345COMPARISON OF RESTORED AND REFERENCE MARSHES ALONG A SALINITY GRADIENT

by

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ABSTRACT

KAYLI JABLONSKI. Comparison of Restored and Reference Marshes Along a Salinity Gradient. (Under the direction of DR. PAOLA LÓPEZ-DUARTE)

Global estimates of salt marsh degradation and loss are between 20-50% by the end of the century especially in Southern Louisiana where this loss is currently one of the highest in the world. Restoration efforts in this area have the goal of creating marsh land that is as similar in function, habitat, and ecosystem services as surrounding reference marshes. Our research takes the next step of quantifying those goals by evaluating the biodiversity and community structure of newly-created sites relative to reference sites along a freshwater siphon-induced salinity gradient. We sampled on-marsh nekton species abundance and composition from two created and four reference sites varying distances from the siphon from 2018 to 2022 using wire mesh traps deployed at marsh subhabitats (ponds, creeks, and edges). We also sampled macroinvertebrate communities through use of nylon mesh bags for two months (May through July) in 2018, 2019, and 2021. Sampling occurred when the siphon was off in 2018 and 2019 and on in 2021 and 2022 causing a salinity gradient from 0 to ~12. The aims of this study were to 1) determine whether there is an associate between environmental factors and the abundance and distribution of on-marsh nekton and macroinvertebrate communities within restored and reference sites, and 2) determine whether population demographics of select on-marsh nekton species difference between restored and reference sites and/or across a salinity gradient. We found that nekton abundance slightly increased between siphon-off and on years with biodiversity minimally declining closest to the siphon. In regards to subhabitats, pond community composition was more stable over time, due to the presence of euryhaline, Cyprinodontiforme fishes. We saw a change in creek and edge communities due to a replacement of shrimp species from brown and grass shrimp to Ohio shrimp in the presence of freshwater input closest to the siphon. When comparing on-marsh nekton and macroinvertebrates, there was only a correlation in Shannon diversity, but not in abundance. Finally, length and biomass population demographics were examined and, although variable between species, minimal differences were found between restored and reference sites and freshwater input did not seem to have a strong effect on these species demographics either. Interestingly, we did find a large amount of differences within the site mid-distance from the siphon through. Information gained here can help determine how combining newly-created sites with reduced salinity sustains species diversity as our results indicate that restored and reference marshes have high similarity less than five years post completion, and that siphon operation mostly affects the sites closest to it.

Key Words: biodiversity, communities, fish, marsh creation, nekton

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DEDICATION

I dedicate this Thesis to my family for their never ending support and encouragement as I pursue the career of my dreams. Especially to my father, Kevin Matsako, who is no longer with us, but is the person who instilled in me a never ending love of nature and the water, and who taught me to never be afraid of a little hard work or getting a little dirty doing what I love. Without all of them, none of this would have been possible.

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INTRODUCTION

Saltmarsh Services

Saltmarshes are dynamic coastal ecosystems that offer a multitude of services to both humans and the environment. Marshes act as natural barriers from coastal erosion and encroaching seas (Adam, 1993; Barbier et al., 2011; Mitsch & Gosselink, 1993; Peterson et al., 2008), and protect the approximately 40% of humans who live in coastal areas from storms and hurricanes (Adam, 1993; Barbier, 2019; Marois & Mitsch, 2015; Sun & Carson, 2020). Saltmarshes are also massive carbon sinks that sequester atmospheric carbon dioxide, which helps address one of the principal contributions to global warming (Quintana-Alcantara, 2014). In terms of biodiversity and fisheries, saltmarshes function as vital nursery grounds for up to 84% of commercially important fishery species in the Gulf of Mexico, such as seatrout, flounder, blue crab, and shrimp species (zu Ermgassen et al., 2021). Saltmarshes also serve as essential refuge and foraging habitats (Boesch & Turner, 1984) due to dense vegetation, shallow waters (Kneib, 1987), high primary productivity (Deegan et al., 2002; Minello et al., 2003), and dynamic environments that provide environmental gradients for species with various tolerances (Kneib, 1997; Layman et al., 2000; Smith & Able, 1994, 2003). Because marshes provide humans with numerous services, from their protective roles to essential habitats for marine animals that humans rely on for sustenance and economic livelihoods, there are strong financial investments to protect and mitigate marsh loss. Since 2007, Louisiana alone has accumulated $21.4 billion to go towards coastal restoration and risk reduction in the next 50 years (*2023 Louisiana's Comprehensive Master Plan for a Sustainable Coast*, 2023).

Marsh Loss and Restoration

Saltmarshes and coastal zones across the world are being degraded and lost (Dahl, 1990; Davidson, 2014; Zou et al., 2016). As of the early 2000's, it was estimated that 1-2% of global saltmarshes were disappearing every year (Adam, 2002; Lotze et al., 2006), but a more recent study that encompasses 2000 to 2019 estimated loss at 0.28% (Campbell et al., 2022). While the rate of global marsh loss seems to be decreasing, we are still experiencing a global net loss with higher rates of loss in some regions of the world. There are very few, if any, "pristine" salt marshes left, due to compounding anthropogenic and environmental effects such as major storms (Elliott et al., 2016; Brock et al., 2013), sea level rise, subsidence (Brock et al., 2013; Chesney et al., 2000; Reed, 1989; Sasser et al., 1986), water way manipulations, dredging, oil exploration, and fishing (Chesney et al., 2000). Specifically, the Mississippi River Delta region has been a location put under stress from human impacts such as dredging, oil transport and levee constructions, erosion, and storms (Penland et al., 1990) with an estimated 1.64% annual loss of marsh land in the area from 1985 to 2006 (CWPPRA, 2019). Hence, there is an immediate need to restore and protect the value of saltmarshes from further degradation for the services they provide (Geist & Hawkins, 2016).

Restoration has been defined as "the act of bringing an ecosystem back into, as nearly as possible, its original condition faster than nature does on its own" (Craft, 2016, p. 348). One common marsh restoration technique that addresses that goal is the build-up of marsh land through transport and placement of dredged materials, which can aid in battling subsidence (Stralberg et al., 2011, Tong et al., 2013) and sea level rise (Ge et al., 2016, Day et al., 2011, Stralberg et la., 2011, Tong et al., 2013). Marsh creation projects have been reported in Tijuana Bay, California (Zedler & Callaway, 1999), Puget Sound, Washington (Simenstad & Thom, 1996), Galveston Bay, Texas (Minello & Webb Jr., 1997), Newport River estuary, North Carolina (Moy & Levin, 1991), Long Island Sound, Connecticut (Warren et al., 2002), and Plaquemines Parish, Louisiana (Keppeler et al., 2023). Success in these studies were defined by damages to habitats being compensated for through creation of these sites through comparison of biotic and abiotic factors between created and reference sites. Factors such as sediment type and height, vegetation (Zedler & Zallaway, 1999, Simenstad & Thom, 1996, Warren et al., 2002, Moy & Levin, 1991), environmental measures (i.e. inundation, salinity, temperature, and dissolved oxygen) (Minello & Webb, 1997, Moy & Levin, 1991), and species richness and functional equivalence (Simenstad & Thom, 1996, Minello & Webb, 1997, Warren et al., 2002, Keppeler et al., 2023) were measured in these studies. These and other studies have reported varying levels of success that include reduced chance of successful creation (Zedler & Callaway, 1999), unpredictable trajectories (Simenstad & Thom, 1996), and variation in time for succession (Mahoney et al, 2021, Warren et al., 2022). This confirms that success is defined differently across projects and varies with what factor is measured which perhaps demonstrates the need to define common objectives and outcomes. Recently, approximately 409 ha of marsh was rebuilt and restored using dredged material from the Mississippi River from 2012 to 2015 in Lake Hermitage to mitigate historic marsh loss.

Freshwater Diversions

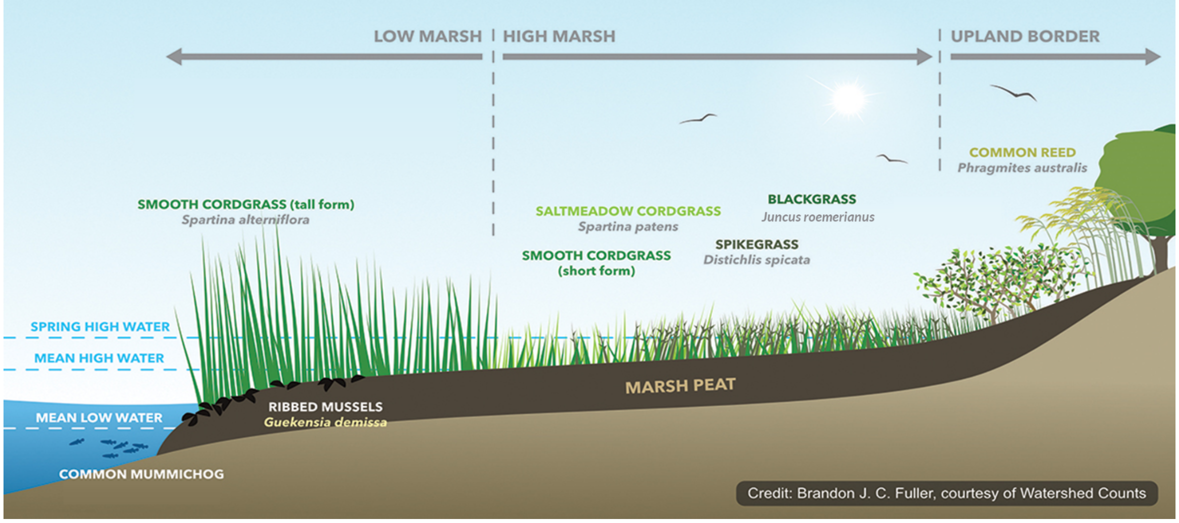
Another saltmarsh restoration technique involves the implementation of siphons or other structures that reintroduce freshwater and sediment to the marsh. Freshwater diversions can also help stabilize salinity, improve water quality, and improve habitat quality for local flora and fauna (Piazza & La Peyre, 2011). This type of restoration has been implemented in places like The Florida Everglades (Lewis & Cook, 2023) and coastal Louisiana (Das et al., 2012; DeLaune et al., 2003) because erosion in these areas has been linked to historic human altered freshwater flow patterns. Approximately 13% of the $21.4 billion investment for coastal restoration in Louisiana has been specifically allocated to new freshwater diversion projects for the next three years (*2023 Louisiana's Comprehensive Master Plan for a Sustainable Coast*, 2023). Findings and outcomes of these projects are necessary in aiding our understanding of how these restoration methods could affect local ecosystems. In 1992, the The Coastal Wetlands Planning, Protection and Restoration Act (CWPPRA) program initiated the construction of the West Point à la Hache Siphon to reintroduce freshwater from the Mississippi River in hopes of restoring ecological function to the area. This siphon is located adjacent to the aforementioned restoration sites in Lake Hermitage. Two goals of this siphon was to 1) reduce surrounding marsh salinity and 2) Increase marsh to open-water ratio (Boshart & Carrell, 2009), but overall goals of siphon constructions are to better manage wildlife and fishery resources through controlled salinity management (Roberts et al., 1992).

Most estuarine species, especially fishes, are able to acclimate to wide salinity ranges (euryhaline), but they can be limited to where they reside based on salinities (Rakocinski et al., 1992). A 10 ppt reduction in salinity is biologically significant by altering the ability of individuals to reside in a location (Das et al., 2012) through increased metabolic cost (Boneragan and Bunn, 1999) or altered feeding behaviors (Dunson et al., 1993) resulting in shifts in community compositions depending on salinity tolerance of the present species with less tolerant species being less common in reduced salinity areas (Das et al., 2012; Hampel et al., 2004; Piazza & La Peyre, 2011; Weinstein et al., 1980; Weinstein et al., 2019). Changes in salinity have been known to reduce biodiversity, alter abundances of species, and affect location demographics which can alter species-species interactions (Das et al., 2012; Hitch et al., 2011;

Layman et al., 2000; Rountree & Able, 2007; Vander Vorste et al., 2019). Many fishery species depend on a salinity gradient for different life stages making the siphon induced salinity gradient vital for fishery production (Boshart and MacInnes, 2000). The occurrence of some marsh species fluctuates based on salinity, but certain species, such as polychaetes (Subrahmanyam & Coultas, 1980), crustaceans (Subrahmanyam & Coultas, 1980) and Gulf killifish (Lipcius & Subrahmanyam, 1986; Mahoney et al., 2021) occur consistently across a wide range of salinities in Gulf of Mexico marshes. Freshwater diversions, paired with marsh creation are ways to mitigate coastal land loss and extend the longevity of coastal marshes, but knowledge of how combining these two methods may affect communities is absent from current literature.

Marsh Food Webs

Saltmarshes and their variety of habitats, ranging from terrestrial to semi- to fully aquatic (**Figure 1. Marsh Habitat**) are home to an array of organisms with diverse niches. The diversity is driven in part by varying topographies (Hitch et al., 2011), elevations (Mitsch & Gosselink, 2008), inundations (Hitch et al., 2011; Mitsch & Gosselink, 2008), and gradients in temperature, dissolved oxygen (Rakocinski et al., 1992), and salinity (Hitch et al., 2011; Mitsch & Gosselink, 2008; Rakocinski et al., 1992). Food web complexity and structure can be used as metrics of habitat health. Saltmarsh food webs are generally controlled in either one of two ways: 1) bottom-up (producer driven) or 2) top-down (predator driven) but there are differing views on which one actually dominates in saltmarshes (Valiela et al., 2004). However, physical and environmental factors can obscure which is in control and make bottom-up control seem like it is actually top-down or vice versa (Breitburg, 2002; Rosemond et al., 2001; Sardá et al., 1996, 1998; Valiela et al., 2004). Factors such as lowered dissolved oxygen can reduce nekton presence even though there is an abundance of available food (Breitburg, 2002), or increased phosphorus levels lead to top-down control but higher overall nutrient levels lead to bottom-up control (Rosemond et al., 2001). There is still a need to understand how species are linked through the food web, especially when there are differing opinions on if top-down or bottom-up control is in control of marsh food webs (Valiela et al., 2004).



Credit: Brandon J. C. Fuller, courtesy of Watershed Counts

Figure 1. Marsh Habitat Representative types of marsh vegetation across different elevations. Original diagram by Brandon J.C. Fuller was downloaded from the Rhode Island Coastal Resource Management Council website. BJCF Image; modifications by Paola López-Duarte.

Key taxa within food webs such as carnivorous fishes, omnivorous snails, and blue crabs, and invertebrate species each play a certain role in food webs and can each be affected differently by environmental factors leading to varying alterations in food web stability during times of environmental stress. (McCann et al., 2017). Much work has been done to describe the trophic links primary producers have in the food web (Nelson et al., 2019; Power, 1992; Rosemond et al., 2001) and identification of individual species diets (Gaines, 2015; Hastings & Yerger, 1971; Rozas & LaSalle, 1990), but there is minimal information on the direct interaction between macroinvertebrates and nekton (McCann et al., 2017; Werme, 1981). Interactions between species have been known to alter community structures (Sardá et al., 1998; Subrahmanyam & Coultas, 1980; Winemiller, 1990), but there is still more detailed information needed as to how functional group roles are filled in times of changing communities. General food webs have been constructed for marsh ecosystems within Louisiana that give a big picture overview of primary producers up to top predators for all areas of the marsh (Davis, 2009; de Mutsert & Cowan, 2012; Lewis et al., 2016), but they do not always include detailed descriptions for areas such as the marsh platform or surface which include groups such as on-marsh nekton and macroinvertebrates.

Nekton

Nekton are marine organisms such as fish, crab, and shrimp, that are able to actively swim against currents. On-marsh nekton include the juvenile and adult stages of various Cyprinodontiforme species and crustaceans. Nekton found in deeper waters off the marsh edge will be referred to as off-marsh nekton. On-marsh nekton species range from herbivorous or planktivorous, to omnivorous, carnivores, and detritivores. They can also be categorized as resident or transient species. Residents, often used as indicator species for marsh health (Mahoney et al., 2021) complete their life cycle in marshes and are often found in higher abundances (Baltz et al., 1993), while transient species only complete part of their life in these areas and typically migrate to offshore and/or deeper water to complete their life cycle. Valued fishery species are often supported by marshes (Kneib, 1997; Subrahmanyam & Drake, 1975) where the marsh platform acts as a feeding ground where they are able to consume shrimp and smaller fishes to maintain links through the food web (Able et al., 2018; Rozas & Reed, 1993), and shrimp and smaller fish species (i.e. on-marsh nekton), in turn, rely on macroinvertebrates, organisms that can be retained by 200 micron mesh and are greater than 1mm in length (Kang & King, 2013; Rosenberg & Resh, 1993), as a food source (Werme, 1981). Demographics such as lengths and biomasses of species can also be indicators for marsh restoration success as this indicates what type of individuals within are utilizing these areas (Hollweg et al., 2020; Zeug et al., 2007; Rozas and Minello, 2009) which may be valuable knowledge for determining fishery value of (Rozas et al., 2005). Physiological conditions of individuals can be affected by factors such as prey availability, competition, and water quality (Mclvor and Odum, 1988), which are all factors that may be affected by restoration efforts (Vincent et al., 2015; Adam 1993). Many species have negative relations to body size in low salinity waters due to energy allocations going towards osmoregulation rather than growth (Ramee et al., 2015) leading to a reduction in lengths of individuals in an area. de Mutsert & Cowan (2012), while looking at both on and off-marsh nekton species, found that the mean weight of species was significantly lower in areas closer to freshwater input than in reference areas, and that species such as sheepshead minnows and grass shrimp were large contributors to dissimilarities in community biomass decreases. With so many types of nekton and environmental factors that can affect them, it is important to understand how individuals, species, and their environments interact.

Marshes are heterogeneous (**Figure 2. Marsh Site Interspersion)** and can include various subhabitats, such as ponds, creeks, and edges, among others (**Figure 3. Marsh Subhabitats**) (Able et al., 2015; Minello et al., 2003; Minello & Rozas, 2002). These subhabitats can provide nekton with varying forms of shelter from stressful conditions, such as lack of food, competition, environmental stress, and predation, among others (Rountree & Able, 2007). Ponds are areas of water on the marsh surface with distinct edges separating the surface from the water (Able et al., 2015). They provide protection from predators and environmental stressors for both early and late life-stage individuals and are vital nursery and feeding grounds (Kneib, 1987, 1997; Rountree & Able, 2007). Creeks are narrow channels dividing the marsh (Able et al., 2015) that act as corridors between aquatic and surface environments (Kimball et al., 2023). Due to their deeper waters, creeks often provide ontogenic refuge for larger, transient individuals from extreme pond environments while still providing adequate food and predator refuge (Gibson, 1973, 1986; Helfman, 1978; Kneib, 1987; Mauchline, 1980; Nordlie, 2006; Peterson & Turner, 1994; Smith & Able, 2003; West & Zedler, 2000). Edges are areas of the marsh that are defined boundaries separating the open water from the marsh surface (Able et al., 2015). Edges tend to have distinct communities from the other subhabitats (Conner & Day Jr., 1982; Hettler, 1989; Rakocinski et al., 1992; Rozas et al., 1988) as a majority of the organisms found there are transient species only present to spawn (Peterson & Turner, 1994). As with environmental factors mentioned above, it is important to understand how individuals and species interact with their physical location as well.

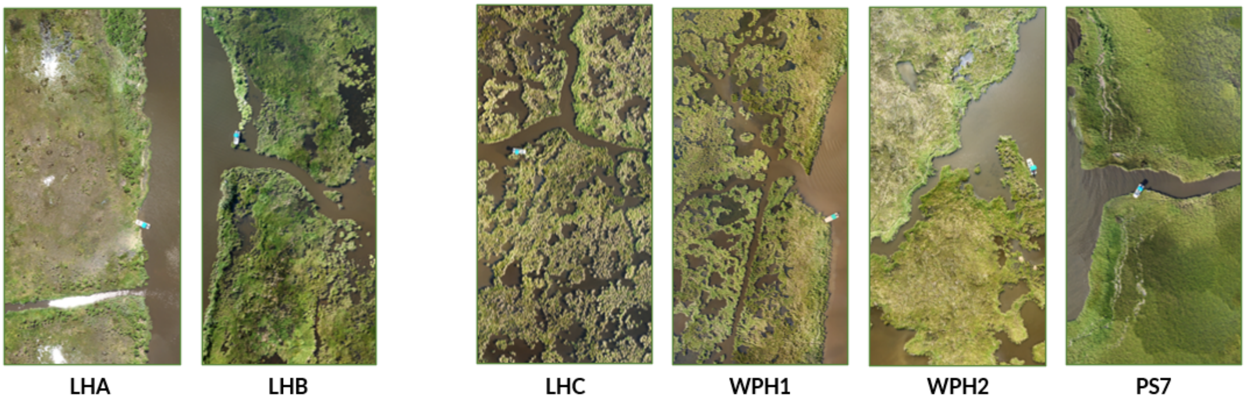


Figure 2. Marsh Site Interspersion Created versus references sites. Each of our 6 sampling sites pictured by drone images, displaying the complexity of the marsh landscape and comparing interspersion of water to land ratio between sites. Images taken by Eddie Weeks (Louisiana State University) and stitched together by Julia Nelson (University of North Carolina at Charlotte.

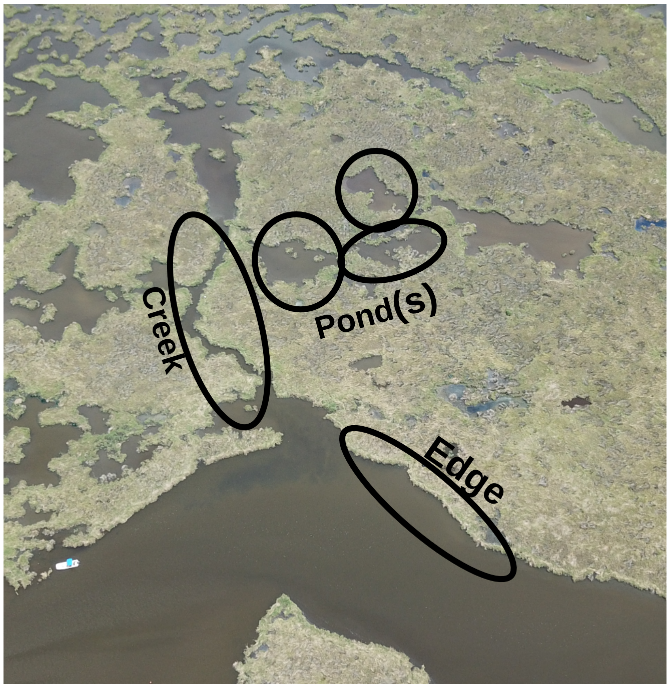


Figure 3. Marsh Subhabitats The marsh landscape can form a complex environment that includes navigable water along marsh edges, shallower tidal creeks, and isolated or semi-isolated ponds. Image taken by Eddie Weeks (Louisiana State University) and modified by Kayli R Jablonski.

Macroinvertebrates

Organisms grouped into the macroinvertebrate category are found in semi-terrestrial and terrestrial areas of the marsh, from just below the marsh surface sediments to just above the surface of present vegetation (Angradi et al., 2001). They can be broadly grouped into insects (larvae), amphipods, oligochaetes, and gastropods (McCormick et al., 2004) and are often used as indicators of marsh health (Weilhoefer, 2011). Many macroinvertebrates are detritivores, scavengers, filter or deposit feeders, or general omnivores who aid in recycling organic material (Barnes, 1980; Odum, 1971; Sanders et al., 1962; Williams, 1965). This helps link the food web between primary producers and higher level consumers, such as birds and fish (Cardoso et al., 2008; Levin et al., 1996; Levin et al., 2001; Levin & Talley, 2002; Moseman et al., 2004; Sacco et al., 1994), as common nekton species are often carnivores or omnivores that feed off macroinvertebrates such as polychaetes, amphipods, nematodes, detrital material, or algae (Hastings & Yerger, 1971; Rozas & LaSalle, 1990; Werme, 1981). Habitat selection of these species is influenced by the structure of the marsh vegetation, soil or sand contents, depth of water, water chemistry (i.e. temperature, salinity, pH, and dissolved oxygen), and inundation (Bolam et al., 2004; Hampel et al., 2009; Levin & Talley, 2002; Mely et al., 2023; Subrahmanyam et al., 1976; Tong et al., 2013). Distance from the edge of the marsh can also influence community composition. If the marsh has a higher elevation with increased distance from the edge, the upper marsh will have a higher occurrence of terrestrial groups (e.g., insects) relative to aquatic organisms (e.g., polychaetes) (Cammen, 1976; Subrahmanyam et al., 1976). As with nekton, macroinvertebrates are useful indicators of marsh health (Mahoney et al., 2021; Mely et al., 2023; Scapin et al., 2019; Tong et al., 2013; Weinstein et al., 1980; Weinstein et al., 2019) and there is a need to understand their role in the marsh in varying environments and in relation to other species.

Aims

Aim 1. To determine whether there is an association between environmental factors (salinity and inundation) and the abundance and distribution of on-marsh nekton and macroinvertebrate communities at restored and reference sites.

Question 1.1: Is there a response in on-marsh nekton communities to changes in salinity and between restored and reference sites?

Hypotheses 1.1: I hypothesize that there will be a shift in the on-marsh nekton communities to more freshwater tolerant species, such as inland silversides (Espinosa-Perez et al., 2015), mosquito fish (Chervinski, 1983), and sheepshead minnows (Bennett et al., 1995), and Ohio

Shrimp (Anderson, 1983), replacing the higher salinity tolerant species, such as grass shrimp (Anderson, 1985), Gulf menhaden (Whitehead, 1985), sailfin molly (Zimmerer, 1983), and various transient species (Stevens et al., 2013). I also hypothesize that when the siphon is on, more nekton will migrate to ponds to seek refuge from changing conditions (Rountree & Able 2007). Finally, I also hypothesize that any changes in community composition attributed to freshwater input will be similar in restored and nearby reference marshes because four to six years is enough to create similar species composition between restored and reference sites (Keppeler et al., 2023) and I predict species found in restored sites, if actually similar to those in reference sites, will act the same when faced with a change in salinity.

Aim 2. To determine whether the population demographics of dominant on-marsh nekton species (i.e. Gulf killifish, bayou killifish, diamond killifish) and those of economic and ecological interest (i.e. sheepshead minnow, rainwater killifish) differ between reference and restored sites and/or across the salinity gradient.

Question 2.1: Are the <u>lengths and frequencies of lengths</u> of these seven species changing with <u>freshwater input</u>?

Hypothesis 2.1: I hypothesize that there will be a shift to smaller individuals as certain Cyprinodontiforme species, such as sheepshead minnows and Gulf killifish have negative relations to growth and size in low salinity waters (Dunson et al., 1998; Ramee et al., 2016).

Question 2.2: Are species <u>biomasses</u> changing with <u>freshwater input</u>?

Hypothesis 2.2: Per the above hypothesis, I predict that as sizes of individuals decrease, their biomasses will follow a similar pattern.

Aim 3: To determine whether there is an association between environmental factors (salinity and inundation) and/or restoration among macroinvertebrate communities.

Question 3.1: Is there a response in macroinvertebrate communities to changes in salinity?

Hypothesis 3.1: I hypothesize that there will be a shift in macroinvertebrate communities to contain more insects than crustaceans in areas of lower salinity (Kang & King, 2012). Per a literature review done by a previous student (Rachel Magallon), I expect a decrease of species in the order Decapoda, the suborder Gammaridae, the class Polychaeta, and the order Pseudoscorpiones in sites closest to the siphon, as they are species with a narrower salinity tolerance range and ones that prefer more brackish waters.

Question 3.2: How do macroinvertebrate communities compare to nekton communities between restored and reference sites in 2018?

Hypothesis 3.2: I hypothesize that macroinvertebrate communities will be similar between restored and reference sites as a recently published study on these exact sites was conducted and found that a four to six year timeframe is long enough to create taxonomy and species compositional comparability between restored and reference sites (Keppeler et al., 2023).

Significance

Louisiana has one of the highest coastal loss rates in the world, as high as 41 miles2/yr (Britsch & Dunbar, 1993). However, freshwater siphons from the Mississippi and restoration of historically present marshes are seen as investments to protect these habitats. Saltmarsh restoration efforts have increased in recent decades, but post-monitoring studies to evaluate their success and impact on communities and ecological interactions are limited (Adam, 2019; Keppeler et al., 2023; Lewis & Cook, 2023). Restoration techniques yield varied and unpredictable outcomes, leading to uncertainties in forecasting their effects, particularly in scenarios involving simultaneous implementation of multiple methods, like combining restoration with freshwater input. Stakeholders, including environmental groups, government agencies, and the public, need clear insights into the ecological impacts and observable changes resulting from successful restoration efforts. Understanding the response of saltmarsh nekton and macroinvertebrates to habitat degradation, alteration, and restoration is crucial due to their ecological roles in the system. My goal is to integrate data from both on-marsh nekton and macroinvertebrates, exploring potential correlations in their abundance, biodiversity, and environmental interactions

METHODS

Study Sites

The study sites are located in Plaquemines Parish, Louisiana, an area in southeastern Louisiana (**Figure 4. Plaquemines Parish**). The area is microtidal (maximum tidal amplitude is 30 cm) with tidal heights heavily influenced by seasonal and daily weather patterns. Tides are diurnal with one high and one low tide per day cycling every 24.8 hours. Salinity can reach 0ppt in the upper parts of the estuary, including our northernmost sites (LHA, LHB, LHC, WPH1), and 25 ppt at the mouth of the estuary (PS7) (Das et al., 2012) because of the influence of the Mississippi River, full strength seawater (35) is found further offshore. Salinity also fluctuates with freshwater input and seasons (Able et al., 2015; Conner & Day, 1987). Vegetation in the area is dominated by smooth cordgrass (*Spartina alternifloria)* and other marsh grasses to a lesser extent, including *Distichlis spicata*, *Juncus roemerianus*, and *Spartina patterns* (Keppeler et al., 2023).

The Mississippi River Delta region is a location put under stress from human impacts such as dredging, oil transport and levee constructions, erosion, and storms (Penland et al., 1990) with an estimated 1.64% annual loss of marsh land in the area from 1985 to 2006 (CWPPRA, 2019). Our study area is strategically located to encompass marsh area that has been the focus of conservation and restoration efforts over the past few decades. In 1992, the The Coastal Wetlands Planning, Protection and Restoration Act (CWPPRA) program initiated the construction of the West Point à la Hache Siphon to reintroduce freshwater from the Mississippi River in hopes of restoring ecological function to the area. Adjacent to West Point à la Hache, approximately 409 ha of marsh was rebuilt and restored using dredged material from the Mississippi River from 2012 to 2015 in Lake Hermitage to mitigate historic marsh loss.

Sampling sites (Figure 3) included two restored sites (LHA, LHB), and four reference sites at varying distances from the siphon; WPH1 (adjacent to the siphon), LHC, WPH2, and PS7 (outside of the area influenced by the siphon). LHC, the closest site to the Lake Hermitage Project, serves as the proximal control. This site was chosen as the control for its proximity to the restored sites as it should have the most similar events occurring through time such as weather and freshwater input from the siphon. PS7, serves as the control for the siphon conditions.

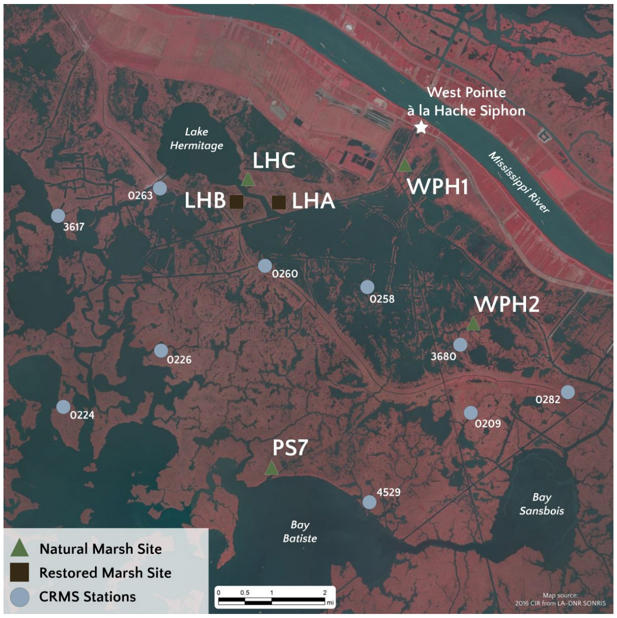


Figure 4. Plaquemines Parish Map of Plaquemines Parish around the West Pointe a la Hache Siphon. Depictions of location and orientation of sites along the salinity gradient and indication of reference (green triangles) and restored (brown squares) sites, along with sites environmental data were collected (blue circle CRMS stations).

Sample Collection, Environmental monitoring, and Processing

On-marsh nekton were sampled at all six sites in May of 2018, 2019, 2021, and 2022. Sampling in 2020 was not possible due to travel restrictions imposed by the COVID-19 pandemic. Wire-mesh traps (4 cm long, 22 cm wide, and an entrance opening of 3cm) were baited with dry dog food and deployed for 1-2 hours at marsh edges (n=9), intertidal creeks (n=9), and ponds (n=9) over 3 days in 2018 and 2019 and 2 days in 2021 and 2022. The target species include various Cyprinodontiforme fishes (i.e., Gulf killifish [*Fundulus grandis*], bayou killifish [*F. pulvereus*], rainwater killifish *[Lucania parva*], sheepshead minnow [*Cyprinodon variegatus*]), blue crabs [*Callinectes sapidus*], and shrimp species). Salinity and temperature were recorded using a portable refractometer (manufactured by Agriculture Solutions) calibrated daily using comparison measurements from a YSI and a waterproof thermometer (manufactured by Extech Instruments, item number 39240), respectively. The catch of each trap was identified, sexed, measured, and counted. A maximum of 30 individuals from each species was weighed (g) and measured (cm) for standard, total, or fork-length for fish or shrimp species, and carapace width for crab species. Individuals too light to weigh using a field scale were batch-weighed to calculate total weight. Fish were sampled in accordance with the guidelines and with the approval of the University of North Carolina at Charlotte’s Institutional Animal Care and Use Committee (IACUC), Protocol Number 20-011.

Macroinvertebrate samples were collected at all six sites in the summers of 2018, 2019, and 2021   At each site, nylon mesh bags (29 x 13.5-centimeter; filled with dried *Spartina* vegetation) were deployed for two months (May through July) at 1m (n=5), 10 m (n=5), 25 m (n=5), 50 m (n=5), and 100 m (n=5) from the edge of the marsh. When the litter bags were retrieved, the contents were preserved in 95% ethanol and dyed with Rose-Bengal (REF) to distinguish between organism and detritus. The contents of each bag were sorted and identified using identification keys. Target macroinvertebrate species include isopods, amphipods, nematodes, harpacticoid copepods, oligochaeta, nematodes, and chironomidae larvae.

Environmental data (i.e. salinity, temperature, and percent time of inundation) was also collected from the 2018 through 2022 sampling years through CRIMS Sonde loggers, which allowed for parameters to be set in both siphon-off and on conditions. Salinity was our main factor of concern, but temperature and inundation can also be confounding factors we expected to have possible influences. Outside of one-point sampling with on-marsh nekton, environmental data was collected daily from all sites from all sampling years, with the exception of temperature and salinity at WPH1 for 2021 and 2022, in which those monitoring data were collected at an outfall canal (OC) located slightly northeast of WPH1. Mean monthly temperature, salinity, and inundation, along with monthly standard error, for each site was found through averaging of daily measurements in each month. **Supplemental Table 1. Salinity, Temperature, and Inundation Averages** displays the environmental parameter averages for each site from March through July of each sampling year. **Supplemental Table 2. Siphon Discharge** displays the dates and associated flow rates of freshwater discharged through the siphon from the Mississippi River during years of sampling. The siphon was operational from March 9, 2021 through June 24, 2021 and again from January 17 to January 26, 2022 and February 24 to June 20, 2022.

Data Analysis

Aim 1. To standardize for deployment time, nekton wire-mesh trap data was transformed to catch-per-unit-effort (CPUE), calculated by taking the number of individuals in a species caught per sample over time duration of trap deployment. The focus on nekton community diversity was aimed at the number of individuals caught within each sample for each site within years, along with the corresponding Shannon diversity of samples. Nekton community diversity was also placed in terms of number of species caught, richness, evenness, and Simpson diversity for a more complete comparison of sites and years.

Aim 2. Due to only obtaining weight and length measurements on 30 individuals per species per catch, we assumed that the proportion of individuals falling into specific categories within the subset could be applied to the whole group. To calculate ratios, the total number of individuals of that species caught were divided by the number of individuals actually measured and then the resulting number was multiplied by the frequency of occurrence to obtain relative frequency.

Aim 3. Macroinvertebrate community data was not transformed as the length of time each litter bag was deployed was roughly equal within each sampling effort, so raw counts for individuals and communities were used. In order to compare on-marsh nekton with macroinvertebrates, since sampling techniques differed, community diversity will be put into the form of community dynamics, such as the number of individuals caught and Shannon Diversity Index (H') for comparison.

Statistical Analysis

Aim 1. Visualizations of biodiversity indices were created using box plots highlighting means and standard deviations of each site within each year of sampling. Biodiversity indices (i.e. number of species caught and Shannon diversity) for restored versus reference sites and subhabitats were compared with one-way ANOVAs. Biodiversity comparisons of sites and subhabitats along the salinity gradient between years was done through repeated measures ANOVAs and year-site interactions along the gradient were analyzed through mixed ANOVAS. Shifts in community composition and abundance for nekton over time or space were visually represented through nonmetric multidimensional scaling plots (nMDS) using Bray-Curtis similarity methods to determine distinctions between sites and years. Vectors (Pearson correlations, >0.2) identify species responsible for distinguishing between sample composition.

Aim 2. Stacked histograms were created to visualize length and biomass frequencies of sites within years of sampling. One-way ANOVAs were used to compare nekton length and biomasses across sites and years for restored versus reference sites (LHA, LHB, and LHC) to compare demographics along the salinity gradient (WPH, WPH2, and PS7) between years.

Aim 3. Shifts in community composition and abundance for macroinvertebrates over time or space were visually represented through nonmetric multidimensional scaling plots (nMDS) using Bray-Curtis similarity methods to determine distinctions between sites and years with Pearson correlations of 0.3 used to identify species responsible for distinguishment between samples. Comparisons between on-marsh nekton and macroinvertebrate number of individuals caught and Shannon diversity were performed through Pearson correlations to a significance level of 5% ($p<0.005$).

RESULTS

Environment Variables

Temperature

Mean monthly temperatures in southern Louisiana (**Figure 5. Temperature per Month)** fluctuate from approximately 15^{o}C in the winter months to 27^{o}C in the summer months. Notably, all reach consistently higher temperatures in the summer months (i.e. May through August) averaging 27.0^{o}C across all sites, compared to winter (i.e. November through February) averaging 17.2^{o}C across all sites. During the nekton sampling efforts in 2018 and 2019, the warmest sites were WPH2 and PS7 (May average 28.3 for both sites), and the cooler sites were WPH1 (May average 23.8^{o}C) and LHB LHC (May average 25.5^{o}C). In the last two years, the pattern was different though, PS7 decreased to an average of 25.3^{o}C in 2022, and, in both years LHB reduced to the coldest sites (2018-2019 May average 27.0^{o}C, 2021-2022 May average 22.7^{o}C) and WPH1 increased (May average 27.0^{o}C). LHA (May average 26.7^{o}C), LHC (May average 24.8^{o}C), and WPH2 (May average 27.6^{o}C) were all consistent between the four years with minimal fluctuations. During the macroinvertebrate deployments (May - July in 2018 and 2021), the warmest sites in 2018 were WPH2 and PS7 (average 29.8^{o}C for both sites), and the cooler sites were WPH1 (average 24.3^{o}C) and LHC (average 26.5^{o}C). Temperatures for 2021 macroinvertebrate sampling was similar to 2018 except LHB (average 23.1^{o}C) became the coldest site and WPH1 (average 26.6^{o}C) temperatures increased.

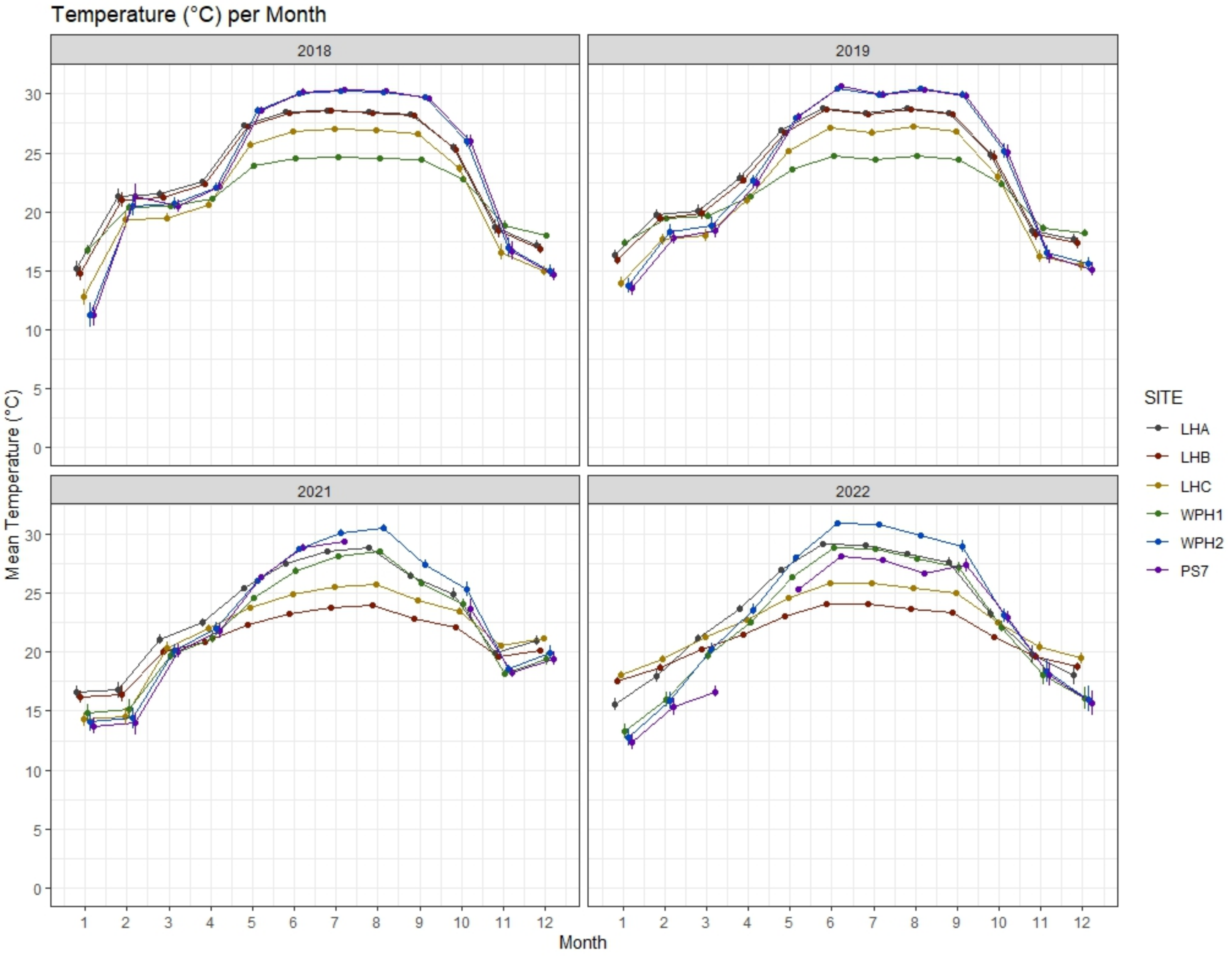


Figure 5. Temperature per Month Monthly mean temperature (°C) at six sites and four years (2018, 2019, 2021, 2022). Mean temperatures were calculated by averaging one measurement for each day of the month. Temperature values were obtained from temperature loggers installed at all sites except 2018 and 2019 WPH1 data which was obtained from a temperature logger located at the outfall canal (siphon), approximately 1,200 meters away from the site. Error bars for each month indicate the standard error of that month's daily measurements.

Salinity

Overall, sites fluctuate in salinity (**Figure 6. Salinity per Month**) with the seasons following an opposite pattern of temperature with winter having the highest and summer having the lowest salinities. In siphon-off years, sites followed similar patterns in all months, but, in siphon-on years, there was more spread among sites, especially in the winter months. In siphon-off years, salinity spread between sites was as high as 7 ppt, whereas in siphon-on years, the spread reached as high as 12 ppt. WPH1, our site closest to the siphon, showed the largest drop in salinity between siphon-off and on conditions. It started as one of the highest salinity sites (average 11.7 ppt), but then dropped to be distinctly separated from the rest of the sites as the lowest salinity (average 3.2 ppt). A gradient is noticeable in siphon-on years with sites closest to the siphon including WPH1, LHA, LHB, LHC, and WPH2 being on the lower end and PS7 having the highest, seemingly most stable between years, salinity farthest away from the siphon (**Supplemental Table 3. May Salinity**).

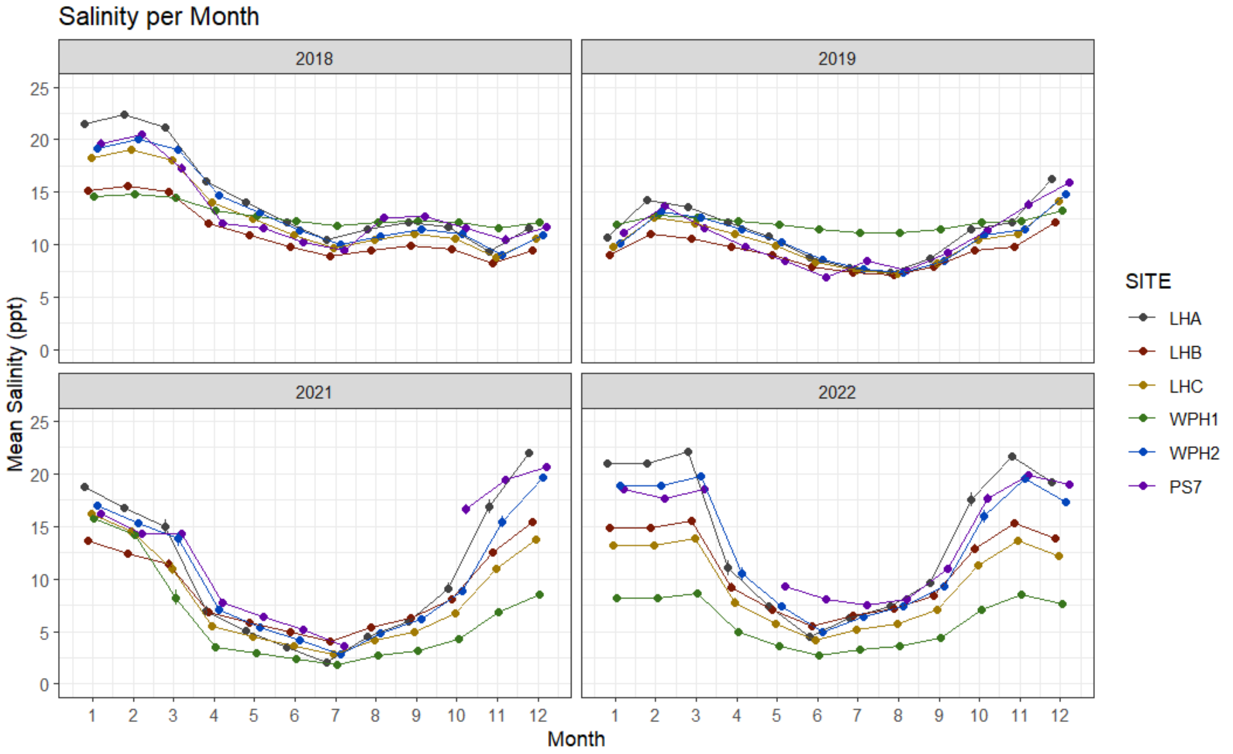


Figure 6. Salinity per Month Monthly mean salinity (ppt) for six sites and four years. Mean salinities were calculated by averaging one measurement for each day of the month. Salinity values were obtained from salinity loggers installed at all sites except 2018 and 2019 WPH1 data which was obtained from a Sonde logger located at the outfall canal (siphon), approximately 1.2 km away from the site. Error bars for each month indicate the standard error of that month’s daily measurements.

Major decreases in salinity, especially at WPH1, began in March 2021 as the siphon began operation. Decreases continued through the year until siphon operation stopped for the year at the end of June, at which point salinity started increasing again until the end of February of 2022, which is when the siphon began continuously discharging freshwater until July 2022. Macroinvertebrate sampling from May through July in 2018 and 2021 showed the same salinity patterns as the nekton with WPH1 starting as the highest (average 12.7) then dropping to the lowest (average 2.7) salinity and PS7 (2018 salinity 11.6, 2021 salinity 6.4) being the highest. LHB showed distinct differences in its ranking compared to other sites between macroinvertebrate sampling as well with an average of 14.0 in 2018 but dropping to one of the lowest 4.6 in 2021.

Marsh Inundation

As with temperature and salinity, the percent of time inundated per day (**Figure 7. Inundation per Month)** fluctuates with seasons. This pattern is slightly more complicated than the previous two as this seems to have bimodal peaks within years. Winter months have the lowest inundation, with peaks in spring and fall months and a dip between those peaks in the summer. WPH1, LHB, LHC, and WPH2 were the most similar in time inundated and ranked highest compared to PS7 and LHA ranking the lowest. However, WPH2 showed a larger separation from WPH1, LHB, and LHC in siphon-on years. For nekton sampling in mid-May 2018 and 2019, LHB (average 72.2%), LHC (average 74.1%), and WPH1 (average 77.4%) have the highest inundation levels with LHA (average 36.%) and PS7 (average 46.%) having the lowest. In 2021 and 2022 LHB (average 67.7%), LHC (average 73.7%), and WPH1 (average 69.6%) stay the highest with LHA (average 28.4%) and PS7 (average 31.4%) still the lowest. WPH2 is highly variable between years but stays in the middle of the upper and lower groups already distinguished. Macroinvertebrate sampling from May through July in 2018 had WPH1 (average 64.5%) flooded the most amount of time with LHB (average 55.0%), LHC (average 54.2%), and WPH2 (average 46.4) in the middle with PS7 (average 28.8%) and LHA (average

25.3) as the lowest. This same ranking goes with 2021 as well except with more distinguishment with WPH2 (average 48.2%) and PS7 (average 40.7%) grouping more closely in between the rest of the sites (average LHA=23.5%, LHB=61.6%, LHC=68.7%, WPH1=63.7%). It should be noted that while the restored sites were being constructed, the platforms were built to the same height, but the sites did not settle the same, leading to LHA staying higher than LHB. The differences in platform height may be the leading cause of LHA's comparatively low inundation to the rest of the sites.

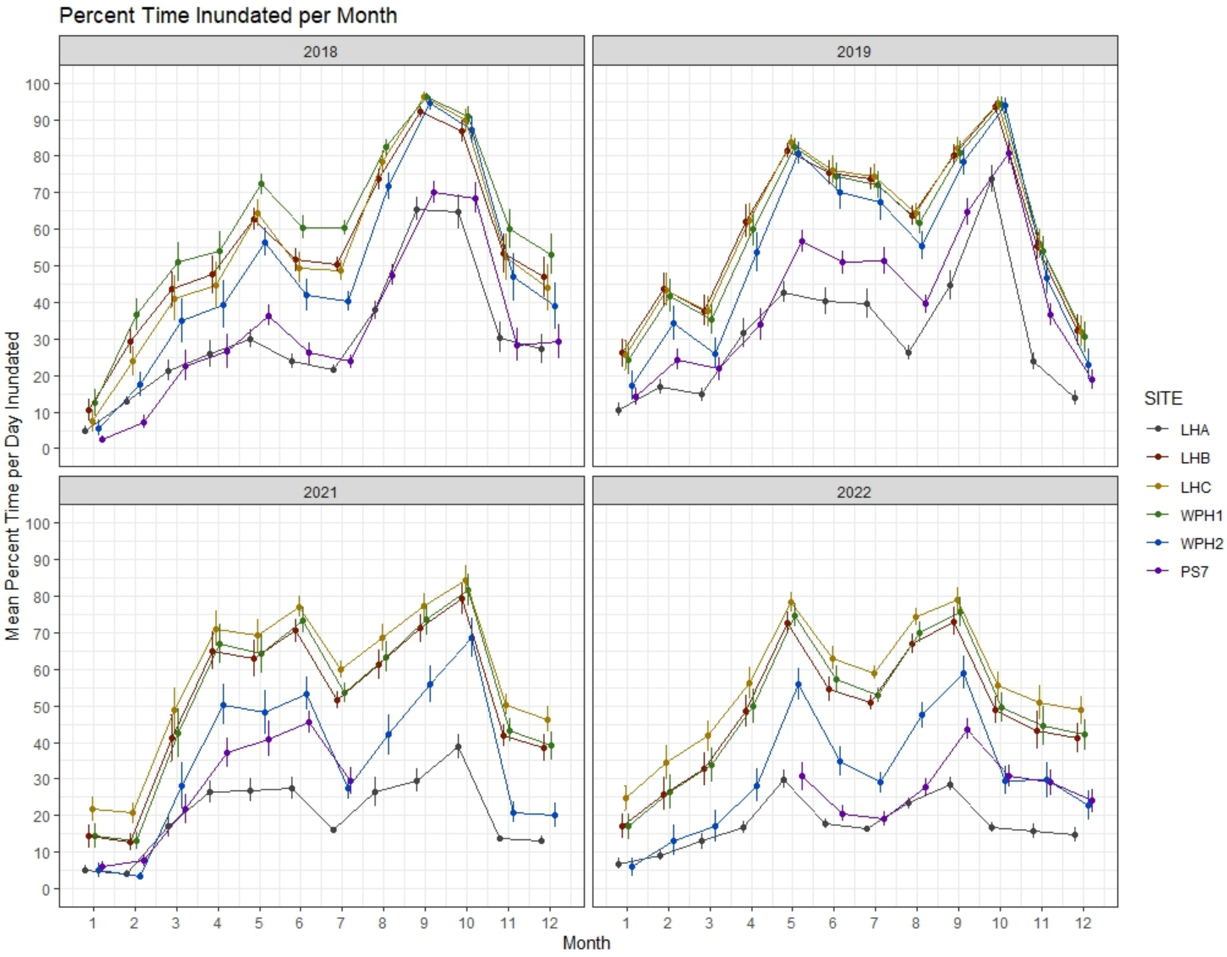


Figure 7. Inundation per Month Monthly mean percent of time inundated for six sites and four years. Mean inundation percentages were calculated by averaging one measurement for each day of the month. Inundation values were obtained from Sonde loggers installed at all sites. Error bars for each month indicate the standard error of that month's daily measurements. Data for PS7 in August 2021 through April 2022 are missing due to loss of the logger, which was replaced in March 2022.

On-Marsh Nekton Site Abundance

There were differences among restored and reference sites and among the salinity gradient sites (**Table 1. Site Number of Individuals Results**, **Figure 8. Site Number of Individuals: Restored vs Reference,**). All sites had their highest means in 2021 (LHA=34.37, LHB=42.35, LHC=30.62) and the lowest in 2022 for LHA (9.05) and LHB (10.21) and in 2018 for LHC (8.17). The only significant differences in total fish abundance among the restored and reference sites was in 2018 ($F_{2,51}$ = 4.60, p=1.46). These differences were between LHA (mean=9.68) and LHB (mean=20.83, p=0.047) and between LHB (mean=20.83) and LHC (mean=8.17, p=0.021).

The three sites along the salinity gradient showed significant differences between siphon-off and on years when comparing the number of species caught among years at each site (**Table 1. Site Number of Individuals**, **Figure 9. Site Number of Individuals: Salinity Gradient**). The highest means for these sites were all in 2022, WPH1=29.28, WPH2=51.48, and PS7=44.39. The lowest mean for all sites were in 2019, WPH1=8.70, WPH2=13.79, and PS7=5.01. Statistical analysis for PS7 day was constrained to day 1 of sampling due to unevenness in sampling in 2021. WPH1 ($F_{3,51}$ = 4.22, p>0.001) was significantly different between 2018 (mean=10.19) and 2022 (mean=29.28, p=0.46) and between 2019 (mean=8.70) and 2022 (mean=29.28, p=0.008), showing distinctions between siphon-off and on conditions. WPH2 ($F_{3,51}$ = 28.1 9, p<0.001) had significant differences between 2019 (mean 13.79) and 2021(mean=34.11, p=0.015) and between 2019 (mean=13.79) and 2022 (mean=51.48 p=0.023), again, between siphon-off and on years, but not all of them. PS7 ($F_{3,24}$ = 28.19, p<0.001) had the most differences found, including between 2018 (mean=44.16) and 2019 (mean=5.01, p<0.001), 2018 (mean=44.16) and 2021 (mean=10.10, p<0.001), 2019 (mean=5.01) and 2022 (mean=44.39, p<0.001), and between 2021 (mean=10.10) and 2022 (mean=44.39, p<0.001).

Table 1. Site Number of Individuals Results Statistical results for the number of individuals caught within sites. Table includes the factor, levels, main statistical tests (One-way ANOVA or Repeated Measures ANOVA [ANOVAR]) and post hoc comparison (Tukey HSD) where applicable, as well as other associated values (degrees of freedom, test statistic, p-value). Alpha level was 0.05 for all tests. Transformations used to meet assumptions of normality or homogeneity of variance are noted. NA = not applicable.

Year & Site	Main Test	DF	Test statistic	P-value (main)	Significant Difference	P-value (Tukey HSD)	Transformation
2018 LHA,B ,C	ANOVA	2, 51	4.60	0.015	LHA and LHB	0.047	NA
					LHB and LHC	0.021	NA
2019, LHA,B ,C	ANOVA	2, 51	3.06	0.056	NA	NA	NA
2021 LHA,B ,C	ANOVA	2, 51	0.77	0.469	NA	NA	NA
2022 LHA,B ,C	ANOVA	2, 51	1.41	0.253	NA	NA	log
WPH1 2018 - 2022	ANOVAR	3, 51	4.22	**<0.001**	2018 and 2022	0.046	NA
					2019 and 2022	0.008	NA
WPH2 2018 - 2022	ANOVAR	3, 51	28.19	**<0.001**	2019 and 2021	0.015	log
					2019 and 2022	0.028	log
PS7 2018 - 2022	ANOVAR	3, 24	28.19	**<0.001**	2018 and 2019	<0.001	log, only day 1
					2018 and 2021	<0.001	log, only day 1
					2019 and 2022	<0.001	log, only day 1
					2021 and 2022	<0.001	log, only day 1

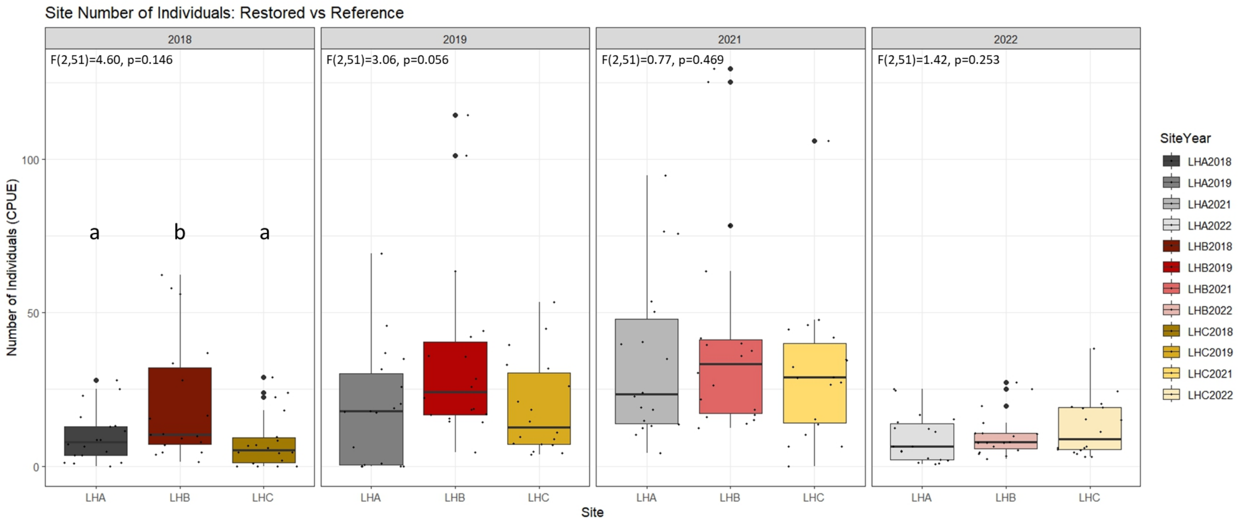


Figure 8. Site Number of Individuals: Restored vs Reference Box plots represent the number of individual on-marsh nekton caught per sample at two restored (LHA and LHB) and reference sites (LHC) over four years (2018, 2019, 2021, 2022). Catch data was transformed into catch per unit effort (CPUE) representing the number of individuals caught in three traps over a one hour period per sampling effort. Individual data points for each sample are represented for each plot.

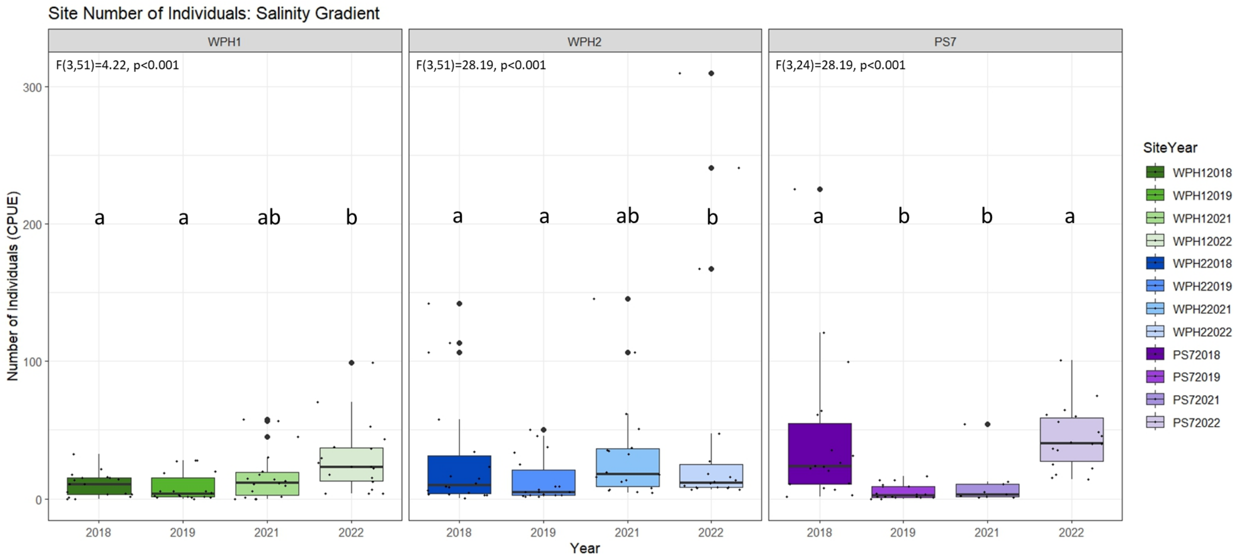


Figure 9. Site Number of Individuals: Salinity Gradient Box plots representing the number of individual on-marsh nekton caught per sample at sites (WPH1, WPH2, and PS7) along the salinity gradient over four years (2018, 2019, 2021, 2022) in which the siphon was on in 2021 and 2022. Catch data was transformed into catch per unit effort (CPUE) representing the number of individuals caught in three traps over a one hour period per sampling effort. Individual data points (n=18 per site per year except 2021 PS7 in which n=9) for each sample are represented for each plot as well.

On-Marsh Nekton Site Diversity

Shannon Diversity Index (H') across space and time per site

Biodiversity was visualized using six different indices (i.e. number of individuals caught (**Figure 10. Site Species Shannon Diversity: Restored vs Reference, Figure 11**. **Site Species Shannon Diversity: Salinity Gradient**), number of species caught (**Supplemental Figure 1. Site Number of Species per Sample**), species richness (**Supplemental Figure 2. Site Species Richness**), species evenness (**Supplemental Figure 3. Site Species Pielou's Evenness**), Shannon diversity (**Figure 10. Site Species Shannon Diversity: Restored vs Reference, Figure 11. Site Species Shannon Diversity: Salinity Gradient**), and Simpson diversity (**Supplemental Figure 4. Site Species Simpson Diversity**) to capture different aspects of community composition and variability across space and time. Only the number of individuals caught and Shannon diversity were further used for statistical analysis. Species richness and evenness was more variable within sites than across sites and years. Notably, species richness and evenness values at the restored sites were consistent with those at reference sites. Similarly, the mean number of species (**Figure 10. Site Species Shannon Diversity: Restored vs Reference, Figure 11. Site Species Shannon Diversity: Salinity Gradient**) did not change at sites nearest to the siphon (LHA, LHB, LHC, and WPH1), where a decrease in salinity was associated with siphon discharge in 2021 and 2022. There was an associated decrease in evenness and the number of species at WPH1 in the first year of siphon operation, but then evenness rebounded in the second year which can also be seen in the number of species caught in some samples. The Shannon diversity index and Simpson index were again more variable within sites across years than across sites within years. Both diversity indices indicate restored sites have similar biodiversity as reference sites. There is also a sustained decrease of biodiversity at WPH1, closest to the siphon, when there is freshwater input at this site over the two years in which it was operational in 2021 and 2022, but these differences were not found to be statistically significant.

Looking at the Shannon diversity for restored and reference sites (**Figure 10. Site Species Shannon Diversity: Restored vs Reference**), there were significant differences among restored and reference, and within reference sites themselves, but only in 2019 ($F_{2,51}$=5.52, p=0.007) with LHA showing the most differences between sites (**Supplemental Table 4. Site Shannon Diversity Results**). Significant differences in 2019 were found specifically between LHA (mean=19.10) and LHB (mean=35.17, p=0.022) and between LHA (mean=19.10) and LHC (mean=19.21, p=0.012).

For sites along the salinity gradient over time (**Figure 11. Site Species Shannon Diversity: Salinity Gradient**), significant differences were only found at PS7 ($F_{3,24}$=4.68, p=0.010) (**Supplemental Table 4. Site Shannon Diversity Results**). Differences at PS7, while containing a singular difference between siphon-off and on years, are not overall consistent with siphon operation, leading us to believe the siphon does not influence Shannon diversity at these sites. PS7 analysis only considered day 1 of sampling due to the unevenness of sampling in 2021, where only one day of sampling occurred compared to two days in other years. Significant differences were found between 2018 (mean=44.16) and 2019 (mean=5.10, p=0.011) and between 2019 (mean=5.10) and 2022 (mean=44.39, p=0.027) using Tukey HSD post hoc tests after an initial repeated measures ANOVA. Interactions between site and year along the salinity gradient were not performed for Shannon diversity again due to the same constraints with 2021 PS7 data and assumptions that results would not be able to encompass true differences limiting to just day 1 sampling data.

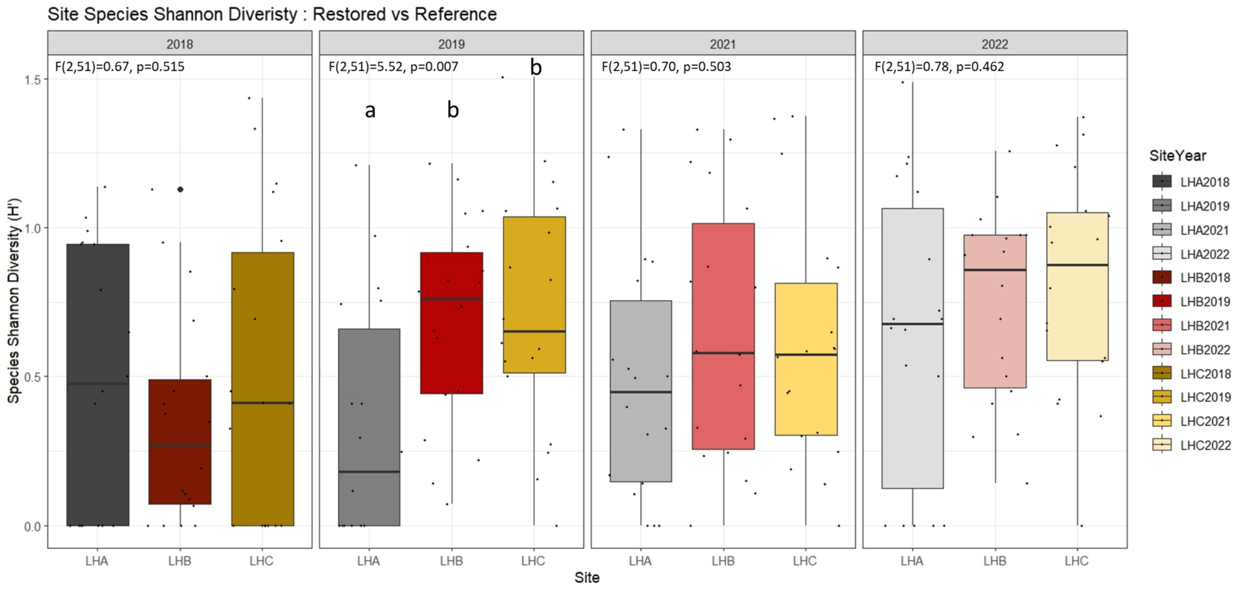


Figure 10. Site Species Shannon Diversity: Restored vs Reference Box plots representing the Shannon Diversity on-marsh nekton caught per sample at two restored (LHA and LHB) and reference sites (LHC) over four years (2018, 2019, 2021, 2022). Catch data was transformed into catch per unit effort (CPUE) representing the Shannon diversity in three traps over a one hour period per sampling effort. Individual data points for each sample are represented for each plot.

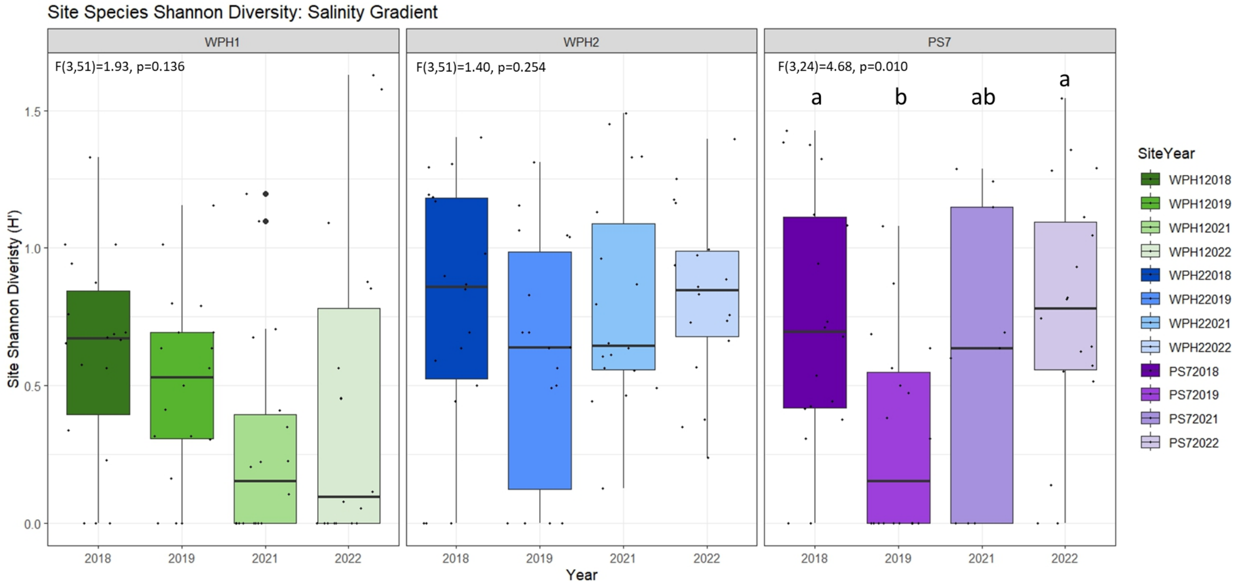


Figure 11. Site Species Shannon Diversity: Salinity Gradient Box plots representing the Shannon diversity on-marsh nekton caught per sample at sites (WPH1, WPH2, and PS7) along the salinity gradient over four years (2018, 2019, 2021, 2022) in which the siphon was on in 2021 and 2022. Catch data was transformed into catch per unit effort (CPUE) representing the Shannon diversity in three traps over a one hour period per sampling effort. Individual data points (n=18 per site per year except 2021 PS7 in which n=9) for each sample are represented for each plot.

Community composition trajectories across space and time per site

Community composition between sites plotted as a trajectory between years through centroids of site-year groupings (**Figure 12. On-Marsh Nekton Community Trajectory nMDS**) displayed similarity between all sites in siphon-on years, including restored and reference sites. Overall, we see interannual variability with shifts between years within all sites, but not one that would lead us to believe in a large change except for WPH1. In 2021, the first year of siphon-on conditions, all sites show a large shift away from their community composition in siphon-off conditions. In the second year of siphon operations, excluding WPH1, all sites remain similar to the previous year but display a “U” shaped shift towards 2018 compositions indicating that, while being affected by the siphon, communities seem to be returning to pre-siphon-on compositions. WPH1, the site closest to the siphon, does not display this same trajectory though. The shift from siphon-off to siphon-on years is much more pronounced and increasing as the siphon remains operational from 2021 to 2022, with seemingly no return to pre-siphon community composition at this site.

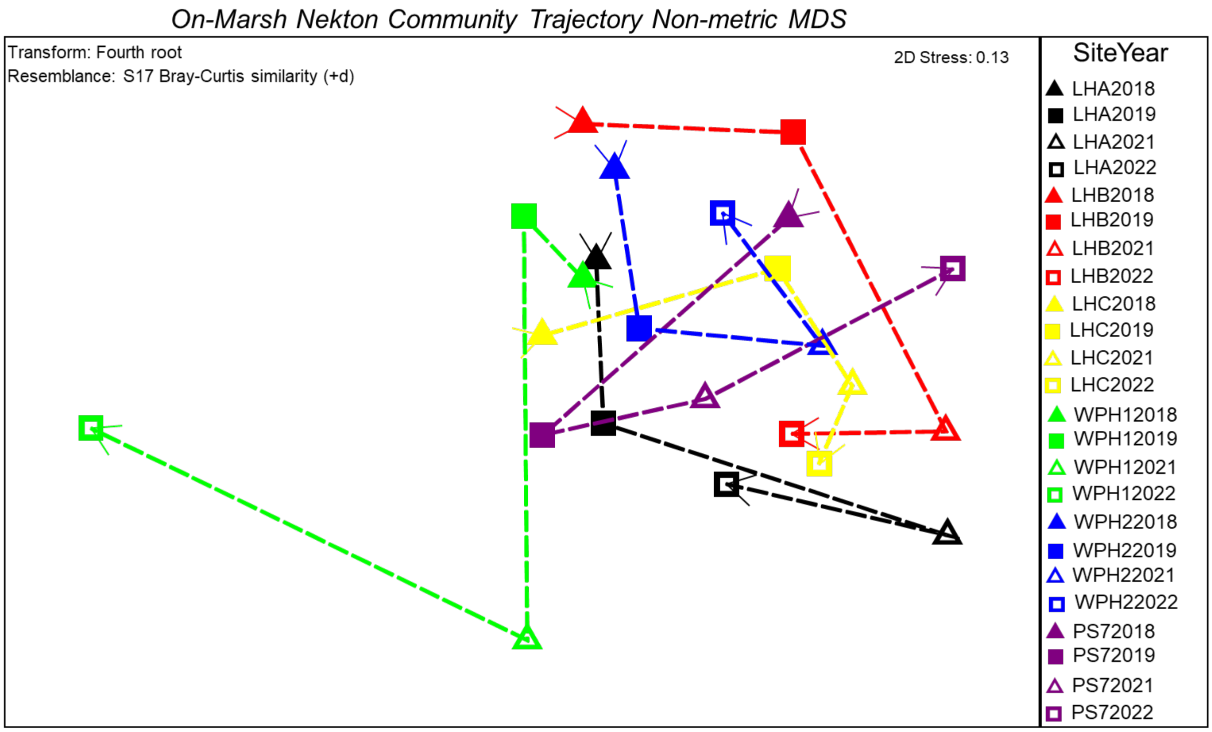


Figure 12. On-Marsh Nekton Community Trajectory nMDS Non-metric multidimensional scaling (nMDS) plot of nekton community composition trajectories displaying shifts between sites over time. Data was standardized to CPUE, fourth root transformed, and Bray-Curtis calculations including a dummy value were used to assemble a resemblance matrix for figure creation. Data points represent the centroids of site-year groupings calculated optimally dividing samples into specific groupings to minimize the within-group sum of squares in a high-dimensional space.

<u>On-Marsh Nekton Subhabitat Abundance</u>

Individual counts across space and time per subhabitat

Plots of restored versus reference sites (**Figure 13. Subhabitat Number of Individuals: Restored vs Reference**) and of sites along the salinity gradient (**Figure 14. Subhabitat Number of Individuals: Salinity Gradient**) displaying distribution of individuals caught between subhabitats revealed that, overall, ponds had the highest abundances of individuals caught through all subhabitats, sites, and years. Looking only at creeks between restored and reference sites, significant differences (**Supplemental Table 5. Creek Number of Individuals Results**) were found between and within restored versus reference groups with LHA having the most differences but only in 2019 ($F_{2,15}$=47.79, p<0.001). Mean abundance for LHA was 19.10 while LHB had a mean of 35.17 individuals caught (p<0.001). Significant differences were also found between LHA's mean of 19.10 and LHC's mean of 19.21 individuals (p<0.001). These results lead us to believe restored and reference sites have minimal differences between them and are highly similar.

Between years within sites along the salinity gradient, only WPH2 ($F_{3,15}$=17.08, p<0.001) and PS7 ($F_{3,6}$=7.17, p=0.021) had significant differences but they were not constrained to just between siphon-off and on years, indicating that siphon operation may have some influence on these sites but that there is still interannual variability unrelated to the siphon that may have caused these differences as well. WPH2 post hoc testing revealed significant differences between 2018 (mean=31.09) and 2021 (mean=34.11, p<0.001), 2019 (mean=13.79) and 2021 (mean=34.11, p<0.001), 2019 (mean=13.79) and 2022 (mean=51.48, p=0.008), and between 2021 (mean=34.11) and 2022 (mean=51.48, p=0.045). PS7 post hoc testing indicated differences between 2019 (mean=5.10) and 2022 (mean=44.39, p=0.028) and between 2021 (mean=10.10) and 2022 (mean=44.39, p=0.038). Interactions between site and year along the salinity gradient were not performed due to the constraint of only being able to utilize one day of data due to the unevenness mentioned previously with PS7 in 2021, assuming results would not be able to encompass true differences with such a limited data set.

Next, looking at edge subhabitats (**Supplemental Table 6. Edge Number of Individuals Results**) between restored and reference sites, significant differences were only found within 2021 ($F_{2,15}$=6.06, p=0.012) between restored sites indicating that edge subhabitats between restored and references are the same in terms of the number of individuals caught. Only the LHA's mean number of individuals of 54.72 was significantly different from LHB's mean of

18.65 (p=0.010). Along the salinity gradient, only the two closest sites, WPH1 and WPH2 showed significant differences which were also between siphon-off and on years indicating that, while not strongly different, there could be possible siphon-induced shifts in the number of species present. WPH1 ($F_{3,15}$=5.75, p=0.008) contained significant differences between 2019's mean of 2.73 and 2021's mean of 29.38 individuals caught (p=0.006). WPH2 (Friedman's test, F_3=13.4, p=0.004) had a mean abundance of 5.04 in 2019 which was significantly different from 2022's mean of 2.22 (Nemenyi post hoc, p=0.002). PS7 ($F_{3,15}$=2.69, p=0.140), only using day 1 data, did not show any significant differences between years. Again, a mixed ANOVA was not performed for the site year interaction along the salinity gradient due to uneven data collection.

Finally, looking at the number of individuals caught within ponds (**Supplemental Table 7. Pond Number of Individuals Results**) between sites and years for restored versus references analysis, there were only a few differences between sites in one year leading us to, once again, say restored and reference sites are extremely similar. 2018 ($F_{2,15}$=21.31, p<0.001) was the only year with significant differences. These were found between the mean number of individuals of 8.32 at LHA and of 45.77 at LHB (p<0.001) and between LHB's mean (45.77) and LHC's mean of 8.43 individuals (<0.001). Next, sites along the salinity gradient had no significant differences between years within any of the sites indicating that the siphon has no detectable influence on pond subhabitat abundances. Again, a mixed ANOVA was not performed for the site year interaction along the salinity gradient due to uneven data collection.

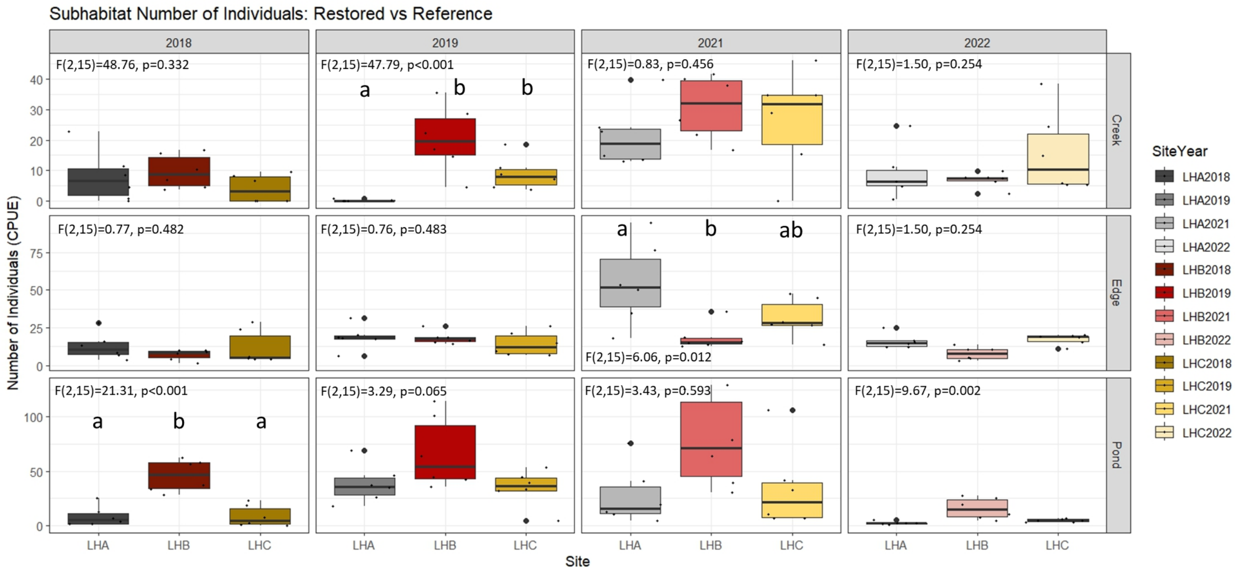


Figure 13. Subhabitat Number of Individuals: Restored vs Reference Box plots representing the number of individual on-marsh nekton caught per sample at two restored (LHA and LHB) and reference sites (LHC) over four years (2018, 2019, 2021, 2022) within three subhabitats (creeks, edges, and ponds). Catch data was transformed into catch per unit effort (CPUE) representing the number of individuals caught in three traps over a one hour period per sampling effort. Individual data points for each sample are represented for each plot.

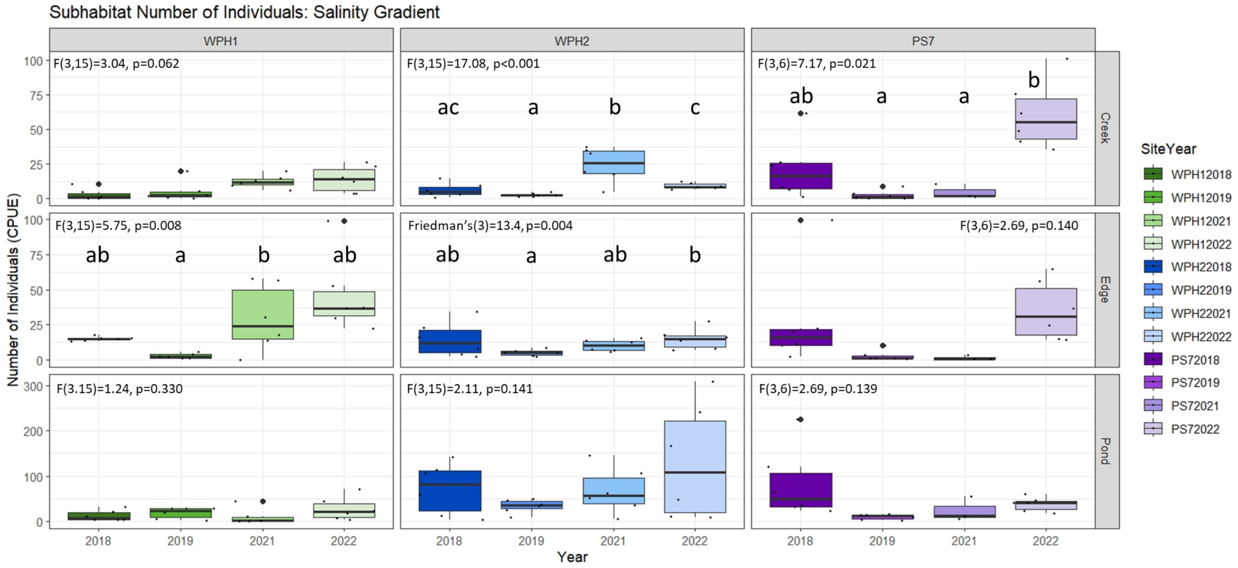


Figure 14. Subhabitat Number of Individuals: Salinity Gradient Box plots representing the number of individual on-marsh nekton caught per sample at sites (WPH1, WPH2, and PS7) along the salinity gradient over four years (2018, 2019, 2021, 2022) within three subhabitats (creeks, edges, and ponds) in which the siphon was on in 2021 and 2022. Catch data was transformed into catch per unit effort (CPUE) representing the number of individuals caught in three traps over a one hour period per sampling effort. Individual data points (n=18 per site per year except 2021 PS7 in which n=9) for each sample are represented for each plot.

On-Marsh Nekton Subhabitat Diversity

Shannon Diversity Index (H') across space and time per subhabitat

Subhabitat biodiveristy was looked at in a simmilar manner to that of site biodiveristy. We visualized six different indices (i.e. number of individuals caught (**Figure 13. Subhabitat Number of Individuals: Restored vs Reference**, **Figure 14. Subhabitat Number of Individuals: Salinity Gradient**), number of species caught (**Supplemental Figure 5. Subhabitat Number of Species**), species richness (**Supplemental Figure 6. Subhabitat Species Richness**), species evenness (**Supplemental Figure 7. Subhabitat Species Pielou's Evenness**), Shannon diversity (**Figure 15. Subhabitat Species Shannon Diversity: Restored vs Reference**, **Figure 16. Subhabitat Species Shannon Diversity: Salinity Gradient**), and Simpson diversity (**Supplemental Figure 8. Subhabitat Species Simpson Diversity**) to capture different aspects of community composition and variability across subhabitat space and time. Again, only the number of individuals caught and Shannon diversity were used for statistical analysis. Biodiversity values (H') at each site exhibited high variability. Upon further examination, the data was grouped by subhabitat (edge, creek, and edge) to evaluate the biodiversity patterns across site and years, while taking into account the spatial heterogeneity of each (**Figure 15. Subhabitat Species Shannon Diversity: Restored vs Reference, Figure 16. Subhabitat Species Shannon Diversity: Salinity Gradient**).

Biodiversity fluctuated across subhabitats over the course of this study within restored and reference sites (**Figure 15. Subhabitat Species Shannon Diversity: Restored vs Reference**). During the first year (2018), the creeks at LHA (mean=0.19), LHB (mean=0.60), and LHC (mean=0.19) had the same levels of biodiversity (p=0.074) (**Supplemental Table 8. Creek Shannon Diversity Results**). During the second year (2019) the biodiversity levels at one restored site LHA (mean=0.00) were significantly smaller relative to the other restored site LHB (mean=0.82, p=0.007), and the control LHC (mean=0.79, p=0.007). However, there were no detectable differences by the end of the study (2021, p=0.192; 2022, p=0.110).

The pattern was different along the edges (**Supplemental Table 9. Edge Shannon Diversity Results**). Differences were detected in the first three years but not the last (2022, p=0.410). In 2018, LHA (mean=0.84) had higher biodiversity relative to LHB (mean=0.27, p=0.038) and was no different than LHC (mean=0.59). In 2019, LHB (mean=0.97) had the highest biodiversity relative to LHA (mean=0.21, p<0.001) and LHC (mean=0.358, p<0.001). In 2021, LHA (mean=0.07) had lower biodiversity than LHB (mean=0.51, p=0.009) and LHC (mean=0.42, p=0.040).

While biodiversity in created creeks and edges seems to have adjusted over time to resemble the biodiversity of the control site, LHC, that was not the case for the pond comparisons (**Supplemental Table 10. Pond Shannon Diversity Results**). There were no differences in 2018 (p=0.182) and 2021 (p=0.337). However, in 2019 the ponds at one restored site LHB (mean=0.32) had lower biodiversity than the other restored site LHA (mean=0.79, p=0.016) and the control LHC (mean=0.79, p=0.002). At the end of the study (2022), the control site LHC (mean=0.98) had higher biodiversity relative to LHA (mean=0.23, p=0.004), but was no different to LHB (mean=0.64).

Along the salinity gradient, Shannon diversity showed no variability at creeks (**Supplemental Table 8. Creek Shannon Diversity Results**), some variability at edges closest to the siphon, and high variability within all site’s ponds that also tended to have the highest diversity out of all subhabitats (**Figure 16. Subhabitat Species Shannon Diversity: Salinity Gradient**). WPH1 (p=0.584), WPH2 (p=0.284), and PS7 (p=0.084) had no significant differences between years within sites indicating that the siphon has no detectable influence on these site's creeks when it is turned on.

At edges, we were able to detect significant differences, but only at the site closest to the siphon and differences were between years of siphon-off and on conditions indicating a high influence at this site when the siphon is turned on (**Supplemental Table 9. Edge Shannon Diversity Results**). WPH1 ($F_{3,15}$=8.53, p=0.005) showed significant differences between the lower diversity (mean=15.15) of 2018 and the higher diversity of 2021 (mean, 29.38) p<0.001), between 2018's lower diversity (mean=15.15) and 2022's higher diversity (mean=17.43, p<0.001), between 2019's lower diversity (mean=2.73) and 2021's higher (mean=29.38, p<0.001), and between 2019's lower (mean=2.73) and 2022 higher diversity (mean=17.43, p<0.001). A mixed ANOVA was not performed for the site year interaction along the salinity gradient due to uneven data collection.

Within ponds along the salinity gradient, there were significant differences between years within all sites, but not strong enough to indicate that the siphon was the main cause of the differences observed, especially at sites farther away from the freshwater influence (**Supplemental Table 10. Pond Shannon Diversity Results**). WPH1 ponds ($F_{3,15}$=8.53, p=0.005) had the highest diversity in 2022 (mean=1.08) compared to 2019 (mean=0.33, p=0.003) and 2021(mean=0.37, p=0.004). WPH2 ($F_{3,15}$=4.50, p=0.019) had significantly higher diversity in 2021 (mean=1.27) compared to 2019 (mean=0.57). PS7 ($F_{3,6}$=5.48, p=0.037), only using data from 1 day sampling, showed significant differences only between 2018's higher diversity (mean=1.15) compared to 2019's lower diversity (mean=0.44, p=0.046). While there were significant differences within all sites, none of them consistently corresponded with siphon-off or on years leading us to conclude that siphon operation has no significant effect on on-marsh nekton Shannon diversity within pond subhabitats. A mixed ANOVA was not performed for the site year interaction along the salinity gradient due to uneven data collection.

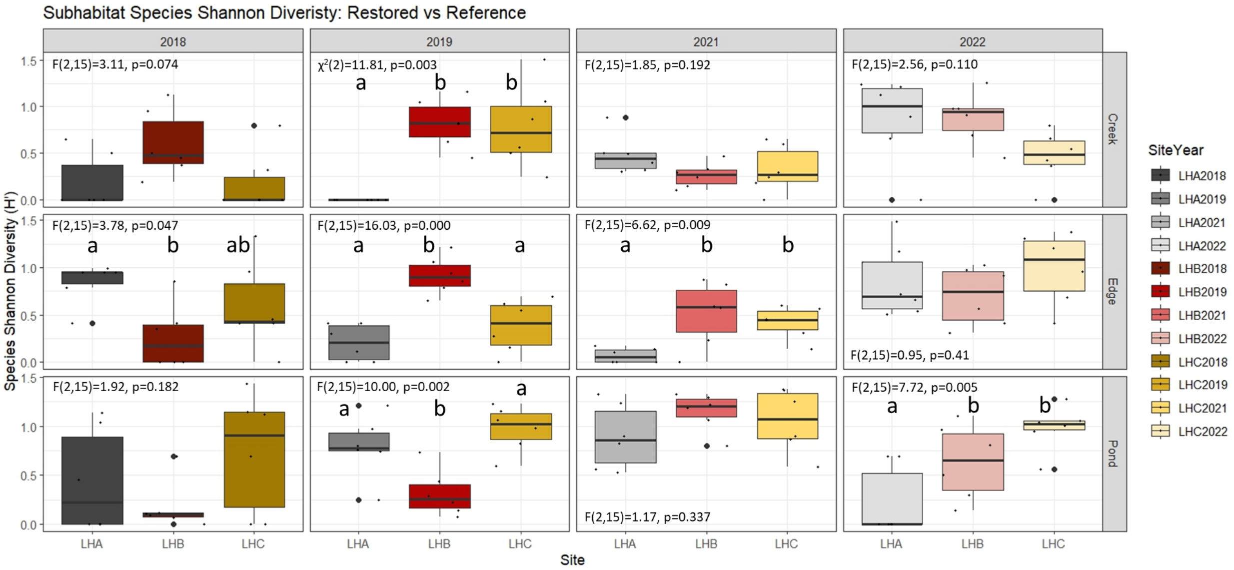


Figure 15. Subhabitat Species Shannon Diversity: Restored vs Reference Box plots representing the Shannon diversity of on-marsh nekton caught per sample at two restored (LHA and LHB) and reference sites (LHC) over four years (2018, 2019, 2021, 2022) within three subhabitats (creeks, edges, and ponds). Catch data was transformed into catch per unit effort (CPUE) representing the Shannon diversity in three traps over a one hour period per sampling effort. Individual data points for each sample are represented for each plot.

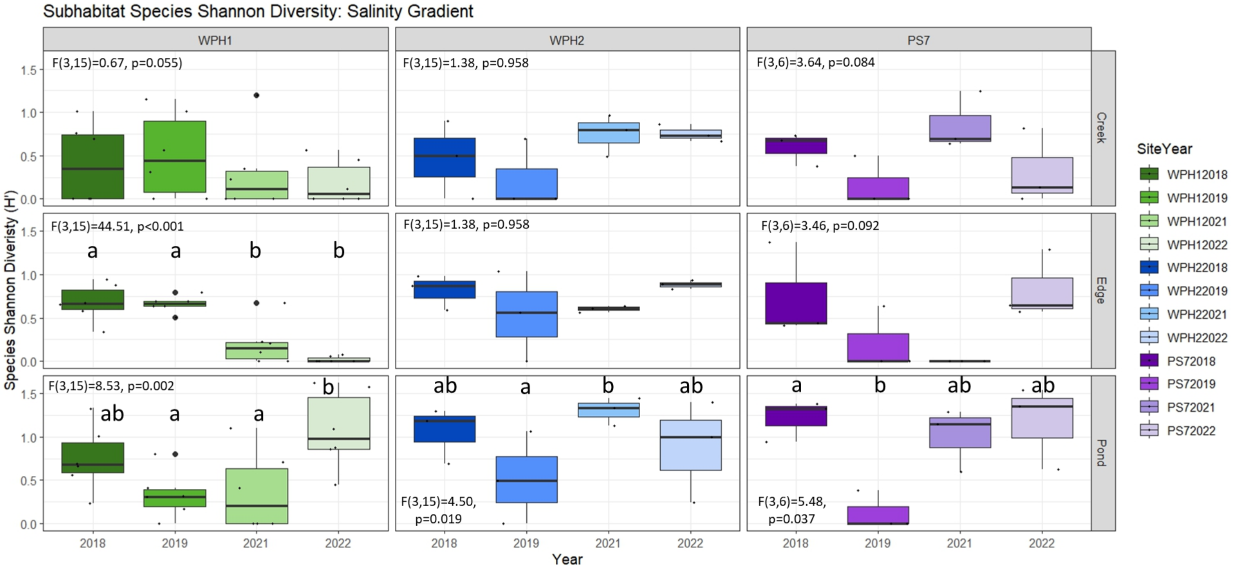


Figure 16. Subhabitat Species Shannon Diversity: Salinity Gradient Box plots representing the Shannon diversity of on-marsh nekton caught per sample at sites (WPH1, WPH2, and PS7) along the salinity gradient over four years (2018, 2019, 2021, 2022) within three subhabitats (creeks, edges, and ponds) in which the siphon was on in 2021 and 2022. Catch data was transformed into catch per unit effort (CPUE) representing the Shannon diversity in three traps over a one hour period per sampling effort. Individual data points (n=18 per site per year except 2021 PS7 in which n=9) for each sample are represented for each plot.

Community composition across space and time per subhabitat

Non-metric multidimensional scaling plots (nMDS) helped visualize the patterns and shifts in communities between subhabitats over time along with determining which species were responsible for groupings and shifts (**Figure 17. On-Marsh Nekton Subhabitat Community Composition**). Between restored and reference sites, pond and creek subhabitats clustered together relatively closely indicating similarity within individual subhabitat samples over time and between creek and edge subhabitats. Ponds, on the other hand, created their own cluster separate from creeks and edges indicating dissimilarity between these subhabitats, but similarity between pond samples through time.

A relatively similar pattern is displayed for sites along the salinity gradient (WPH1, WPH1 and PS7) in which creeks and edges cluster and ponds create their own grouping.

Similarity between years is consistent at WPH2 and PS7, but not at WPH1, particularly in 2022 within creeks and edges. The distance between these polygons and the rest of the group indicate high levels of dissimilarity at creeks and edges in the last year of our study when the siphon was turned on. Within all sites, species correlated to the similarity we saw in creeks and edges were determined to be due to the presence of brown and grass shrimp. Consistency in ponds is attributed to Cyprinodontiforme fish. The dissimilarity and outgrouping of 2022 WPH1 creeks and edges was due to a shift in the shrimp species present in which Ohio shrimp took over in these communities. (**Supplemental Figure 9. On-Marsh Nekton Subhabitat Trajectory nMDS**) also shows this similar grouping pattern and stability over time within ponds with higher variability between years within edges and especially creeks.

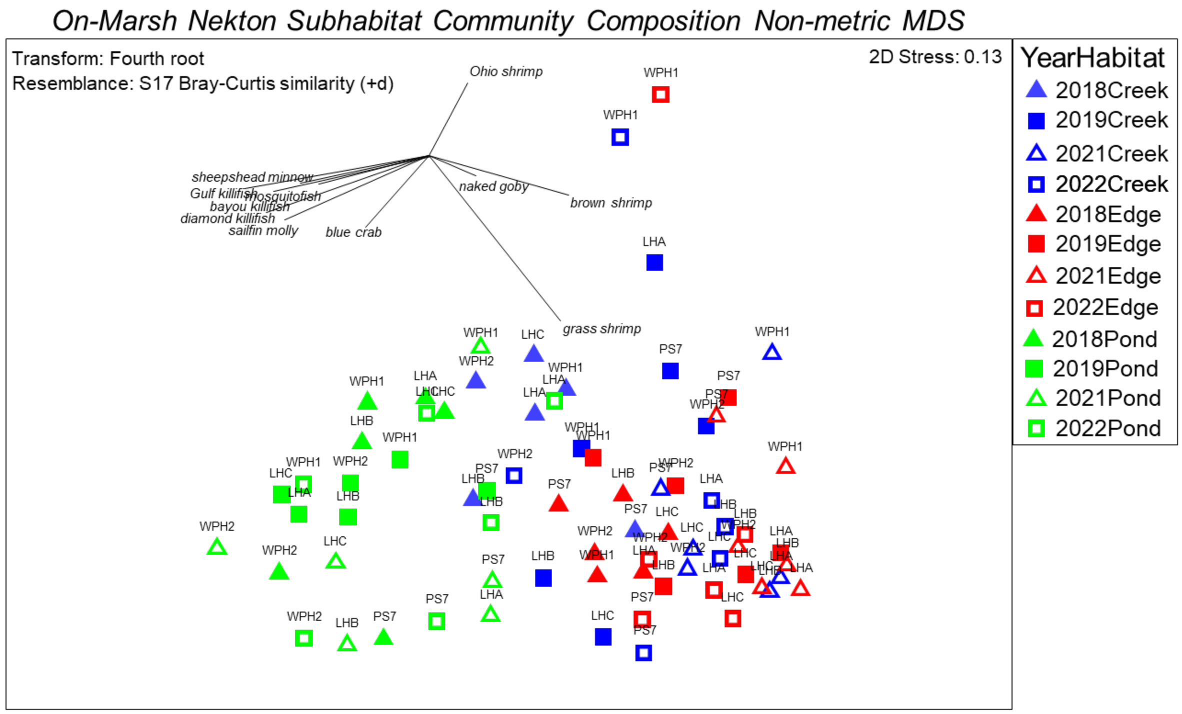


Figure 17. On-Marsh Nekton Subhabitat Community Composition Non-metric multidimensional scaling (nMDS) plot of nekton community composition displaying shifts between subhabitats over time. Data was standardized to CPUE, fourth root transformed, and Bray-Curtis calculations including a dummy value were used to assemble a resemblance matrix for figure creation. An additional vector displaying species responsible for sample differences having a Pearson correlation>0.2 was also added.

Grass Shrimp (Palaemon spp)

Grass shrimp were one of the top species, abundance wise, that we captured through our sites in all years of sampling comprising 29.92% of our total individuals caught. Raw catch numbers can be found within **Supplemental Table 11. Grass Shrimp Population Numbers**. They resided within all three subhabitat types with the highest amount found at the edge (54.94%), then creeks (37.97%), and the least found within ponds (8.57%) While they were generally abundant though our sampling efforts, there were some drastic fluctuations that occurred within years between the restored and reference sites (**Figure 18. Grass Shrimp CPUE: Restored vs Reference**) and within sites along the salinity gradient between siphon-off and on conditions (**Figure 19. Grass Shrimp CPUE: Salinity Gradient**).

During the first two years of sampling within the restored and reference sites, abundance levels of grass shrimp were generally lower than they were in the last two years of sampling in which the siphon was turned on. There was a large spike in the first year of siphon operation (2021) but it was not sustained and CPUE levels dropped down to be just slightly higher than the first two years of sampling. Between restored and reference though, it seems as if most of the sites are similar to one another with mean CPUEs being fairly close and area as follows: 2018 LHA=2.99, LHB=0.90, LHC=3.31; 2019 LHA=5.93, LHB=4.47, LHC=4.94; 2021 LHA=30.06, LHB=17.63, LHC=18.06; 2022 LHA=3.24, LHB=4.23, LHC=6.97. Having such similar mean CPUEs led to only one significant difference being found between restored and reference sites though the years (**Supplemental Table 12**. **Grass Shrimp CPUE Results**). This difference was within the restored sites in 2019 ($F_{2,65}$=4.43, p=0.016), LHA and LHB (P=0.011), indicating that restored and reference sites are inhabited extremely similarly for grass shrimp species.

Sites along the salinity gradient were fairly stable though time except for a large spike in abundance in 2021 for WPH1 and WPH2 and in 2022 for PS7. While this spike does correspond to the first year the siphon was turned on, the increase was not sustained and there was a large decrease back to siphon-off abundances in WPH2 with a complete elimination of grass shrimp at WPH1, the site closest to the siphon. Average CPUEs for these sites through the years sampled are as follows: WPH1=2.85, 0.17, 11.34, 0.00; WPH2=1.28, 0.31, 8.38, 1.98; PS7=10.42, 0.73, 1.93, 22.12. With the large fluctuations found amongst years within sites, it was not surprising to find significant differences along the salinity gradient. WPH1 ($\chi^2(3)=15.26$, p=0.002) had differences between and within siphon-off and on years, but these changes cannot be fully attributed to the siphon as the trends are drastically different within siphon-on years. 2022, the year there were no grass shrimp caught, was significantly different from 2018 (p=0.028) and 2021 (p=0.002) which had much larger CPUEs. WPH2 ($\chi^2(3)=12.01$, p=0.007) was different between only siphon-off and on years but not all combinations of years, just the first year of siphon-on conditions. 2021 CPUE was larger than both 2018 (p=0.016) and 2019 (p=0.014), 2022 was not different from any of the years though. PS7 was different between a siphon-off and one year, but one difference is not strong enough to suggest the siphon influenced this. Within PS7 only 2019 and 2022 ($p<0.001$) differed significantly with 2022 having a much larger CPUE than 2019.

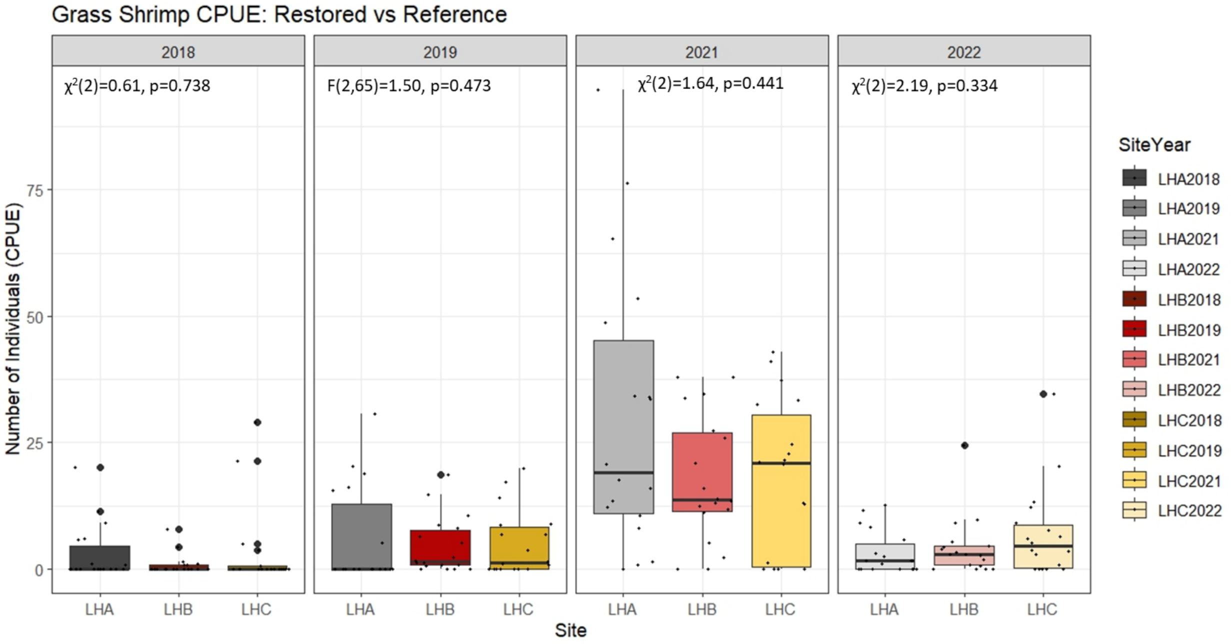


Figure 18. Grass Shrimp CPUE: Restored vs Reference Box plots representing median, upper and lower quartiles, and outliers of grass shrimp caught per sample (CPUE) between restored (LHA and LHB) and reference (LHC) sites within years (2018, 2019, 2021, and 2022) along with individual data points plotted. Color of each site fades from dark to light as years progress.

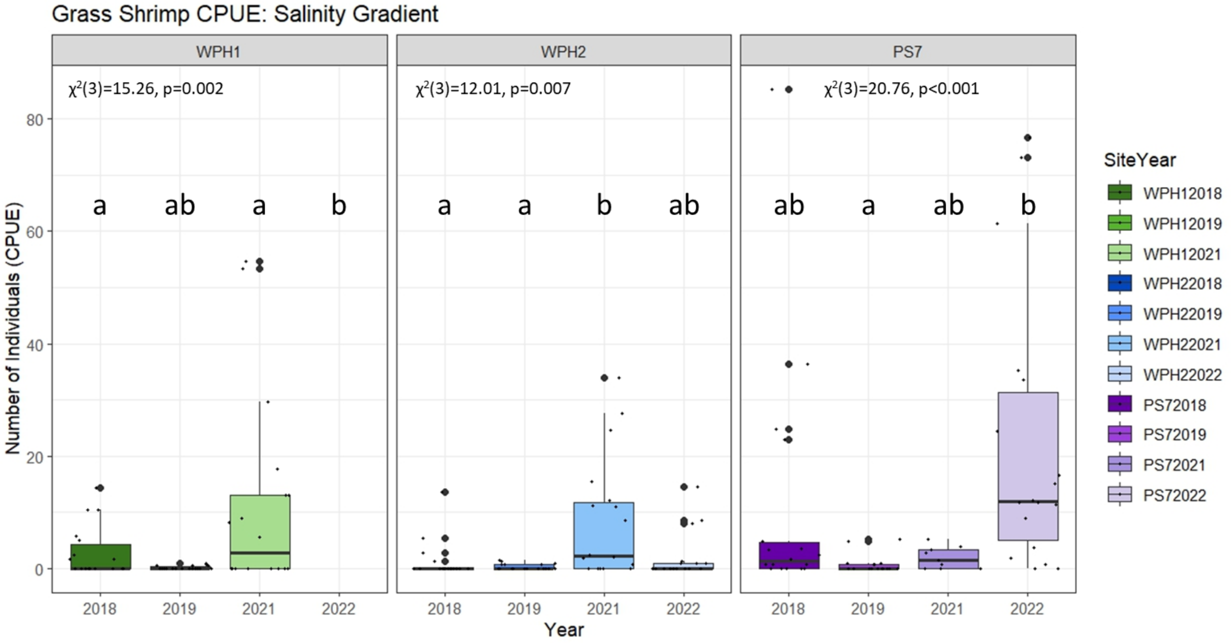


Figure 19. Grass Shrimp CPUE: Salinity Gradient Box plots representing median, upper and lower quartiles, and outliers of grass shrimp caught per sample (CPUE) within sites along the salinity gradient (WPH1, WPH1, and PS7) between years (2018, 2019, 2021, 2022) along with individual data points plotted. Color of each site fades from dark to light as years progress.

Brown Shrimp (Penaeus spp.)

As with grass shrimp, brown shrimp were found in all restored and reference sites **(Figure 20. Brown Shrimp CPUE: Restored vs Reference**) and sites along the salinity gradient (**Figure 21. Brown Shrimp CPUE: Salinity Gradient**), except WPH1 where brown shrimp populations were not found in siphon-on years in 2021 and 2022. While not a large portion of the total individuals caught within samples through all four years, they made up 5.06% of all species found. Raw catch numbers can be found within **Supplemental Table 13. Brown Shrimp Population Numbers**. Brown shrimp were also found in all subhabitat types as well with the majority found at the edges (66.21%) then in creeks (32.07%), then in ponds (1.72%).

Restored and reference sites had lower abundances in the first two years of sampling compared to the last two years in which the siphon was turned on, but LHA remained relatively low until the last year of sampling. LHB had a large spike in 2019 but not due to siphon conditions, and then abundances remained high with slight increases in siphon-on years. LHC saw the largest increase in siphon-on years out of these sites. Mean CPUEs were lowest in 2018 (LHA=0.36; LHB=0.34; LHC= 0.33), 2019 saw slight increases at LHA (0.39) and LHC (0.42) with a large spike at LHB (2.12) that then slightly decreased in 2021 (1.81) and 2022 (1.91). LHA and LHC saw a large increase in CPUE in 2021 (LHA=1.09; LHC=2.17) that then continued to increase into 2022 (LHA=3.74; LHC=3.31). While there were some larger shifts between years, especially between siphon-off and on years, there were no statistically significant differences in brown shrimp CPUE (**Supplemental Table 14. Brown Shrimp CPUE Results**) indicating that brown shrimp utilize restored and reference sites equally through the time we sampled.

Sites along the salinity gradient had lower abundances than did restored and reference sites and salinity gradient sites went through cyclical fluctuations of increasing and decreasing. This was especially evident at WPH2 and PS7 as they increased, decreased, increased, then decreased again but WPH2 had the highest mean abundances (2018=0.31; 2019=1.70; 2021=1.80; 2022=2.20), except in 2018 when PS7 (2018=0.76; 2019=1.22; 2021=0.59; 2022=0.89) was slightly higher. PS7. WPH1 (mean CPUE: 2018=0.13; 2019=0.32) had a unique pattern in which individuals were present when the siphon was off but then were not captured when the siphon was on. This elimination of brown shrimp from our samples in siphon-on years at WPH1 was the only instance of significant difference within sites leading us to believe the siphon strongly affects the presence of brown shrimp at this site. The mean CPUE in 2018 was close to zero but 2019 mean CPUE was significantly larger than zero in both 2021 (p=0.10) and 2022 (p=0.10).

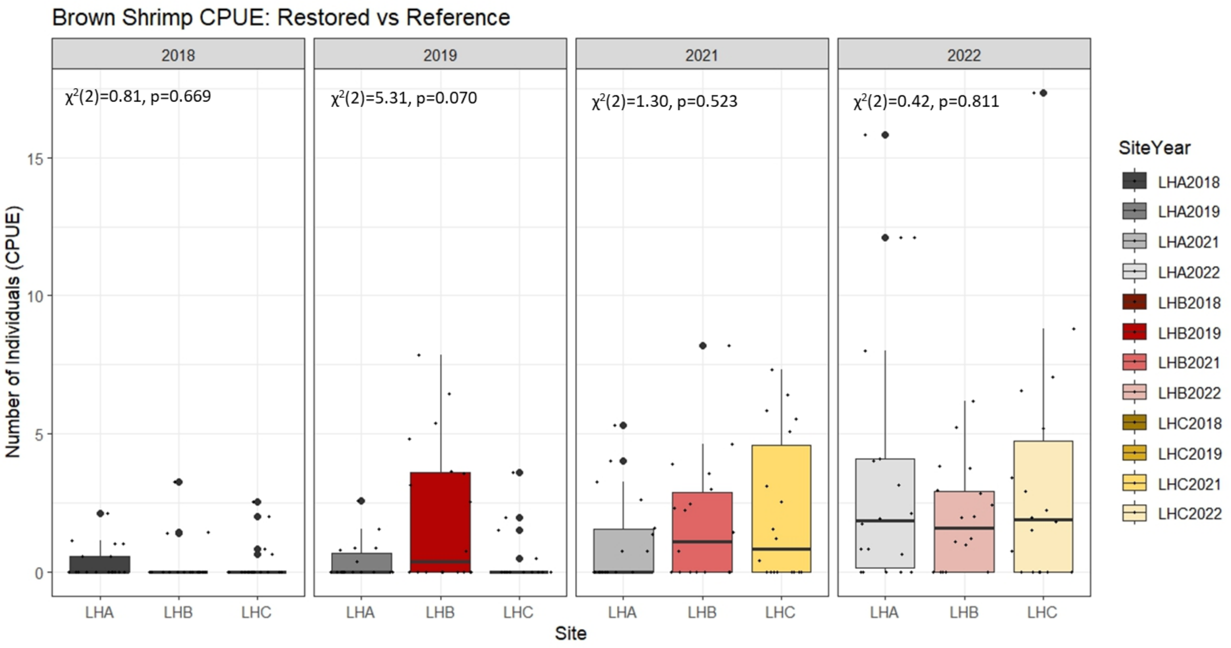


Figure 20. Brown Shrimp CPUE: Restored vs Reference Box plots represent median, upper and lower quartiles, and outliers of brown shrimp caught at two restored (LHA and LHB) and one reference (LHC) sites in 2018, 2019, 2021, and 2022 along with individual data points plotted. Color gradient of each site fades from dark to light as years progress.

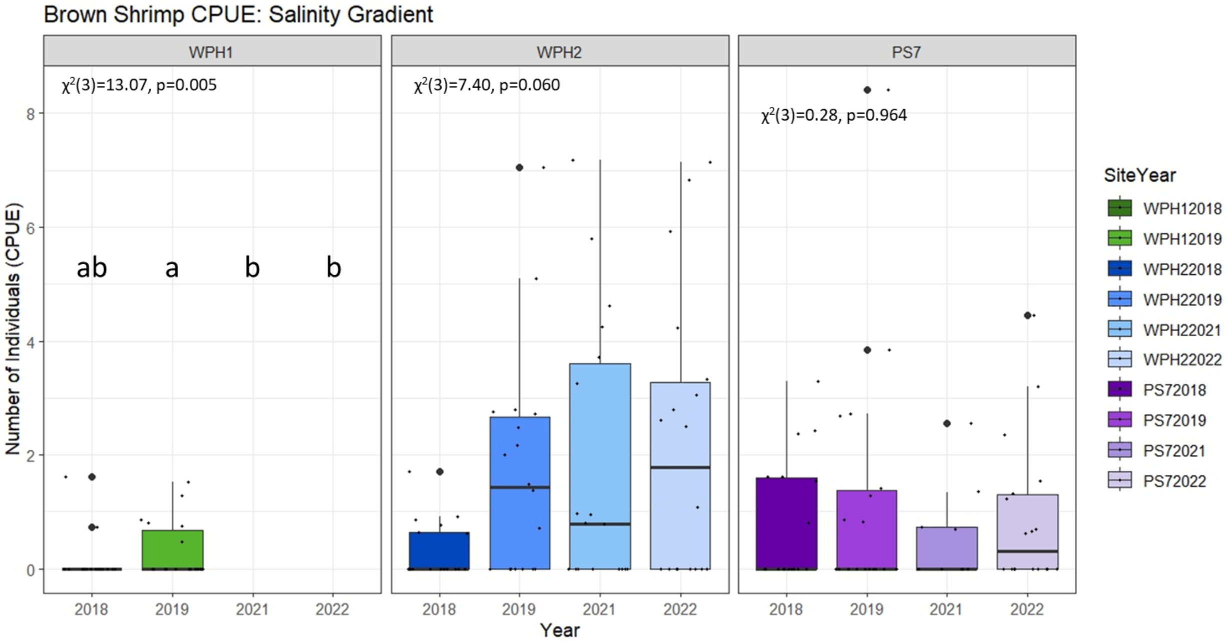


Figure 21. Brown Shrimp CPUE: Salinity Gradient Box plots representing median, upper and lower quartiles, and outliers of brown shrimp caught per sample (CPUE) within sites along the salinity gradient (WPH1, WPH1, and PS7) between years (2018, 2019, 2021, 2022) along with individual data points plotted. Color gradient of each site fades from dark to light as years progress.

Ohio Shrimp (Macrobrachium ohio)

With the previous two shrimp species examined, grass (Figure 18, Figure 19) and brown shrimp (Figure 20, Figure 21), we noticed that while most sites had continual population of each species, although variable in abundance, WPH1 had a complete loss of these species. These losses occurred only when the siphon was turned on in the last two years of our sampling in which grass shrimp were lost in the second year (2022) and brown shrimp were lost as soon as the siphon turned on in 2021. This further motivated us to look at the third shrimp species captured within our sites, Ohio shrimp (Figure 22). These shrimp were only found in siphon-on years and only at WPH1, the site closest to the siphon and the site which lost other shrimp species. They were also only present in creek (27.35%) and, predominantly, edge (72.65%) subhabitats. Ohio shrimp, while only present for half of the study, made up 4.04% of the individuals captured in all four years (total raw count of individuals caught in 2021=68, 2022=501), and their CPUE dramatically increased as the siphon continued to stay on to the last year of sampling. The elimination of other species and the arrival of Ohio shrimp seem to have strong relations to each other and the siphon. The complete elimination of both species in 2022 corresponds to a significant increase in Ohio shrimp in 2022 (mean CPUE=19.70) from what it started at in 2021 (mean CPUE=2.32, p=0.010). This could allude to some interaction of the shrimp species not only with the siphon and freshwater input, but with other incoming species as well.

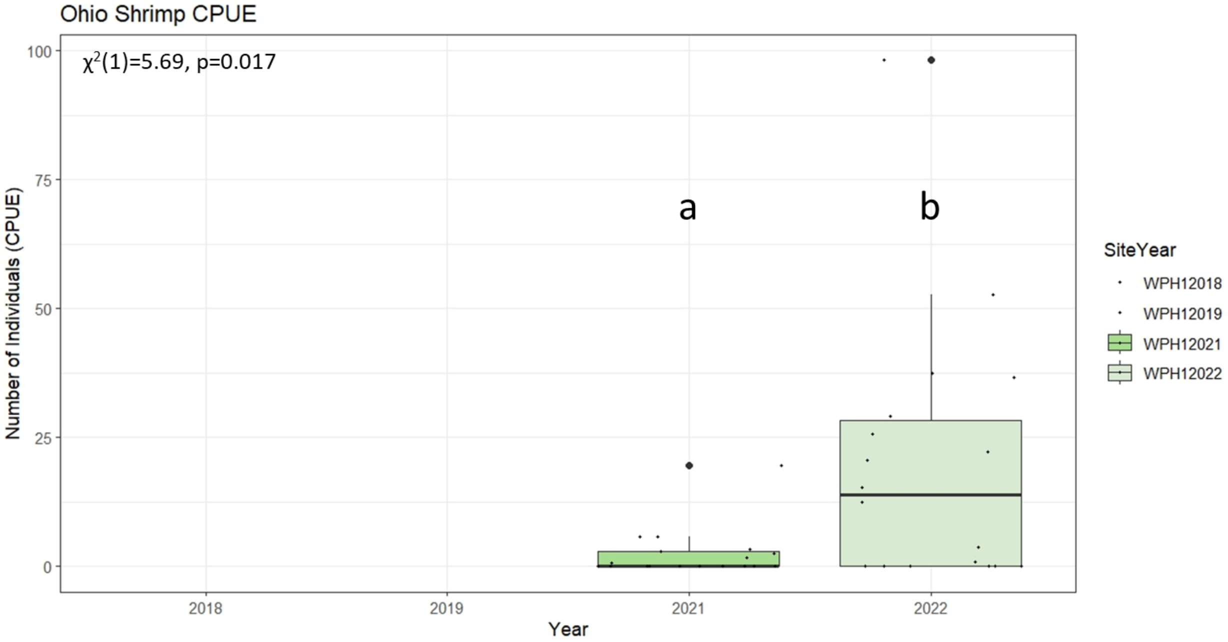


Figure 22. Ohio Shrimp CPUE Ohio Shrimp CPUE Box plots representing median, upper and lower quartiles, and outliers of Ohio shrimp catch numbers per sample (CPUE) aggregated by site (WPH1) between years (2018, 2019, 2021, and 2022) along with individual data points plotted. Color gradient of each site fades from dark to light as years progress

On-Marsh Nekton Population Dynamics - Lengths & Biomass

Gulf Killifish (Fundulus grandis)

Lengths. Gulf killifish were one of the most abundant species captured in our four years of sampling in which 29.15% of all individuals caught were Gulf killifish. Raw catch numbers can be found within **Supplemental Table 15. Gulf Killifish Population Numbers**. Gulf

Killifish Population Numbers Overall, 71.95% of the Gulf killifish were collected in ponds and only 16.08% and 11.97% were collected at creeks and edges, respectively. However, the size distributions across subhabitats differed across sites and years.

During the first two years we observed similar patterns within restored and reference sites (**Figure 23. Gulf Killifish Length Frequency: Restored vs Reference**). At the beginning of the study (2018), Gulf killifish were found across all three subhabitats at LHA, LHB, and LHC. The sizes ranged from 27-106mm at LHA, 9-136mm at LHB, and 22-92mm at LHC. Notably, there were fewer individuals at the control site LHC across all three subhabitats. In 2019, most of the Gulf killifish were still caught in ponds. In fact, they were only caught in ponds at LHA. The size ranges in 2019 (LHA: 22-128mm; LHB: 22-128mm; LHC: 26-109mm) were similar to those recorded in 2018, and the distribution patterns were similar for LHB and LHC. During the last two years, which coincided with the resumption of siphon operations, we recorded a decline in Gulf killifish abundance [means (CPUE): 2018=8.04; 2019=10.10, 2021=4.25, 2022=4.63] and total size ranges trending towards smaller size classes. The sizes in 2021 ranged from 18-62mm at LHA, 27-102mm at LHB, and 18-75mm at LHC; in 2022 they ranged from ranged from 24-117mm at LHA, 31-106mm at LHB, and 24-117mm at LHC.

Along with the abundance and size ranges of individuals being variable between sites, the means lengths of individual Gulf killifish caught were also variable especially in the first three years of sampling but not in the last year (**Table 2. Gulf Killifish Length Results**), indicating that in the first three years of sampling, restored and reference sites were not similar, but they became more similar over time. All sites were significantly different from one another in 2018 ($\chi^2(2)=50.84$, $p<0.001$) with LHB having the largest mean length (72.4mm) and being significantly different from LHA (mean=64.1mm, $p<0.001$) and LHC (mean=53.1mm, $p<0.001$), and LHA individualism (mean=64.1mm) being larger than LHC (mean=53.1mm, p<0.001). 2019 ($\chi^2(2)=22.76$, p<0.001) had the most length differences between LHB having larger individuals (mean=54.9mm) as compared to LHA (mean=51.7mm, p<0.001) and LHC (mean=48.5mm, p<0.001). 2021 ($\chi^2(2)=9.03$, p=0.011) had the least amount of differences between restored and reference sites with LHB's mean length of 42.9mm being significantly larger than LHC's mean length of 38.6mm.

Sites along the salinity gradient showed high interannual variability in regards to Gulf killifish abundance and lengths (**Figure 24. Gulf Killifish Length Frequency: Salinity Gradient**) with there being a decline in lengths and a large decrease in the number of individuals in 2019, but an increase in lengths and abundance in 2022. Individuals were found within all three subhabitats within WPH1, WPH2, and PS7 in all years except 2021 WPH1 in which individuals were only found within ponds. Size ranges in 2018 were 17-108mm at WPH1, 14-110mm at WPH2, and 24-123mm at PS7. In 2019 length ranges decreased to 37-128mm at WPH1, 27-117mm at WPH2, and 31-86mm at PS7. 2021 length ranges were still smaller than in the first year of sampling (2018), 22-40mm at WPH1, 19-88mm at WPH2, and 30-122mm at PS7. The middle two years of sampling had a reduction in both length ranges and in maximum length found except 2019 WPH1 which had one individual at a larger length. The last year of sampling (2022) had a rebound in both length ranges, maxim lengths, and abundance except WPH1 still. Size ranges in 2022 were 25-57mm at WPH1, 14-108mm at WPH2, and 24-112mm at PS7. Mean abundances (CPUE) fluctuated through time as well with 2022 having the highest overall abundance (mean=7.22) then 2018 (mean=7.7), then 2019 (6.10), and the lowest abundance in the first year of siphon-on conditions in 2021 (mean=2.40).

As with the restored and reference sites, there was high variability between years within all sites along the salinity gradient. Significant differences at WPH1, closest to the siphon, indicate that the siphon turning on may have a high impact on Gulf killifish lengths but there may be other factors causing these differences as well. WPH1 ($\chi^2(3)=165.33$, $p<0.001$) had significant differences between all year combinations except between 2021 and 2022. 2018 had the largest mean length of 60.8mm and was significantly different from the mean length of 48.5mm in 2019 ($p<0.001$), from 31.6mm in 2021 ($p<0.001$), and from 34.7mm in 2022 ($p=0.004$). 2019 then had the second longest length mean of 48.5mm and was significantly different from 2021's mean length of 31.6mm ($p<0.001$) and 2022's mean length of 34.7mm ($p<0.001$). WPH2 ($\chi^2(3)=142.73$, $p<0.001$) contained less differences than WPH1 with some differences found between siphon-off and on years but differences within those conditions as well. 2019 had the longest individuals with a mean length of 72.9mm making it significantly different from shorter mean lengths in 2018 (mean=53.0mm, $p<0.001$), 2021 (mean=40.6mm, $p<0.001$), and 2022 (mean=54.0mm, $p<0.001$). 2018 had the second longest length mean of 53.0mm, and besides 2019, was only different from 2021 (mean=40.6mm, $p<0.001$). PS7 ($\chi^2(3)=47.98$, $p<0.001$) had the same year differences as WPH1 with the addition of 2021 and 2022 being different as well. 2018 had the longest individuals at PS7 with a mean length of 69.8mm and was significantly longer than 2019 (mean=50.3mm, $p<0.001$), 2021 (mean=60.3mm, $p=0.003$), and 2022 (mean=65.3mm, p=0.0.17). 2019 had the smallest mean length at 50.2mm and was different from 2021's mean of 60.3 ($p=0.048$) and 2022's mean of 65.3 ($p<0.001$). Both siphon-on years 2021 (mean=60.3mm) and 2022 (mean=65.3mm, $p=0.048$) were different from each other as well. A two-way ANOVA was not conducted for site-year interaction along the salinity gradient due to failure to meet normality and homogeneity of variance and no non-parametric test being known.

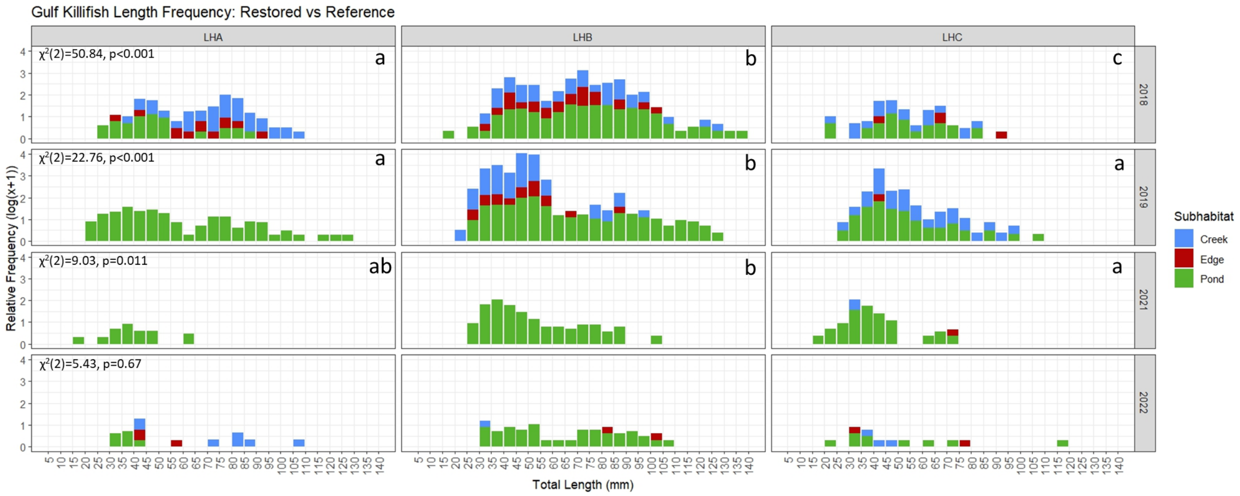


Figure 23. Gulf Killifish Length Frequency: Restored vs Reference Gulf killifish length frequency data between restored and reference sites calculated from relative frequencies (log(x+1) transformed) based on measured number of individuals transformed to reflect the total number of caught individuals. Bins are split into 5mm measurement increments and colors representing subhabitat location of individuals caught spanning all sites and years sampled.

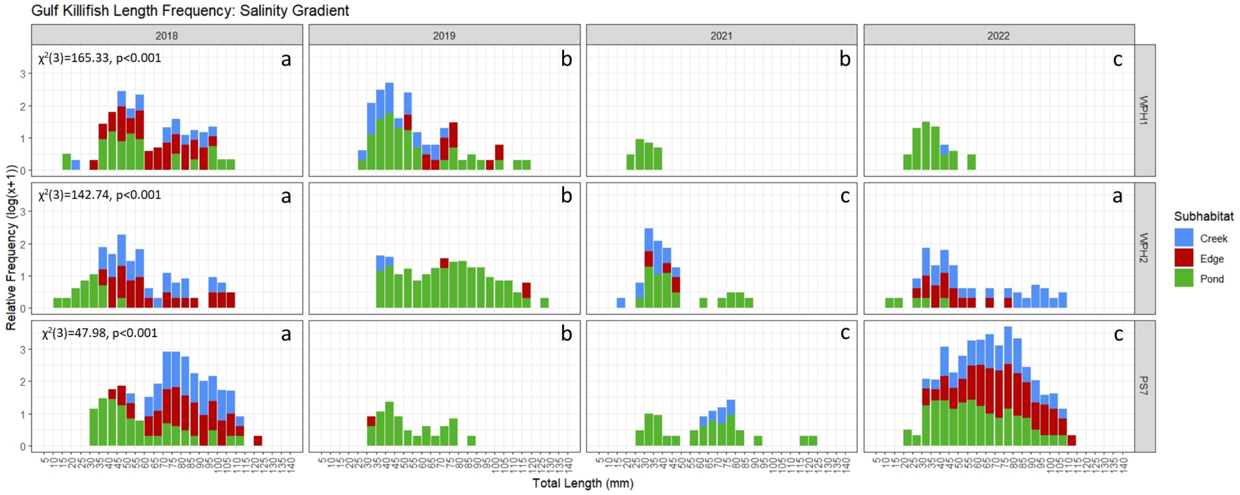


Figure 24. Gulf Killifish Length Frequency: Salinity Gradient Gulf killifish length frequency data between years of sites located along the salinity gradient calculated from relative frequencies (log(x+1) transformed) based on measured number of individuals transformed to reflect the total number of caught individuals. Bins are split into 5mm measurement increments and colors representing subhabitat location of individuals caught spanning all sites and years sampled.

Table 2. Gulf Killifish Length Results Statistical results for Gulf killifish lengths (mm) per each site. The table includes the factor, levels, main statistical test (Kruskal-Wallis) and post hoc comparison test (Dunnett) where applicable, and other associated values (degrees of freedom, test statistic, p-value). Alpha level was set to 0.05 for all tests. No transformations were performed. NA = not applicable.

Year & Site	DF	Test statistic	P-value (Kruskal-Wallis)	Significant Difference	P-value (Dunnett)
2018: LHA, B, C	2	50.84	**<0.001**	LHA and LHB	0.001
				LHA and LHC	0.001
				LHB and LHC	<0.001
2019: LHA, B, C	2	22.76	**<0.001**	LHA and LHB	0.001
				LHB and LHC	<0.001
2021: LHA, B, C	2	9.03	**0.011**	LHB and LHC	0.008
2022: LHA, B, C	2	5.43	0.067	NA	NA
WPH1 (2018 - 2022)	3	165.33	**<0.001**	2018 and 2019	<0.001
				2018 and 2021	<0.001
				2018 and 2022	0.004
				2019 and 2022	<0.001
				2021 and 2022	<0.001
WPH2 (2018 - 2022)	3	142.74	**<0.001**	2018 and 2019	<0.001
				2018 and 2021	<0.001
				2019 and 2021	<0.001
				2019 and 2022	<0.001
				2021 and 2022	<0.001
PS7 (2018 - 2022)	3	47.98	**<0.001**	2018 and 2019	<0.001
				2018 and 2021	0.003
				2018 and 2022	0.017
				2019 and 2021	0.048
				2019 and 2022	<0.001
				2021 and 2022	0.048

Biomass. Plots of restored versus reference sites (**Figure 25. Gulf Killifish Biomass Frequency: Restored vs Reference**) and of sites along the salinity gradient (**Figure 26. Gulf Killifish Biomass Frequency: Salinity Gradient**) for Gulf killifish biomass display the same distributions between subhabitats and biomass frequency patterns as those found in the Gulf killifish abundance figures in the previous section (**Figure 23. Gulf Killifish Length Frequency: Restored vs Reference**, **Figure 24. Gulf Killifish Length Frequency: Salinity Gradient**). The matching pattern between lengths and weights indicate there is no mismatch between these two factors in the presence of created sites or siphon-on conditions indicating that only population numbers at WPH1 may be affected by a decrease in salinity.

Within the first two years of the study (2018 and 2019), we found a larger range of sizes present in restored versus reference sites which then were reduced in 2021 and then most sites rebounded back in 2022. In 2019 the ranges were as follows: 0.3-16.4g at LHA, 0.3-40.5g at LHB with the largest range, and 0.0-12.3g at LHC with the smallest range. In 2021 LHA had the largest range from 0.2-38.0g, LHB at 0.1-25.1g, and LHC having the smallest range again from 0.3-19.6. In 2021, all ranges shrank to be 0.1-3.4g at LHA with the smallest range, 0.3-16.5g at LHB with the largest range again, and 0.1-6.9g at LHC. In 2022 there was some rebound in range with LHA at 0.2-19g, LHB at 0.4-17.5g with a slight increase in range from 2021, but not much, and LHC at 0.2-19.5g.

As the weight ranges fluctuated through time, so did the mean weights of individuals found among sites within years, especially within 2018, 2019, and 2022, but not so much in 2021 (**Supplemental Table 16. Gulf Killifish Biomass Results**). This indicates that there is high variability between restored and reference sites for the use of different weight classes of Gulf killifish. In 2018 ($\chi^2(2)=50.50$, $p<0.001$), all combinations of sites were significantly different from one another with no restored site being similar to the other or to the reference. LHB had the largest massed individuals at 6.9g and was different from LHA's mean mass of 4.7g ($p<0.001$) and different from the LHC ($p<0.001$) with the lowest mean mass of 2.6g. LHA's larger mass (4.7g) was also significantly different from LHC's ($p<0.001$) lower mass (2.6g). In 2019 ($\chi^2(2)=15.97$, $p<0.001$), only the restored sites were different from one another. LHB's mean biomass was 3.8g and was significantly different from LHA's mean mass of 3.2g ($p<0.001$). In 2022 $\chi^2(2)=86.47$, $p<0.001$) though, we have a significant difference between one of the restored and reference sites. LHB is significantly higher with a mean individual mass of 4.8g as compared to LHC's mean of 2.9g ($p=0.025$).

Sites along the salinity gradient (**Figure 26. Gulf Killifish Biomass Frequency: Salinity Gradient**) showed high interannual variability of Gulf killifish biomass as well. No site consistently had the largest or smallest range of masses but, as with restored and reference sites, the pattern of the first two years having the largest ranges then the range dropping and then increasing again from 2021 to 2022 is present, except for PS7 dropping in 2019 then regaining in 2021. Biomass ranges in 2018 at WPH1 were 0.1-18.0g, WPH2 had the smallest range from 0.0-17.8g, and PS7 had the largest range from 0.3-28.9. In 2019, masses ranged from 0.2-21.5 at WPH1, WPH2 had the largest range from 0.7-30.5g, and PS7 had the smallest range this year from 0.2-10.0g. In 2021, where we see the drop in range at the WPH sites, WPH1 was the smallest range from 0.1-0.8 grams, WPH2 from 0.0-9.2g, and PS7 recovered from 2018 having a range of 0.2-28.1g massed individuals. In 2022, WPH1 range did slightly increase from 2021 but not by much, only going from 0.2-2.4g, WPH2 fully rebounded now going from 0.0-18.5g, and PS7 stayed similar to 2021 ranging from 0.0-20.3g.

With these fluctuating ranges in biomasses at sites along the salinity gradient, the means for all sites had significant variation among years. WPH1 ($\chi^2(3)=86.47$, $p<0.001$) had differences between all year combinations except the two years the siphon was turned on (2021 and 2022) indicating that the siphon may have a high influence on the biomass of Gulf killifish at this closest site. 2018 had the highest biomass mean for WPH1 at 4.3g and was different from 2019’s mean biomass of 2.1 ($p<0.001$), from 2021’s mean of 0.4g ($p<0.001$), and from 2022’s mean of 3.4g ($p<0.001$). WPH1’s 2019 mass of 2.1g was also different from the mean mass in 2021 (0.4g, $p<0.001$) and the mean mass in 2022 (3.7g, $p<0.001$). WPH2 ($\chi^2(3)=134.82$, $p<0.001$) had a combination of differences within and between siphon-on and off years indicating the siphon is most likely not a strong influence at this mid-range site. 2019 had the largest biomass mean out of all the years at 7.1g and was different from 2018’s mean biomass of 3.4g ($p<0.001$), 2021’s mean biomass at 1.3g ($p<0.001$), and 2022’s mean biomass at 4.0g ($p<0.001$). 2018 also had a mean biomass (3.4g) different from 2021 (1.3g, $p<0.001$), and 2021 (1.3g) was significantly smaller than 2022 (4.0g, $p<0.001$). PS7 ($\chi^2(3)=42.63$, $p<0.001$) had the fewest differences amongst years and little indication of siphon influence. 2018 had the highest mean biomass at 6.9g and was different from the mean biomass of 2.3g in 2019 ($p<0.001$) and 4.5 in 2021 ($p=0.012$). 2019’s biomass of 2.3g was also significantly lower than 5.0g in 2022 ($p<0.001$).

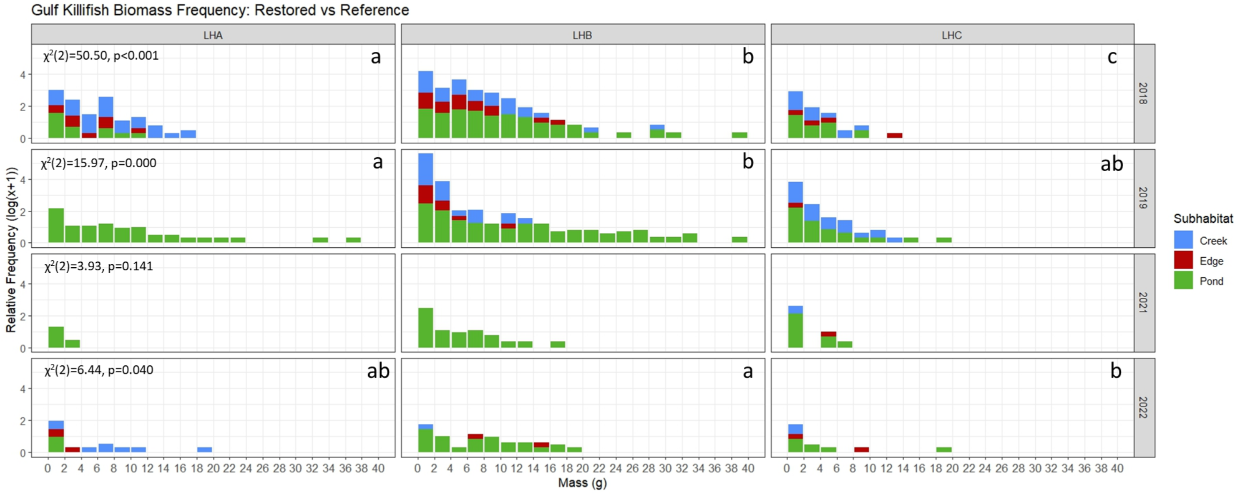


Figure 25. Gulf Killifish Biomass Frequency: Restored vs Reference Gulf killifish biomass frequency data between restored and reference sites calculated from relative frequencies (log(x+1) transformed) based on measured number of individuals transformed to reflect the total number of caught individuals. Bins are split into 2g measurement increments and colors representing subhabitat location of individuals caught spanning all sites and years sampled.

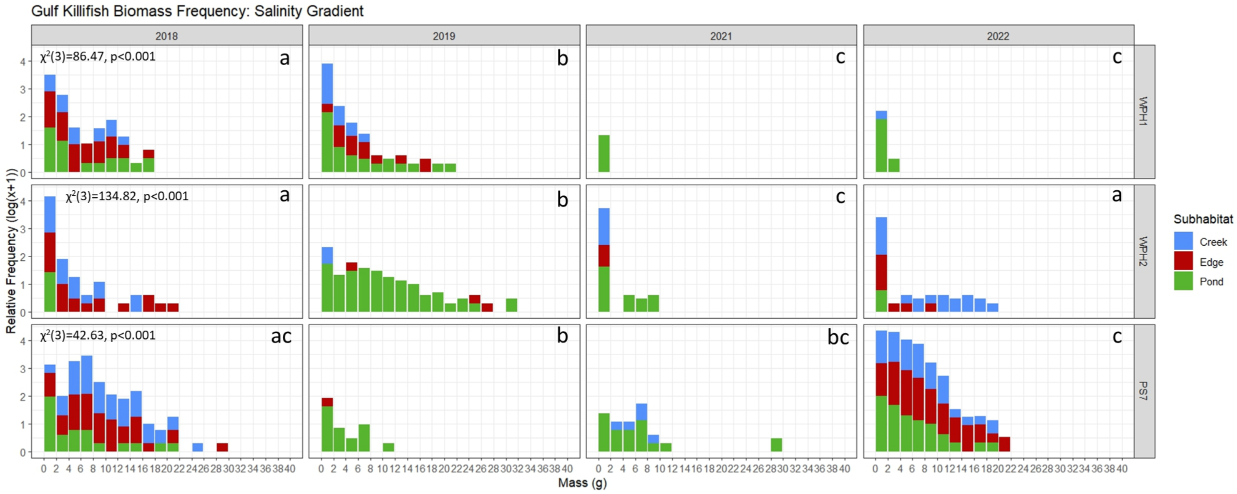


Figure 26. Gulf Killifish Biomass Frequency: Salinity Gradient Gulf killifish biomass frequency data between years of sites located along the salinity gradient calculated from relative frequencies (log(x+1) transformed) based on measured number of individuals transformed to reflect the total number of caught individuals. Bins are split into 2g measurement increments and colors representing subhabitat location of individuals caught spanning all sites and years sampled.

Diamond Killifish (Fundulus xenicus)

Lengths. Out of the species looked at in these analyses, diamond killifish had the second highest fish abundance out of all species caught at 9.75%. Raw catch numbers can be found within **Supplemental Table 17. Diamond Killifish Population Numbers**. They were found almost exclusively in pond subhabitats (99.75%) with very few found in edges (0.18%) and almost none found in creeks (0.07%). In general, 2022 had the highest abundance of diamond killifish (CPUE=3.24 individuals) while 2019 had the lowest (0.96) and 2018 (2.85) and 2021 (2.67) were in the middle leading us to believe, amongst all the sites, the siphon did not strongly influence abundance in this region and may be more site specific. While not as drastic as Gulf killifish, diamond killifish length did fluctuate between sites for restored and reference areas (**Figure 27. Diamond Killifish Length Frequency: Restored vs Reference**) and amongst years for sites along the salinity gradient (**Figure 28. Diamond Killifish Length Frequency: Salinity Gradient**).

For restored and reference sites, 2019 had the largest range in lengths of individuals with LHA ranging from 37-41mm, LHB ranging from 32-41mm, and LHC ranging from 30-42mm. 2021 then had the second largest range amongst sites with LHA lengths being between 30-36mm, LHB from 37-39mm, and LHC being from 36-38mm. 2018 and 2022 had the smallest ranges with LHA being from 38-40mm in 2018 and only one 31mm individual found in 2022, LHB ranging from 36-43 in 2018 and from 30-39 in 2022, and LHC ranging from 33-38 in 2018 and only one 32mm individual found in 2022. Size classes did not visually seem to differ much from year to year but did differ between restored and reference sites within years.

Significant length differences were found in all years sampled except the last year (2022, $F_{2,7}$=1.18, p=0.108) with a reduction in the differences in 2021 leading us to believe these sites may be becoming more similar over time (**Supplemental Table 18. Diamond Killifish Length Results**). In 2018 ($F_{2,20}$=15.74, p<0.001) both restored sites are different from the reference. LHA had a mean length of 38.0mm and was different from the mean length of 38.7mm at LHC (P=0.015), and LHC having the smallest mean length (35.0mm) was different from 39.1mm at LHB with the largest length (<0.001). 2019 ($F_{2,109}$=10.4, p<0.001) had differences between restored sites themselves and only one restored versus the reference. LHA had the shortest mean length at 33.0mm and was different from LHB's mean length of 37.0mm (p=0.013), and LHA (33.0mm) was significantly smaller than the mean length of 36.1 at LHC (p<0.001). 2021 ($\chi^2(2)$=14.66, p<0.001) Only had one significant difference in mean lengths between LHB and LHC, a restored and reference site. LHB had a mean length of 32.6 and was larger than the mean length of 31.5 found at LHC (P<0.001).

Comparing sites along the salinity gradient, WPH2 had the most consistent, largest span of lengths, with PS7 having the next largest spans, although not much larger from WPH1's span, only in 2018. A reduction in max lengths can be seen at WPH1 and somewhat at PS7, but PS7's trend may be hindered due to the abundance dramatically dropping before the siphon turned on. WPH2 saw a steady increase in lengths through time, but not fully attributed to the siphon. Going year by year, lengths of individual diamond killifish in WPH1 ranged from 36mm in 2018 due to only one individual being caught, 31-40mm in 2019, 31-35mm in 2021, and from 29-35mm in 2022. WPH2 had a range of 13-37mm in 2018, 32-41mm in 2019, 27-44mm in 2021, and 26-45 in 2022. PS7 lengths ranged from 23-47 in 2018, only one 45mm individual was found in 2019, a range of 32-39mm in 2021, and a range of 31-39mm in 2022.

Examining the mean lengths of individuals, we found significant differences at WPH2 and PS7, the mid and far distanced from the siphon sites, but none at the closest site, WPH1

($F_{3,61}$=2.11, p=0.108) (**Supplemental Table 18. Diamond Killifish Length Results**). WPH2 (χ^2(3)=42.70, p<0.001) had differences within siphon-off years and between siphon-on and off years as well indicating the siphon may have some influence on this site but there could also be other factors affecting interannual variability. A mean length of 29.5mm in 2018 was significantly shorter than a mean length of 36.2mm in 2019 (p<0.001), of 32.7mm in 2021 (p=0.030), and of 33.4mm in 2022 (p<0.001). 2019 had the largest mean length of 36.2mm and was significantly larger than the mean lengths found in 2021 of 32.7mm (p<0.001) and in 2022 of 33.4mm (p=0.002). PS7 (χ^2(3)=8.38, p=0.039) only contained one difference, and although it was between siphon-off and on conditions, there is not enough evidence to suggest the siphon influenced this change in diamond killifish lengths. For PS7, 2018 had the smallest mean length of 35.2mm and was significantly smaller than 36.1mm in 2022 (p=0.021).

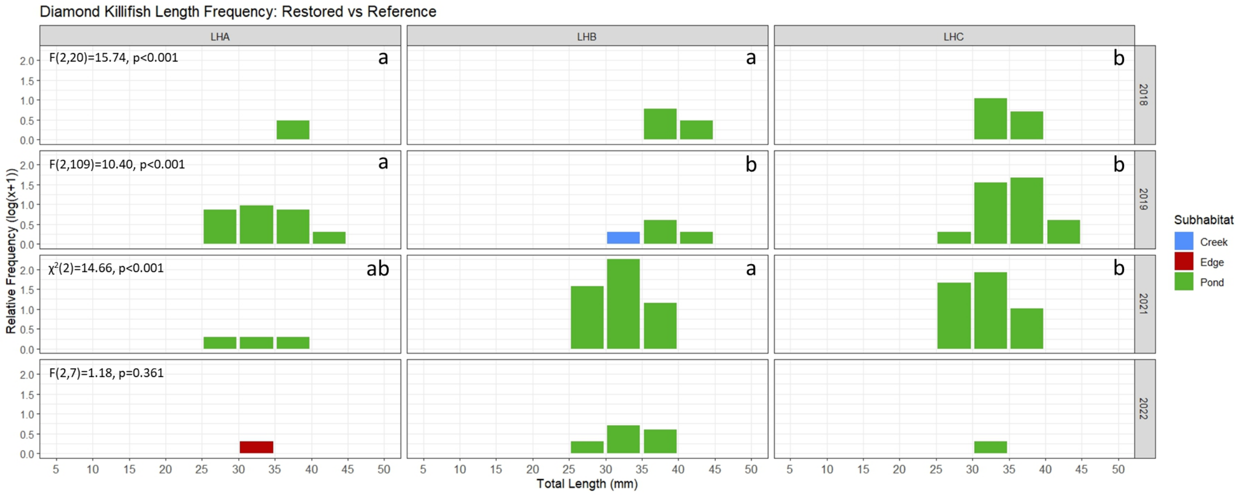


Figure 27. Diamond Killifish Length Frequency: Restored vs Reference Diamond killifish length frequency data between years of sites located along the salinity gradient calculated from relative frequencies (log(x+1) transformed) based on the measured number of individuals transformed to reflect the total number of caught individuals. Bins are split into 5mm measurement increments and colors representing subhabitat location of individuals caught spanning all sites and years sampled.

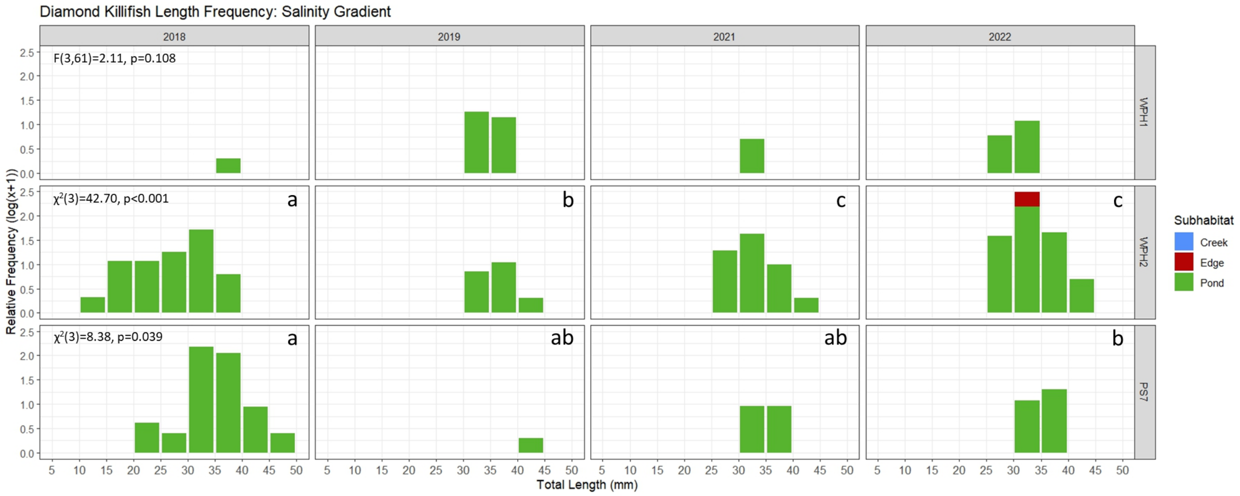


Figure 28. Diamond Killifish Length Frequency: Salinity Gradient Diamond killifish length frequency data between restored and reference sites calculated from relative frequencies (log(x+1) transformed) based on the measured number of individuals transformed to reflect the total number of caught individuals. Bins are split into 5mm measurement increments and colors representing subhabitat location of individuals caught spanning all sites and years sampled.

Biomass. Diamond killifish biomasses do not seem to display any discrepancy between length (**Figure 27. Diamond Killifish Length Frequency: Restored vs Reference**, **Figure 28. Diamond Killifish Length Frequency: Salinity Gradient**) and biomass frequencies between restored and reference marshes (**Figure 29. Diamond Killifish Biomass Frequency: Restored vs Reference**), but along the salinity gradient (**Figure 30. Diamond Killifish Biomass Frequency: Salinity Gradient**), WPH2 does seem to have a larger range of weights in siphon-on years as compared to a narrower range of lengths which may indicate a change in individuals ability to maintain weights in siphon-on conditions in WPH1, the site closest to the freshwater input.

Comparing biomass ranges for restored and reference sites showed that LHA tended to have a shorter range than the other sites but that LHB was more spread out and erratic than LHC from year to year. In 2018, LHA had a biomass range of 0.8-1.2g, LHB was from 1.0-1.6g, and LHC was from 0.7-2.2g. In 2019, these ranges spread out a bit more with LHA having weights between 0.3-1.3g, LHB from 0.3-1.8g, and LHC from 0.3-1.5g. 2021 had a slight reduction in these ranges that was most noticeable at LHA. LHA diamond killifish biomasses ranged from 0.5-0.8g, LHB ranged from 0.2-1.2g, and LHC ranged from 0.3-1.2g. 2022 then had the smallest range out of all year sampled with LHA only having one individual at 0.6g, LHB masses between 0.4-1.2, and LHC only having one individual weighing 0.6g.

Comparing the means of these biomasses for restored and reference sites for diamond killifish (**Supplemental Table 19. Diamond Killifish Biomass Results**), we found that only 2019 and 2021 had significant differences indicating that these sites are fairly similar to one another. LHA was the most different out of the three sites indicating that LHB is the most similar for presence of certain weight classes as the reference site. In 2019 ($\chi^2(2)=18.26$, $p<0.001$), LHA had the smallest biomass at 0.7g, with LHB's biomass of 1.2g being significantly larger ($p=0.007$), and LHB's mean biomass (1.2g) was significantly larger than LHC's mean biomass of 1.0g ($p<0.001$). In 2021, only LHB and LHC differed with LHB having a mean biomass significantly larger (0.7g) than LHC (0.6g, $p=0.003$).

Looking at sites along the salinity gradient, WPH1 and PS7 had highly variable biomass frequency ranges but also abundances which may hide biomass trends, but WPH2 was fairly stable and had the most consistent abundances and biomass ranges out of these three sites. Going year to year, WPH1 only had one individual of 0.9g in 2018, biomasses had the largest range from 0.3-1.3g in 2019, ranged from 0.5-0.7g in 2021, and from 0.2-1.0 in 2022. WPH2 ranged from 0.0-1.1g in 2018, from 0.6-1.4 in 2019, from 0.3-1.2 in 2021, and had the largest range from 0.2-1.6 in 2022. Lastly, PS7 had the largest range in 2018 from 0.1-1.7, the smallest range in 2019 with only one individual measuring 1.9g, which also happened to be the largest individual out of these sites, and diamond killifish biomasses ranged from 0.3-1.3 in 2022.

While ranges of biomasses were variable at sites along the salinity gradient, only a few significant differences in the means were only found at WPH1 and more differences found at WPH2, but none at PS7 ($\chi^2(3)=5.44$, $p<0.142$) (**Supplemental Table 19. Diamond Killifish Biomass Results**). WPH1 ($F_{3,47}=5.44$, $p=0.003$) had only one difference, and while it was between a siphon-off and siphon-on year, it is not enough to say the siphon strongly affected this site. Diamond killifish mean biomass at WPH1 was significantly larger in 2019 at 0.8g than it was in 2022 at 0.6g ($p=0.002$). WPH2 ($\chi^2(3)=40.86$, $p<0.001$) contained more differences from year to year, both within and between siphon conditions so there may be some influence of the siphon at this site. WPH2 had the smallest mean biomass in 2018 at 0.5g which was significantly smaller than the mean biomass in 2019 of 1.9g ($p<0.001$), in 2021 with 0.8g ($p=0.049$), and in 2022 with 0.9g ($p<0.001$). PS7's 2019 mean biomass (1.9g) was significantly larger than in 2021 (0.8g, $p<0.001$) and in 2022 (0.9g, $p<0.001$).

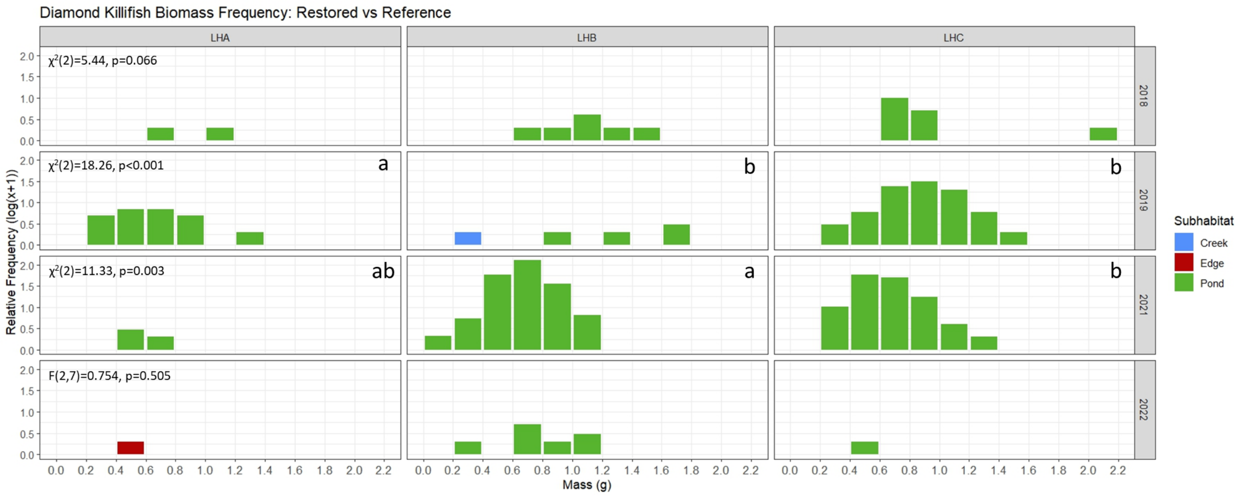


Figure 29. Diamond Killifish Biomass Frequency: Restored vs Reference Diamond killifish biomass frequency data between restored and reference sites calculated from relative frequencies (log(x+1) transformed) based on measured number of individuals transformed to reflect the total number of caught individuals. Bins are split into 0.2g measurement increments and colors representing subhabitat location of individuals caught spanning all sites and years sampled.

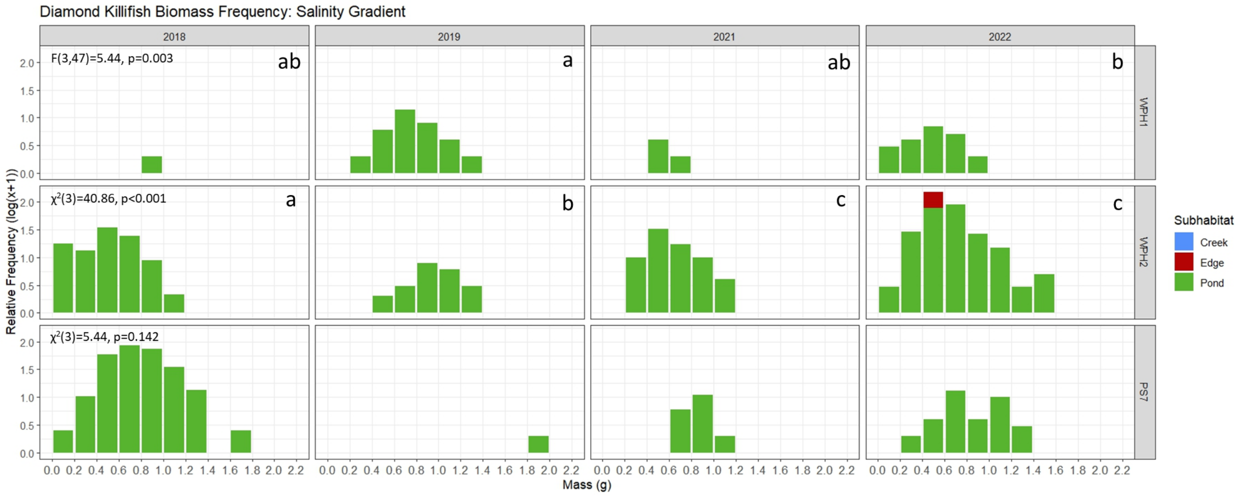


Figure 30. Diamond Killifish Biomass Frequency: Salinity Gradient Diamond killifish biomass frequency data between years of sites located along the salinity gradient calculated from relative frequencies (log(x+1) transformed) based on measured number of individuals transformed to reflect the total number of caught individuals. Bins are split into 0.2g measurement increments and colors representing subhabitat location of individuals caught spanning all sites and years sampled.

Bayou Killifish (Fundulus pulvereus)

Lengths. Bayou killifish, while not the most abundant species in our study, were still one of the top species caught at 6.61% of our total catch out of the four years sampled. Raw catch numbers can be found within **Supplemental Table 20. Bayou Killifish Population Numbers**. Bayou killifish are most present in pond habitats (99.51%) with very few individuals found in creeks (0.22%) or edges (0.27%). Presence of individuals fluctuated from year to year, going from a CPUE of 1.9 in 2019 down to 0.47 in 2019, but then regaining back again in 2021 (1.87) and 2022 (1.94) with seemingly no overall effect of the siphon on our six sites. Length frequencies were relatively stable for this species, when present, compared to Gulf (**Figure 23. Gulf Killifish Length Frequency: Restored vs Reference**, **Figure 24**) and diamond (**Figure 27, Figure 28. Gulf Killifish Length Frequency: Salinity Gradient**) killifish.

Restored and reference sites showed various differences in ranges of lengths captured from site to site and year to year (**Figure 31. Bayou Killifish Length Frequency: Restored vs**

Reference). Overall LHA had the lowest abundance and the narrowest range of lengths. In 2018 LHA ranged from 42-49mm, LHB ranged from 42-45mm, and LHC had the largest range from 21-54mm. In 2019, LHA had the shortest range again from 40-52mm, LHB from 40-58mm, and LHC with the longest range again 35-65. 2021 (LHA: 48-46mm; LHB: 41-52mm; LHC: 32-50mm) and 2022 both reflect this same trend of LHA having the narrowest and LHC having the widest range of measurements with the caveat that no individuals were captured in 2022 at LHA or LHB and only one 57mm individual at LHC. When looking at the means of bayou killifish lengths, no significant differences were found amongst any of the restored or reference sites (**Supplemental Table 21. Bayou Killifish Length Results**) indicating that bayou killifish of various mean lengths ranging from 41.8mm (2021 LHC) all the way up to 57.0mm (2022 LHC) indiscriminately inhabit these sites.

Sites along the salinity gradient showed a slight reduction in the expanse of lengths captured within these sites (**Figure 32. Bayou Killifish Length Frequency: Salinity Gradient**) with a large reduction in 2019, still a siphon-off year, that was accompanied by a large reduction in overall abundance as well. After the drop in 2019, sites rebounded but not fully back to what they were in 2018. WPH1 had the largest length range in 2018 from 22-55mm, the smallest range in 2019 of just 55mm due to only one individual being captured, and a rebound in range from 34-50 in 2021 and from 35-53 in 2022. WPH2 ranged from 18-59mm in 2018, had its smallest range in 2019 from 38-60mm, ranged from 33-66 in 2021, and had its largest ranges in 2022 from 16-60mm. PS7 ranged from 32-55 in 2018, had no bayou killifish captured in 2019, had its largest range from 38-58mm in 2021, then had its smallest range in 2022 from 46-51mm.

Capturing the mean lengths of bayou killifish allowed us to determine that there was no significant differences at the site farthest away from the siphon, PS7 ($F_{2,54}$=1.88, p=0.163), very minimal differences in the site closest to, WPH1, and the most differences at the mid distance site, WPH2. WPH1 ($\chi^2(3)=15.35$, p=0.002) had one set of years different from one another, and while they were between siphon off and on years, is not enough to say the siphon influences bayou killifish lengths at this site. WPH1 ($\chi^2(3)=15.35$, p=0.002) in 2018 had a mean length of 34.9mm which was significantly smaller than the mean length in 2022 of 43.2mm (p<0.001). WPH2 had quite a few significant differences between years with 2018 as the year with the shortest mean lengths (40.6mm) as compared to 2019 (50.5mm, p<0.001), 2021 (43.3mm, p=0.005), and 2022 (42.0mm, p<0.001). 2019 had the longest mean length (50.5mm) and, along with 2018, was significantly larger than 2021 (43.3mm, p<0.001) and 2022 (42.0mm, p<0.001).

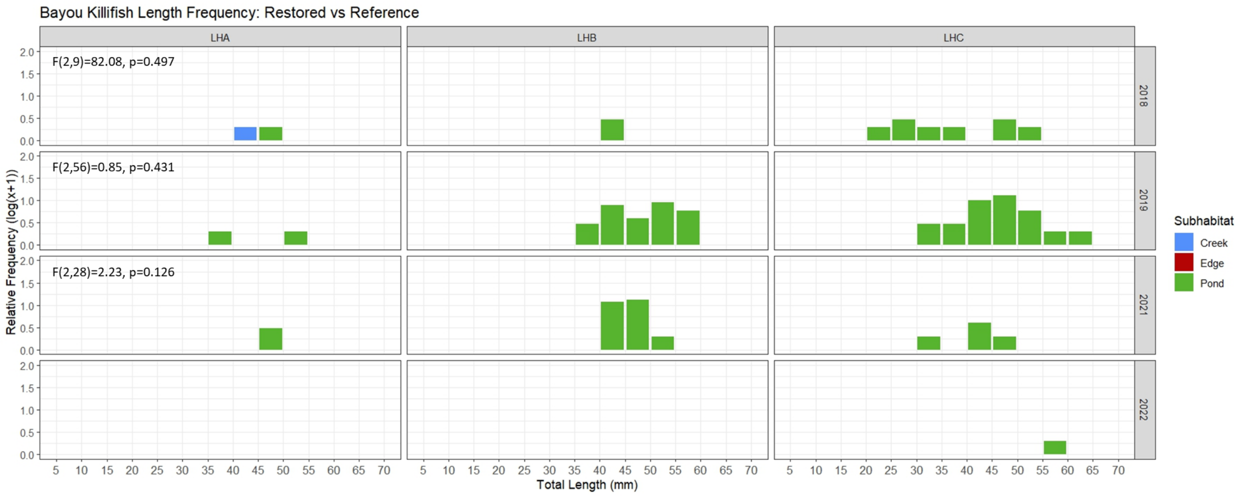


Figure 31. Bayou Killifish Length Frequency: Restored vs Reference Bayou killifish length frequency data between restored and reference sites calculated from relative frequencies (log(x+1) transformed) based on measured number of individuals transformed to reflect the total number of caught individuals. Bins are split into 5mm measurement increments and colors representing subhabitat location of individuals caught spanning all sites and years sampled.

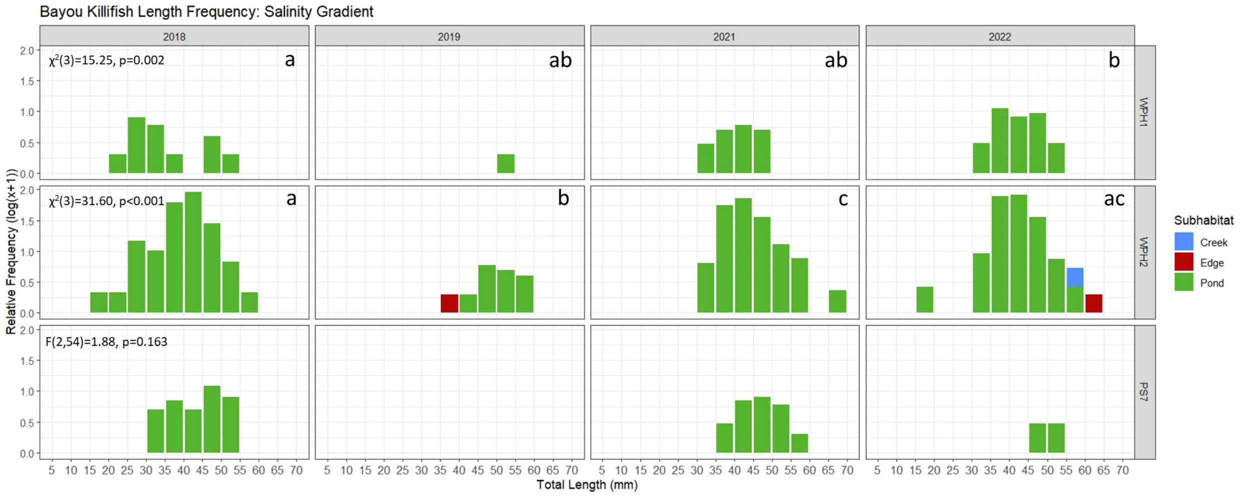


Figure 32. Bayou Killifish Length Frequency: Salinity Gradient Bayou killifish length frequency data between years of sites located along the salinity gradient calculated from relative frequencies (log(x+1) transformed) based on measured number of individuals transformed to reflect the total number of caught individuals. Bins are split into 5mm measurement increments and colors representing subhabitat location of individuals caught spanning all sites and years sampled.

Biomass. Bayou killifish distribution and fluctuation in trends seem to follow those of the length frequency trends (**Figure 31. Bayou Killifish Length Frequency: Restored vs Reference**, **Figure 32. Bayou Killifish Length Frequency: Salinity Gradient**) when considering restored versus reference sites (**Figure 33. Bayou Killifish Biomass Frequency: Restored vs Reference**) and sites located along the salinity gradient (**Figure 34. Bayou Killifish Biomass Frequency: Salinity Gradient**). Restored and reference sites showed that LHA had the least range in masses but, again, the fewest weighed individuals as well. LHB and LHC had wider ranges but abundances still fluctuated from year to year. The same ranking of narrowest to largest range of biomasses fits what was found above for lengths. In 2018 LHA ranged from 0.8-1.5g, LHB from 0.8-1.2g, and LHC from 0.0-2.0g. 2019 (LHA: 0.9-1.5g; LHB: 0.7-3.4g; LHC: 0.5-4.3g) and 2021(LHA: only one individual measured at 1.5g, LHB: 0.9-1.9, LHC: 0.3-2.0g) saw the same trend as in the first year with the note that LHC had an increase in the number of individuals included in the measurements. 2022 had very limited data for these sites as no individuals were captured and or weighed for LHA or LHB and only one 2.8g individuals was noted for LHC. When looking at the means of these biomass measurements, we see the same results as those found for the mean lengths, when present, bayou killifish have no significant differences between restored and reference sites (**Supplemental Table 22. Bayou Killifish Biomass Results**).

The same trends from the length frequency ranges match those of biomass for sites along the salinity gradient as well. The largest ranges were found within WPH2 with WPH1 and PS7 ranges being more constricted, and 2019 showing the lowest abundances but rebounding back in 2021 and 2022. WPH1 had the largest span of biomasses in 2018 from 0.0-2.3g, the smallest in 2018 with only one measurement of 2.6g, and measurements between 0.4-1.87g and 0.5-2.2g in 2021 and 2022. WPH2 2018 had a range of 0.0-2.8g, the smallest range in 2019 from 0.7-2.9g, and a rebound to the largest ranges of 0.4-3.9 in 2021 and from 0.3-3.8 in 2022. PS7 had the largest range and abundance in 2018 from 0.2-1.9g, there were no biomasses in 2019, but a rebound in 2021 (0.8-2.4g) and 2022 (0.9-1.7).

WPH1 ($F_{2,58}$=8.29, p<0.001) had differences between siphon-off and on years but only from 2018 which is not strong enough to indicate that the siphon had a strong influence on bayou killifish biomasses with the input of freshwater. WPH1 had a mean biomass measurement of 0.6g in 2018 which was significantly smaller than that found in 2021 (1.0g, p=0.009) or 2022 (1.0 g, p<0.001) (and **Supplemental Table 22. Bayou Killifish Biomass Results**). WPH2 contained fewer significant differences than it did for length analysis, and biomasses were not consistently different between siphon-off or on conditions ($\chi^2(3)$=30.24, p<0.001). 2018 had the lowest biomass (0.9g), especially compared to 2019 (2.6g, p<0.001) and 2021 (1.1g, p<0.001), while 2019 had the highest (2.0g), especially compared to 2022 (1.1g, p<0.001). PS7 only had one difference ($F_{2,54}$=3.83, p=0.028) , and while between siphon-off and on years, is not enough to attribute to the siphon. PS7 2018 had a biomass that was the smallest of all the years at 1.1gg and was significantly smaller than in 2021 with a biomass of 1.5g (p=0.030).

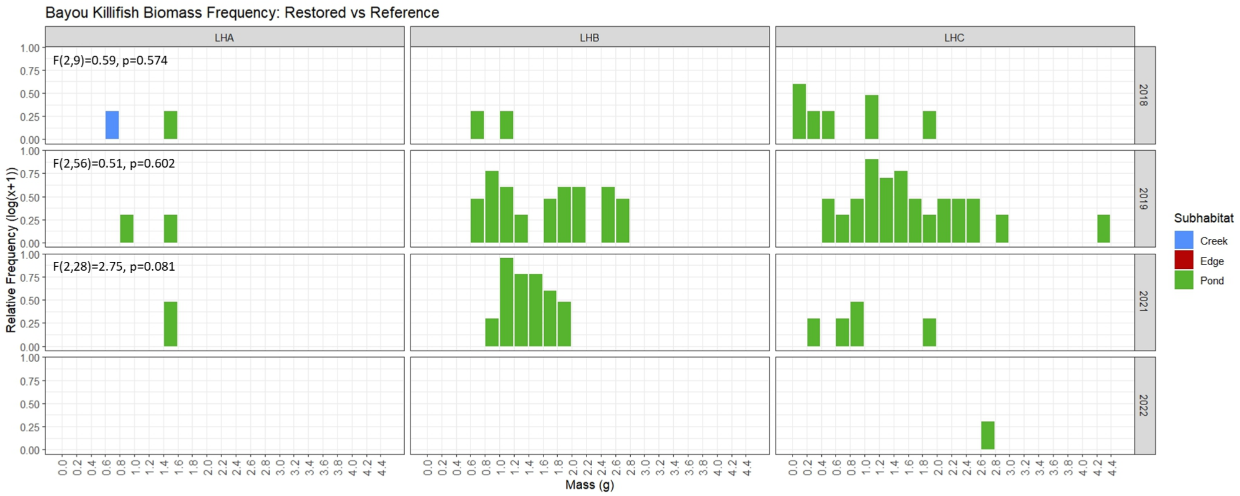


Figure 33. Bayou Killifish Biomass Frequency: Restored vs Reference Bayou killifish biomass frequency data between restored and reference sites calculated from relative frequencies (log(x+1) transformed) based on measured number of individuals transformed to reflect the total number of caught individuals. Bins are split into 0.2g measurement increments and colors representing subhabitat location of individuals caught spanning all sites and years sampled.

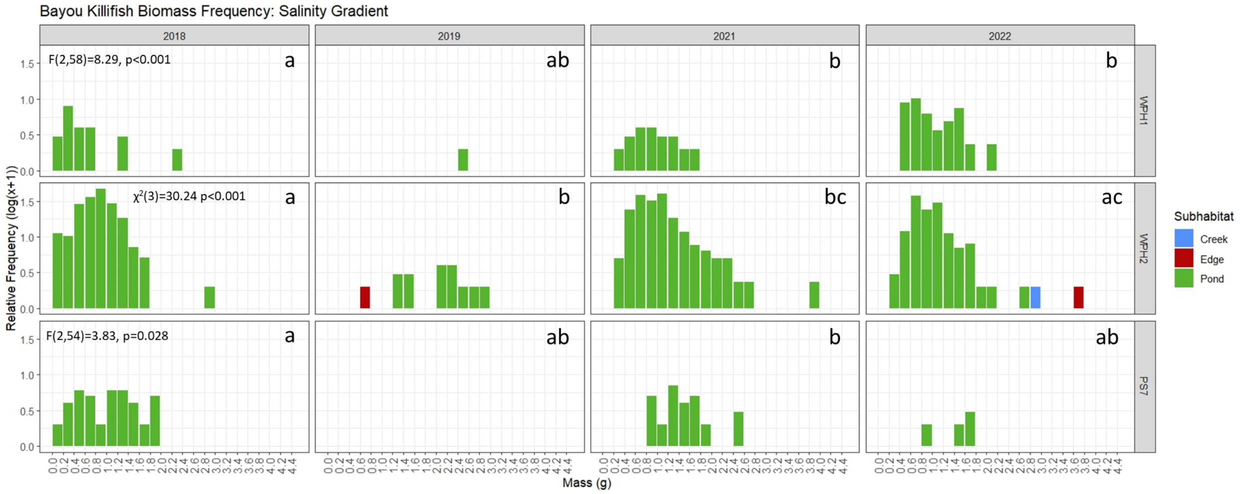


Figure 34. Bayou Killifish Biomass Frequency: Salinity Gradient Bayou killifish biomass frequency data between years of sites located along the salinity gradient calculated from relative frequencies (log(x+1) transformed) based on measured number of individuals transformed to reflect the total number of caught individuals. Bins are split into 0.2g measurement increments and colors representing subhabitat location of individuals caught spanning all sites and years sampled.

Sheepshead Minnow (Cyprinodon variegatus)

Lengths. Continuing population dynamic analysis, sheepshead minnows are even less prevalent in our samples and individuals only occurred in 1.34% of the total catches over the four years sampled. Raw catch numbers can be found within **Supplemental Table 23. Sheepshead Minnow Population Numbers**. As with all the other species previously in this section, they are distributed through all subhabitats but are primarily found in ponds in 92.68% of our catches and only 1.95% and 5.38% are found in creeks and edges. Between all six sites, 2019 had the highest number of individuals with a mean CPUE of 0.55 amongst all taps, with 2021 having the next highest at 0.42, and 2018 and 2019 the lowest at 0.17 and 0.16.

Trends between sites and years are highly variable between restored and reference sites, individuals are not found within edges at these sites (**Figure 35. Sheepshead Minnow Length Frequency: Restored vs Reference**). There are notably more individuals found within LHA but they are absent in 2019 with a trend towards higher numbers of smaller individuals over time. LHB is the only site where individuals are found within creeks in 2019 but there are no individuals anywhere in 2018 or 2022. LHC has an increasing abundance over time especially in the last two years of the study with a wide, sporadic size range of individuals. To compare length ranges of sites within years, LHA had the largest range from 30-51mm and LHC only had one individual at 45mm in 2018. In 2019, LHA again had the largest range from 16-47mm, LHB had the smallest from 22-30mm, and LHC was between 17-32mm. In 2021, LHA had the narrowest range from 15-34mm, LHB was between 26-48mm, and LHC had the largest but most sporadic range from 12-57mm. Individuals were only present at LHC in 2022 with another sporadic length range from 15-51mm.

Restored and reference sites are highly similar in terms of their mean lengths even with such varying ranges (**Supplemental Table 24. Sheepshead Minnow Length Results**) leading us to believe sheepshead minnows of similar lengths are able to utilize restored sites as they are reference sites. There were significant differences found in one year, 2021 ($\chi^2(2)=16.01$, $p<0.001$), one within restored sites and one between restored and reference. Individuals within LHA had a mean length of 24.4mm which was significantly smaller than the mean length at LHB of 36.6mm ($p=0.002$), and LHB was significantly longer than LHC (24.3mm, $p<0.001$) as well.

Sites along the salinity gradient were compared for length differences between years within sites but data was more sporadic at these sites with no individuals at WPH1 in 2019 or 2021, or at PS7 in 2018, 2021, or 2022 (**Figure 36. Sheepshead Minnow Length Frequency**). Sheepshead minnows can be found throughout the salinity gradient in all three subhabitats through but only in 2018, otherwise they were only found in ponds. Lengths within WPH1 and WPH2 were somewhat sporadic and spaced out, similar to LHC. WPH1 ranged from 12-41mm in 2018 but there was a large, unfilled gap between those measurements, and lengths in 2022 ranged from 26-42mm. WPH2 also had a small gap between measurements in the middle of the range from 12-48mm, there was no gap in 2019 ranging from 31-60mm, a large gap was present between obtained lengths of 15-55mm in 2021, and a range of 33-46mm was present in 2022. PS7 only had measurements in 2019 that ranged from 37-42mm. While there is high variability in ranges of these lengths, when looking at the mean length measurements, there are no statistically significant differences of sheepshead minnow lengths between years within sites (**Supplemental Table 24. Sheepshead Minnow Length Results**).

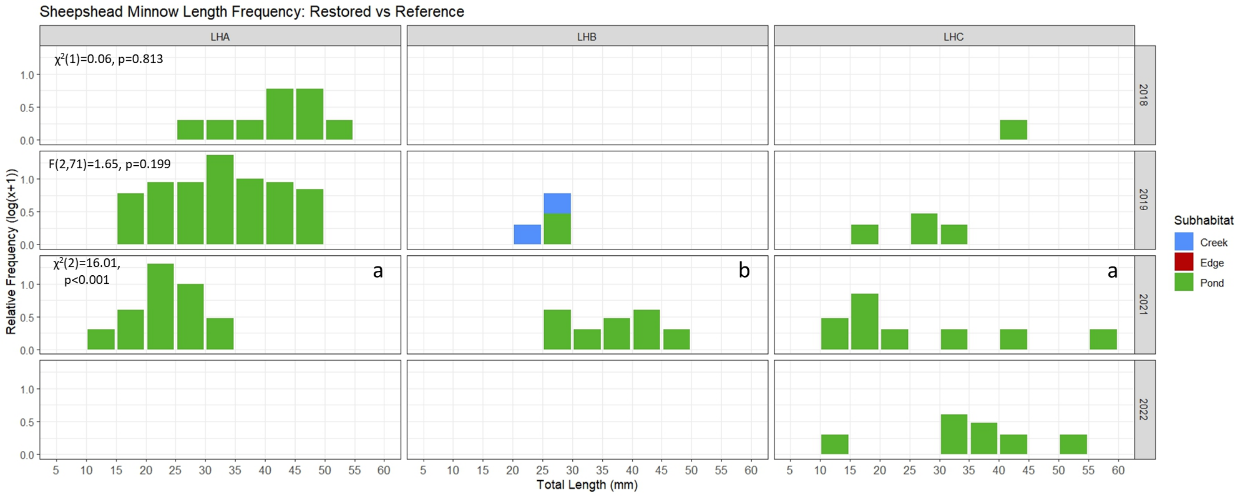


Figure 35. Sheepshead Minnow Length Frequency: Restored vs Reference Sheepshead minnow length frequency data between restored and reference sites calculated from relative frequencies (log(x+1) transformed) based on the measured number of individuals transformed to reflect the total number of caught individuals. Bins are split into 5mm measurement increments and colors representing subhabitat location of individuals caught spanning all sites and years sampled.

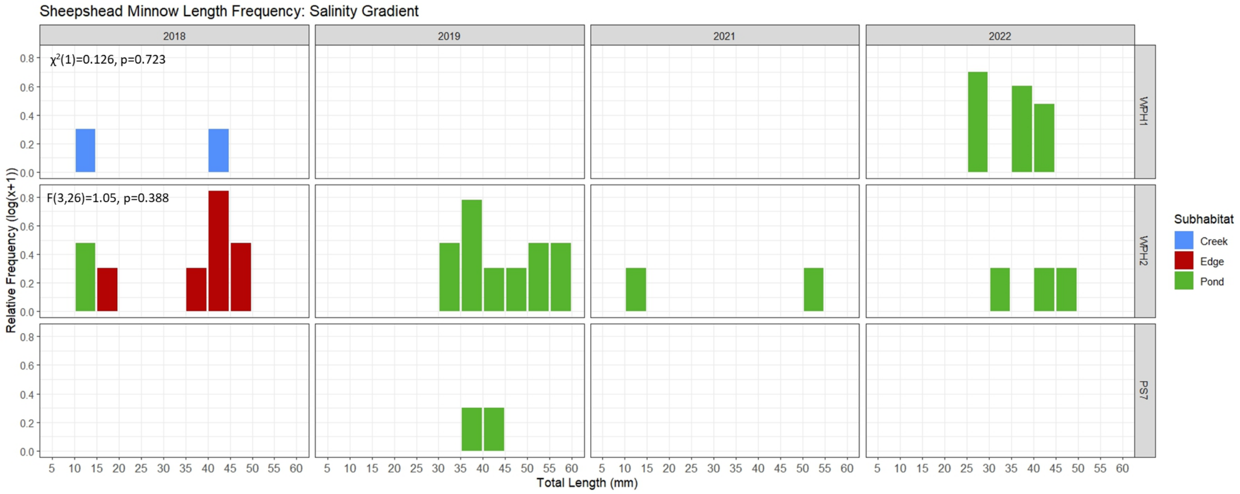


Figure 36. Sheepshead Minnow Length Frequency Sheepshead minnow length frequency data between years of sites located along the salinity gradient calculated from relative frequencies (log(x+1) transformed) based on the measured number of individuals transformed to reflect the total number of caught individuals. Bins are split into 5mm measurement increments and colors representing subhabitat location of individuals caught spanning all sites and years sampled.

Biomass. Sheepshead minnow biomass distribution for restored and reference sites (**Figure 37. Sheepshead Minnow Biomass Frequency: Restored vs Reference**) and within sites between years of siphon-off and on conditions (**Figure 38. Sheepshead Minnow Biomass Frequency: Salinity Gradient**) seems to match fairly closely to those of the length class distributions (**Figure 35. Sheepshead Minnow Length Frequency: Restored vs Reference**, **Figure 36. Sheepshead Minnow Length Frequency**). There were a few discrepancies in which LHA in 2018 seemed to have a wider spread with some minor gaps in biomasses as compared to lengths. This trend was also seen for WPH2 in 2019. There seems to be a discrepancy between lengths and biomasses at WPH1 in 2018 and WPH2 in 2021, but some individuals were not weighed after being measured which may be the cause of this discrepancy.

At LHA, biomass seems to be decreasing over time. In LHB, there were no data in 2018 or 2022 to compare and LHC shows a trend of increasing but sporadic biomasses over time. To compare sites within years, LHA had a biomass range of 0.7-4.0g and LHB only had one individual with a mass of 2.2g. In 2019, LHA had the largest range of 0.1-2.3g, LHB and LHC had the same range of 0.2-0.6g and 0.1-0.5g. In 2021, LHA had the smallest range between 0.1-0.7g, LHB ranged from 0.3-2.6, and LHC had the largest range from 0.0-2.4g. And LHC was the only site that had sheepshead minnows present in 2022 ranging in biomass from 0.0-3.6g.

Looking at the mean of biomasses between restored and reference sites (**Supplemental Table 25. Sheepshead Minnow Biomass Results**), there are more differences than there were with the mean lengths spread between two years now and all together encompassing differences between all combinations of sites. While there are more differences than with the lengths, restored and reference sites are still very similar to one another. The first set of significant differences come in 2019 ($\chi^2(2)=698$, p=0.030) between LHA and LHC (p=0.045) where LHA had a mean biomass of 33.0g and LHC's mean biomass was smaller at 26.8g. The next year of sampling in 2021 ($\chi^2(2)=17.72$, $p<0.001$) also had significant differences both within and between restored and reference sites. LHB had the highest biomass mean at 36.6g and was significantly larger than the 24.4g mean biomass at LHA ($p<0.001$) and the 24.3g mean biomass at LHC ($p<0.001$).

Sites along the salinity gradient showed interesting patterns with sites having high fluctuations between years but there were also sites without sheepshead minnow biomass measurements (WPH1 2019 and 2021; PS7 2018, 2019, 2022) that do not seem to follow siphon-off or on years. WPH1 only had one individual weighted in 2021 at 1.6g and then had a wider range in biomass in 2022 from 0.2-1.9g. WPH2 had the highest abundance and biomass measurements but these were also somewhat spaced out with small gaps between weight classes. 2018 ranged from 0.1-2.9g, 2019 had the largest range from 0.8-4.7, 2021 had the narrowest range but also only had one individual weighed at 4.2g, and 2022 had a range from 0.8-2.4g. PS7 only had a few measurements in 2019 creating a range from 1.3-2.3g with no other years having individuals caught. Although variable and somewhat spread out, mean biomasses between years within sites showed no significant differences (**Supplemental Table 25. Sheepshead Minnow Biomass Results**), indicating that sheepshead minnows utilize all areas along this region with and without a salinity gradient. Also, there are no significant interactions (Two-way ANOVA, $F_{1,32}=0.086$, $P=0.77$).

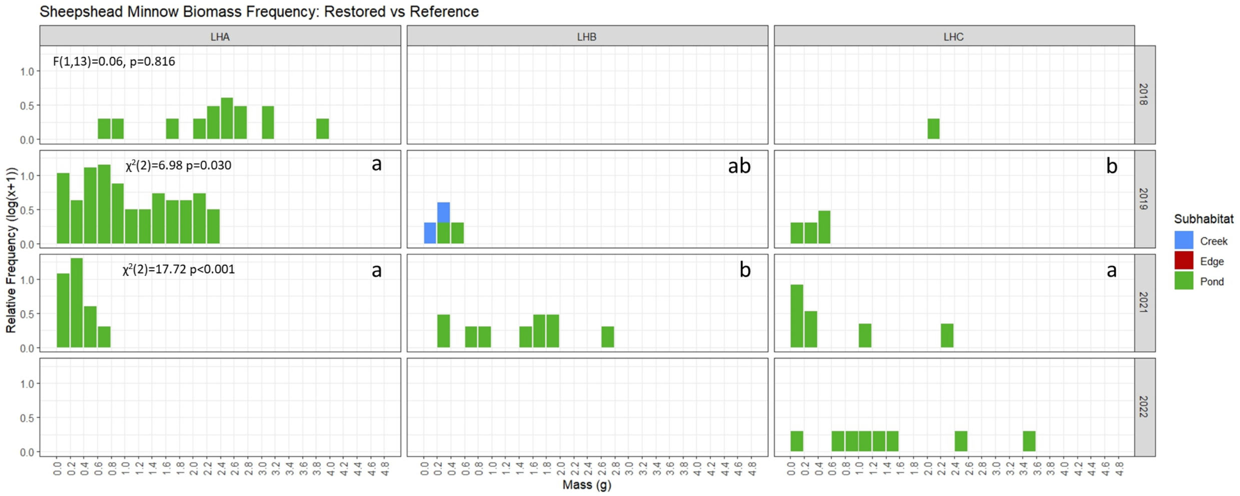


Figure 37. Sheepshead Minnow Biomass Frequency: Restored vs Reference Sheepshead minnow biomass frequency data between restored and reference sites calculated from relative frequencies (log(x+1) transformed) based on the measured number of individuals transformed to reflect the total number of caught individuals. Bins are split into 0.2g increments and colors representing subhabitat location of individuals caught spanning all sites and years sampled.

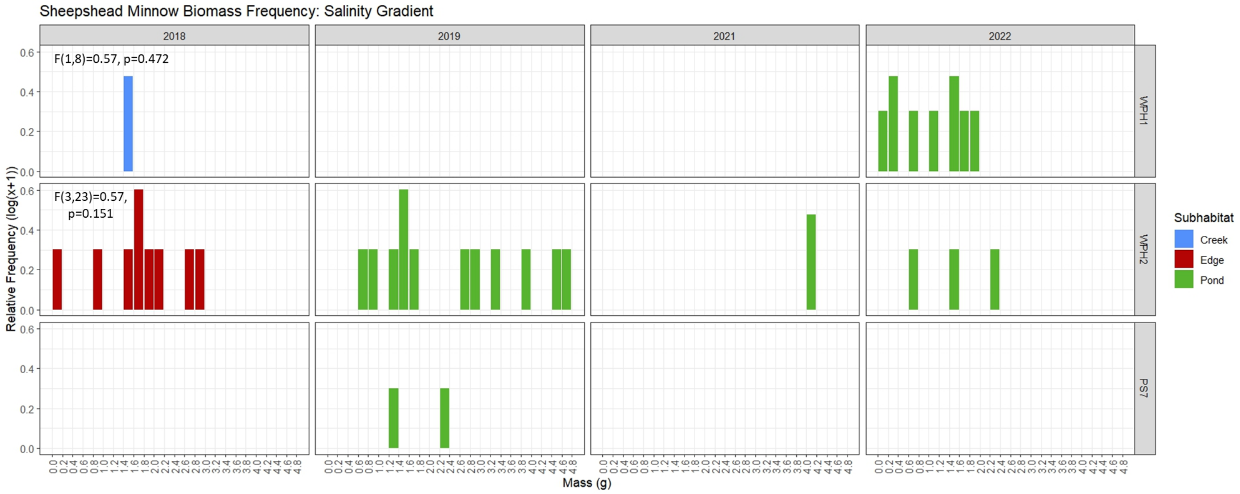


Figure 38. Sheepshead Minnow Biomass Frequency: Salinity Gradient Sheepshead minnow biomass frequency data between years of sites located along the salinity gradient calculated from relative frequencies (log(x+1) transformed) based on the measured number of individuals transformed to reflect the total number of caught individuals. Bins are split into 0.2g increments and colors representing subhabitat location of individuals caught spanning all sites and years sampled.

Rainwater Killifish (Lucania parva)

Lengths. Out of the four specific fish species looked at, the rainwater killifish has the lowest abundance within all of our catch data making up only 0.83% of it through the four years sampled. Raw catch numbers can be found within **Supplemental Table 26. Rainwater Killifish Population Numbers**. While they made up a small fraction of the catches, rainwater killifish had the most unique distribution amongst subhabitats we have looked at thus far. They were present in the lowest percentages within ponds only making up 14.03% of the catches, while 38.98% of individuals were caught in creeks, and the largest percentage, 46.99%, were at the edge. Individuals were found more readily for restored versus reference sites (**Figure 39. Rainwater Killifish Length Frequency: Restored vs Reference**) than there were for sites along the salinity gradient (**Figure 40. Rainwater Killifish Length Frequency: Salinity Gradient**) though time. Overall, for all six sites, mean CPUE for rainwater killifish was lowest in 2021 when individuals were only individual was caught at LHC, next was in 2022 with a mean CPUE of 0.12, then 2018 at 0.29 CPUE, and the highest catches were in 2019 at 0.34 CPEU individuals per sampling event.

No rainwater killifish were obtained in LHB 2018 or 2021 or LHA in 2021 but were found in all other sites and year when looking at restored and reference data. LHA had relatively similar lengths of individuals between years with 2019 having a few slightly smaller individuals. LHB data was scarce with only one individual in 2022 but many were caught in 2019. LHC had individuals caught in all years, the only site to have this occur but catches were still scarce in all years except 2019. Going from site to site within years, in 2018 LHA ranged in lengths from 33-41mm and LHC had the highest range from 33-47mm. In 2019, the highest abundance year, LHA had the largest length range from 25-45mm but the lowest catch, while, LHB and LHC had similar catches but LHB had the largest range from 21-45mm and LHC was from 19-33mm. LHC was the only site that had a singular rainwater killifish in 2019 with a length of 40mm. 2022 had individuals within all sites but only LHA had a range from 31-46mm as LHB and LHC only had one individual at 47mm and 37mm.

With such variability in abundance and some variability in lengths with the individuals that were caught, there was a minute difference between restored and reference sites, when individuals were caught, within just one year (**Supplemental Table 27. Rainwater Killifish Length Results**). This indicated that, when present, rainwater killifish of similar lengths utilize restored and reference sites similarly. The only significant difference found was in 2019 ($\chi^2(2)=6.71$, $p=0.035$) when LHA had a mean length of 35.6mm and was significantly larger than the mean length of 25.7mm at LHC ($p=0.028$).

Sites located along the salinity gradient had very scarce catches except at WPH2 in 2018 (**Figure 40. Rainwater Killifish Length Frequency: Salinity Gradient**). WPH1 had the most expansive data covering all years except 2021, but individuals were only caught in the first and last year of sampling at WPH2, and never at PS7. Trend of catch and abundance do not seem to follow with siphon-off or on conditions leading us to believe there is no distinguishable affect when there is freshwater input to these sites. WPH1 had the largest range in length measurements in 2018 from 27-37mm, the second largest range in 2019 from 32-44mm, and the smallest in 2022 from 36-39mm. WPH2 had the largest, out of all salinity gradient sites, and only range of lengths for this site in 2018 from 19-44mm, and only one individual measuring 44mm in 2022. With the individuals we were able to catch, we found no significant differences between any years within sites for rainwater killifish mean lengths (**Supplemental Table 27. Rainwater**

Killifish Length Results), again indicating that freshwater input does not affect the length of individuals present at this site.

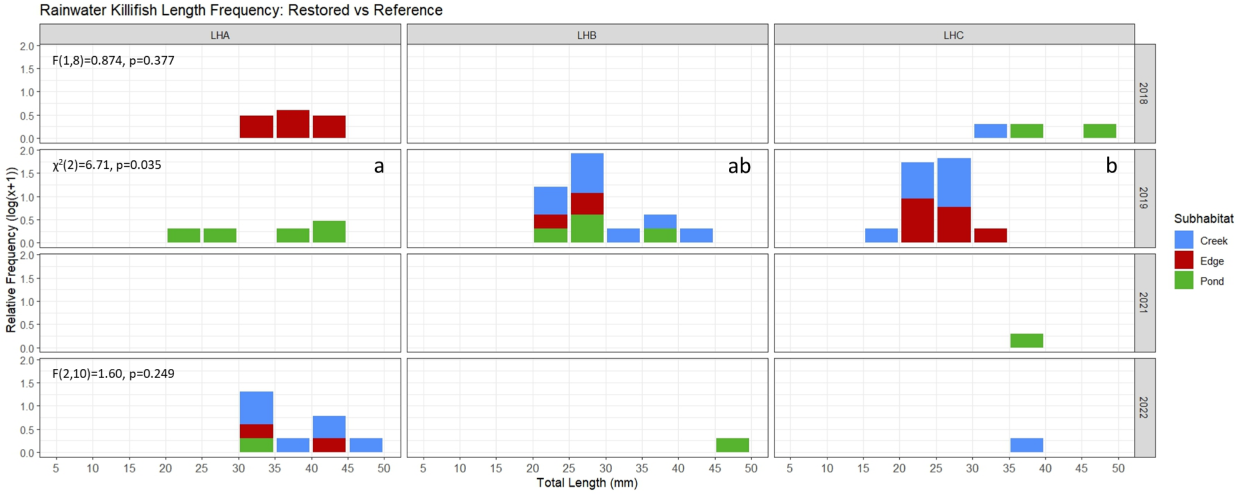


Figure 39. Rainwater Killifish Length Frequency: Restored vs Reference Rainwater killifish length frequency data between restored and reference sites calculated from relative frequencies (log(x+1) transformed) based on the measured number of individuals transformed to reflect the total number of caught individuals. Bins are split into 5mm measurement increments and colors representing subhabitat location of individuals caught spanning all sites and years sampled.

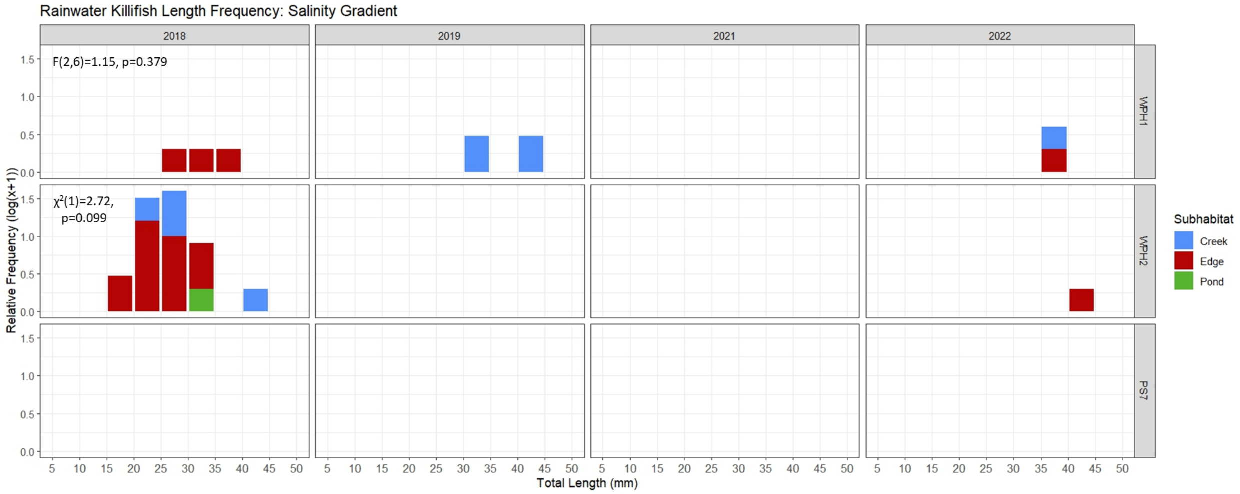


Figure 40. Rainwater Killifish Length Frequency: Salinity Gradient Rainwater killifish length frequency data between years of sites located along the salinity gradient calculated from relative frequencies (log(x+1) transformed) based on the measured number of individuals transformed to reflect the total number of caught individuals. Bins are split into 5mm measurement increments and colors representing subhabitat location of individuals caught spanning all sites and years sampled.

Biomass. Rainwater killifish biomasses (**Figure 41. Rainwater Killifish Biomass Frequency: Restored vs Reference**, **Figure 42. Rainwater Killifish Biomass Frequency: Salinity Gradient**) and length frequency distributions (**Figure 39. Rainwater Killifish Length Frequency: Restored vs Reference**, **Figure 40. Rainwater Killifish Length Frequency: Salinity Gradient**) seem to match fairly well with the caveat that some biomass measurements were missing from the data set that were present for length measurements, specifically edge individuals at LHC in 2019, all 2018 WPH1 individuals, and edge and pond individuals at WPH2 in 2018. Again, data was more complete for restored and reference sites **(Figure 41. Rainwater Killifish Biomass Frequency: Restored vs Reference**). No rainwater killifish biomass data was available for LHB in 2018, or for LHA or LHB in 2021, or for WPH1 in 2018 and 2021, WPH2 in 2019 and 2021, or at PS7 in any year.

LHA had the most consistent range of biomasses but also had the most robust data set through the years. A pattern is hard to distinguish for LHB as there wasn't much data except in 2019 where there was an extremely wide spread of biomasses found. LHC seemed to fluctuate the most through time but stayed within somewhat similar ranges. Looking at each site within the years, in 2018, LHA ranged in biomasses from 0.4-0.8g, and LHC had a slightly larger range from 0.4-1.1g. All sites captured biomasses in 2019 where LHA ranged from 0.2-1.3g, LHB had the largest range from 0.0-1.5g, and LHC had the smallest range from 0.1-0.3g. Only one individual was found in LHC in 2019 weighing 0.9g. And LHA had the largest range in 2022 from 0.3-0.9g, and LHB and LHC only contained one individual at 0.9g and 0.6g.

Restored and reference sites had individuals falling into similar ranges with some variability between years and within sites, so with the mean of the biomasses, we were able to determine that there were slightly more differences between restored and references

(**Supplemental Table 28. Rainwater Killifish Biomass Results**) as compared to their mean lengths. Both of the restored sites were significantly different from one another in a singular year which indicates that although not exactly the same, restored sites are able to accommodate similarly masses rainwater killifish as references. The only difference was in 2019 ($\chi^2(2)=16.39$, $p<0.001$) with LHA having the highest mean biomass at 0.7g and being significantly larger than LHC's biomass at 0.1g ($p=0.007$), and LHC's biomass was also then significantly smaller than LHB's biomass at 0.5g ($p<0.001$).

There seemed to be no shift between siphon-off or on years but we were also not able to capture any individuals within many of the sites and years we attempted to sample (**Figure 42. Rainwater Killifish Biomass Frequency: Salinity Gradient**). WPH2 has a decrease in abundance after 2018 but individuals are captured again in 2022 and seem to maintain larger biomasses. Lack of data led to minimal range data but WPH1 had the largest range in biomass in 2019 from 0.3-0.9g and then a slightly smaller range in 2022 from 0.5-0.8g. WPH1 biomasses only captured one range in 2018 from 0.0 to 1.0 which is the largest range out of these sites, and only one individual was captured in 2022 weighing 0.9g. Comparing the mean rainwater killifish biomasses within sites for years we captured had data, we found no significant differences ($\chi^2(3)=0.06$, $p=0.812$), indicating that freshwater input does not affect the mean biomass of individuals present within WPH1 or WPH2.

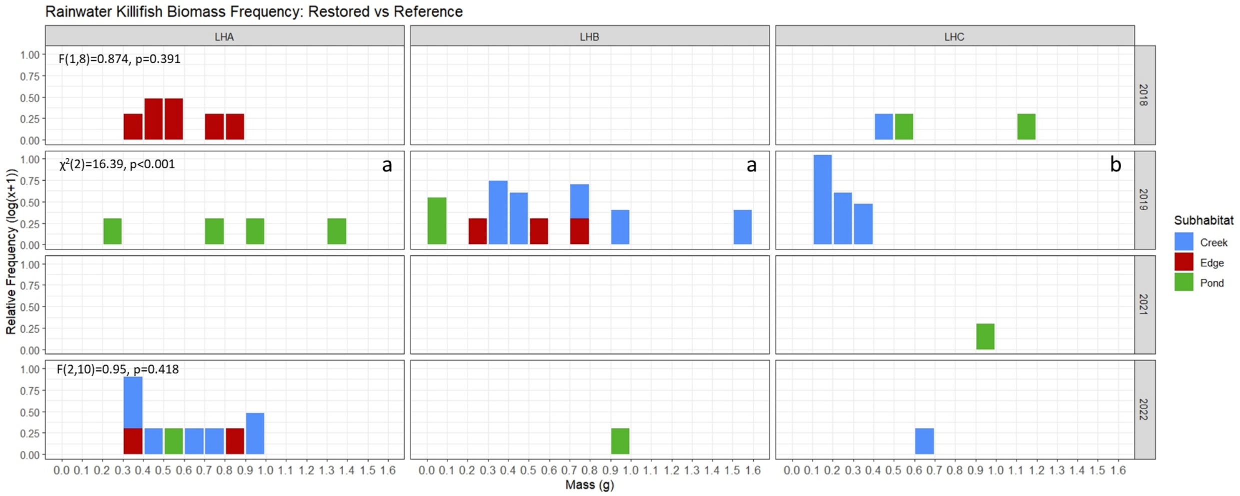


Figure 41. Rainwater Killifish Biomass Frequency: Restored vs Reference Rainwater killifish biomass frequency data between restored and reference sites calculated from relative frequencies (log(x+1) transformed) based on measured number of individuals transformed to reflect the total number of caught individuals. Bins are split into 0.1g measurement increments and colors representing subhabitat location of individuals caught spanning all sites and years sampled.

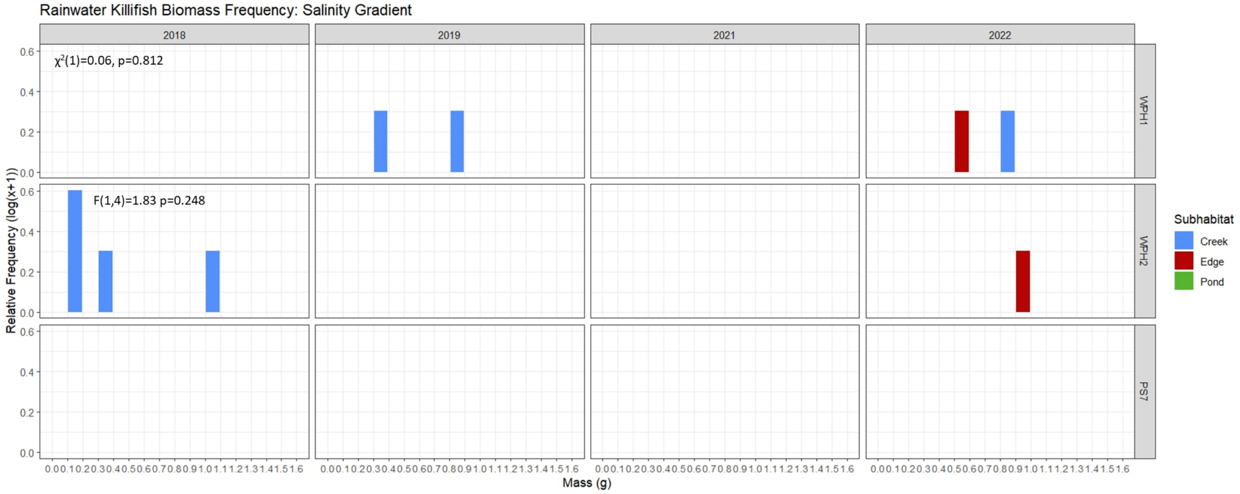


Figure 42. Rainwater Killifish Biomass Frequency: Salinity Gradient Rainwater killifish biomass frequency data between years of sites located along the salinity gradient calculated from relative frequencies (log(x+1) transformed) based on measured number of individuals transformed to reflect the total number of caught individuals. Bins are split into 0.1g measurement increments and colors representing subhabitat location of individuals caught spanning all sites and years sampled.

Macroinvertebrate Community Composition

Utilizing Non-metric multidimensional scaling plots (nMDS) again, we were able to visualize the patterns and shifts in macroinvertebrate communities between restored and reference sites and sites along the salinity gradient (**Figure 43. Macroinvertebrate Community Composition**). Within this nMDS, we notice close clustering of all sites, but LHA, one of the restored sites, seems to have a slightly different community composition from the rest of the sites within some samples as indicated by the distance between its and the rest of the site's polygons. The other restored site, LHB, and the control, LHC, are highly similar, as are WPH1, WPH2, and PS7 along the salinity gradient. Using Pearson Correlations (>0.3), we were able to determine that four groups of macroinvertebrates were able to distinguish the differences in community compositions observed in the nMDS. Organisms categorized in the groups of Family Blattidae, Order Diptera, Family Formicidae, and Family Braconidae were identified as having significantly different enough presences within LHA samples to cause its distinguished community composition.

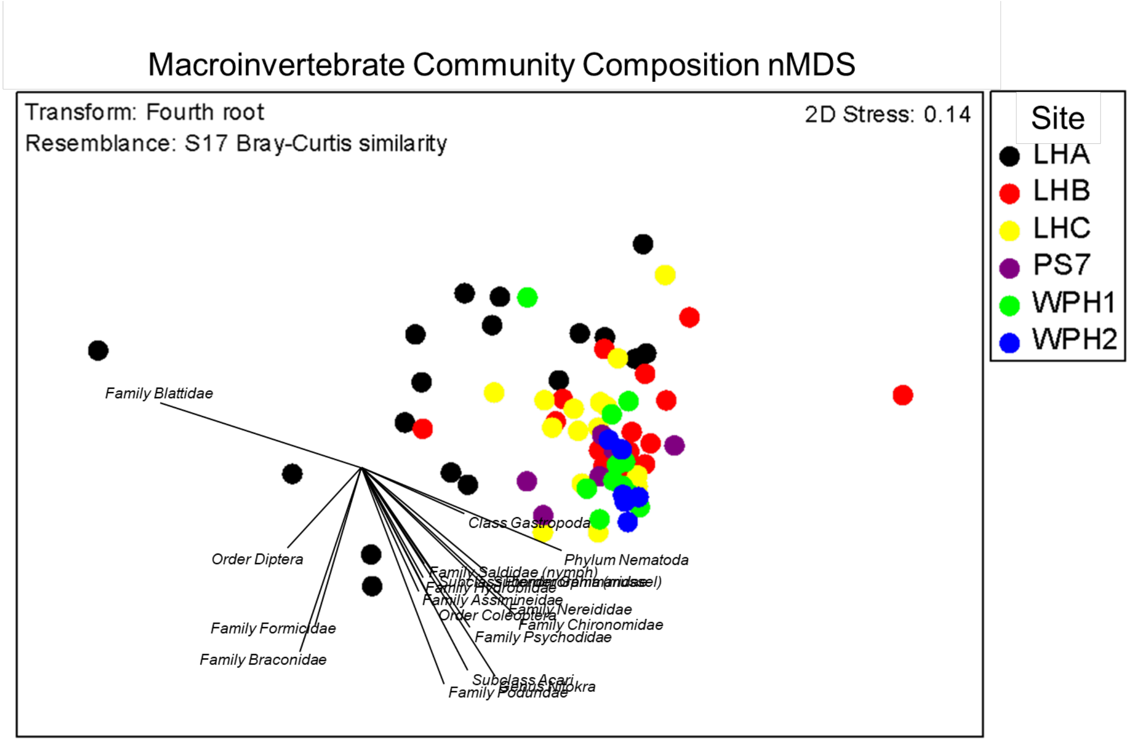


Figure 43. Macroinvertebrate Community Composition Non-metric multidimensional scaling (nMDS) plot of macroinvertebrate community composition displaying shifts between site samples over time. Data was fourth root transformed, and Bray-Curtis calculations including a dummy value were used to assemble a resemblance matrix for figure creation. An additional vector displaying species responsible for sample differences having a Pearson correlation>0.3 was also added.

<u>On-Marsh Nekton and Macroinvertebrate Comparisons</u>

In 2018, there was no correlation between nekton and macroinvertebrate abundance (Pearson's Test: t=-0.005, df=4, p=0.996, r=0.0086) (**Figure 44. 2018 Number of Individuals Correlation**). However, there was a significant, positive correlation between nekton and macroinvertebrate biodiversity (Pearson's Test: t=4.704, df=4, p=0.009, r=0.920) (**Figure 45. 2018 Shannon Diversity Correlation**). Notably, the pattern is such that the lower biodiversity values correspond to restored sites and the higher values to the sites furthest from the siphon.

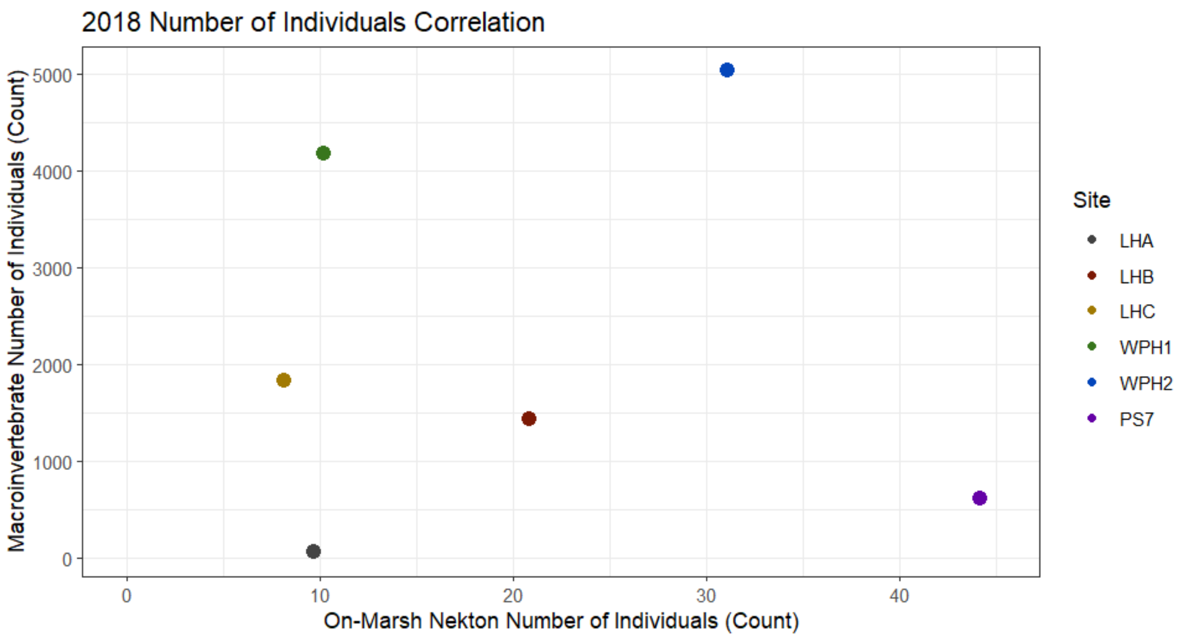


Figure 44. 2018 Number of Individuals Correlation Correlation between on-marsh nekton and macroinvertebrate number of individuals caught within each site (LHA, LHB, LHC, WPH1, WPH1, and PS7) in 2018. Colors of polygons represent each individual site sampled.

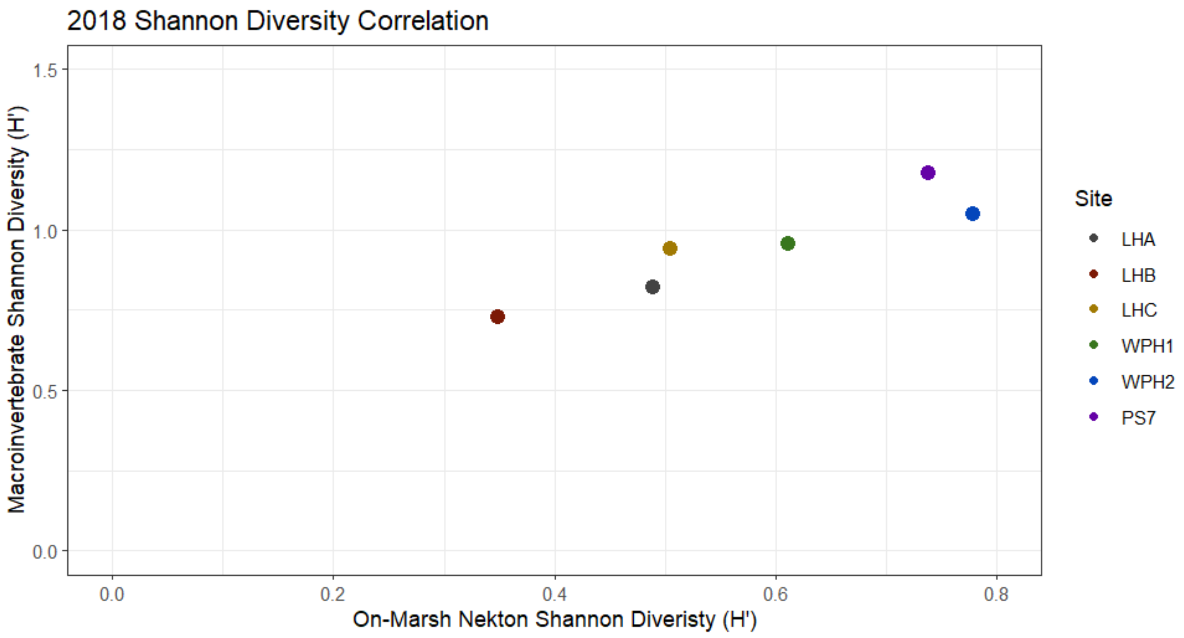


Figure 45. 2018 Shannon Diversity Correlation Correlation between on-marsh nekton and macroinvertebrate Shannon diversity within each site (LHA, LHB, LHC, WPH1, WPH1, and PS7) in 2018. Colors of polygons represent each individual site sampled.

DISCUSSION

Environmental Variables

Looking at environmental factors, there were some interesting results when taking into account restored versus references sites and sites that fell along the salinity gradient created by the siphon, and some interactions that crossed between both factors. In all years, at least during times of sampling, we found that restored and reference sites were similar in temperature to one another, but the order from warmest to coolest site fluctuated when the siphon was turned on. LHA was the warmest and most similar to LHC with LHB just slightly cooler in siphon-off years. In siphon-on years though, LHA became the coolest site and all three sites had slight gaps between their temperatures. A similar trend is noticeable with sites along the salinity gradient in which the two farther sites, WPH2 and PS7 are similar and warmer in siphon on years with WPH1, the closest site, being slightly cooler, but in siphon on years, WPH1 warms up and eventually becomes warmer than PS7 with a slight gap in temperatures between all three of these sites as well.

Salinity told a more dramatic story though with large shifts in salinity mainly constrained between siphon-off and on years. While there was still seasonal variability seen within all years, the magnitude of variability differed. In siphon-off years, salinity was fairly stable, with the site closest to the siphon having the highest salinity most of the year. When the siphon turned on, however, the site closest had decreased salinity by approximately 12 ppt as compared to previous years, which is biologically significant and could lead to effects in the community composition of the area. The other sites saw a decrease in salinity in siphon-on years, as well, but there was a rebound in salinity at other sites in the second year of siphon-on conditions. This trend is what we expected to see with the transportation of freshwater from the Mississippi River into a brackish area, and matches the results of previously determined by Boshart and William (2009), in that only locations closest to the siphon are affected by a drop of up to 10 ppt in salinity, not those farther away.

Inundation, another variable parameter influencing nekton use of an area (Rozas, 1995), was highly variable within years, more so than temperature or salinity. In southern Louisiana, a microtidal system, inundation is highly affected by weather patterns rather than by lunar cycles. We saw more days with higher percentages of the day inundated in the spring and fall months, with lower inundation in summer and winter months. Restored and reference sites were relatively stable across years, with LHB and LHC having roughly the same inundation times, but LHA having the lowest out of all sites due to its high platform height. LHA tended to have a lower number of individuals, species, and biodiversity, possibly correlated to its platform height and low inundation time. Remaining sites along the salinity gradient were slightly more variable though years, especially the sites closest (WPH1) and farthest (PS7) from the siphon, but fluctuations were seemingly not related to the siphon operation.

Spatial and Temporal Pattern of Nekton Abundance and Biodiversity

The number of individuals caught and the biodiversity across sites led to interesting results. Restored and reference sites were relatively similar to each other, with only minor differences seen between restored sites themselves, indicating that restored and reference sites have come to relative equilibrium with each other less than five years after completion. While this was preliminarily shown through results from Keppeler et al. (2023), it contradicts other studies such as Minellow and Webb Jr. (1997) and Minello and Zimmerman (1992), who found that five years was not long enough for restored sites to come to equilibrium with reference sites. Sites along the salinity gradient were more variable, though, with more differences between years, especially in the number of individuals caught that showed distinct differences between siphon-off and on years at sites closest to the siphon, showing an increase in the number of individuals in siphon-on years but a decrease in the biodiversity. Piazza and La Peyre (2007) highlighted similar results, where nekton abundance increased with the introduction of freshwater. This was also found to be true by Roberts et al. (1992), who also looked at effects of freshwater diversions from the Mississippi River in Louisiana. Their results seemed to match with the shift in shrimp populations as well, in which brown and grass shrimp were eliminated from this site in siphon-on years and were replaced by an increasing number of Ohio shrimp, which led to the increase in individuals, but decrease in biodiversity. Increasing catch numbers and decreasing biodiversity due to freshwater input in our results contradicts the conventionally held belief that brackish areas hold more individuals and that they have reduced biodiversity due to fewer species being able to tolerate frequent salinity fluctuations (Elliott and Whitfield, 2011). The site farthest from the siphon, while still variable, was variable within siphon-off and on years, not just between on- and off-conditions, leading to other variables seemingly causing these shifts. The patterns of increasing and decreasing catches, especially at PS7 and WPH1, match patterns of increasing and decreasing percent of time inundated at these sites, possibly indicating a correlation.

Habitat Use Patterns and Biodiversity

Subhabitats catches and biodiversity showed high variability when broken down across subhabitats, but catches in ponds were relatively stable in all sites, matching with these locations typically being habitats for resident species that spend their whole lives within ponds (Hitch et al., 2011), whereas creek and edge catches are more variable, as they are used more often as transportation and areas for transient species (Baltz et al., 1993). Biodiversity between restored and reference sites was only significantly affected within all subhabitats in one year, but, again, there were differences between restored sites themselves, leading us to believe that, even within subhabitats, restored and reference sites have come to equilibrium after less than five years. Matching with the decrease in biodiversity of whole sites, edges were highlighted as the subhabitat where the most change occurred closest to the siphon, and aligns with the change in shrimp communities found in the composition analysis. Every combination of siphon-off and on years showed a significant decrease in edge biodiversity closest to the siphon, giving us strong backing that freshwater input highly affects community composition, specifically at the edge of the marsh.

Spatial and Temporal Patterns of Community Composition

Closer looks into the community composition aligned with what the biodiversity analysis revealed. Restored and reference sites, while communities varied over time, were still similar in composition over time, and changes within sites between siphon-off and on conditions led to changes as well, but one that were not permanent. Communities were able to rebound to their original composition at the beginning of the study, except for the site closest to the siphon, in which it continued to become more dissimilar to its own compositions in previous years and all other sites in general. These differences were highlighted to be most substantial in the second year of siphon operation, and restricted to just creeks and edges where brown and grass shrimp were replaced by Ohio shrimp. Ponds, however, remained stable with the continual presence of Cyprinodontiformes such as sailfin molly, diamond killifish, bayou killifish, Gulf killifish, sheepshead minnows, and mosquitofish. de Mustsert and Cowan (2012) found similar results to this, in which there were significant shifts in community composition with the reduction of salinity. They also saw a similar disappearance of shrimp species, specifically brown and white shrimp, when freshwater was introduced to an area. This matches previous knowledge that brown shrimp are more often found in higher salinity areas (Rozas and Minello 2011) and are directly affected by freshwater diversions (Roberts et al, 1992). Contrary to our findings, Rozas et al. (2005) and Day et al. (2009) found an increase in grass shrimp density and biomass with freshwater input, whereas we found a decrease and subsequent elimination of those species. This contradictory finding may indicate some interaction between Ohio and grass shrimp, in which Ohio shrimp outcompete grass shrimp in freshwater conditions. The presence of a salinity gradient at sites and within subhabitats has the ability to create unique habitats for species with preferential salinity regimes (Montagna and Kalke, 1992).

Associations between Macroinvertebrate and Nekton Communities

Macroinvertebrate community compositions did not have much variability among sites through time with most sites containing highly similar community compositions from the rest of the sites. Sites along the salinity gradient did not show much of a difference between siphon-off or on conditions, opposing the works of others such as Husseneder et al. (2022), Vander Vorste et al. (2018), and Aker et al. (2023) who found significant differences in community structure along a salinity gradient. However, we did find differences in one of the restored sites compared to all other sites. LHA had community compositions containing noticeable increases in the presence of individuals categorized into Family Blattidae, Order Diptera, Family Formicidae, and Family Braconidae. Since LHA has a higher platform height than the other sites, this may be the cause for the community differences we saw. Nordström et al. (2015) conducted a study on the effects of both marsh age and elevation on macroinvertebrate communities and found that age and elevation had a significant effect, but with older, less-elevated marshes having a higher number of Insecta species. The class Insecta contains a wide variety of organisms though with varying characteristics and life histories (Resh and Cardé, 2009). Only identifying down to Family for these organisms may limit our ability to distinguish how individual species and functional groups within area affected as we know effect of sediment heights affect these categories differently (Tong et al., 2013). There were no noticeable differences in the community composition of the other restored site compared to the reference though. Previous studies have noted that it may take anywhere from three to 25 years for macroinvertebrate communities to recover and become similar to surrounding areas (Craft and Sacco, 2003). This study seems to be on par with that assessment on the faster time frame for recovery as this study shows that from completion of the restored sites in 2015 to the start of our study in 2018, these communities are highly similar to one another. Previous studies on sediment slurry additions also show that macroinvertebrate densities were negatively affected by high sediment additions and platform height, but that Shannon-Weiner diversity was not affected (Tong et al., 2013), similar to what we found as well.

Within a marsh, species are not isolated to interacting with just conspecifics –they are interacting with multiple other species, as well. Between on-marsh nekton and macroinvertebrates, there was not a correlation between the number of individuals caught between the groups, but there was a positive correlation between the biodiversity of the groups. These individuals between groups have food web interactions, which may account for increases or decreases in their presence within the marsh. Gulf killifish often consume detrital material along with macroinvertebrates, such as polychaetes and amphipods, and may play an influential part in prey-species distributions and presence in the marsh (Rozas and LaSalle, 1990). For freshwater stream habitats, it is often assumed that macroinvertebrates and fish communities covary in their response to different environmental conditions (Kilgour and Barton, 1999) which, while we studied a brackish saltmarsh system, matches what we found in our saltmarsh sites, as each species responded similarly to their respective site's environmental condition – as biodiversity of one group goes up, so does the other. I was only able to find one study by Kilgour and Barton (1999) that looked at the covariability of these two groups within saltmarsh habitats, leading us to implore future researchers to include these analyses in their studies.

<u>Fish Demographic Patterns - Length & Biomass</u>

The presence of examined species (i.e. Gulf killifish, bayou killifish, sheepshead minnows, rainwater killifish, diamond killifish, grass shrimp, brown shrimp, Ohio shrimp) and their population demographics were highly variable, especially depending on the species. A notable trend between the fish species are the numerous differences found mid-distance from the siphon at WPH2 where the majority of significant length and biomass differences were found. This could mean that, while the abundance of individuals mid-distance isn't highly affected by freshwater input, there are possible physiological effects to those communities residing there. Previous work on 44 Gulf of Mexico estuarine species has shown that juveniles are less sensitive to salinity changes compared to adults (Christensen et al., 1997) so reduction in lengths and biomasses of species may be due to movement of adult individuals away from freshwater areas, leaving only tolerant juveniles in areas closer to the siphon. Various studies have shown differing results to species reactions to freshwater input on growth and biomass (Piazza and La Peyre 2010, Rozas and Minello 2011), but it seems like the specific salinity tolerance of the species is the determining factor on their reaction. While we saw a decrease in Gulf killifish lengths in siphon-on years, matching Ramee et al. (2016) predictions, we did not see a decrease in sheepshead minnows as predicted by results from Dunson et al. (1998). Piazza and L. Peyre (2007) found higher growth and biomass with freshwater flow, while Rozas and Minellow

(2011) found decreases with freshwater. de Mustsert and Cowan (2012) found that weights of individuals were significantly reduced in lowered salinity areas, while some of the species we looked at did have reduced biomasses in siphon-on years, it was not contained to just exclusively those years and was not found to affect all individuals in that area, further reinforcing that the specific tolerances of species may be affecting their reactions.

Gulf killifish, while a staple species found in Gulf of Mexico marshes, had the largest differences out of all the species we looked at, except Diamond killifish, between restored and reference sites where are bayou and sheepshead minnows had no or minimal differences, indicating some species are more tolerant to young, developing marshes than other species. While there were differences between restored and reference marshes though, they were not consistent enough to indicate that they are drastically different from one another and that they are overall similar after less than 5 years of establishment.

Limitations

As with any study, there were limitations to sampling and analysis that we were unable to avoid or overcome. As ecologists, we face unpredictable weather and unforeseen circumstances in the field which may limit our data collection ability. This was the case for PS7 in 202. We were thankful to have one day's worth of data for this site though so that we were able to still run some analysis as consistently as possible with the other sites and years. Another limitation was the comparisons between nekton and macroinvertebrates in which I was unable to find any source that directly compared these communities when they were part of separate samplings, only literature looking at diet studies made direct comparisons between these groups. Within our samples, macroinvertebrates also include species that are not restricted to just aquatic habitats, but include species that are also semi- or fully terrestrial, leading to a habitat mismatch when considering food web interactions. Nekton that reside in water are highly unlikely to interact with a macroinvertebrate that is aerial or terrestrial unless it goes into the water. This may have been the cause for the non-correlation found when comparing the number of nekton and macroinvertebrates caught per site. Looking at correlations between just aquatic macroinvertebrates and nekton may lead to different results.

Implications

The goal of Louisiana's Comprehensive Master Plan (2007) is to find a way to reduce land loss and preserve coastal areas for the ability of people in Louisiana to live, work, and play. This study helps evaluate those goals though understanding the implications it has for the organisms found within coastal salt-marsh habitats that many people rely on for economic and social means. Looking at the communities present within marshes, we can identify and monitor indicator species to assess the health of the habitat and determine changes occurring (Weilhoefer, 2011). This study was able to do that through tracking changes in abundance and community composition to indicate which sites and which species were least and most affected by freshwater input and which subhabitats were affected by these changes as well. We were also able to determine the health of restored marshes through community comparisons which indicated that our sites required less than 5 years to become highly similar in composition, abundance, and species demographics.

Many of the key indicators classified by the US EPA Science Advisory Board (SAB) Essential Ecological Attributes were taken into account for this analysis, such as landscape condition (i.e. land to water ratio and subhabitats), biotic conditions, and physical characteristics of the marsh along with ecological processes (Young and Sanzone, 2002), leading it to be a source of guidance for future restoration projects as to outcome expectations. This study, paired with future development and understanding of indicators of restoration outcomes and success, will allow managers and stakeholders to make more informed decisions in the future when considering restoration projects. Implications to community composition and species demographics, such as those outlined through this Thesis, may be taken into consideration when planning and implementing future projects. As the current goal of freshwater diversions is to manage wildlife and fisheries though salinity and to also increase sediment deposition (Roberts et al., 1992), it is important for future projects to be mindful of species already shown to be affected by these methods Species such as brown shrimp (this study) or oysters (Roberts et al., 1992), which are of high fishery importance, are negatively affected, while other species such as Ohio shrimp are positively impacted (this study) and Cyprintontiform species, also valuable to fisheries, are neutrally affected (this study). These implications may have an effect on an area's economy and should also be taken into consideration when planning future projects.

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APPENDIX: SUPPLEMENTAL TABLES AND FIGURES

Supplemental Tables

Supplemental Table 1. Salinity, Temperature, and Inundation Averages Summary of averaged salinity, temperature, and inundation levels for the month of March through July in each sampling year (2018, 2019, 2021, 2022). Data from all sites was collected through continuous monitoring at each site daily except WPH1 salinity and temperature for 2021 and 2022, instead, samples were collected from an outfall canal (OC) monitor slightly northeast of WPH1 for those data. A) Temperature B) Salinity C) Percent Time of Day Inundated.

a) Temperature (°C)

Year	LHA	LHB	LHC
	WHP1	WHP2	PS7
2018	25.9	25.8	24.2
	23.1	26.6	26.7
2019	25.4	25.2	23.6
	22.7	26.0	25.9
2021	25.4	23.3	23.6
	25.2	26.0	25.9
2022	26.0	23.3	24.1
	25.5	26.7	24.5

b) Salinity

Year	LHA	LHB	LHC
	WHP1	WHP2	PS7
2018	13.4	10.6	12.0
	12.6	12.5	11.0
2019	10.0	8.5	9.0
	11.4	9.6	8.5
2021	4.9	5.4	4.5
	3.0	5.2	6.0
2022	8.0	7.2	6.1
	3.8	7.8	9.0

Supplemental Table 1. Salinity, Temperature, and Inundation Averages (continued)

c) Percent Time of the Day Inundated (%)

Year	LHA WHP1	LHB WHP2	LHC PS7
2018	25.8 62.1	53.4 45.5	52.6 29.3
2019	33.8 64.9	66.2 59.6	66.9 42.9
2021	22.8 60.2	58.2 41.4	65.3 31.8
2022	7.8 22.1	21.9 7.5	31.1 14.1

Supplemental Table 2. Siphon Discharge Summary table of dates and flow rates of freshwater discharge from the West Point a la Hache siphon in 2021 and 2022. The siphon was not operational until March 9, 2021 due to hurricane levee construction. Data was taken from Boshart and MacInnes monitoring series Report BA-040MSTY.

Date(mm/dd/yyyy)	**West Point a la Hache Siphon Discharge (m^3/s)**
03/09/2021	38.5
03/10/2021	40.5
03/11/2021	37.9
03/12/2021	40.3
03/13/2021	41.1
03/14/2021	41.0
03/15/2021	40.1
03/16/2021	39.1
03/17/2021	28.4
03/18/2021	37.0
03/19/2021	42.4

Supplemental Table 2. Siphon Discharge (continued)	
03/20/2021	43.3
03/21/2021	42.7
03/22/2021	40.5
03/23/2021	37.9
03/24/2021	39.9
03/25/2021	37.3
03/26/2021	39.2
03/27/2021	39.9
03/28/2021	40.9
03/29/2021	45.5
03/30/2021	40.9
03/31/2021	41.4
04/01/2021	47.6
04/02/2021	45.5
04/03/2021	44.7
04/04/2021	44.9
04/05/2021	43.7
04/06/2021	42.3
04/07/2021	41.8
04/08/2021	41.4
04/09/2021	42.0
04/10/2021	40.6
04/11/2021	43.8
04/12/2021	44.4

Supplemental Table 2. Siphon Discharge (continued)	
04/13/2021	43.6
04/14/2021	41.6
04/15/2021	42.8
04/16/2021	43.8
04/17/2021	43.7
04/18/2021	43.3
04/19/2021	44.0
04/20/2021	42.0
04/21/2021	42.3
04/22/2021	44.2
04/23/2021	39.9
04/24/2021	37.36
04/25/2021	41.5
04/26/2021	39.7
04/27/2021	38.6
04/28/2021	36.9
04/29/2021	35.2
04/30/2021	35.2
05/01/2021	35.4
05/02/2021	31.6
05/03/2021	28.9
05/04/2021	28.8
05/05/2021	32.1
05/06/2021	37.5
05/07/2021	38.4

Supplemental Table 2. Siphon Discharge (continued)	
05/08/2021	37.4
05/09/2021	34.0
05/10/2021	35.7
05/11/2021	36.7
05/12/2021	42.5
05/13/2021	42.9
05/14/2021	42.7
05/15/2021	42.1
05/16/2021	42.3
05/17/2021	43.1
05/18/2021	44.4
05/19/2021	45.1
05/20/2021	44.7
05/21/2021	44.6
05/22/2021	45.1
05/23/2021	44.2
05/24/2021	43.7
05/25/2021	43.0
05/26/2021	42.4
05/27/2021	41.7
05/28/2021	41.2
05/29/2021	40.9
05/30/2021	41.1
05/31/2021	39.9
06/01/2021	39.3

Supplemental Table 2. Siphon Discharge (continued)	
06/02/2021	39.6
06/03/2021	39.4
06/04/2021	39*.3
06/05/2021	40.0
06/06/2021	39.96
06/07/2021	40.2
06/08/2021	39.9
06/09/2021	39.1
06/10/2021	39.1
06/11/2021	38.5
06/12/2021	38.4
06/13/2021	38.3
06/14/2021	38.9
06/15/2021	39.0
06/16/2021	39.2
06/17/2021	40.3
06/18/2021	40.6
06/19/2021	42.5
06/20/2021	40.0
06/21/2021	40.2
06/22/2021	40.0
06/23/2021	39.5
06/24/2021	39.2
01/17/2022	34.4
01/18/2022	35.7

Supplemental Table 2. Siphon Discharge (continued)	
01/19/2022	38.0
01/20/2022	38.5
01/21/2022	41.0
01/22/2022	40.3
01/23/2022	39.9
01/24/2022	40.1
01/25/2022	41.7
01/26/2022	40.9
02/24/2022	29.6
02/25/2022	30.0
02/26/2022	30.6
02/27/2022	31.9
02/28/2022	33.0
03/01/2022	36.4
03/02/2022	38.2
03/03/2022	39.6
03/04/2022	41.0
03/05/2022	42.0
03/06/2022	42.8
03/07/2022	42.8
03/08/2022	43.2
03/09/2022	43.1
03/10/2022	43.1
03/11/2022	43.0
03/12/2022	43.0

Supplemental Table 2. Siphon Discharge (continued)	
03/13/2022	42.6
03/14/2022	42.6
03/15/2022	44.2
03/16/2022	43.8
03/17/2022	44.1
03/18/2022	44.2
03/19/2022	44.2
03/20/2022	45.2
03/21/2022	44.4
03/22/2022	45.7
03/23/2022	44.8
03/24/2022	44.3
03/25/2022	43.6
03/26/2022	43.0
03/27/2022	42.9
03/28/2022	43.2
03/29/2022	43.2
03/30/2022	44.2
03/31/2022	44.6
04/01/2022	45.3
04/02/2022	44.5
04/03/2022	44.3
04/04/2022	44.0
04/05/2022	43.2
04/06/2022	43.1

Supplemental Table 2. Siphon Discharge (continued)	
04/07/2022	42.2
04/08/2022	41.8
04/09/2022	40.7
04/10/2022	40.3
04/11/2022	40.9
04/12/2022	41.2
04/13/2022	42.3
04/14/2022	42.4
04/15/2022	42.3
04/16/2022	42.7
04/17/2022	42.0
04/18/2022	42.0
04/19/2022	43.0
04/20/2022	41.9
04/21/2022	41.4
04/22/2022	41.4
04/23/2022	41.1
04/24/2022	42.3
04/25/2022	43.0
04/26/2022	42.9
04/27/2022	43.9
04/28/2022	44.2
04/29/2022	44.5
04/30/2022	45.1
05/01/2022	44.4

Supplemental Table 2. Siphon Discharge (continued)	
05/02/2022	44.3
05/03/2022	43.6
05/04/2022	42.9
05/05/2022	42.5
05/06/2022	42.1
05/07/2022	41.5
05/08/2022	41.1
05/09/2022	40.5
05/10/2022	41.1
05/11/2022	40.7
05/12/2022	41.3
05/13/2022	42.0
05/14/2022	42.8
05/15/2022	43.2
05/16/2022	43.7
05/17/2022	43.5
05/18/2022	42.5
05/19/2022	42.3
05/20/2022	42.3
05/21/2022	42.1
05/22/2022	42.7
05/23/2022	43.4
05/24/2022	43.5
05/25/2022	43.7
05/26/2022	44.54

Supplemental Table 2. Siphon Discharge (continued)	
05/27/2022	44.4
05/28/2022	44.1
05/29/2022	43.5
05/30/2022	42.5
05/31/2022	42.0
06/01/2022	41.2
06/02/2022	40.9
06/03/2022	40.4
06/04/2022	40.6
06/05/2022	39.9
06/06/2022	39.5
06/07/2022	38.9
06/08/2022	38.9
06/09/2022	39.0
06/10/2022	39.3
06/11/2022	39.7
06/12/2022	39.9
06/13/2022	39.7
06/14/2022	39.1
06/15/2022	38.9
06/16/2022	38.4
06/17/2022	37.9
06/18/2022	37.3
06/19/2022	37.4
06/20/2022	37.5

Supplemental Table 3. May Salinity Mean and standard deviation of May salinity (ppt) measurements between all six sites (LHA, LHB, LHC, WPH1, WPH1, and PS7) and years (2018, 2019, 2021, and 2022) of sampling.

Site	Year	May Salinity (ppt) Mean±SD
LHA	2018	14.03±0.11
LHA	2019	10.77±0.77
LHA	2021	5.09±1.93
LHA	2022	7.36±1.41
LHB	2018	10.91±0.34
LHB	2019	9.05±0.44
LHB	2021	5.81±1.10
LHB	2022	3.32±0.80
LHC	2018	12.5±0.47
LHC	2019	9.89±0.61
LHC	2021	4.49±1.06
LHC	2022	5.74±0.78
WPH1	2018	12.74±0.15
WPH1	2019	11.94±0.19
WPH1	2021	5.43±1.63
WPH1	2022	3.65±0.47
WPH2	2018	13.00±0.51
WPH2	2019	10.24±0.65
WPH2	2021	5.43±1.63
WPH2	2022	7.35±1.20
PS7	2018	11.56±0.65
PS7	2019	8.45±0.50
PS7	2021	6.39±1.0
PS7	2022	9.33±0.78

Supplemental Table 4. Site Shannon Diversity Results Statistical results for the Shannon diversity of species caught within sites. The table includes the factor, levels, statistical tests (main and post hoc comparison tests where the main test resulted in a p<0.05) and associated values (degrees of freedom, test statistic, p-value). Alpha, or significance level, is set to 0.05 (5%) for all tests. Transformations used to meet assumptions of normality or homogeneity of variance are noted. NA = not applicable.

Sites / Years Tested	Test / Post Hoc Name	DF	Test statistic	p-value	Significant Post-Hoc Interaction	Post Hoc p-value	Transformations
2018 LHA/LHB/ LHC	One-way ANOVA / Tukey HSD	2, 51	0.67	0.515	NA	NA	NA
2019 LHA/LHB/ LHC	One-way ANOVA / Tukey HSD	2, 51	5.52	0.007	LHA and LHB	0.022	NA
					LHA and LHC	0.012	NA
2021 LHA/LHB/ LHC	One-way ANOVA / Tukey HSD	2, 51	0.70	0.503	NA	NA	NA
2022 LHA/LHB/ LHC	One-way ANOVA / Tukey HSD	2, 51	0.78	0.462	NA	NA	NA
WPH1 2018/2019/ 2021/2022	Repeated Measures ANOVA / Tukey HSD	3, 51	1.93	0.136	NA	NA	NA
WPH2 2018/2019/ 2021/2022	Repeated Measures ANOVA / Tukey HSD	3, 51	1.40	0.254	NA	NA	NA
PS7 2018/2019/ 2021/2022	Repeated Measures ANOVA / Tukey HSD	3, 24	4.68	0.010	2018 and 2019	0.011	Only day 1 data used
					2019 and 2022	0.027	Only day 1 data used

Supplemental Table 5. Creek Number of Individuals Results Statistical results for the number of individuals caught within creek subhabitats per each site. The table includes the factor, levels, statistical tests (main and post hoc comparison tests where the main test resulted in a p<0.05) and associated values (degrees of freedom, test statistic, p-value). Alpha, or significance level, is set to 0.05 (5%) for all tests. Transformations used to meet assumptions of normality or homogeneity of variance are noted. NA = not applicable.

Sites / Years Tested	Test / Post Hoc Name	DF	Test statistic	p-value	Significant Post-Hoc Interaction	Post Hoc p-value	Transformations
2018 LHA/ LHB/LHC	One-way ANOVA / Tukey HSD	2, 15	48.76	0.332	NA	NA	NA
2019 LHA/LHB/ LHC	One-way ANOVA / Tukey HSD	2, 15	47.79	<0.001	LHA and LHB	<0.001	log(x+1)
					LHA and LHC	<0.001	log(x+1)
2021 LHA/LHB/ LHC	One-way ANOVA / Tukey HSD	2, 15	0.83	0.456	NA	NA	NA
2022 LHA/LHB/ LHC	One-way ANOVA / Tukey HSD	2, 15	1.50	0.254	NA	NA	NA
WPH1 2018/2019/ 2021/2022	Repeated Measures ANOVA / Tukey HSD	3, 15	3.04	0.062	NA	NA	NA
WPH2 2018/2019/ 2021/2022	Repeated Measures ANOVA / Tukey HSD	3, 15	17.08	<0.001	2018 and 2021	<0.001	log(x+1)
					2019 and 2021	<0.001	log(x+1)
					2019 and 2022	0.008	log(x+1)
					2021 and 2022	0.045	log(x+1)
PS7 2018/2019/ 2021/2022	Repeated Measures ANOVA / Tukey HSD	3, 6	7.17	0.021	2019 and 2022	0.028	log(x+1), only used day 1 data
					2021 and 2022	0.038	log(x+1), only used day 1 data

Supplemental Table 6. Edge Number of Individuals Results Statistical results for the number of individuals caught within edge subhabitats per each site. The table includes the factor, levels, statistical tests (main and post hoc comparison tests where the main test resulted in a p<0.05) and associated values (degrees of freedom, test statistic, p-value). Alpha, or significance level, is set to 0.05 (5%) for all tests. Transformations used to meet assumptions of normality or homogeneity of variance are noted. NA = not applicable.

Sites / Years Tested	Test / Post Hoc Name	DF	Test statistic	p-value	Significant Post-Hoc Interaction	Post Hoc p-value	Transformations
2018 LHA/ LHB/LHC	One-way ANOVA / Tukey HSD	2, 15	0.77	0.482	NA	NA	NA
2019 LHA/ LHB/LHC	One-way ANOVA / Tukey HSD	2, 15	0.76	0.483	NA	NA	NA
2021 LHA/ LHB/LHC	One-way ANOVA / Tukey HSD	2, 15	6.06	0.012	LHA and LHB	0.010	NA
2022 LHA/ LHB/LHC	One-way ANOVA / Tukey HSD	2, 15	1.50	0.254	NA	NA	NA
WPH1 2018/2019/ 2021/2022	Repeated Measures ANOVA / Tukey HSD	3, 15	5.75	0.008	2019 and 2021	0.006	Not homogeneous (p=0.047), transformation did not help
WPH2 2018/2019/ 2021/2022	Friedman's Test / Nemenyi	3	13.4	0.004	2019 and 2022	0.002	NA
PS7 2018/2019/ 2021/2022	Repeated Measures ANOVA / Tukey HSD	3, 6	2.69	0.140	NA	NA	Only used day 1 data

Supplemental Table 7. Pond Number of Individuals Results Statistical results for the number of individuals caught within pond subhabitats per each site. The table includes the factor, levels, statistical tests (main and post hoc comparison tests where the main test resulted in a p<0.05) and associated values (degrees of freedom, test statistic, p-value). Alpha, or significance level, is set to 0.05 (5%) for all tests. Transformations used to meet assumptions of normality or homogeneity of variance are noted. NA = not applicable.

Sites / Years Tested	Test / Post Hoc Name	DF	Test statistic	p-value	Significant Post-Hoc Interaction	Post Hoc p-value	Transformations
2018 LHA/ LHB/LHC	One-way ANOVA / Tukey HSD	2, 15	21.31	<0.001	LHA and LHB	<0.001	NA
					LHB and LHC	<0.001	NA
2019 LHA/ LHB/LHC	One-way ANOVA / Tukey HSD	2, 15	3.29	0.065	NA	NA	NA
2021 LHA/ LHB/LHC	One-way ANOVA / Tukey HSD	2, 15	3.43	0.593	NA	NA	NA
2022 LHA/ LHB/LHC	One-way ANOVA / Tukey HSD	2, 15	9.67	0.002	NA	NA	log(x+1), did not meet homogeneity of variance (p=0.048)
WPH1 2018/2019/ 2021/2022	Repeated Measures ANOVA / Tukey HSD	3, 15	1.24	0.330	NA	NA	NA
WPH2 2018/2019/ 2021/2022	Repeated Measures ANOVA / Tukey HSD	3, 15	2.11	0.141	NA	NA	NA
PS7 2018/2019/ 2021/2022	Repeated Measures ANOVA / Tukey HSD	3, 6	2.69	0.139	NA	NA	Only used day 1 data

Supplemental Table 8. Creek Shannon Diversity Results Statistical results for the Shannon diversity within creek subhabitats per each site. The table includes the factor, levels, statistical tests (main and post hoc comparison tests where the main test resulted in a $p<0.05$) and associated values (degrees of freedom, test statistic, p-value). Alpha, or significance level, is set to 0.05 (5%) for all tests. Transformations used to meet assumptions of normality or homogeneity of variance are noted. NA = not applicable.

Sites / Years Tested	Test / Post Hoc Name	DF	Test statistic	p-value	Significant Post-Hoc Interaction	Post Hoc p-value	Transformations
2018 LHA/ LHB/LHC	One-way ANOVA / Tukey HSD	2, 15	3.11	0.074	NA	NA	NA
2019 LHA/ LHB/LHC	Kruskal-Wallis / Dunnett	2	11.81	0.003	LHA and LHB	0.007	NA
					LHA and LHC	0.007	NA
2021 LHA/ LHB/LHC	One-way ANOVA / Tukey HSD	2, 15	1.85	0.192	NA	NA	NA
2022 LHA/ LHB/LHC	One-way ANOVA / Tukey HSD	2, 15	2.56	0.110	NA	NA	NA
WPH1 2018/2019/ 2021/2022	Repeated Measures ANOVA / Tukey HSD	3, 15	0.67	0.584	NA	NA	NA
WPH2 2018/2019/ 2021/2022	Repeated Measures ANOVA / Tukey HSD	3, 15	1.38	0.287	NA	NA	NA
PS7 2018/2019/ 2021/2022	Repeated Measures ANOVA / Tukey HSD	3, 6	3.64	0.084	NA	NA	Only used day 1 data

Supplemental Table 9. Edge Shannon Diversity Results Statistical results for the Shannon diversity within edge subhabitats per each site. The table includes the factor, levels, statistical tests (main and post hoc comparison tests where the main test resulted in a p<0.05) and associated values (degrees of freedom, test statistic, p-value). Alpha, or significance level, is set to 0.05 (5%) for all tests. Transformations used to meet assumptions of normality or homogeneity of variance are noted. NA = not applicable.

Sites / Years Tested	Test / Post Hoc Name	DF	Test statistic	p-value	Significant Post-Hoc Interaction	Post Hoc p-value	Transformations
2018 LHA/ LHB/LHC	One-way ANOVA / Tukey HSD	2, 15	3.78	0.047	LHA and LHB	0.038	NA
2019 LHA/ LHB/LHC	One-way ANOVA / Tukey HSD	2, 15	16.03	<0.001	LHA and LHB	<0.001	NA
					LHB and LHC	<0.001	NA
2021 LHA/ LHB/LHC	One-way ANOVA / Tukey HSD	2, 15	6.62	0.009	LHA and LHB	0.009	NA
					LHA and LHC	0.040	NA
2022 LHA/ LHB/LHC	One-way ANOVA / Tukey HSD	2, 15	0.95	0.410	NA	NA	NA
WPH1 2018/2019/ 2021/2022	Repeated Measures ANOVA / Tukey HSD	3, 15	44.51	<0.001	2018 and 2021	<0.001	log(x+1)
					2018 and 2022	<0.001	log(x+1)
					2019 and 2021	<0.001	log(x+1)
					2019 and 2022	<0.001	log(x+1)
WPH2 2018/2019/ 2021/2022	Repeated Measures ANOVA / Tukey HSD	3, 15	1.56	0.241	NA	NA	NA
PS7 2018/2019/ 2021/2022	Repeated Measures ANOVA / Tukey HSD	3, 6	3.46	0.092	NA	NA	Only used day 1 data

Supplemental Table 10. Pond Shannon Diversity Results Statistical results for the Shannon diversity within pond subhabitats per each site. The table includes the factor, levels, statistical tests (main and post hoc comparison tests where the main test resulted in a $p<0.05$) and associated values (degrees of freedom, test statistic, p-value). Alpha, or significance level, is set to 0.05 (5%) for all tests. Transformations used to meet assumptions of normality or homogeneity of variance are noted. NA = not applicable.

Sites / Years Tested	Test / Post Hoc Name	DF	Test statistic	p-value	Significant Post-Hoc Interaction	Post Hoc p-value	Transformations
2018 LHA/ LHB/LHC	One-way ANOVA / Tukey HSD	2, 15	1.92	0.182	NA	NA	NA
2019 LHA/ LHB/LHC	One-way ANOVA / Tukey HSD	2, 15	10.00	0.002	LHA and LHB	0.016	log(x+1)
					LHB and LHC	0.002	log(x+1)
2021 LHA/ LHB/LHC	One-way ANOVA / Tukey HSD	2, 15	1.17	0.337	NA	NA	NA
2022 LHA/ LHB/LHC	One-way ANOVA / Tukey HSD	2, 15	7.72	0.005	LHA and LHC	0.004	NA
WPH1 2018/2019/ 2021/2022	Repeated Measures ANOVA / Tukey HSD	3, 15	8.53	0.005	2019 and 2022	0.003	NA
					2021 and 2022	0.004	NA
WPH2 2018/2019/ 2021/2022	Repeated Measures ANOVA / Tukey HSD	3, 15	4.50	0.019	2019 and 2021	0.023	NA
					2018 and 2019	0.046	NA
PS7 2018/2019/ 2021/2022	Repeated Measures ANOVA / Tukey HSD	3, 6	5.48	0.037	NA	NA	Only used day 1 data

Supplemental Table 11. Grass Shrimp Population Numbers Raw total counts of the number of grass shrimp caught within each site (LHA, LHB, LHC, WPH1, WPH2, PS7) and year (2018, 2019, 2021, 2022).

	2018	2019	2021	2022
LHA	67	136	715	87
LHB	19	136	479	87
LHC	73	149	602	188
WPH1	74	6	343	0
WPH2	33	8	178	41
PS7	276	15	25	576

Supplemental Table 12. Grass Shrimp CPUE Results Statistical results for the number of grass shrimp caught (CPUE) per each site. The table includes the factor, levels, statistical tests (main and post hoc comparison tests where the main test resulted in a p<0.05) and associated values (degrees of freedom, test statistic, p-value). Alpha, or significance level, is set to 0.05 (5%) for all tests. Transformations used to meet assumptions of normality or homogeneity of variance are noted. NA = not applicable.

Sites / Years Tested	Test / Post Hoc Name	DF	Test statistic	p-value	Significant Post-Hoc Interaction	Post Hoc p-value	Transformations
2018 LHA/ LHB/LHC	Kruskal-Wallis / Dunnett	2	0.61	0.738	NA	NA	NA
2019 LHA/ LHB/LHC	Kruskal-Wallis / Dunnett	2, 65	1.50	0.473	NA	NA	NA
2021 LHA/ LHB/LHC	Kruskal-Wallis / Dunnett	2	1.64	0.441	NA	NA	NA
2022 LHA/ LHB/LHC	Kruskal-Wallis / Dunnett	2	2.19	0.334	NA	NA	NA
WPH1 2018/2019/ 2021/2022	Kruskal-Wallis / Dunnett	3	15.26	0.002	2018 and 2022	0.028	NA
					2021 and 2022	0.002	NA
WPH2 2018/2019/ 2021/2022	Kruskal-Wallis / Dunnett	3	12.01	0.007	2019 and 2021	0.014	NA
					2018 and 2021	0.016	NA
PS7 2018/2019/ 2021/2022	Kruskal-Wallis / Dunnett	3	20.76	<0.001	2019 and 2022	<0.001	NA

Supplemental Table 13. Brown Shrimp Population Numbers Raw total counts of the number of brown shrimp caught within each site (LHA, LHB, LHC, WPH1, WPH2, PS7) and year (2018, 2019, 2021, 2022).

	2018	2019	2021	2022
LHA	9	9	24	98
LHB	9	60	50	39
LHC	9	12	65	92
WPH1	3	5	0	0
WPH2	7	21	48	45
PS7	17	28	8	23

Supplemental Table 14. Brown Shrimp CPUE Results Statistical results for the number of brown shrimp caught (CPUE) per each site. The table includes the factor, levels, statistical tests (main and post hoc comparison tests where the main test resulted in a p<0.05) and associated values (degrees of freedom, test statistic, p-value). Alpha, or significance level, is set to 0.05 (5%) for all tests. Transformations used to meet assumptions of normality or homogeneity of variance are noted. NA = not applicable.

Sites / Years Tested	Test / Post Hoc Name	DF	Test statistic	p-value	Significant Post-Hoc Interaction	Post Hoc p-value	Transformations
2018 LHA/ LHB/LHC	Kruskal-Wallis / Dunnett	2	0.81	0.669	NA	NA	NA
2019 LHA/ LHB/LHC	Kruskal-Wallis / Dunnett	2	5.31	0.070	NA	NA	NA
2021 LHA/ LHB/LHC	Kruskal-Wallis / Dunnett	2	1.30	0.523	NA	NA	NA
2022 LHA/ LHB/LHC	Kruskal-Wallis / Dunnett	2	0.42	0.811	NA	NA	NA
WPH1 2018/2019/ 2021/2022	Kruskal-Wallis / Dunnett	3	13.07	0.005	2019 and 2021	0.010	NA
					2019 and 2022	0.010	NA
WPH2 2018/2019/ 2021/2022	Kruskal-Wallis / Dunnett	3	7.40	0.060	NA	NA	NA
PS7 2018/2019/ 2021/2022	Kruskal-Wallis / Dunnett	3	0.28	0.964	NA	NA	NA

Supplemental Table 15. Gulf Killifish Population Numbers Raw total counts of the number of Gulf killifish caught within each site (LHA, LHB, LHC, WPH1, WPH2, PS7) and year (2018, 2019, 2021, 2022).

	2018	2019	2021	2022
LHA	123	210	21	20
LHB	404	674	320	63
LHC	70	243	145	15
WPH1	284	211	20	81
WPH2	132	232	74	62
PS7	110	60	54	42

Supplemental Table 16. Gulf Killifish Biomass Results Statistical results for Gulf killifish biomass (g) per each site. The table includes the factor, levels, statistical tests (main and post hoc comparison tests where the main test resulted in a p<0.05) and associated values (degrees of freedom, test statistic, p-value). Alpha, or significance level, is set to 0.05 (5%) for all tests. Transformations used to meet assumptions of normality or homogeneity of variance are noted. NA = not applicable.

Sites / Years Tested	Test / Post Hoc Name	DF	Test statistic	p-value	Significant Post-Hoc Interaction	Post Hoc p-value	Transformations
2018 LHA/ LHB/LHC	Kruskal-Wallis / Dunnett	2	50.50	<0.001	LHA and LHB	0.001	NA
					LHA and LHC	0.001	NA
					LHB and LHC	<0.001	NA
2019 LHA/ LHB/LHC	Kruskal-Wallis / Dunnett	2	15.97	0.000	LHA and LHB	0.000	NA
2021 LHA/ LHB/LHC	Kruskal-Wallis / Dunnett	2	3.93	0.140	NA	NA	NA
2022 LHA/ LHB/LHC	Kruskal-Wallis / Dunnett	2	6.44	0.040	LHB and LHC	0.025	NA
WPH1 2018/2019/ 2021/2022	Kruskal-Wallis / Dunnett	3	86.47	<0.001	2018 and 2019	<0.001	NA
					2018 and 2021	<0.001	NA
					2018 and 2022	<0.001	NA
					2019 and 2021	<0.001	NA
					2019 and 2022	<0.001	NA
WPH2 2018/2019/ 2021/2022	Kruskal-Wallis / Dunnett	3	134.82	<0.001	2018 and 2019	<0.001	NA
					2018 and 2021	<0.001	NA
					2019 and 2021	<0.001	NA
					2019 and 2022	<0.001	NA
					2021 and 2022	<0.001	NA

Supplemental Table 16. Gulf Killifish Biomass Results (continued)

PS7 2018/2019/ 2021/2022	Kruskal-Wallis / Dunnett	3	42.63	<0.001	2018 and 2019	<0.001	NA
					2018 and 2021	0.012	NA
					2019 and 2022	<0.001	NA

Supplemental Table 17. Diamond Killifish Population Numbers Raw total counts of the number of diamond killifish caught within each site (LHA, LHB, LHC, WPH1, WPH2, PS7) and year (2018, 2019, 2021, 2022).

	2018	2019	2021	2022
LHA	2	22	3	1
LHB	7	5	226	8
LHC	14	86	135	1
WPH1	1	30	4	16
WPH2	96	17	69	241
PS7	276	1	16	30

Supplemental Table 18. Diamond Killifish Length Results Statistical results for diamond killifish lengths (mm) per each site. The table includes the factor, levels, statistical tests (main and post hoc comparison tests where the main test resulted in a p<0.05) and associated values (degrees of freedom, test statistic, p-value). Alpha, or significance level, is set to 0.05 (5%) for all tests. Transformations used to meet assumptions of normality or homogeneity of variance are noted. NA = not applicable.

Sites/ Years Tested	Test / Post Hoc Name	DF	Test statistic	p-value	Significant Post-Hoc Interaction	Post Hoc p-value	Transformations
2018 LHA/ LHB/LHC	One-way ANOVA / Tukey HSD	2, 20	15.74	<0.001	LHA and LHC	0.015	NA
					LHB and LHC	<0.001	NA
2019 LHA/ LHB/LHC	One-way ANOVA / Tukey HSD	2, 109	10.40	<0.001	LHA and LHB	0.013	NA
					LHA and LHC	<0.001	NA
2021 LHA/ LHB/LHC	Kruskal-Wallis / Dunnett	2	14.66	<0.001	LHB and LHC	<0.001	NA
2022 LHA/ LHB/LHC	One-way ANOVA / Tukey HSD	2, 7	1.18	0.361	NA	NA	NA
WPH1 2018/2019/ 2021/2022	One-way ANOVA / Tukey HSD	3, 61	2.11	0.108	NA	NA	NA
WPH2 2018/2019/ 2021/2022	Kruskal-Wallis / Dunnett	3	42.70	<0.001	2018 and 2019	<0.001	NA
					2018 and 2021	0.030	NA
					2018 and 2022	<0.001	NA
					2019 and 2021	<0.001	NA
					2019 and 2022	0.002	NA
PS7 2018/2019/ 2021/2022	Kruskal-Wallis / Dunnett	3	8.38	0.039	2018 and 2022	0.021	NA

Supplemental Table 19. Diamond Killifish Biomass Results Statistical results for diamond killifish biomass (g) per each site. The table includes the factor, levels, statistical tests (main and post hoc comparison tests where the main test resulted in a p<0.05) and associated values (degrees of freedom, test statistic, p-value). Alpha, or significance level, is set to 0.05 (5%) for all tests. Transformations used to meet assumptions of normality or homogeneity of variance are noted. NA = not applicable.

Sites / Years Tested	Test / Post Hoc Name	DF	Test statistic	p-value	Significant Post-Hoc Interaction	Post Hoc p-value	Transformations
2018 LHA/ LHB/LHC	Kruskal-Wallis / Dunnett	2	5.44	0.066	NA	NA	NA
2019 LHA/ LHB/LHC	Kruskal-Wallis / Dunnett	2	18.26	<0.001	LHA and LHB	0.007	NA
					LHA and LHC	<0.001	NA
2021 LHA/ LHB/LHC	Kruskal-Wallis / Dunnett	2	11.33	0.003	LHB and LHC	0.025	NA
2022 LHA/ LHB/LHC	One-way ANOVA / Tukey HSD	2, 7	0.754	0.505	NA	NA	NA
WPH1 2018/2019/ 2021/2022	One-way ANOVA / Tukey HSD	3, 47	5.44	0.003	2019 and 2022	0.002	NA
WPH2 2018/2019/ 2021/2022	Kruskal-Wallis / Dunnett	3	40.86	<0.001	2018 and 2019	<0.001	NA
					2018 and 2021	0.049	NA
					2018 and 2022	<0.001	NA
					2019 and 2021	<0.001	NA
					2019 and 2022	<0.001	NA
PS7 2018/2019/ 2021/2022	Kruskal-Wallis / Dunnett	3	5.44	0.142	NA	NA	NA

Supplemental Table 20. Bayou Killifish Population Numbers Raw total counts of the number of bayou killifish caught within each site (LHA, LHB, LHC, WPH1, WPH2, PS7) and year (2018, 2019, 2021, 2022).

	2018	2019	2021	2022
LHA	2	2	2	0
LHB	2	25	24	0
LHC	8	32	5	1
WPH1	18	1	15	29
WPH2	211	14	187	208
PS7	32	0	21	4

Supplemental Table 21. Bayou Killifish Length Results Statistical results for bayou killifish length (mm) per each site. The table includes the factor, levels, statistical tests (main and post hoc comparison tests where the main test resulted in a p<0.05) and associated values (degrees of freedom, test statistic, p-value). Alpha, or significance level, is set to 0.05 (5%) for all tests. Transformations used to meet assumptions of normality or homogeneity of variance are noted. NA = not applicable.

Sites / Years Tested	Test / Post Hoc Name	DF	Test statistic	p-value	Significant Post-Hoc Interaction	Post Hoc p-value	Transformations
2018 LHA/ LHB/LHC	One-way ANOVA / Tukey HSD	2, 9	82.08	0.497	NA	NA	NA
2019 LHA/ LHB/LHC	One-way ANOVA / Tukey HSD	2, 56	0.85	0.431	NA	NA	log(x)
2021 LHA/ LHB/LHC	One-way ANOVA / Tukey HSD	2, 28	2.23	0.126	NA	NA	NA
2022 LHA/ LHB/LHC	Test not performed, only LHC data available	NA	NA	NA	NA	NA	NA
WPH1 2018/2019/ 2021/2022	Kruskal-Wallis / Dunnett	3	15.25	0.002	2018 and 2022	0.004	NA
WPH2 2018/2019/ 2021/2022	Kruskal-Wallis / Dunnett	3	31.60	<0.001	2018 and 2019	0.004	NA
					2018 and 2021	<0.001	NA
					2019 and 2021	0.005	NA
					2019 and 2022	<0.001	NA
PS7 2018/2019/ 2021/2022	One-way ANOVA / Tukey HSD	2, 54	1.88	0.163	NA	NA	log(x)

Supplemental Table 22. Bayou Killifish Biomass Results Statistical results for bayou killifish biomass (g) per each site. The table includes the factor, levels, statistical tests (main and post hoc comparison tests where the main test resulted in a $p<0.05$) and associated values (degrees of freedom, test statistic, p-value). Alpha, or significance level, is set to 0.05 (5%) for all tests. Transformations used to meet assumptions of normality or homogeneity of variance are noted. NA = not applicable.

Sites / Years Tested	Test / Post Hoc Name	DF	Test statistic	p-value	Significant Post-Hoc Interaction	Post Hoc p-value	Transformations
2018 LHA/ LHB/LHC	One-way ANOVA / Tukey HSD	2, 9	0.59	0.574	NA	NA	NA
2019 LHA/ LHB/LHC	One-way ANOVA / Tukey HSD	2, 56	0.51	0.602	NA	NA	log(x)
2021 LHA/ LHB/LHC	One-way ANOVA / Tukey HSD	2, 28	2.75	0.081	NA	NA	NA
2022 LHA/ LHB/LHC	Test not performed, only LHC data available	NA	NA	NA	NA	NA	NA
WPH1 2018/2019/ 2021/2022	One-way ANOVA / Tukey HSD	2, 58	8.29	<0.001	2018 and 2021	0.009	log(x), 2019 data taken out since only one point
					2018 and 2022	<0.001	log(x), 2019 data taken out since only one point
WPH2 2018/2019/ 2021/2022	Kruskal-Wallis / Dunnett	3	30.24	<0.001	2018 and 2019	<0.001	NA
					2018 and 2021	<0.001	NA
					2019 and 2022	<0.001	NA
PS7 2018/2019/ 2021/2022	One-way ANOVA / Tukey HSD	2, 54	3.83	0.028	2018 and 2021	0.030	NA

Supplemental Table 23. Sheepshead Minnow Population Numbers Raw total counts of the number of sheepshead minnow caught within each site (LHA, LHB, LHC, WPH1, WPH2, PS7) and year (2018, 2019, 2021, 2022).

	2018	2019	2021	2022
LHA	14	66	34	0
LHB	0	4	10	0
LHC	1	4	12	8
WPH1	2	0	0	13
WPH2	12	13	2	3
PS7	0	2	0	0

Supplemental Table 24. Sheepshead Minnow Length Results Statistical results for sheepshead minnow length (mm) per each site. The table includes the factor, levels, statistical tests (main and post hoc comparison tests where the main test resulted in a p<0.05) and associated values (degrees of freedom, test statistic, p-value). Alpha, or significance level, is set to 0.05 (5%) for all tests. Transformations used to meet assumptions of normality or homogeneity of variance are noted. NA = not applicable.

Sites / Years Tested	Test / Post Hoc Name	DF	Test statistic	p-value	Significant Post-Hoc Interaction	Post Hoc p-value	Transformations
2018 LHA/ LHB/LHC	Kruskal-Wallis / Dunnett	1	0.06	0.813	NA	NA	NA
2019 LHA/ LHB/LHC	One-way ANOVA / Tukey HSD	2, 71	1.65	0.199	NA	NA	NA
2021 LHA/ LHB/LHC	Kruskal-Wallis / Dunnett	2	16.01	<0.001	LHA and LHB	0.002	NA
					LHB and LHC	<0.001	NA
2022 LHA/ LHB/LHC	Test not performed, onl.y LHC data available	NA	NA	NA	NA	NA	NA
WPH1 2018/2019/ 2021/2022	Kruskal-Wallis / Dunnett	1	0.126	0.723	NA	NA	NA
WPH2 2018/2019/ 2021/2022	One-way ANOVA / Tukey HSD	3, 26	1.05	0.388	NA	NA	NA
PS7 2018/2019/ 2021/2022	Test not performed, only 2019 data available	NA	NA	NA	NA	NA	NA
WPH1/ WPH2/PS7 2018/2019/ 2021/2022	Two-way ANOVA / Tukey HSD	1, 36	0.38	0.540	NA	NA	NA

Supplemental Table 25. Sheepshead Minnow Biomass Results Statistical results for sheepshead minnow biomass (g) per each site. The table includes the factor, levels, statistical tests (main and post hoc comparison tests where the main test resulted in a p<0.05) and associated values (degrees of freedom, test statistic, p-value). Alpha, or significance level, is set to 0.05 (5%) for all tests. Transformations used to meet assumptions of normality or homogeneity of variance are noted. NA = not applicable.

Sites / Years Tested	Test / Post Hoc Name	DF	Test statistic	p-value	Significant Post-Hoc Interaction	Post Hoc p-value	Transformations
2018 LHA/ LHB/LHC	One-way ANOVA / Tukey HSD	1, 13	0.06	0.816	NA	NA	NA
2019 LHA/ LHB/LHC	Kruskal-Wallis / Dunnett	2	6.98	0.030	LHA and LHC	0.045	NA
2021 LHA/ LHB/LHC	Kruskal-Wallis / Dunnett	2	17.72	<0.001	LHA and LHB	NA	NA
					LHB and LHC	<0.001	NA
2022 LHA/ LHB/LHC	Test not performed, onl.y LHC data available	NA	NA	NA	NA	NA	NA
WPH1 2018/2019/ 2021/2022	One-way ANOVA / Tukey HSD	1, 8	0.57	0.472	NA	NA	NA
WPH2 2018/2019/ 2021/2022	One-way ANOVA / Tukey HSD	3, 23	0.57	0.151	NA	NA	NA
PS7 2018/2019/ 2021/2022	Test not performed, only 2019 data available	NA	NA	NA	NA	NA	NA
WPH1/ WPH2/PS7 2018/2019/ 2021/2022	Two-way ANOVA / Tukey HSD	1, 32	0.09	0.771	NA	NA	NA

Supplemental Table 26. Rainwater Killifish Population Numbers Raw total counts of the number of rainwater killifish caught within each site (LHA, LHB, LHC, WPH1, WPH2, PS7) and year (2018, 2019, 2021, 2022).

	2018	2019	2021	2022
LHA	4	5	0	11
LHB	0	20	0	1
LHC	3	31	1	1
WPH1	3	4	0	2
WPH2	35	0	0	1
PS7	0	0	0	0

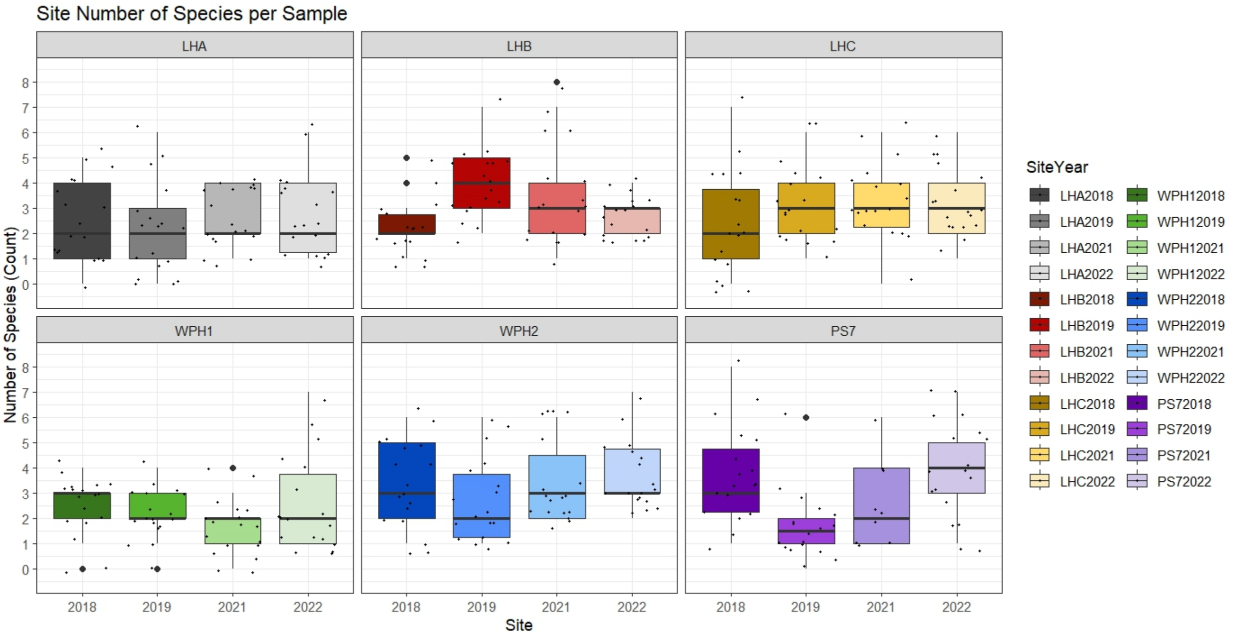
Supplemental Table 27. Rainwater Killifish Length Results Statistical results for rainwater killifish length (mm) per each site. The table includes the factor, levels, statistical tests (main and post hoc comparison tests where the main test resulted in a p<0.05) and associated values (degrees of freedom, test statistic, p-value). Alpha, or significance level, is set to 0.05 (5%) for all tests. Transformations used to meet assumptions of normality or homogeneity of variance are noted. NA = not applicable.

Sites / Years Tested	Test / Post Hoc Name	DF	Test statistic	p-value	Significant Post-Hoc Interaction	Post Hoc p-value	Transformations
2018 LHA/ LHB/LHC	One-way ANOVA / Tukey HSD	1, 8	0.874	0.377	NA	NA	NA
2019 LHA/ LHB/LHC	Kruskal-Wallis / Dunnett	2	6.71	0.035	LHA and LHC	0.028	NA
2021 LHA/ LHB/LHC	Test not performed, onl.y LHC data available	NA	NA	NA	NA	NA	NA
2022 LHA/ LHB/LHC	One-way ANOVA / Tukey HSD	2, 10	1.60	0.249	NA	NA	NA
WPH1 2018/2019/ 2021/2022	One-way ANOVA / Tukey HSD	2, 6	1.15	0.379	NA	NA	NA
WPH2 2018/2019/ 2021/2022	Kruskal-Wallis / Dunnett	1	2.72	0.099	NA	NA	NA
PS7 2018/2019/ 2021/2022	Test not performed, no data available	NA	NA	NA	NA	NA	NA

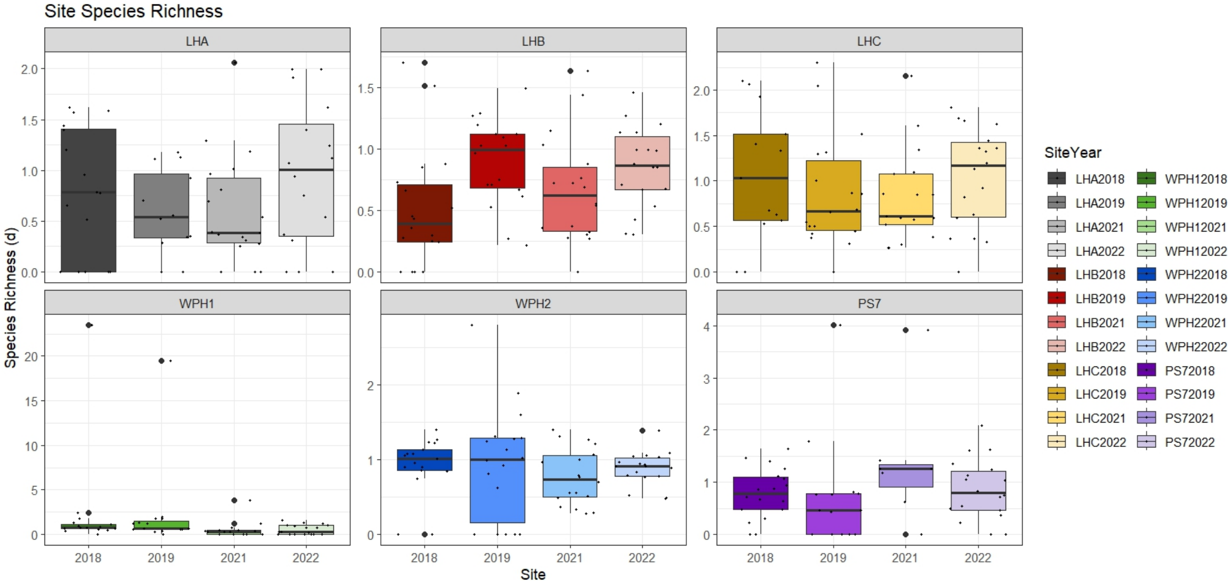
Supplemental Table 28. Rainwater Killifish Biomass Results Statistical results for rainwater killifish biomass (g) per each site. The table includes the factor, levels, statistical tests (main and post hoc comparison tests where the main test resulted in a p<0.05) and associated values (degrees of freedom, test statistic, p-value). Alpha, or significance level, is set to 0.05 (5%) for all tests. Transformations used to meet assumptions of normality or homogeneity of variance are noted. NA = not applicable.

Sites / Years Tested	Test / Post Hoc Name	DF	Test statistic	p-value	Significant Post-Hoc Interaction	Post Hoc p-value	Transformations
2018 LHA/ LHB/LHC	One-way ANOVA / Tukey HSD	1, 8	0.872	0.391	NA	NA	NA
2019 LHA/ LHB/LHC	Kruskal-Wallis / Dunnett	2	16.39	<0.001	LHA and LHC	0.007	NA
					LHB and LHC	<0.001	NA
2021 LHA/ LHB/LHC	Test not performed, only LHC data available	NA	NA	NA	NA	NA	NA
2022 LHA/ LHB/LHC	One-way ANOVA / Tukey HSD	2, 10	0.95	0.418	NA	NA	NA
WPH1 2018/2019/ 2021/2022	Kruskal-Wallis / Dunnett	1	0.06	0.812	NA	NA	NA
WPH2 2018/2019/ 2021/2022	One-way ANOVA / Tukey HSD	1, 4	1.83	0.248	NA	NA	log(x)
PS7 2018/2019/ 2021/2022	Test not performed, data not available	NA	NA	NA	NA	NA	NA

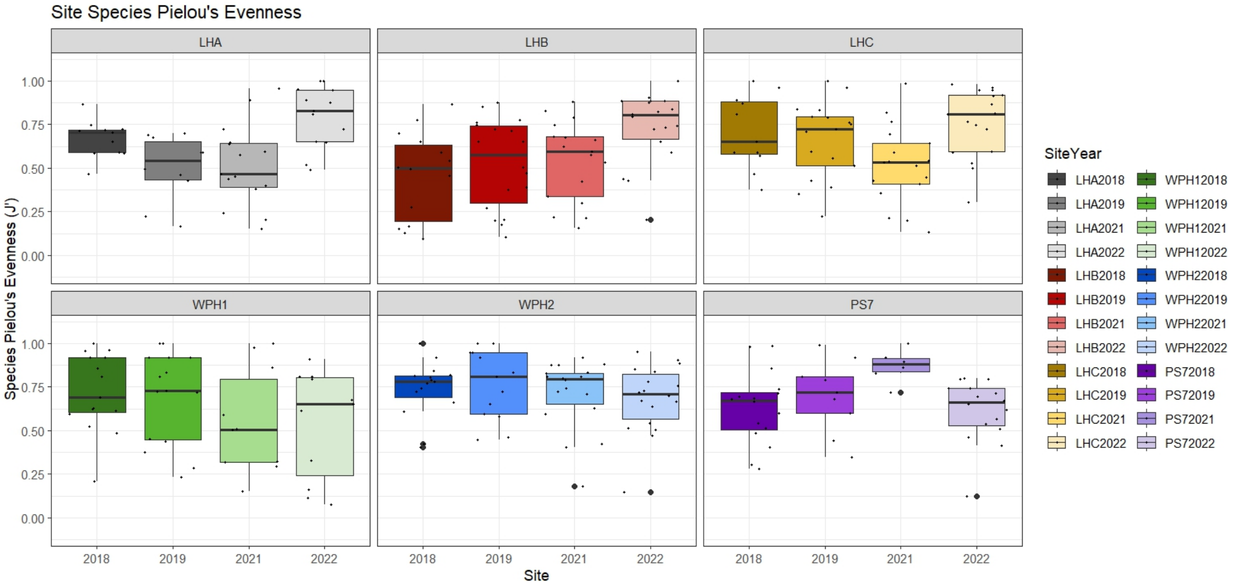
<u>Supplemental Figures</u>



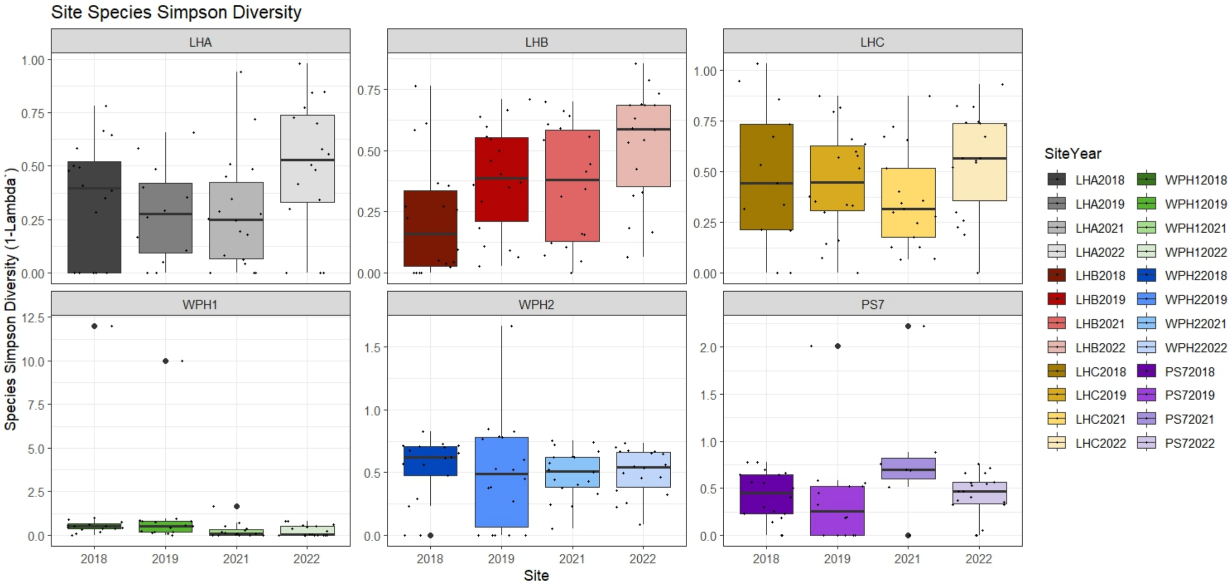
Supplemental Figure 1. Site Number of Species per Sample Box plots representing the number of on-marsh nekton species caught per sample at six sites and four years (2018, 2019, 2021, 2022). Individual data points (n=18 per site per year except 2021 PS7 in which n=9) for each sample are represented for each plot as well.



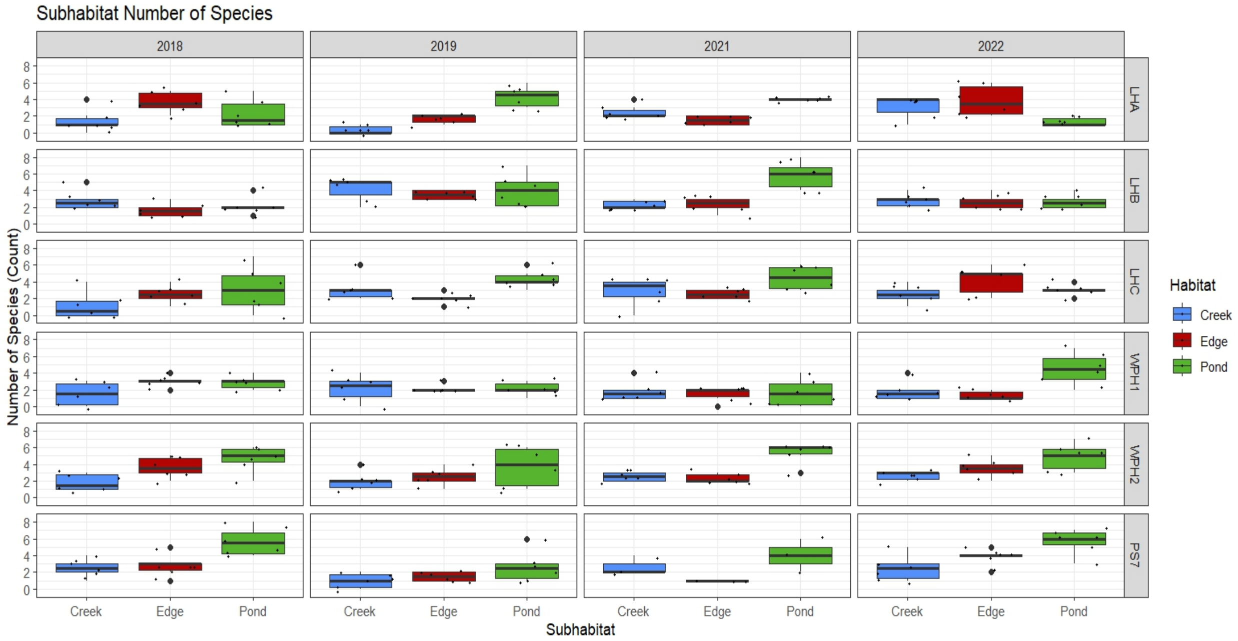
Supplemental Figure 2. Site Species Richness Box plots representing the on-marsh nekton species richness per sample at six sites and four years (2018, 2019, 2021, 2022). Catch data was transformed into catch per unit effort (CPUE) representing the number of individuals caught in three traps over a one hour period. Individual data points (n=18 per site per year except 2021 PS7 in which n=9) for each sample are represented for each plot as well. Y-axes for each site are unique to accommodate for data ranges.



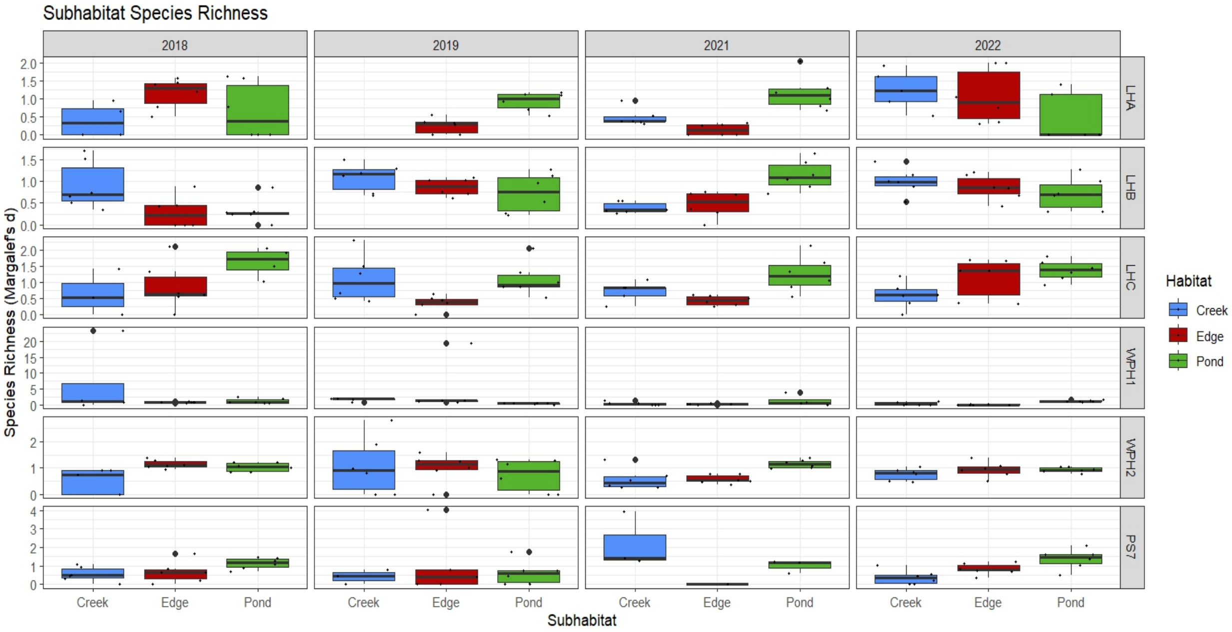
Supplemental Figure 3. Site Species Pielou's Evenness Box plots representing Pielou's Evenness of on-marsh nekton species caught per sample at six sites and four years (2018, 2019, 2021, 2022). Catch data was transformed into catch per unit effort (CPUE) representing the number of individuals caught in three traps over a one hour period. Individual data points (n=18 per site per year except 2021 PS7 in which n=9) for each sample are represented for each plot as well.



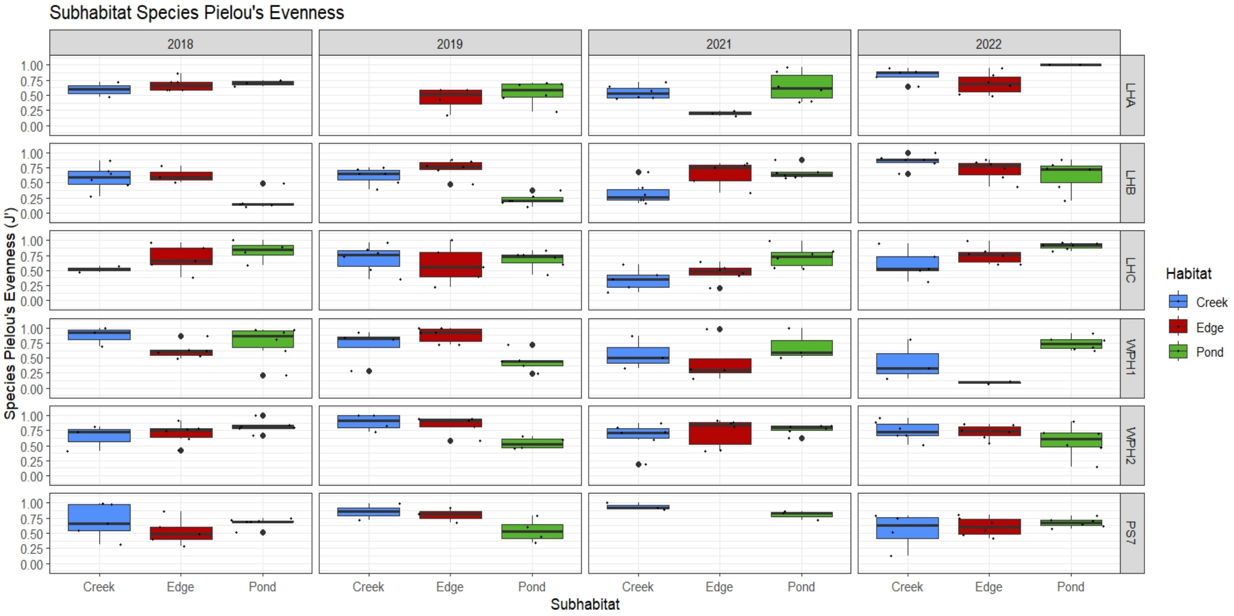
Supplemental Figure 4. Site Species Simpson Diversity Box plots representing the Simpson Diversity of on-marsh nekton species caught per sample at six sites and four years (2018, 2019, 2021, 2022). Catch data was transformed into catch per unit effort (CPUE) representing the number of individuals caught in three traps over a one hour period. Individual data points (n=18 per site per year except 2021 PS7 in which n=9) for each sample are represented for each plot as well. Y-axes for each site are unique to accommodate for data ranges.



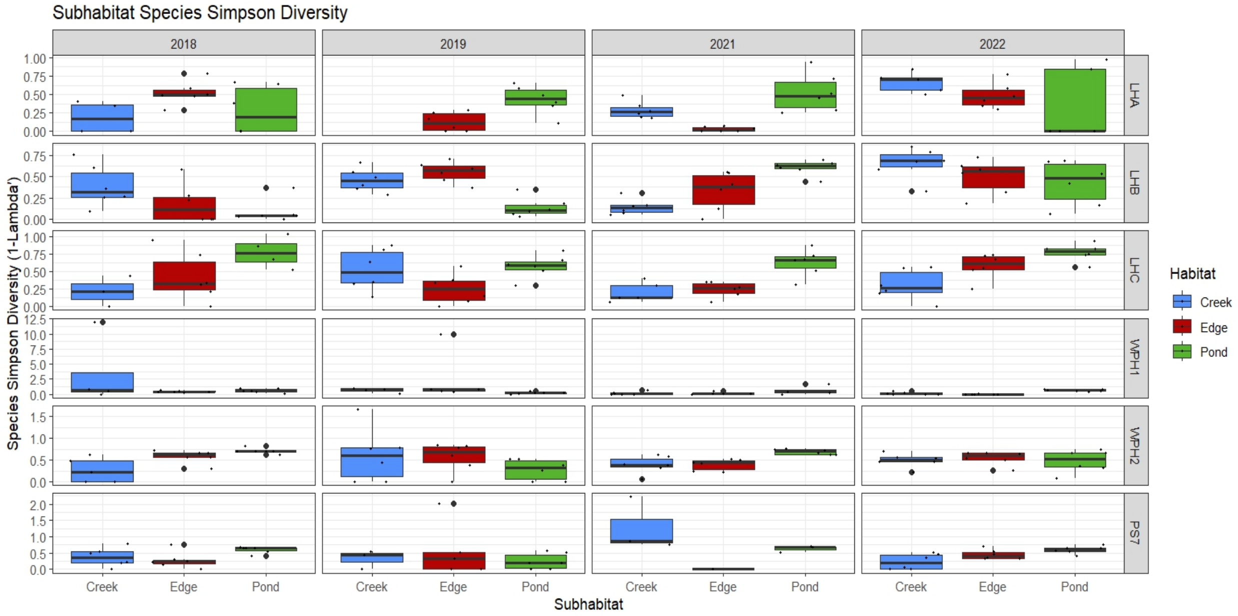
Supplemental Figure 5. Subhabitat Number of Species Box plots representing the number of on-marsh nekton species caught per sample within subhabitats at six sites and four years (2018, 2019, 2021, 2022). Individual data points (n=6 per subhabitat per site per year except 2021 PS7 in which n=3) for each sample are represented for each plot as well.



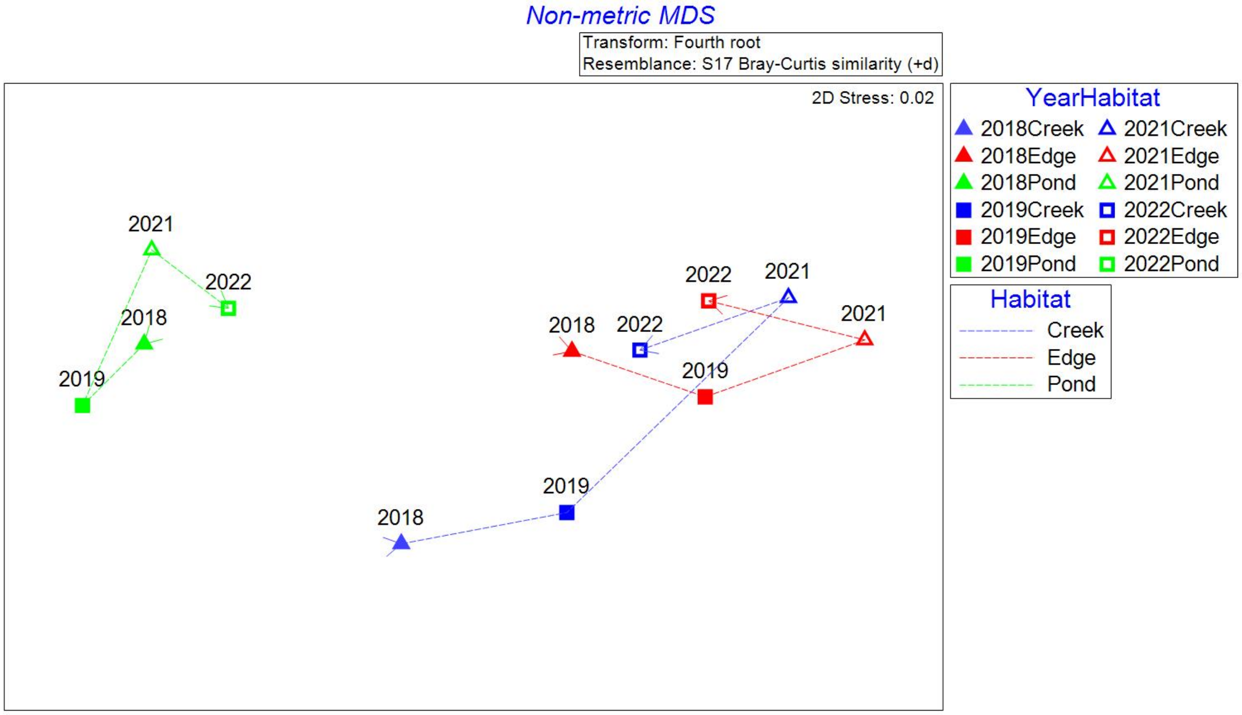
Supplemental Figure 6. Subhabitat Species Richness Box plots representing the on-marsh nekton species richness per sample within subhabitats at six sites and four years (2018, 2019, 2021, 2022). Catch data was transformed into catch per unit effort (CPUE) representing the number of individuals caught in three traps over a one hour period. Individual data points (n=6 per subhabitat per site per year except 2021 PS7 in which n=3) for each sample are represented for each plot as well. Y-axes for each site are unique to accommodate for data ranges.



Supplemental Figure 7. Subhabitat Species Pielou's Evenness Box plots representing the Pielou's evenness of on-marsh nekton per sample within subhabitats at six sites and four years (2018, 2019, 2021, 2022). Catch data was transformed into catch per unit effort (CPUE) representing the number of individuals caught in three traps over a one hour period. Individual data points (n=6 per subhabitat per site per year except 2021 PS7 in which n=3) for each sample are represented for each plot as well.



Supplemental Figure 8. Subhabitat Species Simpson Diversity Box plots representing the Simpson Diversity of on-marsh nekton per sample within subhabitats at six sites and four years (2018, 2019, 2021, 2022). Catch data was transformed into catch per unit effort (CPUE) representing the number of individuals caught in three traps over a one hour period. Individual data points (n=6 per subhabitat per site per year except 2021 PS7 in which n=3) for each sample are represented for each plot as well. Y-axes for each site are unique to accommodate for data ranges.



Supplemental Figure 9. On-Marsh Nekton Subhabitat Trajectory nMDS Non-metric multidimensional scaling (nMDS) plot of nekton community composition trajectories displaying shifts between subhabitats over time. Data was standardized to CPUE, fourth root transformed, and Bray-Curtis calculations including a dummy value were used to assemble a resemblance matrix for figure creation.