EFFECTS OF WET AND DRY WEATHER EVENTS ON BACTERIA (ENTEROCOCCI) AND THE PUBLIC HEALTH THREAT FROM THE RE-SUSPENSION OF SEDIMENT SEQUESTERED ENTEROCOCCI

A Thesis

by

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ABSTRACT

Approximately 66% of Texas surface waters are impaired with bacteria from fecal waste, including several tributaries and segments within the Galveston Bay system. This study was conducted in the waters of the Marina Del Sol marina on Clear Lake in Kemah, Galveston County, Texas, USA. A series of hypothesis were tested; 1) rainfall and subsequent runoff from stormwater is the primary cause of elevated *Enterococcus* levels in the waters in Marina Del Sol, 2) hotspots of *Enterococcus* will be present in the waters in Marina Del Sol and 3) the concentration of *Enterococcus* will increase from the marina entrance to the rear of the marina. Sampling was conducted at 10 stations between 0800 and 1100 every Monday, Thursday, and Saturday over five weeks in June and July 2013. Enterococci concentrations were quantified using the IDEXX Enterolert method for detection and enumeration estimation, Fluorogenic Substrate *Enterococcus* Test, Multi-well procedure and three-day rainfall accumulation prior to sampling was recorded from NOAA's Climate Data Online. Eleven dry weather and four wet weather events occurred during the sampling period with the largest rainfall accumulation at 1.39 inches. The geometric means of wet versus dry weather samples were not significantly different (Mann Whitney). Two hotspots were found yielding geometric means of 42.98 and 41.25 MPN, which exceed the U.S. EPA primary contact recreation limit of 35 MPN. Additionally, the EPA single sample maximums (104 CFU/100 mL) were exceeded at nine out of ten sampling stations at least once, including a spike of 1,445 MPN and 1,198 MPN. A low to high gradient of Enterococci, from the entrance to the

back portion of the marina, was evident. The results from the initial summer study indicated that the stormwater retention pond to the west of the marina could be a possible source of Enterococci. The fourth hypothesis, states that sediments are a source of elevated *Enterococcus* concentrations in the water at the Marina Del Sol marina, was tested during a follow up study. Sediment and water samples were collected on the 13th of November, 2013 between the hours of 0900 and 1400. Six stations in the stormwater retention pond were sampled. In addition, three of the original sampling stations in the marina were sampled. A stormwater outfall was found to be a concentrated source of Enterococci into the retention pond (12,098 MPN/100 mL). Data from these two studies indicate that there are numerous sources that contribute to the concentrations of Enterococci in the marina. A gateway effect is occurring between the increasingly built environment of the Galveston Bay marinas and the natural environment.

To my son, Grayson. May you find beauty and wonder in nature.

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NOMENCLATURE

CFU	Colony Forming Unit
CWP	Clean Water Program
ENT	*Enterococcus*
FIB	Fecal Indicator Bacterium
GB	Galveston Bay
GBF	Galveston Bay Foundation
MDS	Marina Del Sol
MPN	Most Probable Number
TCEQ	Texas Commission of Environmental Quality

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CHAPTER I

INTRODUCTION

Approximately 66% of Texas surface waters are impaired with bacteria from fecal waste (Texas 303d list 2012), including several tributaries and segments within the Galveston Bay watershed. According to the EPA, Enterococci are the preferred indicator bacterium to determine the level of health risk of fecal contamination in marine waters used for recreation (US EPA 2012).

There are five distinct groups of *Enterococcus* (ENT) consisting of *E. faecalis*, *E. faecium, E. avium*, *E. gallinarum* and *E. cecorum*. The *E. faecalis* species group is of particular concern because they are known to inhabitat surface water (Byappanahalli et al. 2012). Their tendency to inhabitat surface waters places *E. faecalis* in the direct path of humans entering Galveston Bay (GB) and the bays surrounding watersheds.

Enterococcus (ENT) bacteria reside in the gastrointestinal tract and oral cavity of warm-blooded animals. Fecal waste may be introduced directly to surface waters from wastewater treatment facility effluents and indirectly from leaking residential and commercial sewage pipes. As well as from stormwater runoff containing pet, wildlife, and human waste. *Enterococcus* bacteria are not only indicators of health risk; they can also cause infection.

Most enterococcal infections are caused by *Enterococcus faecalis* (90%) and the rest by *Enterococcus faecium* (Jett and Huycke 1994). Human infections caused by *Enterococcus* can occur in the oral cavity, urinary tract, blood stream, abdomen, wounds, and heart (Byappanahalli et al. 2012; Jett and Huycke 1994). Human infections caused by *Enterococcus* include urinary track and abdominal infections, wound infections and endocarditis (Arias et al. 2013). Due to the ability of *Enterococcus* to persist on the hands of health care workers, many of these infections are nosocomial. *Enterococcus* are increasingly becoming more resistant to antibiotics (Arias et al. 2013), making the presence of *Enterococcus* and associated fecal waste in the marine environment a main concern for contact recreational use. In a recent study, humans who were randomly assigned to bathe in *Enterococcus*-contaminated marine waters, reported an increase in gastrointestinal, respiratory and skin illnesses when compared to non-bathers (Sinigalliano et al. 2013).

The ability of *Enterococcus* to persist in the marine environment makes these fecal indicator bacteria (FIB) an ideal candidate for fecal contamination source tracking. However, *Enterococcus* are thought to decrease in concentration or 'die off' the longer they are outside of their animal host. The primary mechanism for die off is Ultraviolet (UV) light exposure. UV light is absorbed by the *Enterococcus* DNA which renders the ENT inactive (Byappanahalli et al. 2012; Fujioka et al. 1981).

However, the detrimental effects of UV light may be less damaging when the ENT are attached to sediment particles. Suspended solids (i.e. particles) in the water column also provide shading and protection from sunlight for free-living as well as particle-attached bacteria (Anderson et al. 2005). Deposition of particle attached *Enterococcus* to bottom sediments has been well documented (Fries et al. 2006). Bottom sediments are an important reservoir and source of these bacteria during storm events or other physical disturbances that cause their resuspension back into the water column (Yamahara 2009; Fries et al. 2006).

Organic matter in the form of fecal waste is also thought to increase the rate of *Enterococcus* survival in the marine environment (Byappanahalli et al. 2012). Enterococcus is known to survive longer at lower salinities typical of marine coastal environments rather than at the average 35 ppt salinity of open ocean seawater. Disinfection, starvation and predation are factors that can lead to reduced survival time of *Enterococcus* in the environment (Byappanahalli et al. 2012; Fujioka et al. 1981).

The presence of *Enterococcus* can indicate other impacts on the water body such as, a high level of inorganic nutrients (i.e. nitrogen and phosphorus). Excesses nutrients can cause eutrophication. Eutrophication occurs when excess nutrients fuel a phytoplankton bloom. When large amounts of the phytoplankton die off in unison and sink to the bottom of the water column their decomposition uses up available dissolved oxygen in the water column and depresses oxygen levels (Parel 1997). The decreased oxygen levels can lead to die off of higher organisms such as, fish and invertebrates. The higher organisms then sink and use more oxygen as they decompose. This exacerbates the hypoxic conditions and continues to suppress oxygen levels eventually rendering the water body unsuitable for life.

For my thesis I examined concentrations of *Enterococcus* in the surface water of Marina Del Sol boat marina on Clear Lake in Kemah, Galveston County, Texas in summer 2013 for an internship with the Galveston Bay Foundation (GBF). The GBF is monitoring surface water in the Galveston Bay watershed for the Texas Commission of

Environmental Quality's (TCEQ) Clean Water Partnership Program. The overall goal of the Clean Water Partnership program is to reduce the amount of fecal bacteria entering Galveston Bay. Marina Del Sol agreed to work towards the goal of reducing the amount of fecal bacteria that enters the bay and have voluntarily monitored the marina since 2012 for *Enterococcus* and supporting water quality parameters.

Hypothesis and Objectives

Over the summer of 2013 I held a Water Quality Internship position with the GBF. The following three research questions regarding water quality were proposed 1) does rainfall and subsequent runoff affect the levels of *Enterococcus* in Marina Del Sol?, 2) Are there hotspots of *Enterococcus* in Marina Del Sol? and 3) do higher levels of *Enterococcus* correlate with lower areas of water movement in Marina Del Sol due to marina design? From these research questions the following three hypothesis and objectives were generated.

Hypothesis 1

Rainfall and subsequent runoff from stormwater is the primary cause of elevated *Enterococcus* levels in Marina Del Sol (Hueiwang et al. 2005).

Objective 1

Determine if stormwater outfalls are the primary source of Enterococci in the marina by collecting samples during wet and dry weather events.

Hypothesis 2

Hotspots of *Enterococcus* will be present in Marina Del Sol.

Objective 2

Design a study that will comprehensively represent the waters in the marina to detect any locations that have a consistently high concentration of *Enterococcus*.

Hypothesis 3

A gradient will exist with higher levels of *Enterococcus* at the landward or back side of the marine and lower levels at the entrance/exit to Clear Lake (Guillen et al. 1993).

Objective 3

Design a study that will test for an *Enterococcus* gradient.

A follow up study was conducted in November of 2013 to determine the following hypothesis and objective.

Hypothesis 4

Sediments are a source of elevated *Enterococcus* concentrations in the water at the Marina Del Sol marina.

Objective 4

Determine *Enterococcus* in concentrations in sediments at the Marina Del Sol marina.

CHAPTER II

ENTEROCOCCUS CONTAMINATION OF MARINA DEL SOL

Marina Del Sol is a privately-owned marina situated in coastal Texas in an area that is popular for recreational boating. It provides an excellent model for studying fecal contamination factors of boat marinas since the Clear Lake area has the third largest concentration of privately owned marinas in the United States (TCEQ 2008).

Boat marinas themselves are considered to be non-point source discharges into Galveston Bay (TCEQ 2008). Of the thirty seven marinas located on Clear Lake and Galveston Bay, only twelve have boat sewage pump out stations (TCEQ 2008). All marinas, Clear Lake, and Galveston Bay are non-discharge zones. However, primary witnesses claim that some boat owners still directly dump their sewage into the water.

Other potential inputs of *Enterococcus* into Marina Del Sol are: contamination from leaking sewer pipes, sewer overflows, septic tank systems, boat sewage, wildlife, (i.e ducks), household pets, and stormwater runoff. Land use in the surrounding area was given a cursory examination and no commercial livestock facilities were found. The marina is surrounded by impervious surfaces on all sides, i.e. roads, parking lots, single-family houses, and commercial buildings. These impervious surfaces prevent rainwater from penetrating into the soil and can facilitate large influxes of stormwater runoff into the marina during rainfall events.

Marina Del Sol Operational History

The marina was established in 1992 and has been operational for 21 years. Marina Del Sol is a private company that is classified as a marine basin. The marina is owned and managed by Marinas International. Amber Faubion is the current marina manager of Marina Del Sol and is a certified Texas Stream Team Water Quality Monitor with the Galveston Bay Foundation. She played a crucial role in entering Marina Del Sol as the first partner in the GBF's Clean Water Partnership program.

Marine Del Sol is the only marina in the Clear Lake area that has a boat sewage pump-out station and mobile pump-out cart (Figure 2.1). It has been operational since 2001. Pump-out stations have the potential to spill boat waste into the marina if proper maintenance is not provided or misuse occurs. Two boat waste collection companies, Maritime Sanitation and Redfish Island Marine, contract with the marina to provide mobile boat waste pump-out services to residents and recreational boat users of the marina.

Figure 2.1. Pump-out station and mobile pump-out cart at Marina Del Sol.

Problem Statement

Pollution in marinas can come from either point or non-point sources. Examples of point sources include wastewater treatment facility effluents or chemical plant discharges. Non-point sources cannot be directly identified at their source. They are also known as diffuse sources of pollution because they consist of more than one source. Therefore, they are not easily identified and remedied. Boat sewage is considered a non-point source unless the dumping event is witnessed (31 TAC §523.1). While stormwater outfalls are technically point sources, the many types of individual pollutants that enter stormwater (i.e. pet waste, fertilizer, pesticides, etc.) having unidentifiable origins are considered non-point sources, thus making them more difficult to address.

Potential stormwater inputs with *Enterococcus* into Marina Del Sol include contamination by human, wildlife and domestic animal sources. *Enterococcus* contamination may be amplified by the impervious ground cover, such as roads, parking lots, housing and commercial developments that surround Marina Del Sol (see Figure 2.2). The impervious ground cover prevents rainwater from penetrating into the soil and disrupts the natural hydrologic cycle by causing large influxes of rainwater to flow into the marina and surrounding areas after rainfall events.

Additionally, the many residential yards in the neighborhood surrounding the marina decrease the amount of rainwater that can infiltrate into the soil significantly due to their high slopes and because sod yards tend to compact the soil due to their shallow root systems. Instead of being absorbed into the soil, rainwater flows over this ground cover and washes pet, bird waste, and other contaminants directly into the marina or indirectly through the stormwater retention pond and storm drains (Figure 2.2).

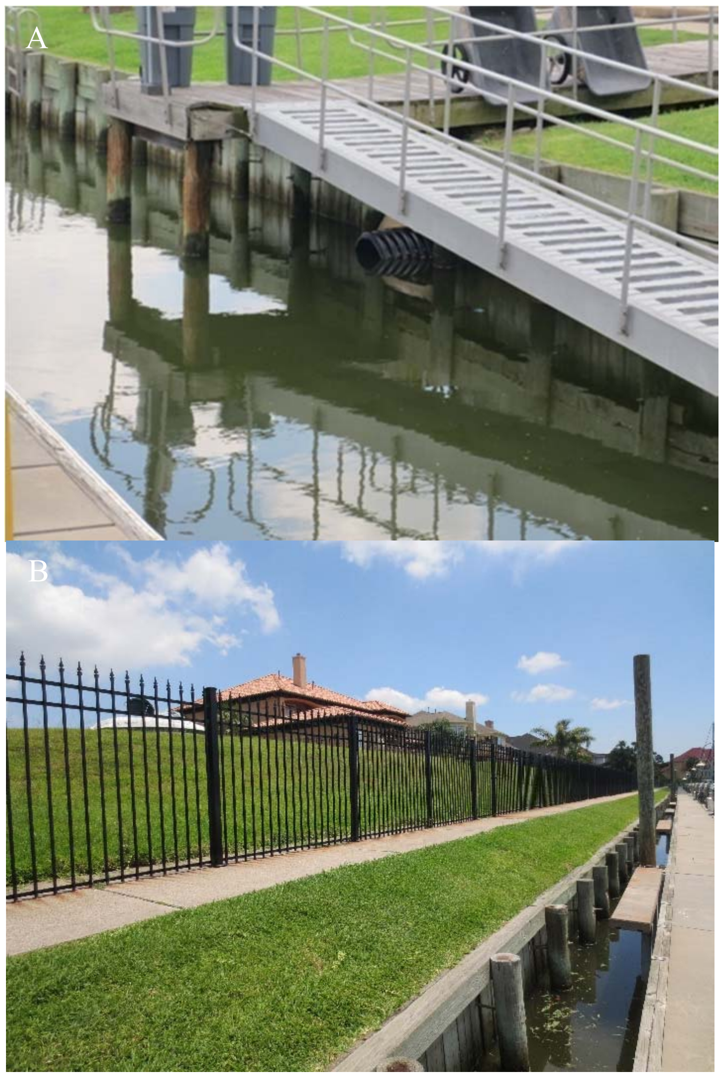

Figure 2.2. Stormwater outfall (A) and sloped residential yard on the northern border (B) of Marina Del Sol.

In general, marina managers and boaters in the Clear Lake and Galveston Bay region have expressed concerns to the GBF regarding pollution from boat sewage and stormwater runoff in local marinas. Quantifying the inputs from these sources is difficult due to the fact they are both non-point sources, and only one local study has taken place in marinas that is known (Guillen et al. 1993). However, having this information could prove valuable in decision making on the part of marinas and environmental groups like GBF.

Specifically, staff and tenants at Marina Del Sol have discussed their concerns with GBF and expressed an interest in analyzing water quality in the marina in more detail. There are currently three certified Texas Stream Team Water Quality Monitors who test for ENT at Marina Del Sol. These monitors use the same testing methods as described in this thesis, however only one sample is collected on the 15th of each month. Ten samples have been collected thus far between March 2013 and January 2014. Figure 2.3 is a graph of the *Enterococcus* data collected so far that shows a trend of increasing concentration (MPN/100 mL) over time.

These volunteer water quality monitors and marina users have reported potential boat sewage discharge and debris collection in western portions of the marina (Figure 2.4). These claims as well as the existing data indicating presence of detectable concentrations of *Enterococcus* in the water highlighted the pollution concerns in Marina Del Sol. These concerns led to the initiation of this thesis.

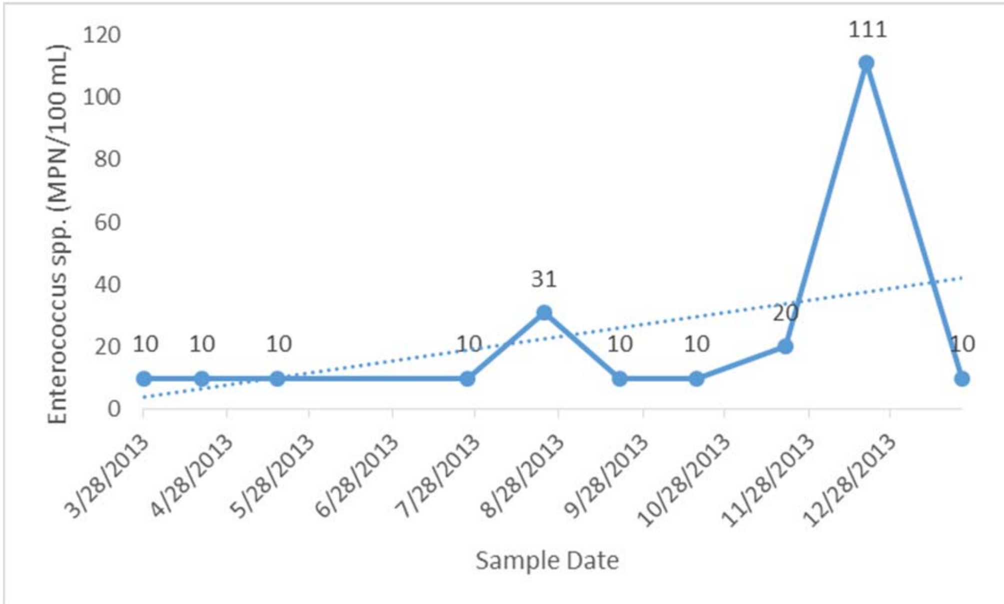

Figure 2.3. MPN of *Enterococcus* at volunteer monitoring site in Marina Del Sol.

Figure 2.4. Organic debris collecting in poorly circulated areas of Marina Del Sol.

Sampling Area Location

Marina Del Sol is directly connected to Clear Lake via an inlet on the marina's eastern border. The marina and Clear Lake are connected to the Galveston Bay system by the Clear Creek Channel under Highway 146 (Figure 2.5).

Figure 2.5. Geographic location of Marine Del Sol.

Marina Description

Currently, Marina Del Sol houses 205 boats in wet slips (62% occupancy), and 164 in two dry dock storage buildings (73% occupancy). One pump-out station and one pump-out cart are located on the property (Figure 2.1). On-site structures can be seen in

Figure 2.6. They consist of two small buildings (clubhouse and office), a pool and a gazebo.

Marina Del Sol occupies 11.92 acres within a residential land use area. The sampling area is bordered on the north by a housing division, on the west by a stormwater retention pond and marina parking lot, on the south by a cement-covered lot that was the former site of a marina apartment complex, and on the east by the inlet from Clear Lake. The specific location of the sampling sectors is shown in figure 2.6.

Water samples were considered to be contaminated if *Enterococcus* concentrations were above primary or secondary contact recreation limits set by Texas Administrative Code (30 TAC §307.7) for surface water quality standards. The primary contact (i.e. swimming, water skiing, surfing, and diving) limit is 35 colony forming units (CFU) per 100 mL for a geometric mean (i.e. multiple samples over a month time period) and 104 CFU/100 mL for single grab samples. The secondary contact (i.e. boating, canoeing, and kayaking) limit is 104 CFU/100 mL. The goal of the study was to determine if the marina contained fecal contamination so that best management practices can be identified and implemented, such practices will be determined upon the completion of the study. Subsequent studies will be required to determine if the contamination issue(s) have been resolved. The resulting data of this study will also be utilized to monitor temporal trends in environmental conditions in Marina Del Sol.

The primary objectives of this study were to 1) determine if hot spots of Enterococci bacteria exist in the surface water at Marina Del Sol that are indicative of an identifiable source and 2) determine whether or not the marina is safe for primary and secondary contact recreation use. If hotspots of *Enterococcus* are identified, best management practices will be recommended.

Materials and Methods

Sectors and sampling stations. In order to adequately document the levels of *Enterococcus* in Marina Del Sol from various potential inputs, the marina was divided into four sectors (A, B, C and D) based on a stratified judgmental sampling design (Figure 2.6). This type of sampling design was chosen for this preliminary study for several reasons. In order to discover potential hotspot(s) of *Enterococcus* the marina needed to be comprehensively represented. Prior knowledge of the sampling area was used to determine the appropriate borders of the sectors and the placement of the sampling stations. The four sectors for the study were chosen to comprehensively represent the potential pathways that Enterococci can enter Marina Del Sol. Each sector was designed to be homogenous and isolate the reflected input as best as possible. An exception was boat sewage.

Ten sampling stations were used to determine the presence of *Enterococcus* in the surface water of Marina Del Sol. The sampling stations were also chosen based on a judgmental sampling design. Prior knowledge of the sampling area was used to select sampling stations that best represent their corresponding sector. Within each sector the sampling stations were placed at an approximately equal distance to ensure the sector was sampled in its entirety and to capture data from the reflected potential bacteria input. Proportional allocation was used to determine the number of sampling stations per sector.

Figure 2.6 Marina Del Sol divided into four sectors (A-D).

A total of ten inputs were determined to potentially contribute *Enterococcus* into Marina Del Sol's surface water. Sector A has six individual inputs and therefore, is comprised of 60% of the total inputs. The primary inputs of *Enterococcus* for sector A are the four stormwater outfalls, the stormwater retention pond and boater sewage waste. Two stormwater outfalls are located on the western border and two on the southern border of the marina. The first stormwater outfall on the western border lies directly beneath dock D, closest to Slip 2. The second stormwater outfall on the western border is located on B dock, nearest slip 19 (Figure 2.7). The two stormwater outfalls on the southern border are located on A dock. The first stormwater outfall is nearest Slip 4 and the second closest to Slip 26. The culvert that connects the storm water retention pond to Marina Del Sol is located under the pathway that joins docks A and B on the south west border of the marina, as seen in figure 2.7. Because sector A has the highest percentage of inputs, six sampling stations were allocated to sector A.

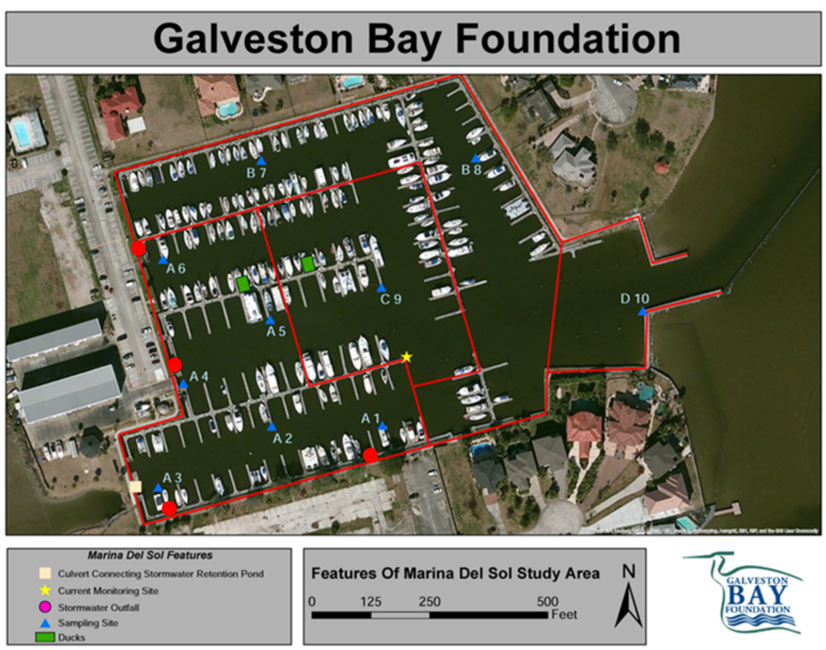

Figure 2.7. Sampling stations (blue triangles) and stormwater outfalls (red circles) in Marina Del Sol.

Two sampling stations were allocated to sector B because it contains two of the ten source inputs to the marina. Sector B's two inputs are from potential boater sewage waste and stormwater runoff from residential yards located on the northern, north east and south east borders of the marina (Figure 2.7). Sector C and D each have one direct input, accounting for 10% each of the total inputs, therefore, one sampling site was allocated to each sector. Clear Lake is the direct source input of *Enterococcus* to sector D, while boat sewage is the direct source input to sector C.

While the number of sampling stations per sector was determined by the number of associated inputs. The sampling stations also reflect the decreasing flow gradient from the eastern border of the marina to the western. The higher flow of sector D, the eastern most sector, allows for more adequate mixing of any present *Enterococcus* in the water column. Due to the reduced flow rate in sector A, less mixing occurs, and so additional samples were collected.

The letter of each of the ten sampling stations coordinates with the associated sector. Sampling site A1 is located in sector A on A dock on the end of the finger piling nearest slip 29 and was chosen to represent the stormwater outfall that is located on the southern border of the marina, nearest slip 26 (Figure 2.7). Sampling site A2 is located in the middle of dock B on the end of the finger piling nearest slip 32. It was selected because the sampling site is midway between stations A1 and A3 and will best represent the fairway between A and B docks. Sampling station A3 is located in the south western corner of Marina Del Sol on the finger piling between slips 2 and 3. It was chosen to determine the influence of the stormwater outfall and input from the stormwater retention pond to the west of the marina (Figure 2.7). A4 is located on the dock nearest slip 19 and represents the stormwater outfall under B dock (Figure 2.7). A5 is located on C dock at the end of the finger piling nearest slip 20. It was selected to represent the fairway between B and C docks. A6 is located on D dock at the end of the finger piling nearest slip 4. A6 represents the stormwater outfall under dock D.

Sampling station B7 is located in sector B in the middle of E dock on the end of the finger piling between slips 17 and 18. Site B8 is located in the middle of G dock at the end of the finger piling between slips 9 and 10 (Figure 2.7). The two sampling stations in sector B were chosen to detect any potential hotspot(s) created by the stormwater runoff from the residential development to the north, north east and south east.

The sampling station in sector C (C9) is located on C dock at the end of the finger piling nearest slip 44 (Figure 2.7). This sampling site was chosen to be representative of the fairway between C and B dock as well as, C and F docks. The sampling site in sector D (D10) is located on the south eastern corner of the piling nearest slip 1 on I dock (Figure 2.7). Sampling site D10 was chosen to best represent the fairway between H and I docks. Incoming surface water from Clear Lake create a flow pattern that forces the surface water close to site D10.

Sample collection. Samples were collected from June 27 to July 29, 2013 between the hours of 0800 and 1100. Each sample was taken at a depth of 0.3 meters (m) with a volume of 7.6 Liters (L). The water samples were analyzed for dissolved oxygen (mg/L) (DO), pH, salinity (ppt), temperature (°C), and the concentration of *Enterococcus*

(MPN/100 mL). Total water depth (m), water transparency (m), and air temperature (°C) were recorded at each site. Algal cover, water color, water clarity, water surface, water conditions and water odor were observed and recorded for each sample taken. All samples were taken at the end of the finger pilings to best represent the marinas fairways and not the niche within the slips.

The environmental parameters measured by this study were chosen because they potentially provide further insight into the health of the marina. These parameters are also the same parameters that the current volunteering water quality monitors are using at Marina Del Sol and can be compared with past data. The observational data is gathered to further support the bacteria and environmental data.

Field observations and water quality data. All water quality sampling was performed according to the Texas Stream Team water quality monitor methods (TST 2009). Prior to collecting the water sample, field observations were recorded at each sampling site. Field observations consisted of flow severity, algal cover, water surface, water conditions, and present weather. To ensure consistency, flow severity was determined on a scale of one to six. A number one indicates no flow and six indicates a high amount of flow. Algal cover was determined on a scale of one to five. Ranging from 1) absent to 5) dominate. Water surface was given a value from one to five. A value of 1) indicates clear, 2) scum, 3) foam, 4) debris and 5) an oil or sheen is present on the water surface. The water conditions are represented on scale ranging from 1) calm to 4) whitecaps. The present weather was assessed on a scale ranging from one to four.

With 1) indicating clear sky, 2) cloudy, 3) overcast and 4) rain. Three-day rainfall accumulation prior to sampling was recorded from NOAA's Climate Data Online.

The water color, water clarity and water odor of the samples were recorded based on the following numeric scales. For water color: 1) no color, 2) light green, 3) dark green, 4) tan, 5) red, 6) green/brown and 7) black. For water clarity: 1) clear, 2) cloudy and 3) turbid. For water odor: 1) none, 2) oil, 3) acrid (pungent), 4) sewage, 5) rotten egg, 6) fishy and 7) musky. The samples water color and water odor were observed using a 100 mL clear beaker. The water clarity was taken prior to the bucket grab.

All water samples were collected using an 11.4 L bucket. Before collecting the water, the bucket was rinsed twice with water from the sampling site. To prevent an increase in dissolved oxygen levels from agitating the sampled water the bucket was gently lowered into the water and allowed to fill at an angle. A Secchi disk was then used to collect water transparency and total water depth.

Aliquots for measurement of DO, pH, salinity and temperature were immediately subsampled from the bucket grab. DO was determined using a modified Winkler method. The pH was determined using a liquid Wide Range indicator and Octo-Slide Viewer. Two different mercury thermometers were used to measure the temperature of the water and the air. Salinity?

Bacterial analysis. The IDEXX method for Enterococci detection and enumeration estimation was used to quantify the MPN/100 mL of fecal indicator bacteria in each water sample (Budnick et al. 1996; Chen et al. 1996). Disposable nitrile gloves were worn at all times to protect against infections and contamination of the bacteria samples. Prior to collecting the bacteria sample from the bucket grab, gloves were sterilized using rubbing alcohol. A 100 mL beaker was sterilized with rubbing alcohol and allowed to air dry. The beaker was then used to transfer 100 mL of water from the bucket grab to a Whirl–pack containing a sodium thiosulphate tab. The tab was then crushed to release the thiosulphate and neutralize any free chlorine. The water sample was placed on ice for transfer to the GBF lab (Figure 2.8).

All bacteria samples were processed at the GBF lab using a Fluorogenic Substrate *Enterococcus* Test, Multi-well procedure. A sterilized, disposable IDEXX Quanti-Tray was used to perform the test. The β-D-glucosidase enzyme hydrolyzes the substrate and causes enterococci to fluoresce under long-wavelength (366-nm) UV light (Budnick et al. 1996). The MPN is estimated by the number of wells that fluoresce after a 24 hour incubation period has occurred (Figure 2.8).

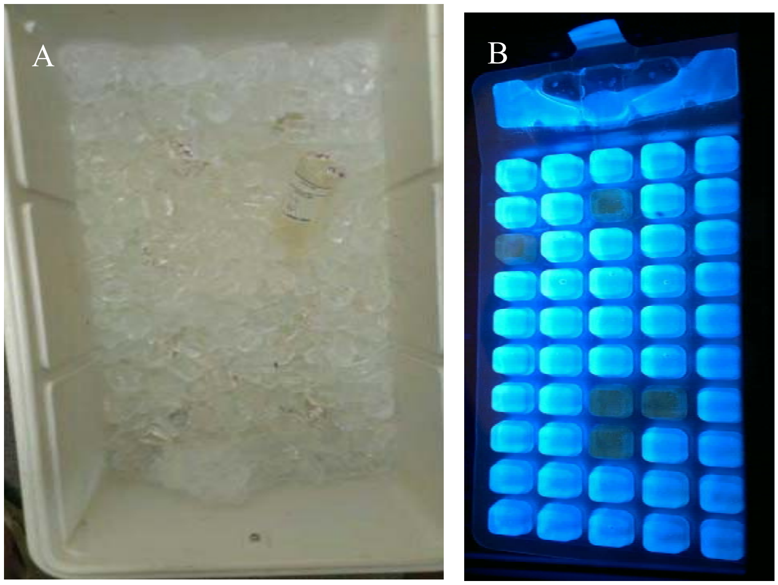

Figure 2.8. Ice chest containing Whirl-packs (A) and IDEXX Quanti-Tray fluorescing, indicating positive wells (B).

Data Quality Indicators (DQIs)

Data review and validation. Data review and verification was performed using data management checklist and self-assessments, as appropriate to the project task, followed by automated database functions that will validate data as the information is entered into the database. The data to be verified are evaluated against project specifications and are checked for errors, especially errors in transcription, calculations, and data input. Potential errors are identified by examination of documentation and by manual and computer assisted examination of corollary or unreasonable data. If a question arises or a potential error or anomaly is identified, the Clean Water Program (CWP) Volunteer Monitor responsible for generating the data is contacted to resolve the issue. Issues that can be corrected are corrected and documented. If there are errors in the calibration log, expired reagents used to generate the sampling data, or any other deviations from the field or *Enterococcus* data review checklists the corresponding data is flagged in the database.

Data management. The data management system for this project is detailed in GBF's EPA-approved Quality Assurance Project Plan. Electronic data sheets are received by GBF from all CWP Volunteer Monitors, and saved on GBF staff computers and a secure network drive. These files are saved in the original format and other than changing the name of a file, remains unchanged. Original field sheets are kept by the CWP Volunteer Monitor. Field calibration and Quality Control reports, and COCs are reviewed by the CWP Project Manager/QAO before any data entry is made. If there are nonconformance issues such as failed calibration, the CWP Project Manager/QAO writes instructions in a different colored ink on the related field form regarding data entry and the instructions are initialed and dated. Data is processed by the CWP Project Manager/QAO or the GBF Water Quality Program Assistant/Intern, entered into the database, and saved on a secure network drive. It is reviewed for accuracy and completeness by either the CWP Project Manager/QAO or GBF Water Quality Program Assistant/Intern (but not the person who performed the original data entry). All changes, validation, and verification actions on the data are documented and saved with the original electronic data sheets. Weekly backups are completed on GBF's server. All electronic data is maintained for at least eight (8) years by GBF. GBF maintains several networked computers to store and manage CWP Volunteer Monitoring data

Assessment oversight. The procedures that were used to implement the Quality Assurance Project (QAP) program for this project are detailed in GBF's EPA-approved Quality Management Plan and Quality Assurance Project Plan. This includes oversight by the Quality Assurance (QA) Officer, and how often a QA review of the different aspects of the project, including audits of field and laboratory procedures, use of performance samples, review of laboratory and field data, etc., will take place. It also describes how the QA Officer will ensure that identified field and analytical problems are corrected and the mechanism by which this will be accomplished.

Results

For the duration of the study air temperature ranged from 21.5° C to 29° C. The maximum water temperature was 31° C while, the minimum was 27° C. Dissolved oxygen (DO) levels ranged from a low of 1.7 mg/L at site A3 to a high of 7.1 mg/L at stations B8 and C9 (Figure 2.9). The pH remained relatively stable and ranged from 7.5 to 8.5. Average water transparency ranged from a low of 0.18 meters at A4 to a high of 0.56 meters at sampling station A3. The salinity levels varied per site and over the sampling period the highest salinity was 24 ppt at station A6 while, the lowest was 11.7 ppt at station B8. Station A5 had the greatest depth of 1.74 meters. The shallowest was station A3 at an average depth of 1.25 meters. There was a 24 day period of no precipitation during the study. The highest amount of rainfall that accumulated over a three day period was 1.39 inches. There were a total of 11 days with no precipitation and 4 days with precipitation over the course of the study.

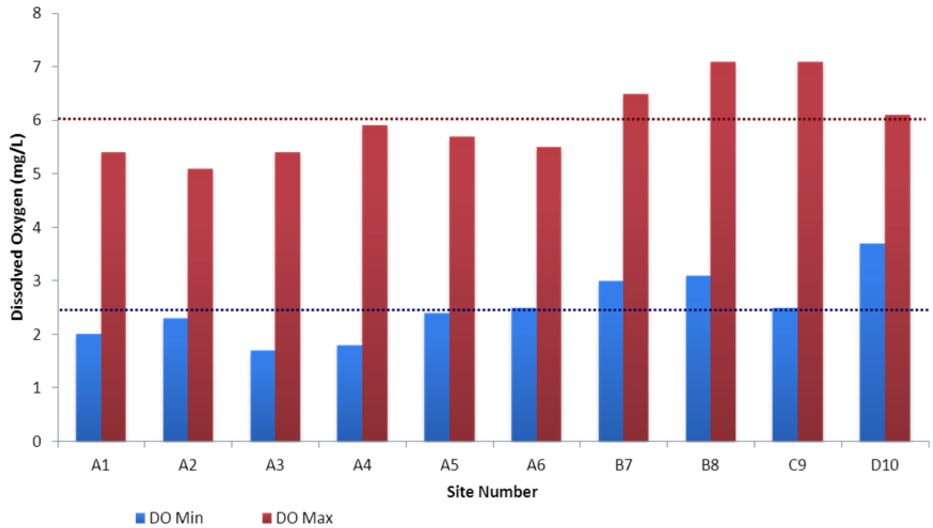

Figure 2.9. Minimum and maximum dissolved oxygen levels (mg/L) at the sampling stations, horizontal lines indicate average high (red) and low (blue) DO values.

For the duration of the sampling period the DO was on average 3.42 mg/L. There is a positive correlation between DO readings and wet weather events. The highest DO levels were recorded the sampling day after the largest accumulation of rainfall occurred.

Over the 5 week sampling period 149 bacteria samples were collected. Out of the fifteen days that sampling was conducted, 11 dry weather and 4 wet weather sampling days occurred. All stations except site A3 and A4 had samples with <10 MPN (table 2.1). The highest single sample concentration of Enterococci was located at site B7 (1,445 MPN/100 mL). Two hotspots were found at stations A3 and A4 yielding geometric means of 42.98 and 41.25 MPN, which exceed the U.S. EPA primary contact recreation limit of 35 MPN. Site C9 also yielded the second highest MPN of Enterococci at 1,184.

Table 2.1. Concentrations of Enterococci at the Marina Del Sol marina.

Site ID	**n**	**Min**	**Max**	**Geometric Mean**
A1	15	<10	164	14.92
A2	15	<10	99	25.07
A3	**15**	**10**	**271**	**42.98**
A4	**15**	**10**	**624**	**41.25**
A5	15	<10	137	22.56
A6	14	<10	110	16.21
B7	15	<10	**1445**	20.4
B8	15	<10	591	11.86
C9	15	<10	**1184**	27.23
D10	15	<10	110	10.36

The geometric mean of *Enterococcus* concentrations were not found to be signifcantly different, when wet and dry weather samples were compared (Figure 2.10).The P value was 0.224 while, the U statistic was 1654. The P value of 0.224 was not significant enough to correlate an increase in *Enterococcus* with an associated wet weather event. While the Mann-Whitney U statistic indicates that wet weather is not the primary factor contributing to an increase of *Enterococcus* in Marina Del Sol.

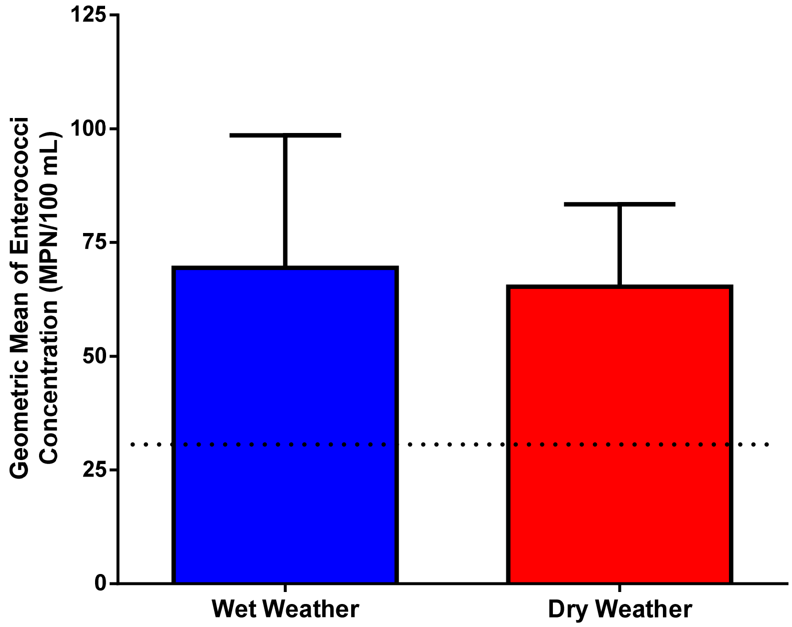

Figure 2.10. Geometric mean of *Enterococcus* vs. wet and dry weather.

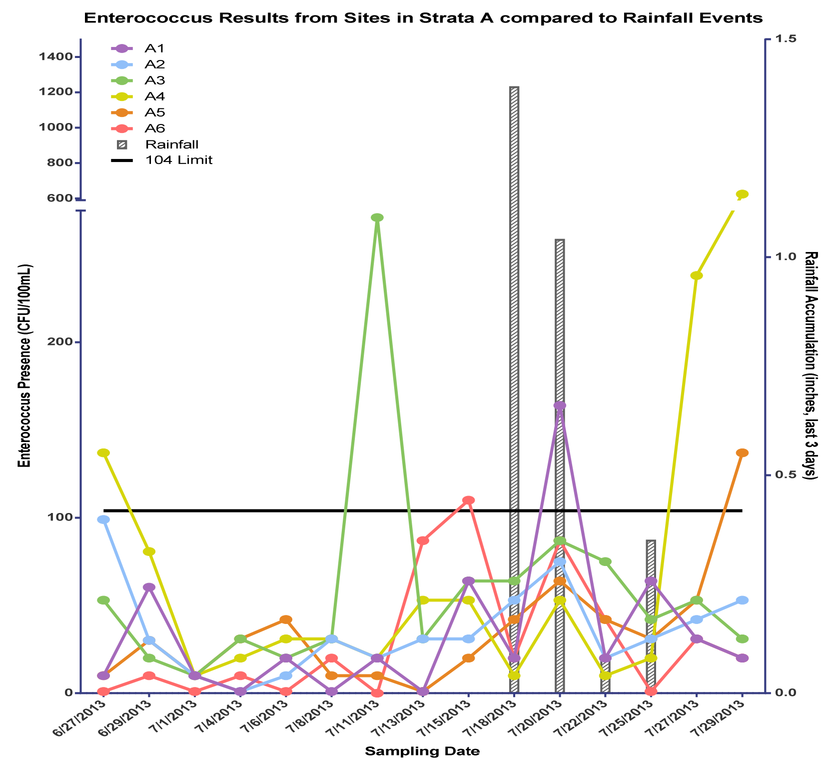

Figure 2.11. *Enterococcus* vs. rainfall stormwater runoff in sector A.

Figure 2.11 represents the presence of *Enterococcus* from all sampling stations within sector A compared to rainfall events. The EPA primary contact limit (104 CFU/100 mL) for a single grab sample was exceeded a total of six times in sector A. All stations within sector A exceeded the single grab sample limit at least once throughout the sampling period except station A2, which did not exceed the primary contact limit. Sampling station A4 exceeded the primary contact limit for a single grab sample the most at three times (20%). *Enterococcus* concentrations were recorded at 137, 238 and

624 MPN for site A4. Sampling site A3 yielded an *Enterococcus* spike from 31 MPN to an MPN of 271 (Figure 2.11) and exceeded the EPA single grab sample limit 7% of the time.

The three day rainfall accumulation peaked at 1.39 inches on 7/18/13. The three day rainfall accumulation then decreased to 1.04 inches. The lack of rain lowered the rainfall accumulation further to 0.08. A subsequent rainfall event on 7/22/13 increased the three day rainfall accumulation to 0.35 inches before returning to 0.0 inches on 7/27/13. In order for the original hypothesis to be supported the increase of *Enterococcus* concentrations should correlate during or post peak rainfall events. For sector A this is not the case.

Figure 2.11 suggests sporadic *Enterococcus* concentrations in sector A. Increases in *Enterococcus* concentrations occurred prior to during and after rain events, opposed to a uniform positive correlation of *Enterococcus* concentrations increasing with rainfall events. For example, a spike from 31 MPN to an *Enterococcus* concentration of 271 MPN occurred at sampling site A3 on 7/11/13. This value was recorded 20 days from the last precipitation event and seven days prior to the first rainfall event. From 7/18/13 to 7/20/13 site A1 increased from 20 to 164 MPN. This increase corresponds with a rainfall accumulation of 1.04 inches. Sampling site A4 peaked at an MPN of 624 on 7/29/13. Four days after a rainfall accumulation 0.35 inches was recorded.

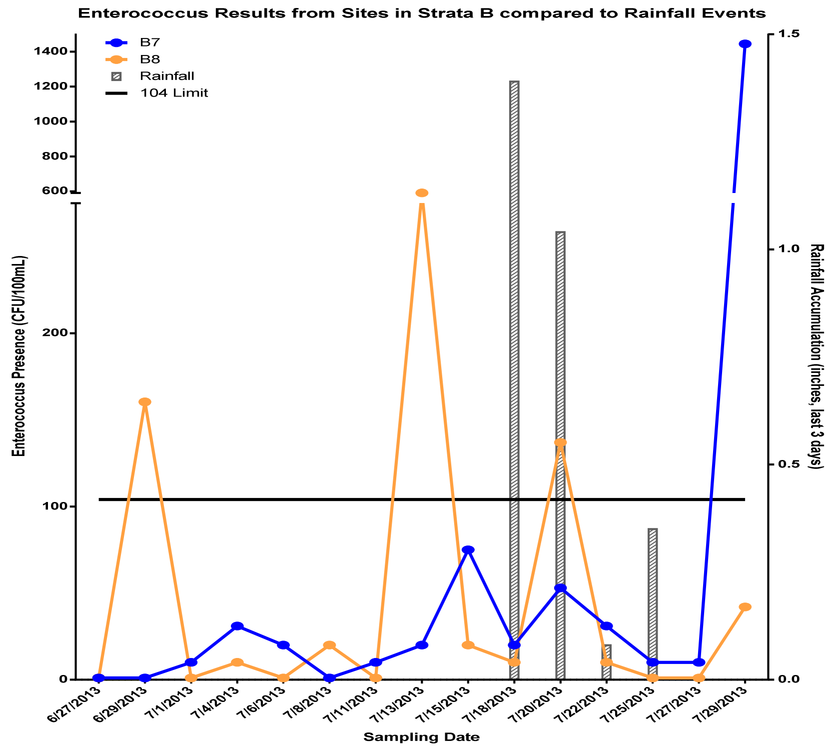

Figure 2.12. *Enterococcus* vs. rainfall stormwater runoff in sector B.

Figure 2.12 shows the concentration of *Enterococcus* compared to rainfall events in sector B. The EPA primary contact limit (104 CFU/100 mL) for a single grab sample was exceeded a total of four times in sector B. Sampling site B8 produced the most sampling dates where the *Enterococcus* concentrations exceed the single grab sample limit 20% of the time. The first breach occurred on 6/29/13 at 160.4 MPN, the second on 7/13/13 at 591 MPN and the third on 7/20/13 at an MPN of 137. One exceedance of the primary contact single grab sample limit was recorded during a wet weather event with a three day rainfall accumulation of 1.04 inches and *Enterococcus* concentration of 137 MPN. The other three breaches of the EPA's primary contact limit (104 CFU/100 mL) for a single grab sample did not occur on sampling days with any recorded rain events. Two were recorded prior to wet weather events and one exceedance was recorded after the wet weather events.

Site B7 exceed the single grab sample limit once at a concentration of 1,445 MPN. This is also the highest *Enterococcus* value recorded during the study. This spike increased from an *Enterococcus* concentration of 10 MPN on 7/27/13 to 1,445 MPN on 7/29/13 (Figure 2.12). There was no rainfall recorded for four days prior to this increase.

Figure 2.13 shows the MPN of *Enterococcus* from site C9, in sector C, compared to rainfall events. Site C9 exceeded the EPA primary contact limit (104 CFU/100 mL) for a single grab sample three times (20%). The first breach occurred on 7/8/13 at an MPN of 110. The second on 7/20/13 at an MPN of 1,184 and the third on 7/29/13 at an MPN of 1,091. The first breach occurred ten days prior to the first recorded rainfall event, seventeen days from the last recorded precipitation. The second occurred two days after the peak rainfall event took place (1.39 inches) and during a three day accumulation of 1.04 inches. The third spike of *Enterococcus* concentrations that breached the EPA primary contact standard occurred four days from a rainfall accumulation of 0.35 inches.

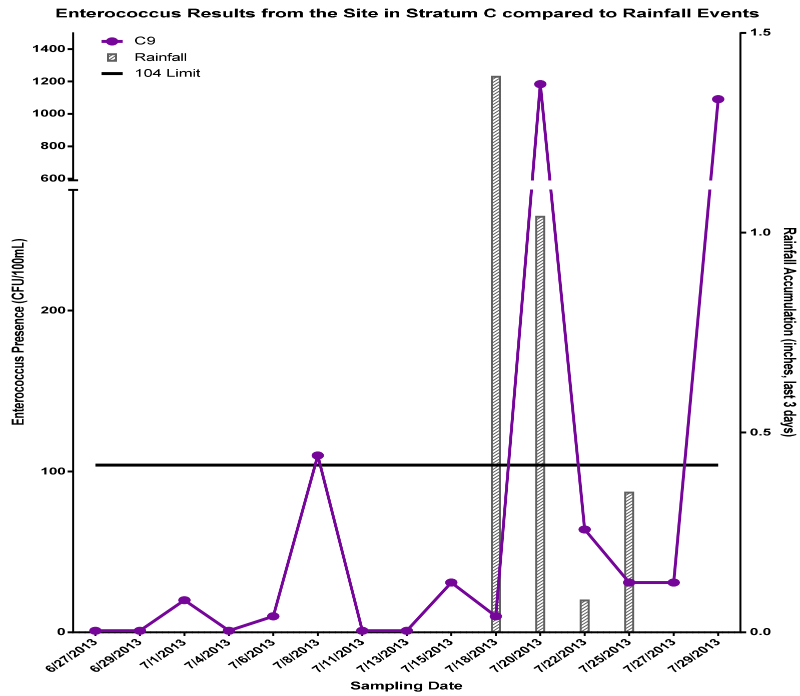

Figure 2.13. *Enterococcus* vs. rainfall stormwater runoff in sector C.

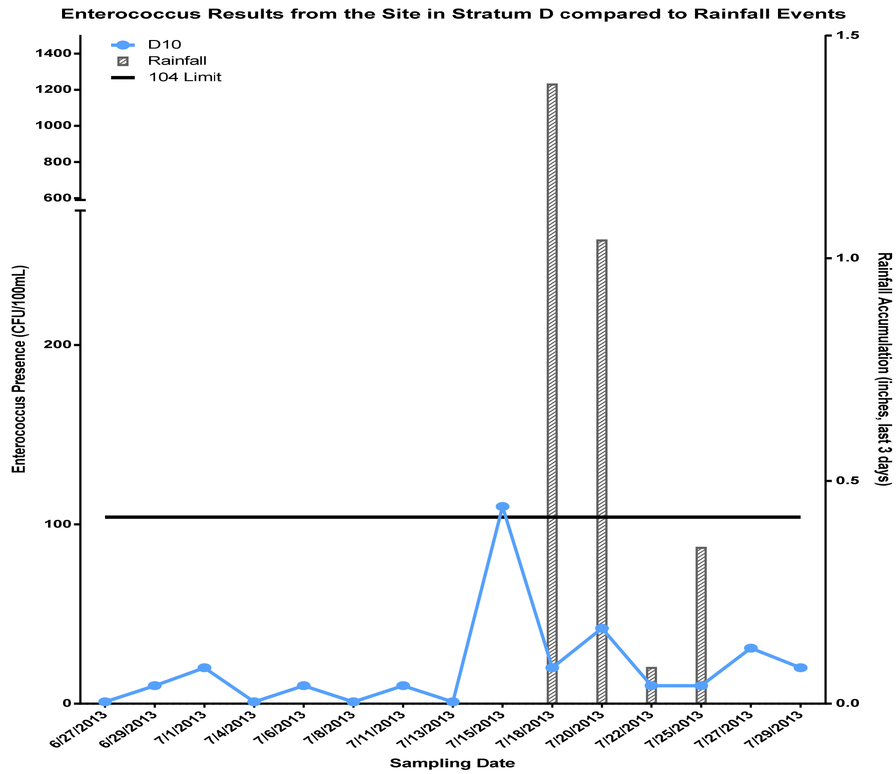

Figure 2.14. *Enterococcus* vs. rainfall stormwater runoff in sector D.

Figure 2.14 represents the one sampling site in sector D (D10) compared to rainfall events. Site D10 exceeded the EPA primary contact limit once on 7/15/13 at an MPN of 110. This breach of the primary contact limit occurred prior to a wet weather event. When the sample was taken twenty-four days had passed from the last recorded precipitation.

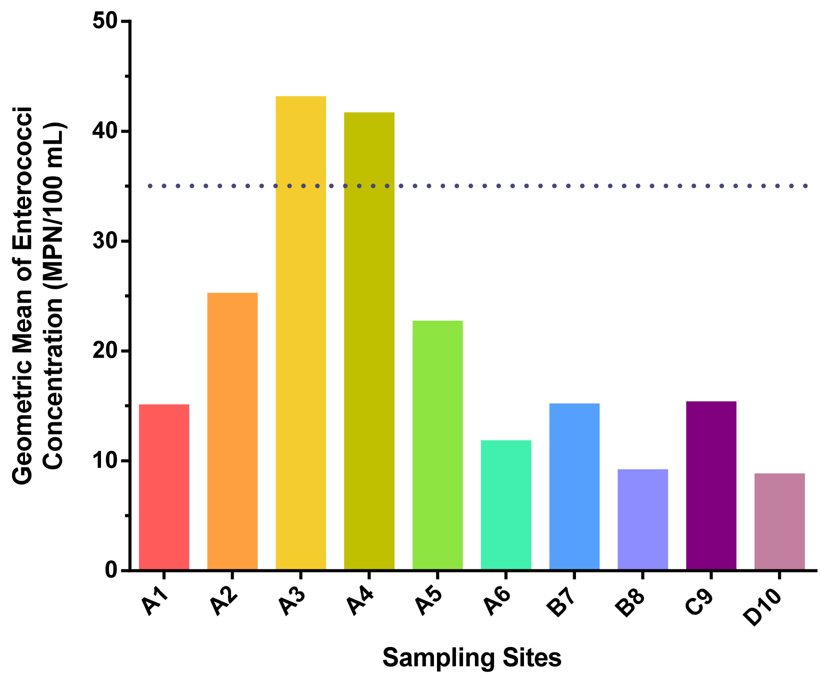

Figure 2.15. Geometric means of *Enterococcus* for all sampling sites.

Figure 2.15 compares the geometric mean of *Enterococcus* concentrations at each sampling station. Figure 2.16 depicts the geometric mean of *Enterococcus* concentrations in sector A with sector B, C and D. Both of these figures relate to the second hypothesis that states hotspots of *Enterococcus* will be present in Marina Del Sol. Figure 2.15 also reveals that two hotspots of *Enterococcus* were present in Marina Del Sol at sampling stations A3 and A4. Site A3 exceeded the EPA primary contact recreation limit for a geometric mean (35 MPN/100 mL) by 8 MPN (43 MPN). Site A4 exceeded the EPA primary contact recreation limit for a geometric mean by an MPN of 6.5 (41.5 MPN/100 mL). Sampling stations B8 and D10 had the lowest geometric means of *Enterococcus* at 12.3 and 10.4 MPN/100 mL, respectively.

The geometric means for the six sampling stations in sector A almost doubled the geometric mean of *Enterococcus* concentration for the other four stations combined (Figure 2.16). This indicates that any present hotspots of *Enterococcus* will be located in sector A.

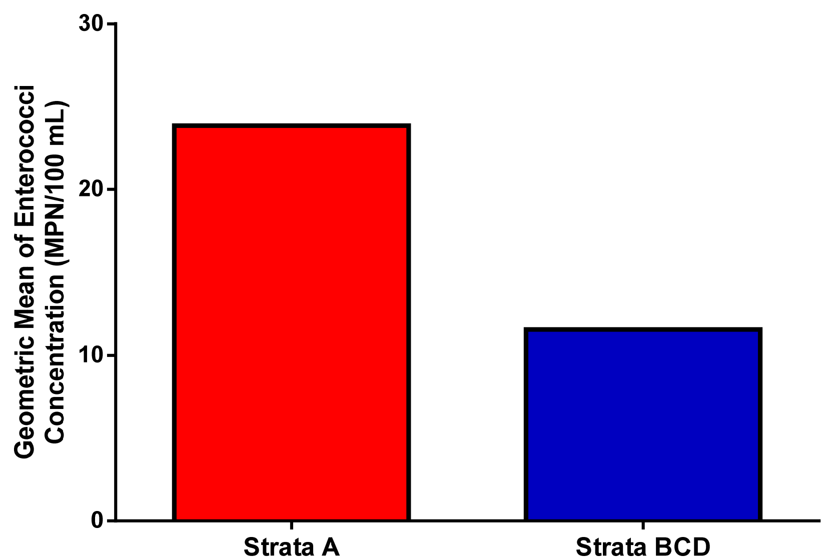

Figure 2.16. Geometric mean of Enterococci sector A vs. sectors B,C and D.

The third hypothesis states that *Enterococcus* concentrations will occur along a gradient. A comparison of the geometric means of *Enterococcus* concentrations at sampling stations throughout Marina Del Sol supports this hypothesis (Figure 2.17). A gradient is evident, from the marina entrance to the back portions of the marina. *Enterococcus* concentration were considered high (red) if they exceeded the EPA primary contact recreation limit for a geometric mean of 35 MPN, medium (yellow) if the geometric mean concentration was between 15 and 34.9 MPN and low (green) if the geometric mean was below 14.9 MPN (Figure 2.17).

To identify any positive correlations, regression analysis was performed on water temperature, DO and transparency vs. bacteria data. The water temperature and DO test yielded low coefficient of determination values and were concluded to not be significantly correlated to the level of *Enterococcus* bacteria in Marina Del Sol. However, the coefficient of determination value for transparency ($R^2 = 0.44$) (Figure 2.18) indicates a moderate correlation.

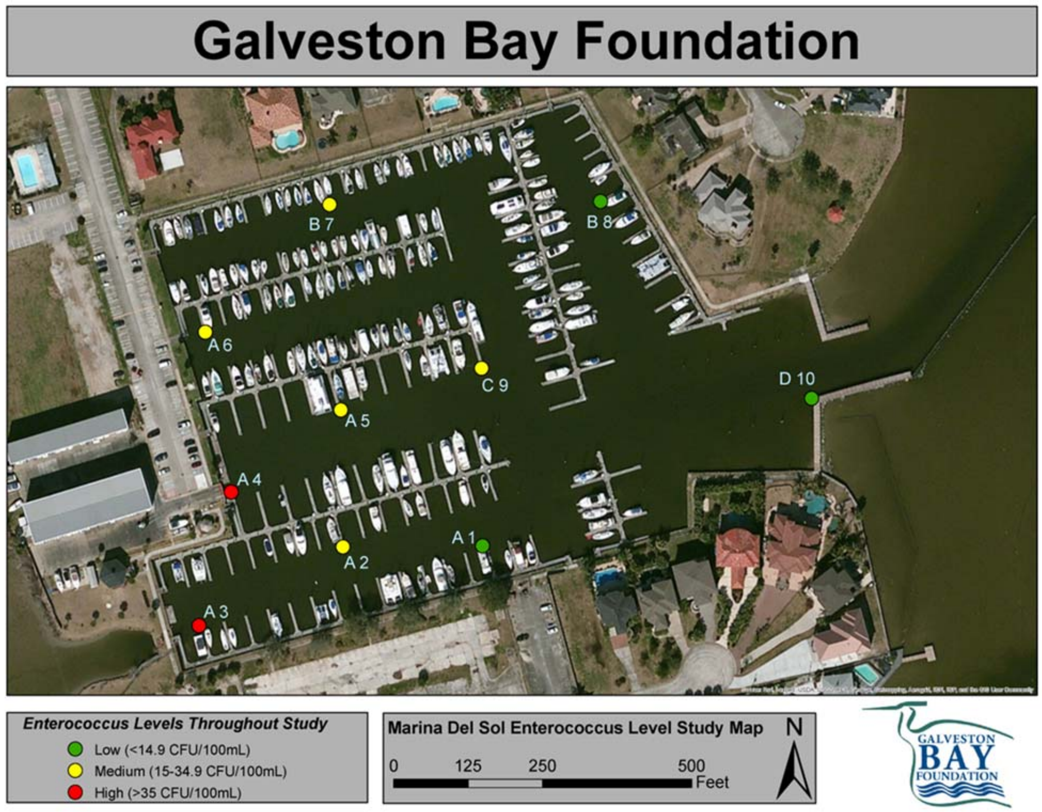

Figure 2.17. Gradient of Enterococci concentrations. Highest concentrations are represented by red dots, intermediate by yellow dots, and lowest by green dots.

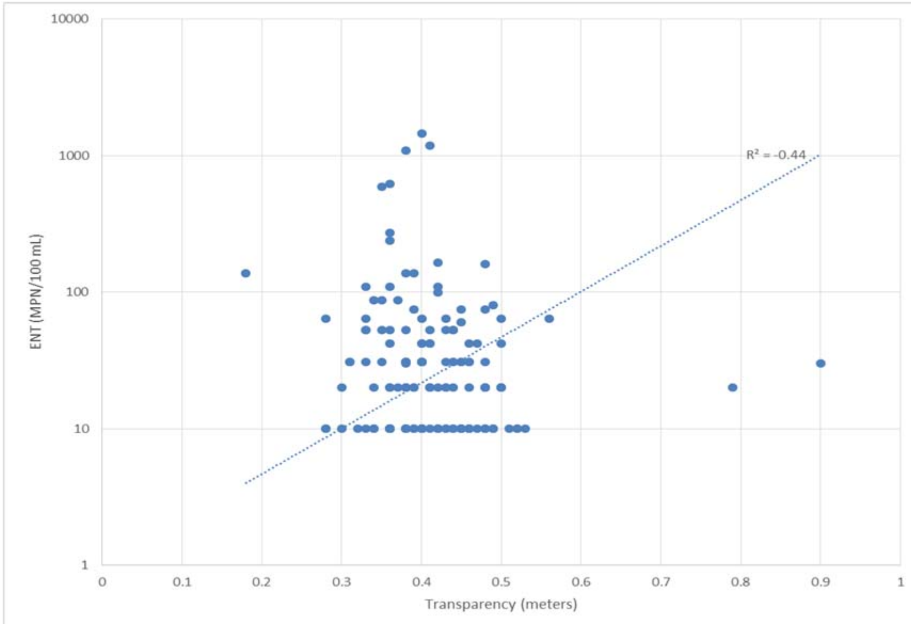

Figure 2.18. ENT (MPN/100 mL) vs. Transparency (m).

Discussion

Initially, it was presumed that the *Enterococcus* levels would be uniform throughout the marina. Early on in the sampling process this was tested. It was discovered that not only were the *Enterococcus* levels highly variable in the marina, they varied from site to site. The level of *Enterococcus* varied from day to day as well as, on the same day.

The widely variable concentrations of *Enterococcus* in the marina suggested that either the bacteria was replicating during periods of hospitable environmental conditions such as a lower salinity and temperature; or a direct input of *Enterococcus* was being introduced into the marina prior to sampling or the *Enterococcus* was being sequestered in the marinas sediments and re-suspended. The persistence of *Enterococcus* in estuarine and marine environments has been well documented (Byappanahalli et al. 1998; Desmarais et al. 2002; Solo-Gabriele et al. 2000). The evidence suggested that the *Enterococcus* was not only persisting but remaining viable in the water column (Anderson et al. 2005). Anderson et al. (2005) also suggested that *Enterococcus* may be able to multiply in warm subtropical waters.

Multiple studies have documented an inverse relationship between salinity and the concentration of *Enterococcus* present in the environment (Byappanahalli et al. 2012; Youngsul et al. 2005). The water of the marina is brackish and averaged 16.1 ppt over the course of the study. A relationship between salinity and the concentration of environmental *Enterococcus* could not be discerned based on the available data. A brackish water environment could have contributed to an increased survival of *Enterococcus* once the fecal indicator bacterium has been introduced into the marina. The trend may not be identifiable due to the brief duration of the study period. However, the average 16.1 ppt of salinity could have allowed background populations of *Enterococcus* to persist longer in the environment.

Another factor that could affect the background population of *Enterococcus* in the marina is temperature. The average water temperature over the duration of the study was 29.3°C. The water temperature did not exceeded 31°C. A third environmental factor that has been known to influence the persistence of *Enterococcus* in the marine environment is UV radiation (Byappanahalli et al. 2012; Fujioka et al. 1981; Gameson and Saxon 1967). It is not likely that temporal environmental factors within Marina Del

Sol have an effect on the reported concentrations of *Enterococcus*, with the exception of UV light rendering the FIB inactive. Therefore, other explanations for the variance of *Enterococcus* concentrations in the marina were explored.

Upon analyzing the data further I hypothesized that if the *Enterococcus* could bind to particulates it could be sequestered in the sediments of the marina (Anderson et al. 2005; Fries et al. 2006; Yamahara et al. 2009). The sediments of the marina and associated stormwater retention pond consist of fine silt sediments. These sediments create an ideal refuge for *Enterococcus* bacteria to bind and settle out of the water column. Furthermore, the survival rate of *Enterococcus* is known to increase in darkness (Lessard and Sieburth 1983). The sequestration of *Enterococcus* has been well documented. I further hypothesized that if the *Enterococcus* was capable of being sequestered in the marina sediments, it was also capable of being re-suspended into the water column. The re-suspension could occur from a variety of anthropogenic and environmentally influenced events.

The marina is on average 1.5 meters in depth. Because the marina is a shallow water body the effects of strong wind events could re-suspended the *Enterococcus*. A high level of wind and waves was observed on 7/15/2013. The highest concentration of *Enterococcus* was recorded at station D10 (110 MPN) during this wind event. The shallowness of the marina also places the benthic environment at the mercy of the waves and tides. The marina is tidally influenced and has been observed to fluctuate by 0.5 meter with low and high tides. These tides also allow transfer of water and possibly the fine sediments of the stormwater retention pond and the marina via the culverts located on the western border of the marina (Figure 2.19).

Figure 2.19. Marina side, culverts connecting marina and stormwater retention pond.

The scoured bottom directly in front of the culverts connecting the stormwater retention pond is evidence that water and sediments are transferred between the marina and pond. The area directly in front of the culverts is the deepest portion of the stormwater pond. Rock and chunks of concrete were recovered in this area with the sediment grab. No fine sediments were recovered in the area directly in front of the culverts. This suggests that the influence of the tides allows water and sediment to be transferred from the stormwater pond to the marina and vice versa.

Along with the environmentally influenced re-suspension of sequestered *Enterococcus*, it is possible that the sediments were agitated by anthropogenic influences. The high amount of boat and associated boat traffic in the shallow marina suggest that the sediments could be re-suspended by the revving of a boat engine. The thrust from the propeller could stir and re-suspend the *Enterococcus*. This could lead to an increase of *Enterococcus* levels in the area of the incident. However, no incidents similar to this were observed during the sampling period. Therefore, it is not likely that it played a part in the spikes of *Enterococcus* during the sampling period.

Hardhead catfish (*Ariopsis felis*) and stripped mullet (*Mugil cephalus*) were frequently observed during the sampling periods. It is possible that bioturbation of the marina sediments by these species re-suspend marina sediments into the water column. The Hardhead Catfish is the most likely candidate due to their size and benthic tendencies.

Another possibility of the *Enterococcus* spikes is a direct input prior to the samples collection. This input could have a variety of origins. One possibility is the direct contamination of the sample by a boater waste dumping event. The direct purging of fecal waste into the water could lead to an isolated increase in *Enterococcus* concentration at a site in close proximity to the dumping event (Mallin et al. 2010). The dumping of boater waste into Marina Del Sol has been witnessed by residents and is one of the primary complaints leading to the necessity of this study.

Contrary to Hueiwang et al. 2005, Youngsul et al. 2005 and my own initial predictions rainfall and subsequent non-point source runoff is not the primary factor that effects the levels of *Enterococcus* in Marina Del Sol. The results from the wet and dry weather events show that a wet weather event does not increase the concentrations of *Enterococcus* in this marina. This supports the hypothesis that the *Enterococcus* must persist to some degree in the marina or a direct input is in close proximity.

The spikes of *Enterococcus* could also be caused by a direct input of fertilizer and domestic or wild animal waste. No cattle or other large domestic animals were identified in the vicinity of the marina during the sampling period. A more likely cause of the spikes is the duck population of the marina. Ducks were observed in close proximity to the sampling stations on fifteen occasions. A flock of sixteen ducks was observed on the day prior to a spike of 1,184 MPN at sampling site C9. A considerable amount of duck fecal waste was seen on the docks of the marina.

The ducks congregated on and around dock C. They made use of dingy platforms in slips C17 and C33 to perch, allowing fecal waste to accumulate on C dock and the dingy platforms. The platforms are approximately 74.5 and 38 meters from sampling station C9 where *Enterococcus* concentrations frequently fluctuated. A considerable amount of fecal waste from the resident population of ducks accumulated on C dock prior to the 7/18/2013 rain event. This was the largest wet weather event at 1.39 inches. A spike of 1,184 MPN was recorded at sampling station C9 during the sampling period after the rain event.

The potential for bird waste to contribute to *Enterococcus* levels has been previously documented by Fleming and Fraser 2001. They show that on average a duck is capable of producing 110 grams of fresh manure per day. They found this 110 grams of fresh manure to contain approximately 180 x 10^6 fecal coliform bacteria colonies. In another study high fecal coliform levels resulting in beach closures in Madison, Wisconsin, were attributed to a permanent mallard duck population of 100 to 200 ducks (Standridge et al. 1979). These studies are supported by the fresh duck manure that was sampled from dock C at Marina Del Sol. This sample yielded an approximate MPN of 4.4 x 10^9 and was highly concentrated. The fresh duck manure is a potential contributing factor to the bacteria loading of Marina Del Sol.

However, due to the ability of UV light to render the bacteria inactive it is not likely that the duck fecal waste seen on the docks significantly contributed to the spikes of *Enterococcus* found in the marina. Ducks defecating directly into the water around the sampling stations could have contributed significantly to *Enterococcus* concentrations. While ducks and other birds observed have been known to contribute to the levels of FIB bacteria in water bodies they are likely only one piece to the puzzle.

Despite prior studies the highest concentration of *Enterococcus* were not recorded at the sampling stations adjacent to the four stormwater outfalls (Youngsul et al. 2005). This goes against popular belief that stormwater outfalls are the primary source of *Enterococcus* and fecal waste contaminants in marinas.

Additionally, the exceedance of EPA’s primary contact recreation limit of 35 MPN/100 mL at site A4 located by the boater waste pump out station suggests that the pump out station may be adding to the contamination of the marina waters. Leaking pipes or improper use could be a source of fecal waste into the marina from the pump out station or pump out cart. Future investigations should focus on site A4 and the area immediately surrounding the pump out station.

CHAPTER III

STORMWATER RETENTION POND AS A SOURCE OF *ENTEROCOCCUS*

After considering the potential for *Enterococcus* to remain viable and enter a replication phase with the onset of ideal environmental parameters and the possibility of *Enterococcus* to be re-suspended I was not satisfied. The stormwater retention pond came under further scrutiny. I hypothesized that the stormwater retention pond (Figure 3.1) was serving as a source of *Enterococcus*.

The results from the initial summer study indicated that the stormwater retention pond to the west of the marina could be a possible source of Enterococci. Dr. Robin Brinkmeyer and I completed a follow up study to determine the potential for the *Enterococcus* bacteria to be sequestered in the marine sediments of the stormwater retention pond. The stormwater retention ponds potential to be acting as a source of *Enterococcus* to Marina Del Sol was to be evaluated. The main objective of this follow up study was to determine the public health threat from the re-suspension of stored Enterococci which indicates the presence of pathogenic microorganisms and sewage contamination.

The two hotspots identified by the summer sampling, stations A3 and A4 in the marina were closet to the culverts, which connect the stormwater retention pond to the marina. The marina and stormwater retention pond are especially susceptible to tidal influence due to their shallow nature. If higher levels of Enterococci are found in marine sediments the current method of quantifying Enterococci, present only in the water column, to indicate the presence of fecal contamination may not be an accurate indicator for the amount of fecal waste in the environment. This issues is of particular concern in shallow marine environments, such as Galveston Bay, that are subject to sediment disturbance by recreation, wildlife or environmental factors. Including bioturbation, wind, and tides.

To determine if sediments were the source of the Enterococci hotspots we revisited the study site to collect sediment samples from the stormwater retention pond and marina (Figure 3.1). The stormwater retention pond is a tidally influenced water body surrounded by scrub brush and cord grass. The retention pond is bordered on the north, northwest and southwest by single-family homes, on the south by residential land, on the west by a park and playground and on the east by Marina Del Sol. The culverts connecting the marina and retention pond are located on the western border of the stormwater pond (Figure 3.2).

Figure 3.1. Stormwater retention pond located west of Marina Del Sol.

Figure 3.2. Stormwater retention pond side, culverts connecting marina and stormwater retention pond.

A study that consisted of the stormwater retention pond (Figure 3.1) and storm sewers in close proximity was conducted by Dr. Robin Brinkmeyer and myself. The survey revealed a stormwater outfall on the western border of the pond. The outfall is directly connected to the stormwater pond via a narrow creek. The creek is approximately 0.3 meters wide at the mouth of the stormwater outfall. The creek gradually widens. The creek is approximately 2.0 meters wide at its mouth were contact to the retention pond is made (Figure 3.3). At the mouth of the stormwater outfall, the sediment consists of coarse gravel. The sediments at the mouth of the stormwater outfall were wet at the time the sample was taken. The majority of the creek consists of the same silt and fine sediments as the marina.

Materials and Methods

Sediment and water samples were collected on the 13th of November, 2013 between the hours of 0900 and 1400 from five stations in the storm water retention pond including a stormwater outfall that drains into the retention pond. An additional three sediment and water samples were taken at three of the original sampling stations in the marina (A3, A4 and C9) (Figure 3.4).

Figure 3.3. Mouth of creek facing northeast (A), station 3 (B), station 2 (C).

Figure 3.4. Locations of sampling stations for the November study.

Corresponding water samples were taken prior to the sediment samples at eight of the nine sampling stations. A water sample was not taken at the first sampling site because the sediment was not submerged. A canoe was used to collect water and sediment samples at stations that were not accessible by land. Samples were handled as described above in Chapter II for transport to the Galveston Bay Foundation lab.

Water sampling. Water sampling consisted of the surface waters in the stormwater retention pond and marina to a depth of 0.3 meters. A YSI multi sonde probe was used to record the following parameters air temperature (°C), water temperature (°C), Dissolved Oxygen (mg/L) (DO) and salinity (ppt) (Figure 3.5). The IDEXX method for Enterococci detection and enumeration estimation was used to quantify the MPN/100 mL of fecal indicator bacterium in each water sample. See the above Bacteria Sampling section 2.6 for details.

Figure 3.5. Thesis author sampling at station 2 in the stormwater retention pond.

Sediment sampling. Sediments at station numbers 1, 2, 3, 4 and 5 were collected where the water was shallow enough using a sterile 50 mL polypropylene conical tube. Due to depth a box corer was used at sites 6, 7, 8 and 9 to retrieve the sediment sample. The sample was then taken from the sediment grab with a 50 mL polypropylene conical tube (Figure 3.6).

The sediment samples were processed by weighing 2 to 5 grams of sediment that were transferred to a new sterile 50 mL polypropylene conical tube. Up to 50 mL sterile DI water was then added. A sample of duck fecal waste was collected on November 15th, 2014 from dock C in Marine Del Sol. The sample was taken to compare with the bacteria sample from station 9, which is in close proximity.

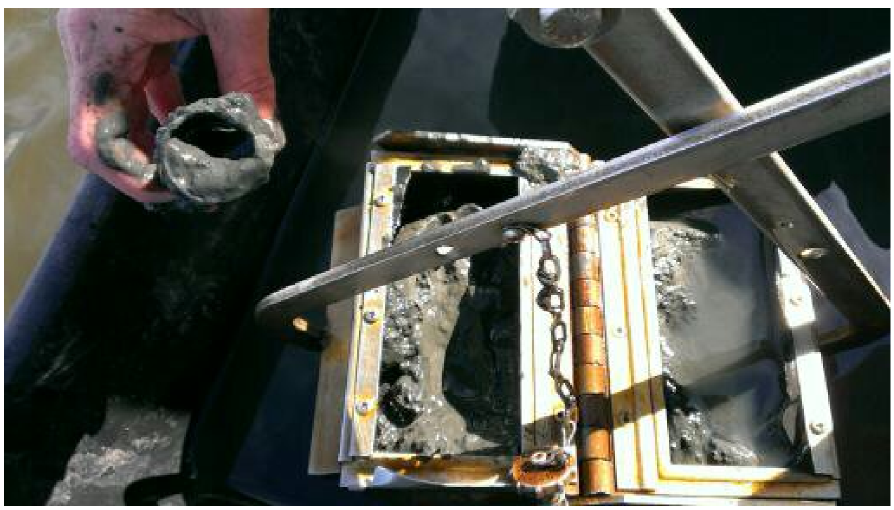

Figure 3.6. Sediment sample at station 8.

The IDEXX method for Enterococci detection and enumeration estimation resulted in all of the wells on the Quanti-trays fluorescing for stations 1 and 9. The sediment samples from stations 1 and 9 were further diluted to obtain the actual upper limit of the *Enterococcus* concentrations in the sediment. The MPN of the duck fecal waste was compared to the *Enterococcus* concentration in the sediments at station 9. Two grams of duck fecal waste was used for the comparison because the fecal waste is a highly concentrated source of *Enterococcus*.

Five grams of the original sediment samples were used for further dilution from stations 1 and 9. The 5 grams was transferred to a new 50 mL conical tube by a sterilized spatula. The 5 grams of sediment was then diluted by 45 mL of distilled water. 2 grams of duck fecal waste was transferred to a 50 mL conical tube using the same sterilization technique. 48 mL of distilled water was then added. All samples were shaken by a Vortex GeniE2 at a speed of 10 for approximately one minute. All three samples were then placed in a holding rack and allowed to settle.

All three samples were diluted four more times. After the initial dilution 5 grams of sediment from station 9 was transferred to a 50 mL conical tube and diluted with 45 mL of distilled water. This process was repeated three more times for the sediment sample from stations 1, 9 and the original duck fecal waste sample. The IDEXX method for Enterococci detection and enumeration estimation was then applied to the final solution of diluted samples (Figure 3.7). The Quanti-trays were counted on November the 25th, 2014. Sample 9 had no positive fluoresced wells. This indicates that the sediment sample from station 9 was over diluted. The Quanti-tray containing the sediment sample from station 1 had 5 positive wells. While the duck fecal waste sample had 86 positive wells. The MPNs were calculated using the factors that the samples were diluted by.

Results

The follow up study collected 9 sediment and 8 water bacteria samples. The concentration of ENT in the sediment samples ranged from a low of 195 MPN at station 4 to high of 12,098 MPN at station 1. The concentration of water borne *Enterococcus* was significantly lower and ranged from <10 MPN at stations 4, 6 and 8 to a high of 105 MPN at station 2 (Table 3.1). Between the hours of 0900 and 1400 the air temperature ranged from 10.3 °C at station 2 to 13.2 °C at station 1. The water temperature varied during the sampling period from 12.3 °C at station 2 to a high of 17.4 °C at station 4. The temperature of the sediment was recorded at four stations and ranged from 8.8 °C to 15.6 °C. Dissolved Oxygen varied from 11.4 mg/ L to 18.2 mg/L. The amount of Total Dissolved Solids in the water varied from 0.001 at station 4 to 0.51 at station 3 (table 3.1).

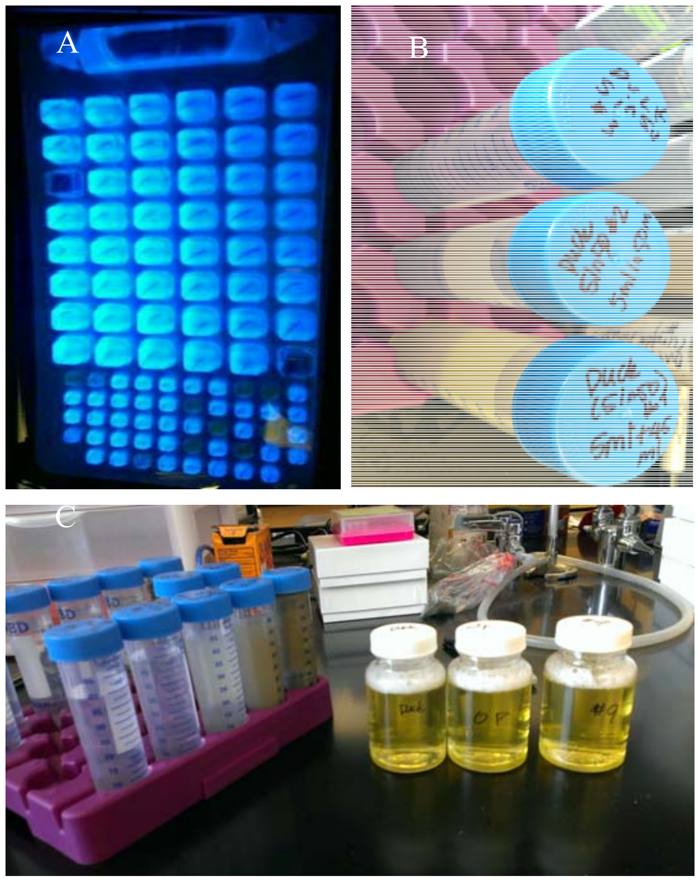

Figure 3.7. IDEXX Quanti-Tray wells fluoresce indicating positives (A), conical tubes after diluting duck sample (B), diluted samples ready for Quanti-Trays.

Table 3.1. Sediment and water quality results from November study.

Sampling Station	*Enterococcus* spp. Sediment (MPN/100 mL)	*Enterococcus* spp. Water (MPN/100mL)	Air Temp (°c)	Water Temp (°c)	Sediment Temp (°c)	DO (mg/L)	Salinity	TDS
1	12098		13.2		8.88			
2	1538	105	10.3	12.3		13.49	0.04	0.056
3	6049	10	11.3	16.55		18.2	0.04	0.51
4	195	<10	11.4	17.4	15.56	13	0.04	0.001
5	317	10	11.8	13.6		17	0.04	0.049
6	406	<10	11.8	15.45	13.33	12.4	0.03	0.049
7	1039	10	12	15.3	14.44	11.43	0.03	0.049
8	263	<10	12	15.4		12.29	0.03	0.049
9	879	20	12.1	15.75		15.01	0.03	0.049

Regression analysis of the sediment *Enterococcus* concentration yielded an R^2 value of 0.3544. This suggests a positive correlation between the concentrations of *Enterococcus* and distance from the stormwater outfall located in the retention pond. This positive correlation can be seen in figure 3.8. The concentration of *Enterococcus* is highest at the mouth of the stormwater outfall, reaches the lowest concentration in the center of the pond. Then once again climbs on the marina side of the culverts.

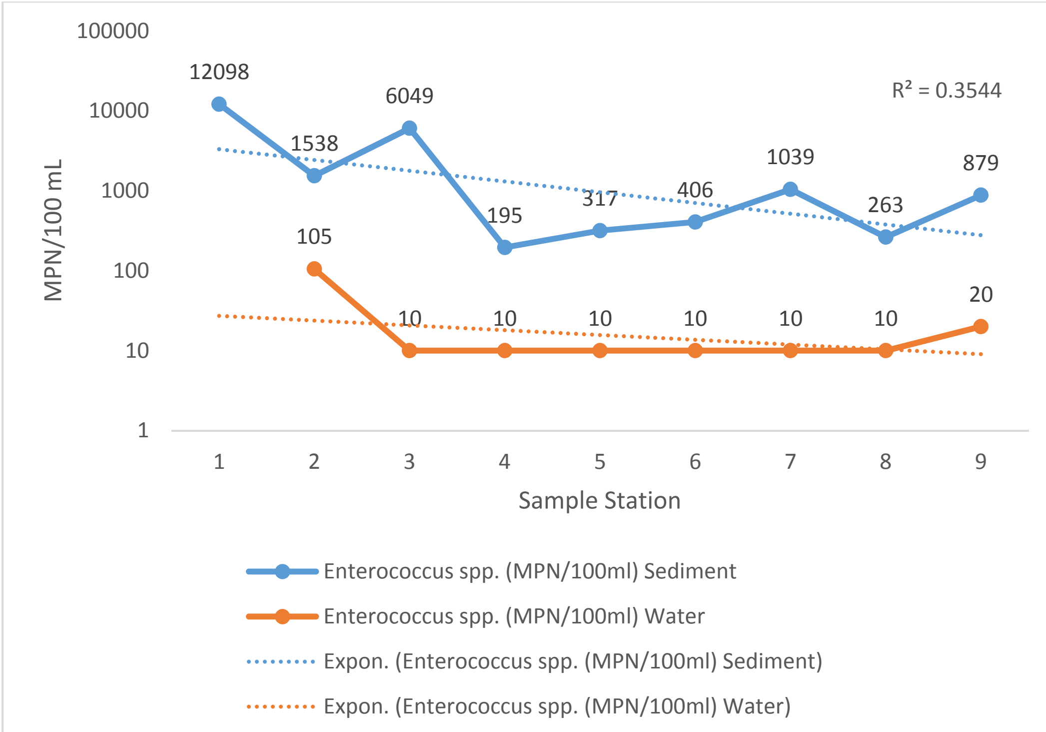

Figure 3.8. Sediment and water sample results from the November 2013 study.

Discussion

The follow up sampling reviles further insights into the study marina. It appears that a concentrated input of *Enterococcus* is the stormwater outfall located on the southwest border of the pond. The concentration of *Enterococcus* decreases as distance from the outfall increases. However, the levels of *Enterococcus* in the sediments start to rise in the three sediment samples that were taken from the marina. The increase is most likely due to other inputs of *Enterococcus* entering the marina.

To further investigate the high levels of *Enterococcus* detected at the stormwater outfall I obtained the original master stormwater drainage plans for the Twin Oaks subdivision, Marina Del Sol and the surrounding area from the GIS coordinator of League City, TX, (Figure 3.9). I examined the stormwater drainage plan for the Park at Marina Del Sol, the area in close proximity to the stormwater outfall. This plan indicates that the stormwater outfall leading into the retention pond drains approximately eight single family homes directly via two stormwater inlets. The stormwater water inlets drain 0.71 acres (ac) (inlet one) and 0.40 ac (inlet two). The stormwater inlets connect directly to the stormwater retention pond outfall via an existing storm sewer (Figure 3.9).

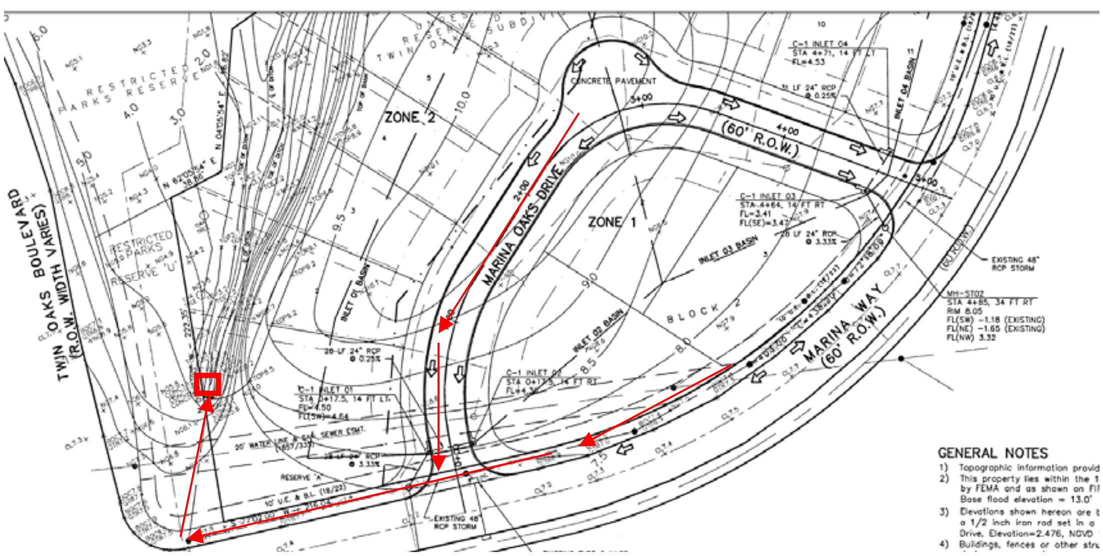

Figure 3.9. Path of stormwater to retention pond stormwater outfall.

In addition to the direct input of stormwater the retention pond collects indirect runoff from approximately 3.9 acres of the single family houses to the southwest. Figure 3.10 depicts the topography of the area in question. The arrows indicate the direction of the overland runoff. The runoff from the northeast face of the 10.0 foot ridge flows to the northeast, into the channel that connects to the retention pond. While the runoff from the northwest face of the ridge flows away from the retention pond to the northwest.

To evaluate rainfall prior to the November 13th sample date the record of climatological observations for the League City station was accessed on NOAA's Climate Data Online. No precipitation was recorded for the three day acclimation period prior to the sampling date. However, two rain events did occur for the month of November prior to the 13th. The first rainfall event occurred on November the 2nd and totaled 0.35 inches. The second rain event occurred on November the 5th and totaled 0.04 inches. The climatological record suggest that the *Enterococcus* was able to persist in the moist sediments of the stormwater outfall for a period of at least 7 days. From the last recorded precipitation event to the date the sample was taken.

Due to the high levels of *Enterococcus* that are harbored in the sediments of the pond it is plausible that the pond is acting as a net source of *Enterococcus* into Marina Del Sol. The stormwater outfall that drains the residential area to the west of the retention pond should be sampled after rainfall events to assess the potential for the runoff to be contributing *Enterococcus* and the associated fecal matter to the pond and ultimately the marina. A survey could be conducted of the residents surrounding the pond and marina to gain further insight into the source of *Enterococcus*. A survey could gather information such as number of residents with a domestic animal and the type of fertilizers that residents use on their yards.

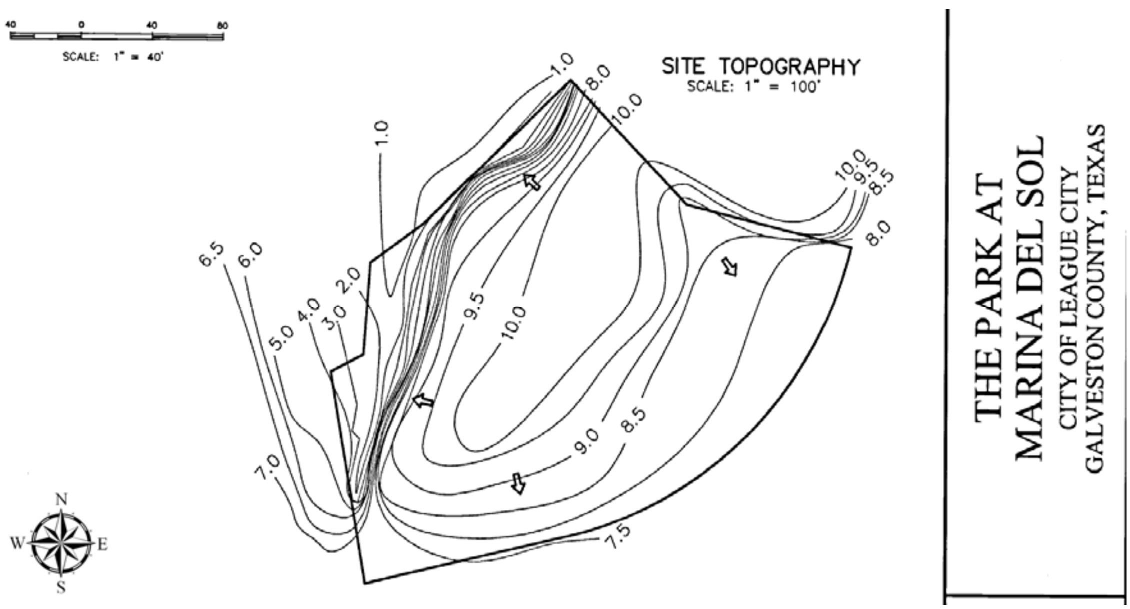

Figure 3.10. Topography of the Park at Marina Del Sol.

CHAPTER IV

CONCLUSION

Of the original hypothesis two were supported (H: 2 and H: 3) and one was inconclusive (H: 1). The first hypothesis states that an increase in the concentration of *Enterococcus* in the marina will correlate with wet weather events. The study shows that this is inconclusive, wet weather events are not the primary driver affecting the levels of *Enterococcus* in Marina Del Sol. The second hypothesis states that hotspots of *Enterococcus* will be evident in the marina. This hypothesis was supported by locating two hotspots that exceed the EPA primary contact recreation standard for a geometric mean of 35 MPN/100 mL. The third hypothesis states that the MPN/100 mL of *Enterococcus* will increase from the marina entrance to the back portion of the marina. This hypothesis was supported. There was a gradient of *Enterococcus* concentrations from the marina entrance to the rear of the marina.

A fourth hypothesis was tested during the follow up study. The fourth hypothesis states that the sediments in the retention pond are acting as a net input of *Enterococcus* into Marina Del Sol. This hypothesis was supported by the elevated levels of *Enterococcus* in the sediments of the marina and retention pond. Furthermore, one source of the *Enterococcus* in the retention ponds sediments is the stormwater outfall on the ponds southwest border (Figure 3.8). A gradient was evident, the MPN of *Enterococcus* decreased while distance from the stormwater outfall increased.

Based on this study there are numerous sources that contribute to the concentrations of *Enterococcus* in the marina. The most likely contributors are the pump out station, the resident population of ducks and the adjoining retention pond via the stormwater outfall. Illegal fecal waste discharge from boaters is another likely contributing factor, but can be difficult to prove. In the future source tracking should be conducted on *Enterococcus* samples from the marina to determine the exact source of the contamination.

Marina Del Sol and other marinas located on Galveston Bay (GB) serve as the hubs for the GB recreational community. They are launching points for recreational fishing, swimming and boating activities in the bay and therefore are the venues for concentrated recreational activities. To assess their impacts on the health of the bay and the Houston- Galveston region marinas should continue to be monitored for elevated levels of *Enterococcus*. A gateway effect is likely occurring between the increasingly built environment of GB marinas and the natural environment.

The frequent and heavy use of many GB marinas makes them especially susceptible to the ever present and increasing population pressures of the Houston-Galveston region. Due to frequent use marinas are likely locations for the public to come into contact with water that may be impaired by bacteria. Marinas in GB are under constant pressure from a variety of anthropogenic and environmental stressors.

It cannot be denied that, although altered from their natural state, marinas are part of the GB environment. As the study of environmental implications of GB marinas increases, so will the resolution of the associated impairments. To assess their part in contributing *Enterococcus* and fecal waste to the Galveston Bay system, the marinas should continue to be studied. The primary focus should be on pinpointing sources of *Enterococcus* and fecal waste inputs to marinas, the flux of concentrated *Enterococcus* between the bay and marinas and the sediment to water pulse of *Enterococcus* in the often flow restricted water bodies of marinas.

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