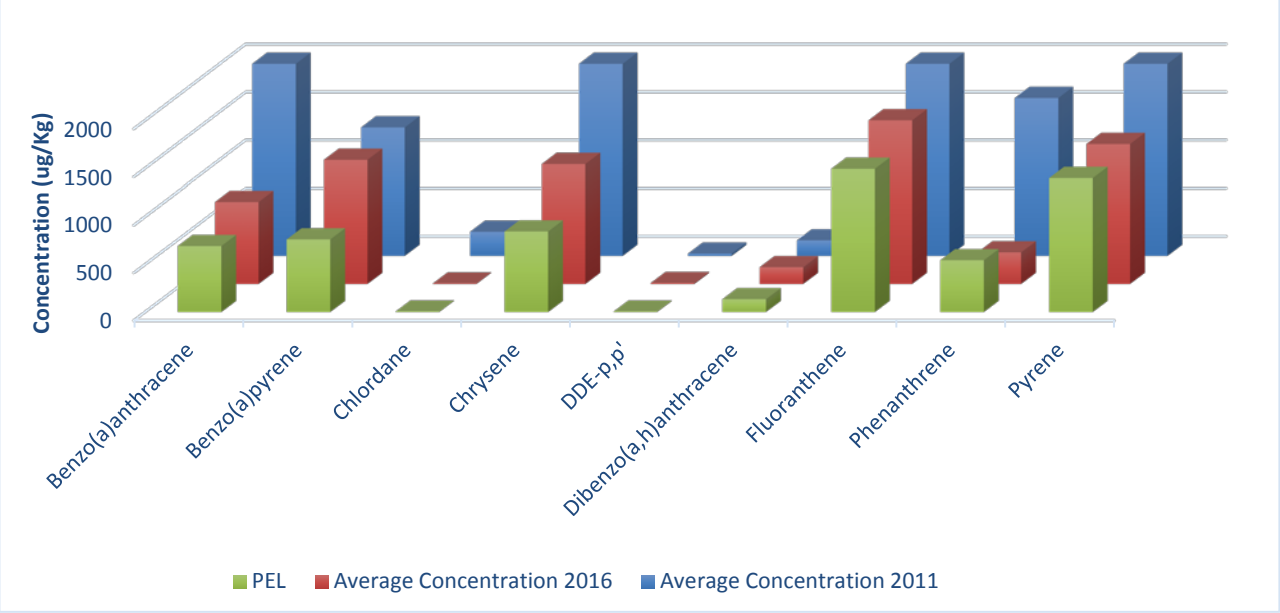


Figure 7. Mean surface sediment concentrations in 2011 and 2016 for compounds that exceeded the Probable Effect Level (PEL) for either year.

Fish Tissue

Chemical analysis of fish tissue showed that eighty-seven percent (87%) of the 63 chemicals analyzed were below the minimum detection limit (MDL) for all 36 fish samples. In 2011, Total PAHs and dieldrin exceeded the target concentration in all three species and chlordane exceeded the TFC in the common snook and striped mullet. In the current study, all Total PAH constituents were detected below the MDL for all species. Using the MDL replacement technique described in the Methods section, the mean Total PAHs for each of the three species was <0.0005 mg/kg, which is below the TFC (0.00093 mg/kg) (**Table 8**). Based on the chlordane water quality criterion (annual average < 0.0059 µg/L), a target fish tissue concentration of 0.0083 mg/kg was estimated and was not exceeded in any of the three species (**Table 8**). In 2011, all three species exceeded the TFC for cis and trans-chlordane. No mean concentration exceeded the TFC (0.0316 mg/kg) for DDE or dieldrin (0.0007 mg/kg). In 2011, all species exceeded the target concentration for dieldrin. Note that chlordane was banned from use in the U.S. in 1988 and dieldrin was banned from agricultural use in 1974, and therefore, these levels represent legacy inputs. Trans-Nonachlor indicates the presence of chlordane; however, because there is no water quality criterion for Trans-Nonachlor a TFC could not be calculated.

Table 8. Analytical results for fish tissue samples collected in Clam Bayou on March 30, 2016. Values represent mean results for each fish species (n=12 for each).

	Archosargus probatocephalus (Sheepshead) Results	*Centropomus undecimalis* (Common snook) Results	*Mugil cephalus* (Striped mullet) Results	Target Fish Concentration
Metals (mg/Kg)				
Arsenic	5.268	0.8023	2.444	-
Chromium	0.060	0.303	0.0588	-
Mercury	0.1614	0.1758	0.0052	-
Selenium	0.5225	0.4433	0.1758	-
Polycyclic Aromatic Hydrocarbons (mg/Kg)				
Acenaphthylene	<0.0005	<0.0005	<0.0005	≤0.00093
Benzo(a)anthracene	<0.0005	<0.0005	<0.0005	≤0.00093
Benzo(a)pyrene	<0.0005	<0.0005	<0.0005	≤0.00093
Benzo(b)fluoranthene	<0.0005	<0.0005	<0.0005	≤0.00093
Benzo-(ghi)perylene	<0.0005	<0.0005	<0.0005	≤0.00093
Benzo(k)fluoranthene	<0.0005	<0.0005	<0.0005	≤0.00093
Chrysene	<0.0005	<0.0005	<0.0005	≤0.00093
Dibenzo-(a,h)anthracene	<0.0005	<0.0005	<0.0005	≤0.00093
Indeno(1,2,3-cd)pyrene	<0.0005	<0.0005	<0.0005	≤0.00093
Phenanthrene	<0.0005	<0.0005	<0.0005	≤0.00093
Total PAH	<0.00005	<0.00005	<0.00005	≤0.00093
Chlorinated Pesticides (mg/Kg)				
Cis and trans-Chlordane	<0.0083	<0.0083	0.00013	≤0.0083
DDE-p,p'	0.0006	0.0008	0.0008	≤0.0316
Dieldrin	<0.0007	<0.0007	0.00007	≤0.0007
Trans-Nonachlor	0.0047	0.0050	0.00047	-
Organonitrogen and Phosphorus Pesticides (mg/Kg)				
None detected	-	-	-	-

Biological Health

Benthic Macroinvertebrates

The results of the benthic invertebrate sampling were provided by Hillsborough County Environmental Protection Division (Karlen *et al.* 2017). The Tampa Bay Benthic Index (TBBI) was calculated for each site following the methods established in Janicki Environmental (2005) and Malloy *et al.* (2007). The TBBI threshold scores for "Degraded" (< 73), "Intermediate" (between 73 to 87) and "Healthy" (> 87) benthic habitats were established by Janicki Environmental (2005) and Malloy *et al*. (2007). The following results are from Karlen *et al.* (2017).

A total of 149 taxa were identified in the 2016 Clam Bayou samples, while 108 taxa were identified in 2008. Annelids were the most diverse taxonomic group in both the 2016 and 2008 samples with 72 taxa (48% of total) identified in 2016 and 44 taxa (41% of the total) identified in 2008. Mollusks were the next most diverse group with 48 taxa in 2016 and 34 taxa in 2008 representing approximately 32% of the species total in both years. Species richness (*S*) ranged from 3 taxa at site 16CLB03 to 65 taxa at site 16CLB06 in 2016 (**Table 9**). The maximum number of taxa at any site in 2008 was 44 at 08CLB06 (**Table 9**). Species richness increases between 2016 and 2008 were most evident at sites CLB06, CLB07 and CLB08, while several other sites showed a decrease in species richness (**Table 9**). The mean number of taxa per site was slightly higher in 2016 (**Table 9**), but there was no significant difference in species richness between the two sampling years (**Table 9**; p = 0.295).

The overall abundance (raw count) was similar between 2016 and 2008 with 1,996 individual organisms counted in 2016 versus 1,745 individual organisms in 2008. Annelids were the most abundant phylum in both years, accounting for approximately 63% of the total abundance in 2016 and 50% in 2008. The top three most abundant taxa accounted for a third of the overall abundance in both 2016 and 2008. The most abundant species in 2016 was the polychaete *Laeonereis culveri,* which accounted for 16% of the total abundance and was found at 70% of the sites. The bivalve *Parastarte triquetra* (Brown Gem clam) was the second most abundant species in 2016, making up 10% of the total abundance and found at 80% of the sites, and unidentified Oligochaetes (Tubificinae) ranked third, making up 6% of the total abundance and present at 70% of the sites. Unidentified Oligochaetes were the dominant taxon in 2008, accounting for 13% of the abundance and found at 100% of the sites, while *Laeonereis culveri* and an unidentified gastropod (Rissooidea) each accounted for 10% of the overall abundance. Sample abundances (*N*) ranged from 125/m^2 at site 16CLB03 to 11,325/m^2 at site 16CLB07 (**Table 9**). Site CLB03 also had the lowest abundance in 2008, with 225/m^2, while the maximum abundance of 9,625/m^2 was at site 08CLB04 (**Table 2**). There was no significant difference in the mean abundance between 2008 and 2016 (p = 0.564), with decreases in abundance observed at sites CLB04 and CLB05 and notable increases at sites CLB06 and CLB07 (**Table 9**).

The Shannon diversity index (H'_{ln}) was not significantly different between 2008 and 2016 (**Table 9**; p = 0.703). Several sites did show decreases in the Shannon diversity, notably CLB01 and CLB12, which mirrored similar decreases in species richness (**Table 9**). There was a relatively large increase in Shannon diversity at site CLB02, however the species richness and abundance at this site was much lower than most of the other sites (with the exception of CLB03) in both 2008 and 2016. The mean evenness index (*J'*) was similar between 2008 and 2016 and was not significantly different (**Table 9**; p = 0.344). Site CLB02 had a large increase in *J'* in 2016, which was due to the low abundance at this site distributed across only 5 taxa (**Table 9**).

The mean Tampa Bay Benthic Index (TBBI) scores were not significantly different between 2008 and 2016, however 60% of the sites did show an increase in their TBBI scores (**Table 9**). The TBBI scores in 2008 were generally near or below the "Degraded" threshold value of 73 and none were above the "Healthy" threshold value of 87, while in 2016 two sites had "Healthy" TBBI scores (CLB07 & CLB08) and two sites (CLB06 and CLB 10) were just below the "Healthy" threshold (**Table 9**).

Table 9. Clam Bayou benthic community summary metrics (modified from Karlen *et al.* 2017).

Site	Spp. Richness (*S* = # of taxa)		Abundance (*N* = #/m^2)		Shannon Diversity (H'_{ln})		Evenness ($J' = H'_{ln}/\ln S$)		TBBI	
	2008	2016	2008	2016	2008	2016	2008	2016	2008	2016
CLB01	24	18	3750	5000	2.82	1.87	0.89	0.65	**76.61	***53.87
CLB02	2	5	650	175	0.16	1.55	0.24	0.96	***64.69	***68.39
CLB03	5	3	225	125	1.30	0.95	0.81	0.86	***72.38	***67.15
CLB04	30	23	9625	4850	2.38	2.12	0.70	0.68	***72.24	***45.65

CLB05	14	11	9425	6900	1.92	1.80	0.73	0.75	**76.38	***51.84
CLB06	44	65	5601	9075	3.17	3.26	0.84	0.78	***62.75	**86.73
CLB07	42	62	3750	11325	2.89	3.03	0.77	0.73	**84.86	*91.41
CLB08	32	49	3776	4975	3.01	3.25	0.87	0.83	***38.56	*92.75
CLB10	22	32	1925	2875	2.70	2.70	0.87	0.78	**81.51	**85.75
CLB12	31	20	4900	4600	2.97	1.96	0.87	0.65	***71.70	**79.80
Mean	24.6	28.8	4362.7	4990.0	2.33	2.25	0.76	0.77	***70.17	***72.33
Median	27	21.5	3763	4912.5	2.76	2.04	0.82	0.76	***72.31	**74.10
Min	2	3	225	125	0.16	0.95	0.24	0.65	***38.56	***45.65
Max	44	65	9625	11325	3.17	3.26	0.89	0.96	**84.86	*92.75

TBBI: ***= “Degraded” (Red) (<73); **= ”Marginal”(Yellow) (73-87); *=”Healthy” (Green)(>87)

Bird Observations

Potential adverse changes in aquatic or shoreline-dependent birds in Clam Bayou were evaluated using a subset of the data collected as part of the National Audubon Society’s annual Christmas Bird Counts for the St. Petersburg, Florida Circle (January 2017, **Figure 8**). In the 2011 report, between 21 and 32 different species of birds utilized the bayou from 1998 through 2010, including good representation from taxa with probing, wading, diving, and dabbling habits. This number of species increased since the completion of the restoration activities to 29 - 33 different species from 2011 through 2016. There is a small amount of inter-annual variability and a slight increasing trend over time (**Figure 8**).

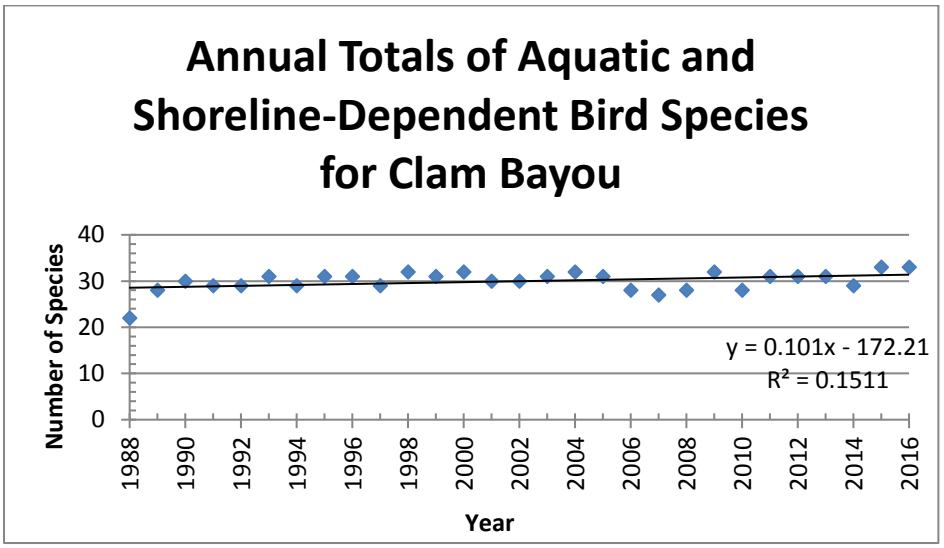


Figure 8. Annual taxa richness of birds that consume fish and aquatic invertebrates in Clam Bayou.

Discussion

Microbial Source Tracking and Bacterial Indicators

Due to a rare rain event in terms of duration and magnitude, more than 30 million gallons of untreated or partially treated sewage was released into the stormwater ponds of Clam Bayou in the summer of 2015. Local municipalities responded by conducting daily and weekly sampling of standard fecal bacterial indicators (*e.g.*, *E. coli*, enterococci, fecal coliforms). These indicators are commonly found in the feces of humans and other animals, but they do not discriminate between enteric (from the gut of a host animal) and environmental bacteria (free-living bacteria not associated with fecal waste or elevated health risks).

DEP has been developing a suite of additional indicators that can be used to identify potential sources of elevated levels of fecal indicators in a waterbody. By using a combination of molecular (HF183 and Gull2), microbiological

(*E. coli and* enterococci), and chemical (sucralose and acetaminophen) methods and a weight of evidence strategy, DEP has successfully implemented the toolbox approach in assessing waterbodies. In this study, a variety of chemical tracers unique to human wastewater, all with different characteristics, were used to allow for better differentiation amongst potential sources.

Sucralose was detected at all sites on all sampling dates, but because sucralose is not broken down during the wastewater treatment process, the detection of sucralose without acetaminophen present may simply reflect the wastewater reuse in the watershed. When acetaminophen was detected, the levels were typically one-tenth ($1/10^{th}$) or one order of magnitude less than the corresponding sucralose levels. Two notable exceptions occurred on May 5, in the Central Stormwater Pond and at the Channel North site, where the levels of acetaminophen were roughly half of the sucralose levels. Since acetaminophen is typically reduced by three orders of magnitude by most common wastewater treatment processes and sucralose is typically not reduced at all, any sucralose to acetaminophen ratios less than 1:100 strongly suggest the presence of untreated wastewater. As stated earlier, acetaminophen is not commonly detected in final wastewater treatment plant effluents and should therefore be absent or rarely detected in surface waters.

The human specific HF183 marker was detected at low levels in the Central Pond during May, and enterococci and *E. coli* exceeded the respective marine and freshwater TPTV at all sampling locations in May. The May sampling event followed a significant rain event (3.41 inches) that broke stormwater containment booms and overwhelmed the treatment system. While these results indicate that untreated sewage may have been present during the May event, there were no reported sewage spills that would have explained the high bacteria and acetaminophen levels. Markers were undetected or measured near the detection limit for all other dates and sites in the study. On all other dates and locations, TPTV values were achieved for *E. coli* and enterococci.

DEP is now assessing fecal coliforms in Class II (shellfish harvesting areas) only. As Clam Bayou is not a Class II water, the results for fecal coliforms are presented for informational purposes as they are still being used by the City of Gulfport and other municipalities until the new criteria are approved by EPA.

The Gull2 marker was detected at North Pond and Channel South sites on May 5, which was likely introduced during the heavy rains and sediment stirring. Given the large numbers of coastal birds in the area, this is not unexpected.

Water Chemistry

Although several significant rain events and an increasing trend in groundwater levels occurred in the Clam Bayou area from the onset of the study to the rainy season sampling on July 27, 2016, individual analytes did not vary greatly among the sampling events or among sites. While there was insufficient data to calculate annual geometric means to assess numeric nutrient criteria, individual total phosphorus values were above the peninsula threshold in the ditches discharging into the bayou. Other than the bacteriological exceedances mentioned above, there were no exceedances of Class III freshwater or marine water quality standards.

Sediments

Eight of the nine compounds that exceeded the PELs in 2011 also exceeded the PELs in 2016 (**Figure 7**). One compound, dibenzo(a,h)anthracene, was slightly higher in 2016 (163.3 ug/Kg vs. 176 ug/Kg, a 7% increase), while the remaining compounds were, on average, 61.8% lower in 2016 compared to 2011. Restoration activities completed since 2011 sampling events included the construction of stormwater retention ponds and the removal of limited amounts of sediment to increase tidal flushing (**Figure 9**).

EPCHC sediment results were comparable to DEP findings. Karlen *et al*. (2017) found that, while the concentration of PAHs in Clam Bayou sediments remained high in 2016, with many threshold effect level (TEL) and PEL exceedances; however, most PAHs were significantly lower in 2016 compared to 2008. In their study, the mean Total Low Molecular Weight PAHs decreased by 51.8%, the mean High Molecular Weight PAHs decreased by 57%, and the mean Total PAHs by 56.65%.

Fish Tissue

Cis and trans-chlordane, DDE, dieldrin, and trans-nonachlor were detected in some individual fish tissue samples, but none of the mean concentrations exceeded their respective TFCs. In contrast, mean concentrations of cis and trans-chlordane and dieldrin were above the TFCs in 2011. Also, in 2011, all species tested exceeded the TFC for Total PAHs, but none were exceeded in the current study. Individual PAH compounds were evaluated and no

exceedances were found. Collectively, these results suggest an improvement in environmental conditions from 2011.

Biological Health

The benthic macrofaunal community has shown indications of improvement since the previous study conducted by EPCHC in 2008 (Karlen *et al.* 2017). There were more taxa found in 2016 (149 taxa) than in 2008 (108 taxa) and while the mean number of taxa per site was not found to be significantly different, the total number of species identified in 2016 represents a 38% increase since 2008 and is a strong indicator of improving water and sediment quality conditions. The other measured benthic community indices (abundance, H', J' and TBBI) were very similar between 2016 and 2008 and were not significantly different. The TBBI did show some overall improvements in 2016, with two sites having "Healthy" index scores. These two sites (CLB07 and CLB08) are near the mouth of Clam Bayou and are well-flushed by tidal exchange with Boca Ciega Bay. The species composition of the benthic community was generally similar between the two sampling years, with annelids and mollusks dominating in terms of species richness and abundance and crustaceans comprising a relatively minor proportion of the species richness and abundance.

Recent bird survey data suggest that species richness and bird populations may be increasing over time, and that the bayou has sufficient food sources and habitat to support a healthy natural community of wildlife.

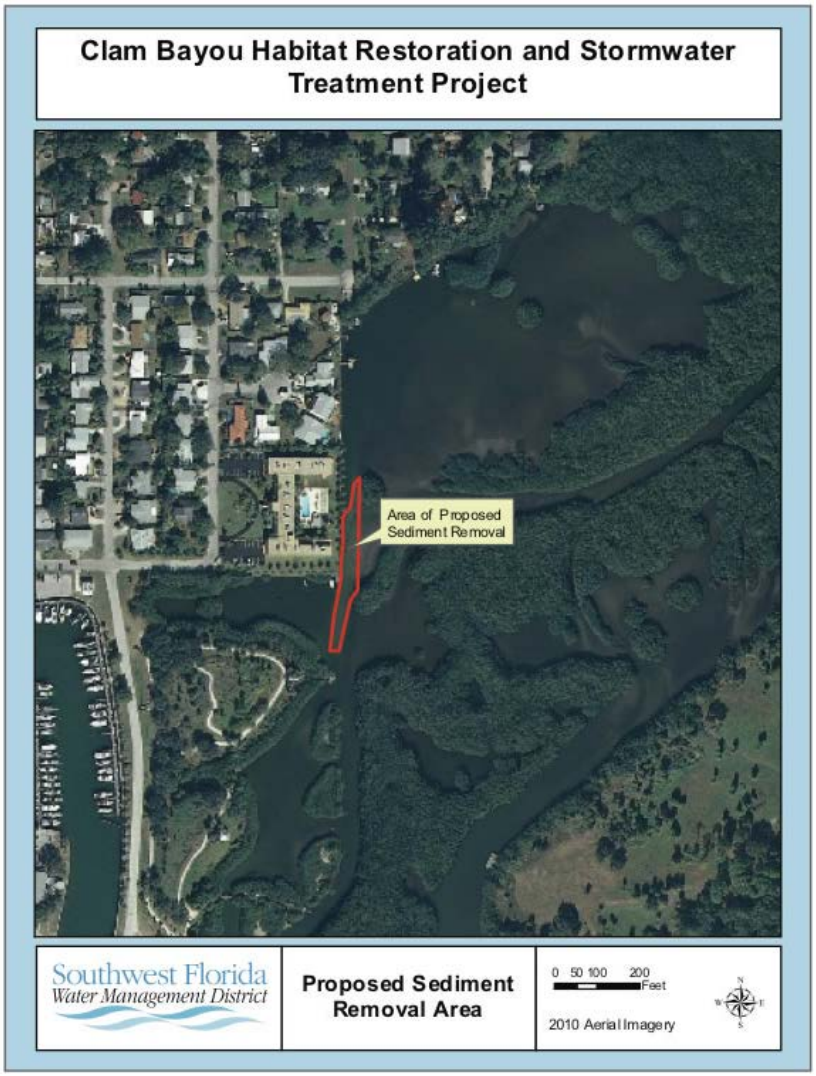


Figure 9. Study site with area of sediment removal highlighted (www.swfwmd.state.fl.us/projects/clambayou).

Conclusions

The following conclusions are based on the information DEP collected during this study:

- The concentrations and ratios of sucralose to acetaminophen at multiple locations in May and July, along with the detection of low levels of the human source marker, HF183, indicate that partially treated or untreated wastewater reaches Clam Bayou during heavy rain events and may contribute to the elevated bacteria levels observed and the exceedances of bacteria criteria.

- Gull and shorebirds specific marker, Gull2, was detected at multiple sampling sites during the May 5 event following the rain event, indicating that birds likely contributed to the exceedances for *Escherichia coli* and enterococci.

- Although sediment contaminant levels remain above the Florida state sediment quality guidelines for eight pollutants, a decrease in PAH concentrations from previous studies is an indication that the sediments are improving in Clam Bayou.

- Fish tissue concentrations did not exceed safe limits for any PAHs in the current study. No exceedances were detected in the individual PAHs or the combined Total PAH. Four priority pollutants were detected in individual fish tissue samples, but the mean concentrations did not exceed the target fish concentration.

- Decreasing sediment contaminant levels along with an increase in the number of macroinvertebrate and bird species is a positive sign that Clam Bayou has benefited from the restoration and habitat enhancement activities and that the ecosystem is starting to recover.

Literature Cited

Du, B., Price, A., W.C. Scott, W., Kristofco, L.A., Ramirez, A. J., Chambliss, C. K., Yelderman, J. C., Brooks, B. W. 2014. Comparison of contaminants of emerging concern removal, discharge, and water quality hazards among centralized and on-site wastewater treatment system effluents receiving common wastewater influent. Science of the Total Environment 466-467 (2014) 976-984.

Clarke, R.K and Ainsworth, M. 1993. A method of linking multivariate community structure to environmental variables. Marine Ecology Progress Series 92: 205-219.

Clarke, K.R. and Gorley, R.N. 2015. PRIMER v7: User Manual/Tutorial. PRIMER-E Ltd. Plymouth, U.K.

Clarke, K.R., and Warwick, R.M. 2001. Change in marine communities: an approach to statistical analysis and interpretation, 2nd edition. PRIMER-E ltd. Plymouth, U.K.

Clarke, K.R., Somerfield, P.J. and Chapman, M.G. 2006. On resemblance measures for ecological studies, including taxonomic dissimilarities and a zero-adjusted Bray-Curtis coefficient for denuded assemblages. Journal of Experimental Marine Biology and Ecology 330: 55-80.

[ECT] Environmental Consulting and Technology, Inc. 2015. Clam Bayou Central Pond Assessment. Assessment of Short and Long Term Effects of Event Driven Fecal Coliform Loading. ECT No. 150715-0100. Prepared for City of St. Petersburg. December 2015. 69 pp.

[FDEP] Florida Department of Environmental Protection. 2012. Antidegradation Study of Clam Bayou (Pinellas County). [48 pages]

Janicki Environmental, Inc. 2005. Development of a benthic index to establish sediment quality targets for the Tampa Bay estuary. Final Report. TBEP Tech. Pub. #01-06.

MacDonald, D.D. 1994. Approach to the Assessment of Sediment Quality in Florida Coastal Waters. Report prepared for FDEP. [140 pages]

Karlen, D.J., T.L. Dix, B.K. Goetting, and S.E. Markham. 2009. The Benthic Macrofaunal Community and Sediment Quality Conditions in Clam Bayou, Pinellas County, Florida. Environmental Protection Commission of Hillsborough County. Prepared for Tampa Bay Estuary Program. March 2009. 61 pp.

Karlen, D.J., T.L. Dix, B.K. Goetting, and S.E. Markham. 2017. A reassessment of the Benthic Macrofaunal Community and Sediment Quality Conditions in Clam Bayou, Pinellas County, Florida. 2008 vs. 2016. Environmental Protection Commission of Hillsborough County. Prepared for Tampa Bay Estuary Program. March 2017. 62 pp.

Kositch, M. S. Batt, A. L. Lazorchak, J. M. 2014. Concentrations of prioritized pharmaceuticals in effluents from 50 large wastewater treatment plants in the US and implications for risk estimation. Environmental Pollution 184 (2014) 354-359.

Malloy, K.J., Wade, D. Janicki, A. Grabe, S.A., and Nijbroek, R. 2007. Development of a benthic index to assess sediment quality in the Tampa Bay Estuary. Marine Pollution Bulletin 54: 22-31.

Wilson T.R.S. (1975) The major constituents of seawater. In: (J.P. Riley and G. Skirrow, eds.) Chemical Oceanography, 2nd Ed., Vol. 1, p. 365-413. Academic Pres