Continued assessment of early invasion dynamics of Asian swamp eels in the Lake Pontchartrain Estuary

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ABSTRACT

Cuchia (*Amphipnous cuchia*) are air-breathing freshwater fish in the family Synbranchidae. Native to Southeastern Asia, these nocturnal, opportunistic predators are capable of surviving in a wide variety of harsh environments and even possess the ability to travel across land when necessary. Cuchia were discovered in Bayou St. John in New Orleans in June 2019, though a population may have been established in Bayou St. John before their initial discovery. The presence of Cuchia in Bayou St. John is of concern because this waterway is hydrologically connected to Lake Pontchartrain, which in turn is connected to the vast Mississippi River and its connected waterways. To better understand the invasion dynamics of this species, we used throw traps, seines, and dip nets to monitor the abundance and distribution of Cuchia at 12 sampling sites across Bayou St. John and two nearby sites in Lake Pontchartrain during the summers of 2019 through 2023. Abiotic factors (e.g., salinity, dissolved oxygen, conductivity) and biotic factors (e.g., plant biomass, species richness, abundance) were also quantified to correlate with the abundance of Cuchia. A rough estimate of the average density of Cuchia in Bayou St. John is 0.038 individual Cuchia per m^2, with an estimated total of around 1,000 Cuchia currently occupying Bayou St. John. Adult and young of year Cuchia were found during each summer, indicating Cuchia have established a reproducing population in Bayou St. John. Fortunately, neither the abundance nor distribution of Cuchia has changed much during the past five years of monitoring. This recently established population of Cuchia is growing slowly and remains in the lag phase of a potential invasion curve. Although their distribution and abundance have not drastically increased, Cuchia may already be having negative effects on the overall abundance of a number of native taxa, including Western Mosquitofish, grass shrimp, and crawfish. This report is based on an honors thesis submitted by Miranda Buckheit in partial fulfillment of the requirements for a Bachelor of Science degree in Biological Sciences with a concentration in Marine Biology.

INTRODUCTION

When referring to a species that is not native to a particular area, it is incorrect to use the terms "introduced" and "invasive" interchangeably. The major distinction between the two is whether or not said species is having a negative impact on its new surroundings. The U.S. National Park Service (2020) defines an introduced species as an organism that does not naturally occur in an area but is introduced as the result of deliberate or accidental human activities. The change from introduced to invasive is instantaneous once that species begins to cause harm to native species or affects socioeconomic systems. Some notable aquatic invasive species include the Burmese Python (*Python molurus bivittatus*) in the Florida Everglades (Dorcas et al., 2012) and lionfishes (*Pterois volitans* and *P. miles*) in the western Atlantic and Caribbean (Finch et. al, 2024). Rio Grande Cichlids (*Herichythys cyanoguttatus),* Nutria (*Myocastor coyous*), and Water Hyacinth (*Eichhorina crassipes*) are examples of aggressive aquatic invasive species in Louisiana.

Invasive species pose a great threat to the stability of ecosystems because they compete directly with local predators, reduce the abundance of native animals and plants, alter the genetics of native populations, and transmit non-native parasites and diseases that can infect and decimate native taxa (Bunkley-Williams and Williams, 1994). In addition to introducing new parasites, some invasive species also increase transmission of native parasites (Chalkowski et al., 2018).

Invasion curves (Figure 1) are commonly used to depict the progression of a species once it has been introduced into a new area. At the beginning of the curve – known as the "lag phase" – populations of introduced species are relatively small and occupy a small enough area that eradication may be possible and economically feasible. As time progresses, the cost of managing an introduced species increases exponentially and the ability to eradicate the introduced species becomes more difficult. Eventually, the introduced population reaches a long-term phase where removal is no longer an option and management is aimed at suppressing abundance and further spread (Figure 1). Billions of dollars are spent each year in attempts to repair the damage caused by invasive species, making it all-the-more important to aggressively manage introduced species as soon as they are discovered (Havel et al., 2015; Zenni et al., 2021).

Cuchia (*Amphipnous cuchia*) are one of several fish species commonly known as Asian swamp eels (Figure 4) that belong to the Family Synbranchidae. Cuchia is native to Southeast Asia, including Bangladesh, India, and Nepal (Nico et al., 2019) and are fossorial (i.e. burrowing), nocturnal, opportunistic predators that feed on a wide variety of aquatic invertebrates, small fishes, and amphibians (Jordan et al., 2020). Cuchia are able to survive in harsh environments, tolerating rapid changes in salinity even in their young-of-year stage, and going without food for up to a year if resources dwindle (Nico et al., 2019; Schofield and Nico, 2009). Most swamp eels are facultative air breathers, but Cuchia is unique in that they are obligate air breathers. They possess posterodorsal extended pouches of the pharynx (Munshi et al., 1989) that allow them to breathe air (Figure 4). Cuchia are therefore able to tolerate low oxygen environments, and even relocate to new aquatic habitats when environmental conditions become inhospitable (Nico et al., 2019). Cuchia are benthic spawners, with their breeding season occurring during the spring and summer months.

Cuchia were first discovered in Bayou St. John in June of 2019, with both adult and young-of-year collected along the bayou's littoral zone. It is unclear how Cuchia were first introduced into Bayou St. John, but it is hypothesized that they were illegally sold at Asian food markets in

New Orleans and then either used as fishing bait or released during Buddhist prayer release rituals (Nico et al., 2019).

The presence of Cuchia in Bayou St. John is of great concern because the waterway is hydrologically connected to Lake Pontchartrain, which in turn is connected to the coastal wetlands of the Gulf of Mexico, and then the Mississippi River Basin. Salinity studies conducted on another genus of synbranchids, *Monopterus*, found that swamp eels can tolerate rapid salinity shifts from 0-16 ppt, and even survive in areas with consistent salinities as high as 14 ppt for extended periods of time (Schofield and Nico, 2009). Cuchia appear to be equally tolerant to elevated salinities (unpublished data, Frank Jordan). Seeing as the salinity of the Lake Pontchartrain estuary ranges from 1.5 – 8 ppt (Smith, 2015), it may be possible for Cuchia to spread and establish a population within the lake, which could negatively alter its overall biodiversity. This tolerance to a wide variety of salinities would also benefit Cuchia if they were able to spread from the Lake Pontchartrain Estuary into the Mississippi River Basin, as they would then have access to up to 41% of the continental U.S.'s freshwater habitats.

The abundance and distribution of *Monopterus* spp. introduced into the Florida Everglades have grown and spread to the point that they are considered invasive. That is, they are at the point along the invasion curve where they are negatively affecting on a number of native Everglades taxa, including Flagfish (*Jordanella floridae)*, Marsh Killifish (*Fundulus confluentus)*, and commercially important crawfishes (Pintar et al., 2023). Invasive *Monopterus* populations have also been found in Georgia, with adults being collected in the vegetated marshes of the Chattahoochee River (Taylor et al., 2021).

Amphipnous cuchia are similar ecologically to *Monopterus* spp., making the findings in the Florida Everglades and the Chattahoochee River relevant to this project. The research done in the

Everglades “*highlights how little is known about the environmental drivers of non-native fish populations following introduction and establishment, or the ability to predict impacts of invasive fishes in wetlands*” (Pintar et al., 2023), further emphasizing the importance of understanding Cuchia as their population persists in Bayou St. John.

For my thesis, I oversaw all aspects of field and laboratory research during the fifth and final year of a five-year monitoring project carried out in Dr. Jordan’s lab. The overall objective of this project was to monitor the abundance and distribution of Cuchia and associated nekton across Bayou St. John and to characterize the early invasion dynamics of a newly introduced aquatic species. My thesis will focus largely on results of year five. Data from the five years of monitoring will be synthesized and combined with information on salinity tolerance and molecular taxonomy into a peer-reviewed publication and, more importantly, will be shared with Louisiana Department of Wildlife and Fisheries (LDWF) and other interested parties to inform and develop a plan to manage this emerging threat to aquatic ecosystems in Louisiana and beyond.

METHODS

Field sampling

We sampled at a total of fourteen sites each summer from 2019 through 2023, with twelve sites spanning across Bayou St. John and two sites nearby Lake Pontchatrain. Sampling began by measuring salinity (mg/l), conductivity ($\mu mhos$/cm), temperature (Cº), and dissolved oxygen (D.O.) at each site using a YSI ProSolo Professional Series Digital Meter. A 1-m^2 aluminum throw trap was then placed over a patch of vegetation within the littoral zone. The throw trap was pushed down hard enough into the soil so as to create a mud seal at the bottom of the trap and ensure that no specimens escaped the 1 m^2 sampling area.

Canopy height (cm), water depth (cm), and relative coverage (%) of plant species were all measured within the throw trap before all vegetation was removed, with a portion stored for further processing. The tallest plant within the throw trap was used to measure canopy height (cm), while total plant % cover and species % cover were estimated visually. Water depth was measured at each corner and the middle of the throw trap, and then averaged to the nearest centimeter. All vegetation was rinsed them within the trap to dislodge any attached nekton, and then removed. Any large obstructions were also removed from the trap before nekton sampling began.

A bar seine with 3-mm mesh was then passed through the trap to remove Cuchia and other nekton. We repeated this process until we obtained three consecutive empty passes of the bar seine. Three throw trap samples were collected from each site for a total of 3 x 14 = 52 throw trap samples per summer sampling period. We used a combination of dip nets and bar seines to qualitatively sample a larger area at each site for 15 minutes.

All collected samples were euthanized using MS-222 and preserved in 10% formalin. The number of Apple snails (*Pomacea maculata*) and fishing spiders (*Dolomedes* spp.) found at each site were also recorded.

Laboratory processing

All vegetation samples were dried at 60º C for 2-3 days. Once dried they were then weighed to the nearest kg to determine total plant biomass of the trap.

Nekton samples collected at each site were first washed under water and then preserved in 50% isopropyl alcohol. Samples were later sorted by species and measured to the nearest mm using standard length measurements for fishes and nearest mm total length or width for macroinvertebrates. Samples were then stored again in 50% isopropyl alcohol for future reference. Cuchia samples were individually stored and frozen for future DNA sampling. A single adult Cuchia, affectionately named “Snoopy”, was kept alive in captivity for approximately two months in order to observe it’s behavior (eating patterns, breathing patterns etc.). All data were recorded in Excel, and then transferred to JMP Pro 17 for data analysis and graph building.

RESULTS

Our data shows that Cuchia distribution has not significantly increased over the past five years (Figure 6). In 2023, we captured 14 young of year and 1 adult Cuchia at site 7E. This marks the only year where Cuchia were not caught at multiple sites. The first two years of sampling saw the largest distribution and abundance of Cuchia across Bayou St. John, with samples collected at sites 5, 5S, and 7E in 2019, and 5, 6, 7E and 8 in 2020 (Figure 6). In 2021 and 2022, Cuchia were only caught between sites 5 and 7E, which is a noticeable decline in both the abundance and distribution of the Cuchia population. Over the past 5 years, the only consistent sampling site for Cuchia has been site 7E. We have not caught a single Cuchia at our sites at Lake Pontchartrain (sites 14 and 15) in the past 5 years of sampling.

Similarly, the abundance of Cuchia has not significantly increased (Figures 5 and 7). 31 Cuchia were collected in 2019 and 2020, marking 2020 the year with the highest distribution and abundance of captured Cuchia. The 2021 and 2022 sampling years saw a large decrease in abundance, with only 4 caught in 2021 and 5 caught in 2022. Young-of-year and adult Cuchia were caught during every year of sampling.

We found that over the past five years there were both temporal and spatial differences in abiotic variables such as salinity and conductivity, while temperature and dissolved oxygen remained relatively stable (Figure 8). Compared to the 2022 sampling year, there was a decline in conductivity, D.O, and salinity across all sites. Temperature was the only relatively consistent variable between 2022 and 2023, with the data showing only a slight increase at all sites. These variations are all typical of an estuarine environment, and while not statistically significant, do show expected trends over our past five years of sampling. We also saw temporal and spatial differences in total nekton species abundance, species richness, and plant biomass (Figures 9). A positive statistically significant ($p \leq 0.05$) correlation was shown between average plant biomass and overall species richness (Figure 11).

One of our biggest goals for this experiment was to find out if Cuchia were having a negative impact on a number of native species of fish and inverts. Figure 12 depicts the overall mean abundance of a number of native taxa (Western Mosquitofish, Least Killifish, Sailfin Mollies, Grass Shrimp, and crawfish) over the past five years. Utilizing MANOVA testing, our data indicates that there are weakly statistically significant ($p \leq 0.05$) declines in the abundance of Least Killifish, Western Mosquitofish, and crawfish.

DISCUSSION

To sum up our findings, we've determined that the established population of *Amphipnous cuchia* in Bayou St. John has not increased significantly in abundance or distribution over the past five years, indicating that they are still in the lag phase of the invasion curve (Figure 1). Both young-of-year and adult Cuchia were collected during all five years of sampling, indicating that this population is not only established, but *reproducing* in Bayou St. John. It is important to note that given the size distribution of adult and young-of-year individuals found in 2019, it is likely that this population had already been reproducing for some time before our study began. If this population were to move up the curve in the future, eradication would become increasingly more unlikely, until long-term management of a now permanent population would be the only option.

One of the major concerns with the discovery of Cuchia in Bayou St. John in 2019 was their potential expansion into Lake Pontchartrain and subsequently the Mississippi River Basin. Bayou St. John is hydrologically connected to Lake Pontchartrain, and through that the Mississippi River Basin, which drains about 41% of the continental U. S.'s water each year, giving Cuchia potential access to a majority of the continent if they were to spread. Fortunately, no Cuchia have been found at our sites at Lake Pontchartrain (Sites 14, 15) throughout the entirety of this project, indicating that they have yet to spread outside of Bayou St. John (Figure 5).

As seen in Figures 5 and 6, there was a massive decline in the abundance of Cuchia in 2021, with an ~87% decrease in the amount of Cuchia caught in 2020 versus 2021. The Cuchia's distribution also seemed to diminish in 2021, narrowing from Sites 5, 6, 7E, and 8 in 2020 to Sites 5 and 7E in 2021, further narrowing to only 7E in 2023.

The decline in abundance and distribution of Cuchia from 2020 to 2021 is likely due, in part, to a decrease in their preferred habitat (especially Water Hyacinth). During that year, there were combined efforts with volunteers and the Louisiana Department of Wildlife to take control of the spread of water hyacinth through physical removal and also spraying of herbicides in Bayou St. John (Guerra, 2022). Cuchia use hyacinth for shelter alongside their burrows, which when removed may disrupt their typical behavior. My predecessors in the Jordan lab suggested that removal of the Water Hyacinth may have contributed to the overall decline of the Cuchia population. However, given the time frame of this study, and the seeming lack of correlation between presence of Water Hyacinth and abundance of Cuchia in 2023, I cannot say that this hypothesis has been supported or refuted by our data.

Another major goal of this project was to determine if Cuchia are affecting native taxa in Bayou St. John. We were especially concerned about taxa such as Western Mosquitofish, Least Killifish, Sailfin Mollies, grass shrimps, and crawfishes because research in the Florida Everglades indicates that ecologically similar *Monopterus* are negatively affecting these taxa, most likely via predation (Taylor et al., 2021). It is concerning that some taxa are already showing negative trends in sites that contain Cuchia given their relatively recent introduction into Bayou St. John. Correlation does not imply causation, so future research should use lab and/or field experiments to determine the effect of Cuchia on native prey taxa.

Throughout the five years of this study, both throw traps and dip nets have been used to sample Cuchia populations. However, dip net data were excluded from several of our analyses because they were intended only to support our throw trap sampling, and have proven inconsistent over the course of the study. Throw traps sample a known area of habitat, and therefore are more helpful and accurate in estimating the total population of Cuchia in Bayou St. John, as well as accurately capturing the surrounding nekton. Dip net sampling was utilized only to capture additional Cuchia in the littoral zone, and therefore cannot be considered in analysis comparing Cuchia to species of native taxa.

Our current method of sampling Cuchia is labor intensive and only samples a small amount of habitat. As mentioned previously, Cuchia are a burrowing nocturnal species, and while we've been successful at collecting samples in the littoral zones of the sites we work in, our equipment doesn't dig deep enough for us to penetrate any of the burrows and tunnels these animals may be hiding in. This leads me to believe that another reason why we have been seeing a decline in Cuchia abundance may not be that there are actually fewer individuals, but rather that our methods haven't allowed us to accurately sample them given their fossorial behavior. A study conducted in Georgia along the Chattahoochee River also came to a similar conclusion, stating that "low captures of adult *Monopterus albus* eels in the study area have been the result of low detectability, not low abundance" (Taylor et al., 2021). We've also conducted our sampling primarily in the morning and early afternoon, and while it's true that Cuchia are somewhat active during the day (as evident by the ones we've collected previously), it would be interesting to see how our sample numbers would differ if we switched to night sampling, given that Cuchia are nocturnal. We haven't conducted night sampling in Bayou St. John for reasons including lack of resources and potential threats from alligators, New Orleanians, and other dangerous wildlife.

So far, Cuchia populations have been successfully established in both New Orleans and Texas (Best et al., 2022), while established populations of *Monopterus* spp. have been found throughout the Florida Everglades and the Chattahoochee River. Data from my thesis indicates that the Cuchia population in Bayou St. John has not increased in abundance or distribution much over the past five years and that they remain at the beginning of the invasive curve.

However, there are signs that Cuchia already may be affecting native taxa. My hope is that our data will motivate LDWF to adopt a more aggressive management plan while Cuchia are remain in the lag phase of a potential invasion curve in Louisiana.

ACKNOWLEDGMENTS

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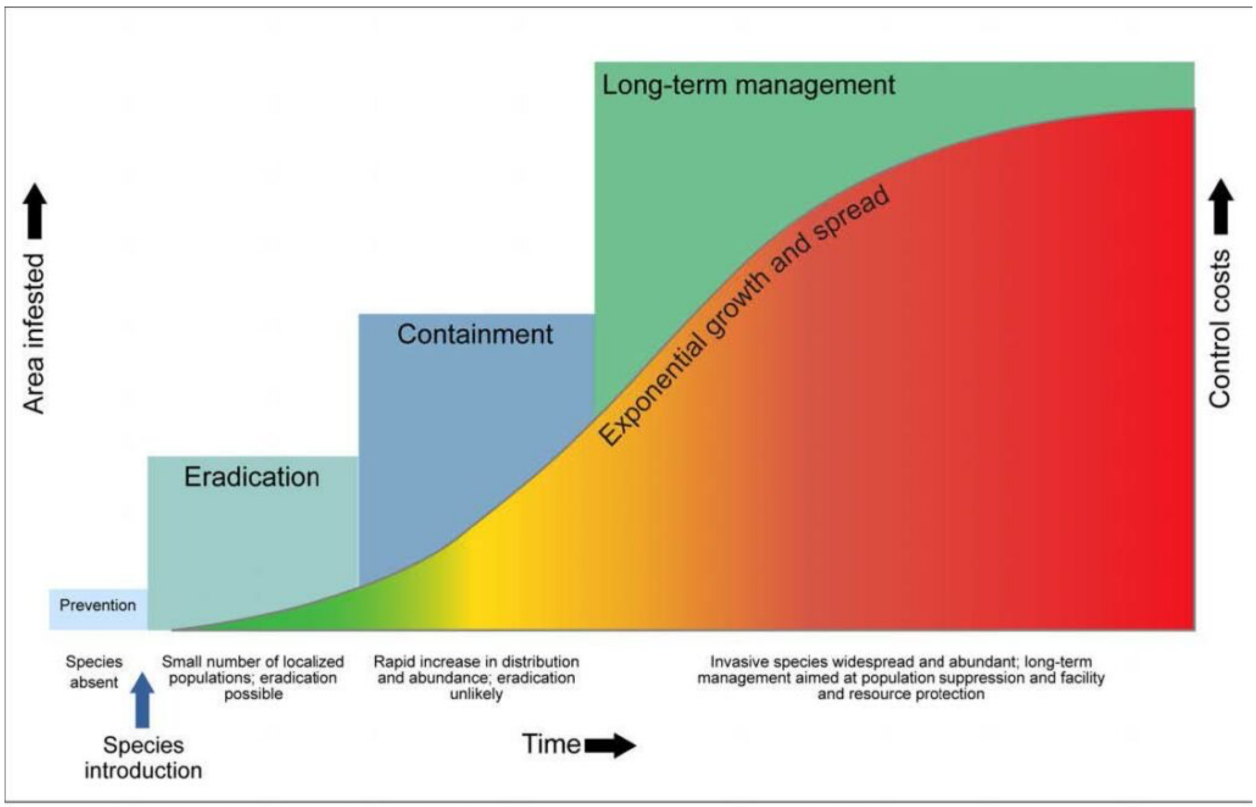
Figure 1. Invasion curve demonstrating that as time progresses following a non-native species introduction, the cost of control increases exponentially, along with the effort required to manage the species. This curve shows that when a species is in the Eradication or Containment phase, it's still possible (even if unlikely) to remove said species from an area. Once it reaches the Long-Term Management phase, the option of removing the species no longer is possible. Image borrowed without permission from the internet- actual sources cited at bottom of figure.



Sources: National Invasive Species Council; U.S. Department of Agriculture; National Park Service; U.S. Fish and Wildlife Service; Rodgers, L, South Florida Water Management District; Department of Primary Industries, State of Victoria, Australia; and GAO. | GAO-16-49

Figure 2. Map depicting the locations of all 14 sampling sites along Bayou St. John and Lake Pontchatrain.

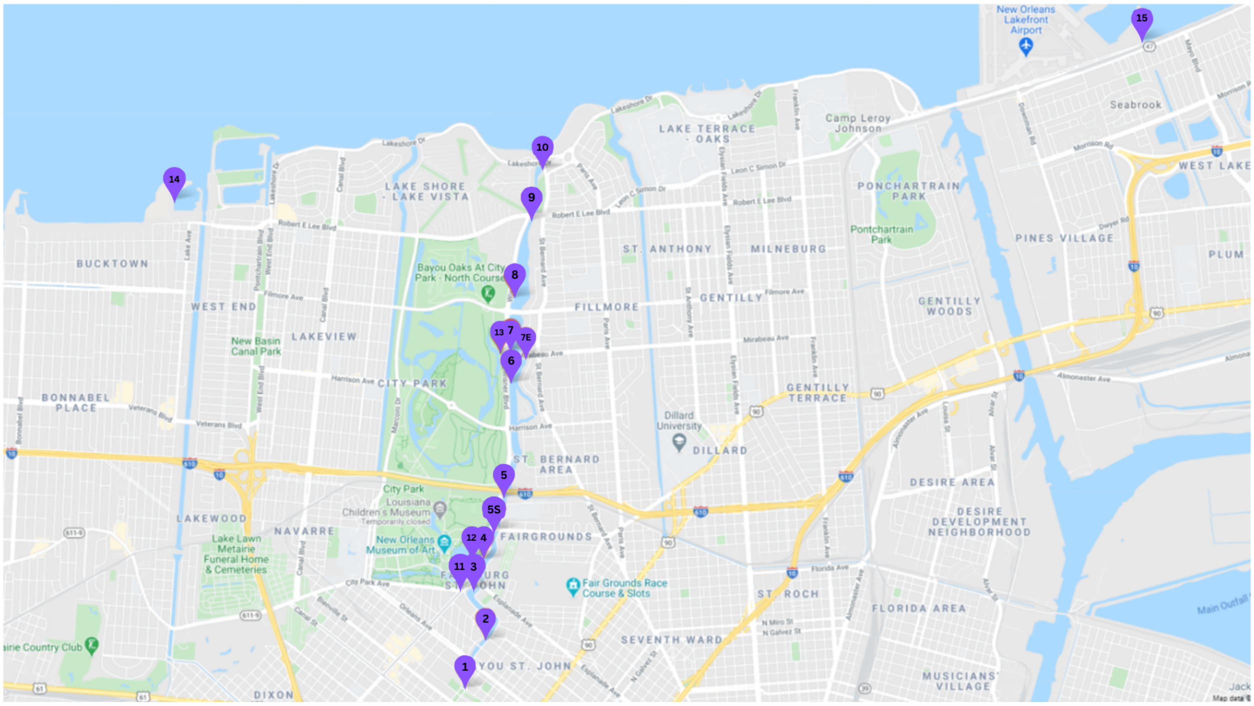


Figure 3. A) Quantitative sampling done via bar seining in a $1m^2$ throw trap B) Qualitative sampling done via dip netting across each sites littoral zone C) Collected samples being euthanized using MS-222 and 10% formalin. Pictures taken by Mojo Williams.

Figure 4. A) Image of an adult Cuchia B + C) Red arrows point towards the Suprapharyngeal pouches Cuchia use to breathe atmospheric oxygen. Image borrowed without permission from the internet.

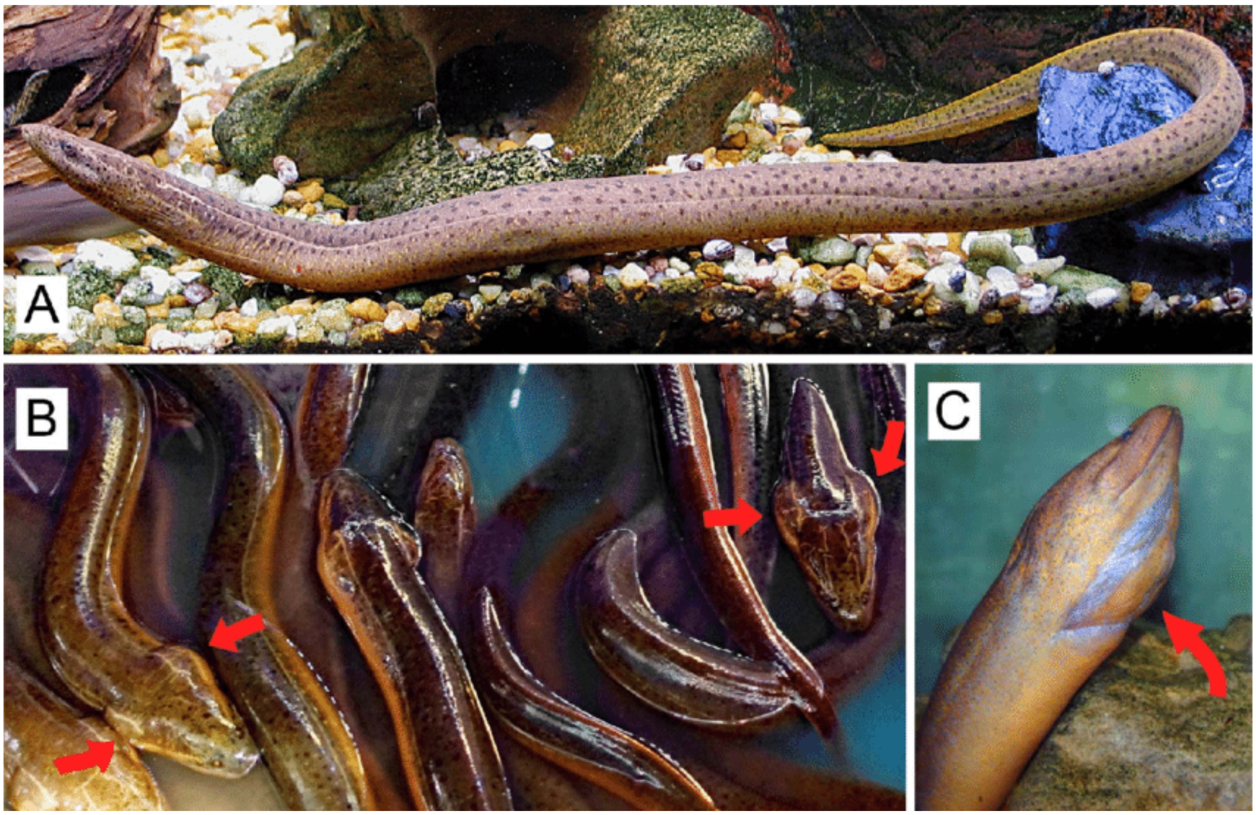


Figure 5. Quantitative and Qualitative amounts of Cuchia caught per year, equaling the total amount of Cuchia caught each year.

Year	Quantitative	Qualitative	Total Cuchia Caught
2019	31	33	64
2020	31	55	86
2021	5	0	5
2022	4	0	4
2023	15	0	15

Figure 6. Distribution and abundance of Cuchia across sites along Bayou St. John and Lake Pontchartrainfrom 2019 through 2023. Only quantitative data (Cuchia caught in throw trap) depicted. The red brackets indicate the edges of the Cuchia populations distribution, as Cuchia were only caught at Sites 5 through 8 over the past five years. The red circles on Sites 14 and 15 indicate that no Cuchia were caught in Lake Pontchartrain.

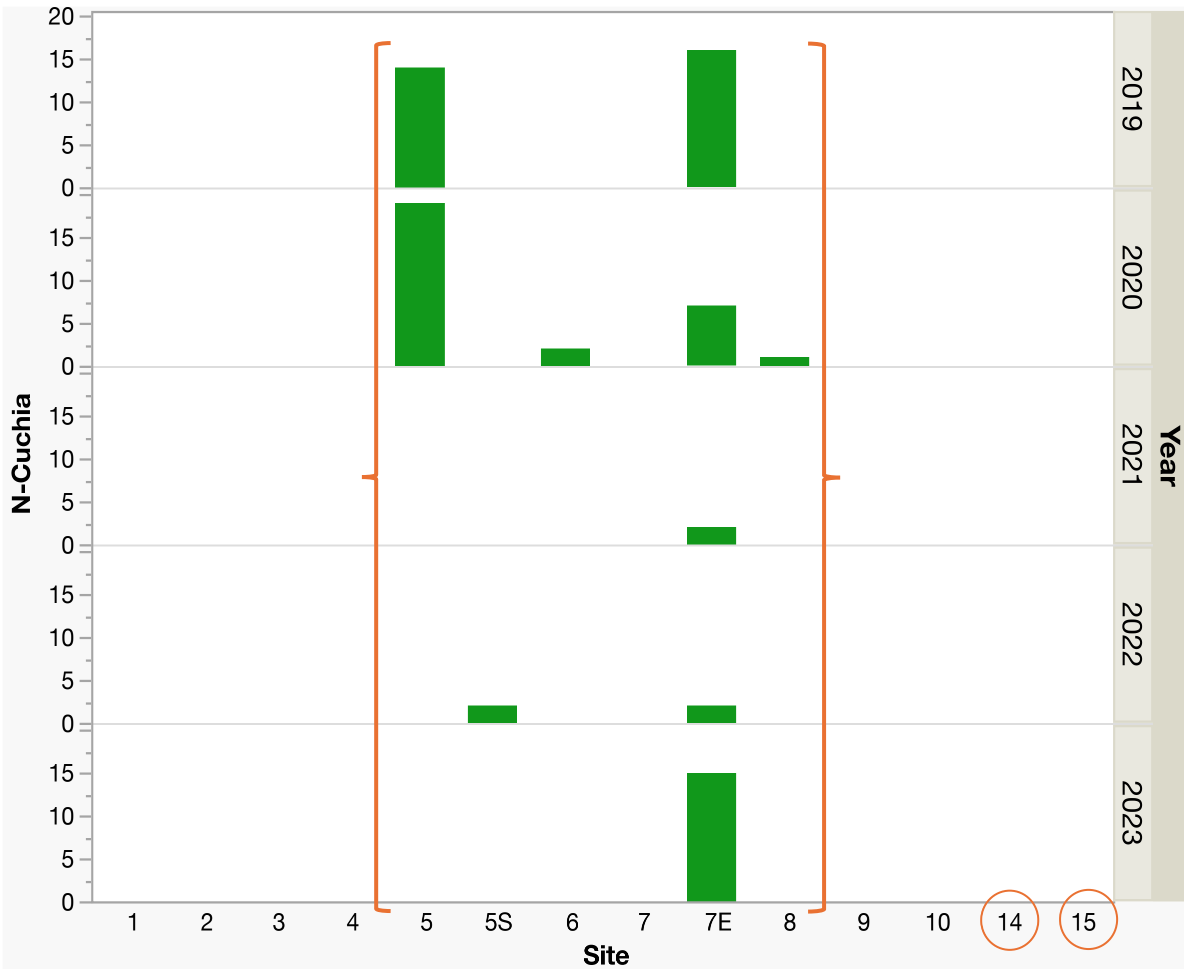


Figure 7. Mean abundance (± 1 $Standard\ Error$) of Cuchia per $m^2$by year. The black line depicts the mean Cuchia per m^2 over the past five years (0.0381 per m^2).

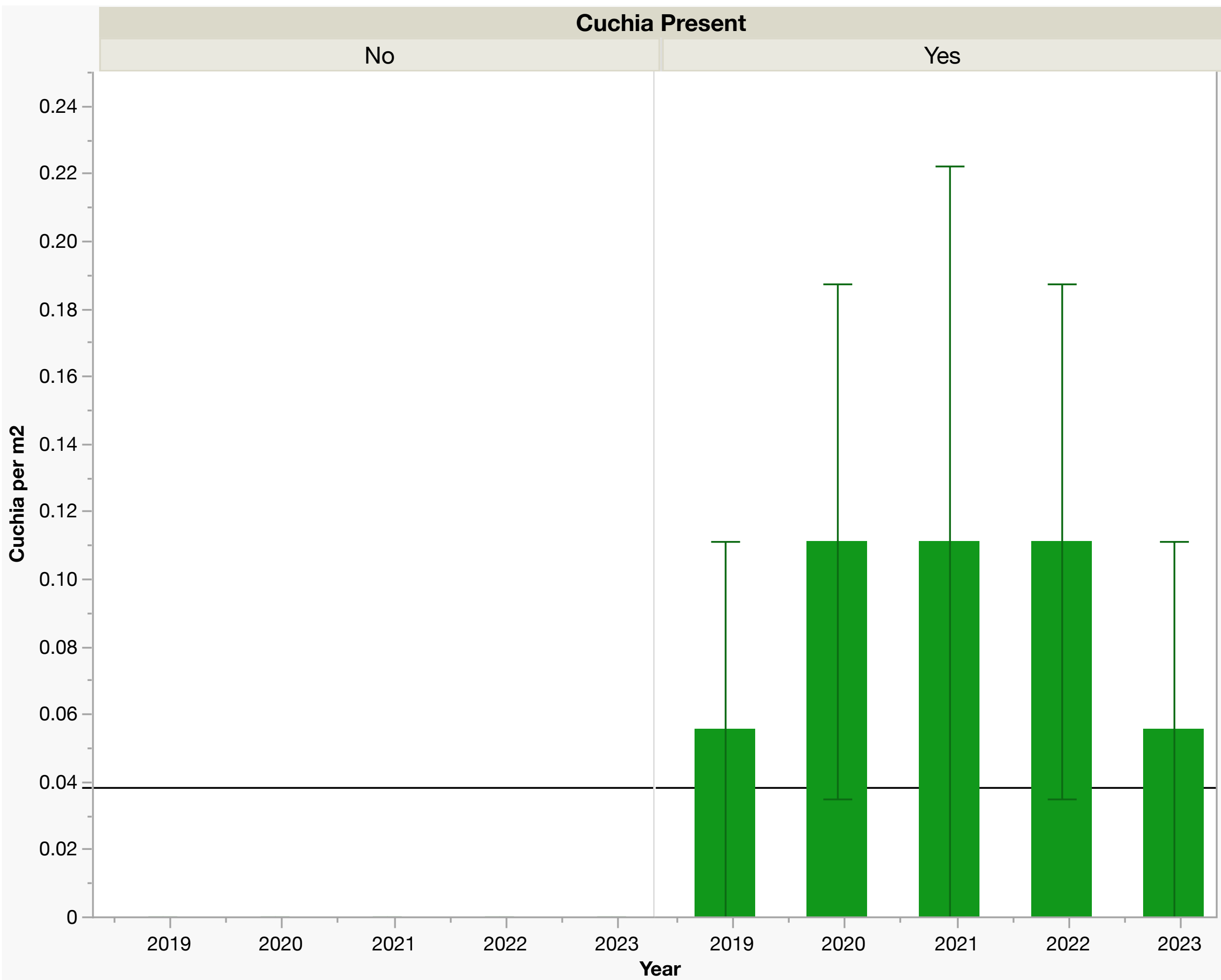


Figure 8. Bar Chart showing average Conductivity ($\mu mhos$/cm), Temperature (°C), Salinity (psu), and Dissolved Oxygen (mg/L) at each site by year.

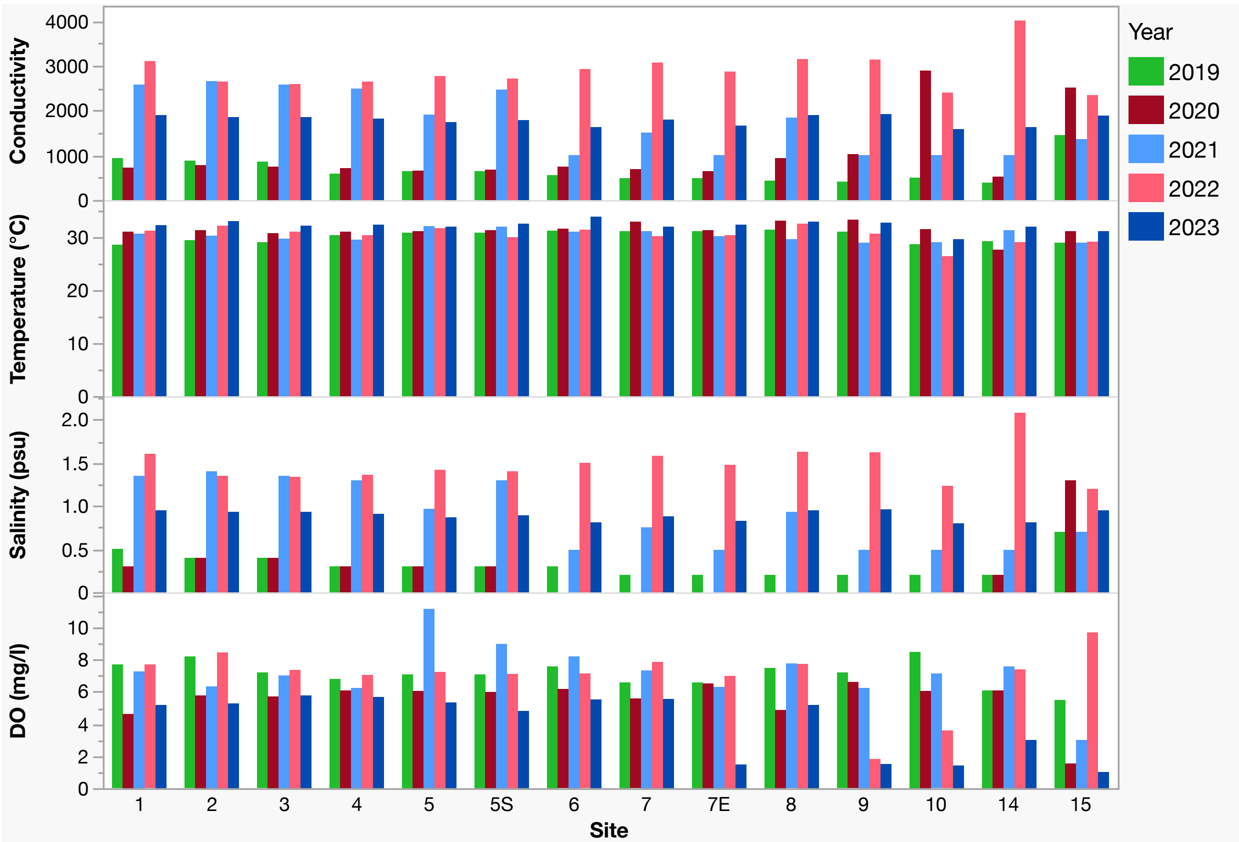


Figure 9. Bar Chart indicating average fish abundance, total species abundance, and species richness at each site over the past five years.

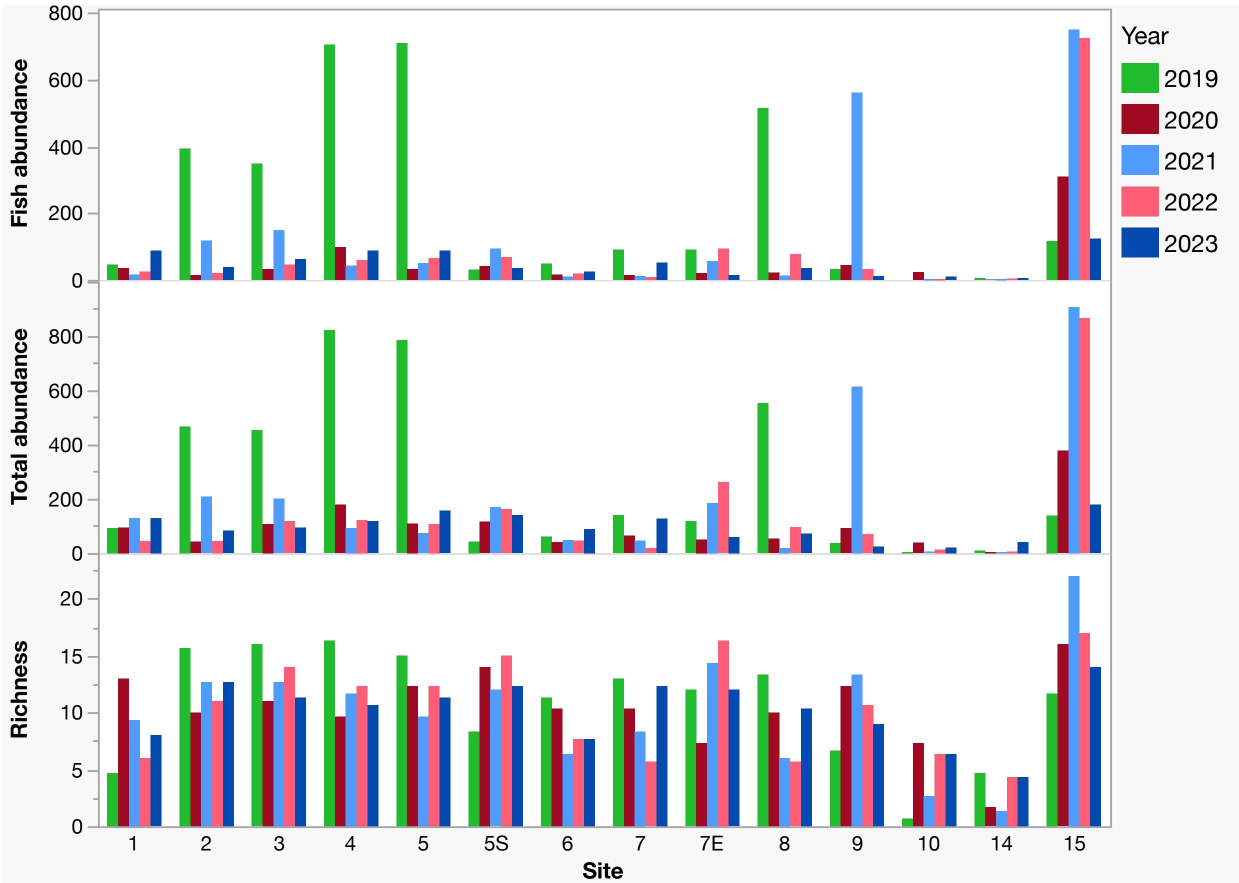


Figure 10. Bar chart (± 1 $Standard\ Error$) showing variations between the average plant biomass vs year. While not statistically significant, the overall trend shows an increase in average plant biomass from the year 2022 to 2023.

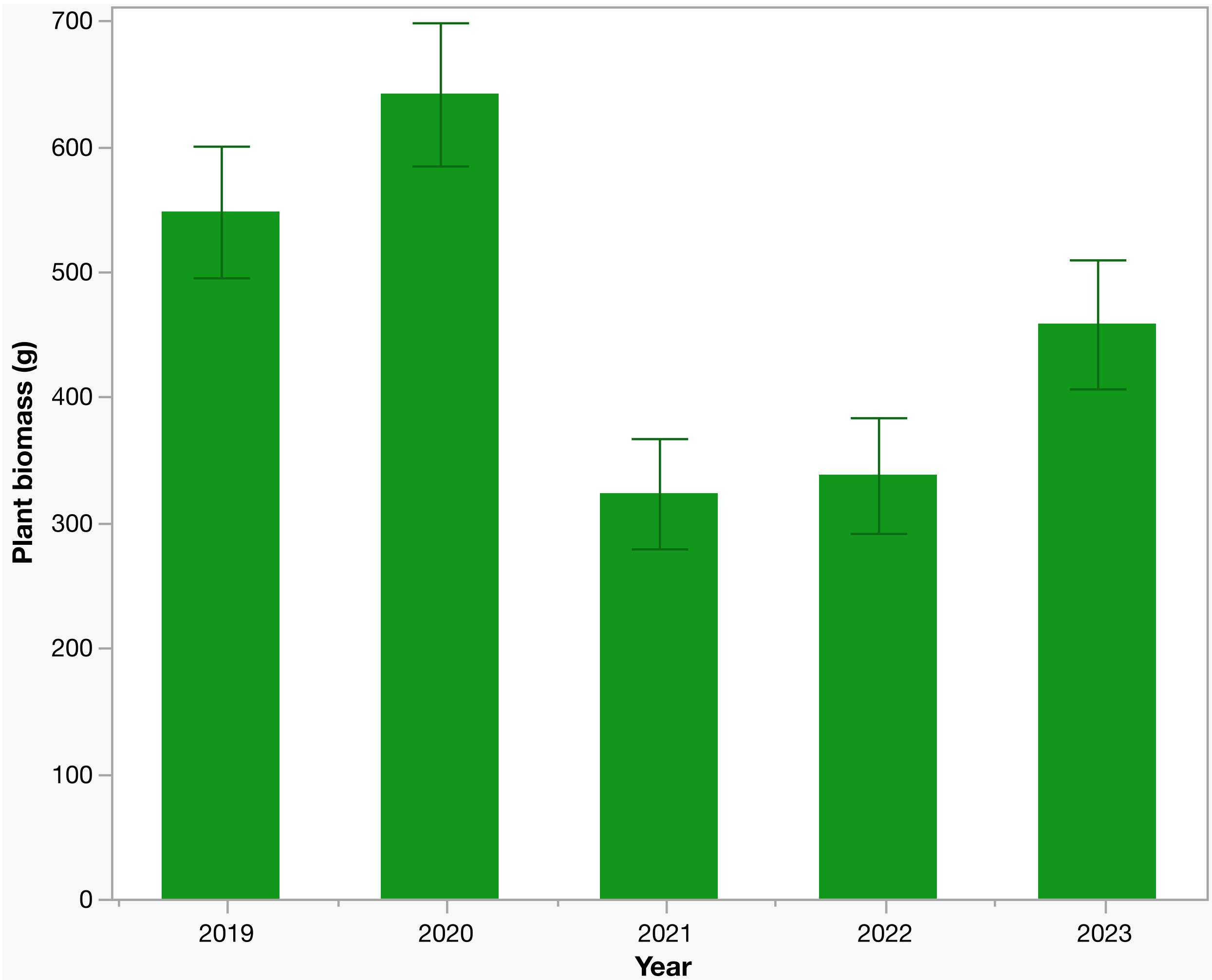


Figure 11. Linear regression showing a statistically significant positive relationship between plant biomass (g) and species richness ($R^2 = 0.048$, p = 0.0015).

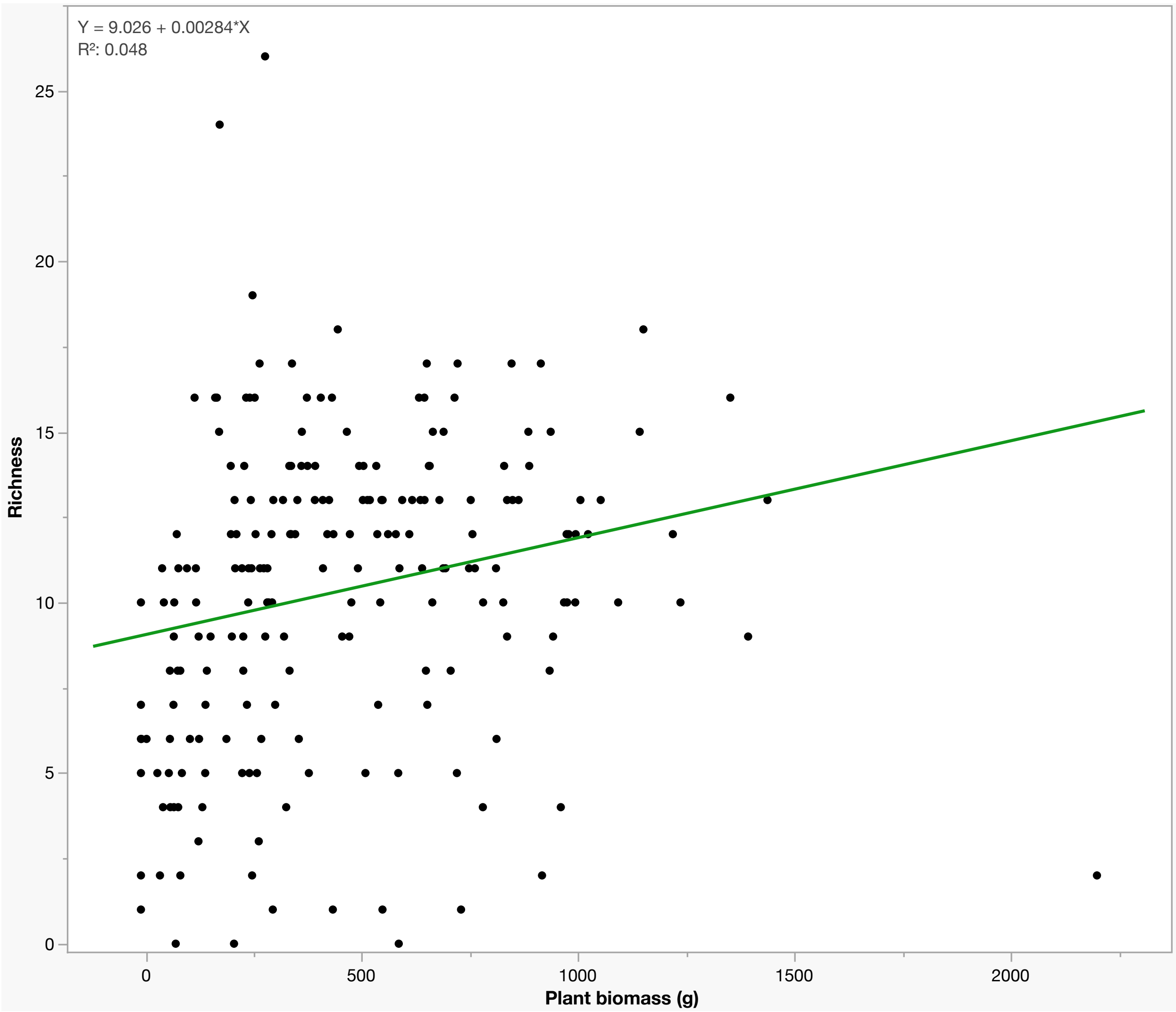


Figure 12. Mean abundances ($\pm 1\ Standard\ Error$) of Western Mosquito Fish (GAMAFF), Least Killifish (HETFOR), Sailfin Mollies (POELAT), Grass Shrimp (PALSPE), and Crawfishes (PROSPE) decline at various degrees in sites where Cuchia were present compared to sites where they were absent. Additional variations of decline or incline in mean abundances can been seen across the past 5 years, with the overall trend showing a decline in abundance where Cuchia were present. Data were analyzed using multivariate analyses of variance (MANOVA) ($p < 0.05$).