DESCRIBING THE IMPACTS OF WASTEWATER EFFLUENT ON THE PHYTOPLANKTON COMMUNITY COMPOSITION IN GALVESTON BAY

An Undergraduate Research Scholars Thesis

by

JAKE BALLARD

Submitted to the LAUNCH: Undergraduate Research office at
Texas A&M University
in partial fulfillment of requirements for the designation as an

UNDERGRADUATE RESEARCH SCHOLAR

Approved by
Faculty Research Advisor: Dr. Antonietta Quigg

May 2022

Major: Marine Biology

RESEARCH COMPLIANCE CERTIFICATION

Research activities involving the use of human subjects, vertebrate animals, and/or biohazards must be reviewed and approved by the appropriate Texas A&M University regulatory research committee (i.e., IRB, IACUC, IBC) before the activity can commence. This requirement applies to activities conducted at Texas A&M and to activities conducted at non-Texas A&M facilities or institutions. In both cases, students are responsible for working with the relevant Texas A&M research compliance program to ensure and document that all Texas A&M compliance obligations are met before the study begins.

I, Jake Ballard, certify that all research compliance requirements related to this Undergraduate Research Scholars thesis have been addressed with my Research Faculty Advisor prior to the collection of any data used in this final thesis submission.

This project did not require approval from the Texas A&M University Research Compliance & Biosafety office.

TABLE OF CONTENTS

ABSTRACT

Describing the Impacts of Wastewater Effluent on the Phytoplankton Community Composition in Galveston Bay

Jake Ballard
Department of Marine Biology
Texas A&M University

Research Faculty Advisor: Dr. Antonietta Quigg
Department of Marine Biology and the Department of Oceanography
Texas A&M University

The treatment of wastewater has been a dilemma since humans began building and inhabiting cities. Globally, wastewater is released into oceans, bays, streams, lakes and rivers, in many cases, without much consideration for the potential ecological impacts. The impact of wastewater effluent on the phytoplankton community is not well understood. Changes in the phytoplankton community composition are related to the physiochemical parameters of the environment. Phytoplankton are essential to the marine ecosystem as they are the foundation of the food chain and support higher trophic levels. Thus, a deep understanding of the relationship between human activity and wastewater effluent impacts on the ecosystem is necessary for identifying potential environmental degradation due to human activities. As the human population increases, so will the amount of wastewater that is produced. Understanding the best methods of disposal and treatments will help ensure that the best practices are being employed. Seemingly small factors such as release rate, release time and surrounding environments of the wastewater effluent input have the potential to influence how the treated wastewater navigates

through the waterway. I *hypothesize* that changes in the phytoplankton community will be correlated to rapid and large increase in human population associated with human events. In order to test this hypothesis, I determined this relationship by establishing baseline environmental parameters and phytoplankton assemblages to data collected during large-scale human activities in Galveston Bay, Texas. My data suggests that there are no changes in the concentration of chlorophyll-*a*, a proxy for phytoplankton biomass, between baseline periods and large-scale human activities. This implies that the large-scale human activities did not impact the concentration of phytoplankton. Why a noticeable change was not observed in this study will be discussed, as well as suggestions for future directions of such research.

ACKNOWLEDGEMENTS

Contributors

I would like to thank my faculty advisor, Dr. Quigg, and, Jessica Hillhouse, for their guidance and support throughout the course of this research.

Thanks also go to my friends and colleagues and the department faculty and staff for making my time at Texas A&M University a great experience. Finally, thank you Nalu Martin and Hagen Klobusnik for their encouragement and help with sample collection.

The IFCB and Lab equipment used for describing the impact of wastewater effluent on the phytoplankton community composition in Galveston Bay were provided by Dr. Quigg. The analyses depicted were conducted in part by Jessica Hillhouse and are currently unpublished. All other work conducted for the thesis was completed by the student independently.

Funding Sources

Undergraduate research was supported in part by an
Institutional Grant NA18OAR4170088, to the Texas Sea Grant College Program from the National Sea Grant Office, National Oceanic and Atmospheric Administration, U.S. Department of Commerce.

1. INTRODUCTION

Galveston Bay is heavily influenced by human activity as shown in the various studies and assessments **[1]**. It is home to the Houston Ship Channel which is the 7^{th} largest estuary in the United States and is the 12^{th} largest port in the Us when ranked by tonnage **[2]**. Because of the human traffic in the area the waters of Galveston Bay are heavily polluted by the human activities **[1]**. Additionally, some of the largest rivers in Texas empty into the bay. Since Galveston Bay catches run off from so many watersheds of Texas, there are massive implications regarding salinity and turbidity. These hydrodynamic features of Galveston Bay impact what the baseline community looks like and the extent by with the effluent impacts the community. If the physiochemical and hydrologic features of Galveston Bay select for an abundance of species that can function in a wide range of nutrients, salinity, and turbidity, then the impact of the wastewater treatment effluent will have a lesser degree of impact because the current community is not being exposed to conditions that are completely out of their functional threshold.

1.1 Wastewater treatment facilities

To emphasize major factors, there are five wastewater treatment facilities around Galveston Bay [**3**]. These facilities typically use any combination of treatment techniques that include physical, chemical, biological means of water purification. Yet these methods are not without error and there are many instances where the contaminate in the wastewater effluent contaminate the surrounding aquatic environment **[4]**. For example, the water that is being released from the wastewater treatment facility is fresh water that is diluting the saline bay water.

This difference in salinity may impact on the species that inhabit the waters because of this dilution effect caused by the effluent. A secondary consideration is the method by which the effluents are released into the bay: in Figure 1, effluent is discharged into a marsh before going into the bay while in Figure 2,site B, the treated wastewater is dumped directly into the bay. The surrounding area lacks vegetation, and the banks are composed of shell and clay. The structure of the water in site is more like a small cay. This is not the case for site A where the entire path of effluent from input to bay interface is loaded with marsh vegetation. The soil is rich in organic matter and microbially active. These two very different methods have the potential to impact the effect that the effluent has on the phytoplankton community.

Other impacts to the environment are due to emergency situations. Texas has witnessed a few recent natural disasters that could have impacted the wastewater treatment facilities. Hurricane Harvey in 2017 inundated the Houston- Galveston area with 11.1×10^9 m^3 precipitation over a 5 day period, that is, about three times the volume of the Bay **[5].** Natural disasters can deteriorate the treatment plant's infrastructure by cracking pipes and overflowing capacity and impact the amount of waste that is coming in and the ability of the facility to treat that waste. In cases of emergency, facilities must sometimes release raw sewage into the surrounding environment so that the facility does not exceed maximum capacity. Raw sewage can overload the water with nutrients causing phytoplankton blooms in addition to creating an unsanitary place for human recreation. Understanding exactly how the presence or absence of effluent might influence the phytoplankton community composition is key to refining the method of wastewater treatment and effluent release such that we are able to minimize the ecological effects.

Additionally, developing our understanding about how the rate of human activity, and thus the increase in the amount of wastewater, correlates to changes in phytoplankton community composition can influence the methods by which wastewater treatment facilities approach influxes of people to an area during things like holidays or festivals. For example, Galveston Island hosts the largest event in Texas the Lone Star Biker rally with an average visiting population of 400,000 **[6]**. Comparing the differences in phytoplankton communities from baseline data collected year round and prior to such an event to phytoplankton community information during these large-scale human events will help to establish the impacts of the wastewater effluent on the phytoplankton community because of these gatherings.

1.2 Phytoplankton

Phytoplankton are microscopic photosynthetic organisms that live in aquatic environments **[7]**. Although phytoplankton are tiny, they are ecologically important and produce roughly half of the earth's primary productivity **[8]**. The productivity of the marine ecosystem is dependent on the health of phytoplankton **[9]**. Phytoplankton are also a massive carbon sink acting as a forest converting $1.5x10^{10}$ up to 20.8×10^{10} tons of atmospheric carbon into bioavailable forms **[10]**. With a rise in atmospheric carbon dioxide causing global climate change, phytoplankton have the ability to play a key role in the removal of some of this carbon dioxide by converting it to sugars that can be used by other species. This role that phytoplankton play in the carbon cycle has the potential to help mitigate the negative effect of burning fossil fuels. Moreover, phytoplankton greatly influence the health and mortality of larval pelagic fish species that rely on them for food **[11]**. The survivorship of larval pelagic fish is directly correlated with the abundance of phytoplankton at the time of their hatching. This impacts the population sizes of pelagic fish for years thus changing the entire ecosystem. If the larval fish do not have an abundance of phytoplankton to feed on their will be very large amounts of mortality. Not only does the entire trophic system depend on the phytoplankton, but humans rely on the economic benefit of pelagic fish, therefore humans suffer economically when phytoplankton are in low abundance and larval pelagic fish populations decrease.

Moreover, phytoplankton are also pertinent to the deep-sea ecosystems as they help to form marine snow **[12].** Marine snow is in part a combination of organic matter *and* particulate materials **[13]** that sink to the bottom of the ocean. As the snow sinks, many deep pelagic species rely on it for food **[14]**. Phytoplankton and bacteria secrete different exopolymeric substances that entrap other organics like fecal matter which then sink to the benthos. Phytoplankton typically produce marine snow as a normal process, but stress can impact certain characteristic of the marine snow. This sinking of marine snow to the benthos results in their sequestering of atomistic carbon into deep sea sediments thereby converting carbon into a more recalcitrant form **[15]**.

There are many different factors that influence the phytoplankton community composition in the marine environment. Nitrogen and phosphorus are the most limiting nutrient to phytoplankton **[16]**. Because phytoplankton rely so heavily on the availability of these nutrient changes, population can be related to the nutrients in the water column even in complex trophic interactions **[17]**. Human waste, like any type of fecal waste, is very high in phosphorus and nitrogen thereby making a great nutrient source for phytoplankton. This increase in nitrogen and phosphorous availability can result in large blooms of phytoplankton species. These blooms then quickly die as they cannot be sustained. Once the blooms die, they can deplete the surrounding water of oxygen through biochemical oxygen demand (BOD). This in some cases results in hypoxic zones which can cause fish kills and disrupt the ecosystem. Because of the potential for the wastewater to have the nutrients it is important that nutrients are monitored in the case that is the driving mechanism behind any potential community composition shifts.

Phytoplankton are not only influenced by the nutrients in the water column but are also impacted by other physical parameters such as pH, salinity, turbidity, and temperature. Different phytoplankton species have a variety of tolerance thresholds for each of these parameters. Understanding the baseline fluctuations in the phytoplankton community can help to quantify the impact that a given parameter might have on the phytoplankton.

The pH of water is a very important factor in the determination of what phytoplankton species can inhabit a given area **[18]**. For example, some phytoplankton species such as coccolithophores have a shell made of calcite that might make them sensitive to changes in pH **[19]**. As more carbon dioxide enters the atmosphere due to the burning of fossil fuels there is more diffusion of carbon dioxide gas into the ocean. Once carbon dioxide dissolves into aqueous solution it forms carbonic acid that depresses the pH of the ocean making it more acidic. This process is known as ocean acidification and can impact the ability for phytoplankton to reproduce and also has the potential to change the community composition based on the ability of some species' abilities to live in a range of pH's.

Salinity has one of the most studied trends with respect to the role that it plays with habitat determination of phytoplankton. There are many different species of phytoplankton that can inhabit a variety of salinities, and the phytoplankton community composition is reflective of the salinity in any given area. Some marine phytoplankton have small ranges of salinity preferences. For example, estuarine phytoplankton can tolerate lower salinities much better than coastal or pelagic species. In contrast, other species such as *Porphyra umbilicalis* are euryhaline and can tolerate a range of salinity from 7 ppt to 52 ppt [**20].**

Phytoplankton are photosynthetic organisms which means that they use light to make food for themselves. The amount of dissolved solids (turbidity) in the water column can impact the amount of light that reaches a given depth, so water with a high turbidity will select for phytoplankton that are able to remain metabolically active under light limiting conditions. Sediment input from surrounding streams and rivers can end in up in the bay thus increasing the turbidity. This can be exacerbated during large rain events when large volumes of water are carrying sediment from land into the water. Moreover, the closer that the water is to the shore the more suspended sediments there are as this region as it is very energetic and constantly re-suspending sediments. The culvert of flowing water from the wastewater treatment facility also has the ability to suspend a lot of sediments and cause a great deal of erosion from the surrounding bank resulting in higher turbidity in the surrounding water column.

Temperature can also alter the ability for certain phytoplankton species to live in a given environments. The higher the temperature the lower the concentration of oxygen, thus higher water temperatures can create low oxygen environments that are not as favorable as higher oxygenated environments. Temperature also impacts the metabolic activity of phytoplankton the metabolism of these ectothermic organisms' scales with the rising and falling water temperature. Water is known for its high specific heat that is the amount of energy to raise one gram of water one degree Celsius. Water has an ability to resist quick fluctuations in water temperature. This means that different sample sites might have different rates of change based on the surrounding environments. Shallow pools will tend to get hot and stay hot while deeper darker water will have more of gradients of warmer and cooler waters. Moreover, if water from a wastewater treatment plant is being dumped into a given environment, and is a different temperature than the environmental water, there will be convection at this interface resulting in the persistence of temperature that is not the same as the rest of the ecosystem. These factors can impact the community composition as a function of the temperature of the water column. Given the importance of the phytoplankton community on the health of the ecosystem and the sensitivity of phytoplankton to environments changes there is a great value in understanding the impacts that human activity has on the productivity and community assemblage within a marine environment.

1.3 Objective

In Texas, from 30-60% of wastewater can end up in a marine environment such as Galveston Bay **[21]**. Understating the impacts that this wastewater effluent has on the community composition is essential to negate the environmental impacts that humans have on their environment. The objective of this research is to describe the direct impacts of wastewater effluent on Galveston Bay phytoplankton community in relation to large gatherings for human activities such as the Lone Star biker rally. It has been established that the phytoplankton dynamics and community are essential for a healthy ecosystem. Moreover, there are many factors that are associated with wastewater treatment facilities that are also responsible for changing the phytoplankton community. Therefore, the connection between the phytoplankton community and the wastewater treatment facility must be understood so that one can make the appropriate moves to ensure that the phytoplankton have the ability to support the ecosystem. I *hypothesize* that there will be shifts in the baseline phytoplankton community as a result of sudden influx of people to the area because of the increased amount of effluent from wastewater treatment facilities

2. METHODS

2.1 Field Sampling

Sampling took place monthly during the winter, spring, and fall from February in 2020 to November in2021 with special collection days for Lonestar Biker rally which occurred in November of 2020. In total, 129 samples were collected from two different locations shown in Figures 1 and 2 over the course of five sampling trips. Site A is located on Pelican Island and treats the wastewater for the Texas A&M University at Galveston campus with a total of six sample sites. Site B is located on Galveston Island and treats the wastewater for the City of Galveston with a total of four sample sites. The treatment facilities were selected based on how they release their treated wastewater. Whereas site A releases relatively small volumes along a vegetated marsh, site B processes vast quantities (millions of gallons) of effluent that are released directly into the Galveston Bay. At each collection site, physical water parameters such as turbidity, temperature, dissolves oxygen, depth, and salinity were measured. Additionally, a one-liter water sample was collected in a brown bottle that had been triple washed with water from that sample location. These samples were placed on ice for 1-2 hours until lab processing.

Figure 1: The wastewater treatment facility in the image above treats the wastewater for the city of Galveston. The water is dumped directly into Galveston Bay from the treatment facility after processing. Samples were collected from four locations adjacent to this facility.

Figure 2: The wastewater treatment facility that is responsible for processing the wastewater of Texas A&M University at Galveston. There is a heavily vegetated marsh effluent stream that takes a longer route to the bay interface (A5). The shell beach face has a highly dynamic erosion/deposition cycle that can block the effluent stream from direct mixing of the stream and bay water.

2.2 Imaging Flow Cytometry (IFCB)

A 5-milliliter sample was processed by the IFCB to determine the phytoplankton community composition. The sample was run until a minimum of 200 images were taken. The IFCB was washed with filtered seawater before any samples were processed to ensure that the system was clean. Additionally, filtered sea water flushes were also used in between processing samples so that there would not be any contamination between the samples. After all the samples were ran, an additional filtered sea water run was done followed by an anti-biofouling measure which is a wash of sodium azide. This helped to keep the IFCB from any biofouling or contamination that might impact the results of the sampling endeavor.

2.2.1 Identification of Phytoplankton

Once the IFCB images were acquired, the images were then manually classified. This process was accomplished by determining the most abundant species that were present in all the samples collected. These abundant species were the enumerated within in each set of images for a given site. Abundant species were defined as the species of phytoplankton that were present in notable abundance in the set of IFCB images regardless of the sampling site. Several in house guides were used for the identification of the phytoplankton species from the IFCB images.

2.2.2 Analysis of IFCB data

During the study period, five selected species were quantified by counting the number of images. The number of each individual species' images was then divided by the total amount of images to give the proportion of that species in the population. Additionally, a portion of each of the five abundant species to the total number of abundant species was calculated to determine how the phytoplankton species changes in their composition with respect to other abundant species through time because of the environmental factors resulting from large-scale human activity. The five species that I focused on during the study.

A. *Skeletonema*
B. *Phormidium*
C. *Asterionellopsis*
D. D*inophysis*
E. *Entomoneis*

2.3 Chlorophyll-*a* (Chl-*a*) analysis

The water sample was homogenized by inversion three times before filtering. The samples were filtered on to a 47mm GF/F Whatman filter. The sample was filtered until the filter was stained and the volume was recorded. Filters were put into centrifuge tubes and stored in -20’C freezer until later processing. The filters were processed according to EPA method 445 according to **[8]** using the 10-AU turner benchtop fluorometer.

2.4 Water chemistry

The water samples that were collected from the wastewater effluent locations were then processed for physiochemical water parameters. These parameters consist of pH, dissolved oxygen, salinity, temperature, and turbidity. The Dissolved oxygen and temperature were recorded in situ with a HQ 40d multi Hach LDO probe. The salinity was collected using a refractometer. Turbidity was approximated *in situ* using a Secchi disk. pH of the water was determined with a tabletop AB15 Lab probe (Probe # 13-620-631). The probe was calibrated before every use.

2.4.1 Nutrient analysis

To obtain the data regarding the nutrients that were in the wastewater the filtrate of the Whatman GF/F filters were saved and stored in the -20’C until they were sent to the Geochemical and Environmental Research Group in Texas A&M College Station to be processed using standard protocols.

2.5 Dilution calculation

To determine the amount of effluent that was present at each site a simple dilution calculation was performed. Because the effluent has a salinity of 0 ppt the salinity at any sampling site was equal to the salinity of the bay diluted with the effluent water. The calculation was performed with the final volume of 1L (Final volume(V2) = Volume of Wastewater + Volume of Bay water(V1)). The Bay salinity (C1) and the site salinity was defined as (C2) with a volume of (V2) the following operation was performed to calculate the Volume of Bay(V1) water in 1L sample from any given site.

$$\frac{C_2 x\, V_2}{C_1} = V_1 \qquad \text{(eq1)}$$

After V1 was calculated it was subtracted from V2 in order to give the volume of wastewater (Vw) in 1L of sample from any given site. The Vw was the divided by 1000 ml and multiplied by 100 to give the percent of 1L that was effluent. This was applied to all sample sites to indicate the presence of effluent at a given time.

2.6 General statistical analysis

All the data was compiled into a master sheet and all the units were converted to be the same form so that values could be compared directly summarized and computed in R and excel to run the statistical analysis required. Two sample T-tests were run with an alpha value of 0.05. These tests measured the significance between the Chl-a concertation as a function of site and season as well as a series of comparison across site and season about physiochemical water parameters. These same factors were computed with respect to the Lone Star biker rally to measure large scale differences. Trends in the Chl-a were graphed, and relative abundance graphs were generated to see trends in the community of phytoplankton.

3. RESULTS AND DISCUSSION

3.1 Imaging Flow Cytometry (IFCB)

3.1.1 Identification of Phytoplankton and analysis of community

The identification of phytoplankton from the collected data indicated that *Entomoneis*, *Skeletonema*, *Phormidium*, *Asterionellopsis* (*Figures 2,4,5, and 6 respectively)* were some of the most abundant genera among all sites. This was a qualitative determination based on observations of the data set. Additionally, there was a large and abrupt presence of the species in *Figure 3* in site A6 during the Fall sampling however it was not seen in site B. This particular species has not yet been identified as it was not in the identification guides being used. *Dinophysis* (*Figure 7*) a known harmful algae bloom species in Galveston bay, was observed to be located at site B.

More intense quantification of the prevalent species needs to be done so that the changes in relative abundances can be presented graphically in addition to more rigorous statically analysis to elucidate significate differences between the Lone Star biker rally and typical fall community composition. There was difficulty with the identification of certain species that were very similar or when the quality of the image was not ideal. These instances were excluded from the overall analysis.

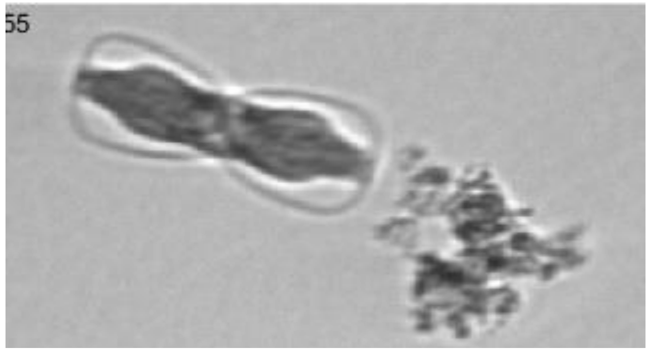

Figure 2 Entomoneis and debris to the right

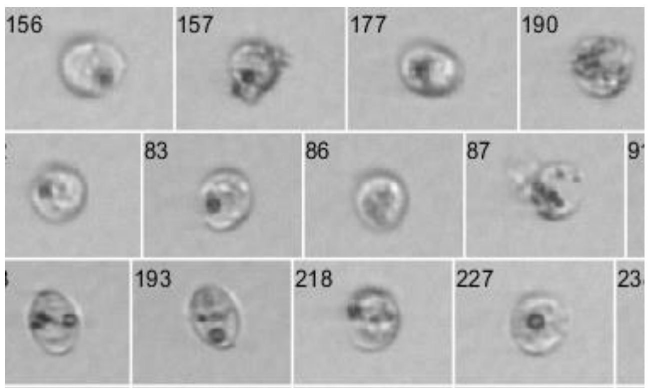

Figure 3 All of the images are of the same unidentified species

Figure 4 Skeletonema

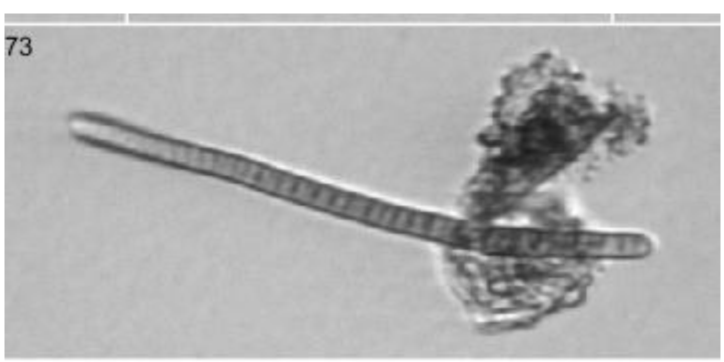

Figure 5 Phormidium

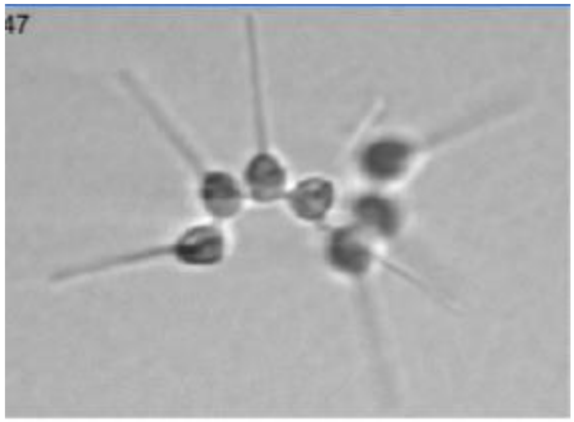

Figure 6 Asterionellopsis

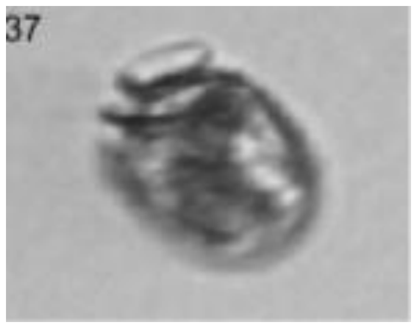

Figure 7 Dinophysis

Because each of the above species has a set of metabolic and/or trophic niches they can be indicative of the quality of the environment. Once the quantification of these species has been achieved one can then describe the changes in community composition and relate these potential changes to the changes in environments that selected for a particular species metabolism. These changes are the related to human activities that altered the chemical environment at the effluent-sea water interface. Moreover, the specific monitoring of HAB species will enable one to draw conclusions about the specific anthropogenic impacts on HAB species that follow from effluent exposure.

3.2 Chlorophyll-*a* (Chl-*a*) analysis

The relative concentration of Chl *a* was compared at each site and over a period of time (Fig. 8). In doing so, it was observed that there was no major distinction in the relative concentration of Chl-a on the day of the biker rally when compared to the Chl-a at other time points

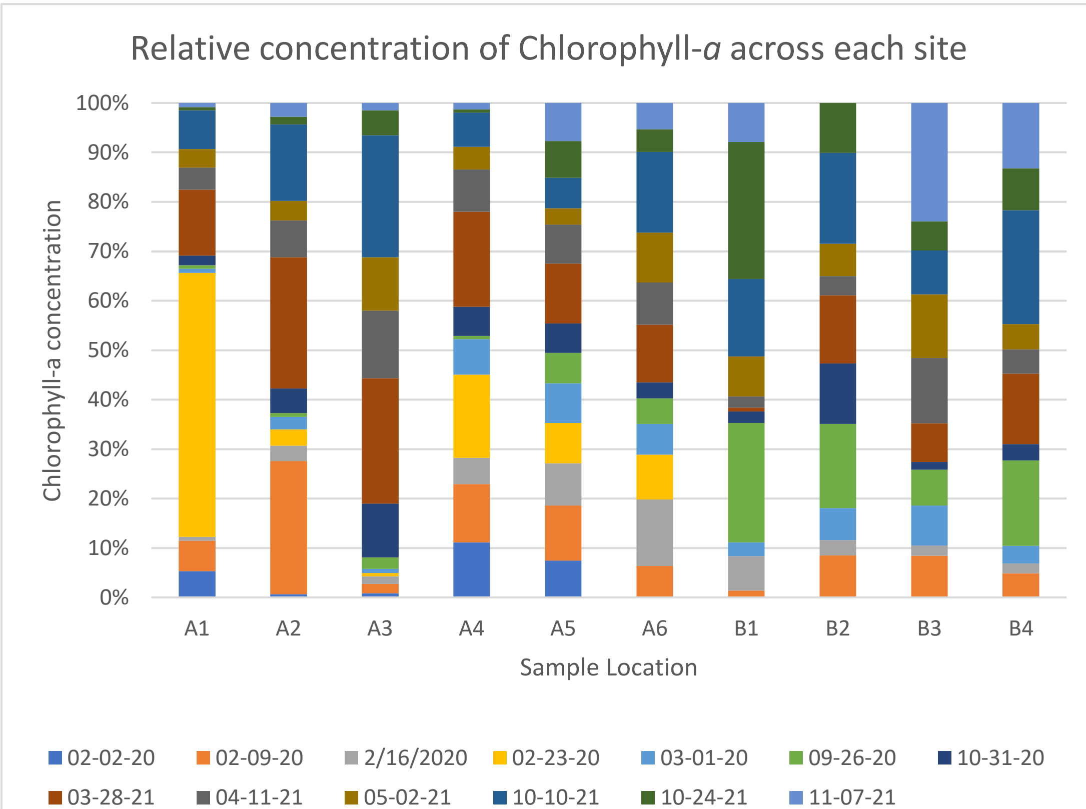

Figure 8 The relative abundance graph shows the proportion of Chl-a for a given collection date to the overall sum of Chl-a concentrations across all sample location and dates.

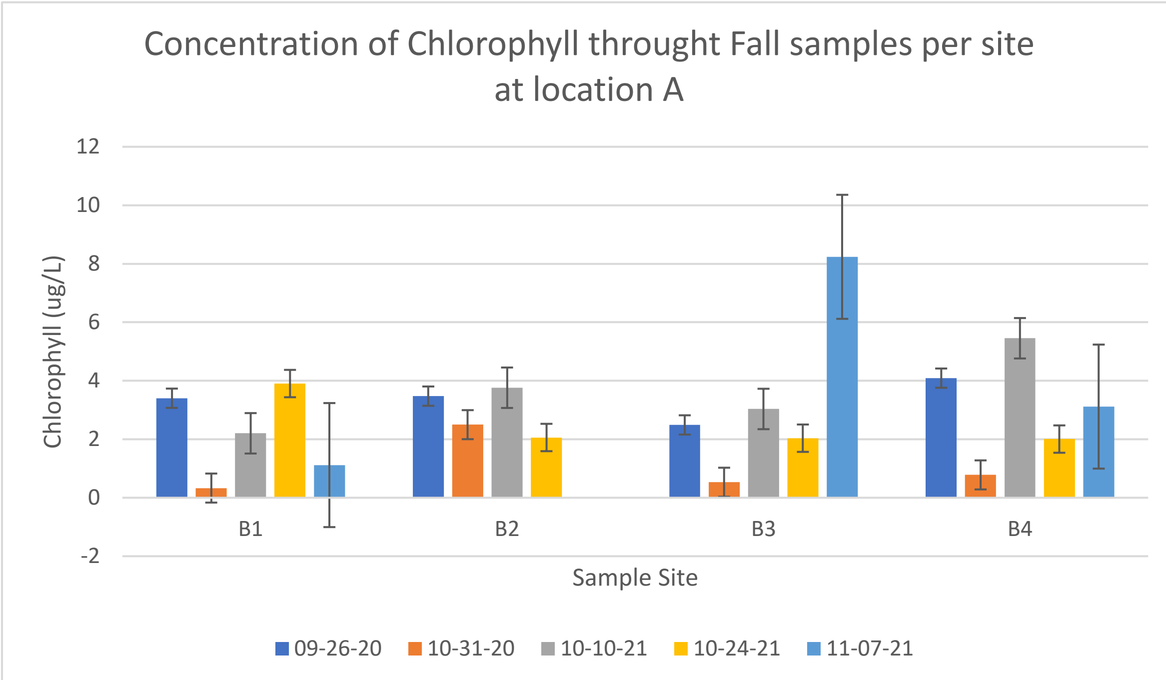

Figure 9 The changes in concentration(ug/L) of Chl-a through Fall sampling dates at site A across each sampling site. Site B2 is missing data from 11-07-2021 because the site was not accessible.

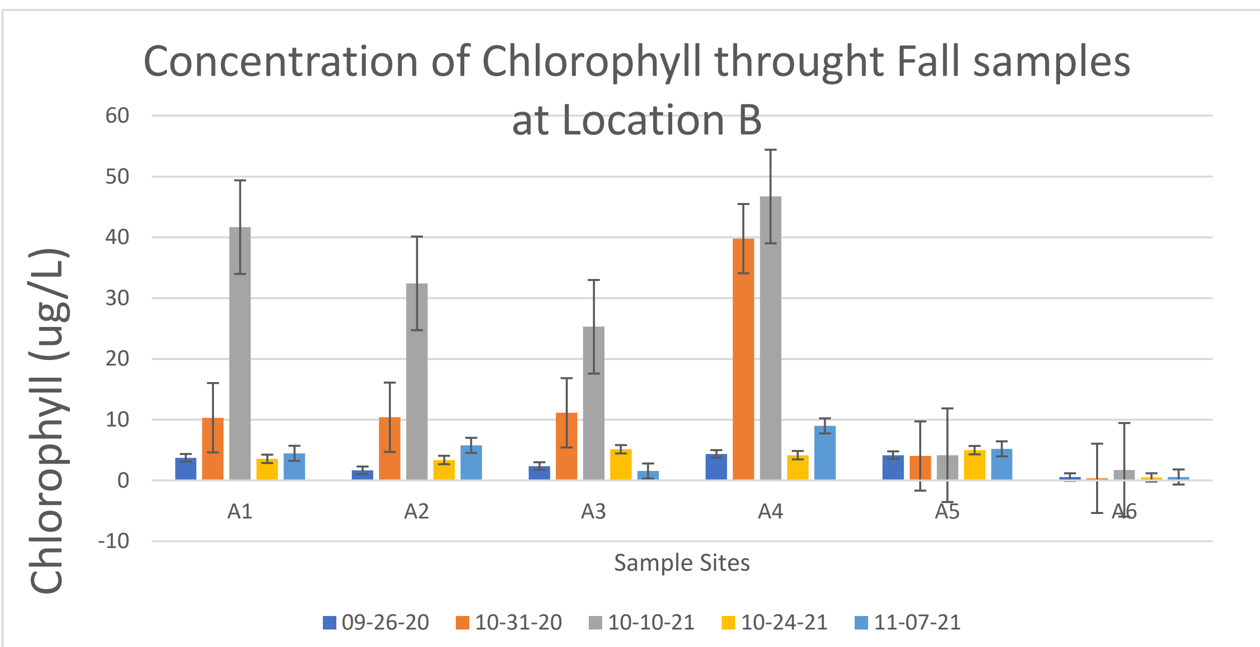

Figure 10 The changes in concentration of Chl-a through Fall sampling dates at Site B across each sampling site.

When the Lone Star biker rally which took place in 11-07-2021 as compared to all other Fall samples within the same locations there were varying results.

When the Lone Star biker rally samples for site B were compared to all other samples collected during the fall there was a statistically significant difference when a t-Test was ran assuming unequal variances with an alpha value equal to 0.05. Yet this was not the case when the variances were assumed to be equal. A Breusch-Pagan test is needed to determine the presence of heteroscedastic features thus indicating which assumption better suits the data. This is the case for all of the t-Tests that were ran on the data. Over all the data does not suggest significant differences in the Chl-a concentrations between fall and the Lone Star biker rally. Moreover, it came to our attention that the weekend of 10-24-2021 was the weekend of Oktoberfest in Galveston. That is another popular event in Galveston that introduces another aspect that will need to be accounted for when conducting further statistical analysis. Additionally, one can see that in *figure 8* there are not any major sampling dates that appear to stick out, yet when one compares only Fall samples one can see that locations as shown in *figure 10* have very significant contributions during 10-21-20 and 10-10-21 as is not the case in *figure 9* location B where the data seems to be more consistent.

3.3 Water chemistry

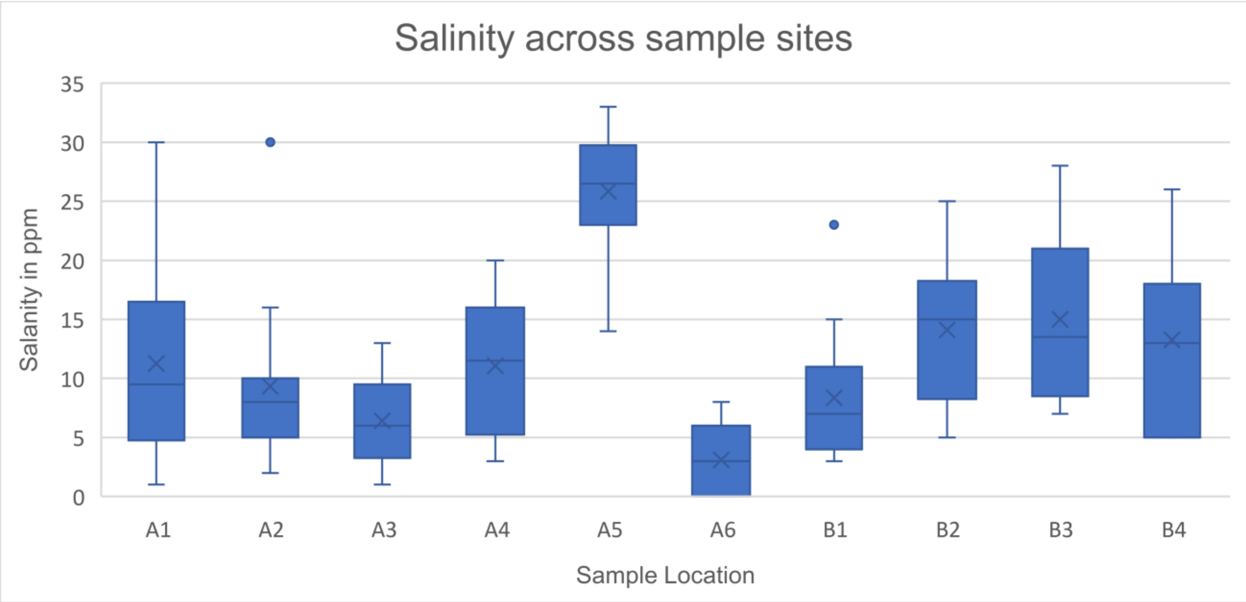

Figure 11 The box and whisker plots compare the ranges of salinities at given sampling sites within locations A & B. The Dots show outliers outside the range of data for a given collection site.

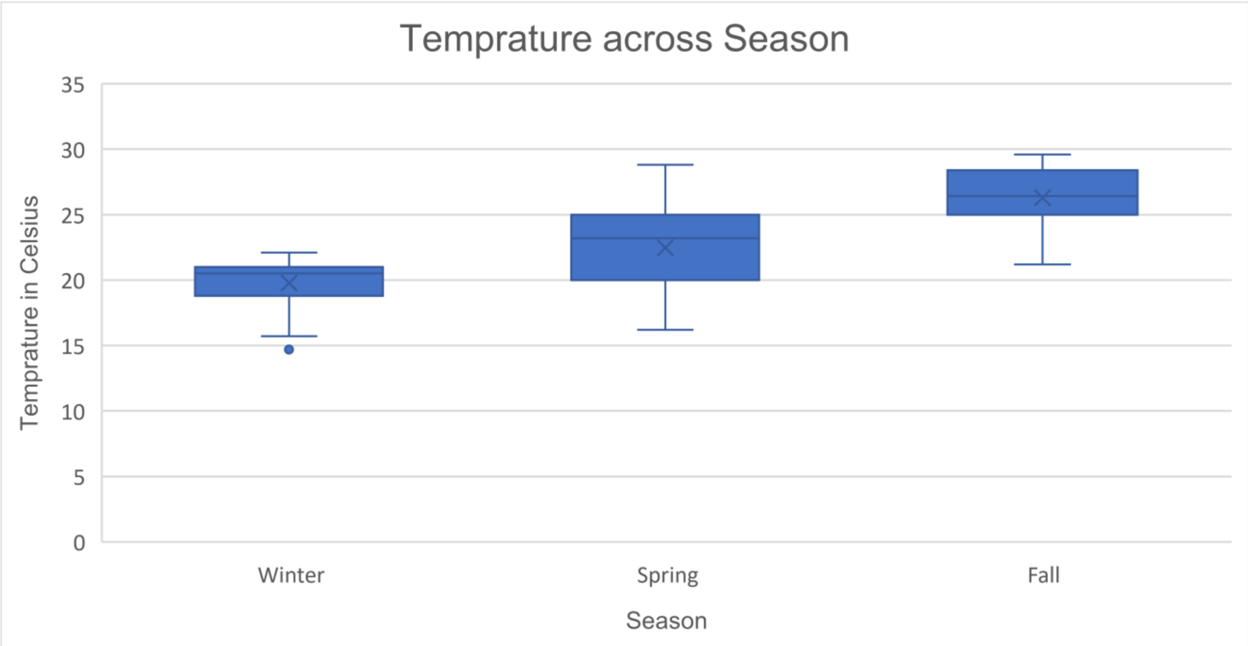

Figure 12: Comparing water temperature between the different seasons that the samples were collected in.

Figures 11 & 12 Included data that was across all sampling points including the Fall. The reason that these figures include all of the data that was collected at the sample location as opposed to just Fall is founded in the fact, we wanted to present the total range of salinities at a given site, so that one can see if any persistent species within a site might be explained by the range of salinity that a site might have. Moreover, a t-Test with an alpha value = 0.05 reveled that salinity was not a significant influence of Chl-a concentrations (Data not shown). This is the same in the case of Temperature across sites with regard to the data that is represented in graph. *Figure 12* represents the whole set of temperatures collect across all sampling sites. *Figure 12* indicates the expected trend of warming waters with increase ambient temperatures. Yet given this trend there was still a great deal of overlap with the season.

3.3.1 Nutrient analysis

Figure 13 graphically represents the Nutrient data across selected sampling locations in the Fall. Sites that have higher amounts of mixing with effluents have higher amounts of silicate present (not shown). It also seems that the Nutrient data of high mixing sites of location A have more Nitrate than is the case for location B yet more advanced analysis will have to be conducted to make sure that the trends in the current data are significant. A t-Test assuming unequal variance with an alpha value of 0.05 indicates a significant difference between the phosphate concentration on the day of the Lone Star biker rally compared with the normal fall phosphate values.

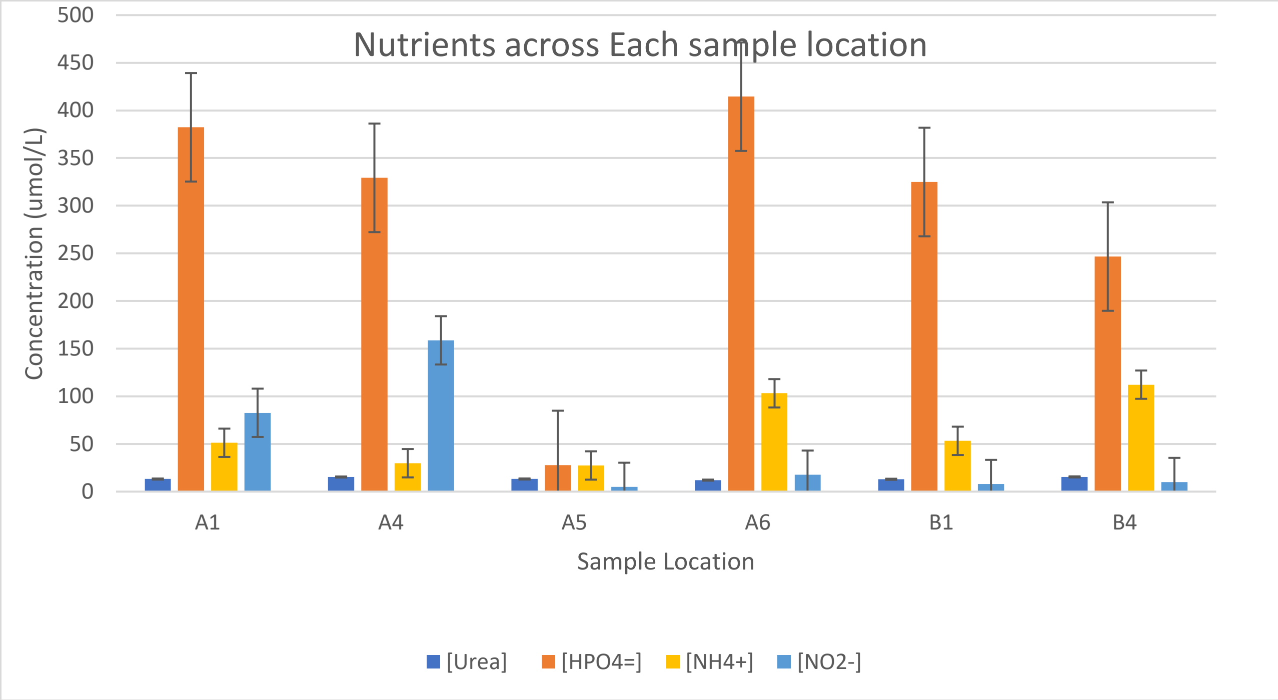

Figure 13 Comparing the concentration of nutrients Urea, Phosphate, Hydrogen Silicate, Ammonium, and Nitrite respectively across all sampling sites at both locations (n=49)

3.4 Dilution calculation

The graphs below indicate the amount of mixing with efflunet that is at a given time of sampling. Location B has a high level of consistancey across sampling sites with regards to the amount of efflunet that is present at a given time. This might be due to the fact that the sampling sites are all very close to the output and mix relativly thorughly with the saltwater from the bay. Moreover, B1 has the largest amount of mixing when compared across all sampling sites which is to be expected given that is the input for the efflunet. Location A (*figure 16*) has a much greater level of variance in the amount of mixing at each sampling site along the effluent stream. This is due to the fact that the mixing is not homogenous throught the system. The mixing in the system is dependant on the proximity of the water to the bay. As the sampling site gets closer to the bay it decrases in the amount of effluent present bacause of dilution.

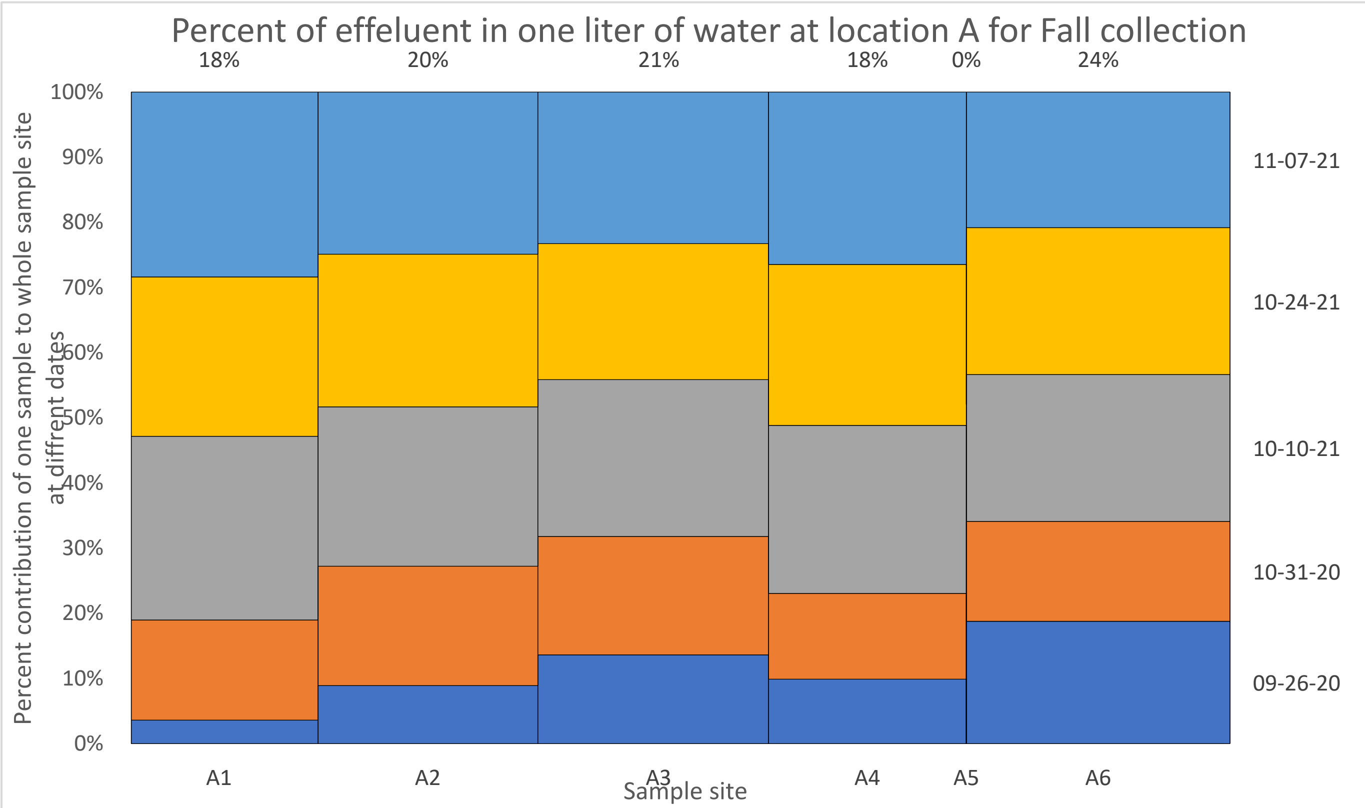

Figure 14 Showing the changes in percent of effluent present in 1liter at a given sampling site within location B for a given time point. (n=24)

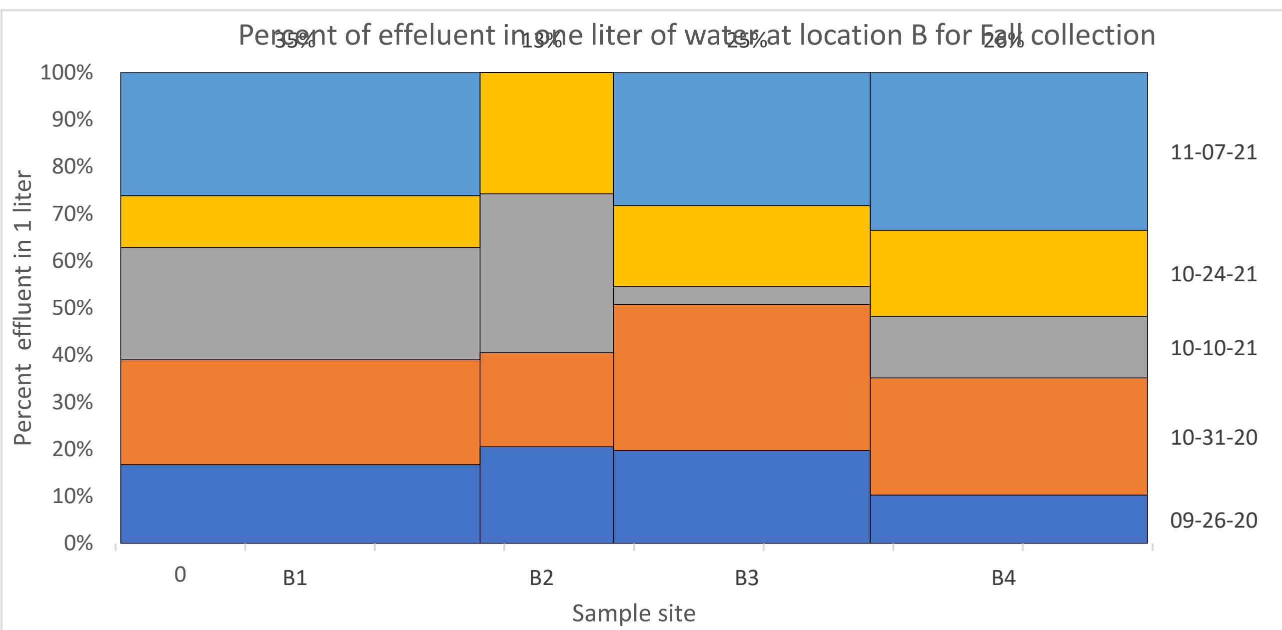

Figure 15 Showing the changes in percent of effluent present in 1liter at a given sampling site within location A for a given time point. (n=25)

4. CONCLUSION

4.1 Conclusion

This endeavor has produced a lot of data to process in order to parse out the mechanisms driving community composition of phytoplankton. Fundamentally one is discussing this process through the mixing of effluent with the bay water. The calculations for mixing indicated the presence of effluent at a given location. The mixing that occurred at the two different sampling sites differed based on the volume and hydrological nature of the effluent stream. At sampling site A as shown in *figure 2* the effluent travels along a small water way lined with marsh grasses before dumping into the Bay. This in contrast to sampling site B in *figure 1* where the effluent is dumped directly into the bay. The hydrologic features of the stream impact the amount effluent that persists in the environment. The nutrient data also supports this claim. There is a very large amount of phosphate in the areas with the highest amount of effluent preset. There also seems to be more phosphate located in location A than in Location B. This may be due to the surrounding system or the method that is used to process the effluent. It also appears that the BR does not impact the amount of mixing that is occurring at site A but in site B the BR seems to be making up a slightly larger portion of the mixing as shown by *figure 17*. This could be due to the fact that more effluent is being processed in response to the increase of people attending the BR. The potential increase in the amount of effluent could be creating some type of flushing effect on the system as a whole. Because the sheer volume of effluent that is being pumped through the culvert it might be flushing the system removing any impacts that the effluent might have on the system. That is potentially why we do not see a distinct increase in the Chl-a concentration at site B as a response to the Lone Star Biker Rally. There might be hydrologic features about the system at site B that could explain why the peak was so high for location B3. It is possible that as the culvert pumps water out the drop of to the side of the moving body of water creates a dead space that is impacted heavily by the effluent. If this is true that means site B3 should have lower salinity on the day of the Lone Star Biker Rally compared to the other locations nearby.

The nutrient analysis data suggested that on the day of the Lone Star Biker Rally there was a significant difference in the concentration of effluent that was present at site B. It is not apparent as to why this was the case. One could assume that fecal matter is rich in nitrogen and phosphorus, therefore an increase in the amount of fecal matter would result in an increase in the amount of Nitrogen in the system. This was not the case however with the same t-Test resulting in no significant difference in ammonia, nitrate, nitrite on the day of the Lone Star Biker Rally. It might be possible that the beer consumed in the area is responsible for the increase in phosphate with beer having 120-735 mg P/L **[22]** one might be able to make the argument that this is a substantial amount when one considers the amount of people in the area that are drinking at the event. This increase in phosphate with the current assessment does not seem to have an impact on the phytoplankton concentration.

The current data does not suggest that effluent has a major impact on the phytoplankton in the surrounding area. Because of the lacking data with respect to the community composition a claim cannot be made about the impact of the wastewater on the community composition. That being said the data does suggest that there were changes in the water chemistry on the day of the biker rally at site B. Further analysis between the two locations might revile some driving mechanism. Although an answer to the major question of the project can only be speculated based on the current analysis. There were major differences in the systems of location A and location B. Further analysis could explore the impact of the wastewater quantity and surrounding environment has on the phytoplankton community. This research could imply that there are more environmentally responsible methods for releasing treated waste. The current analysis did elucidate that human activity could result in an increase in the amount of phosphate in the environment. Further research is needed to see what is causing this change in concentration of phosphate and if the impact of the phosphate on the phytoplankton community is masked by flushing effects of the system.

REFERENCES

[1] GALVESTON *BAY REPORT CARD 2015*.

[2] Pulich Jr., Warren (2002). "Seagrass Status and Trends in the Northern Gulf of Mexico: Galveston Bay System" (PDF). United States Geological Survey. Retrieved March 29, 2020.

[3] "Adapting Galveston Island's Wastewater Infrastructure to Sea Level Rise." *Urban Water Atlas*, 9 Dec. 2020, www.urbanwateratlas.com/2020/12/09/adapting-galveston-islands-wastewater-infrastructure-to-sea-level-rise/. Accessed 4 Mar. 2022.

[4] Toze, Simon. "Reuse of effluent water—benefits and risks." *Agricultural water management* 80.1-3 (2006): 147-159.

[5] Du, Jiabi, et al. "Dramatic hydrodynamic and sedimentary responses in Galveston Bay and adjacent inner shelf to Hurricane Harvey." *Science of the Total Environment* 653 (2019): 554-564.

[6] "Lone Star Rally 2022 - Galveston Bike Rally | LightningCustoms.com." *LightningCustoms.com Motorcycle Rallies Information*, www.lightningcustoms.com/events/lone-star-rally/. Accessed 4 Mar. 2022.

[7] Gao, Drs, and Daniel Roelke. *A Prototype Information System for Monitoring and Predicting Phytoplankton Productivity over Galveston Bay a REPORT FUNDED by a TEXAS COASTAL MANAGEMENT PROGRAM GRANT APPROVED by the TEXAS LAND COMMISSIONER pursuant to NATIONAL OCEANIC and ATMOSPHERIC ADMINISTRATION AWARD NO. NA15NOS4190162*. 2015.

[8] Arar, E. J. & Collins, G. B. *Method 445.0: In vitro determination of chlorophyll a and pheophytin a in marine and freshwater algae by fluorescence*. (United States Environmental Protection Agency, Office of Research 1997).

[9] Beardall, J., Allen, D., Bragg, J., Finkel, Z. V., Flynn, K. J., Quigg,A., Rees, T. A. V., Richardson, A., and Raven, J. A.: Allom-etry and stoichiometry of unicellular, colonial and multicellu-lar phytoplankton, New Phytol., Tansley Review, 181, 295–309,https://doi.org/10.1111/j.1469-8137.2008.02660.x, 2008.

[10] Falkowski, Paul. "Ocean science: the power of plankton." *Nature* 483.7387 (2012): S17-S20.

[11] Cloern, J. E., S. Q. Foster, and A. E. Kleckner. "phytoplankton primary production in the world's estuarine-coastal ecosystems." *Biogeosciences Discussions* 10.11 (2013): 17725-17783.

[12] Tappan, Helen. "Primary production, isotopes, extinctions and the atmosphere." *Palaeogeography, Palaeoclimatology, Palaeoecology* 4.3 (1968): 187-210.

[13] Ohman, M. D., and H-J. Hirche. "Density-dependent mortality in an oceanic copepod population." *Nature* 412.6847 (2001): 638-641.

[14] Shanks, Alan L., and Jonathan D. Trent. "Marine snow: sinking rates and potential role in vertical flux." *Deep Sea Research Part A. Oceanographic Research Papers* 27.2 (1980): 137-143.

[15] Alldredge, Alice. "The carbon, nitrogen and mass content of marine snow as a function of aggregate size." *Deep Sea Research Part I: Oceanographic Research Papers* 45.4-5 (1998): 529-541.

[16] Turner, Jefferson T. "Zooplankton fecal pellets, marine snow, phytodetritus and the ocean's biological pump." *Progress in Oceanography* 130 (2015): 205-248.

[17] Abell, Jonathan M., Deniz Özkundakci, and David P. Hamilton. "Nitrogen and phosphorus limitation of phytoplankton growth in New Zealand lakes: implications for eutrophication control." *Ecosystems* 13.7 (2010): 966-977.

[18] Vanni, Michael J. "Effects of nutrients and zooplankton size on the structure of a phytoplankton community." *Ecology* 68.3 (1987): 624-635.

[19] Hinga, Kenneth R. "Effects of pH on coastal marine phytoplankton." *Marine ecology progress series* 238 (2002): 281-300.

[20] Richier, Sophie, et al. "Response of the calcifying coccolithophore Emiliania huxleyi to low pH/high pCO2: from physiology to molecular level." *Marine biology* 158.3 (2011): 551-560.

[21] Kirst, G. O. "Salinity tolerance of eukaryotic marine algae." *Annual review of plant biology* 41.1 (1990): 21-53.

[22] Fernandes, Sílvia MV, José LFC Lima, and António OSS Rangel. "Spectrophotometric flow injection determination of total phosphorus in beer using on-line UV/thermal induced digestion." *Fresenius' journal of analytical chemistry* 366.1 (2000): 112-115.