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Fish Community Composition and Structure Near a Freshwater River Diversion in Southeastern Louisiana

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FISH COMMUNITY COMPOSITION AND STRUCTURE NEAR A FRESHWATER RIVER DIVERSION IN SOUTHEASTERN LOUISIANA

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This thesis is dedicated to the light of my life: my dog, Klaus. He has been with me since the very beginning of my time at LSU, and he is the reason I get out of bed every single day. Klaus helped preserve my sanity throughout this process, and he deserves all the milk bones in the world for that. Even though he doesn't know what a marsh is, this thesis would not have been possible without him.

Ten out of ten, the best boy.

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Abstract

Gulf of Mexico estuaries, particularly in Louisiana, are among the world's most productive, with landings of commercially- and recreationally-important species exceeding all other contiguous US states. Coastal wetlands are disappearing at an alarming rate because they have been impounded from Mississippi River water and sediment. Although controversial, one proposed solution is to re-route the Mississippi River through diversions and siphons to supply the freshwater and sediments necessary to rebuild vanishing wetlands, particularly in Barataria Bay and Breton Sound, LA. This strategy is one approach outlined in the Louisiana Coastal Master Plan. This project aimed to describe the composition and structure of the fish community associated with the Caernarvon freshwater diversion in three microhabitats within Big Mar pond. These three habitats included Bayou Bonjour (shallow, slow-moving, protected habitat), Center Big Mar (shallow, slow-moving, submerged aquatic vegetation dominated habitat), and SW Big Mar (open water habitat). The fish community was dominated by a few ubiquitous species, and no differences were detected between the three sites. A single-species approach was adopted to determine how the three locations support the common species. Though the community did not vary, four of the five common species show evidence of an ontogenetic shift in habitat within Big Mar Pond. This work will inform fishers and policymakers on the impacts of the Mississippi River diversions and siphons on fish communities, especially as new diversion projects are proposed to restore eroding coastal wetlands and marshes.

Chapter 1. Literature Review and Introduction

1.1. Coastal Wetlands

Coastal wetlands are found at the land-ocean interface and include many habitats that support biodiverse communities. Examples of coastal wetlands include marshes (saltwater, freshwater, and brackish), swamps, mudflats, seagrass meadows, and mangrove forests. Though these ecosystems occur within different environmental parameters, they all provide important ecosystem services and are vital to human society. Intact biotic communities in wetlands can remove high concentrations of nitrates from fertilizers, improving water quality and decreasing nutrient loading in coastal waters (Mitsch 2000). Slow moving currents create a depositional environment, contributing to the largest terrestrial pool of biological carbon, despite wetlands only contributing to 5-8% of the world's total terrestrial landscape (Chmura 2003, Mitsch 2013). Coastal wetlands are critical for the protection of coastal communities from hurricanes and other natural disasters. They are sometimes referred to as "horizontal levees", attenuating wave energy and mitigating floods. Using a multiple regression analysis and hurricane frequency data, Constanza et al estimated that coastal wetlands provide over $23 billion in storm protection services to the United States each year (Constanza 2008). Wetlands are the buffer that protect inland areas from hurricane damage and storm surge.

In addition to storm protection, coastal wetlands support diverse communities of organisms in a wide range of taxa, including birds, plants, fish, and shellfish. Wetlands provide nesting ground for many species of birds, including Red-winged Blackbirds, Yellow-headed Blackbirds, and Seaside Sparrows (Tozer 2010). Plant community composition is one of the defining features of many wetlands, as they are heavily influenced by environmental conditions, such as temperature and salinity (Casanova 2000). Wetland plants contribute to ecosystem

function by stabilizing the shoreline, attenuating wave energy, and aerating the soil (Brix 1994). They also are major primary producers, supporting the base of the food web.

As water is typically shallow and slow-moving, coastal wetlands are able to support diverse fish populations. Wetlands are considered essential fish habitat by the National Oceanic and Atmospheric Administration (NOAA), because they provide vital habitat for fish to breed, spawn, feed, and grow. In particular, these areas are nursery grounds for juvenile fish, contributing to the production of offshore fisheries. Nursery grounds provide habitat, food resources, refuge, and favorable environmental conditions for juvenile fish to grow and develop before undergoing ontogenetic migrations to their adult habitats (Sheaves 2015).

In addition to the ecological necessity of healthy wetlands, coastal Louisiana is home to nearly 2 million people who have a deep cultural connection to the wellbeing of both their land and their water resources (Coastal Protection and Restoration Authority of Louisiana 2017). This is nearly half of the state's population. Louisiana is home to five of the top twelve ports in the United States by cargo volume. Louisiana's wetlands protect and support the sources of hundreds of billions of dollars that are contributed to the United States economy each year by providing ecosystem services (Coastal Protection and Restoration Authority of Louisiana 2017). Protecting coastal Louisiana is vital from all perspectives: ecological, economic, or cultural.

1.2. Coastal Erosion and Land Loss

Coastal wetlands are facing many threats, particularly due to erosion and land loss. The development of coastal areas has led to a dramatic change in the hydrography of the land-ocean interface. Land loss occurs gradually, as productive marsh land is converted to open water, the shoreline shifts inward, and the dry upland area loses elevation. Louisiana is home to 40% of the wetlands in the United States, yet these vital lands are currently undergoing unprecedented rates

of land loss. Between 25 and 35 square miles of marsh are lost each year in Louisiana, and more than a million acres have been lost since 2000 (Couvillion 2011, Day 2007). A combination of natural and anthropogenic forces is responsible for direct impacts to coastal land and indirect impacts that can be felt throughout the state and even the country.

Hurricanes, saltwater intrusion, and wave erosion naturally lead to wetland loss, but human activities have accelerated coastal land loss by altering the hydrology of the landscape. The construction of channels and canals for boat use converts shallow wetlands into less productive open water systems, leading to the habitat fragmentation of surviving wetland areas. The levees around the Mississippi River have stifled the flow of freshwater, nutrients, and sediment into the surrounding wetlands, allowing for saltwater intrusion and the subsequent transition from freshwater marsh to brackish and salt marsh communities.

The coastal land loss over the century has undisputedly altered the ecology of the landscape. In addition to damage to the coastal community, storm damage can increase in inland areas as a result of wetland loss. This puts both natural and manmade resources, including ecosystem services and economic activity, at risk.

1.3. Land Loss Mitigation Strategies

Louisiana is the nation’s primary provider of shrimp, oysters, blue crabs, crawfish, and alligators (Louisiana Seafood n.d.). As 75% of Louisiana’s commercial fisheries depend on wetlands for nursery habitat, spawning sites, and feeding grounds, wetland health is vital for the economy of the state (Chesney 2000). In Louisiana's Comprehensive Master Plan for a Sustainable Coast, $50 billion in projects are proposed to protect and restore Louisiana’s coasts. Of that sum, $17.8 billion is to be allocated to use dredged material to rebuild marshes lost to erosion, and an additional $5.1 billion to restore marshes through the use of diversions. This plan

recommends a total of 124 projects, which are projected to create and preserve more than 800 square miles of land over the next 50 years (Coastal Protection and Restoration Authority of Louisiana 2017).

One strategy for mitigating coastal land loss is the strategic deposition of dredged material that is removed from the Mississippi River, nearby bodies of water, or offshore shoals. The application of dredged material can increase the rates of subsidence (e.g. loss of elevation) and the compaction of shallow sediments (Wiegman 2018). This strategy is seen by some as only a temporary solution, as it does not slow down the rate of erosion, but simply prolongs the root issue. If dredging is the sole method of marsh creation, more frequent dredging will be necessary to keep pace with accelerating sea level rise and erosion, as accretion via the ecological community will be unable to keep up in most areas. Hydraulic dredging can expensive, and it is predicted to get more expensive over the next century. It is unlikely that marsh creation through dredging alone can mitigate coastal land loss (Wiegman 2018).

The construction of diversions to reconnect the Mississippi River with adjacent marshes is another commonly used mitigation strategy to slow wetland loss and restore habitats to a more natural state. These can function as water diversions or sediment diversions, though both types have the ability to restore wetland habitat with varying degrees of success (Renfro 2018). River diversions are considered to be a cheaper strategy over a long period of time, as sediment is continuously being delivered as opposed to a one-time deposition previously discussed. Water is re-routed from the Mississippi River and allowed to flow through different types of terrain, such as marshes and bayous, in a designated outfall area. The marshes and bayous are typically shallow and slow-moving, creating a depositional environment for the sediment in the river water to build new land, combatting the coastal land loss that has been occurring for decades.

The reintroduction of freshwater and nutrients changes the ecological structure of the area, and the effects on the fish communities are not well understood and documented.

1.4. Understanding Fish Communities

The biological integrity of aquatic ecosystems, as defined by Karr and Dudley (1981), is the "capability of supporting and maintaining a balanced, integrated, adaptive community of organisms having a species composition, diversity, and functional organization comparable to that of natural habitat of the region." The fish community within an aquatic ecosystem reflects the environmental conditions of the region, and can be used as a biological indicator when monitoring ecosystem health (Fausch 1990). Traditionally, single taxa indicators have been used to assess ecosystem health, but a community approach has become more widely applied in the last few decades. To accomplish this, fish community metrics are calculated and compared between locations to understand the response to environmental gradients or stressors.

Typically, community metrics, such as richness, evenness, and diversity, are calculated and compared across groups. These groups can be based on location to understand how the fish community changes over spatial scales, or a time-series can be used to determine how the community has changed over time. These metrics are commonly employed, as they can reduce huge amounts of fish data into a single number, which simplifies comparisons. However, the use of these methods alone results in a loss of relevant data, such as species composition, which are important for understanding changes in community. There is no scientific consensus regarding the "perfect" method for answering these questions, so many scientists opt for a combination of several metrics.

Multivariate methods, such as multidimensional scaling and principal components analysis, are also commonly used to compare species composition. An advantage of this strategy

is that the community data is kept intact compared to analyzing community metrics. A disadvantage of using these analyses is that the result is typically a two-dimensional plot. The conclusions from said plots can be subjective rather than objective and can be heavily influenced by the author's interpretation (Rochet and Trenkel 2003).

Lastly, resampling statistical methods, such as bootstrap and jackknife methods, are iterative techniques that can result in more precise estimations of community metrics. The basis for these techniques is that each sample is a random assemblage from a larger, unknown community. Typically, ecologists are limited in the number of samples they can perform within time and budget constraints, so it is impractical and nearly impossible to fully enumerate the community. Bootstrap and jackknife techniques iteratively use the sample distribution to resample the population to estimate community metrics with greater precision, and are often accompanied by confidence intervals. Though these methods may not be the most statistically tractable, they can provide ecologically useful coefficients, and are commonly used to estimate bias and variance of a statistic (Dixon 1993).

Fish community data must be accompanied by contextual data to correlate changes in community structure with characteristics of the ecosystem. Marsh, pond, seagrass and open water habitats provide different elements that are more suited for some species, or even certain individuals or age classes, than others. Understanding how changes in habitat type affect fish communities is vital to the development of successful restoration projects that aim to enhance fish populations and fisheries production. A study conducted in a New Jersey estuary compared eelgrass, macroalgae and saltmarsh creek habitats to determine impacts to epibenthic fish and decapod communities, and found that habitat type affected the abundance and diversity of both groups of species. They found higher fish densities in vegetated sites compared to adjacent

nonvegetated sites and compared habitat utilization for different fish species. The authors concluded that vegetated habitats are important for small fishes and may function as nursery habitat within the estuary (Sogard and Able 1991).

In addition to community metrics, ecosystem-based modeling has been employed as a multi-variate approach to account for the complexity of ecosystems. Mutsert *et al* used an Ecopath with Ecosim model to explore community response in the Breton Sound estuary to the Caernarvon freshwater diversion. In particular, they were interested in modeling the effects of changing salinity on species biomass distributions (SBD). They did not find a significant difference in the estuary-wide SBD before and after the opening of the diversion, which the authors interpreted as a redistribution of the community rather than the replacement of species within the community (Mutsert *et al*. 2012).

1.5. Effects of Salinity and Hypoxia Gradients on Fish Communities

As there are very few true euryhaline fish species, changes in salinity inevitably lead to changes in the abundance and distribution of fish within the ecosystem. Estuaries are the most commonly encountered ecosystem when discussing how salinity affects fish communities, as they are typically characterized by salinity gradients that are formed as freshwater from a river meets saltwater from the ocean. Salinity tolerances of species determines their distribution throughout the estuary. When freshwater diversions are built to increase delivery of freshwater to wetlands, the salinity gradient of the estuary is affected, which subsequently affects the structure and composition of the ecological community.

In the Tampa Bay and Charlotte Harbor estuaries (both in Florida), there was rapid change in the nekton community at very low salinities (0 to 2 ppt), as well as at high salinities (31 to 35 ppt). At intermediate salinities, there was comparatively slow change in the community

along the gradient. This study found little evidence of defined salinity zones except at the extreme ends of the salinity gradient, which likely reflect the contributions of stenohaline species (Greenwood 2007). Near freshwater diversions, salinity is close to 0 ppt, and an ecotone likely exists in the outfall area.

Though salinity is one of the most well-studied estuarine gradients, hypoxia can also affect the structure of fish communities in estuaries. Hypoxia is caused by eutrophication, or nutrient pollution. Inorganic nutrients, such as nitrogen and phosphorous, are limited in many estuaries, which controls primary productivity. Freshwater flow from rivers to estuaries carries nutrients from all over the watershed, with a significant source being from agricultural fertilizer. This causes surface algal blooms, leading to hypoxic and anoxic conditions as oxygen is consumed when the algae decompose (Howarth *et al.* 2011). Like salinity, fish species have differing tolerances for reduced dissolved oxygen concentrations. Hypoxia gradients caused by freshwater input, coupled with salinity gradients, also have been shown to shape fish communities in estuaries (*e.g.* Maes *et al.* 1998).

1.6. The Caernarvon Freshwater Diversion

The Caernarvon Freshwater Diversion Project was authorized by the Flood Control Act of 1965 (PL 89-298), the Water Resources Development Act of 1974 (PL 93-251), and Water Resources Development Act of 1986 (PL 99-622). Construction took place between 1988 and 1991, and operations began in August of 1991. The diversion is located in both Plaquemines Parish and St. Bernard Parish on the eastern bank of the Mississippi River. It is capable of delivering a flow of up to 8,000 cubic feet of water per second into 77,000 acres of surrounding marshes and wetlands (Kaintz, 2010). The goal of the diversion was to increase flood protection while restoring the wetlands by reducing salinity, building land, and increasing the production of

fisheries and wildlife. Additionally, freshwater diversions have been shown to reduce inorganic nutrient (e.g. nitrate) load as the water flows through marshes and wetlands (DeLaune *et al.* 2005; Lane *et al.* 1999).

From December 2009 through the end of 2012, approximately 202,000 cubic meters of sediment were delivered from the Caernarvon diversion to the receiving basin. The deposition of this sediment was sufficient to support a permanent community of emergent vegetation within Big Mar Pond in the outfall area. From 1998 to 2011, a total of 600 acres of land was created in Big Mar as a result (Fig. 1; Lopez *et al.* 2014). The original intention of the diversion was to move freshwater into the adjacent wetlands. The diversion was designed to minimize the amount of retained sediment, so the potential sediment delivery in future diversions is much greater. As Caernarvon was the first diversion of its kind built in Louisiana, the delta-building capabilities are promising for the future of coastal restoration in the Mississippi River delta and in eroding deltas around the world.

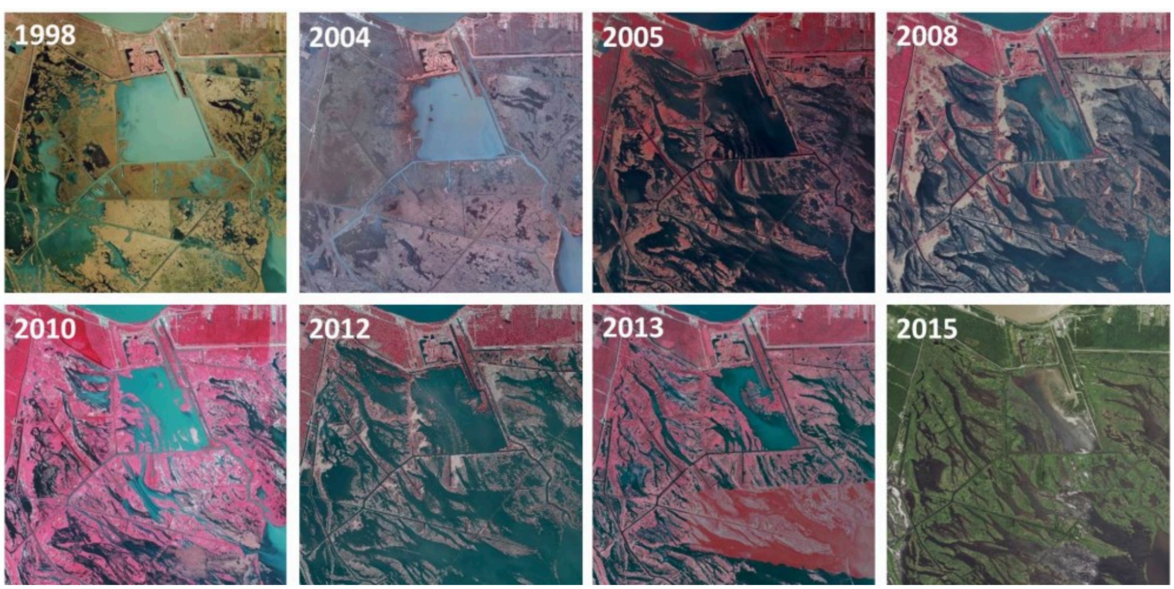

Figure 1. Satellite imagery of land change in Big Mar pond from 1998 to 2015. Visual land-building did not occur until 2005, when sediment deposition reached the height to support emergent vegetation. (From the Lake Ponchartrain Basin Foundation; saveourlake.org).

Chapter 2. Methods

2.1. Study Site Description

Big Mar is a small (2,040 acre) freshwater pond within the Caernarvon freshwater diversion outfall area (29.83022 N, -89.90905 E) located in Southeastern Louisiana in Plaquemines Parish, east of the Mississippi River and southeast of New Orleans (Figs. 2 & 3). As of 2010, the average depth was 1.2 meters, and the maximum depth was 3.7 meters (Kaintz 2010). Big Mar was fed by the Mississippi River before levee construction began in the 18^{th} century. Then, the new levees negatively affected the flow of freshwater from the river to the wetlands, which resulted in a tidally influenced, saltwater-dominated system. In the early 20^{th} century, the marsh was drained by the United States Department of Agriculture to convert Big Mar to a 2,040-acre agricultural field. This field was susceptible to flooding and therefore unsuccessful for agriculture and was subsequently abandoned and allowed to return to a more natural state.

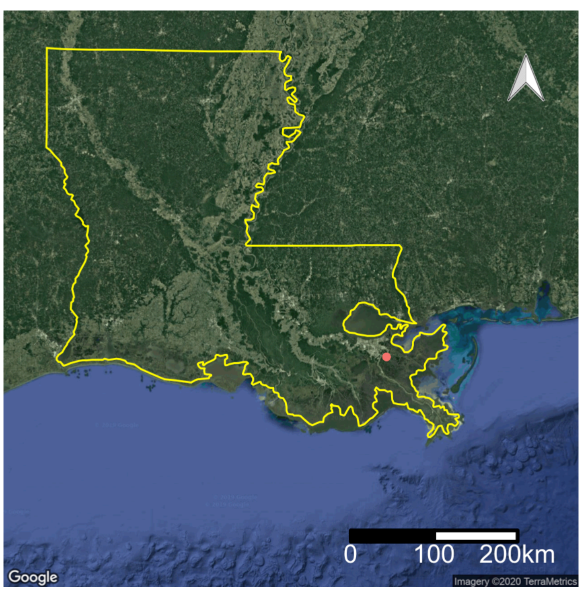

Figure 2. Map of Louisiana with Big Mar pond indicated by the pink point.

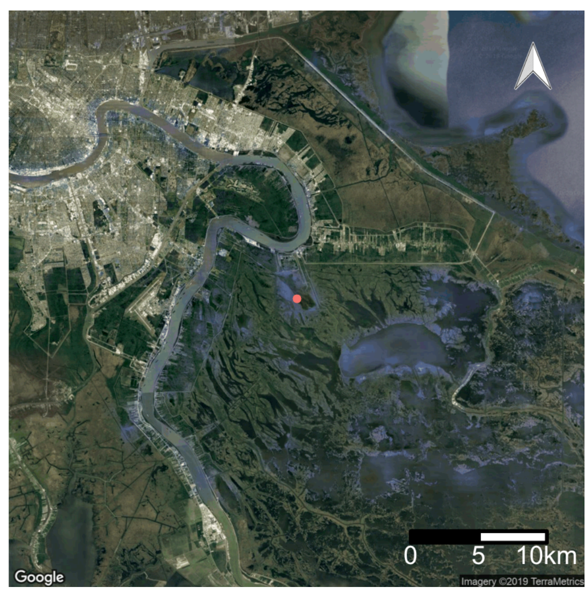

Figure 3. Map of Southeastern Louisiana with Big Mar study site represented with a pink dot 20 km SE of New Orleans.

Sampling was carried out within three distinct locations in Big Mar (Fig. 4) on May 7-9, 2019, July 29-30, 2019, and October 10, 2019. Each site varied in environmental conditions such as plant community (both emergent and submerged), flow rate, and depth. I did not directly measure any of these conditions, but the sites were chosen to represent different habitats within the pond.

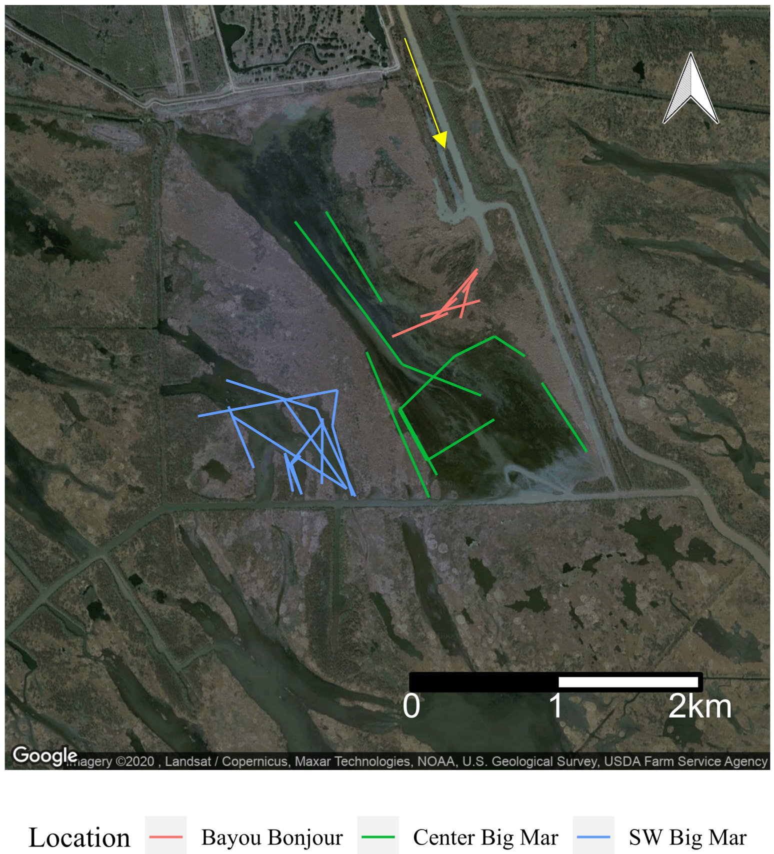

Figure 4. Big Mar map with electrofishing transects shown in the three areas, Bayou Bonjour, Center Big Mar, and SW Big Mar. The yellow arrow indicates the direction of the flow from the Caernarvon River Diversion.

The first site, Bayou Bonjour, was the closest in proximity to the Caernarvon diversion (Fig. 5a). It is a narrow, shallow, slow-moving bayou that flows from east to west. It is heavily vegetated and dominated by smooth sawgrass (*Cladium mariscoides*), giant cutgrass (*Zizaniopsis miliacea*), and water hyacinth (*Eichhornia crassipes*). Bayou Bonjour likely has the most influence

from the diversion, as it is where the nutrient-rich sediment settles out as the freshwater flows through the bayou.

Center Big Mar is the second location moving away from the diversion (Fig. 5b). It is slightly deeper and more open than Bayou Bonjour. It is approximately 30 to 45 centimeters deep, and the water is clear enough to see the bottom in most places. It is dominated by submerged aquatic vegetation (SAV), with fragmented patches of emergent vegetation. The only shade is along the edges of the sawgrass and under the cypress, willow and tupelo trees on the bank.

SW Big Mar is the furthest location from the diversion (Fig. 5c). This habitat is mainly open water. It is the deepest of the three locations. There is very little vegetation in the center, though both sides are lined by emergent vegetation. The water flows quickly, likely driven by the wind and flow from the adjacent navigable channel.

Figure 5. Representative images of the three study sites: (A) Bayou Bonjour; (B) Center Big Mar, and (C) SW Big Mar.

2.2. Hypotheses

With the general descriptions of the three locations, I hypothesized that Bayou Bonjour and SW Big Mar support different fish communities due to having different habitat characteristics. Center Big Mar seems to be an intermediate habitat between the other two, and will likely have the highest species richness and diversity as a result. This hypothesis is based on the nested-subset

hypothesis by Patterson and Atmar (1986), which states that in fragmented habitats, the species composition in the lower richness fragments are subsets of the species composition in the higher richness fragments. Species evenness with likely be the highest in SW Big Mar, as I expect it to have mainly large, solitary adult fish rather than schools of small fish. Due to its high plant productivity and sheltered environment, I expect Bayou Bonjour to act as a nursery habitat for juvenile fish. This is where I expect to find the highest relative abundance of fish, with high densities of small fish using the habitat's rugosity as a refuge from predation.

2.3. Collection Methods

The fish community was sampled by electrofishing (Smith-Root, Vancouver, WA) within the three locations of Big Mar. I used the Smith-Root Streambank Generator Powered Pulsator (GPP) electrofisher. This electrofisher is ideal for sampling within low to medium conductivity water, though it can also work in high conductivity water. It is a barge electrofisher, which I towed alongside an airboat for each sampling event. I used a 28-centimeter stainless steel electrode ring which was submerged in the water during sampling. This ring was the cathode, and the anode was located on the underside of the electrofisher barge, which is how the electrofisher is able to maintain an electric current to stun the fish.

Before most sampling events, I recorded environmental data: salinity, temperature, dissolved oxygen, and conductivity. Unfortunately, our equipment had some glitches; I did not collect these data before every transect (Table A.1.). I conducted the surveys in transects and standardized them by time. I electrofished in a relatively straight line for approximately 15 minutes per transect. For some transects, many fish were caught at once, and the transect was cut short to minimize fish mortality. I recorded the GPS location at the beginning and end of each transect. I treated each transect as a replicate sample of the fish community in the location.

As the collection team electrofished along the transect, one person used the cathode wand, one person was responsible for catching the stunned fish using a dip net. We used a course dipnet and a fine mesh dipnet on different rounds based on the size of the fish encountered during collection. Once fish were onboard, two people identified all individuals to the lowest taxonomic level possible and recorded the total length and weight (when possible) of the individuals before placing the fish into a bucket of water. The individuals that could not be identified to species level onboard were placed in a labeled, zippered bag and stored on ice until they could be transferred to a freezer in the laboratory to be identified at a later date. Only after the transect was completed, either after 15 minutes or when there were too many fish caught to process in a timely, humane manner, the collected fish were returned to the water to avoid repeat sampling of a single individual in the same transect. I avoided resampling the same transect on the same day. All data was recorded in a weather-proof data book.

After the sampling event was over, the data was entered into a spreadsheet by one of the people who collected the data. When possible, a second person double-checked all data entered to ensure accuracy. Any fish that could not be identified to species level was photographed and collected and were identified later using morphologies and morphometrics. Unfortunately, some individuals could not be identified using this method, and were analyzed in an "unknown" group of the lowest taxonomic level possible.

2.4. Data Analysis

All data analysis was done in R, and figures were created using the package ggplot2 (R Core Team, 2013; Wickham 2009). Once raw data was uploaded into R, data were cleaned to remove transects that did not meet the standard for inclusion in the study. Two transects from SW Big Mar were removed from the July 29, 2019 sampling date, as I was not electrofishing for the entire

transect while attempting to catch large Common Carp (*Cyrpinus carpio carpio)*. One transect from SW Big Mar was removed from the October 10, 2019 sampling date, as the electrofishing wand malfunctioned and was not delivering electric charge throughout the entire transect. Additionally, any invertebrates that were caught in the survey (i.e. blue crabs, crawfish) were removed from the data prior to analysis.

Next, the data for each individual fish were aggregated into a community matrix, where each transect is assumed to be an independent sample of a fish assemblage from a larger community. For each transect, I measured water quality, GPS location of the beginning and end, site, and electrofishing duration. The community matrix was standardized as catch per unit effort, which I defined as the number of individuals per minute of electrofishing. Therefore, the community metrics, with the exception of species richness, are all calculated per unit effort. The vegan package was used to calculate alpha diversity metrics (species richness, Shannon diversity, Simpson Diversity, inverse Simpson diversity, and Pielou's evenness) from the community matrix (Oksanen *et al.* 2019).

I used the "specaccum" function in the vegan R package to produce a species accumulation curve for the individual locations and pooled across all locations (Oksanen *et al.* 2019). This visualized the accumulation of unique species with an increasing number of transects. I used the random method, so the function added transect samples randomly. It completed 100 permutations to calculate the associated standard error for adding each additional transect to the curve.

Asymptotic species richness was predicted for the entire pond (all locations pooled) and for each individual location using multiple non-parametric methods from the fossil package in R (Vavrek, 2011). Parametric extrapolations of true species richness can be difficult to fit to empirical data, and there are many concerns with comparing estimates from different models and

distributions, particularly when there are multiple assemblage samples. Non-parametric methods are considered more robust and allow for comparison across assemblages.

The purpose of this extrapolation is to estimate the undetected species that were not sampled to understand the true species richness of the community. The non-parametric methods used were Chao1, Chao2, Abundance-based Coverage Estimator (ACE), Incidence-based Coverage Estimator (ICE), Bootstrap, and second-order Jackknife. Chao1, ACE, Bootstrap and Jackknife methods for estimating true species richness use raw abundance data, which was not standardized as catch per unit effort. Chao2 and ICE each use incidence (presence/ absence) data. As there is no perfect method for this estimation, all of these can be used in conjunction to get a more complete understanding of the species richness of the community (Gotelli & Chao, 2013).

The Chao1 method uses the observed richness with the number of singletons and doubletons (considered rare species) to obtain the lower bound of species richness (Chao 1984). This is based on the concept that rare species carry the most information regarding the number of undetected species. The Chao2 method is similar to the Chao1 method, but it converts abundance data to incidence data and incorporates a sample-size correction factor.

The ACE method for predicting total species richness considers all rare species rather than just singletons and doubletons. It is a more general approach than Chao1 and Chao2, allowing a cutoff value to be set to classify all species as either rare or abundant. The default value is ten individuals to be considered rare. The coverage is the proportion of individuals in the assemblage that are represented by the species recorded in the sample, which is a measure of heterogenous richness. The estimator is adjusted to account for this heterogeneity to predict the species richness. This method does not perform well for species rich and highly heterogenous assemblages (e.g. species richness > 1000), but this is not a concern for these data. Similar to the ACE method, the

ICE method uses coverage to estimate species richness, but instead uses incidence data. This method also uses a cutoff value to categorize species as either frequent or infrequent. ICE adjusts the estimator based on the probability of detecting rare species, which is the basis for the estimation (Chao *et al.* 2005).

The Bootstrap method is a data resampling technique that has been adapted for multiple disciplines, including the estimation of true species richness (Smith & van Belle, 1984). It independently samples from the dataset with replacement to estimate summary statistics, such as mean and standard deviation. The Jackknife method is similar to the Bootstrap, but instead it iteratively deletes a random observation from the dataset and recalculates the desired statistic. In general, the Jackknife method tends to be more conservative and better suited for smaller sample sizes than the Bootstrap method (Efron, 1982). Higher-order Jackknife estimators give increasingly less weight to the most common species, so I chose to use the second-order Jackknife, which successively deletes two individuals from the dataset for each iteration (Heltshe & Forrester, 1983).

I compared the total catch per unit effort, or relative abundance, and species richness between the three sites. Both sets of pooled data failed the Shapiro-Wilkes test of normality from the stats R package, so the data are not normally distributed, and a non-parametric statistical analysis was necessary (R Core Team, 2018). I used the Kruskal-Wallis test to determine if sample relative abundance and species richness from the three locations were different from each other (R Core Team, 2018).

The sample values for Shannon and Simpson diversity passed the Shapiro-Wilk test, so they likely come from a normal distribution and the assumptions of a parametric statistical analysis are met. I used an ANOVA to determine differences between the three locations. The sample values for inverse Simpson diversity did not pass the Shapiro-Wilk test, so they do not come from a normal

distribution and a non-parametric statistical analysis was necessary. I used the Kruskal-Wallis test to determine if samples from the three locations were different from each other.

To compare beta diversity between the three sites, I used nMDS from the vegan R package with the square-root transformed relative abundance data to down-weight the importance of hyperabundant species (Oksanen *et al.* 2019). I used Bray-Curtis dissimilarity test with two dimensions and set a limit of 100 random starts or iterations. I visualized the results with 95% confidence circles to illustrate how clustered the samples from the three locations are. I used an analysis of similarity, or ANOSIM, to determine if distances between groups are greater than differences within groups. I also used a PermANOVA to determine if distances varied between groups. To determine which species were driving the similarity between the three locations, I used SIMPER from the vegan package (Oksanen *et al.* 2019).

The relative abundance of Bluegill, Mosquitofish, Sailfin Mollie, Spotted Gar, and Striped Mullet per sample each failed the Shapiro-Wilk test, and a non-parametric statistical analysis was necessary to compare the relative abundance per sample between the three locations. The Kruskal-Wallis test was used.

The lengths of Bluegill passed the Shapiro-Wilk test of normality. ANOVA and a post-hoc LSD test were used to determine differences in the length of individuals by location. The lengths of individual Striped Mullet, Spotted Gar, Mosquitofish, and Sailfin Mollies failed the Shapiro-Wilk test, and a non-parametric statistical analysis was necessary to compare the lengths of each species between the three locations. The Kruskal-Wallis test was used, and the *post hoc* Dunn test (R package FSA) of multiple comparisons using rank sums was used to determine pairwise differences in lengths by location, as locations did not have an equal number of observations (Zar, 2010; Ogle,

2020). The p-values of the Dunn test were adjusted using the Holm's sequential Bonferroni procedure, because there were multiple pairwise comparisons being made (Holm, 1979).

Chapter 3. Results

3.1. Community Description

In total, we collected 21 fish species in our survey (Table 1). Of the species caught, 10 are only found in freshwater. Four are found in both freshwater and brackish water. Eight are considered euryhaline species that are found in freshwater, brackish, and marine habitats. Two of the euryhaline species, gulf menhaden and red drum, are primarily brackish and marine species that only occasionally enter freshwater habitats. In addition, we were not able to positively identify some individuals to the species level. These were categorized as unknown Goby (family *Gobiidae*, possibly *Gobiosoma bosc*) and unknown sunfish (*Lepomis* sp.). The unknown Sunfish were juveniles and were likely Bluegill or Green Sunfish, or a hybrid species.

Table 1. List of species caught in survey with Latin name and salinity affinity (Y- Yes, N- No, S- Sometimes).

Common Name	**Scientific Name**	**Fresh-Water**	**Brackish**	**Marine**	**Reference**
Alligator Gar	*Atractosteus spatula*	Y	S	N	(Carlander, 1969)
Bay Anchovy	*Anchoa mitchilli*	Y	Y	Y	(Riede, 2004)
Bluegill	*Lepomis macrochirus*	Y	N	N	(Beitinger and Bennett, 2000).
Common Carp	*Cyprinus carpio carpio*	Y	N	N	(Riede, 2004)
Golden Topminnow	*Fundulus chrysotus*	Y	N	N	(Baensch and Riehl, 1985)
Green Sunfish	*Lepomis cyanellus*	Y	N	N	(Stuber *et al.*, 1982)
Gulf Menhaden	*Brevoortia patronus*	S	S	Y	(Whitehead, 1985)
Inland Silverside	*Menidia beryllina*	Y	Y	S	(Espinosa-Perez, 2015)
Largemouth Bass	*Micropterus salmoide s*	Y	N	N	(Glover *et al.,* 2013)
Marsh Killfish	*Fundulus confluentus*	Y	Y	Y	(Griffith, 1974)

(table cont'd.)

Common Name	Scientific Name	Fresh-Water	Brackish	Marine	Reference
Mosquitofish	*Gambusia affinis*	Y	Y	N	(Chervinski, 1983)
Orange Spotted Sunfish	*Lepomis humilis*	Y	N	N	(Kilby, 1955)
Red Drum	*Sciaenops ocellatus*	S	Y	Y	(Riede, 2004)
Redbreast Sunfish	*Lepomis auritus*	Y	N	N	(Kilby, 1955)
Redear Sunfish	*Lepomis microlophus*	Y	S	N	(Bailey *et al.*, 1954)
Redspotted Sunfish	*Lepomis miniatus*	Y	N	N	(Peterson, 1991)
Sailfin Mollie	*Poecilia latpinna*	Y	Y	Y	(Nordlie, *et al.,*1992)
Sheepshead Minnow	*Cyprinodon variegat us*	Y	Y	Y	(Bennett *et al.*, n.d.)
Spotted Gar	*Lepisosteus oculatus*	Y	Y	N	(Carlander, 1969)
Striped Mullet	*Mugil cephalus*	Y	Y	Y	(Riede, 2004)
Threadfin Shad	*Dorosoma petenense*	Y	Y	Y	(Whitehead, 1985)
Unknown Goby	-	-	-	-	-
Unknown Sunfish	*Lepomis sp.*	Y*	N*	N*	-

*Assumed based on genus characteristics

We collected a total of 1,637 individuals in the three locations within Big Mar (Table A.2.). We collected the most individuals in Center Big Mar (n = 709) over 15 transects. We collected 565 individuals in Bayou Bonjour over 15 transects. We collected 363 individuals in SW Big Mar over 7 transects. The most overall abundant species was mosquitofish, with 753 total individuals. Mosquitofish were the most abundant species encountered in Bayou Bonjour and SW Big Mar (Figs. 6 & 7). Striped Mullet were the most abundant species in Center Big Mar.

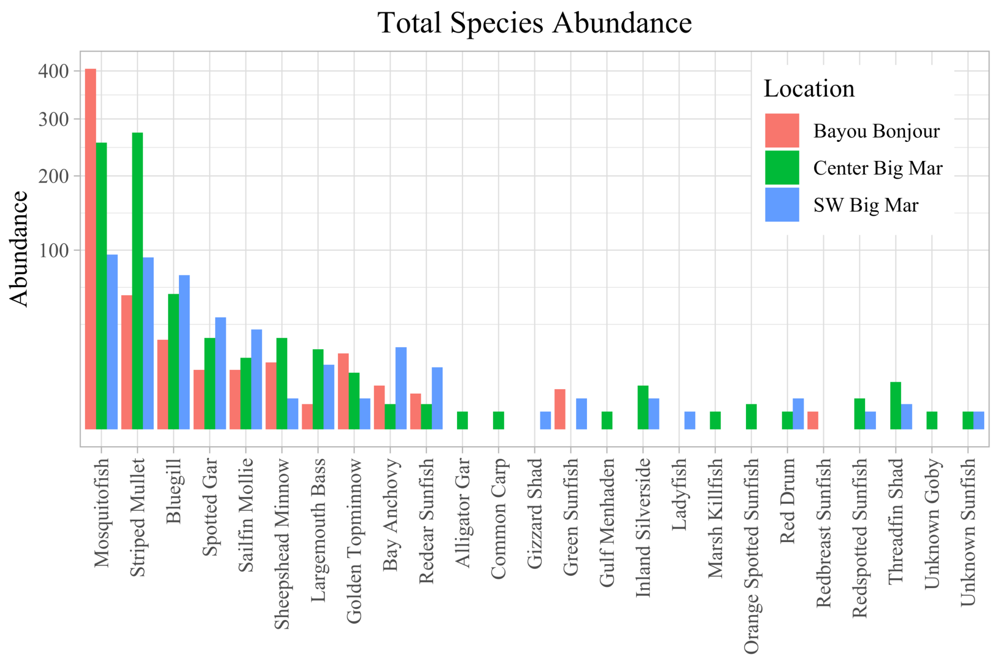

Figure 6. Total abundance of each species by location. Note: the y-axis are true abundances with a square root scale to emphasize the rare species.

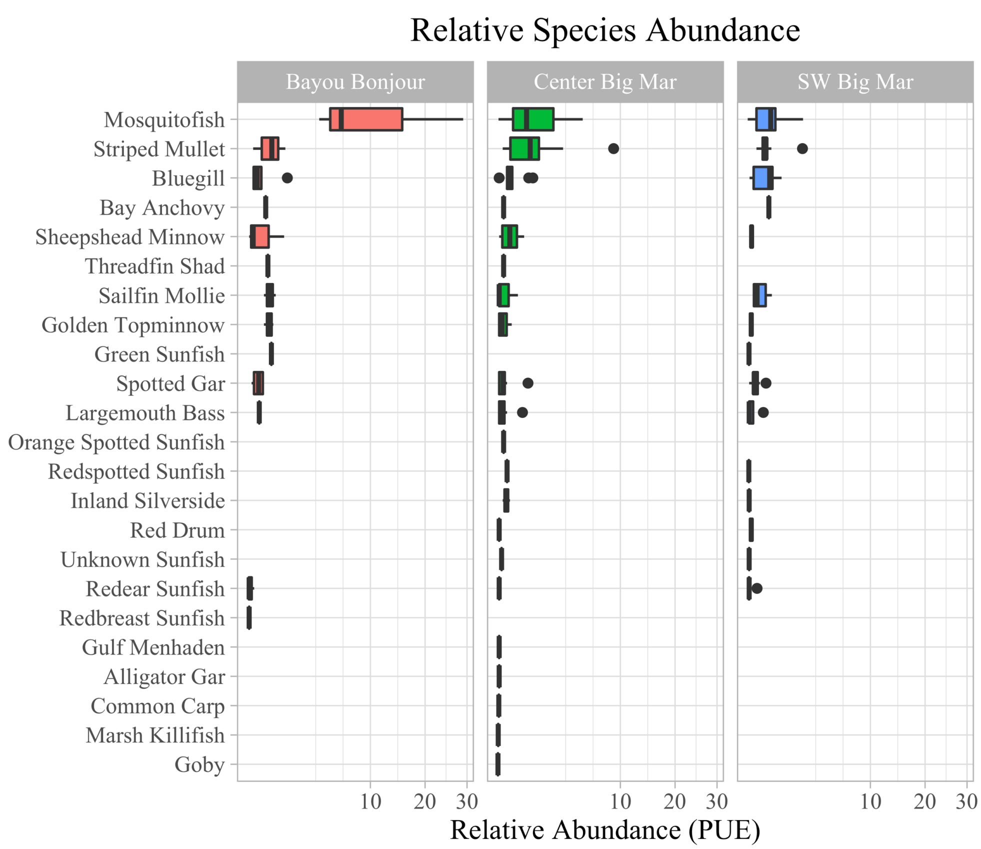

Figure 7. Relative species abundance (per unit effort) for each site and species. Note: the x-axis is on a square-root scale due to large range of value.

A species accumulation curve was calculated for each of the three locations, as well as for the combined Big Mar richness with all locations pooled together (Fig. 8). The curves for SW Big Mar and all locations pooled seem to be leveling off to an asymptote, indicating that nearly all species were encountered during samples. In contrast, the curves for Bayou Bonjour and Center Big Mar appear to still be increasing. This suggests that there are additional species in these locations that were not encountered during this sampling effort. This is further enumerated by comparing the various estimates of total species richness calculated using both abundance- and incidence-based methods (Table 2).

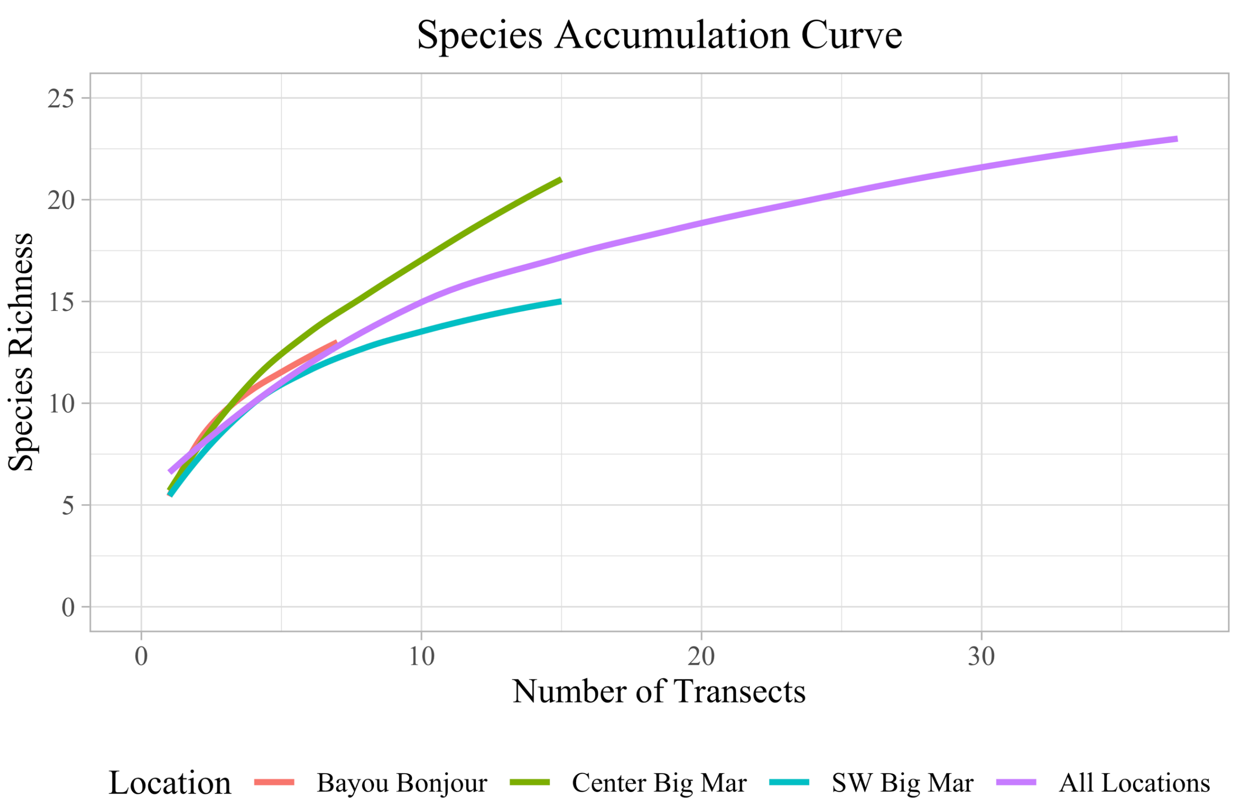

Figure 8. Species accumulation curve by each location and total species accumulation curve for all locations.

Table 2. Observed and estimated species richness for the three locations all locations pooled.

Location	Observed	Chao1	Chao2	ACE	ICE	Bootstrap	Jacknife
All (pooled)	22	32	31.17	25.11	25.87	25.5	32.98
Bayou Bonjour	13	13.5	25.5	13.25	15.17	20.14	14
Center Big Mar	21	27.125	51.25	24.63	25.54	25.66	30.96
SW Big Mar	15	17	16.12	15.48	15.71	22.28	17.98

3.2. Community Metrics

Relative abundance, species richness, and Pielou's evenness were compared across the three locations in Big Mar (Fig. 9). The relative abundance of individuals in samples from each of the three locations were not significantly different from each other (Kruskal-Wallis rank sum test; chi-squared = 4.186; DF = 2; p-value = 0.12). Relative abundance did not vary across the three locations in Big Mar. The number of species per sample, or species richness, of each transect was compared between the three locations. There was no difference in species richness observed between the locations (Kruskal-Wallis rank sum test; chi-squared = 0.66; DF = 2; p-value = 0.72). Pielou's evenness was compared between the three sites to determine effect of location. Evenness did not vary significantly across the three locations (ANOVA; F-value = 2.473; DF = 2; p-value = 0.1).

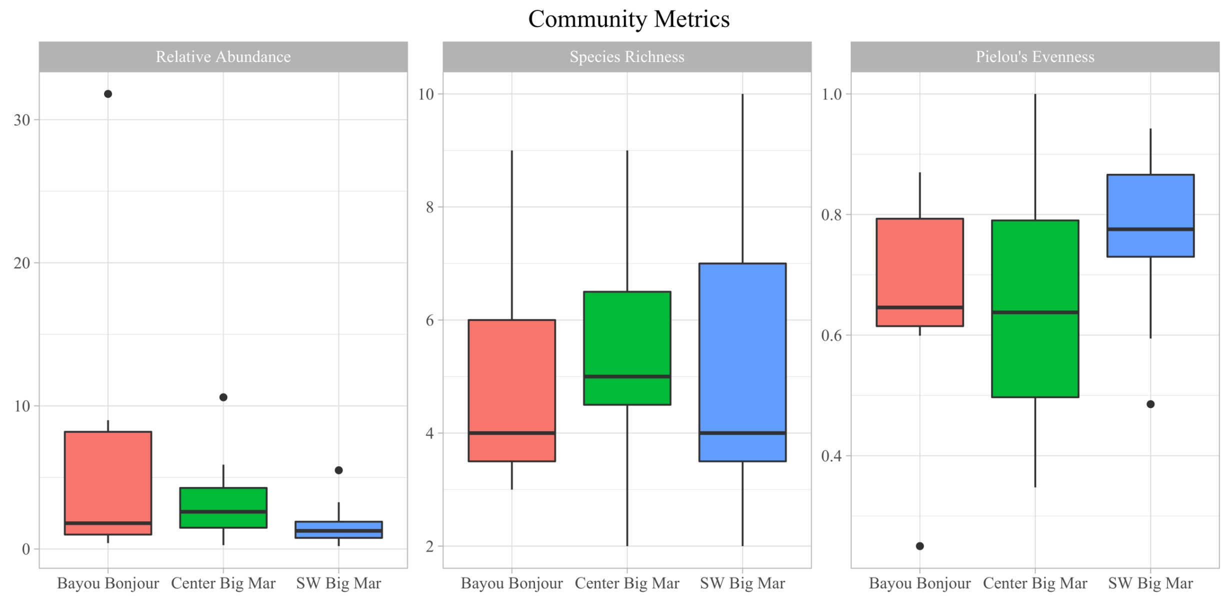

Figure 9. Relative abundance, species richness, and Pielou's evenness for each site.

Three diversity indices were used to determine effect of location on the community: the Shannon index, Simpson’s index, and inverse Simpson’s index (Fig. 10). The Shannon diversity index was not significantly different across the three locations (ANOVA; F-value = 0.716; DF = 2; p-value = 0.496). Simpson’s diversity also did not vary by location (ANOVA; F-value = 1.131; DF = 2; p-value = 0.335). Lastly, location did not affect the inverse Simpson’s diversity (Kruskal-Wallis rank sum test; chi-squared = 3.12; DF = 2; p-value = 0.21).

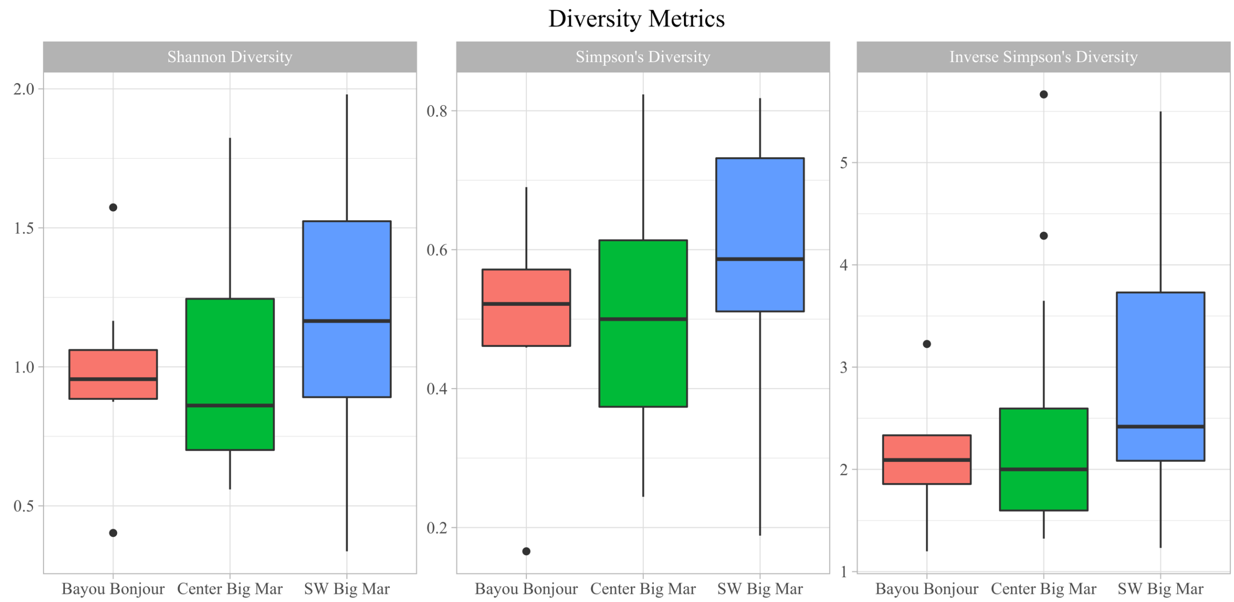

Figure 10. Diversity metrics compared between the three locations.

3.3. Beta Diversity (nMDS) and SIMPER

The two-dimensional nonmetric multidimensional scaling (NMDS) method was used to visualize beta diversity and patterns of community abundance within the three locations of Big Mar (Fig. 11). The stress value of the nMDS was 0.13, which is within an acceptable range of less than 0.2 (Kruskal 1964). The ellipses shown are 95% confidence intervals for each site. There is no apparent clustering of any location and the confidence interval ellipses overlap, indicating no difference between the communities that can be detected with the NMDS method. Further, a non-parametric analysis of similarity (ANOSIM) found no difference in the communities (significance = 0.12). The R value was 0.056, indicating high similarity between groups. A PermANOVA was used to assess differences in the centroids and dispersion of the three location clusters, which did not vary by the three locations (F.Model = 1.44; DF = 2; p-value = 0.14).

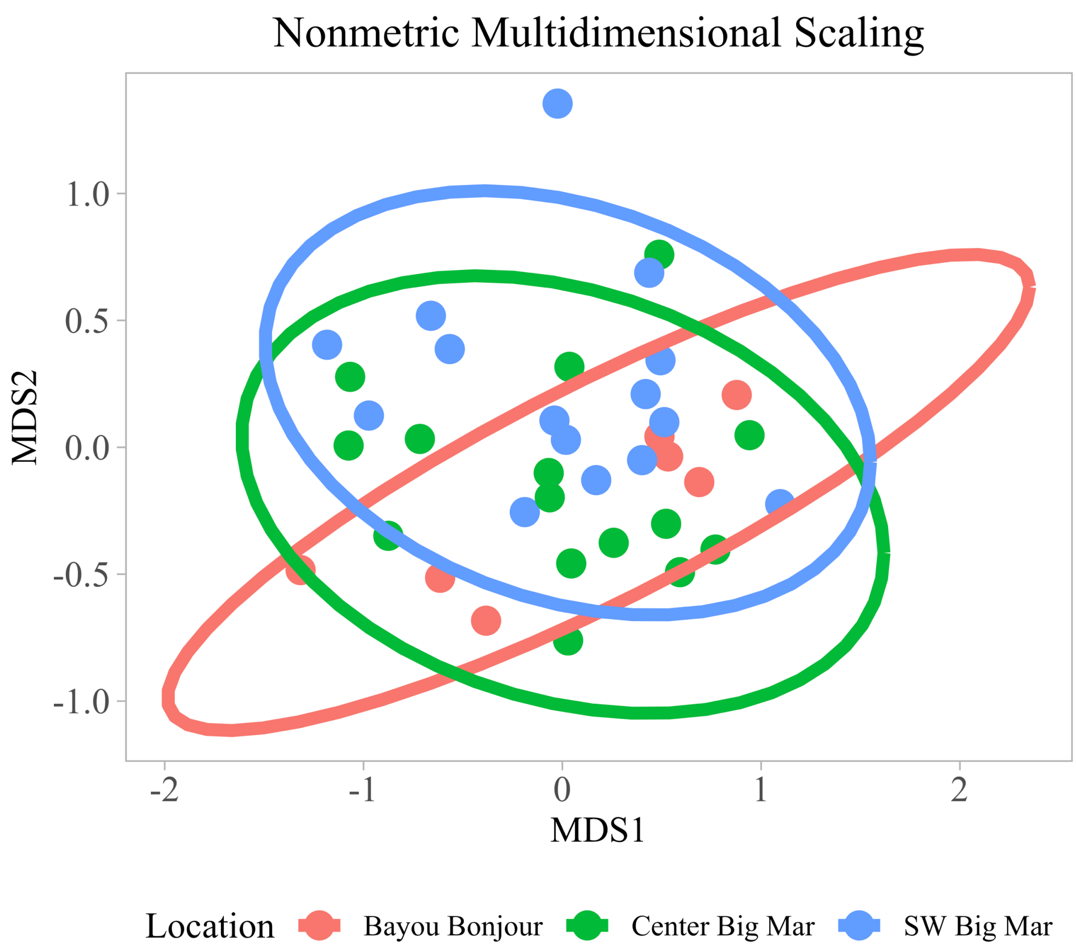

Figure 11. Two-dimensional nonmetric multidimensional scaling ordination of samples comparing the communities within the three locations. Each point is one transect. The ellipses represent 95% confidence intervals. (Stress = 0.13)

An analysis of similarity percentages (SIMPER) was used to identify the most influential species driving the similarity reported by the ANOSIM and PermANOVA. The SIMPER reported the cumulative contributions of each species to the overall similarity. As expected, the most abundant species contributed the largest amount to the pairwise similarities of the three locations (Table 3). Like the nMDS, ANOSIM, and PermANOVA, the SIMPER analysis was also applied to the square-root transformed data.

Table 3. Pairwise cumulative contributions of most influential species from SIMPER analysis.

Bayou Bonjour & Center Big Mar		**Bayou Bonjour & SW Big Mar**		**Center Big Mar & SW Big Mar**	
Species	**Cumulative Contribution**	**Species**	**Cumulative Contribution**	**Species**	**Cumulative Contribution**
Mosquitofish	0.29	Mosquitofish	0.28	Striped Mullet	0.21
Striped Mullet	0.44	Striped Mullet	0.42	Mosquitofish	0.41
Bluegill	0.53	Bluegill	0.54	Bluegill	0.53
Spotted Gar	0.61	Spotted Gar	0.62	Spotted Gar	0.61
Sheepshead Minnow	0.67	Sailfin Mollie	0.70	Sailfin Mollie	0.68
Golden Topminnow	0.73	Golden Topminnow	0.75	Largemouth Bass	0.74

3.4. Single Species Abundance

A single species approach was adopted for the five most abundant species: Bluegill, Mosquitofish, Sailfin Molly, Spotted Gar, and Striped Mullet. Relative abundance of these species was compared using the Kruskal-Wallis rank sum test (Fig. 12). Relative abundance did not vary by location for any of these species (Table 4).

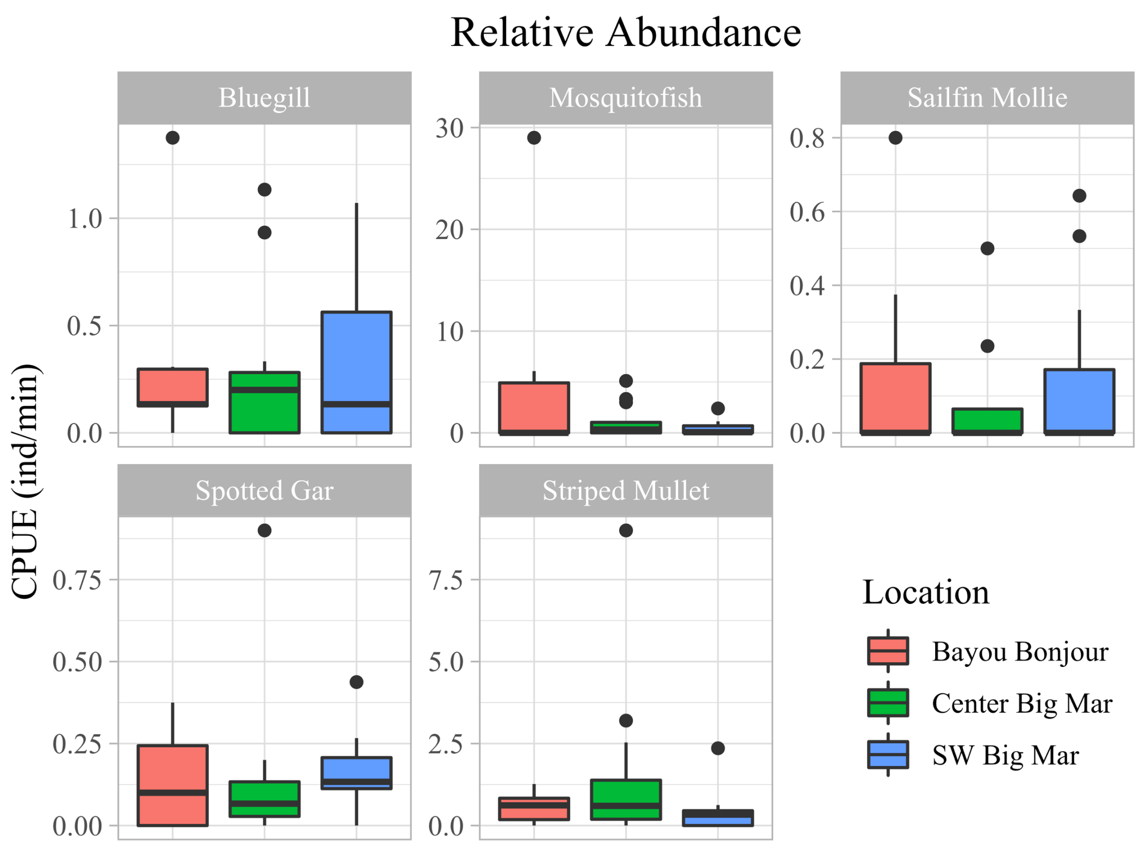

Figure 12. Comparison of relative abundance by location for five common species. No differences were significant.

Table 4. Kruskal-Wallis rank sum test p-values for the relative abundance of five common species. The relative abundance of each species did not vary by location.

Species	Chi-Squared	DF	P-value
Bluegill	0.30	2	0.86
Mosquitofish	0.60	2	0.74
Sailfin Mollie	1.07	2	0.59
Spotted Gar	2.88	2	0.24
Striped Mullet	3.07	2	0.22

3.5. Length Variation by Species

As there was no variation in community-level metrics and species abundance, within-species variation was compared between the three locations for the five most abundant species, Bluegill,

Mosquitofish, Sailfin Mollie, Spotted Gar, and Striped Mullet. First, the length distributions of individuals caught in each location were compared (Fig. 13).

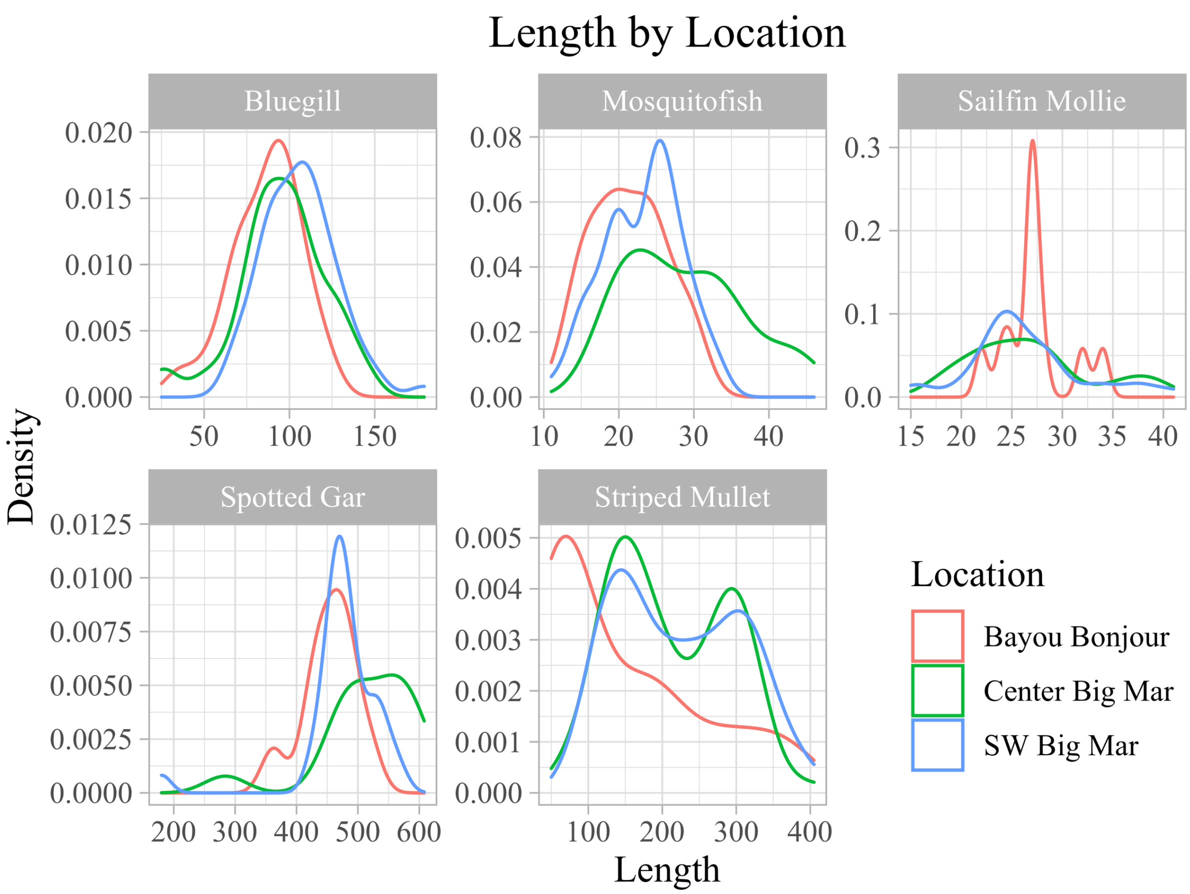

Figure 13. Density plot of the length distributions of the five most abundant species for each location.

Next, the total length of individuals for each of the five most abundant species were compared between the three locations using ANOVA or the Kruskal-Wallis rank sum test (Fig. 14). Lengths of all species except Sailfin Mollie varied significantly by location (Table 5).

Bluegill individuals were significantly larger in SW Big Mar than in both Center Big Mar and Bayou Bonjour according to a post-hoc Fisher-LSD. Striped Mullet were significantly smaller in Bayou Bonjour than in Center Big Mar and SW Big Mar according to a post-hoc Dunn test. The largest Mosquitofish and Spotted Gar were caught in Center Big Mar, and were significantly larger than individuals caught in both Bayou Bonjour and SW Big Mar (Table 6).

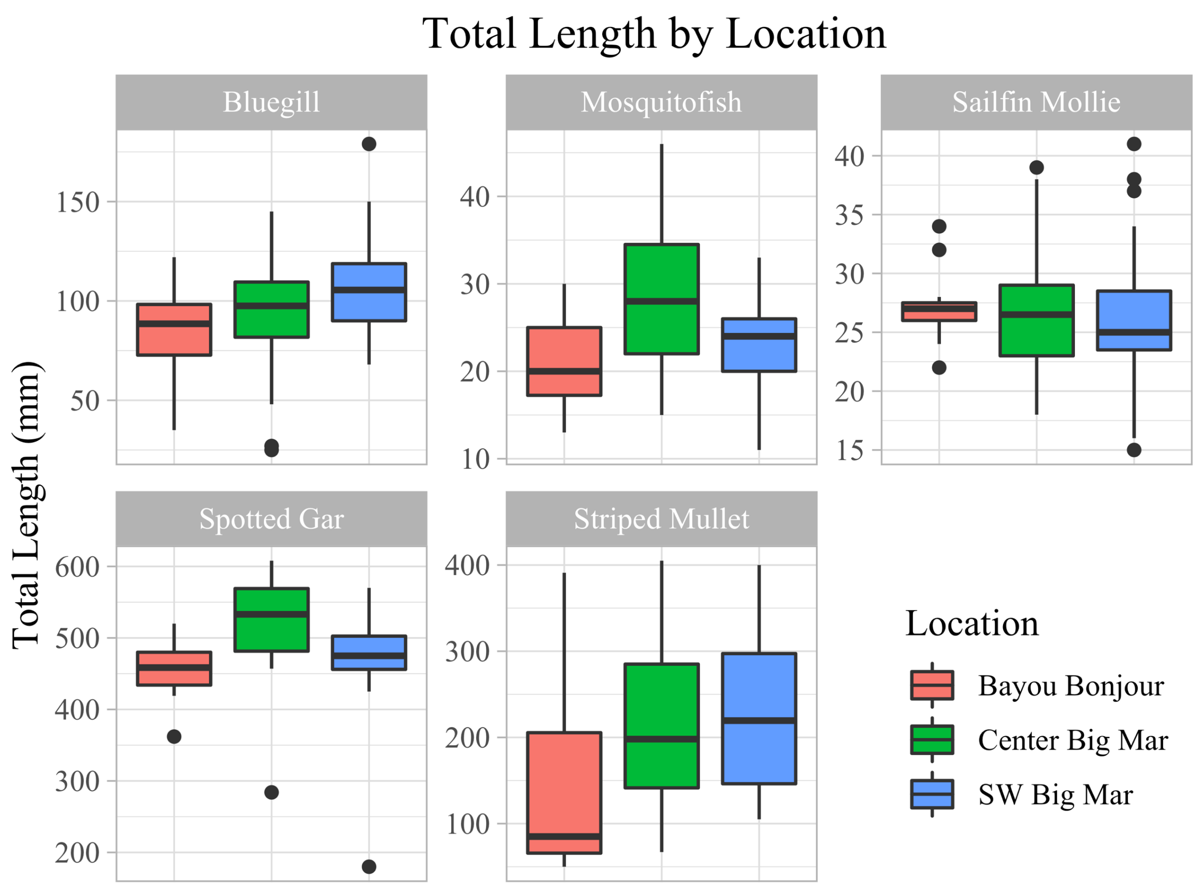

Figure 14. Length comparison of the five most abundant species for each location.

Table 5. Statistical analysis used to compare the lengths of individuals in the three locations, as well as the test statistic (F-value for ANOVA and chi-squared for Kruskal-Wallis), the degrees of freedom (DF) and the p-value.

Species	Statistical Test Used	Test Statistic	DF	P-value
Bluegill	ANOVA	6.911	2	0.001
Mosquitofish	Kruskal-Wallis	25.431	2	< 0.0001
Sailfin Mollie	Kruskal-Wallis	0.0705	2	0.703
Spotted Gar	Kruskal-Wallis	11.007	2	0.004
Striped Mullet	Kruskal-Wallis	21.906	2	< 0.0001

Table 6. Post-hoc Dunn test results for Mosquitofish, Spotted Gar, and Striped Mullet. P-values adjusted using the Holm method.

	Bayou Bonjour & Center Big Mar		Bayou Bonjour & SW Big Mar		Center Big Mar & SW Big Mar	
Species	**Z-Score**	**P-value (adj.)**	**Z-Score**	**P-value (adj.)**	**Z-Score**	**P-value (adj.)**
Mosquitofish	-4.54	< 0.0001	-1.47	0.14	3.69	0.0004
Spotted Gar	-3.123	0.0054	-1.27	0.203	2.58	0.020
Striped Mullet	-3.92	0.0001	-4.38	< 0.0001	-0.75	0.45

Chapter 4. Discussion

Based on visual differences in environmental conditions, I hypothesized that fish community structure would vary across the three locations in Big Mar. There was insufficient evidence from the data presented to support this hypothesis, as alpha and beta diversity were not significantly different in any of the locations. There were no observed differences in abundance, richness, evenness, and diversity based on the metrics calculated. The communities were dominated by a few species (Mosquitofish, Striped Mullet, and Bluegill), which appear to be ubiquitous throughout Big Mar. The abundance of these species vastly outnumbered the moderately abundant and rare species within the unique areas of the pond. This, as well as a relatively low sample size, prevented the rejection of the null hypotheses that the communities varied between the three locations based on the metrics presented above.

The nMDS analysis also showed no differences in communities. The ANOSIM resulted in an R-value of 0.053, showing that there is very low dissimilarity between the locations. Further, the PermANOVA, which is considered to be more robust than ANOSIM, resulted in an insignificant p-value, so there is no difference in the centroid and dispersion of the points for each location. The SIMPER results show that the similarity between the three locations is vastly influenced by the most abundant species. Increasing the sampling effort with a greater number of transects may increase the richness and abundance of rare species and make potential differences between the three sites apparent.

Based on the species accumulation curve (Fig. 8), nearly all fish species present in Big Mar (as a whole) were encountered in this sampling effort. There were 21 species positively identified, with one additional species that was not identified to species level (unknown Goby family). The unknown Sunfish were juveniles whose species could not be distinguished, but were likely one of

the sunfish species that were included in the study, or potentially a hybrid of two species (Birdsong and Yerger 1967). The highest estimation of total species richness was 33 species, and the lowest was 25 species, suggesting that with an increasing sampling effort, only an additional three to ten species could be detected.

Of the three locations, all estimation methods suggest that the highest species richness occurred in Center Big Mar. A comparison of the shape of the species accumulation curves, as well as the observed species richness with the estimated true richness, indicates that there could be the highest number of undetected species in Center Big Mar compared with the other two locations. In contrast, sampling in Bayou Bonjour had the least number of species captured, and our sampling likely detected nearly all the species present, though the estimations were comparable to SW Big Mar. There were no apparent differences in the abundance-based and the incidence-based estimation methods.

Differences in the fish community began to emerge when using a single-species approach. Though the relative abundance of the five most abundant species did not vary by location, the mean length of individuals from four species (Bluegill, Striped Mullet, Mosquitofish, and Spotted Gar) were significantly different between the three locations. The non-parametric Kruskal-Wallis rank sum test was used to determine differences in relative abundance of each species. This analysis is non-parametric and employs a ranking system to determine differences between groups, which is less powerful than using an ANOVA and may have contributed to the insignificant (p-value > 0.05) results of the effect of location on relative abundance. ANOVA was not used because the data were not normally distributed and therefore did not meet the assumptions.

Sailfin Mollie were the only species that did not have a significant effect of location on length. This could be attributed to the wide range of temperature, salinity, and dissolved oxygen

tolerances of the species, as Mollies are considered habitat generalists (e.g. Nordlie *et al.* 1992; Timmerman and Chapman 2004; Fischer and Schlupp 2009). Males achieve sexual maturity at approximately 51 mm, and female maturity is attained at 48 mm. The maximum length individual encountered of this species was 41 mm, categorizing all individuals as either juveniles or sub-adults. It is possible that adult Sailfin Mollies inhabit Big Mar, but were not encountered in our study. Larval and neonate Mollies have exhibited a wider tolerance for shifts in salinity than adults, which may contribute to the distribution of the species in close proximity to the Caernarvon freshwater diversion (Bachman and Rand 2008).

Though their length distributions varied, Bluegill and Striped Mullet lengths varied by location with a similar pattern. Both species had the smallest individuals in Bayou Bonjour and largest individuals in SW Big Mar. Length of fish seems to increase with depth of water available and distance from the freshwater diversion at the three locations for these species. Juvenile Bluegill have been shown to prefer calm water with lateral vegetation cover in laboratory experiments, which is similar to the type of habitat in Bayou Bonjour. Additionally, juvenile Bluegill actively avoid areas with adult Bluegill, which may affect the distribution of the individuals in Big Mar (Casterlin and Reynolds 1978). Additionally, juvenile Bluegill have been shown to alter their habitat preference and foraging rate in the presence of a predator which may have affected distribution within Big Mar (Gotceitas and Colgan 1990). In this study, predators of Bluegill may include Largemouth Bass, Spotted Gar, and Red Drum. These piscivorous fish were mainly encountered in Center Big Mar and SW Big Mar, which may have contributed to the use of Bayou Bonjour as a refuge for juvenile Bluegill.

Like Sailfin Mollies, Striped Mullet are considered habitat generalists and can withstand wide varieties of environmental conditions (Collins 1985). In Louisiana, Striped Mullet are

caught for their roe and for use as live bait. They are the only commercially relevant species frequently encountered in Big Mar during sampling, although Mullet caught in saltwater are more desired than those in freshwater. Eggold and Motta (1992) found evidence of an ontogenetic dietary shift in Striped Mullet that is consistent with the observed patterns in Big Mar. They found that smaller individuals of similar length to those caught in Bayou Bonjour primarily fed on surface film. Larger individuals of similar length to those caught in Center Big Mar and SW Big Mar were benthic grazers, which dig into the sediment to feed (Eggold and Motta 1992). This study did not find evidence of an ontogenetic habitat shift, which may occur in Big Mar, as the individuals in Bayou Bonjour were primarily juveniles (TL < 75 mm) and those in Center Big Mar and SW Big Mar appear to represent two distinct age classes, potentially age one (TL ~150 mm) and age two to three (TL > 250 mm) (Broadhead 1956). No Mullet smaller than 50 mm TL were encountered, which is consistent with evidence that larval Mullet (< 40 mm SL) cannot tolerate freshwater (Collins 1985). Length at maturity for the species has been estimated at 200 to 300 mm standard length (SL), indicating that Big Mar could provide suitable habitat for juvenile, sub-adult, and mature adult Striped Mullet.

Instead of increasing with distance from the diversion like Mullet and Bluegill, Mosquitofish and Spotted Gar were longest in the intermediate habitat, Center Big Mar. There was no difference in length of individuals caught in Bayou Bonjour and SW Big Mar for both Mosquitofish and Gar. In laboratory experiments, Mosquitofish have expressed active habitat preference. They preferred calm water with submerged vegetation and avoided floating cover, which may limit access to the surface (Casterlin and Reynolds 1977). Center Big Mar likely provides these habitat preferences for mature Mosquitofish. Bayou Bonjour had submerged vegetation, but also had emergent vegetation that may have provided surface cover that discouraged Mosquitofish habitat

use. The water likely flows too quickly in SW Big Mar for Mosquitofish, and larger individuals are able to move to a different area to avoid turbulence.

Spotted Gar are important predators in freshwater systems such as Big Mar, and have very few predators of their own (Snedden *et al.* 1999). Therefore, their distribution may be driven by prey densities within Big Mar. Spotted Gar have been shown to avoid turbulent areas in favor of calm, shallow habitats with submerged vegetation including conditions similar to those in Bayou Bonjour and Center Big Mar. Spotted Gar may avoid turbidity in SW Big Mar and are likely too large to navigate the narrow channels and emergent vegetation in Bayou Bonjour. When they are large enough to hunt and eat fish, Spotted Gar first prey upon Mosquitofish and small Sunfish, which are abundant within Bayou Bonjour, where habitat complexity does not affect Spotted Gar predatory behavior (Redmond 1964; James and Heck 1994). Additionally, few individuals caught in Big Mar were less than 350 mm in length, and nearly all can be considered mature (mean = 482.5 mm TL; standard error = 9.18; N = 56) based on age at maturity estimations from other areas of the Lake Pontchartrain estuary with similar environmental conditions (Love 2004). Only two Spotted Gar encountered were likely juveniles based on their lengths (284 mm in Center Big Mar and 180 mm in SW Big Mar). There is little evidence that Big Mar functions as a nursery habitat for Spotted Gar, though a greater sampling effort could encounter a greater number of juveniles.

All species but Sailfin Mollie had the smallest individuals in Bayou Bonjour. This provides evidence that Bayou Bonjour may be an important nursery habitat for several species of fish in Big Mar and the surrounding wetlands. Shallow waters of vegetated marshes provide refugia from predators for juvenile fishes during this critical life history stage (Boesch and Turner 1984). In an experimental pond study, Werner et al. (1983) found that in the presence of a predator (Largemouth Bass), juvenile Bluegill selected vegetated habitats, even though those sites were less profitable than

other available habitats. This behavior presumably occurred because the harborage protected the bluegill from predation. Mature bluegill sunfish had higher growth rate in non-vegetated, open water habitats, likely attributed to reduced competition with juvenile sunfish (Werner et al. 1983).

The five most abundant species are considered generalist species with a wide range of tolerances, particularly to low oxygen, or hypoxic, environments. The majority of the dissolved oxygen measurements in Big Mar were below the 2 mg/L threshold, indicating that many of the areas were experiencing hypoxia when I sampled (See Table A.1.). All of the most abundant species encountered (except Bluegill) have adaptations that allow them to thrive in these conditions. Mosquitofish have physiological traits that allow them to thrive in increased heat and decreased dissolved oxygen conditions (Stoffels *et al*. 2017). Sailfin Mollies have a superior mouth shape, allowing them to ventilate their gills with water from the air-water interface with a higher concentration of dissolved oxygen (Kramer and Mehegan 1981). Further, Striped Mullet are thought to jump out of the water to aerate their gills to cope with low dissolved oxygen concentrations, though most of the evidence is circumstantial (Hoese 1985). Lastly, Spotted Gar are bimodal breathers, meaning that they can gulp air from the surface into their swim bladder for gas exchange underwater, as well as exchanging oxygen from the water through their gills (Rimoldi *et al*. 2016).

Bluegill have shown an intermediate tolerance for hypoxia compared to other species of sunfish, but they have been stocked in Big Mar by the Louisiana Department of Wildlife and Fisheries, which may have contributed to its status as a common species (Farwell *et al.* 2007; LDWF report). Further, the hypoxia we measured might be temporary, in which case the dissolved oxygen concentration may increase before Bluegill populations are affected. Largemouth Bass, Channel Catfish, and Common Carp were also stocked, but they were not frequently encountered in Big Mar, likely due to their size not being sensitive to the electrofishing equipment we used.

Largemouth Bass have the lowest hypoxia tolerance of several species of sunfish (Farwell *et al.* 2007). As Big Mar can be considered a disturbed system, tolerant species are more likely to survive changing conditions and outcompete other species for resources, potentially hindering rare species from becoming common and abundant.

A study of estuarine fish throughout the Biloxi Marsh Complex described the species present in Lake Lery, which is near Big Mar within the Breton Sound Estuary, during the 1960s (Fontenot *et al.* 1970). Lake Lery is closer to the Gulf of Mexico with greater saltwater influence, making it a more brackish habitat. Many of the freshwater affinity species from this study were found in Big Mar, indicating that they were present in the system before the diversion was constructed. There were also several brackish and saltwater affinity species present in this study that were not found in Big Mar during the sampling efforts of this study. This suggests that the Caernarvon freshwater diversion affected the distribution of the species within the estuary, but likely did not change the species composition. The fish community was likely not negatively or positively affected by the diversion, but simply, it is different within Big Mar.

As the Caernarvon diversion continues to deliver freshwater, sediment, and nutrients from the Mississippi River, open-water habitats like SW Big Mar may be converted to shallow, slow-moving bayous, marshes and swamps, and narrow creeks with both emergent and submerged vegetation to provide refugia for small fish. I observed the submerged aquatic vegetation trapping sediment which will accelerate the accretion process. This habitat change from open water to land will increase the habitat available for fish species and support a more diverse fish community than if it were to remain an open water habitat. Further, open water channels will likely continue to be filled in as sediment arrives from the diversion. This will limit the access of larger species to Big Mar, and the habitat will likely match that of Bayou Bonjour and Center Big Mar throughout the

entire pond. This will hopefully enhance the nursery function of Big Mar, which may contribute to more productive commercial and recreational fisheries in other areas of Breton Sound Estuary.

Additionally, saltwater intrusion is a pressing issue in many previously freshwater marshes that are impounded by the levee system and no longer receiving water from the Mississippi River. Big Mar was especially affected by saltwater intrusion after Hurricanes Katrina and Rita in 2005, as storm surge pushed saltwater high into the freshwater marshes and wetlands (Kaintz, 2010; Steyer *et al.* 2007). In fact, low-salinity wetlands saw greater land loss than high-salinity wetlands during Hurricane Katrina (Howes *et al.* 2010). Further, significant sedimentation and vegetation growth did not occur in Big Mar until after the 2005 hurricane season (Fig. 1).

In order to save Louisiana's vanishing wetlands, $2.2 billion of settlement fees from BP and Transocean following the Deepwater Horizon Oil drilling disaster are going to be used to fund two proposed sediment diversion projects: the Mid-Barataria diversion and the Mid-Breton diversion, located on opposite banks of the Mississippi River in Plaquemines Parish (Coastal Protection and Restoration Authority of Louisiana, 2017). These projects are a significant focus of the 2017 Coastal Master Plan's $50 billion budget to restore and protect coastal marshes and wetlands. There is little known about how these will affect the inshore ecological communities as the wetlands are reconnected with the Mississippi River. It is expected that the inshore brackish habitats with moderate salinities will be converted to freshwater, which would support a fish community similar to that in Big Mar. Additionally, sediment deposition will hopefully create new land, turning open water areas into swamps (with trees), and fresh water marshes, and subsequently creating more productive fish habitat.

This study had many limitations. Weather and access to an airboat limited the number of sampling days we were able to complete. The initial study design included three days per season in

May, July, and October, for a total of nine sampling days, instead of the six sampling days completed. With an increased sampling effort in all areas, particularly Bayou Bonjour, the differences in the fish communities may have been perceived. Additionally, this study would have been able to address seasonal variances in the fish communities, but with only five electrofishing transects completed in October because of inclement weather, any comparisons would have lacked the statistical power to reliably address hypotheses. Big Mar is a dynamic system; it is frequently disturbed by storms and subject to variation in temperature (due to its shallow depth) and freshwater input from the diversion. The close proximity to the Mississippi River makes Big Mar susceptible to pollution carried from the entire watershed, particularly pesticides. These data provide a narrow, but valuable perspective of the community, and a sampling effort of greater spatial and temporal scope is necessary to fully describe the fish community and how it may change over time.

The lack of environmental and vegetation data also limited the ability to address the variety of conditions in the three locations. There were several issues with equipment so we didn't trust all the measurements I was able to obtain and some instances where the equipment failed completely. With consistent temperature, salinity, and dissolved oxygen measurements, the fish community could have been compared with the changes in environmental factors that shape the distributions within Big Mar. In the future, multi-variate statistical models can be developed with a more reliable environmental condition dataset, making this study more robust with quantitative conclusions about the relationships between the fish community and the environmental gradient created by the Caernarvon diversion.

Lastly, electrofishing is a suitable sampling method for certain areas of Big Mar, such as Bayou Bonjour and Center Big Mar, but it comes with its own limitations. Electrofishing has a low mortality rate, when employed correctly, and it is a non-destructive fishing method. It can be used

in shallow, slow-moving water that is not suitable for certain sampling methods, such as trawling, seining, or deploying gill nets, as most of Big Mar is only accessible by air boat. The sediment is silty and soft, limiting the options for deploying fishing gear by wading in the water. In SW Big Mar, the electric field created by the model of electrofisher I used to attract and stun fish may not reach to the bottom of the water column. This may exclude demersal species from the collection. Further, the power capabilities of the electrofisher are limited, and the largest fish in the community may not be affected. In order to catch larger fish, the electrofisher's power would have had to be increased, which would have increased the mortality of the smaller fish in Big Mar. Even at the highest power possible of the barge fisher I used, some large fish were not stunned. For example, when attempting to catch Red Drum in SW Big Mar, individuals would recover from the electric shock very quickly, if they were affected at all. Some individuals, like large Spotted Gar, would simply swim away from the electrofisher instead of being stunned like the smaller fish. This introduces a potential bias to the sampling method, and likely limited the individuals and species we were able to sample in Big Mar. To account for this, we attempted to deploy gill nets to capture the larger individuals in SW Big Mar, but it proved nearly impossible to do so from an airboat. I deployed a gill net in SW Big Mar and caught Red Drum, Striped Mullet and Common Carp. However, those fishes were not included in this study as we only deployed the nets twice and in one location.

More work is needed to answer key questions about how the Caernarvon freshwater diversion affects fish communities in Big Mar, as well as throughout the Lake Ponchartrain Basin. This study served as the preliminary guide of the various fish species and their relative abundances, but was limited in its ability to make strong conclusions. Future projects should focus on increasing the sampling effort, both spatially and temporally, to improve statistical power. Though it is likely

all of the species in Big Mar were encountered, the species accumulation curve for each of the three locations indicate that there are rare species that have not been accounted for (Fig. 8). Additionally, environmental conditions and water quality data should be collected to attribute any variances in the fish communities to the abiotic factors that affect fish biology and physiology in Big Mar, especially as conditions change over time. Vegetation data would also aid in future studies to correlate fish occurrences with habitat type. A variety of fishing methods should be employed to reduce bias and ensure that the sampling effort captures as much of the true biological community as possible.

Additionally, as there were no differences in the fish communities in the three locations, it was necessary to adopt a single-species approach to determine how the three habitat types may be supporting different size classes of fish. A traditional approach was not sufficient to describe differences in community, though a greater sampling effort may reveal community-level variation. The communities were dominated by a few species, but the evidence in this study suggests that there may be an ontogenetic shift in habitat for these species in Big Mar. Though fish were not categorized by age class during sampling, a comparison of the length distributions with the estimated length at maturity allowed speculation regarding the function of Bayou Bonjour as a nursery habitat for species of recreational and commercial value. Studying within-species variation in addition to traditional methods of investigating community ecology may be necessary to fully describe how freshwater diversions affect fish communities in adjacent wetlands.

In summary, freshwater marshes supported by river diversions appear to support a diverse community of fishes. Due to the small size and close proximity of Bayou Bonjour, Center Big Mar, and SW Big Mar, no differences were observed between locations at the community level. The fish community was dominated by a few ubiquitous species. A single-species analysis of the most

common species provided evidence of an ontogenetic shift in habitat within Big Mar Pond, as the locations supported different age classes of the species. This work will inform fishers and policymakers on the impacts of the Mississippi River diversions and siphons on fish communities, especially as new diversion projects are proposed to restore eroding coastal wetlands and marshes. More work is needed to understand how disturbance over time affects the fish community within Big Mar and other wetlands within the upper Breton Sound estuary.

Appendix. Data

Table A.1. Date and location of each transect included in the analysis with the associated environmental data.

Date	Location	Conductivity (µS)	Temperature (°C)	Dissolved Oxygen (mg/L)	Salinity (ppt)
5/7/19	Bayou Bonjour	371.5	20.1	4.5	0.2
5/7/19	Bayou Bonjour	371.5	20.1	4.5	0.2
5/7/19	Bayou Bonjour	348.3	-	-	-
5/7/19	Bayou Bonjour	341	30	9.5	-
5/8/19	Center Big Mar	369	26	1.75	0
5/8/19	Center Big Mar	358.3	-	2.69	-
5/8/19	Center Big Mar	-	-	-	-
5/8/19	Center Big Mar	-	-	-	-
5/8/19	Center Big Mar	365	27.4	17.95	0.2
5/8/19	Center Big Mar	-	-	-	-
5/8/19	Center Big Mar	-	-	-	-
5/9/19	SW Big Mar	475	24.7	2.2	0.2
5/9/19	SW Big Mar	-	-	-	-
5/9/19	SW Big Mar	436.5	25.2	1.2	0.2
5/9/19	SW Big Mar	-	-	-	-
5/9/19	SW Big Mar	-	-	-	-
7/29/19	SW Big Mar	454.2	27.7	0.49	0.2
7/29/19	SW Big Mar	-	27.1	1.55	0.2
7/29/19	SW Big Mar	485	27.1	1.43	0.2
7/29/19	SW Big Mar	-	29.6	1.72	0.2
7/29/19	SW Big Mar	-	30.1	2.07	0.2
7/29/19	SW Big Mar	497	31.6	2.47	0.2
7/29/19	SW Big Mar	-	30.9	1.46	0.2
7/30/19	Center Big Mar	542	29.4	0.52	0.3
7/30/19	Center Big Mar	-	28.5	0.82	0.3
7/30/19	Center Big Mar	-	27.5	1.36	-
7/30/19	Center Big Mar	359	29.6	1.65	0.2
7/30/19	Center Big Mar	-	30.3	1.84	0.2
7/30/19	Center Big Mar	-	30.4	1.96	0.2
7/30/19	Center Big Mar	529	30	1.57	0.3
7/30/19	Bayou Bonjour	421	28.8	1.72	0.2
7/30/19	Bayou Bonjour	-	27.3	1.75	-
10/10/19	SW Big Mar	-	28.3	5.1	-
10/10/19	SW Big Mar	-	-	-	-
10/10/19	SW Big Mar	-	-	-	-
10/10/19	Center Big Mar	-	-	-	-
10/10/19	Bayou Bonjour	-	-	-	-

Table A.2. Total number of individuals for each species with relative abundance, mean CPUE with associated standard deviation (SD) for each site, as well as a grand total caught of each species. There were 7 transects in Bayou Bonjour, 15 transects in Center Big Mar, and 15 transects in SW Big Mar.

	Bayou Bonjour			**Center Big Mar**			**SW Big Mar**			
Common Name	**Total Caught**	**Mean CPUE**	**SD**	**Total Caught**	**Mean CPUE**	**SD**	**Total Caught**	**Mean CPUE**	**SD**	**Grand Total Caught**
Alligator Gar	0	0	0	1	0.004	0.017	0	0	0	1
Bay Anchovy	6	0.061	0.162	2	0.009	0.034	8	0.036	0.138	16
Bluegill	25	0.336	0.47	57	0.258	0.339	70	0.301	0.387	152
Common Carp	0	0	0	1	0.004	0.016	0	0	0	1
Golden Topminnow	18	0.235	0.308	10	0.04	0.087	3	0.013	0.037	31
Green Sunfish	5	0.089	0.236	0	0	0	2	0.009	0.023	7
Gulf Menhaden	0	0	0	1	0.004	0.017	0	0	0	1
Inland Silverside	0	0	0	6	0.026	0.073	3	0.013	0.028	9
Largemouth Bass	2	0.036	0.094	20	0.092	0.17	10	0.045	0.095	32
Marsh Killifish	0	0	0	1	0.004	0.014	0	0	0	1
Mosquitofish	405	5.546	10.619	256	1.008	1.555	92	0.414	0.663	753
Orange Spotted Sunfish	0	0	0	2	0.009	0.034	0	0	0	2
Red Drum	0	0	0	1	0.004	0.017	3	0.013	0.037	4
Redbreast Sunfish	1	0.01	0.025	0	0	0	0	0	0	1
Redear Sunfish	4	0.038	0.055	2	0.009	0.023	10	0.044	0.054	16
Redspotted Sunfish	0	0	0	3	0.013	0.052	1	0.004	0.016	4
Sailfin Mollie	11	0.168	0.312	16	0.062	0.136	31	0.141	0.208	58
Sheepshead Minnow	14	0.199	0.444	26	0.109	0.217	3	0.014	0.038	43
Spotted Gar	11	0.137	0.16	26	0.133	0.222	37	0.16	0.11	74
Striped Mullet	56	0.558	0.468	274	1.43	2.3	89	0.391	0.585	419
Threadfin Shad	7	0.071	0.189	2	0.009	0.034	0	0	0	9
Unknown Goby	0	0	0	1	0.004	0.014	0	0	0	1
Unknown Sunfish	0	0	0	1	0.007	0.026	1	0.004	0.017	2
Sums	565	-	-	709	-	-	363	-	-	1637

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Vita

Rachel Snider was born and raised in Charlotte, North Carolina. She is a member of the 2014 graduating class of Weddington High School. She attended the University of North Carolina at Chapel Hill where she graduated in 2018 with Highest Honors, earning the degrees of Bachelor of Science in Environmental Sciences and Bachelor of Arts in Biology. Her undergraduate thesis was titled, "A Fish Eat Fish World: Trophic Structure of the Fish Communities on Artificial and Natural Reefs in Onslow Bay, NC." After graduating from Louisiana State University, she plans to work in science communication to connect scientists, policymakers, stakeholders, and constituents during the coastal restoration decision-making process. Outside of the classroom and laboratory, Rachel is an avid surfer, SCUBA diver, dog mom, and hiking enthusiast.