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MONITORING THE EFFECTS OF POULTRY WASTE ON FISHES AND MACROINVERTEBRATES IN THE SABINE RIVER

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MONITORING THE EFFECTS OF POULTRY WASTE
ON FISHES AND MACROINVERTEBRATES IN THE SABINE RIVER

by

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Freshwater is a vital resource that provides life and sustainability for almost all organisms on Earth. It is important to maintain its health and protect it from emerging pollutants that pose a threat to the organisms that use it. Pollution continues to threaten the well-being of the environment's freshwater sources all around the world that could lead to damaging effects in the future. The Sabine River is a major freshwater resource in the east Texas and western Louisiana areas that provides a habitat for thousands of organisms as well as other domestic uses for humans. In 2019, a waste discharge pipe was placed underwater by Sanderson Farms chicken factory dispels minimally treated chicken remains into the Sabine River near Hawkins, TX (Smith Co). A study was conducted by collecting fish and macroinvertebrate species at various dates and at upstream and downstream locations from the pipe to determine if the placement of the pipe was influencing the biodiversity of the organisms inhabiting the river as well as the health of the organisms over time. Many statistical analyses were performed including an IBI, B-IBI, habitat quality index, Shannon's diversity index, Hilsenhoff biotic index, EPT index, and NMDS Plot where it was found that there was a significant difference in samples over time but not by site. There are many factors that could have accounted for this change such as drought patterns, geological differences in the

substrate, and hydrological data over the years, so it is inconclusive whether the difference can be attributed to the placement of the pipe or some other factor. More data will be collected in later studies to examine any potential changes in health or diversity in the Sabine River near Hawkins, TX.

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CHAPTER 1

HISTORY OF THE SABINE RIVER

The Sabine River has a great historical significance in the development of land, animals, and people across eastern Texas piney woods and western Louisiana (Long, 2017). It is known to have the second largest watershed of any river basin in Texas because of the high precipitation and low evaporation rates of the East Texas region (Texas Water Development Board, https://www.twdb.texas.gov). The river begins its headwaters in Hunt County and ends at Sabine Lake where it eventually drains into the Gulf of Mexico (Texas Water Development Board, https://www.twdb.texas.gov). Archaeological evidence has shown human inhabitance in the Sabine River basin spanning across all stages of southeastern Native American development prior to 780 A.D. with the Caddos being the predominant tribe in the area (Long, 2017). Much of the historical significance of the Sabine River comes from geological data that is available (Conner and Suttkus, 1986). It is part of the coastal plains region of Texas which is characterized by its inner zone of erosional hills or slopes and outer zones of depositional plains (Conner and Suttkus, 1986). Tertiary and Cretaceous formations have developed a series of lowlands on weaker rocks and a formation known as “wolds” on the stronger rocks (Conner and Suttkus, 1986). Bernard and Leblanc (1965) described the region to have five terrace levels with four stemming from the Pleistocene era and one from more recent occurrence (Conner and Suttkus, 1986). These terraces tend to decrease in altitude and age as they get closer to the sea (Conner and Suttkus, 1986). These most likely occurred due to changes in glacial and interglacial periods (Conner and Suttkus, 1986). The soil makeup of the Sabine in the coastal plains region has also been known to include clays, sands, and soft limestones (Conner and Suttkus, 1986).

In the early days of the Republic of Texas, the Sabine River acted as a transportation route for lumber and cotton from southeast Texas (Long, 2017). Once the rafts would reach the Sabine River, their cargo was transferred to larger ships to be transported to New Orleans, Galveston, and other ports, which eventually lead to the boom of Port Arthur and Orange (Long, 2017). The first steamboats came around in the mid 1840s and stayed in business up until around the 1900s where they were eventually replaced by railroad transportation (Long, 2017). The first steamboat that traveled up the Sabine was in the fall of 1843 captained by John Clemmons (Texas Water Development Board, https://www.twdb.texas.gov). During the nineteenth and twentieth century, the middle of the Sabine River was a major logging operation with many sawmills built along the banks of the river as well as the connecting tributaries (Long, 2017). The downstream portion of the river became a large proponent in crop irrigation as well as a major site of a large-scale oil exploration which unfortunately, quickly lead to increasing amounts of pollutants in the water (Long, 2017). Oil refineries and chemical plants discharged copious amounts of ammonia, phenol, sulfides, zinc, lead, and other chemicals into the Sabine River (Long, 2017). In more recent years, there have been efforts to clean up the polluted areas of the stream, but the pollution continued in 1990 by increased waste discharge by neighboring factories and facilities (Long, 2017). To this day, the Sabine River basin contributes to the health and well-being of many large cities such Marshall, Orange, Port Arthur, and Longview, which is reported to be the largest city on the basin with a population of 81,762 according to the 2021 census (Texas Water Development Board, https://www.twdb.texas.gov).

CHAPTER TWO

INTRODUCTION

Freshwater systems provide the foundation for life such as energy, food, and health (Arya, 2021). They allow for the growth and development of humans as well as provide crucial regulatory services for the environment like water purification, flood mitigation, and waste treatment (Darwall et al., 2018). Rivers and streams are the veins, arteries, and capillaries of the world that allow for all the terrestrial environmental systems of earth to occur and the transportation of vital resources (Arya, 2021). Being such an incomparable resource regarding the sustainability of life, freshwater systems are important to maintain and preserve for the organisms that inhabit them as well as the organisms that must utilize them for survival.

The health of a freshwater system is largely dependent on the surrounding ecosystem and the organisms that inhabit the area (Darwall et al., 2018; Arya 2021). Specifically, freshwater systems have a riparian buffer zone that surrounds the perimeter with vegetation and terrestrial elements that can interact with the organisms of the water to either help or harm the system (Angeler et al., 2014). Organisms inhabiting the river mainly consist of fish, aquatic macroinvertebrates, and mussels (Darwall et al., 2018). Fish come in all shapes and sizes depending on the size of the freshwater system as well as the resources available to them (Darwall et al., 2018; Schlosser 1995). A freshwater system with a high flow rate will attract certain fish while a low flow rate could allow for a different species to be present (Worthington et al., 2014; Darwall et al. 2018). Similarly, aquatic macroinvertebrates also inhabit many freshwater systems and often do much of the work to maintain the balance of the system (Luiza-Andrade et al., 2017). There are many different types of aquatic macroinvertebrates that fall into various functional feeding groups, such as, shredders, collectors, and grazers, along with many other types that work together to keep leaf litter and other potentially harmful substances out of the water (Vannote et al., 1980; Tierno de Figueroa et al., 2019). Many freshwater systems also are home to numerous freshwater mussel species that filter out toxins and harsh chemicals (Vaughn and Hakenkamp, 2001). Without the interactions between these aquatic organisms, freshwater systems would not be able to function in the capacity they do now, so it is important that they are preserved and protected along with the fish, aquatic macroinvertebrates, and mussels as they all have specific roles to play in the environment (Darwall et al., 2018).

River Continuum Concept, Functional Feeding Groups and Community Assemblages

The River Continuum Concept (RCC) describes the interactions between all the fish, macroinvertebrates, and mussels with the riparian buffer zones and vegetation to create a continuously integrated system throughout the entire length of the river that changes physically and chemically from the headwater stream to the mouth (Vannote et al., 1980; Doretto et al., 2020). The RCC includes all the dynamics of a changing environment as a river ebbs and flows through a particular region because of the differences in vegetation, substrate type, and nutrients in the water (Doretto et al., 2020). As the river runs through regions of high forestation or through high anthropogenic activity, the interactions between the fish and macroinvertebrates present in certain areas tends to change (Roebuck et al., 2019). The river continuum concept can be applied to the cycling of nutrients, transitioning biological communities, influx of organic matter, and the expenditure of energy to describe the entire river as a continuously integrating series of biological adjustments from beginning to end (Doretto et al., 2020). The RCC tends to group rivers according to certain patterns they show in stream flow, tree coverage, and other physical characteristics. For example, it is common to see coarse particulate organic material (CPOM) in the headwater streams where there tends to be more overhead tree coverage and low overall production compared to the fine particulate organic material (FPOM) found in the more downstream areas that tend to be wider and more open canopied with a deeper water column (Jiang et al., 2011). Within each of these contrasting communities, there tends to be specific functional feeding groups and assemblages that are more adapted to the organic material present (Jiang et al., 2011). The primary feeding groups for macroinvertebrates consist of shredders, collectors, grazers, and predators which maintain the various organic material sizes present in the water (Jiang et al., 2011). The shredders feed on the coarse organic material; whereas collectors feed on the fine organic material (Jiang et al., 2011). The grazers, however, tend to feed on the algae on stones, branches, and the zooplankton, also helping to filter the water (Jiang et al., 2011).

Freshwater Fish and their Sensitivity to Changing Water Conditions

Fish have been deemed long term indicators of water quality as they have much longer lifespans comparatively to other aquatic organisms such as macroinvertebrates (Kuklina et al., 2013). There are an incredible variety of fish species in freshwater ecosystems taking on various shapes, sizes, colors, and behaviors (Schlosser, 1995). Fish are one of the first indicators of water quality, as fish can be sensitive to their surroundings and the environment they are inhabiting, shown by external markings such as lesions or growths in cases of poor water quality as well as lessened community biodiversity (Eklöv et al., 1998). This is especially true of intolerant species such as the Freckled Madtom (*Noturus nocturnus*) as well as many darter and cyprinid species along with more endangered or threatened species such as the Pallid Shiner (*Hybopsis amnis*) in the Sabine River, specifically. There are many different types of fish inhabiting the Sabine River including catfish, bass, sunfish and numerous minnow and shiner species that rely on this water source as their habitat. When chemicals and harsh materials penetrate the river and linger throughout the water column, fish begin to absorb them, through their skin and gills, and ingest them into their digestive system and blood streams (Kelly et al., 2004). This exposure can eventually lead to skin rot, fin rot, lesions, and cancerous growths leading overall to a lack of biodiversity amongst fish in a particular region (Kelly et al., 2004).

Macroinvertebrates and Their Roles within Freshwater Ecosystems

While fish can show external issues on their individual bodies that indicate poor water quality over time, they are not the only indicators of good water quality and typically cannot account for the short-term changes in aquatic conditions (Durance and Ormerod, 2009). Freshwater macroinvertebrates are sensitive to invading chemicals and environmental waste that causes them to migrate towards a better environment altogether or leaves them unable to survive poor water conditions (Luiza-Andrade et al., 2017). Many macroinvertebrates are benthic organisms that live primarily in or near the substrate of the river. In many cases, harmful chemicals that are discharged into a freshwater source will eventually begin to settle into the river bottom where a large majority of these macroinvertebrates reside. Macroinvertebrates can take the form of insect larvae that have very limited range in mobility such as amphipods to larger more developed species such as crayfish and aquatic spiders that can traverse much longer distances. Therefore, in many cases, when the water is in poor condition, some macroinvertebrates can move elsewhere, but many will be unable to acclimate and will eventually die. Specifically, aquatic macroinvertebrates such as those in the groups Ephemeroptera, Plecoptera, and Trichoptera (EPT) are sensitive to poor water quality and can be used as biological indicators by performing what is known as an EPT index to determine abundance and diversity of such insects because of their intolerance of pollutants (Whiles et al., 2000). This type of index is generally used in the instance of high anthropogenic pollution (e.g. near freshwater systems such as wastewater treatment plants, oil refineries, and chicken slaughterhouses and packing facilities) to assess water quality for aquatic life (Whiles et al., 2000).

The Effects of Pollutants on Freshwater Systems

Approximately one percent of the world's water is accessible freshwater that can be used for human consumption, recreation, domestic necessities, and maintenance of all living organisms in the environment (Darwall et al., 2018). Being such an important element of life, there are naturally many factors in the world that pose a threat to the health of water as well as the organisms that inhabit it (Darwall et al., 2018). Water itself plays an incredibly important role in nature as it is a necessity for all organisms to survive, so it is pertinent that it is maintained from potentially pathogenic invaders (Darwall et al., 2018). Many species are fragile to harsh chemicals and contaminants when pollution is unregulated (Amoatey and Baawain, 2019). These harsh chemicals that are introduced through waste include nitrogen and phosphorus which are typically naturally in low concentrations for most freshwater systems but can cause eutrophication in high quantities (Rabalais et al., 2002). When wastes are dumped into these freshwater systems, the nitrogen and phosphorus are taken in by the aquatic plants, increasing their growth and ultimately creating much more plant waste once they die because of the overall increase in plant biomass (Rabalais et al., 2002). Once the plants die and start to decay, they trap much of the oxygen that was available in the freshwater causing issues with the other organisms present such as fish and macroinvertebrates that require oxygen to survive (Rabalais et al., 2002). Therefore, when freshwater ecosystems such as rivers, lakes, and wetlands are degraded, the species that they support can suffer and become threatened (Amoatey and Baawain, 2019).

There are many different types and methods of pollution that can occur by various means. Point source pollution is the act of one single, identifiable source of pollution that is expelling waste in an ecosystem such as a pipe discharging pollutants, large ships dumping oil, factories eliminating waste into freshwater, and sewage from wastewater treatment plants (Morrison et al., 2001). Sometimes these factors of point source pollution can be combined to form large contamination sites within a freshwater ecosystem. Specifically, agriculture can pose a detrimental and harmful effect on freshwater sources by dumping all their waste into one pipe to be expelled to one location (Morrison et al., 2001). In east Texas, Sanderson Farms implemented a processing plant in Tyler in 2019 where the chicken processing waste is run through a retention pond, treated with bleach, and expelled through a single pipe that dumps directly into the Sabine River. This processing plant is located north of Tyler near the Sabine River approximately 0.4 miles west of the intersection of FM 2015 and CR 313. According to their public, corporate website this processing plant slaughters 1.3 million chickens every week (Sanderson Farms Chicken Factory, https://sandersonfarms.com). Just northeast in Lindale, Tx, southeast of the Tyler, Tx processing plant, Sanderson Farms also has opened a hatchery facility where wastewater and harmful toxins are introduced into neighboring water systems within the Sabine watershed as well (Sanderson Farms Chicken Factory, https://sandersonfarms.com). These pollutants are dumped directly into the Sabine River and are being ingested and absorbed through the macroinvertebrates, fish, and nearby vegetation, potentially causing a loss of biodiversity and causing issues within the health of the organisms (Amoatey and Baawain, 2019).

River Systems and Minimally Regulated Waters

Many river systems across the world are impacted by anthropogenic effects and lack of regulation that prohibits such actions from taking place (Darwall et al., 2018). According to Texas Commission on Environmental Quality (TCEQ, 2009), there is very little defined in the way of disposing of industrial waste when it comes to how companies and factories eliminate it from the facility. There is adequate information about how to handle toxins in the workplace, but once it leaves the workplace, there is no regard for where it goes (TCEQ, 2009). Most factories are only required to separate the chemicals according to whether they are hazardous or non-hazardous, but there is no clear definition for the vague statement of a non-naturally occurring chemical being hazardous (TCEQ, 2009). A chemical that may not be hazardous to humans also may not be safe for the thousands of organisms beneath the surface of the river where it is being released. This policy, or lack thereof, is causing many species to quickly die from not being able to withstand the harsh environment or rather develop tumors, lesions, and rotting exteriors that are lessening biodiversity altogether (Amoatey and Baawain, 2019). In the case of the TCEQ regarding Texas poultry facilities, there are general guidelines according to how and where companies are not to dispose of poultry waste including limitations on dumping into surface water such as the Sabine River (Losses, 2009). However, many poultry facilities have been able to bypass this rule with permits issued by TCEQ. With 46 chicken plants in Texas and 10 of them being in East Texas, there are some major concerns with low regulatory dumping (ProPublica, https://projects.propublica.org/chicken/states/TX/). The Sanderson Farms Chicken Factory has obtained a permit issued by the Texas Commission of Environmental Quality, allowing them to dump mildly treated wastewater into the Sabine River through an underwater pipe unseen by the public (EPA I.D. No. TX0137740). This permit allows for disposal of poultry waste directly into the Sabine River with very little regulation concerning biochemical oxygen, suspended solids, ammonia (as nitrogen concentration), total nitrogen, fecal coliforms, *E. coli*, oil contents, and there is no defined limit of phosphorus concentration (EPA I.D. No. TX0137740). Much of this waste is also expected to contain toxic levels of bleach that could pose harm to the freshwater organisms in the Sabine River downstream from the pipe. A chicken processing plant typically will slaughter the chickens, pluck, clean, and cut them up to then be frozen, packaged, and distributed Poultry Processing, https://www.osha.gov/poultry-processing/hazards-solutions). The process of cleaning them leaves behind large quantities of feathers, organs, blood, excrement, and bones that are usually disposed of altogether with very little treatment Poultry Processing, https://www.osha.gov/poultry-processing/hazards-solutions). Chicken plants that do use some form of treatment will typically use chemicals such as ammonia, chlorine, hydrogen peroxide, carbon dioxide and peracetic acid which have all been known to cause health problems in humans when used at high volumes (Poultry Processing, https://www.osha.gov/poultry-processing/hazards-solutions) While it is typical for Sanderson Farms to use their wastewater as a spray treatment fertilizer for crops, the Tyler plant specifically must go through the process of being routed into a covered anaerobic lagoon, anoxic basin, aeration basin, clarifier, and then put through UV disinfection (EPA I.D. No. TX0137740).

As the freshwater crisis across the world continues to rise, it is even more important that every water system is protected from harmful contaminants and regulated for the safety of everyone that encounters it. The Sabine River is only one scenario of many threats to a freshwater system. The continuation of minimally regulated dumping from the Sanderson Farms chicken factory will likely result in a decrease in biodiversity for fish and macroinvertebrates as well as water quality if nothing is changed (Wear et al., 2021). This research project aims to study the species inhabiting the Sabine River north of Hawkins, Tx to determine whether this discharge pipe is causing damage to the environment by examining the species found and comparing them to data collected prior to the pipe's installation in 2019.

Research Significance

Understanding the role of the local fish and freshwater macroinvertebrates is key in determining the significance of protecting them and their environment. By examining the biodiversity of the fish and macroinvertebrate assemblages over multiple sites at different times of the summer, changes can be determined over time according to various community indices. Depending on the results, the effects could become catastrophic to the survival of many fragile species creating potential for invasive species or a cascade of local extinctions in the area. This research attempts to document any changes that may be occurring and to propose solutions that could prevent further damage to the Sabine watershed.

Objectives

I. **Determine if the chicken factory waste affects overall biodiversity in fish and macroinvertebrates in the Sabine River**

Chicken factory waste can consist of a mixture of feathers, blood, feces, and other chicken parts that bring about abundant levels of nitrogen, phosphorus, and anoxic chemicals that pose a real threat to the fish and macroinvertebrate species that inhabit the river.

II. **Establish a plan of action for the state to outline the potential effects of the pollution present in the Sabine River because of the newly added pipe.**

As the pipe is loosely regulated, it is important to highlight reasoning behind providing stricter regulations on dumping into the river.

III. **Determine future areas of concern for the organisms inhabiting the Sabine River because of the long-term effects of the drainage pipe.**

It is important to understand what these added nutrients and pollutants could do to community assemblages and function.

CHAPTER 3

MATERIALS AND METHODS

Study Site – The Sabine River

The Sabine River is a geographically unique water system that flows through much of upper East Texas and western Louisiana over a span of approximately 925.4 km (Long, 2017). It is filled with an abundance of fish and aquatic macroinvertebrate species along with being surrounded in many areas by riparian vegetation. Unfortunately, in years past the Sabine has been lined with oil refineries and chemical plants that discharged ammonia, sulfides, heavy metals, phenols, and other chemicals into the river (Long, 2017). While efforts have been made to regulate this pollution, a pipe from the local Sanderson Farms chicken factory was placed beneath the surface of the river in 2019 at the approximate location indicated in Fig. 1 (Sanderson Farms chicken factory, https://sandersonfarms.com). This pipe is known to have very little regulations as to what can spill out into the river, which has the potential to do irreparable damage to the surrounding vegetation and wildlife. Furtula et al. (2010) conducted a study to examine the microbial contamination in Canada. Waste or “litter” from chicken factories can contain but is not limited to, harmful nitrates, bacteria, antibiotics and viruses within the feathers, guts, chicken blood, feces, and bedding that is swept into the water systems. The lack of monitoring of this pipe spilling gallons of waste material directly into the river could pose catastrophic consequences to the freshwater quality and organisms inhabiting the river and nearby streams (Bustillo-Lecompte et al., 2016).

The sampling site on the Sabine River was off the Highway 14 boat ramp near Hawkins, Texas (Smith County) (Fig 1). The geology of the site was noted to have a sandy substrate upstream from the overpass bridge and a rocky substrate just downstream from the overpass bridge. The water flowed from North to South at quite a steady pace with a median flow rate of 1.8 m^3/s on June 1, 2022 and 2.2 m^3/s on August 12, 2022(Fig 2 and Fig 3) with rich riparian vegetation atop the banks of each side. The precipitation for one month prior to each sampling trip according to USGS near Hawkins, TX was recorded to account for the potential dispersal patterns of fish and macroinvertebrates as the water levels settle (Fig 4 and Fig 5). Throughout the river upstream, there were many large logs that had fallen along with vines hanging down from overhead trees. The downstream side of the river had many large rocks mainly dispersed within the middle of the river fairly centralized near the thalweg. The river was approximately 39.62 m in width for the entirety of the sampling site.

Sampling of Macroinvertebrates and Fish

Collection of macroinvertebrates and fish was conducted on June 1, 2022 and August 12, 2022 from approximately 0900 - 1500 hours. At the time of sampling, the National Weather Service estimated water levels of the Sabine River to be 1.51m. The latitude-longitude coordinates for each site were four: (32.54983526966787, -95.17844921556141), three: (32.5532070, -95.1994592), two: (32.55659342046775, -95.2031661107943), and one: (32.56905820465679, -95.2032979578961). This was the order at which each site was sampled as sites two through four were downstream from the drainage pipe from Sanderson Farms and site one was upstream from the pipe.

The crew was divided into two groups as three to four people collected fish and two people sampled for macroinvertebrates. The two methods used for macroinvertebrate sampling for each site were the D-frame kick neck and the Surber sampler. Those using the D-frame kick net firmly placed the flat part of the net parallel onto the substrate bottom facing against the river current and performed twenty passes of kicking the substrate in front of the net allowing the current to push all the loose substrate into the net to be collected and preserved in ethanol (EtOH). The Surber sampler was used to collect macroinvertebrates as one would position it on the river bottom against the current and steadily scoop in as much substrate into the pyramidal shaped net as possible for five minutes. The collected sample was placed in a separate bag and preserved with EtOH.

Fish were collected using a Smith Root barge electrofisher. Electrofishing consisted of one person maneuvering the craft, one person with the anode pole, and one person with a large fishing net catching the stunned fish. The barge was run downstream first and then back upstream to make sure a significant area was covered. The fish were captured and MS – 222 and formaldehyde were used to anesthetize and preserve the fish. Larger fish were recorded and released.

The same techniques were performed for all four sites with slight variation in shocking methods as passes were performed upstream and downstream at different amounts according to the width and depth available as well as considering any impeding elements such as fallen trees or large rocks. Site three consisted of three passes for the electroshocking barge while sites one, two, and four only allowed for two passes because of the factors mentioned previously.

Once sampling had been completed, the collected and preserved fish and macroinvertebrate samples were taken back to the UT Tyler campus where sorting, identifying and analyses were performed. The fish were drained of the formalin and rinsed with water. After the washes were performed, the fish were sorted, identified, and preserved in 70% ethanol. The fish were then counted and inspected for any lesions or abnormalities. Each macroinvertebrate sample was thoroughly inspected to make sure every specimen was retrieved from the substrate and placed in jars according to which site they came from as well as separated according to which method they were collected (surber sampler or d-frame kick net). They were then sorted and identified using a dissecting microscope to then be used for further analysis.

Environmental Sample at Each Site

An environmental survey also was performed to determine the habitat quality index, which measures bottom substrate stability, channel flow status, bank stability, aesthetics of reach and many other categories. A clinometer and a densiometer were used to measure the angle of banks and relative canopy density, respectively. A Hydrolab was used to determine the pH, salinity, temperature, conductivity, ammonium, nitrate, dissolved oxygen, and turbidity levels. These data collected were used in the statistical analyses.

Statistical Analysis

A Benthic Index of Biotic Integrity (B-IBI) and IBI were performed from the data collected for macroinvertebrates and fish, respectively. These scores were then compared by site and by year to compile multiple linear regressions in order to determine the sites trend over time. An ANOVA was also performed for both DNET and Surber samples according to site and date using the B-IBI scores. A Shannon's Diversity Index and Percent Similarity Index also were performed on macroinvertebrate data to compare June 2022 to August 2022, June 2020 to June 2022, August 2019 to August 2022, and a compiled list of 2019 and 2020 averages compared to the 2022 averages. Finally, an NMDS plot was created using Bray-Curtis distances to determine the community similarities between macroinvertebrates at each site by year (Matthews et al., 2013). A multiresponse permutation procedure was used to test for differences in macroinvertebrate communities by site and year.

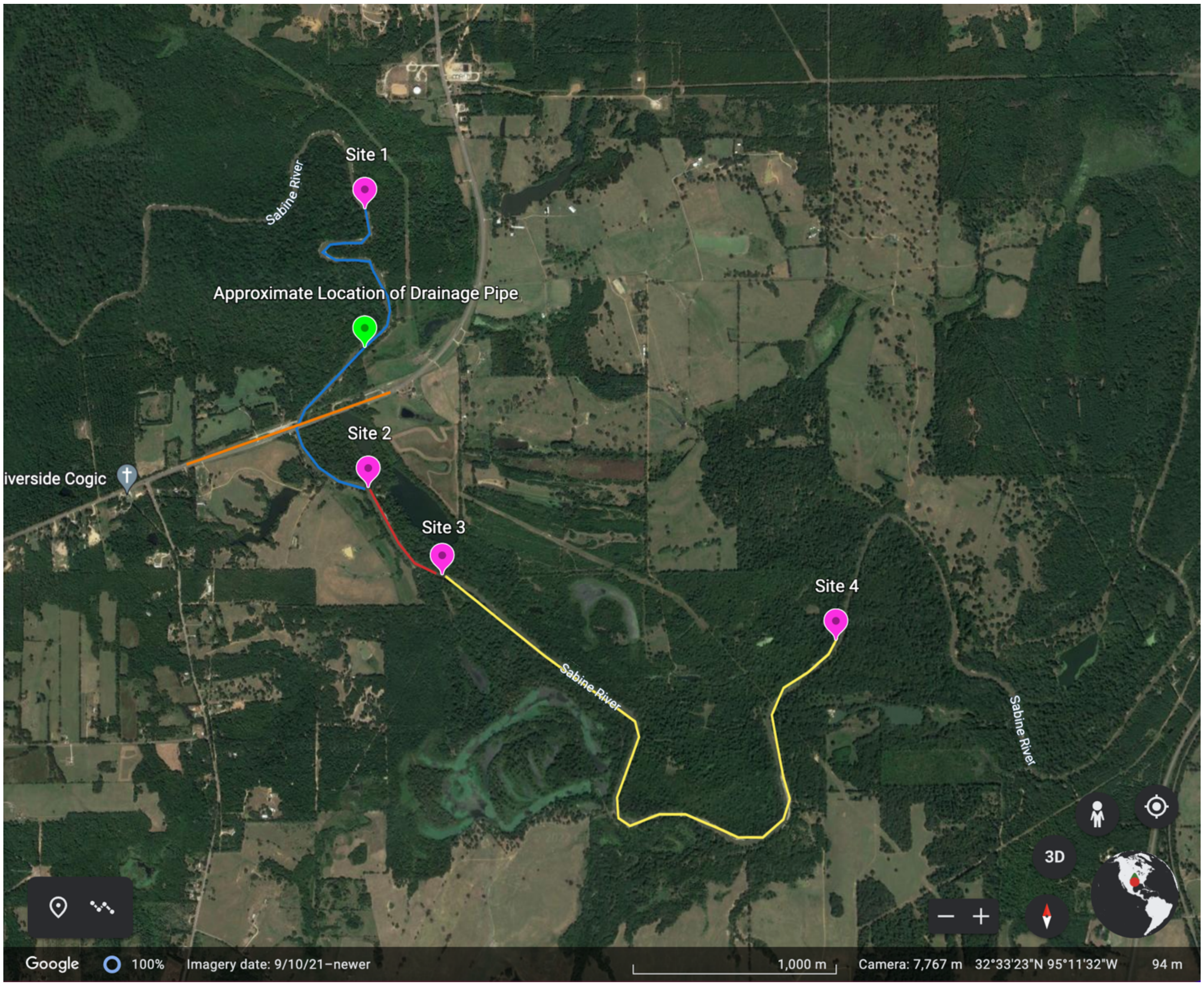


Figure 1. The locations of the four sampling sites on the Sabine River (Smith County, TX). This image was obtained from Google Earth using the coordinates for each site. The approximate location of the underwater drainage pipe for Sanderson Farms is shown with a green marker along with the intersection of highway 14 indicated by the orange line.

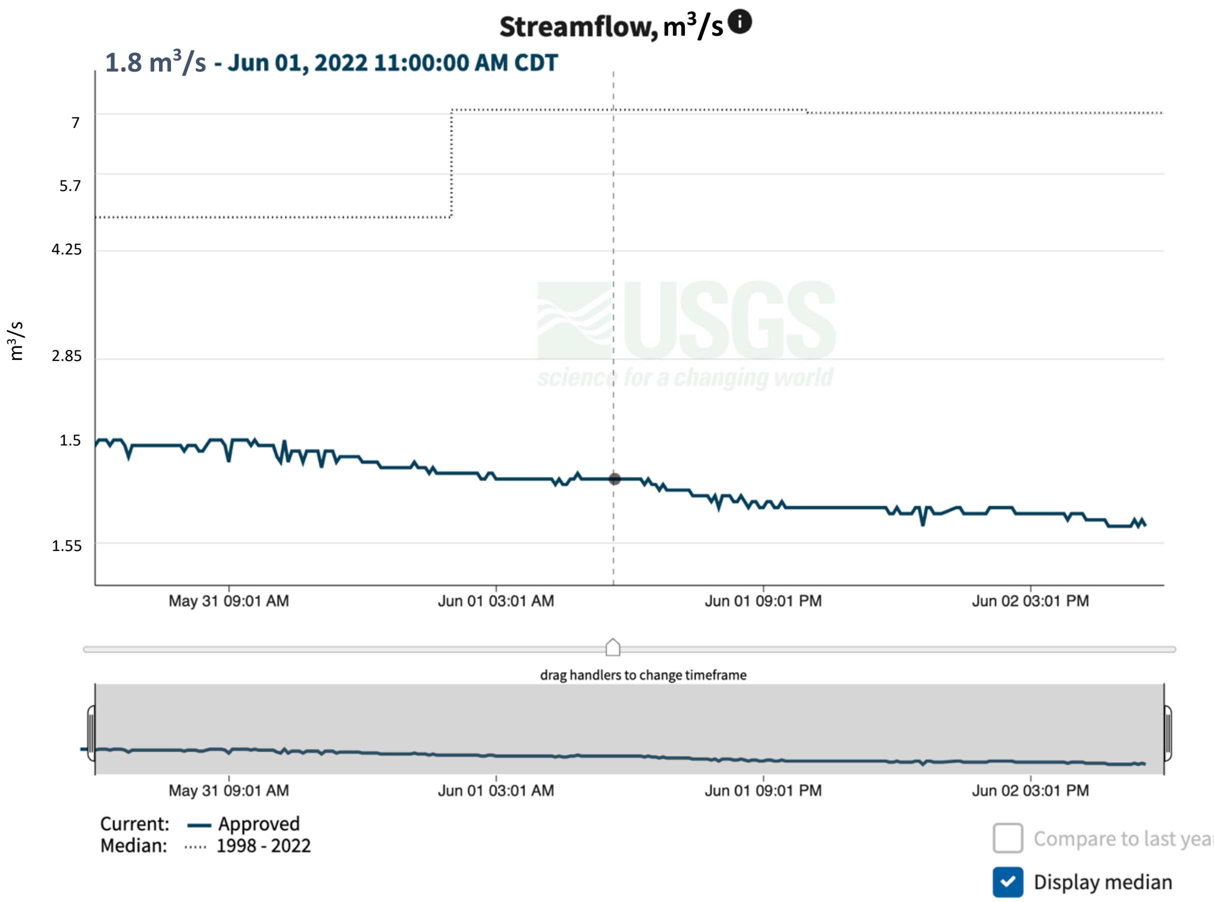


Figure 2. The median flow rate for the Sabine River near Hawkins, TX (Smith Co.) in cubic meters per second for June 1 around 11:00 am. This shows the median streamflow to be 1.8 m^3/s, which was collected using real-time data from the Sabine River Water Authority.

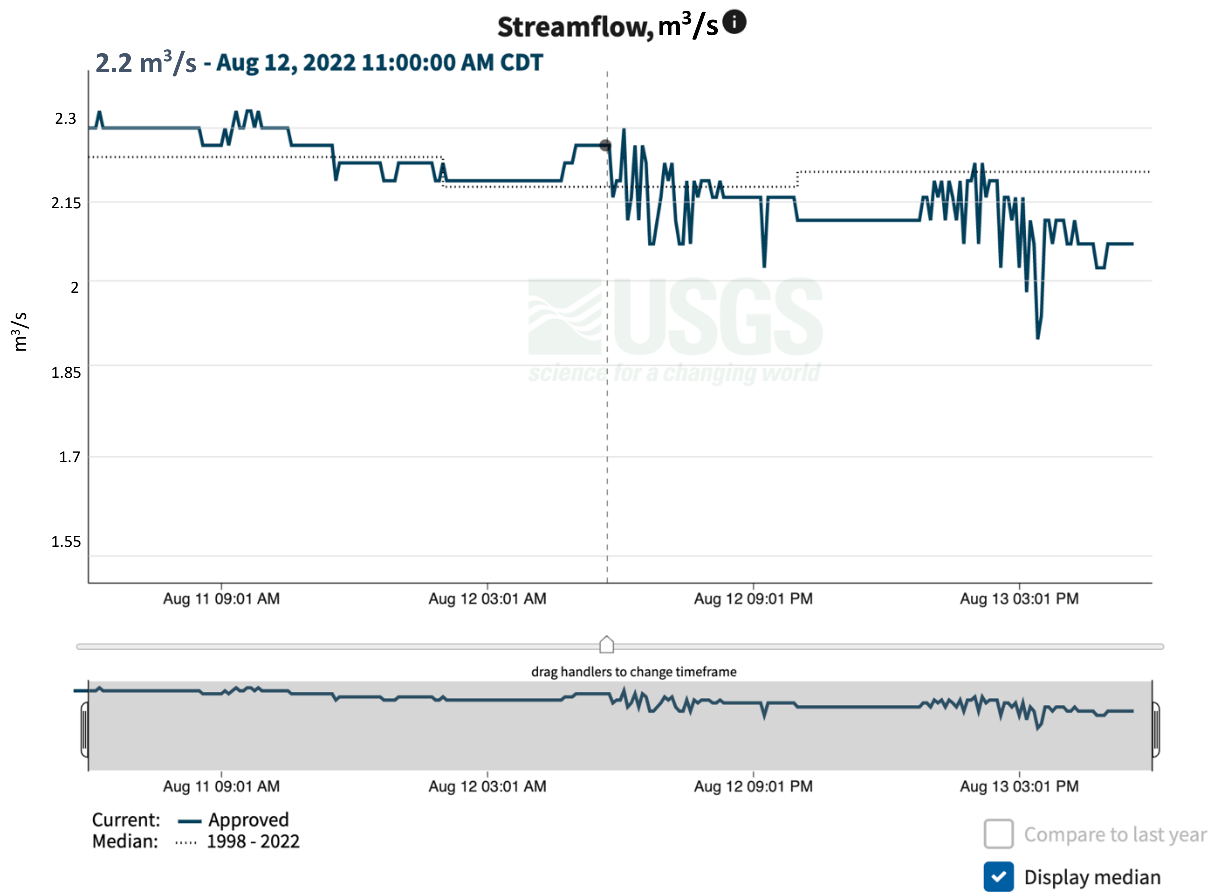


Figure 3. The median flow rate for the Sabine River near Hawkins, TX (Smith Co.) in cubic meters per second for August 12 around 11:00 am. This shows the median streamflow to be 2.2 m^3/s, which was collected using real-time data from the Sabine River Water Authority.

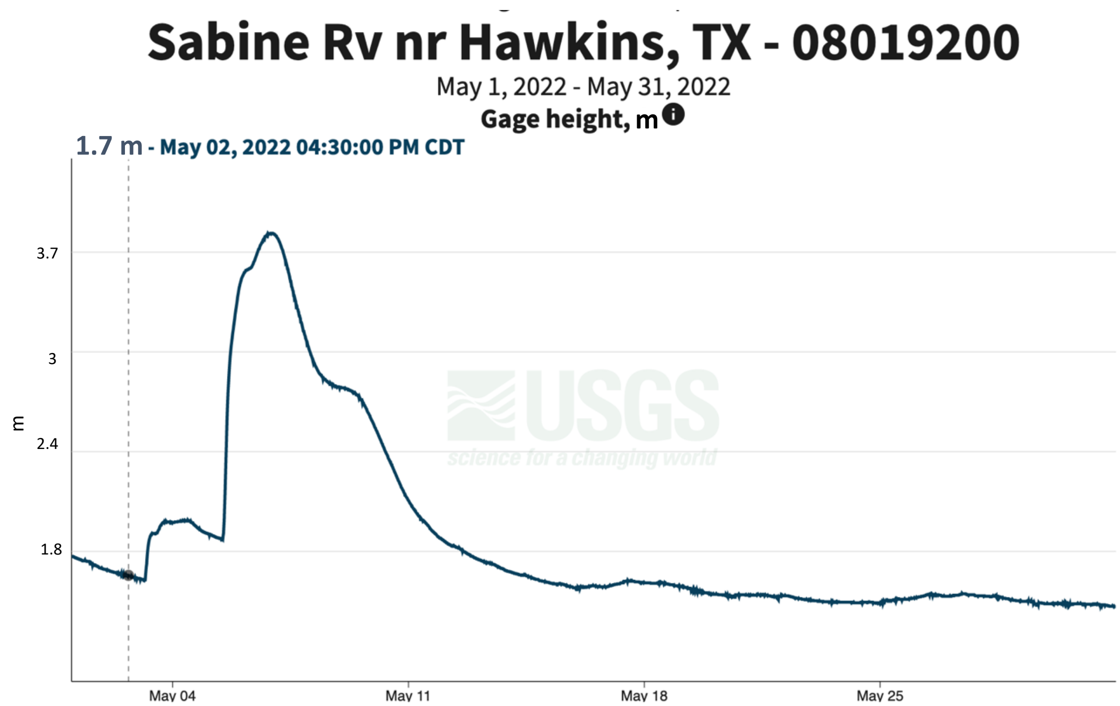


Figure 4. The gage height for the Sabine River near Hawkins, TX (Smith Co.) one month prior to the June 1, 2022 sampling trip to account for the hydrology of fish and macroinvertebrate dispersal as it relates to precipitation.

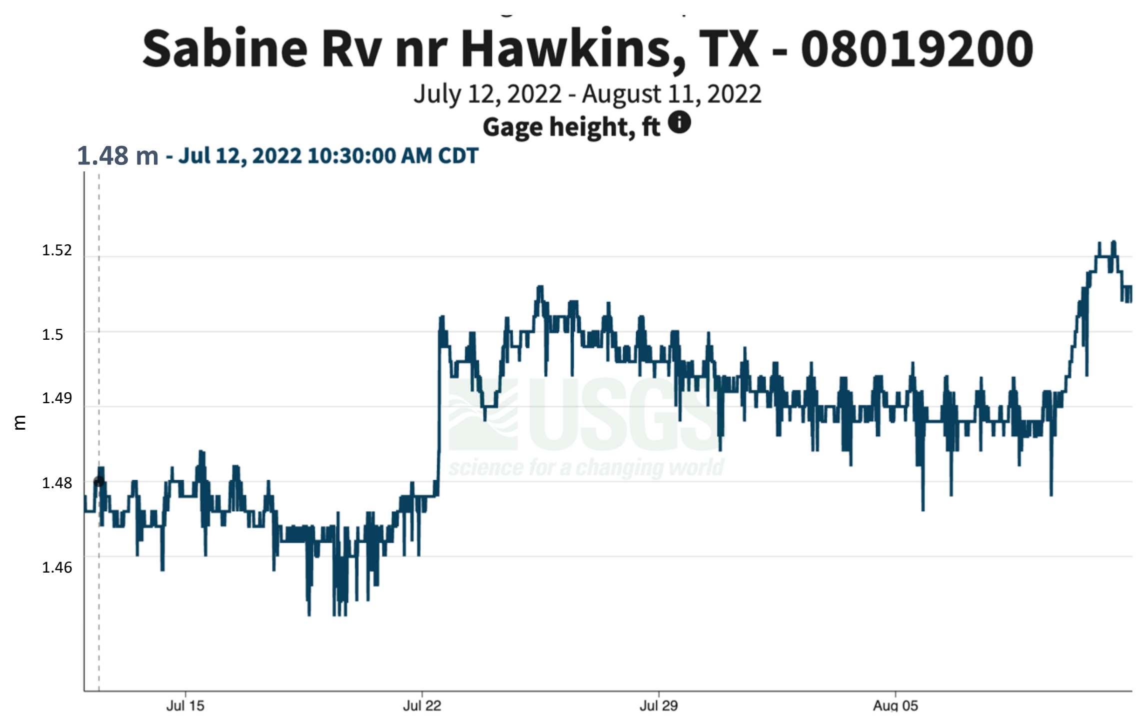


Figure 5. The gage height for the Sabine River near Hawkins, TX (Smith Co.) one month prior to the August 12, 2022 sampling trip to account for the hydrology of fish and macroinvertebrate dispersal as it relates to precipitation.

CHAPTER 4

RESULTS

Analyses were performed to examine the data collected to determine whether the fish and macroinvertebrates of the Sabine River near Hawkins, TX (Smith Co) were being affected by the discharge of chicken pollution from the underwater pipe placed by Sanderson Farms. During the counting process, the fish bodies were examined for any anomalies that may be present with the downstream sites showing more abundance of abnormal growth (Fig 6), sores or wounds (Fig 7), and gill or fin rot from poor water quality conditions (Fig 8). Much of the raw data were compiled and organized to calculate the B-IBI for macroinvertebrates and the IBI for fish that was then used to create regression plots and ANOVAs as well as paired t-tests. The compilation of D-Frame Kicknet results (Fig 9) from B-IBI scores across each site for each sampling trip showed a positive trend towards a healthier aquatic life use score as the sites progressed downstream. Similarly, the ANOVA (Fig 10), showed this same trend with a positive increase in B-IBI score in a downward direction; however, the ANOVA was not statistically significant. The Surber B-IBI showed three sites with little fluctuation, but site one was increasing in scores and site four showed to decrease but remained in the high category (Fig 9). The ANOVA, again, was not significant, but also showed a trend of increasing water quality moving downstream (Fig 10).

Analysis of Macroinvertebrates Over Time

Each site was analyzed on its own as well according to the various sampling methods for August 2019, June 2020, June 2022, and August 2022. Site one D-frame kicknet stayed in the limited range throughout each sampling trip with a slight increase from 2019 to 2020 but a drastic decline in August 2022 (Fig 9). The Surber Sampler for site one, however, showed the opposite effect with a positive trend over time, moving from an intermediate aquatic life use to a high aquatic life use as August 2022 showed to have the highest B-IBI score comparatively (Fig 9). The graph for site two D-frame kicknet showed a fairly stable range of B-IBI scores with a slight increase from 2019 to 2020, decrease from 2020 to June 2022 and then it levels back out in August of 2022 in the limited range of aquatic life use (Fig 9). The Surber Sampler for site two showed to be stable for all four sampling trips as it remained within the high range of B-IBI values for each one (Fig 9). Similar to that of the site two D-frame kicknet, the site three D-frame kicknet also showed to be dynamic in site stability with an incline from 2019 to 2020, drastic decline from high to limited from 2020 to June 2022 and another increase from June 2022 to August 2022 where it remained in the Intermediate range of B-IBI scores (Fig 9). The graph correlated to site three Surber Sampler showed to a slight incline from 2019 to 2020 and then remained in the high category of B-IBI scores for the remainder of the sampling trips indicating a stable community (Fig 9). The site four D-frame kicknet graph showed to also be fluctuating as it increased and then decreased, eventually returning back to the intermediate range (Fig 9); whereas the site four surber sampling B-IBI showed a fairly stable trend amongst the high and exceptional ranges, but it made a drastic decline in August 2022 into the lower portion of the high aquatic life use category of B-IBI scores (Fig 9) The compiled graph of all trends shown between B-IBI scores of each sampling trip tends to show an overall increase as time progresses with some exceptions to this occurring in the August 2022 sampling trip where a few samples showed decline in aquatic life use (Fig 9). The data were then examined and used to determine the definite trends shown for each site according to sampling technique used as either stable, declining, or increasing in overall B-IBI scores and thus aquatic life use (Table 1). It was shown that many of the sites remained stable; however, site one using the D-Frame Kicknet showed to be declining and site one using the Surber Sampler showed to be increasing and site four using the Surber Sampler also showed to be declining (Table 1).

Analysis of Fish Over Time

The fish data collected were used to create an IBI that was then utilized in statistical tests and graphed according to sampling trip date. Each of the four sites showed trends of increasing in aquatic life use and IBI scores as the summer progressed from June 2022 to August 2022. Site one showed a positive increase from intermediate in June 2022 to high in August 2022 (Fig 11); whereas site two also showed increases from limited in June 2022 to high in August 2022 (Fig 11). Sites three and four both stayed stable from June 2022 to August 2022 with both IBI scores falling within the high range for both sites (Fig 11). These graphs were then used to determine the trends over time as either stable, declining, or increasing. It was determined that site one and site two showed to be both be increasing in IBI scores and aquatic life use; whereas sites three and four remained stable (Table 1).

When performing an ANOVA regarding the number of fish that showed some type of impurity, it was determined that site one showed the least number of fish with issues whereas site two showed the largest amount and the largest variability between sampling trips (Fig 13). Sites three and four seemed to decrease in number of fish with outward imperfections gradually as they became further from the pipe (Fig 13). A Tukey test was performed comparing each individual site and it was determined that there was not significant difference between any of the site pairs, however, sites one and two showed the least similarity (Table 3).

Community Analysis

The Shannon's diversity (H') measurement and percent similarity index (PSI) were also performed comparing multiple sites and sampling dates for macroinvertebrates. The first comparison among June 2022 and August 2022 showed the Shannon scores to be 0.760974 and 0.8205884, respectively with a PSI of 0.6173107 or 61.73% (Table 2). This indicates that both sampling trips showed good diversity in their species as the Shannon's values were close to one (Table 2). The next comparison performed was between June 2020 and June 2022 to determine the differences over the course of two years within the same month. The June 2020 sampling trip had a H' score of 0.876496 with a PSI of 0.752264 or 75.23% indicating similar communities between the June sampling trips and high species diversity in 2020 (Table 2). A comparison of communities was performed between August 2019 and August 2022 that showed the similarity and differences over a three-year period. The August 2019 sampling trip showed to have a H' of only 0.5983549, being the lowest of all sampling trips in diversity (Table 2). The PSI between the two August trips showed to also be quite low at 0.5359005 or 53.59% (Table 2). The last comparison done was a compilation of all 2019 and 2020 data which were averaged together and all the 2022 data which were averaged together. This showed an overall H' of 0.7140521 and 0.8370957 to 2019-20 and 2022, respectively and an overall PSI of 0.6614001 or 66.14% (Table 2).

NMDS Plot and Bray Curtis Distances

Non-metric multidimensional scaling (NMDS) was used to compare macroinvertebrate communities over time. Fish were not analyzed because data was only retrieved for 2022. Bray Curtis was used as the distance measure in the analysis. Site 1 showed the most variability in macroinvertebrate communities over time. In site 4, 2022 samples are starting to circle back to 2019 samples indicating that site 4 may be the most stable. Multi-response permutation procedure (MRPP) showed that macroinvertebrate communities differed across time ($p=0.03$) but did not differ as much by site ($p=0.08$). The latter analysis would be significant at an α of 0.1.

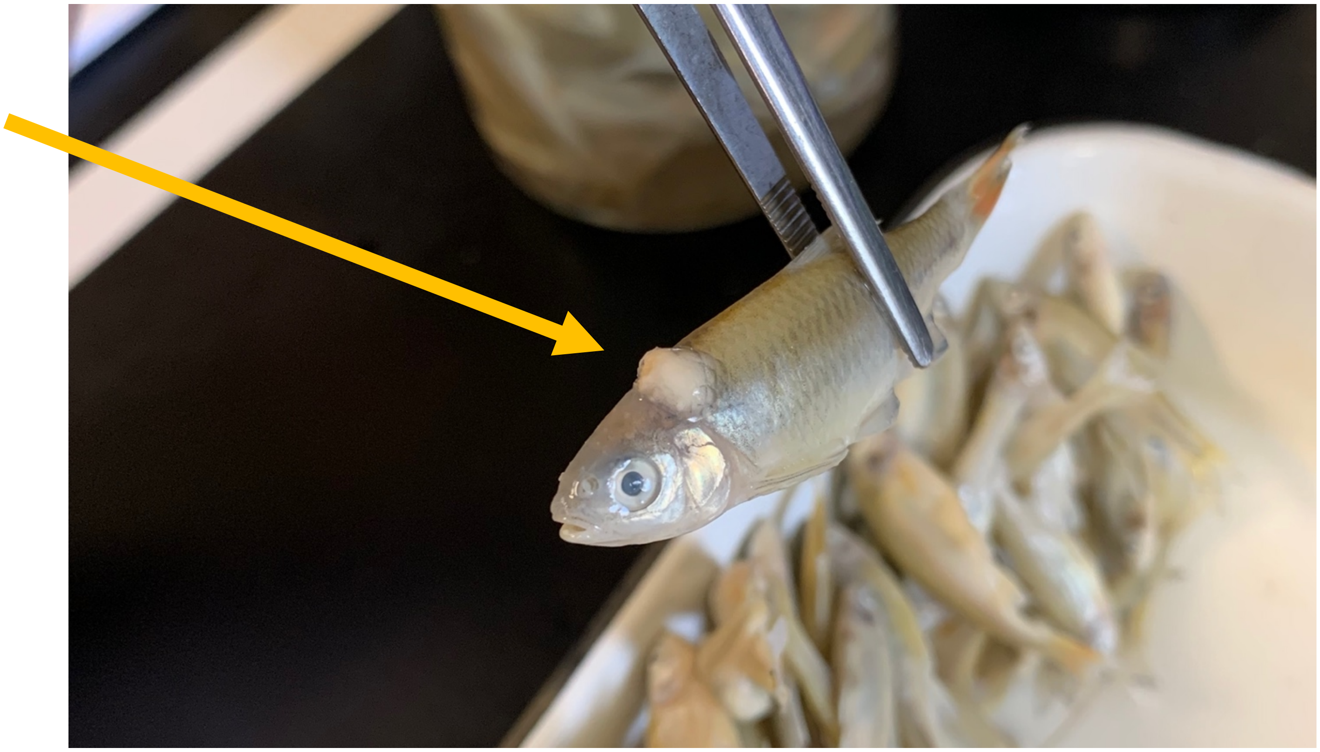


Figure 6. A large growth found on a Red Shiner (*Cyprinella lutrensis)* individual during the counting process collected during the June 2022 sampling trip.

Figure 7. A wound on the dorsal side of a Flathead Catfish (*Pylodictis olivaris)* found at site four of the Sabine River near Hawkins, TX (Smith Co) on August 12, 2022.

Figure 8. Caudal and dorsal fin rot next to healthy fin on a Centrarchidae species examined during the sorting process from the August 12, 2022 sampling trip near Hawkins, TX (Smith Co).

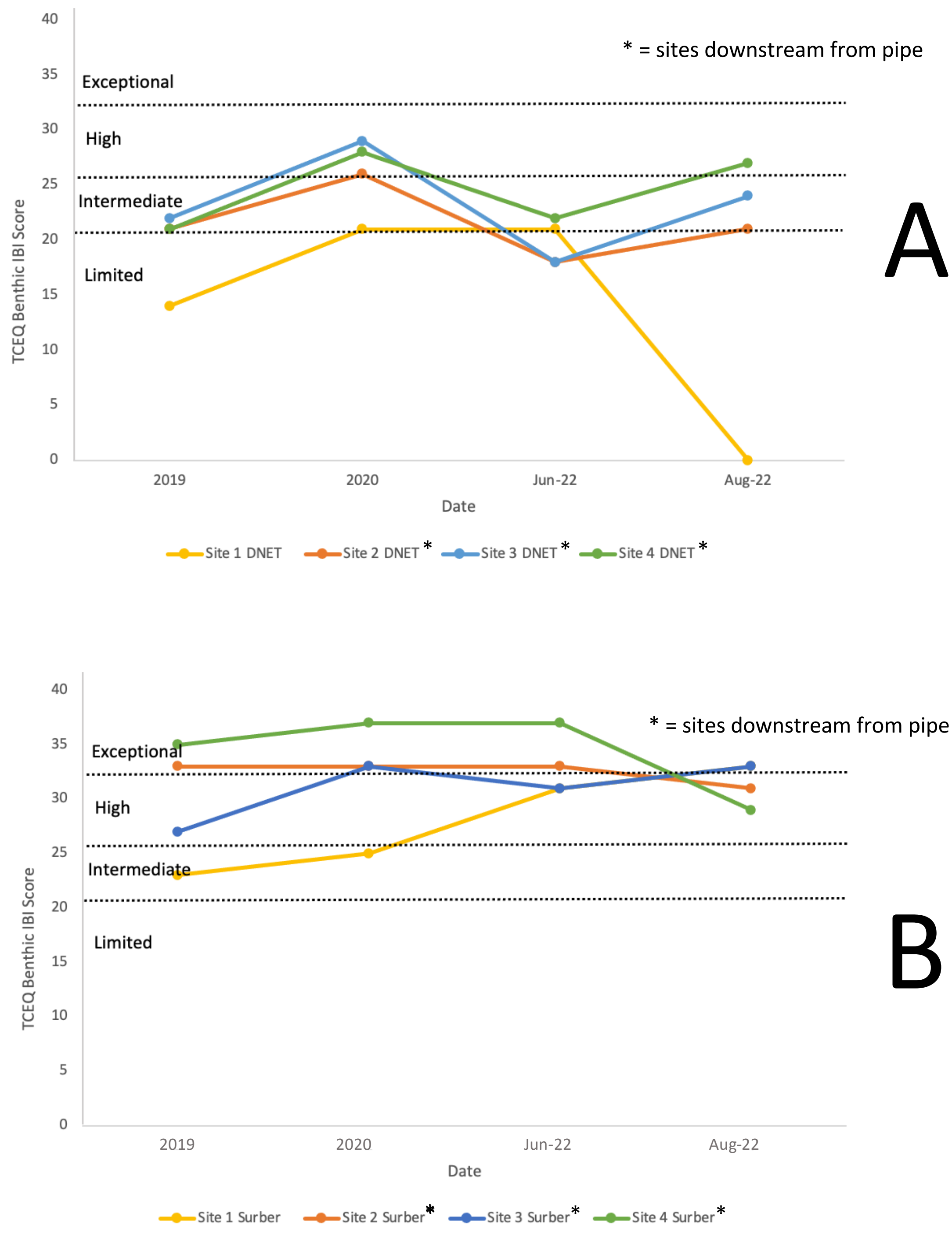
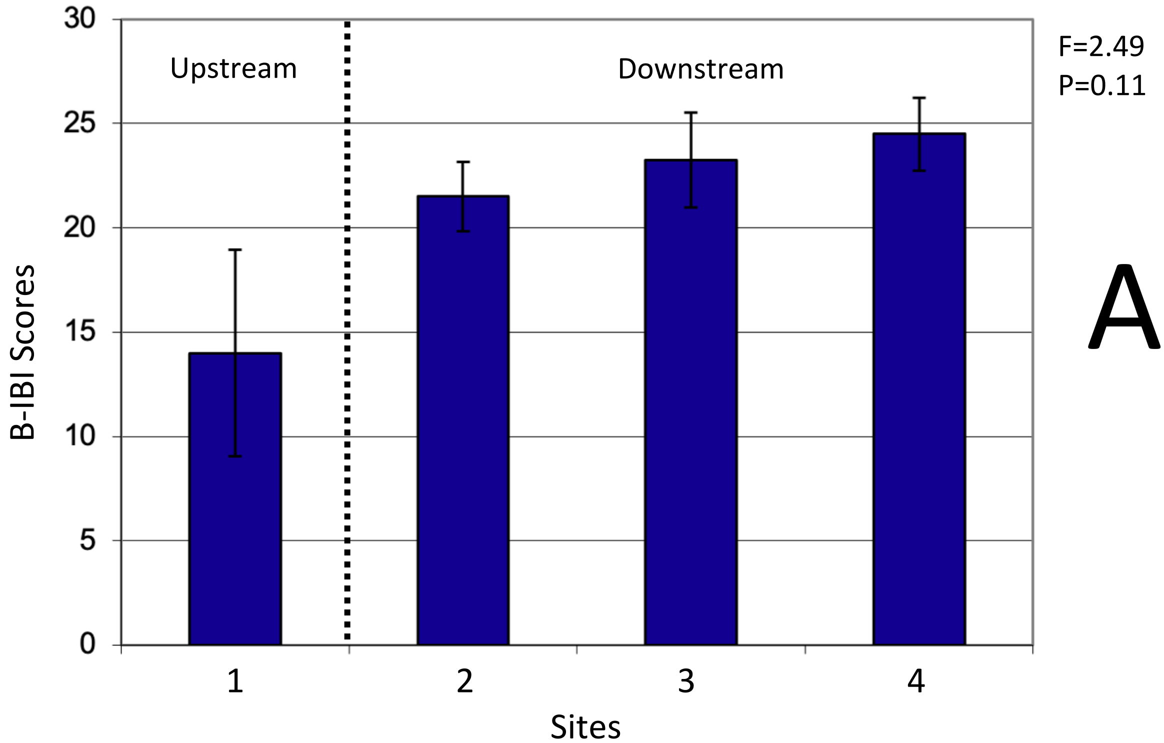


Figure 9. Compilation of Benthic IBI Scores according to sampling date and site for D-Frame Kicknet (A) sampling and Surber sampling (B) for macroinvertebrates to determine the aquatic life use over time as well as significant trends of change.



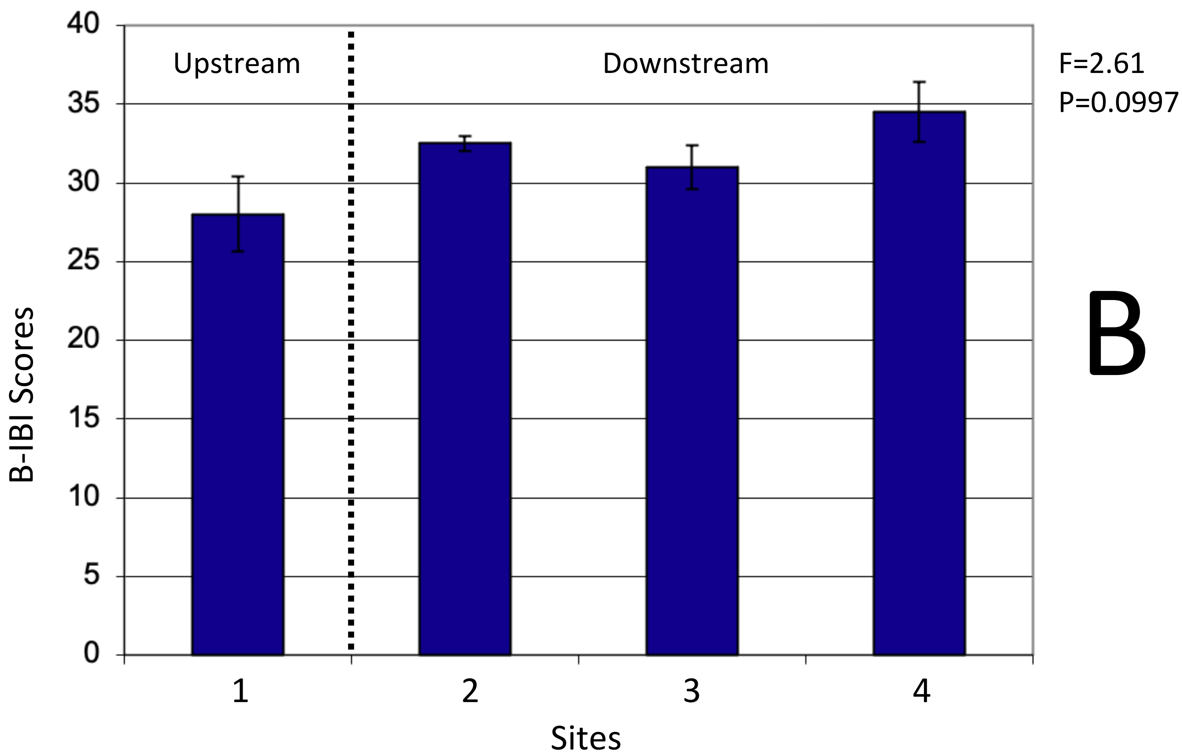


Figure 10. Analysis of Variance for the means between each sampling trip and sites to determine any significant differences for the D-Frame Kicknet (A) and Surber Sampler (B) macroinvertebrate sampling methods.

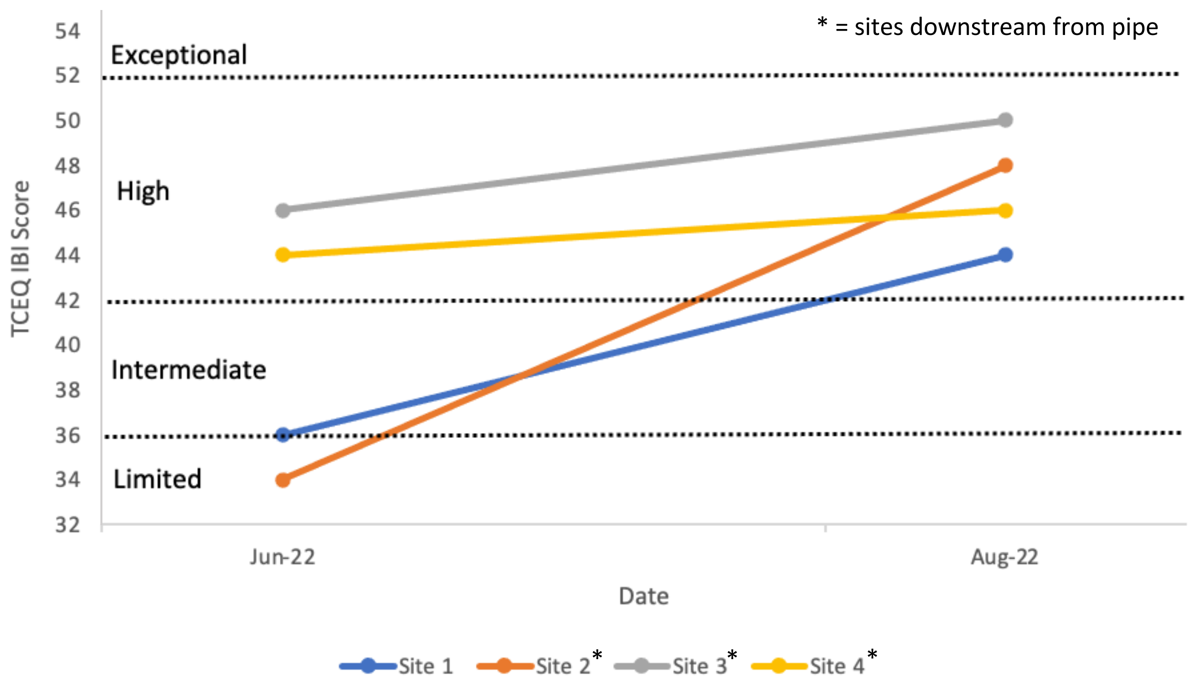


Figure 11. Compilation of all IBI Scores for fish from June 2022 and August 2022 to determine any visible trends in the data over time.

Table 1. Water quality trends per site for each sampling method using the B-IBI, IBI, and Habitat Quality Index scores over time

B-IBI Correlated Trends		
Site	**Sampling Method**	**Trend**
1	DNET	declining
1	Surber	increasing
2	DNET	stable
2	Surber	stable
3	DNET	stable
3	Surber	stable
4	DNET	stable
4	Surber	declining

IBI Correlated Trends				
Site	**June-IBI**	**Aug-IBI**	**HQI**	**Trend**
1	36	44	High	increasing
2	34	48	High	increasing
3	46	50	Exceptional	stable
4	44	46	Exceptional	stable

Table 2. Shannon’s Diversity values and Percent Similarity Index for multiple comparisons

Shannon's Diversity and Percent Similarity Index Comparisons			
	H'	H'	PSI
June 2022 to Aug 2022	0.760974	0.8205884	0.6173107
June 2020 to June 2022	0.876496	0.760974	0.752264
Aug 2019 to Aug 2022	0.5983549	0.8205884	0.5359005
2019-20 to 2022 Averages	0.7140521	0.8370957	0.6614001

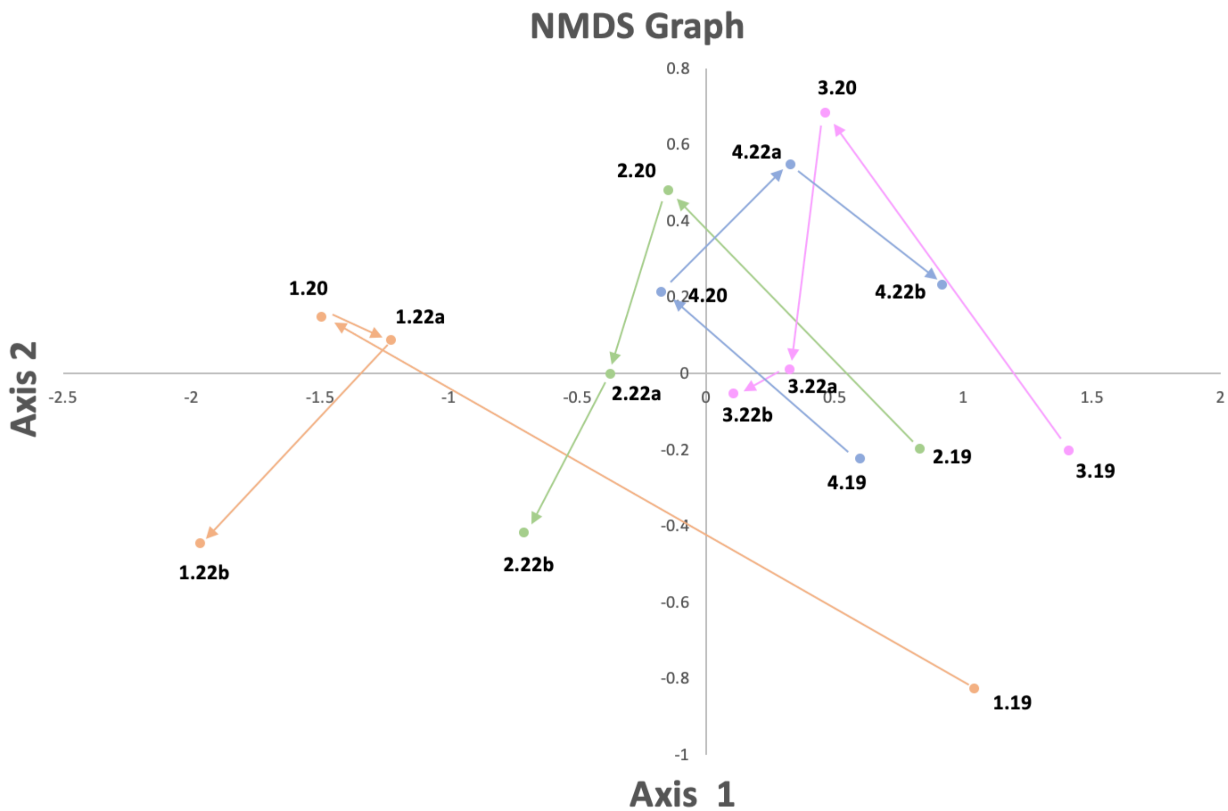


Figure 12. Non-metric Multi-Dimensional Scaling graph representing multiple variables to create a scatter plot that shows the similarity of sites and dates. The arrows indicate the next site in chronological order and the various colors represent new sampling dates.

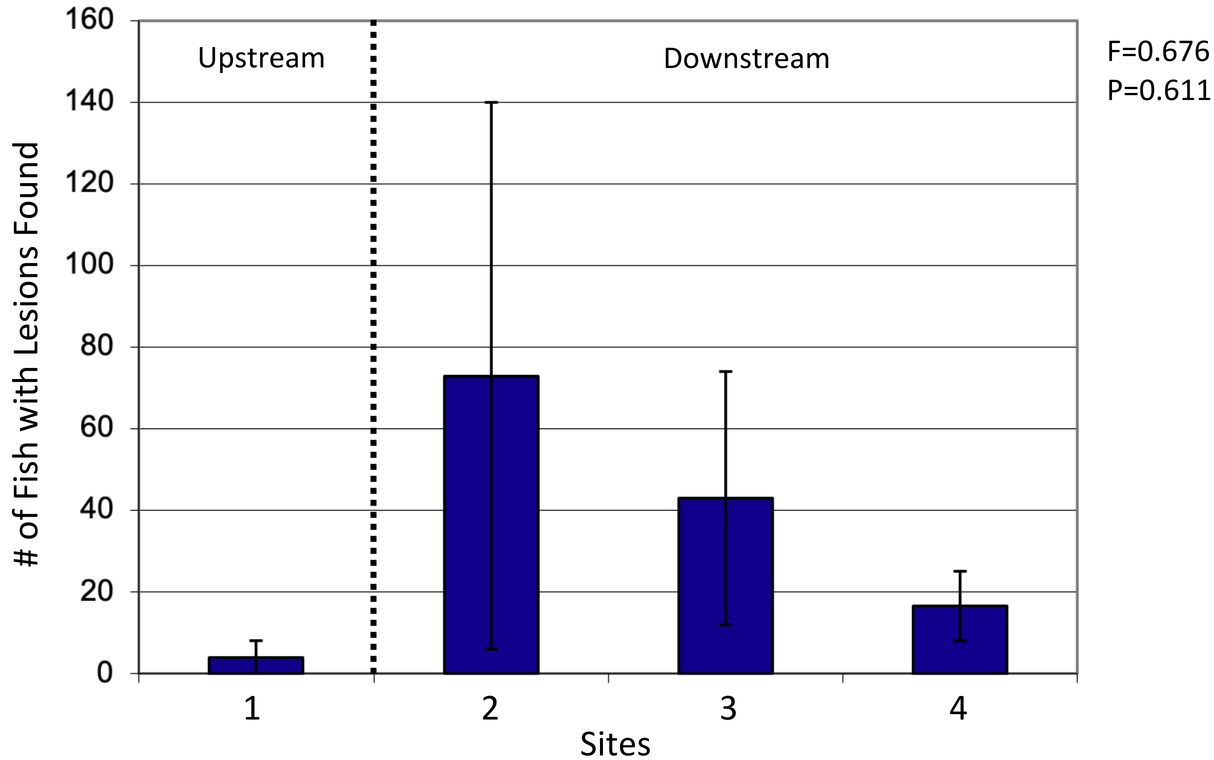


Figure 13. Analysis of Variance for the means between sites to determine any significant differences for the impurities of fish. Impurities consist of lesions, growths, scale rot, gill rot, fin rot.

Table 3. Tukey's Test comparing one site to another site individually to determine any significant differences in sites according to fish impurities.

TUKEY'S TEST RESULTS FOR FISH IMPURITIES	
Pair	**P-Value**
1 x 2	0.6026
1 x 3	0.8763
1 x 4	0.9945
2 x 3	0.9361
2 x 4	0.7219
3 x 4	0.954

CHAPTER 5

DISCUSSION

The results suggested that the pipe placed by Sanderson Farms in 2019 has not had a significant impact on the water quality scores and abundance of species inhabiting the Sabine River. With a few exceptions, B-IBI and IBI scores have remained stable if not increased in aquatic life use; however, there were a couple of sites that emerged as areas of concern as they were shown to be declining for macroinvertebrates diversity and abundance (Table 1). Site one for D-frame Kicknet Sampling and site four for Surber Sampling showed a decline over time and are areas to continue watching. However, site one is upstream from the pipe and is most likely declining because of other environmental factors; whereas site four is the furthest from the pipe and should also not have the pipe's placement attributed to its sudden decline. Site one has a dense, clay substrate and low flow rate that may attribute to the lower diversity, and site four has such a higher flow rate and rocky substrate. With such different substrates and mesohabitats, site one showed to be a poor control when looking to compare habitats for diversity and water quality. The lack of gravel in the substrate and lack of riffles does not make this site a good area for many organisms and therefore, makes it somewhat incomparable to the downstream sites with higher quality geological makeup. In August of 2022, Texas was under an extreme drought and may have contributed to lower index scores in site four. Despite the relative stability in aquatic health, there were some notable abnormalities in fish tissue (e.g., lesions, fin rot, etc.) which is something that should be monitored over time.

The Shannon's Diversity Index showed most of the sampling trips yielded similar values close to one apart from the August 2019 sampling trip that may have lower been because of the recent placement of the pipe or other external factors that are unknown. With such high diversity values, it is hard to attribute the placement of the pipe to any known declines in community structure. Likewise, the percent similarity index showed the sites to have similar communities over time. While they were all above 50% the same, none were above the 80th percentile in comparison. This indicates that while many of the species that have been collected are still abundant and of good health, some are not the same over time and the communities may have shifted to favor more tolerant species over the years. This was represented by the community data for macroinvertebrates as the ratio of intolerant to tolerant species changed drastically. Site one in June 2020 showed the ratio to be approximately 2.4 with site four having a ratio of 5.4 whereas site one in June 2022 had a ratio of 0.67 with sites three and four having a ratio of 4. The EPT Index also represents a shift as there were more taxa that fall within the EPT orders as the sites progressed downstream. In August 2019, the EPT Index was found to be 62.5% in site one and 64% in site four. However, site one showed to have approximately 12.5% EPT taxa in June 2022 whereas site four reached approximately 61% EPT taxa illustrating a positive trend in water quality as the sites progress downstream. The MRPP results indicated that while the individual sites are not significantly different from each other during a single sampling trip, it can be concluded that each sampling trip does show to be significantly different over time, solidifying this change in community structure and diversity as time progresses. The NMDS graph suggests that site four is the most stable of the sites that were sampled as the August 2022 data had almost circled back around the first data set from August 2019. Meanwhile, site one showed the largest variation with August 2019 being isolated from the other three sampling dates.

As much of the results showed the sites were relatively stable in aquatic life use, it may be a case of the ecotoxicology paradigm that “the solution to pollution is dilution” (Floehr et al., 2013). This has been the idea for years and has been slowly phased out by the idea that “what goes around, comes back around”, however, the dilution factor of chicken waste from the pipe discharge may be so great because of the fluidity of the river and rockiness of the substrate as it progresses downstream, that it may attribute to the overall health, abundance, and biodiversity of the downstream sites (Floehr et al., 2013). It can be seen that the trends obviously point towards sites three and four having higher habitat quality when compared to sites one and two. This was demonstrated by the HQI results that showed sites three and four in the exceptional habitat quality category. As the water flows faster and the substrate becomes more rocky, the chemicals in the water likely diluted as water moved downstream (Floehr et al., 2013). Site four also showed greater sinuosity when compared to the other sites, which may have contributed to there not being as much waste at this site (Xiao et al., 2020). The curves and winding of the river can slow down the transportation of substances in the water and potentially inhibiting it from going as far from the source of pollution (Xiao et al., 2020). The hydrodynamic force of the river strongly controls the distribution of these pollutants and the flow at a channel bend can lead to the deposition of substances more on the inner bend near the surface essentially stopping its flow (Xiao et al., 2020).

The substrate and hydrology of the river seemed to play a large role in the quality and abundance of the samples. There is an obvious shift in substrate just above the Highway 14 overpass that acts as a border between sandy substrate downstream and clay substrate above the bridge. Sites two, three, and four were all very rocky substrate with the rocks gradually getting larger and larger as the river progressed downstream. Fish and macroinvertebrates have been known to build nests and habitats utilizing the rockier substrate as it can also be good for spawning and camouflage from predators (Armbruster and Page, 1996). Rocky substrate provides more diversity in habitat for the majority of freshwater organisms than a sandy substrate (Armbruster and Page, 1996). For instance, many macroinvertebrates in the order Trichoptera tend to build casings out of the rocky substrate available to them (Prestidge, 1977). A rockier substrate does not trap chemicals and harsh matter for long periods of time as they can get easily washed over the gravels whereas a sandy substrate can hold onto it causing it to be a more toxic habitat for benthic organisms (Wang et al., 1997). Cordero-Umaña and Santidrián-Tomillo (2020) found that two different fish and invertebrate communities in a rocky substrate and a sandy substrate will utilize their surroundings to their advantage with different substrates providing different functions for the organisms. The rocky substrate showed a higher biodiversity in aquatic organisms that was found to be better suited for breeding and recruitment, whereas, the sandy substrate had a higher biomass but less diverse trophic groups with piscivores and planktivorous being the more dominant groups. This is somewhat true of the sites sampled in the Sabine River where the sites with a rockier substrate showed to have the more diverse samples. The site one substrate was sandy and silty making it hard to sample and it had many species that were tolerant of poor conditions such as Chironomidae, Red Shiner, and Channel Catfish. The hydrology also may have played a role in the abundance and quality of samples collected. The gage height a month prior to each sampling trip showed very different results as it rained much more in May that it did in late July/early August. The extra rain in May that caused the river to reach approximately four meters two weeks prior to sampling could have caused many of the fish and macroinvertebrates to spread out making them harder to capture. Meanwhile, the month prior to the August sampling trip remained fairly stable with the gage height never getting above five feet. The areas being sampled near Hawkins, TX were in a drought period during this time that most likely attributed to the lower water height and sampling outcome for site one where no macroinvertebrates were collected with the D-frame kicknet. This also may have had an effect on sampling as a narrower water column has fish more confined and macroinvertebrates much tighter together making them easier to catch, which could attribute to the much higher abundance in organisms overall.

While much of the data were to be not significant based on the statistical tests, it is important to have continuous monitoring of the quality and health of these habitats because of the Sabine River being such a main component to the wildlife of East Texas and western parts of Louisiana. It is an important natural freshwater source that many organisms rely on, and while the pipe may not have an obvious immediate effect, change in aquatic species can occur over long periods of time and is hard to reverse once the damage has been done. The Sabine River holds so much history and life that its significance to the region is invaluable. This can be said of all freshwater sources as the quality and quantity of freshwater systems is rapidly declining all across the United States and the rest of the world. It is key to continue testing the quality of these habitats routinely as pollution of any kind can have a long-lasting impact.

APPENDIX A

Site 1 – sandy, silty substrate with low flow rate and little to no riffles. There were many large logs from fallen trees in the middle of the river and low hanging vines from riparian vegetation.

Table A.1 The macroinvertebrates collected for site one over each sampling trip according to sampling method.

Year	Sampling Method	Order + Family	Abundance
2019	DNET	Ephemeroptera Caenidae	192
2019	DNET	Diptera Chironomidae	1710
2019	DNET	Ephemeroptera	1
2019	DNET	Ephemeroptera Baetidae	5
2019	DNET	Ephemeroptera Heptageniidae	27
2019	DNET	Coleoptera Dytiscidae	5
2019	DNET	Hemiptera Corixidae	1
2019	DNET	Megaloptera Corydalidae	5
2019	DNET	Coleoptera Gyrinidae	2
2019	DNET	Annelida Hirudinea	3
2019	DNET	Trichoptera Leptoceridae	1
2019	DNET	Ephemeroptera Leptohypidae	40
2019	Surber	Diptera Chironomidae	216
2019	Surber	Ephemeroptera Caenidae	6
2019	Surber	Coleoptera Elmidae	4
2019	Surber	Trichoptera Hydropsychidae	2
2019	Surber	Ephemeroptera Baetidae	1
2019	Surber	Trichoptera Leptoceridae	1
2019	Surber	Odonata Gomphidae	1
2019	Surber	Ephemeroptera Leptohypidae	4
2020	DNET	Diptera Chironomidae	11
2020	DNET	Diptera Ceratopogonidae	4
2020	DNET	Coleoptera Elmidae	8
2020	DNET	Ephemeroptera Baetidae	1
2020	DNET	Amphipoda Gammaridae	2
2020	DNET	Ephemeroptera Leptohypidae	2
2020	DNET	Ephemeroptera Caenidae	6
2020	DNET	Annelida Oligochaete	2
2020	DNET	Trichoptera Hydropsychidae	1
2020	DNET	Decapoda Palaemonidae	1
2020	Surber	Trichoptera Hydropsychidae	5

2020	Surber	Amphipoda Gammaridae	2
2020	Surber	Diptera Chironomidae	6
2020	Surber	Ephemeroptera Leptohypidae	3
2020	Surber	Ephemeroptera Baetidae	1
2020	Surber	Ephemeroptera Caenidae	9
2020	Surber	Annelida Oligochaete	5
2022a	DNET	Megaloptera Corydalidae	1
2022a	DNET	Diptera Chironomidae	7
2022a	DNET	Coleoptera Elmidae	2
2022a	DNET	Odonata Gomphidae	1
2022a	DNET	Ephemeroptera Caenidae	5
2022a	DNET	Ephemeroptera Polymitarcyidae	2
2022a	DNET	Diptera Simuliidae	1
2022a	DNET	Ephemeroptera Leptohyphidae	1
2022a	Surber	Coleoptera Elmidae	15
2022a	Surber	Trichoptera Hydropsychidae	37
2022a	Surber	Diptera Chironomidae	13
2022a	Surber	Diptera Ceratopogonidae	2
2022a	Surber	Amphipoda Gammaridae	8
2022a	Surber	Ephemeroptera Caenidae	9
2022a	Surber	Ephemeroptera Leptohyphidae	1
2022a	Surber	Ephemeroptera Baetidae	2
2022b	Surber	Odonata Gomphidae	1
2022b	Surber	Coleoptera Elmidae	15
2022b	Surber	Trichoptera Hydropsychidae	3
2022b	Surber	Ephemeroptera Leptohyphidae	2
2022b	Surber	Ephemeroptera Caenidae	1
2022b	Surber	Ephemeroptera Baetidae	1
2022b	Surber	Diptera Chironomidae	2
2022b	Surber	Megaloptera Corydalidae	1

Table A.2 The fish species collected for site one over each sampling trip with number of fish that showed to have imperfections.

Date	Common Name	Scientific Name	Abundance	Impurities
2022a	Longear Sunfish	*Centrarchidae Lepomis megalotis*	3	0
2022a	Red Shiner	*Cyprinidae Cyprinella lutrensis*	6	0

2022a	Bullhead Minnow	*Cyprinidae Pimephales vigilax*	9	0
2022a	Harlequin Darter	*Percidae Etheostoma histrio*	1	0
2022a	Dusky Darter	*Percidae Percina sciera*	1	0
2022b	Dusky Darters	*Percidae Percina sciera*	2	0
2022b	Frecked Madtoms	*Ictaluridae Noturus nocturnus*	2	0
2022b	Channel Catfish	*Ictaluridae Ictalurus punctatus*	3	0
2022b	Red Shiner	*Cyprinidae Cyprinella lutrensis*	17	2
2022b	Bullhead Minnows	*Cyprinidae Pimephales vigilax*	266	6
2022b	Spotted Bass	*Centrarchidae Micropterus punctulatus*	4	0
2022b	Large mouth Bass	*Centrarchidae Micropterus salmoides*	1	0
2022b	Sabine Shiners	*Cyprinidae Notropis sabinae*	4	0
2022b	Blacktail Shiner	*Cyprinidae Cyprinella venusta*	2	0
2022b	Longear Sunfish	*Centrarchidae Lepomis megalotis*	8	0
2022b	Dollar Sunfish	*Centrarchidae Lepomis marginatus*	7	0

Table A.3 Site one B-IBI and IBI raw scores for each sampling trip

	DNET	Surber	Fish
2019	14	23	N/A
2020	21	25	N/A
2022a	21	31	36
2022b	0	33	44

APPENDIX B

Site 2- rocky, gravel substrate with moderate flow rate and one distinct riffle. The water column was deep in some places with very little sinuosity.

Table B.1 The macroinvertebrates collected for site two over each sampling trip according to sampling method.

Year	Sampling Method	Order + Family	Abundance
2019	DNET	Diptera Chironomidae	669
2019	DNET	Ephemeroptera Caenidae	403
2019	DNET	Coleoptera Dytiscidae	2
2019	DNET	Plecoptera Perlidae	3
2019	DNET	Trichoptera Hydropsychidae	69
2019	DNET	Diptera Ceratopogonidae	3
2019	DNET	Amphipoda Gammaridae	22
2019	DNET	Coleoptera Elmidae	47
2019	DNET	Ephemeroptera Heptageniidae	32
2019	DNET	Ephemeroptera Baetidae	26
2019	DNET	Annelida Oligochaete	1
2019	DNET	Annelida Hirudinea	1
2019	DNET	Trichoptera Leptoceridae	6
2019	DNET	Odonata Coenagrionidae	6
2019	DNET	Odonata Gomphidae	1
2019	DNET	Coleoptera Hydrophilidae	1
2019	DNET	Megaloptera Corydalidae	28
2019	DNET	Trichoptera	1
2019	DNET	Ephemeroptera Leptohypidae	549
2019	Surber	Diptera Chironomidae	130
2019	Surber	Ephemeroptera Caenidae	91
2019	Surber	Amphipoda Gammaridae	7
2019	Surber	Trichoptera Hydropsychidae	77
2019	Surber	Coleoptera Elmidae	17
2019	Surber	Diptera Ceratopogonidae	1
2019	Surber	Ephemeroptera Heptageniidae	4
2019	Surber	Ephemeroptera Baetidae	16
2019	Surber	Trichoptera Leptoceridae	1
2019	Surber	Odonata Coenagrionidae	1
2019	Surber	Ephemeroptera Leptophlebiidae	4
2019	Surber	Megaloptera Corydalidae	10
2019	Surber	Trichoptera Polycentropodidae	1

2019	Surber	Ephemeroptera Leptohypidae	59
2020	DNET	Trichoptera Hydropsychidae	89
2020	DNET	Coleoptera Elmidae	23
2020	DNET	Amphipoda Gammaridae	7
2020	DNET	Ephemeroptera Heptageniidae	7
2020	DNET	Ephemeroptera Baetidae	16
2020	DNET	Diptera Chironomidae	27
2020	DNET	Diptera Ceratopogonidae	11
2020	DNET	Ephemeroptera Leptohypidae	35
2020	DNET	Ephemeroptera Caenidae	24
2020	DNET	Annelida Oligochaete	22
2020	DNET	Diptera Simuliidae	19
2020	DNET	Megaloptera Corydalidae	1
2020	DNET	Trichoptera Leptoceridae	4
2020	Surber	Trichoptera Hydropsychidae	149
2020	Surber	Amphipoda Gammaridae	36
2020	Surber	Coleoptera Elmidae	31
2020	Surber	Ephemeroptera Heptageniidae	13
2020	Surber	Diptera Chironomidae	21
2020	Surber	Ephemeroptera Leptohypidae	56
2020	Surber	Ephemeroptera Baetidae	4
2020	Surber	Ephemeroptera Caenidae	6
2020	Surber	Diptera Ceratopogonidae	1
2020	Surber	Annelida Oligochaete	3
2020	Surber	Annelida Hirudinea	1
2020	Surber	Trichoptera	2
2020	Surber	Trichoptera Leptoceridae	1
2022a	DNET	Trichoptera Hydropsychidae	71
2022a	DNET	Procambarus Dupratzi	1
2022a	DNET	Coleoptera Elmidae	14
2022a	DNET	Diptera Chironomidae	98
2022a	DNET	Amphipoda Gammaridae	3
2022a	DNET	Ephemeroptera Leptohyphidae	31
2022a	DNET	Diptera Ceratopogonidae	5
2022a	DNET	Ephemeroptera Caenidae	4
2022a	Surber	Diptera Chironomidae	31
2022a	Surber	Coleoptera Elmidae	11
2022a	Surber	Trichoptera Hydropsychidae	21
2022a	Surber	Ephemeroptera Baetidae	5

2022a	Surber	Coleoptera Gyrinidae	5
2022a	Surber	Ephemeroptera Caenidae	11
2022a	Surber	Ephemeroptera Leptohyphidae	26
2022a	Surber	Amphipoda Gammaridae	2
2022b	DNET	Diptera Chironomidae	57
2022b	DNET	Coleoptera Elmidae	11
2022b	DNET	Ephemeroptera Caenidae	32
2022b	DNET	Ephemeroptera Leptohyphidae	15
2022b	DNET	Amphipoda Gammaridae	4
2022b	DNET	Araneae Tetragnathidae	1
2022b	DNET	Odonata Coeagrionidae	9
2022b	DNET	Odonata Gomphidae	1
2022b	DNET	Hempitera Nepidae	1
2022b	DNET	Annelida Oligochaeta	2
2022b	DNET	Annelida Polychaeta	3
2022b	DNET	Ephemeroptera Heptageniidae	5
2022b	DNET	Hemiptera Corixidae	6
2022b	DNET	Coleoptera Hydrophilidae	11
2022b	DNET	Coleoptera Haliplidae	1
2022b	DNET	Coleoptera Gyrinidae	2
2022b	DNET	Trichoptera Hydropsychidae	3
2022b	Surber	Coleoptera Elmidae	20
2022b	Surber	Diptera Chironomidae	3
2022b	Surber	Ephemeroptera Caenidae	12
2022b	Surber	Ephemeroptera Leptohyphidae	10
2022b	Surber	Ephemeroptera Isonychiidae	1
2022b	Surber	Ephemeroptera Heptageniidae	1
2022b	Surber	Ephemeroptera Baetidae	6
2022b	Surber	Ephemeroptera Baetiscidae	1
2022b	Surber	Trichoptera Hydropsychidae	1
2022b	Surber	Amphipoda Gammaridae	1

Table B.2 The fish species collected for site two over each sampling trip with number of fish that showed to have imperfections.

Date	Common Name	Scientific Name	Abundance	Impurities
2022a	Red Shiner	*Cyprinidae Cyprinella lutrensis*	95	1

2022a	Bullhead Minnow	*Cyprinidae Pimephales vigilax*	57	4
2022a	Channel Catfish	*Ictaluridae Ictalurus punctatus*	1	0
2022a	Freckled Madtom	*Ictaluridae Noturus nocturnus*	13	1
2022a	Harlequin Darter	*Percidae Etheostoma histrio*	3	0
2022a	Dusky Darter	*Percidae Percina sciera*	5	0
2022b	Channel Catfish	*Ictaluridae Ictalurus punctatus*	19	0
2022b	Bullhead Minnows	*Cyprinidae Pimephales vigilax*	999	133
2022b	Freckled Madtoms	*Ictaluridae Noturus nocturnus*	86	0
2022b	Red Shiner	*Cyprinidae Cyprinella lutrensis*	169	5
2022b	Dollar Sunfish	*Centrarchidae Lepomis marginatus*	71	2
2022b	Longear Sunfish	*Centrarchidae Lepomis megalotis*	34	0
2022b	Spotted Bass	*Centrarchidae Micropterus punctulatus*	9	0
2022b	Largemouth Bass	*Centrarchidae Micropterus salmoides*	2	0
2022b	Blacktail Shiner	*Cyprinidae Cyprinella venusta*	9	0
2022b	Ribbon Shiner	*Cyprinidae Lythrurus fumeus*	2	0
2022b	Sabine Shiner	*Cyprinidae Notropis sabinae*	1	0
2022b	Western Mosquitofish	*Poeciliidae Gambusia affinis*	1	0
2022b	Harlequin Darter	*Percidae Etheostoma histrio*	1	0
2022b	Dusky Darter	*Percidae Percina sciera*	11	0
2022b	Redspot Darter	*Percidae Etheostoma artesiae*	3	0
2022b	Pallid Shiner	*Cyprinidae Hybopsis amnis*	1	0
2022b	Freshwater Drum	*Sciaenidae Aplodinotus grunniens*	1	0

Table B.3 Site two B-IBI and IBI raw scores for each sampling trip

	DNET	Surber	Fish
2019	21	33	N/A
2020	26	33	N/A
2022a	18	33	34
2022b	21	31	48

APPENDIX C

Site 3- very rocky, gravel substrate with high flow rate and many riffles. The water column was fairly shallow throughout and there was high substrate stability with good riparian vegetation.

Table C.1 The macroinvertebrates collected for site three over each sampling trip according to sampling method.

Year	Sampling Method	Order + Family	Abundance
2019	DNET	Diptera Chironomidae	2991
2019	DNET	Amphipoda Gammaridae	25
2019	DNET	Coleoptera Hydrophilidae	4
2019	DNET	Ephemeroptera Caenidae	336
2019	DNET	Trichoptera Hydropsychidae	986
2019	DNET	Odonata Calopterygidae	2
2019	DNET	Megaloptera Corydalidae	73
2019	DNET	Trichoptera Philopotamidae	1
2019	DNET	Coleoptera Dytiscidae	1
2019	DNET	Coleoptera Scirtidae	5
2019	DNET	Coleoptera Elmidae	70
2019	DNET	Ephemeroptera Baetidae	39
2019	DNET	Ephemeroptera Heptageniidae	8
2019	DNET	Diptera Ceratopogonidae	18
2019	DNET	Trichoptera Hydroptilidae	3
2019	DNET	Odonata Coenagrionidae	5
2019	DNET	Dolomedes Pisaridae	2
2019	DNET	Trichoptera Leptoceridae	24
2019	DNET	Trichoptera Molannidae	2
2019	DNET	Lepidoptera Noctuidae	2
2019	DNET	Diptera Tipulidae	2
2019	DNET	Trichoptera	1
2019	DNET	Ephemeroptera Leptohypidae	431
2019	Surber	Diptera Chironomidae	552
2019	Surber	Amphipoda Gammaridae	15
2019	Surber	Ephemeroptera Caenidae	68
2019	Surber	Trichoptera Hydropsychidae	283
2019	Surber	Diptera Ceratopogonidae	6
2019	Surber	Coleoptera Elmidae	20
2019	Surber	Ephemeroptera Heptageniidae	3
2019	Surber	Ephemeroptera Baetidae	19
2019	Surber	Megaloptera Corydalidae	10

2019	Surber	Coleoptera Scirtidae	1
2019	Surber	Trichoptera Hydroptilidae	1
2019	Surber	Trichoptera Leptoceridae	2
2019	Surber	Diptera Empididae	1
2019	Surber	Odonata Coenagrionidae	1
2019	Surber	Ephemeroptera Leptohypidae	63
2020	DNET	Coleoptera Elmidae	20
2020	DNET	Amphipoda Gammaridae	33
2020	DNET	Ephemeroptera Heptageniidae	23
2020	DNET	Ephemeroptera Baetidae	151
2020	DNET	Trichoptera Hydropsychidae	368
2020	DNET	Ephemeroptera Leptohypidae	77
2020	DNET	Diptera Chironomidae	156
2020	DNET	Ephemeroptera Caenidae	35
2020	DNET	Annelida Oligochaete	12
2020	DNET	Megaloptera Corydalidae	4
2020	DNET	Coleoptera Hydrophilidae	1
2020	DNET	Trichoptera Leptoceridae	3
2020	DNET	Coleoptera Chrysomelidae	1
2020	DNET	Diptera Simuliidae	110
2020	Surber	Diptera Chironomidae	28
2020	Surber	Coleoptera Elmidae	21
2020	Surber	Trichoptera Hydropsychidae	361
2020	Surber	Ephemeroptera Baetidae	75
2020	Surber	Ephemeroptera Heptageniidae	46
2020	Surber	Ephemeroptera Leptohypidae	57
2020	Surber	Ephemeroptera Caenidae	5
2020	Surber	Amphipoda Gammaridae	22
2020	Surber	Diptera Ceratopogonidae	1
2020	Surber	Megaloptera Corydalidae	10
2020	Surber	Annelida Oligochaete	2
2020	Surber	Diptera Simuliidae	6
2020	Surber	Ephemeroptera Leptohypidae	2
2020	Surber	Coleoptera Gyrinidae	1
2020	Surber	Plecoptera Perlidae	1
2020	Surber	Collembola	1
2022a	DNET	Diptera Chironomidae	152
2022a	DNET	Procambarus Dupratzi	13
2022a	DNET	Trichoptera Hydropsychidae	67

2022a	DNET	Coleoptera Elmidae	18
2022a	DNET	Ephemeroptera Caenidae	21
2022a	DNET	Ephemeroptera Leptohyphidae	110
2022a	DNET	Amphipoda Gammaridae	7
2022a	Surber	Diptera Chironomidae	198
2022a	Surber	Coleoptera Elmidae	25
2022a	Surber	Trichoptera Hydropsychidae	78
2022a	Surber	Amphipoda Gammaridae	44
2022a	Surber	Trichoptera Polycentropodidae	17
2022a	Surber	Ephemeroptera Leptohyphidae	155
2022a	Surber	Ephemeroptera Heptageniidae	1
2022a	Surber	Ephemeroptera Caenidae	41
2022a	Surber	Ephemeroptera Baetidae	8
2022a	Surber	Ephemeroptera Baetiscidae	1
2022b	DNET	Diptera Chironomidae	32
2022b	DNET	Coleoptera Elmidae	11
2022b	DNET	Trichoptera Hydropsychidae	11
2022b	DNET	Ephemeroptera Caenidae	19
2022b	DNET	Ephemeroptera Leptohyphidae	9
2022b	DNET	Plecoptera Perlidae	2
2022b	DNET	Plecoptera Chloroperlidae	1
2022b	DNET	Ephemeroptera Heptageniidae	2
2022b	DNET	Ephemeroptera Baetidae	4
2022b	DNET	Odonata Gomphidae	9
2022b	DNET	Odonata Coeagrionidae	2
2022b	DNET	Annelida Hirudinea	1
2022b	DNET	Ephemeroptera Ephemeridae	1
2022b	DNET	Coleoptera Hydrophilidae	4
2022b	DNET	Odonata Macromiidae	6
2022b	DNET	Hemiptera Corixidae	15
2022b	DNET	Ephemeroptera Ameletidae	2
2022b	DNET	Amphipoda Gammaridae	4
2022b	Surber	Coleoptera Elmidae	81
2022b	Surber	Megaloptera Corydalidae	5
2022b	Surber	Diptera Chironomidae	86
2022b	Surber	Coleoptera Gyrinidae	11
2022b	Surber	Odonata Coeagrionidae	1
2022b	Surber	Odonata Gomphidae	1
2022b	Surber	Ephemeroptera Oligoneuriidae	11

2022b	Surber	Coleoptera Corixidae	1
2022b	Surber	Trichoptera Hydropsychidae	91
2022b	Surber	Trichoptera Polycentropodidae	3
2022b	Surber	Ephemeroptera Heptageniidae	21
2022b	Surber	Ephemeroptera Caenidae	127
2022b	Surber	Ephemeroptera Baetidae	18
2022b	Surber	Ephemeroptera Leptohyphidae	222

Table C.2 The fish species collected for site three over each sampling trip with number of fish that showed to have imperfections.

Date	Common Name	Scientific Name	Abundance	Impurities
2022a	Dollar Sunfish	*Centrarchidae Lepomis marginatus*	3	0
2022a	Longear Sunfish	*Centrarchidae Lepomis megalotis*	11	0
2022a	Largemouth Bass	*Centrarchidae Micropterus salmoides*	2	0
2022a	Red Shiner	*Cyprinidae Cyprinella lutrensis*	124	5
2022a	Blacktail Shiner	*Cyprinidae Cyprinella venusta*	4	1
2022a	Ribbon Shiner	*Cyprinidae Lythrurus fumeus*	1	0
2022a	Ghost Shiner	*Cyprinidae Notropis buchanani*	6	0
2022a	Sabine Shiner	*Cyprinidae Notropis sabinae*	3	0
2022a	Weed Shiner	*Cyprinidae Notropis texanus*	9	0
2022a	Bullhead Minnow	*Cyprinidae Pimephales vigilax*	103	2
2022a	Channel Catfish	*Ictaluridae Ictalurus punctatus*	14	3
2022a	Freckled Madtom	*Ictaluridae Noturus nocturnus*	17	1
2022a	Flathead Catfish	*Ictaluridae Pylodictis olivaris*	2	0
2022a	Longnose Gar (let go)	*Lepisosteidae Lepisosteus osseus*	1	0
2022a	Scaly Sand Darter	*Percidae Ammocrypta vivax*	1	0

2022a	Redpot Darter	*Percidae Etheostoma artesiae*	1	0
2022a	Mud Darter	*Percidae Etheostoma asprigene*	1	0
2022a	Harlequin Darter	*Percidae Etheostoma histrio*	5	0
2022a	River Darter	*Percidae Percina schumardi*	1	0
2022a	Dusky Darter	*Percidae Percina sciera*	10	0
2022b	Bullhead Minnow	*Cyprinidae Pimephales vigilax*	766	60
2022b	Freckled Madtom	*Ictaluridae Noturus nocturnus*	119	0
2022b	Blacktail Shiner	*Cyprinidae Cyprinella venusta*	5	0
2022b	Channel Catfish	*Ictaluridae Ictalurus punctatus*	119	1
2022b	Blackstripe Topminnow	*Fundulidae Fundulus notatus*	4	0
2022b	Harlesquin Darter	*Percidae Etheostoma histrio*	2	0
2022b	Dusky Darter	*Percidae Percina sciera*	22	0
2022b	River Darter	*Percidae Percina schumardi*	2	0
2022b	Mimic Shiner	*Cyprinidae Notropis volucellus*	1	0
2022b	Weed Shiner	*Cyprinidae Notropis texanus*	2	0
2022b	Ghost Shiner	*Cyprinidae Notropis buchanani*	1	0
2022b	Sabine Shiner	*Cyprinidae Notropis sabinae*	2	0
2022b	Red Shiners	*Cyprinidae Cyprinella lutrensis*	184	5
2022b	Redear Sunfish	*Centrarchidae Lepomis microlophus*	1	0
2022b	Longear Sunfsih	*Centrarchidae Lepomis megalotis*	20	2
2022b	Dollar Sunfish	*Centrarchidae Lepomis marginatus*	97	6
2022b	Bluegill	*Centrarchidae Lepomis macrochirus*	1	0

2022b	Spotted Bass	*Centrarchidae Micropterus punctulatus*	2	0

Table C.3 Site three B-IBI and IBI raw scores for each sampling trip

	DNET	Surber	Fish
2019	22	27	N/A
2020	29	33	N/A
2022a	18	31	46
2022b	24	33	50

APPENDIX D

Site 4- rocky, gravel substrate with high flow rate and many riffles throughout. There was a small riparian zone in the middle of the site that provided a break in the width of the river. The water column seemed to stay shallow in most of the areas with some drop offs scattered throughout.

Table D.1 The macroinvertebrates collected for site four over each sampling trip according to sampling method.

Year	Sampling Method	Order + Family	Abundance
2019	DNET	Diptera Chironomidae	717
2019	DNET	Ephemeroptera Caenidae	129
2019	DNET	Coleoptera Scirtidae	1
2019	DNET	Plecoptera Perlidae	14
2019	DNET	Trichoptera Hydropsychidae	100
2019	DNET	Trichoptera Philopotamidae	1
2019	DNET	Megaloptera Corydalidae	13
2019	DNET	Amphipoda Gammaridae	41
2019	DNET	Ephemeroptera Heptageniidae	44
2019	DNET	Diptera Ceratopogonidae	2
2019	DNET	Ephemeroptera Baetidae	39
2019	DNET	Coleoptera Elmidae	38
2019	DNET	Trichoptera Limnephilidae	2
2019	DNET	Odonata Coenagrionidae	3
2019	DNET	Plecoptera	1
2019	DNET	Trichoptera	2
2019	DNET	Trichoptera Leptoceridae	23
2019	Surber	Ephemeroptera Leptohypidae	248
2019	Surber	Ephemeroptera Baetidae	8
2019	Surber	Ephemeroptera Caenidae	31
2019	Surber	Ephemeroptera Heptageniidae	8
2019	Surber	Coleoptera Scirtidae	2
2019	Surber	Diptera Chironomidae	66
2019	Surber	Coleoptera Elmidae	5
2019	Surber	Trichoptera Hydropsychidae	18
2019	Surber	Odonata Calopterygidae	1
2019	Surber	Diptera Tipulidae	1
2019	Surber	Trichoptera Polycentropodidae	1
2019	Surber	Trichoptera Philopotamidae	1
2019	Surber	Plecoptera Perlidae	1

2019	Surber	Trichoptera Leptoceridae	3
2019	Surber	Ephemeroptera Leptohypidae	45
2020	DNET	Ephemeroptera Baetidae	11
2020	DNET	Amphipoda Gammaridae	8
2020	DNET	Ephemeroptera Heptageniidae	7
2020	DNET	Coleoptera Elmidae	14
2020	DNET	Trichoptera Hydropsychidae	38
2020	DNET	Ephemeroptera Leptohypidae	43
2020	DNET	Ephemeroptera Caenidae	7
2020	DNET	Diptera Ceratopogonidae	1
2020	DNET	Diptera Chironomidae	24
2020	DNET	Annelida Hirudinea	2
2020	DNET	Coleoptera Gyrinidae	1
2020	DNET	Megaloptera Corydalidae	1
2020	DNET	Trichoptera Leptoceridae	2
2020	DNET	Diptera Simuliidae	1
2020	DNET	Odonata Calopterygidae	1
2020	DNET	Trichoptera Molannidae	1
2020	Surber	Ephemeroptera Heptageniidae	35
2020	Surber	Amphipoda Gammaridae	21
2020	Surber	Coleoptera Elmidae	34
2020	Surber	Trichoptera Hydropsychidae	99
2020	Surber	Ephemeroptera Baetidae	20
2020	Surber	Ephemeroptera Leptohypidae	72
2020	Surber	Diptera Chironomidae	25
2020	Surber	Ephemeroptera Caenidae	20
2020	Surber	Annelida Oligochaete	8
2020	Surber	Diptera Simuliidae	1
2020	Surber	Coleoptera Scirtidae	3
2020	Surber	Megaloptera Corydalidae	1
2020	Surber	Plecoptera Perlidae	2
2020	Surber	Trichoptera Leptoceridae	2
2020	Surber	Coleoptera Amphizoidae	2
2022a	DNET	Diptera Chironomidae	150
2022a	DNET	Coleoptera Gyrinidae	4
2022a	DNET	Coleoptera Elmidae	26
2022a	DNET	Ephemeroptera Oligoneuriidae	1
2022a	DNET	Trichoptera Hydropsychidae	163
2022a	DNET	Trichoptera Polycentropodidae	6

2022a	DNET	Amphipoda Gammaridae	4
2022a	DNET	Ephemeroptera Heptageniidae	2
2022a	DNET	Ephemeroptera Leptohyphidae	24
2022a	DNET	Ephemeroptera Baetidae	6
2022a	DNET	Ephemeroptera Caenidae	2
2022a	DNET	Procambarus Dupratzi	2
2022a	DNET	Plecoptera Perlidae	1
2022a	Surber	Coleoptera Elmidae	95
2022a	Surber	Ephemeroptera Heptageniidae	21
2022a	Surber	Diptera Chironomidae	47
2022a	Surber	Coleoptera Gyrinidae	1
2022a	Surber	Coleoptera Dytiscidae	1
2022a	Surber	Ephemeroptera Oligoneuriidae	4
2022a	Surber	Plecoptera Perlidae	4
2022a	Surber	Megaloptera Corydalidae	1
2022a	Surber	Trichoptera Hydropsychidae	503
2022a	Surber	Diptera Simuliidae	1
2022a	Surber	Amphipoda Gammaridae	5
2022a	Surber	Trichoptera Polycentropodidae	7
2022a	Surber	Ephemeroptera Caenidae	15
2022a	Surber	Ephemeroptera Leptohyphidae	60
2022a	Surber	Ephemeroptera Baetidae	48
2022b	DNET	Coleoptera Elmidae	19
2022b	DNET	Diptera Stratiomyidae	1
2022b	DNET	Diptera Chironomidae	40
2022b	DNET	Trichoptera Hydropsychidae	61
2022b	DNET	Ephemeroptera Leptohyphidae	49
2022b	DNET	Ephemeroptera Caenidae	13
2022b	DNET	Ephemeroptera Baetidae	10
2022b	DNET	Ephemeroptera Heptageniidae	3
2022b	DNET	Coleoptera Hydrophilidae	2
2022b	DNET	Hemiptera Corixidae	1
2022b	DNET	Plecoptera Perlidae	2
2022b	DNET	Trichoptera Glossosomatidae	2
2022b	DNET	Diptera Muscidae	1
2022b	DNET	Odonata Macromiidae	1
2022b	DNET	Araneae Pisauridae	1
2022b	DNET	Araneae Tetragnathidae	1
2022b	DNET	Megaloptera Corydalidae	3

2022b	Surber	Diptera Chironomidae	317
2022b	Surber	Trichoptera Glossosomatidae	4
2022b	Surber	Coleoptera Gyrinidae	4
2022b	Surber	Coleoptera Hydrophilidae	1
2022b	Surber	Trichoptera Hydropsychidae	384
2022b	Surber	Coleoptera Elmidae	111
2022b	Surber	Ephemeroptera Ephemerellidae	3
2022b	Surber	Amphipoda Gammaridae	3
2022b	Surber	Ephemeroptera Baetidae	48
2022b	Surber	Ephemeroptera Baetiscidae	79
2022b	Surber	Ephemeroptera Heptageniidae	8
2022b	Surber	Ephemeroptera Caenidae	343
2022b	Surber	Ephemeroptera Leptohyphidae	1062

Table D.2 The fish species collected for site four over each sampling trip with number of fish that showed to have imperfections.

Date	Common Name	Scientific Name	Abundance	Impurities
2022a	River Carpsucker	*Catostomidae Carpiodes carpio*	1	1
2022a	Bluegill	*Centrarchidae Lepomis macrochirus*	1	0
2022a	Longear Sunfish	*Centrarchidae Lepomis megalotis*	6	2
2022a	Largemouth Bass	*Centrarchidae Micropterus salmoides*	1	0
2022a	Red Shiner	*Cyprinidae Cyprinella lutrensis*	77	0
2022a	Pallid Shiner	*Cyprinidae Hybopsis amnis*	2	0
2022a	Ribbon Shiner	*Cyprinidae Lythrurus fumeus*	4	0
2022a	Shoal Chub	*Cyprinidae Macrhybopsis hyostoma*	1	0
2022a	Bullhead Minnow	*Cyprinidae Pimephales vigilax*	48	4
2022a	Channel Catfish	*Ictaluridae Ictalurus punctatus*	3	1
2022a	Freckled Madtom	*Ictaluridae Noturus nocturnus*	8	0

2022a	Flathead Catfish	*Ictaluridae Pylodictis olivaris*	1	0
2022a	Longnose Gar (let go)	*Lepisosteidae Lepisosteus osseus*	1	0
2022a	Harlequin Darter	*Percidae Etheostoma histrio*	13	0
2022a	River Darter	*Percidae Percina schumardi*	2	0
2022a	Dusky Darter	*Percidae Percina sciera*	2	0
2022b	Freckled Madtom	*Ictaluridae Noturus nocturnus*	36	1
2022b	Red Shiners	*Cyprinidae Cyprinella lutrensis*	256	6
2022b	Bullhead Minnows	*Cyprinidae Pimephales vigilax*	267	7
2022b	Channel Catfish	*Ictaluridae Ictalurus punctatus*	13	4
2022b	Harlequin Darter	*Percidae Etheostoma histrio*	9	0
2022b	Ribbon Shiner	*Cyprinidae Lythrurus fumeus*	1	0
2022b	Scaly Sand Darter	*Percidae Ammocrypta vivax*	1	0
2022b	Mud Darter	*Percidae Etheostoma asprigene*	1	0
2022b	Pallid Shiner	*Cyprinidae Hybopsis amnis*	21	0
2022b	Largemouth Bass	*Centrarchidae Micropterus salmoides*	5	0
2022b	Dollar Sunfish	*Centrarchidae Lepomis marginatus*	82	2
2022b	Longear Sunfish	*Centrarchidae Lepomis megalotis*	17	0
2022b	Flathead Catfish	*Ictaluridae Pylodictis olivaris*	1	1
2022b	Spotted Bass	*Centrarchidae Micropterus punctulatus*	10	1
2022b	Dusky Darter	*Percidae Percina sciera*	15	1
2022b	Blacktail Shiner	*Cyprinidae Cyprinella venusta*	53	2
2022b	Blackspot Shiner	*Cyprinidae Notropis atrocaudalis*	5	0

Table D.3 Site four B-IBI and IBI raw scores for each sampling trip

	DNET	Surber	Fish
2019	21	35	N/A
2020	28	37	N/A
2022a	22	37	44
2022b	27	29	46

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