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Evaluating Marsh Restoration Success Using Structural and Trophic Metrics on Deer Island, MS

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EVALUATING MARSH RESTORATION SUCCESS USING STRUCTURAL AND TROPHIC METRICS ON DEER ISLAND, MS

by

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ABSTRACT

Coastal marshes in the northern Gulf of Mexico provide essential habitat for various consumer species, however, land loss has severely degraded marsh habitat in this region. Few studies have examined restored black needlerush (*Juncus roemerianus*) marshes, such as those found in Mississippi (MS), and how they affect faunal inhabitants.

Restoration of *Juncus*-dominated marshes on Deer Island, MS sought to reestablish ecological functions with the intention of supporting natural consumer assemblages. To test this, quadrat and minnow trap sampling were used to compare invertebrate and nekton abundance, species richness, and diversity of two restored marshes (5+ yrs and 15+ yrs) with a natural reference marsh (100+ yrs old). Stable isotope analysis was also used to compare basal carbon sources and trophic support between sites. Quadrat sampling showed invertebrate abundance did not reach natural levels in either restored site, but minnow trap sampling showed abundance and species richness at the younger 5+ yr site surpassed that of the natural marsh. A comparison of community assemblage and stable isotope analysis showed similarity between the 5+ yr restored site and the natural site. The 5+ yr site better resembled a natural marsh than the 15+ yr site in many ways, suggesting that certain ecological processes are recovering faster in the younger site.

Our assessment of consumer community structure, combined with previous studies evaluating environmental and vegetative characteristics provide a thorough assessment of restoration efforts on Deer Island, MS. It also gives insight into future Beneficial Use restoration projects on *Juncus*-dominated marshes in this area.

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DEDICATION

I would like to thank my friends and family for providing emotional support and encouragement throughout my project. This work is dedicated to my parents.

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CHAPTER I - INTRODUCTION

1.1 Importance of Wetland Habitat.

Coastal wetlands are vital ecosystems for many species and provide ecologically and economically important services including water quality regulation, carbon sequestration, commercial and recreational fisheries support, and storm surge protection (Mitsch et al. 2009; Barbier et al. 2011; Engle 2011a; Hollweg et al. 2019). In the Gulf of Mexico, marshes are characterized by extremely high primary productivity which provides essential habitat and trophic support for consumers at varying life stages (Craft 2001a; Barbier et al. 2011; Engle 2011b; Pritzker et al. 2015). In 2018, commercial fisheries in the Gulf of Mexico landed over 690,000 tons valued at $887 million (Hollweg et al. 2019; NMFS 2019). Around 95% (by weight) of these species are estuarine-dependent at some point in their life, with many of these species relying on coastal wetlands as nursery habitat (Lellis-Dibble et al. 2008).

Dense vegetation cover allows consumers to occupy almost all spaces within the marsh based on the consumers' life history (Craft and Sacco 2003). The marsh edge is used by both resident and transient species, while the interior is primarily used by marsh residents who complete their entire life cycle within the shallow estuary (Thompson and Forman 1987; Peterson and Turner 1994b; Kneib 2003). Residents utilize the entire marsh including the interior ponds and channels at high tide and have adapted to surviving the adverse effects of low tide such as extreme heat and low dissolved oxygen (Peterson and Turner 1994a). Some common marsh residents found throughout the northern Gulf of Mexico are periwinkles (*Littorina irrorata*), killifish (*Fundulus spp.*), sheepshead minnow (*Cyprinodon variegatus*), grass shrimp (*Palaemonetes spp.*), and

fiddler crabs (*Uca spp.*)(Kneib 2003). Transient consumers are found primarily along the marsh edge with high connectivity to the open water (Peterson and Turner 1994b; Kneib 2003). A few common Gulf of Mexico species include mullet (*Mugil spp.*), red drum (*Sciaenops ocellatus*), and penaeid shrimp (*Penaeus aztecus* and *P. setiferus*) who use the edge as juveniles before returning to open water to spawn (Peterson and Turner 1994a). Blue crabs (*Callinectes sapidus*), another transient marsh species, also spawn out in open waters but return to the marsh throughout their life to feed and mate (Shakeri et al. 2020). Marshes have been estimated to support 66% of penaeid shrimp and 25% of blue crab production in the Gulf of Mexico (Zimmerman et al. 2002). In fact, the presence of marsh vegetation, regardless of species, supports significantly more consumers than non-vegetated areas (Shakeri et al. 2020). A study conducted in Grand Bay National Estuarine Research Reserve (GBNERR) in Mississippi found that grass shrimp (*Palaemonetes pugio)* and blue crabs were significantly more abundant in vegetated marsh edge than non-vegetation bottom (NVB) likely due to lower predation rates in marsh grass structure compared to non-vegetated habitats (Shervette et al. 2011).

1.2 Wetland Loss and Restoration in the Gulf of Mexico.

For decades, wetland loss in the Gulf of Mexico has been accelerated by stressors such as coastal development, hydrologic modifications, natural disasters, and limited estuarine marsh retreat options in the face of sea level rise (Turner 1990; EPA Report 2015; EPA Report 2017). Most notably, these stressors have led to physical degradation of vegetative communities across the northern Gulf of Mexico, thus increasing marsh fragmentation and altering how organisms utilize the marsh (Shakeri et al. 2020). In the contiguous U.S., from 2004 to 2009, the Gulf of Mexico lost more vegetated estuarine

wetlands (95,300 acres) than any other region (Dahl and Stedman 2013; Hollweg et al. 2019). This accounted for 99% of the total saltwater wetland loss to open water habitat in the contiguous U.S. (Dahl and Stedman 2013). As certain habitat niches are reduced, the degree to which marsh inhabitants are affected may vary depending on their life history. An increase in marsh edge could positively affect species that use the edge for nursery habitats (Minello et al. 1994; Zimmerman et al. 2002; La Peyre et al. 2007), negatively affect species that rely on the marsh interior for building nests above high tide (Rozas et al. 2007), or have relatively minimal effects on species that are able to occupy other structured habitats such as submerged aquatic vegetation (Shakeri et al. 2020).

In response, wetland restoration efforts have been widespread throughout the Gulf of Mexico and have primarily focused on restoring sediment and tidal hydrology to promote rapid succession of marsh vegetation (Mendelssohn et al., 2017). According to the U.S. Fish and Wildlife Services, approximately $1.74 billion has been allocated towards restoring wetlands, coastal, and nearshore habitat in the Gulf of Mexico since the Deepwater Horizon oil spill in 2010. Many restoration projects involve the creation of new marshes through dike breaching, river diversions, marsh terraces, and the beneficial use of dredged material (Brasher 2015; Herbert et al. 2015; Martin et al. 2019). Beneficial use (BU) is a restoration technique that involves relocating dredged sediment from shipping channels, harbors, and other construction sites to restore marsh habitat at locations within the same estuary systems, with the intention that local sediment and nutrients will encourage natural succession. Some studies, however, have found that dredged material lacks essential organic content needed for the recolonization of plants due to low proportions of fine material such as silt and clay (Fearnley 2008).

Since the 1950s, coastal Mississippi has lost approximately 9,000 acres of salt marsh and habitat loss is predicted to increase (Romero-Lankao et al. 2014; EPA 2015). Marshes dominated by smooth cordgrass (*Spartina alterniflora*) are extremely widespread throughout the U.S. Atlantic coast and Gulf of Mexico, and their restoration success has been extremely well studied (Moy and Levin 1991b; Levin et al. 1996; Craft et al. 1999; Craft and Sacco 2003; Wozniak et al. 2006; Craft et al. 2007; Snedden et al. 2015). Fewer studies have been conducted on the marshes of the northcentral Gulf of Mexico which are largely dominated by black needlerush (*Juncus roemerianus*) to understand how they will respond to impending habitat loss and restoration efforts (LaSalle 1996; Sparks et al. 2013). The majority of Mississippi, Florida, and Alabama marshes consist of *J. roemerianus* with average percent composition being 91%, 59% and 52% respectively (Eleuterius 1976). A small scale restoration project in Grand Bay National Estuarine Research Reserve in Mississippi found that re-planted *J. roemerianus* sods grew to natural levels in terms of above and belowground biomass within 3 years (Sparks et al. 2013). However, when conducted on a larger scale in Pascagoula, MS, *J. roemerianus* belowground biomass did not recover in an 8-year-old restored marsh (LaSalle 1996). Success of *J. roemerianus* recolonization may depend on planting material, and should be monitored for a sufficient amount of time to allow for succession (Murphy 2020). It is imperative that more long-term, large scale monitoring programs be conducted to understand how *Juncus*-dominated marshes will respond to habitat changes.

1.3 Evaluating Restoration Success: Landscape and Community Structure.

Restoration goals typically involve establishing natural levels of various ecosystem attributes such as elevation (Snedden et al. 2015), landscape (Kneib 2003),

hydrology (Minello et al. 1994), soil nutrients (Craft 2001b; Fearnley 2008), vegetation succession (Craft et al. 2007), consumer composition (Levin et al. 1996; Craft and Sacco 2003; La Peyre et al. 2007; Llewellyn and La Peyre 2011; Baumann et al. 2018) and ecosystem services (Moy and Levin 1991b; Engle 2011a; Staszak and Armitage 2013). It is thought that reestablishing these structural and trophic metrics in a created marsh will restore ecological processes to natural levels, reaching functional equivalence with natural marshes.

Elevation is a key landscape characteristic that establishes zonation in the marsh and controls the range of plant and animal species through abiotic and biotic stressors (Eleuterius 1972; Snedden et al. 2015). Pennings et al. (2005) found that in a Georgia salt marsh, the lower limit of *J. roemerianus'* range was controlled by physical stress such as flooding and salinity likely due to waterlogged soil increasing hypoxic conditions and sulfide toxicity (Pennings et al. 2005). This suggests that planting *J. roemerianus* in low elevations where flooding is more frequent could cause it to fail in restored sites. Sediment grain size can also influence plant succession as it is known to correlate with soil organic content (SOC) (Moy and Levin 1991a). Areas with large grain size (e.g., sand) are porous, resulting in higher drainage, higher oxygen, and lower levels of SOC whereas areas with small grain size (e.g., silt and clay) have poor drainage, lower oxygen, and thus higher retention of SOC (Bradley and Morris 1990). Developing the soil carbon and nitrogen pool is also crucial to support plant growth and recolonization of benthic consumers in created *S. alterniflora* marshes (Moy and Levin 1991a; Craft 2001a; Craft and Sacco 2003). Moy and Levin (1991a) showed low recolonization of subsurface-deposit feeding oligochaetes in created marshes (1–3 years old) where they would

normally be abundant in natural marshes, likely due to lower organic matter in the created marsh soil. Additionally, they observed lower rates of predation within the restored marshes, suggesting that the created marshes did not support secondary consumers (Moy and Levin 1991a). Elevation and SOC levels may be good metrics to assess structural equivalence in restored marshes, however, it may take 20+ years to reach natural levels and has been known to vary by geographic region (Moy and Levin 1991b; Craft et al. 1999; Craft 2001a)

The vegetation community, on the other hand, typically reestablishes in restored marshes relatively rapidly. Studies on *Spartina*-dominated marshes show that aboveground biomass may reach natural levels within 2-3 years, which provides numerous essential functions for consumers, such as refuge and feeding grounds (Boesch and Turner 1984; Craft et al. 1999; L.P. Rozas et al. 2007; Staszak and Armitage 2013). Belowground biomass, however, typically takes longer to establish in restored marshes, which is concerning because it is strongly correlated to marsh accretion and accumulation of organic matter (Craft et al. 1999; Staszak and Armitage 2013). For this reason, belowground biomass may be the best indicator of long term health in a created marsh (Turner et al. 2004). Restored *J. roemerianus* marshes in Mississippi have been known to reach aboveground biomass comparable to natural levels within 2-3 years, but fewer studies have been conducted on belowground biomass (Sparks et al. 2013). Hunter et al. (2015) found that *J. roemerianus* undergoes lower leaf herbivory and slower decomposition compared to *S. alterniflora* and, therefore, contributes most of its biomass to the detrital food source and organic matter pool (Staszak and Armitage 2013; Hübner et al. 2015; Hunter et al. 2015). This suggests that *Juncus*-dominated marshes could

develop belowground C reservoirs relatively quickly and contribute to faster marsh accretion (Hunter et al. 2015). The importance of a *Juncus* carbon pool has been shown in an oyster restoration study in Mississippi's Grand Bay National Estuarine Research Reserve (GBNEER) where constructed reefs of eastern oysters (*Crassostera virginia*) reached natural levels of oyster density and consumer trophic support within 2 years of construction. Stable isotopes of the oysters showed that *Juncus*-derived carbon in the form of detritus was the primary food source for the functionally equivalent reefs (Dillon et al. 2015). Monitoring the health of aboveground and belowground vegetation biomass, along with their associated organic matter pools, is essential to tracking functional equivalence in restored sites.

Consumers reestablish in constructed marshes at different rates depending on soil characteristics (e.g., organic content) and succession of the vegetation community (Moy and Levin 1991b; Craft and Sacco 2003). Therefore, consumer biomass, density and diversity can be used to reflect the succession of essential resources and the habitat's overall suitability (Minello and Webb 1997; La Peyre et al. 2007; Baumann et al. 2018). A long term restoration study conducted in North Carolina looked at the establishment of benthic infauna on constructed *S. alterniflora* marshes and found that surface feeding deposit feeders achieved natural levels within ~8 years while subsurface deposit feeders took ~25 years (Craft and Sacco 2003), highlighting the importance of aboveground vegetation and belowground soil characteristics in community composition. The colonization of higher trophic marsh consumers, like mummichogs and blue crabs, are affected by the succession of prey benthic infauna (Moy and Levin 1991b; Llewellyn and La Peyre 2011). Another study used periwinkle and amphipod density as an indicator of

constructed *S. alterniflora* marsh health because they facilitate ecological processes such as nutrient cycling by grazing algae and fungi from live *S. alterniflora* biomass (Kneib 2003; Baumann et al. 2018). By doing so, they allow nekton predators to more effectively capture marsh production before it is decomposed by microbes (Kneib 2003). In a meta-analysis of restored *Spartina*-dominated marshes throughout the northern Gulf of Mexico, they found that epifaunal periwinkle density recovered in ~ 4-6 years while infaunal amphipod density developed much more slowly and reflected the slow recovery of soil metrics and nutrient pools (Baumann et al. 2018). Other tools that can be used to evaluate a marsh restoration project are models such as a resource equivalency analysis (REA) and Ecosystem Index score (%) which can quantify species benefits and relate environmental variables to species occurrence (Hirzel and Le Lay 2008; Staszak and Armitage 2013; Baumann et al. 2018). Common marsh-surface invertebrates and nekton species serve as an effective measurement of marsh restoration because they reflect the succession of key ecosystem functions including nutrient cycling and vegetation success. They also serve as the link between basal resources and higher trophic commercial species (Silliman and Bertness 2002; Wozniak et al. 2006; Olin et al. 2017; Baumann et al. 2018).

As a newly created marsh develops, it is expected to slowly undergo succession and community stabilization until it reaches ecological and functional equivalence with its natural counterparts (Llewellyn and La Peyre 2011)(Table 1.1). It is important to consider all facets of ecosystem attributes when evaluating a restored site as they recover to natural levels at different rates (Craft et al. 1999; Strange et al. 2002). Restoration

trajectories may also vary by region and community compositions, which can restrict their applicability to other studies (Hollweg et al. 2019).

Table 1.1 Trajectory of restoration success in Spartina-dominated marshes.

Marsh Age	Location of Marsh	Description
~0-5	SW Louisiana; North Carolina	Natural hydrology, sediment nutrients and OM, and vegetation not equivalent; trophic support not equivalent (Moy and Levin 1991a; Wozniak et al. 2006; Llewellyn and La Peyre 2011)
~4–6	Northern GOM	Periwinkles may take between 4 and 6 year to establish natural biomass and density in constructed *Spartina* marshes
~8	North Carolina; SW Louisiana	Infauna density and species richness equivalent, abundance of surface feeding deposit feeders equivalent (Craft & Sacco, 2003); Soil OM not equivalent (Llewellyn and La Peyre 2011)
~5–10	North Carolina	Aboveground vegetation colonizes to natural levels (Craft et al., 1999); Soil OM not equivalent (Llewellyn and La Peyre 2011)
~10–15	North Carolina	Macro-organic matter (MOM) equivalent (Craft & Sacco, 2003)
10+	Galveston Bay, Texas	Belowground biomass reaches natural levels (Staszak and Armitage 2013)
~15–25	North Carolina	Benthic infauna density and species richness greater than natural levels (Craft et al., 1999)
~20–25	North Carolina	Soil OM not equivalent (Craft et al., 1999) Near surface sediment C and N levels equivalent (Craft, 2000).
25+	North Carolina	Abundance of subsurface feeding deposit feeders nearing equivalence (Craft & Sacco, 2003)
42	Sapelo Island, Georgia	Biogeochemical properties, such as C, N, C:N ratio, and N:P, equivalent to natural marshes (Craft, 2001)

Benchmarks for soil characteristics, landscape, hydrology, vegetation, and consumer communities based on restored Spartina-dominated marsh studies. Restoration trajectories are known to vary by region and baseline resources.

1.4 Evaluating Restoration Success: Trophic Transfer.

It is apparent that landscape, soil, and vegetative characteristics are useful indicators of restoration success, but it is less clear whether these indicators lead to trophic support for consumers. To better understand how these resources are allocated to consumers in restored marshes, restoration studies have used stable isotopes to characterize niche properties and predator-prey interactions (Post 2002; Perkins 2007; Fry et al. 2008; Rush et al. 2010; Boecklen et al. 2011; Llewellyn and La Peyre 2011)

Consumers acquire their isotopic signal from their diet which has been integrated over time and space in a specific marsh (Fig. 1.1) (Peterson and Fry 1987; Post 2002; Llewellyn and La Peyre 2011; Olin et al. 2017). Carbon isotope values differ between primary producers based on their photosynthetic pathway and CO_2 fixation (Choi et al 2001, Deines 1980). Marsh grasses such as *Spartina* spp. utilize a C4 pathway while rushes such as *Juncus* spp. utilize a C3 pathway, with ^{13}C being more depleted in C3 plants (around -28‰) relative to C4 plants (around -14 ‰) (Hobbie and Werner 2004). Consumer carbon ratios, $\delta^{13}C$, can be used to differentiate primary carbon sources as it fractionates very little between trophic levels, making it possible to determine whether a marsh is supported primarily by C3 or C4 plants (Post 2002; Wozniak et al. 2006). Carbon ratios may also differ within tissue compartments of a plant such as belowground or aboveground biomass (Hobbie and Werner 2004). Consumer nitrogen ratios, $\delta^{15}N$, can be used to determine trophic position because it fractionates at a predictable rate relative to the base of the food web as it moves through trophic levels (Post 2002). A mean ^{15}N

fractionation rate of around 3.4‰ has been documented between trophic levels, however the exact value depends on the species involved.

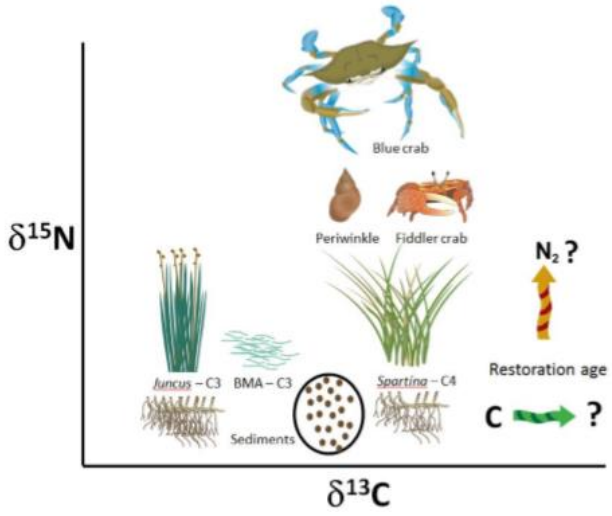


Figure 1.1 *Conceptual diagram of trophic food reflected by stable isotopes.* All symbols courtesy of Integration and Application Network, University of Maryland Center for Environmental Science (ian.umces.edu/imagelibrary/).

Stable isotope analysis (SIA) has been used in many studies to track the trophic support of *S. alterniflora* marshes (Levin et al. 1996; Kwak and Zedler 1997; Wozniak et al. 2006; Fry et al. 2008) while less stable isotope research has been conducted on *Juncus*-dominated marshes (Dillon et al., 2015). A study conducted by Llewllyn and La Peyre (2011) used SIA techniques to evaluate trophic support for blue crabs in created *S. alterniflora* marshes. Blue crabs are a common marsh inhabitant that feed opportunistically at multiple trophic levels throughout their life, making them an interesting species to track for trophic support studies (Llewellyn and La Peyre 2011). They found that the created marshes were structurally equivalent to their paired reference marsh in terms of nekton abundance and emergent vegetation, but only the oldest

marshes (8, 14, and 24 years old) matched their reference marsh in terms of blue crab stable isotope values. This highlights the differences between structural metrics and stable isotopes as indicators of functional equivalence (Llewellyn and La Peyre 2011). Their findings suggest that trophic support for higher order consumers may take longer to restore than structural metrics. Similar SIA approaches were used to compare food web support for *Fundulus* spp. in marshes of different tidal restriction in southern New England (Wozniak et al. 2006) and in constructed oyster reefs in southern Mississippi (Dillon et al. 2015).

Sullivan and Moncreiff (1990) emphasized differences in the stable isotopes of primary producers and consumer found in Mississippi marsh versus those found in Atlantic salt marshes. Results from other studies conducted in coastal Mississippi found stable isotope values of *S. alterniflora* and *J. roemerianus* to be within the ranges reported by Sullivan and Moncreiff (1990) (Hackney and Haines 1980; Dillon et al. 2015). Stable isotopes are a powerful tool to investigate functional equivalence in terms of food web support from producers to higher level consumers but should be compared carefully across regions and systems.

Studies that compare restored sites with natural counterparts in terms of site landscape, community structure, and food web support provide a good understanding of the restored site's trajectory because they track the succession of physical habitat and basal resources that encourage consumer colonization.

1.5 Restoration of Deer Island, MS.

This study takes place at created and natural marshes on Deer Island off the coast of Biloxi, MS (Fig. 1.2). Historically, Deer Island has protected the Biloxi area from

long-term erosion by reducing wind and wave energy and buffering major storm damage (Schmid and Otvos 2003). It has lost approximately one third of its original footprint and gradually shifted landward since the 1850s (Schmid and Otvos 2003; Sloan and Schmid 2003). Its severe land loss, vital storm protection services, and proximity to dredging projects make Deer Island an ideal candidate for BU restoration.

Restoration projects funded by Mississippi Department of Marine Resources (MDMR) and U.S. Army Corps of Engineers (USACE) began in 2003 with the addition of Deer Island to the Coastal Preserves program and the onset of the first created marsh, Deer Island Multi-year Restoration 1 (DIMR 1), along the eroded north-eastern end of the island (Fig. 1.2) (Schmid and Otvos 2003; Roth et al. 2012). The outline of DIMR 1 was delineated with a sand-berm built and filled with dredged material from the Biloxi Lateral Channel, creating approximately 16 hectares of new tidal marsh (Lang 2012; Roth et al. 2012; Jennifer et al. 2015; Biber 2016). After dewatering and stabilizing the fill, natural marsh vegetation such as *S. alterniflora, J. roemerianus,* and saltmarsh hay (*Spartina patens*) were planted throughout DIMR 1 by volunteers. Planting was conducted in spring of 2005; however, efforts were set back due to the damages from Hurricane Katrina in August that same year. Additional planting took place in 2009 along with a second round of berm construction and filling in 2010 and 2013 (Roth et al. 2012; Biber 2016). After this second round of maintenance, semi-annual qualitative vegetation monitoring of DIMR 1 was conducted by the MS Habitat Stewards program. The second created marsh, DIMR 2, was constructed in 2015 to the immediate west of DIMR 1, almost doubling the total area of created marsh (Fig. 1.2). DIMR 2 was constructed by the same methods and filled with dredged material from the Biloxi Lateral Channel and

other dredging projects in Jackson County, MS. Approximately 36,000 marsh plants were planted by volunteers in spring 2016, including *S. alterniflora, J. roemerianus, S. patens,* sea oats (*Uniola paniculata*), and bitter panicgrass (*Panicum amarum*) (Biber 2016). Based on results from Murphy (2020) and anecdotal information by Dr. Patrick Biber, *J. roemerianus* largely failed to survive in both created marshes (Murphy 2020). A natural reference marsh, DIN, was delineated directly south of DIMR 1 and DIMR 2 (Fig. 1.2). All sites are within 1 mile of each other to minimize hydrological differences due to along-island changes that could complicate statistical comparisons over time and to minimize travel time between sites.

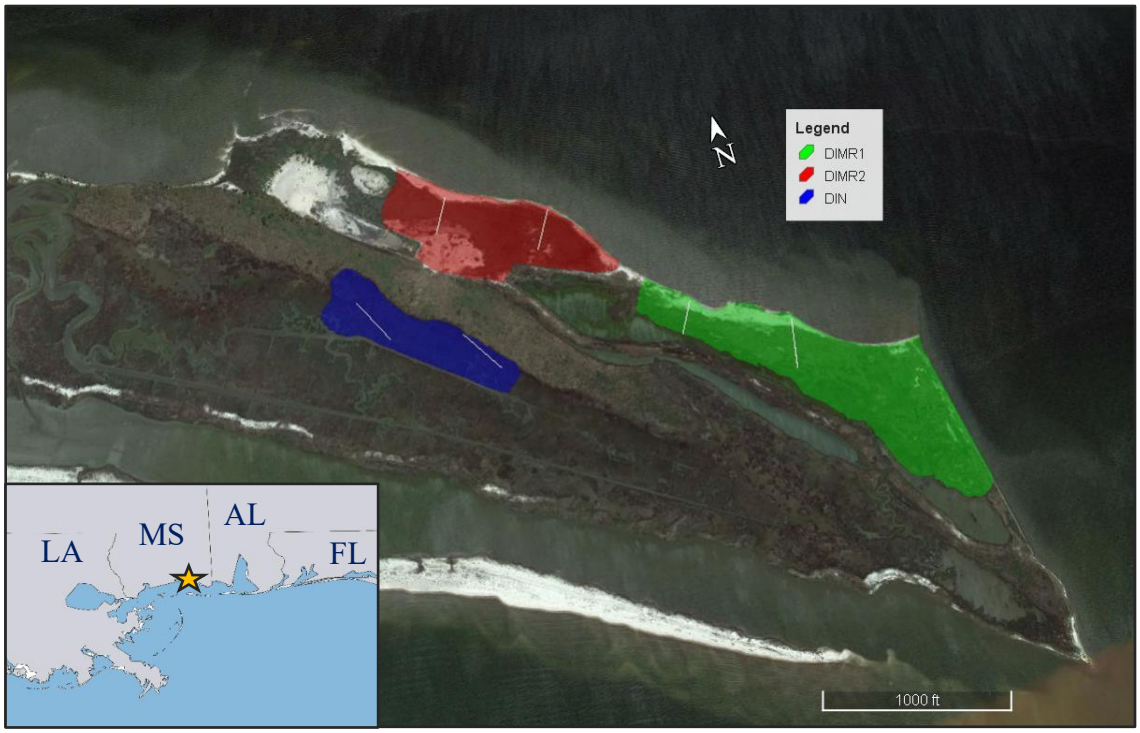


Figure 1.2. *Deer Island off the coast of Biloxi and Ocean Springs, MS.* Sampling took place at the constructed sites DIMR1 and DIMR2 (Green and Red) and natural site DIN (Blue). Each site had two 100 m long sampling transects to represent the entire elevation and vegetation gradient throughout the site.

A few studies have monitored structural and vegetative traits at the restored and natural marshes on Deer Island and found significant differences between them, beginning at the foundational level (Lang 2012; Murphy 2020)(Table 1.2). Methods can be found in their papers. To distinguish between the sites more easily, DIMR1 will hereafter be referred to as the 15+ yr constructed site, DIMR2 as the 5+ yr constructed site, and DIN as the 100+ yr natural site.

Murphy (2020) found that the 15+ yr site on Deer Island had the highest and most dynamic elevation, higher portions of larger sand grain sizes, and the lowest soil organic content (SOC) which follows the trend of some *S. alterniflora* restored marshes (Craft 2001a; Murphy 2020). Other studies have found that fewer flooding events and slow development of sediment characteristics, as seen in the 15+ yr constructed site, led to a slower nekton recovery (Minello and Webb 1997; Hollweg et al. 2019). Despite being almost a decade younger, the 5+ yr site was more similar to the natural site in that it had lower elevation and higher levels of SOC, suggesting it could have a faster nekton recovery time compared to the 15+ yr site.

There was significantly more belowground plant biomass in the natural site than the constructed sites, suggesting that development of the rhizosphere has yet to reach natural levels in either of the constructed sites (Murphy 2020). A well-developed rhizosphere has been known to increase resiliency to disturbances and storm events, increase blue carbon burial, and cycle nutrients. Belowground biomass is also known to positively affect marsh accretion rates (Turner et al. 2004).

The vegetative community at the 15+ yr old site was also more dissimilar to the natural site compared to the younger 5+ yr site, which is expected given the landscape

and soil characteristics (Murphy 2020). The primary vegetation cover in 100+ yr natural site on Deer Island is composed of 36% *J. roemerianus* and 62% *S. alterniflora*. The 5+ y and 15+ y constructed sites were abundant in *S. alterniflora* (63% and 34% coverage respectively) but relatively absent of *J. roemerianus* (1% and 2% coverage respectively). Instead, dune grasses such as saltgrass (*Distichlis spicata*) and *S. paten*s were flourishing in the restored sites likely due to higher elevations consistent with the 15+ y and 5+ y constructed sites' landscapes (Eleuterius 1972). The absence of *J. roemerianus* in the restored marshes could cause dissimilar consumer communities and result in different consumer support in the form of stable isotopes compared to the natural site. Murphy (2020) also showed that the vegetation communities in the 5+ yr and 15+ yr constructed sites were more similar to each other than to the natural site, likely due to higher elevation creating different vegetation assemblages. The constructed sites had significantly higher species richness than the natural site which was uniformly dominated by only a few indicator species, mainly *J. roemerianus* and *S. alterniflora* (Murphy 2020).

It is important to track the success of environmental characteristics, such as elevation, grain size, and soil organic content (SOC), and the succession of primary producers because they establish the foundation for consumers such as crustaceans, mollusks, fish, and birds, whose success is a primary goal of marsh restoration. Overall, the results from Murphy (2020) suggest that the restored and natural sites will support different assemblages of consumer species.

Table 1.2 *Summary of landscape, soil, and vegetation characteristics at the constructed and natural marshes on Deer Island, MS.*

Variables	**5+ yr constructed**	**15+ yr constructed**	**100+ yr natural**
Mean elevation and Elevation Range	0.54 m Range: 0.35 m	0.76 m Range: 0.8 m	0.27 m Range: 0.05 m
Sediment Bulk Density (g/cm3)	1.02 to 1.21 (g/cm^3)	1.12 to 1.23 (g/cm^3)	0.44 to 1.09 (g/cm^3)
Grain size	Very fine sand	Very fine sand	Silt/clay
Soil organic content (SOC)	Intermediate	Low	High
Vegetation Species Richness	n = 16	n = 32	n = 5
Juncus/*Spartina* coverage – based on quadrats	1% / 63%	2% / 34%	36% / 62%
Aboveground biomass (g/m^2)	Intermediate	Low	High
Belowground biomass (g/m^2)	Low	Low	High

For more information, data analysis and results can be found in Murphy (2020).

1.6 Goals and Objectives

The goal of this study is to evaluate the functional equivalence of restored marshes on Deer Islands, MS in terms of consumer support to better inform future Beneficial Use restoration decisions in the northern Gulf of Mexico. While previous studies have focused on vegetation and sediment characteristics, this study focuses on the faunal community and the food web, using both structural and trophic metrics of consumers to compare sites of different ages. Specific objectives are as follows:

1) Compare the abundance of marsh-surface invertebrate species among the two restored sites (5+ yr and 15+ yr) and natural site.
2) Compare the abundance, species richness, and diversity of nekton species among the two restored sites (5+ yr and 15+ yr) and natural site.

3) Investigate the community assemblage and diversity of nekton communities at the two restored sites (5+ yr and 15+ yr) and natural site.
4) Compare food web structure in terms of $\delta^{13}C$ and $\delta^{15}N$ stable isotopes, focusing on various feeding strategies and lifestyle of consumer species.

CHAPTER II – METHODS

2.1 Study area

This study takes place at created and natural marshes located at the northeast end of Deer Island off the coast of Biloxi, MS (Latitude: 30°22'5.40"N, Longitude: 88°49'33.20"W)(Fig. 1.1). Deer Island is a mainland remnant that stretches approximately 5.7 km (NW to SE). The island is located at the mouth of the Biloxi Back Bay and is hydrologically affected by drainage from the Biloxi River (Moncreiff and Sullivan 2001). Diurnal tidal range in this area is approximately 0.55 m.

2.2 Sampling design

Sampling began in Fall 2018 along two 100-m long transects at each study site with approximately 250 m between starting points. Transects in the created marshes ran perpendicular to the shoreline to capture adequate zonation patterns that represent different conditions for marsh vegetation to thrive. Transects in the natural marsh ran diagonal as there was no shoreline to base transect orientation off (Fig. 2.1). Sampling took place every spring and fall from 2017–2019 unless otherwise stated.

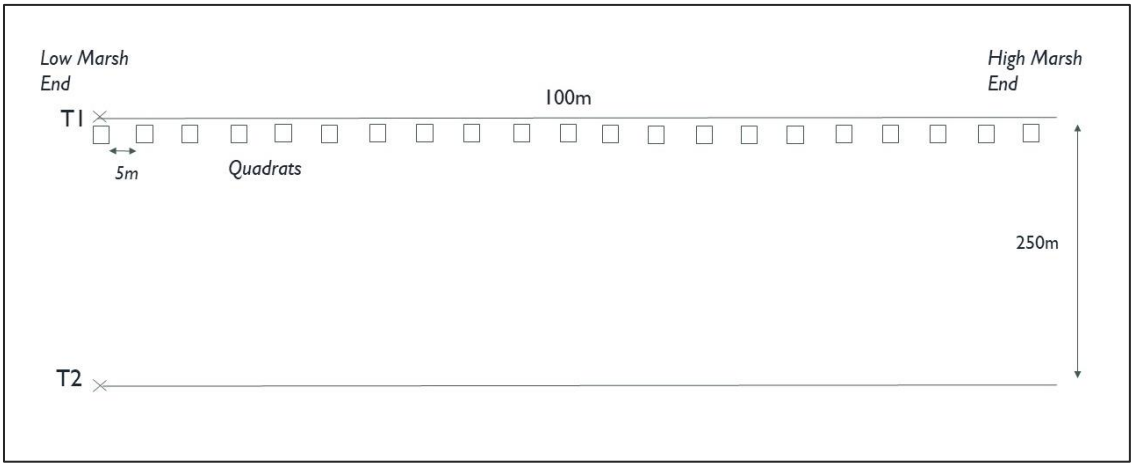


Figure 2.1 *Transect sampling layout for marsh-surface invertebrates.*

2.3 Sample Collection

2.3.1 Vegetation and Sediment

Emergent marsh and dune vegetation and sediment sampling was conducted under the direction of Dr. Patrick Biber and details can be found in Murphy (2020). Results from their analyses are included in this study for ecological context. Vegetation and sediment sampling were conducted from Spring 2017 through Spring 2019 for a total of 5 seasons (Table 2.1).

2.3.2 Benthic Microalgae

Benthic microalgae (BMA) were sampled using glass plate collectors. Collectors consisted of two 10 cm x 10 cm glass plates glued together with marine silicone adhesive, with a 3 mm gap between the plate faces. The plates were secured to a PVC pipe with monofilament to mark them in the field. Three collectors were deployed in each site in standing water and inserted half-way into the sediment so that BMA could collect on the light exposed surface above the sediment. After at least 3 weeks in the field, the plates were collected and put on ice until returned to the lab for processing. Once back to the lab, the glass plates were frozen until ready for SIA processing. BMA were sampled from Fall 2018 through Fall 2019 including Summer 2019 for a total of 4 seasons (Table 2.1).

2.3.3 Consumers

Consumers were collected using two methods: quadrat sampling and minnow traps. Quadrat sampling was used to collect marsh-surface invertebrates within a 0.25-m^2 quadrat every 5 m along the two outer-most transects (Fig. 2.2.1). All invertebrates within the quadrat were identified and counted, and all fiddler crab burrows were counted with their diameters measured. Fiddler crabs typically spend much of the day deep inside

burrows and are rarely seen during sampling, therefore, burrow abundance served as a proxy for fiddler crab abundance (Mouton and Felder 1996; Staszak and Armitage 2013). The ratio of burrow abundance to animals is approximately 1:1 in vegetated marshes, which provides a close estimate of fiddler crab presence (Mouton and Felder 1996). Any fiddler crabs caught within the quadrat were brought back to the lab to be identified by sex and species. Four to five individuals of each unique invertebrate species were bagged and put on ice until returned to the lab and frozen. Transect sampling was conducted from Spring 2017 through Fall 2019 for a total of 6 seasons (Table 2.1).

Minnow traps were used to collect nekton inhabiting shallow rivers and ponds within each site. Traps were deployed for ~24 h during neap tides to ensure they remained in standing water for the duration of the deployment. Traps were baited with frozen shrimp and deployed in an area with standing water deep enough for the entry to be submerged. After 24 hours, contents were bagged and put on ice until retuned to the lab. Once back to the lab, organisms were stored in a -20˚C freezer until further analysis. Minnow trap collection was conducted from Spring 2018 through Fall 2019 including Summer 2019 for a total of 5 seasons (Table 2.1).

Table 2.1 *Overview of sample collection timeline*

	2017		**2018**		**2019**		
Sample type	**Spring**	**Fall**	**Spring**	**Fall**	**Spring**	**Summer**	**Fall**
Vegetation	X	X	X	X	X		X
Sediments		X	X	X	X		
Transect	X	X	X	X	X		X
Minnow Trap			X	X	X	X	X
BMA				X	X	X	X

2.4 Sample Processing

2.4.1 Vegetation

Vegetation and sediment samples were processed under the direction of Dr. Patrick Biber and details can be found in Murphy (2020). Vegetation percent cover and species present were measured in the field during sample collection. Vegetation biomass was processed in the lab and separated by belowground (BG), aboveground green living (AL) and aboveground yellow-brown dead (AD) tissue fractions. Dried tissues of BG, AL, and AD for each species were coarsely cut using scissors, then ground to a fine powder in a Wiley mill to pass a #40 sieve. Sediment was also processed in the lab and separated into surface (S) and deep (D) core depths. Soil organic content was measured using loss on ignition techniques. Ground plant tissue and sediment cores were stored in 20 ml glass scintillation vials for later stable isotope analysis.

Vegetation samples that were processed for stable isotope analysis included *S. alterniflora*, *J. roemerianus*, and BMA. Only quadrats that composed of exclusively *S. alterniflora* and/or *J. roemerianus* were considered for isotope analysis of aboveground living biomass (AL) and surface (S) and deep (D) sediment core fractions.

2.4.2 BMA

Outer surfaces of BMA plates were rinsed under gently running tap water to remove sediment, separated using a razor blade, and rinsed again on the inside surfaces of the plates to further remove sediment. The inside surfaces were scraped with a razor blade to remove microalgae and placed in a tin weigh boat. Only the area that was above the sediment was scraped for BMA to avoid collecting additional carbonates. Tissue was dried at 70˚C to constant weight and stored in glass scintillation vials in a desiccator.

Dried tissue was ground to a fine powder in the glass vial to prevent losing tissue during the grinding process.

2.4.3 Consumers

Marsh-surface invertebrates collected in quadrats were identified and counted for abundance in the field. Nekton from minnow traps were brought back to the lab and identified to species level and counted for abundance.

A subset of each invertebrate and nekton species was processed for stable isotope analysis. Gastropod, bivalve, and crustacean tissues were removed from their shells for processing, except for grass shrimp, which were processed whole due to size. Fish were descaled and carefully dissected for dorsal muscle tissue to avoid bones. For smaller fish (<25 mm total length) the scales, head, and tail were removed then the rest was processed whole. All samples were rinsed thoroughly with deionized water to remove particulates then dried at 60˚C and ground to a fine powder with a mortar and pestle. All ground samples were stored in glass scintillation vials in desiccators.

Samples that contained inorganic carbon components were measured for isotopic values both before and after acid washing. The purpose of sample acidification is to remove inorganic, non-dietary carbonates found in skeletal material and shells in the form of calcium carbonate ($CaCO_3$) (Bunn et al. 1995). Samples that were acid washed included sediment, grass shrimp, and fish run whole. Running samples twice allows us to measure $\delta^{15}N$ values that typically change due to acid washing (Kennedy et al. 2005; Levin and Currin 2012; Schlacher and Connolly 2014).

For isotope analysis, approximately 0.3–0.8 mg of animal muscle tissue, 2–3 mg of plant and BMA tissue, and 25–30 mg of sediment were weighed on a Mettler Toledo

XP26 microbalance (0.001-mg accuracy). Samples were placed into tin capsules, folded, and pressed for combustion. All samples were analyzed for $\delta^{13}C$ and $\delta^{15}N$ values using a Costech 4100 Elemental analyzer coupled to a Thermo Delta V Advantage stable isotope ration mass spectrometer via a Thermo Conflo IV interface at The University of Southern Mississippi's Gulf Coast Research Lab.

Carbon and nitrogen isotope ratios are expressed in delta notation $\delta^{13}C$ and $\delta^{15}N$ where R is the ratio of heavy to light isotopes (^{13}C:^{12}C or ^{15}N: ^{14}N) in the sample and in the standard material:

$$\delta X = \left[\left(\frac{\text{Rsamples}}{\text{Rreference}}\right) - 1\right] \text{x } 1000$$

The standard for carbon was referenced to Vienna Pee Dee Belemnite (VPDB) and the standard for nitrogen was referenced to atmospheric nitrogen. Results are expressed in units of per mil (‰) ± standard deviation.

Table 2.2 *Sample replication of structural metrics and stable isotopes*

Analysis	**Sample Type**	**Replication**	**Total samples**
Structural metrics	Quadrats	21 quadrats per transect × 2 transects per site × 3 sites × 6 sampling seasons	756
	Minnow Traps	6 MT per site × 3 sites × 5 sampling seasons	90
Trophic metrics	Sediment Cores	1 shallow and 1 deep cores per quadrat × 2 veg species per quadrat* × 1 quadrats per transect × 2 transects per site × 3 sites × 1 sampling season	24
	Veg Biomass Cores	2 veg species per core* × 2 cores per quadrat × 2-3 quadrats per transect × 2 transects per site × 3 sites × 4 sampling seasons	~48–72
	BMA Plates	3 plates per site × 3 sites × 4 sampling seasons	36

Table 2.2 (continued).

	Quadrat Invertebrates	3 – 4 invert species per site × 2 – 3 individuals per species × 3 sites × 3 sampling seasons	~54–108
	Minnow Trap Nekton	4 – 7 species per trap × varying individuals per species × 3 sites × 5 sampling seasons	157

* J. roemerianus was not always present in a quadrat.

2.5 Data Analysis

2.5.1 Quadrat

Marsh surface invertebrates sampled using quadrats included periwinkles, olive snails, fiddler crabs (using burrow counts), and ribbed mussels. Abundance of each species and total abundance per quadrat were analyzed for effects of site, season, and year using a suite of different models. The presence/absence of quadrats was also analyzed for effects of site using a generalized linear model. The presence of species in a site was used to indicate favorable qualities such as environmental conditions, interactions with other species, or accessibility that allows the species to grow and thrive (Hirzel and Le Lay 2008). Model parameters are described in the table below (Table 2.4). No transformations were performed on abundance data, as transformations were found to perform poorly compared to GLM distributions (O'Hara and Kotze 2010). Instead, different distributions were used depending on the model fit for each species.

Model selection was conducted by using the Akaike Information Criterion (AIC) to rank models. Poisson, negative binomial, zero-inflated Poisson, and zero inflated negative binomial distributions were fitted to each model and the "best" performing model was chosen based on diagnostics such as dispersion, QQ-plot, and normality of residuals and the lowest AIC value. Once a distribution was chosen, then a stepwise regression was used to select the best combination of fixed effects and interactions. This

was done in R by iteratively removing predictors until the AIC value did not improve by removing them. Fixed effects included site, year, and season with interactions.

Total quadrat abundance was modeled using a zero-inflated negative binomial. Fixed effects incorporated in the final model included site, season, year, site × year interaction, and season × year interaction.

Fiddler crab burrow abundance was modeled using a zero-inflated negative binomial. Fixed effects incorporated in the final model included site, season, year, and all interactions except the 3-way (site × season × year) interaction.

Olive snail abundance was modeled using a zero-inflated negative binomial. Fixed effects incorporated in the final model included site, season, year, and season × year interaction.

Periwinkles and ribbed mussels were not present in the constructed sites and only present at the natural site, therefore no models were created.

An ANVOA was used for the presence/absence model and a type III Wald chi-squared test was used for the abundance models to test for significance of the fixed effects. Significance was set at $\alpha < 0.05$. Pairwise comparison of least-squares means with Sidak adjustment was performed on fixed effects when necessary.

2.5.2 Minnow Traps

Nekton collected in minnow traps were measured for abundance, species richness and diversity at the trap level and analyzed for effects of site and season using a suite of multiple regression models. Diversity was calculated using Simpson's index, which indicates the probability that two randomly chosen individuals belong to different species (Kathryn Morris et al. 2014).

Abundance and species richness were modeled using separate negative binomial generalized linear mixed models (GLMMs) with site and season as fixed effects, and year as a random effect (Table 2.4). Presence/absence of organisms in minnow traps was modeled using a GLMM with a binomial error distribution (Table 2.4). Presence/absence of any organism was used as the response variable with site and season as fixed effects, and year as a random effect. Fixed effect P-values were obtained using Type II or Type III Wald Chi Square Test. Significance was set at $\alpha < 0.05$. Pairwise comparisons of least-squares means with Sidak adjustment was performed on fixed effects when necessary.

Consumer community assemblage was also compared among sites using an ANOSIM (permutations = 5000) derived from a Bray-Curtis dissimilarity matrix, with the null hypothesis being no distance greater than zero between sites. To visualize results from the ANOSIM, minnow traps were plotted in ordination space with a nMDS (k = 3) plot.

2.5.3 Stable Isotopes

Stable isotopes $\delta^{13}C$ and $\delta^{15}N$ were measured from sediment, primary producers, and consumers at the constructed and natural sites and analyzed using linear regression models. Sediment stable isotope data were first tested for an effect of core depth (surface vs deep). A linear model was created for each site with mean $\delta^{13}C$ and $\delta^{15}N$ as the response variable and depth as the fixed effect. All sites showed no difference between surface and deep stable isotopes; therefore, sediment samples were pooled by site for the remainder of the analysis. Sediment isotopes were then modelled using a linear model (LM) with mean $\delta^{13}C$ and $\delta^{15}N$ as the response variable and site as a fixed effect.

Vegetation stable isotopes were taken from alive aboveground biomass of *J. roemerianus, S. alterniflora,* and benthic microalgae (BMA). Mean $\delta^{13}C$ and $\delta^{15}N$ of was modelled using a LM with mean $\delta^{13}C$ and $\delta^{15}N$ as the response variable and site and vegetation as fixed effects (Table 2.4).

Consumer stable isotopes were first analyzed for effects of acid washing. Carbon stable isotope values of acid washed samples were plotted against values of non-acid washed samples, and we found no difference in $\delta^{13}C$ between the two treatments, therefore, we used $\delta^{13}C$ and $\delta^{15}N$ isotope values from the original, non-acid washed runs for the remainder of the analyses. Consumer isotopes were modelled using a LM with $\delta^{13}C$ and $\delta^{15}N$ as the response variable and site, feeding strategy, and lifestyle as fixed effects (Table 2.4). Life history groupings of consumers were based on classifications from Thompson and Forman (1987). We classified consumers as either resident or transient marsh species to investigate habitat quality between sites (Nyman 2017). Feeding groups included filter feeders, grazers, and omnivores (Table 2.3).

Table 2.3 *List of species classified by feeding strategy and lifestyle.*

Species	**Scientific name**	**Feeding strategy**	**Lifestyle**
Ribbed mussel	*Geukensia demissa*	Filter Feeder	Resident
Carolina marsh clam	*Polymesoda caroliniana*	Filter Feeder	Resident
Common marsh snail	*Melampus bidentatus*	Grazer	Resident
Fiddler crab	*Uca virens*	Grazer	Resident
Periwinkle	*Littoraria irrorata*	Grazer	Resident
Olive snail	*Neritina usnea*	Grazer	Resident
Striped mullet	*Mugil cephalus*	Grazer	Transient
White mullet	*Mugil curema*	Grazer	Transient
Grass shrimp	*Palaemonetes spp.*	Grazer	Resident
Brown shrimp	*Farfantepenaeus aztecus*	Omnivore	Transient
White shrimp	*Litopenaeus setiferus*	Omnivore	Transient
Blue crab	*Callinectes sapidus*	Omnivore	Transient

Table 2.3 (continued).

Sailfin molly	*Poecilia latipinna*	Omnivore	Resident
Eastern mosquitofish	*Gambusia affinis*	Omnivore	Resident
Western mosquitofish	*Gambusia holbrooki*	Omnivore	Resident
Diamond killifish	*Adinia xenica*	Omnivore	Resident
Sheepshead minnow	*Cyprinodon variegatus*	Omnivore	Resident
Gulf killifish	*Fundulus grandis*	Omnivore	Resident
Bayou killifish	*Fundulus pulvereus*	Omnivore	Resident

A one-way ANOVA was used to compare mean $\delta^{13}C$ and mean $\delta^{15}N$ values of sediment, vegetation, and consumers across sites. Average $\delta^{13}C$ values must be >1 per mil different to be considered biologically meaningful as it allows for a minimum difference of 2 standard deviations (Dillon et al. 2015). Multiple comparison of means with Tukey contrasts was used if significant differences were noted. Ellipses were drawn around 40% core of consumer data using SIBER and ellipse overlap was calculated using NicheRover. Ellipses were used to characterize isotopic niche space of consumer feeding groups because they are less influenced by extreme values, represent a reliable niche extension, and are unbiased to sample size compared to convex hulls (Jackson et al. 2011).

All analyses were run in R, v4.0.2.

Table 2.4 *Parameters of statistical models.* *Details of models used in quadrat invertebrate analyses, minnow trap nekton analyses, and stable isotope analysis.*

Data	Analysis	Response variable	Fixed effect	Random effect	Distribution
Quadrat	GLM	Presence/absence	Site	n/a	Binomial
Quadrat	Zero Inflated Negative Binomial	Total abundance	Site + Season + Year + Site × Year + Season × Year	n/a	Negative binomial
Quadrat	GLMM	Olive snail abundance	Site + Season + Year + Site × Season + Site × Year + Season × Year + Site × Season × Year	n/a	Negative binomial
Minnow Trap	GLMM	Olive snail abundance	Site + Season + Year	n/a	Negative binomial
Quadrat	Zero Inflated Negative Binomial	Fiddler crab burrow abundance	Site + Season + Year + Site × Season + Site × Year + Season × Year	Transect nested in Site	Negative binomial
Minnow trap	GLMM	Presence/absence	Site	Season nested in Year	Binomial
Minnow trap	GLMM	Abundance	Site + Season + Site × Season	n/a	Negative Binomial
Minnow trap	GLMM	Species richness	Site + Season + Site × Season	Year	Negative Binomial
Sediment isotope separated by site	LM	$\delta^{13}C$	Depth	n/a	n/a
Sediment isotope separated by site	LM	$\delta^{15}N$	Depth	n/a	n/a
Sediment isotope *Spartina* only	LM	$\delta^{13}C$	Site	n/a	n/a
Sediment isotope *Spartina* only	LM	$\delta^{15}N$	Site	n/a	n/a

LM indicates Linear Model

LMM indicates Linear Mixed-effect Model

GLM indicates Generalized Linear Model

GLMM indicated Generalized Linear Model

CHAPTER III - RESULTS

3.1 Quadrat Results

3.1.1 Present Absence

The likelihood of finding at least one marsh surface invertebrate in a quadrat differed significantly among the sites ($\chi^2 = 441.49$, $P < 0.0001$). At the 5+ yr site, only 41 quadrats contained organisms which accounted for 17.4% of the total quadrats in the site (Table 3.1). Similarly, at the 15+ yr site only 28 quadrats contained organism which accounted for 13.5% of the total quadrats in the site. Majority of quadrats at the 100+ yr natural site contained organisms, which accounted for 97.95% of the total quadrats in the site. When looking at the frequency distribution of individuals per quadrat, the created marshes have more quadrats with low abundance while the natural site has a more uniform distribution (Fig. 3.1).

Table 3.1 *Summary of quadrat presence/absence per site.*

	Number of quadrats sampled	Number of quadrats with organisms	% of quadrats that contained organisms
5+ yr restored site	236	41	17.37%
15+ yr restored site	207	28	13.53%
100+ yr natural site	195	191	97.95%

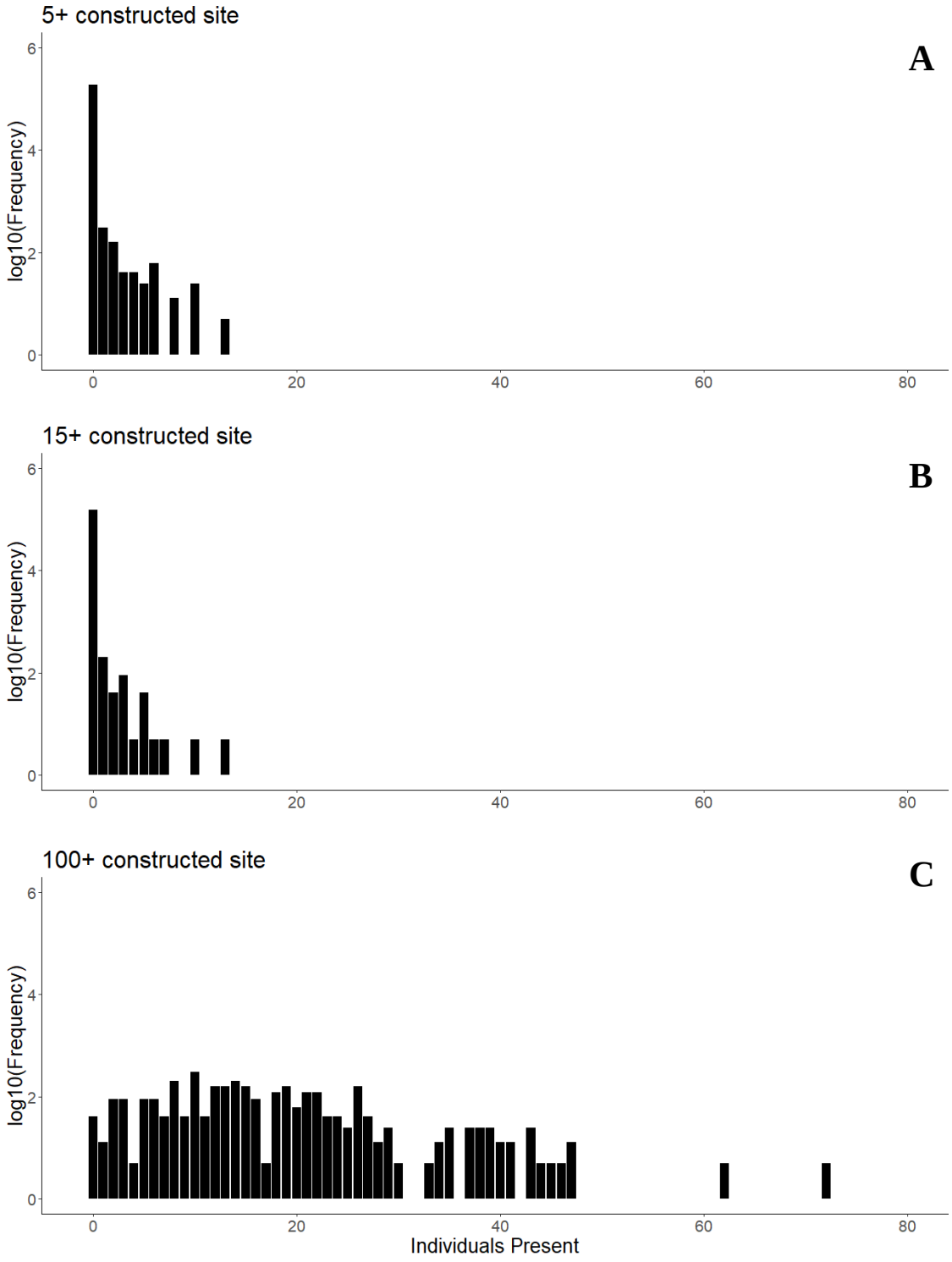


Figure 3.1 *Frequency of individuals present in a quadrat.*

(A) 5+ yr constructed site, (B) 15+ yr constructed site, and (C) 100+ yr natural site. Frequency has been log-transformed

3.1.2 Total Quadrat Abundance

Total abundance of organisms per quadrat differed among the three sites (χ^2= 37.86, $P < 0.0001$)(Table 3.3). The 100+ yr site had significantly higher quadrat abundance than the 5+ yr ($P < 0.001$) and 15+ yr ($P < 0.001$) constructed sites (Table 3.4). The 5+ yr site had the next highest quadrat abundance, followed by the 15+ yr site with the lowest quadrat abundance (Fig 3.2). Periwinkles and ribbed mussels were not present in either of the constructed sites, and only present in the natural site (Table 3.3).

There was also a significant site × year interaction ($P = 0.014$)(Table 3.3). Within each site, total abundance did not differ significantly across years. Between sites, however, quadrat abundance was significantly higher at the 5+ yr site in 2019 than at the 15+ yr site in 2017. This distinction is ecologically irrelevant though as these abundances do not come close to reaching natural levels.

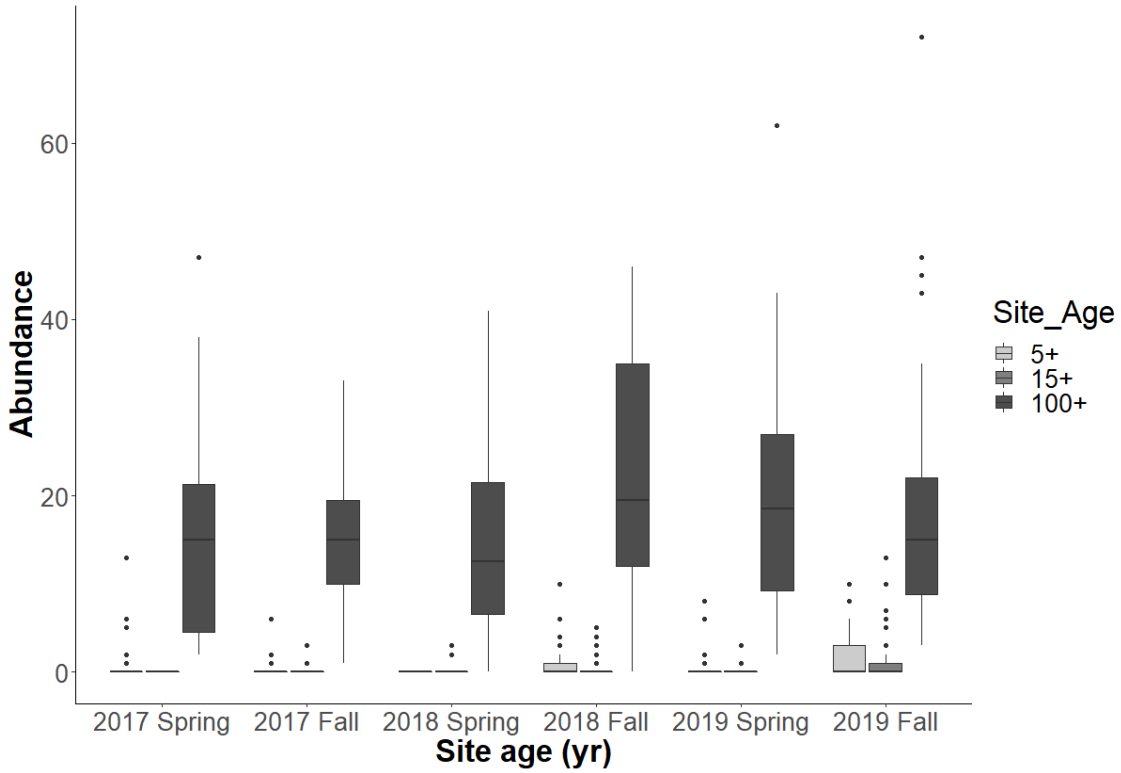


Figure 3.2 Total abundance per quadrat site and sampling event.

Letters indicated significant groupings determined by Least Square Mean. Line through the box indicates the median, while the upper and lower limits of the box indicate the 25th and 75th percentiles, respectively. Upper and lower whiskers indicate the largest and smallest value no further than the 1.5* IQR. Dots plotted outside of the whiskers indicate outliers

Table 3.2 *Summary of quadrat abundance.* *Abundance per quadrat (0.25 m^2) of fiddler crab burrows, periwinkles, olive snails, ribbed mussels, and total quadrat abundance per site presented as mean ± S.E.*

	Total	Fiddler crab burrows	Olive snails	Periwinkles	Ribbed mussels
Spring 2017					
5+ yr	1.1 ± 0.54	1.07 ± 0.54	0	0	0
15+ yr	0	0	0	0	0
100+ yr	15.7 ± 2.36	2.62 ± 0.55	1.58 ± 0.44	10.92 ± 2.02	0.58 ± 0.23
Fall 2017					
5+ yr	0.2 ± 0.15	0.21 ± 0.15	0	0	0
15+ yr	0.2 ± 0.1	0.19 ± 0.1	0	0	0
100+ yr	14.8 ± 1.59	0.57 ± 0.29	1.17 ± 0.51	11.3 ± 1.23	1.78 ± 1.06
Spring 2018					
5+ yr	0	0	0	0	0
15+ yr	0.2 ± 0.11	0.16 ± 0.11	0	0	0
100+ yr	14.6 ± 2.34	3.5 ± 0.9	3.58 ± 0.91	4.77 ± 2.25	0.41 ± 0.22
Fall 2018					
5+ yr	0.8 ± 0.3	0.67 ± 0.27	0.14 ± 0.14	0	0
15+ yr	0.6 ± 0.21	0.57 ± 0.21	0	0	0
100+ yr	21.8 ± 1.97	2.19 ± 0.41	1.64 ± 0.31	14.86 ± 1.35	3.14 ± 0.86
Spring 2019					
5+ yr	0.4 ± 0.24	0.41 ± 0.24	0	0	0
15+ yr	0.1 ± 0.09	0.11 ± 0.09	0	0	0
100+ yr	19.8 ± 2.1	1.79 ± 0.28	0.98 ± 0.23	15.86 ± 1.93	1.17 ± 0.39
Fall 2019					
5+ yr	1.7 ± 0.43	1.31 ± 0.37	0.36 ± 0.25	0	0
15+ yr	1.6 ± 0.51	1.49 ± 0.51	0.08 ± 0.08	0	0
100+ yr	19 ± 2.1	0.17 ± 0.11	0.12 ± 0.05	13.33 ± 1.06	5.33 ± 1.58

Table 3.3 *Significance test of total abundance per quadrat.*

Source	df	χ^2	*P*
Site	2	37.86	<0.0001*
Season	1	0.08	0.777
Year	2	5.63	0.060
Site × Year	4	12.39	0.015*
Season × Year	2	3.09	0.213

Summary table of Type III Wald Chi-squared test for total quadrat abundance by site, season, and year and interactions.

Table 3.4 *Post hoc test for total abundance per quadrat.*

Contrast	Estimate	*P*
(5+) – (15+)	0.22	0.32
(5+) – (100+)	-16.9	<0.0001*
(15+) – (100+)	-17.2	<0.0001*

Pairwise comparison of site with Sidak adjustment for total abundance per quadrat.

3.1.3 Fiddler Crab Burrow Abundance

Fiddler crab burrow abundance per quadrat differed among the three sites ($P < 0.0001$) (Table 3.5). The 100+ yr natural site had significantly higher fiddler crab burrow abundance per quadrat than the 5+ yr ($P < 0.0001$) and 15+ yr ($P < 0.0001$) constructed sites (Table 3.6).

There was also a significant site × year interaction ($P < 0.0001$) (Table 3.5). In 2017, the 100+ yr site had significantly more burrows per quadrat than the 15+ yr site, but not the 5+ yr site. In 2018 the natural site had significantly more burrows per quadrat than both the constructed sites. However, in 2019 the sites did not differ in burrow abundance. The 5+ yr and 15+ yr constructed sites did not differ significantly in fiddler crab burrow abundance across years. The 100+ yr natural site, on the other hand, had a significantly higher abundance in 2018 than in 2017 or 2019 (Fig. 3.4).

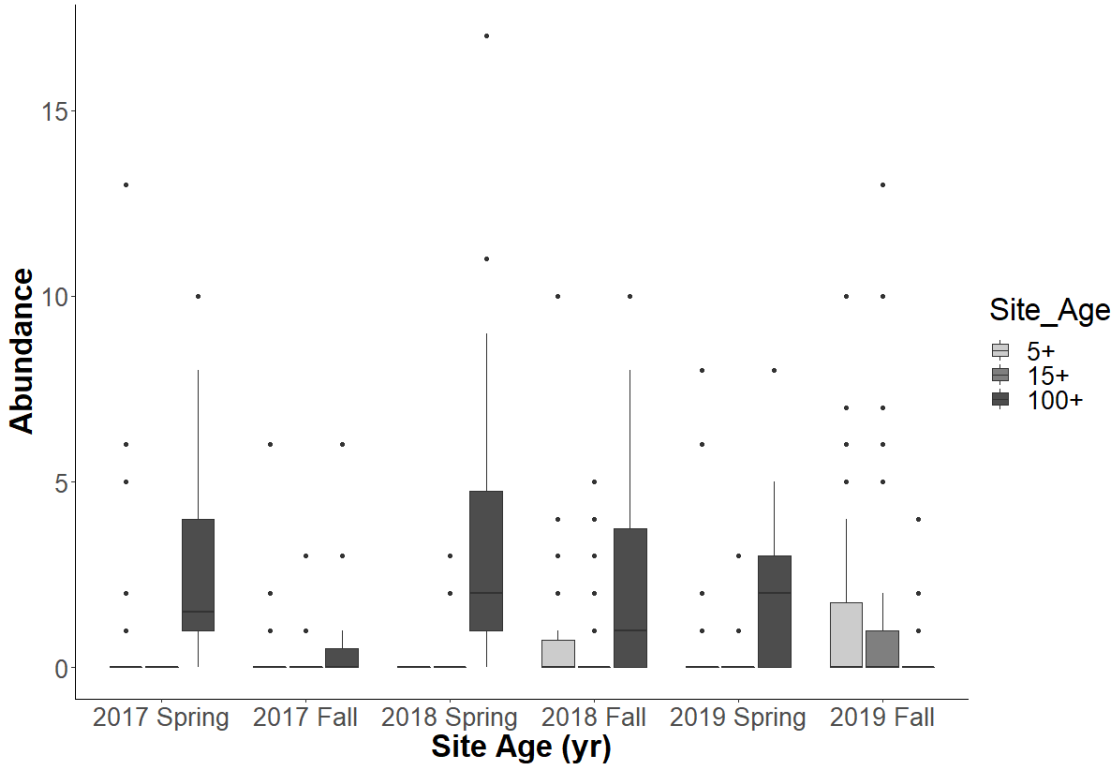


Figure 3.3 *Fiddler crab burrow abundance across sampling events by site.*

Table 3.5 *Significance test of fiddler crab burrow abundance per quadrat.*

Source	df	χ^2	*P*
Site	2	39.69	<0.0001*
Season	1	0.56	0.45
Year	2	0.27	0.87
Site × Season	2	5.98	0.05
Site × Year	4	24.77	<0.0001*
Season × Year	2	0.37	0.83

Summary table of Type III Wald Chi-squared test for fiddler crab burrow abundance by site, season, and year and interactions.

Table 3.6 *Post hoc test for fiddler crab burrow abundance per quadrat.*

Contrast	Estimate	*P*
(5+) – (15+)	0.20	0.51
(5+) – (100+)	-1.22	< 0.0001*
(15+) – (100+)	-1.42	< 0.0001*

Pairwise comparison of site with Sidak adjustment for fiddler crab burrow abundance per quadrat by site.

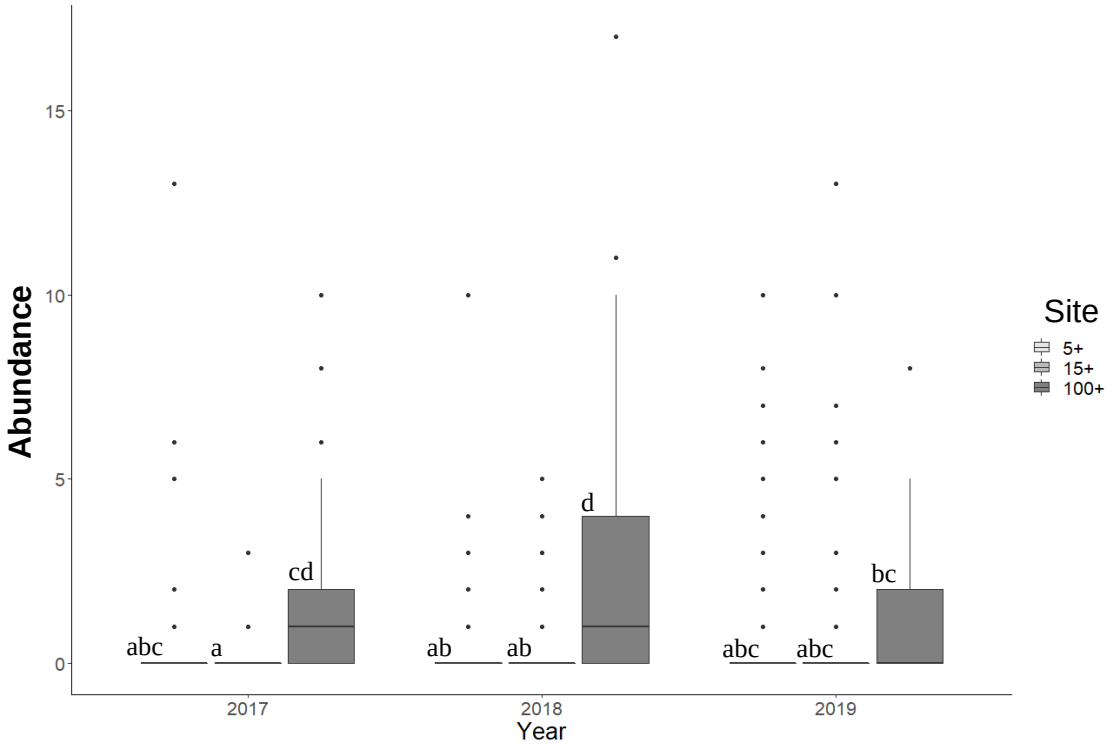


Figure 3.4 *Fiddler crab burrow abundance by site and year.*

3.1.4 Olive Snail Abundance

Olive snails are semi aquatic gastropods, spending much of their time submerged to feed on the marsh floor (Heard 1982). For this reason, we considered both quadrat and minnow trap sampling methods to assess olive snail abundance by site.

Olive snail abundance per quadrats differed by site ($P < 0.05$) and year ($P < 0.05$) (Table 3.7). The 100+ yr natural site had significantly higher abundance per quadrat than the 5+ yr ($P < 0.001$) and 15+ yr ($P < 0.001$) constructed sites (Table 3.8). Abundance per quadrat was also significantly higher in 2018 compared to 2017 ($P = 0.37$*).

There was also a significant season × year interaction (Table 3.7). Between sites, there was no significant difference in olive snail abundance except in Fall 2018 and

Spring 2019. In Fall 2018, the 100+ yr site had significantly more olive snails per quadrat than the 15+ yr site, but not the 5+ yr site. In Spring 2019, the 100+ yr site had significantly more olive snails per quadrat than both the 15+ yr and 5+ yr sites.

Olive snail abundance per minnow trap also differed by site and year, but showed an opposite trend seen by quadrat sampling. Abundance was significantly higher in the 5+ yr site compared to the 15+ yr site ($P = 0.003$) and the 100+ yr site ($P = 0.02$). There was no difference between the 15+ yr and 100+ yr sites ($P = 0.92$). Abundance was also significantly higher in 2019 than 2018 ($P = 0.0026$)

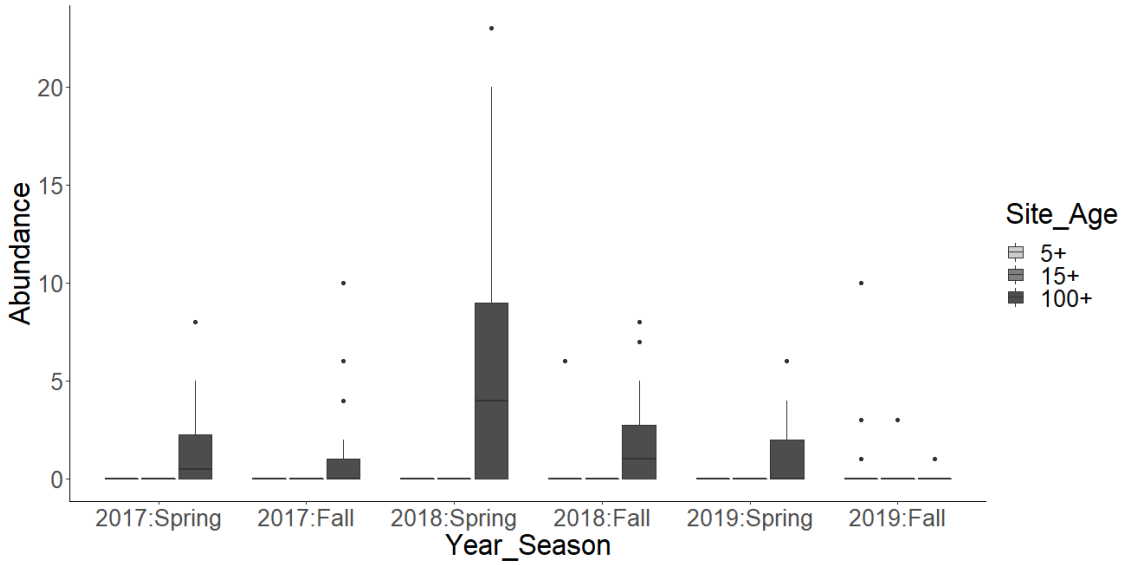


Figure 3.5 *Olive snail abundance per quadrat across sampling events by site*

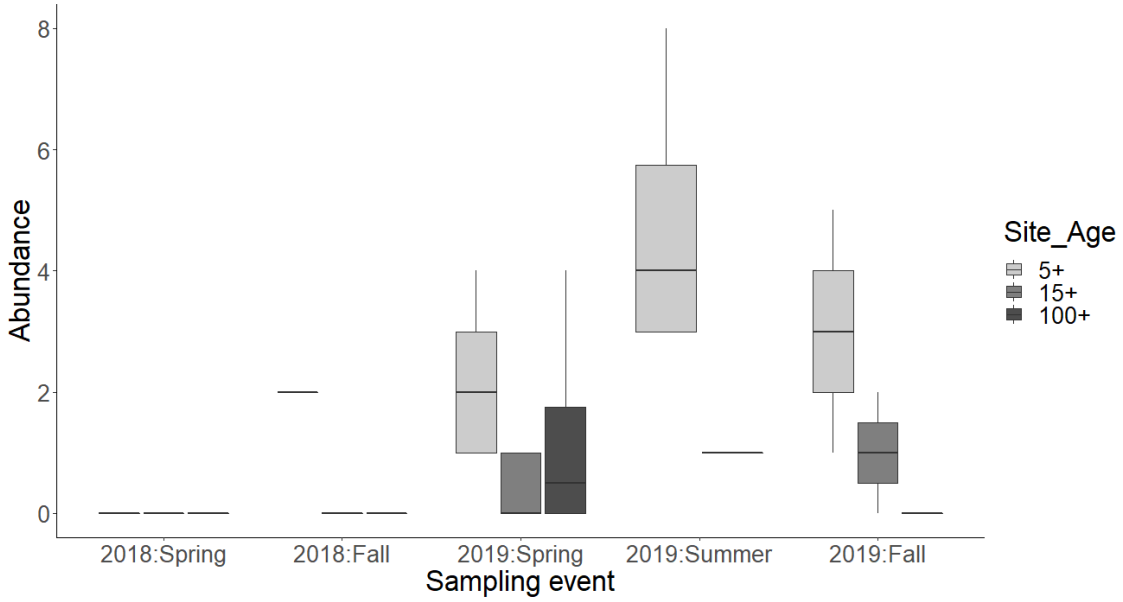


Figure 3.6 *Olive snail abundance per minnow trap across sampling events*

Table 3.7 *Significance test of olive snail abundance per quadrat.*

Source	df	χ^2	*P*
Site	3	123.2	< 0.001*
Season	3	0.03	0.86
Year	5	7.73	0.02*

Summary table of Type III Wald Chi-squared test for olive snail abundance per quadrat by site, season, and year and interactions.

Table 3.8 *Significance test of olive snail abundance per minnow trap.*

Source	df	χ^2	*P*
Site	2	18.7	< 0.001*
Season	2	4.02	0.13
Year	1	16.3	< 0.001*

Summary table of Type III Wald Chi-squared test for olive snail abundance per minnow trap by site, season, and year and interactions.

3.2 Minnow Trap Results

3.2.1 Presence Absence

The likelihood of capturing at least one animal in in a minnow trap differed significantly among the sites ($\chi^2 = 12.37$, $P < 0.002$). The 5+ yr site had the highest percentage of minnow traps with organisms present, which accounted for 93.1% of deployed traps. The 15+ yr and 100+ yr sites did not differ in the number of minnow traps with organisms present ($P = 0.25$). At the 15+ yr site, 66.7% of deployed minnow traps had organisms present. At the 100+ yr natural site only 46.4% of deployed minnow traps had organisms present. When looking at the frequency distribution of individuals per minnow trap, older restored site and natural site have more traps with low abundance while the younger restored site has a more uniform distribution with an outlier (Fig 3.2.1).

Table 3.9 *Number of empty minnow traps per site*

	Number of minnow traps sampled	Number of minnow traps with organisms	% of minnow traps that contained organisms
5+ yr restored site	29	27	93.1%
15+ yr restored site	30	20	66.7%
100+ yr natural site	28	13	46.4%

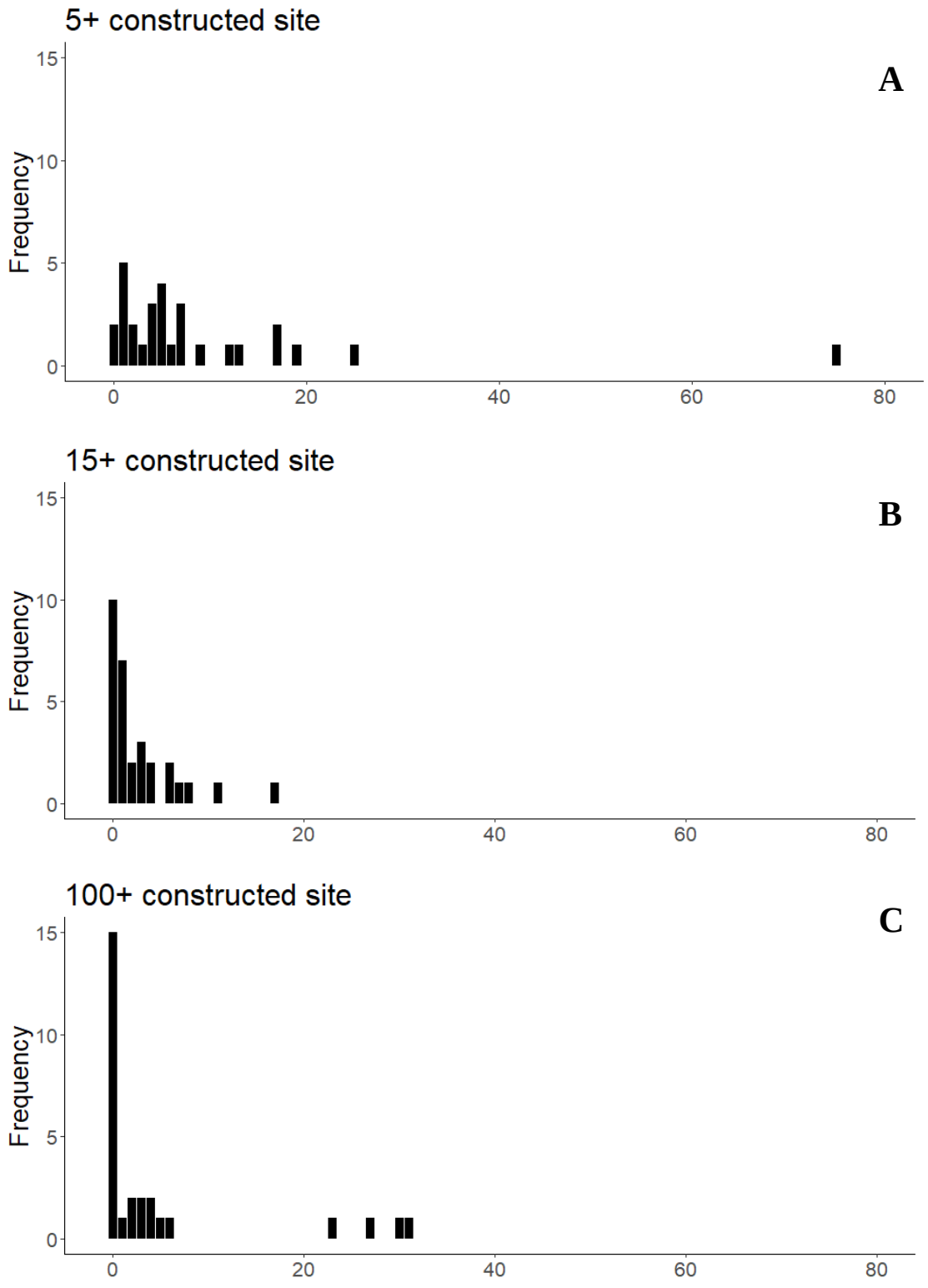


Figure 3.7 *Histogram of total abundance of nekton caught in minnow traps.*

(A) 5+ yr constructed site, (B) 15+ yr constructed site, and (C) 100+ yr natural site. Frequency has been log-transformed on y-axis

3.2.2 Total Minnow Trap Abundance

Mean nekton abundance (all species combined) caught in minnow traps differed significantly among the sites ($\chi^2 = 14.22$, $P < 0.0001$), with the 5+ year site having the most individuals per trap, followed by the 100+ yr site, and the 15+ yr site with the least (Table 3.11). There was also a significant site × season interaction ($\chi^2 = 17.19$, $P = 0.002$). In Spring and Fall, the 5+ yr site had significantly higher mean abundance than the 15+ yr site and 100+ yr site respectively (Fig. 3.8). In Summer however, there was no significant different in total nekton abundance among sites.

Table 3.10 *Summary of minnow trap metrics.*

	Number of traps sampled	Mean Nekton Abundance	Mean Species Richness	Mean Simpson's Diversity
5+ yr				
Spring	12	5.3 ± 1.61	2.2 ± 0.37	0.55 ± 0.08
Summer	6	6 ± 1.51	2.5 ± 0.43	0.38 ± 0.08
Fall	11	14.4 ± 6.54	2 ± 0.3	0.31 ± 0.08
15+ yr				
Spring	12	0.3 ± 0.14	0.3 ± 0.14	0.67 ± 0.14
Summer	6	5.8 ± 1.3	3 ± 0.37	0.56 ± 0.06
Fall	12	3.7 ± 1.37	1.3 ± 0.28	0.34 ± 0.11
100+ yr				
Spring	12	1.6 ± 0.62	0.9 ± 0.36	0.77 ± 0.09
Summer	4	22.8 ± 6.64	3.2 ± 0.48	0.43 ± 0.07
Fall	12	2.6 ± 1.9	0.4 ± 0.19	0.67 ± 0.14

Abundance, species richness, and Simpson's diversity index by site and season presented as mean ± S.E. Spring and Fall samples were collected in 2018 and 2019. Summer samples were collected in 2019 only.

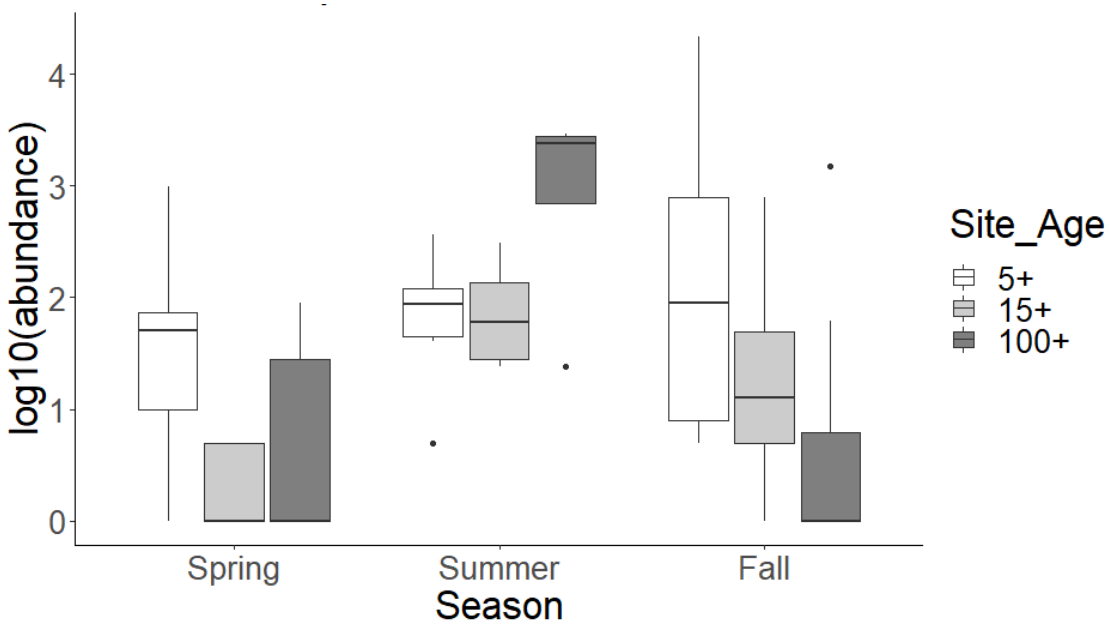


Figure 3.8 *Minnow trap abundance by site and season.*

Abundance on the y-axis has been log-transformed. Letters indicated significant groupings determined by Least Square Mean

Table 3.11 *Significance test of total abundance per minnow trap.*

Source	df	χ^2	*P*
Site	2	17.57	<0.0001*
Season	2	4.71	0.095
Site × Season	4	17.26	0.002*

Summary table of Type III Wald Chi-squared test for nekton abundance per minnow trap by site, season, and site × season interactions.

3.2.3 Species Richness

Species richness per trap differed significantly among the sites (χ^2 = 16.4, *P* = 0.0002) (Table 3.13). There is also a significant site × season interaction (χ^2 = 17.8, *P* = 0.001). In Spring, the 5+ yr site had significantly higher species richness per trap than the 15+ yr site (*P* = 0.01) (Fig 3.9). In Summer and Fall, species richness did not differ significantly between sites.

Species richness at the 15+ yr and 100+ yr sites also differed across seasons. The 15+ yr site had significantly lower species richness in Spring compared to Summer (*P* = 0.002*)*, and the 100+ yr site had significantly lower richness in Fall compared to Summer (Estimate = -2.05, *P* = 0.004*)*. The 15+ yr site did not change across seasons.

Minnow traps at the natural site had the highest mean Simpson's diversity (D = 0.68), followed by the 15+ yr constructed site (D = 0.51), and lastly the 5+ yr site (D = 0.42) (Table 3.10).

Table 3.12 *List of species collected at each site during each season.*

	Spring	Summer	Fall
5+ yr	*Adinia xenica* *Cyprinodon variegatus* *Fundulus spp.* *Gambusia holbrooki* *Mugil curema* *Neritina usnea* *Poecilia latipinna*	*Adinia xenica* *Callinectes sapidus* *Cyprinodon variegatus* *Fundulus spp.* *Gambusia holbrooki* *Neritina usnea* *Poecilia latipinna* *Uca virens*	*Callinectes sapidus* *Cyprinodon variegatus* *Fundulus spp.* *Gambusia affinis* *Neritina usnea* *Palaemonetes spp.* *Poecilia latipinna*
15+ yr	*Cyprinodon variegatus* *Neritina usnea* *Palaemonetes spp.*	*Callinectes sapidus* *Cyprinodon variegatus* *Fundulus spp.* *Gambusia holbrooki* *Neritina usnea* *Palaemonetes spp.* *Poecilia latipinna*	*Callinectes sapidus* *Farfantepenaeus aztecus* *Fundulus spp.* *Litopenaeus setiferus* *Neritina usnea* *Palaemonetes spp.* *Poecilia latipinna*
100+ yr	*Cyprinodon variegatus* *Farfantepenaeus aztecus* *Mugil cephalus* *Neritina usnea* *Palaemonetes spp.* *Uca virens*	*Adinia xenica* *Cyprinodon variegatus* *Fundulus spp.* *Gambusia holbrooki* *Poecilia latipinna* *Uca virens*	*Cyprinodon variegatus* *Fundulus spp.* *Litopenaeus setiferus* *Neritina usnea*

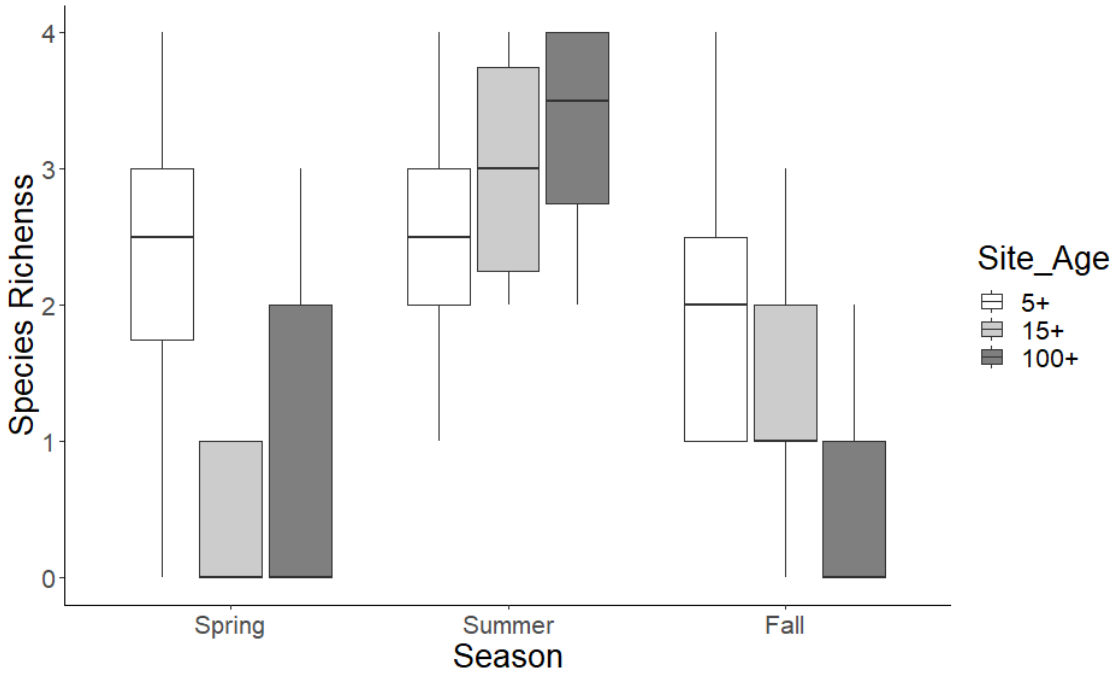


Figure 3.9 *Minnow trap species richness by site and season.*

Table 3.13 *Significance test of species richness per minnow trap.*

Source	df	χ^2	*P*
Site	2	16.41	<0.0001*
Season	2	0.45	0.80
Site × Season	4	17.8	0.0013*

Summary table of Type III Wald Chi-squared test for species richness per minnow trap by site, season, and site season × interactions.

Table 3.14 *Post hoc test for species richness per minnow trap.*

Contrast	Estimate	*P*
(5+) – (15+)	0.70	0.009*
(5+) – (100+)	0.72	0.007*
(15+) – (100+)	0.02	0.99

Pairwise comparison of site with Sidak adjustment for species richness per minnow trap by site.

3.2.4 Community Assemblage

The ANOSIM indicated a weak relationship between site and consumer community assemblage (permutations = 5000, R = 0.205, Significance = 0.0002). There is also a weak relationship between sampling event and community assemblage (R = 0.108, Significance = 0.01). Mean of ranks at the 5+ yr site was 728.34, at the 15+ yr site was 778.95 and at the 100+ yr site was 915.27. Mean of ranks between the groups was 948.99.

To visualize this relationship, we created an nMDS (k = 3) based on a Bray Curtis dissimilarity matrix, however, we received a warning of nearly zero stress (Fig 3.2.4). When plotted, the nMDS showed an outlier sample caused by the presence of *Gambusia affinis* at that sample only. For that reason, we combined *G. affinis* and *Gambusia holbrooki* for the rest of the analysis. We created another nMDS (k = 3, stress = 0.115) with a non-metric fit $R^2 = 0.987$ and linear fit $R^2 = 0.921$. The three axes in the nMDS explain 45.3% of the estimated variance, with the first axis explaining 20.9% and the second axis explaining 15.8%. The NMDS plot does not show strong separation between samples from the 3 sites. We also plotted species that had significant ($P < 0.1$) correlation along the first two axes, with vector length representing magnitude of significance. Species excluded from the nMDS plot were *Poecilia latipinna (P* = 0.63*), Adinia xenica (P* = 0.20*), Uca spp. (P* = 0.31*),* and *Mugil cephalus (P* = 0.352*).*

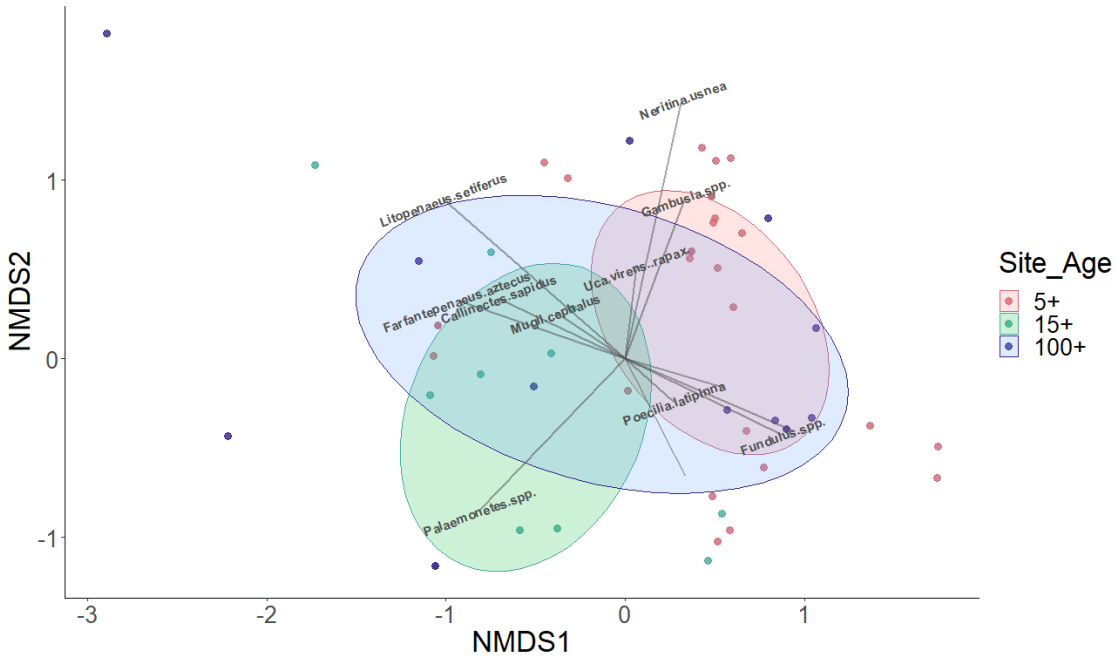


Figure 3.10 *Non-metric multidimensional scaling plot of consumers collected in minnow traps.*

Samples are separated by site, represented by color. Centroids are darker points within 40% confidence intervals around samples by site. Species vectors length corresponds to significance with along the first 2 axes.

3.3 Stable Isotope Results

3.3.1 Sediment

Mean $\delta^{13}C$ and $\delta^{15}N$ stable isotopes of *S. alterniflora* sediment differed significantly by site (Table 3.16). *S. alterniflora* sediment $\delta^{13}C$ at the 100+ yr site was significantly more enriched compared to the 15+ yr site ($P = 0.017$) and the 5+ yr site ($P < 0.001$), with no difference between the constructed sites ($P = 0.33$). *S. alterniflora* sediment $\delta^{15}N$ at the 15+ yr site was significantly more enriched compared to the 5+ yr site ($P = 0.01$) and 100+ yr site ($P = 0.008$), with no difference between the 5+ yr and 100+ yr sites (Table 3.17). *J. roemerianus* sediment, only collected at the natural site, had

mean $\delta^{13}C$ values of -24.1‰ ± 1.5 and mean $\delta^{15}N$ of 2.7‰ ± 0.8 (Fig 3.3.1). There was no significant difference in C:N ratio between sites (P = 0.16) (Fig. 3.11)

Table 3.15 *Summary table for mean sediment isotope values.*

Species	‰ $\delta^{13}C$ ± SD	Range $\delta^{13}C$	‰ $\delta^{15}N$ ± SD	Range $\delta^{15}N$	C:N (mass:mass)
5+					
Juncus	–	–	–	–	–
Spartina	-23.4 ± 1.1	4.6	2.8 ± 0.9	3.1	14.4
15+					
Juncus	–	–	–	–	–
Spartina	-22.0 ± 1.0	2.3	4.1 ± 0.1	0.3	12.4
100+					
Juncus	-24.1 ± 1.5	3.8	2.7 ± 0.8	2.3	16.5
Spartina	-18.7 ± 2.6	7.0	2.6 ± 0.6	1.5	13.6

Stable isotopes δ13C, δ15N, and C:N ratio by site and primary vegetation species present in the sample.

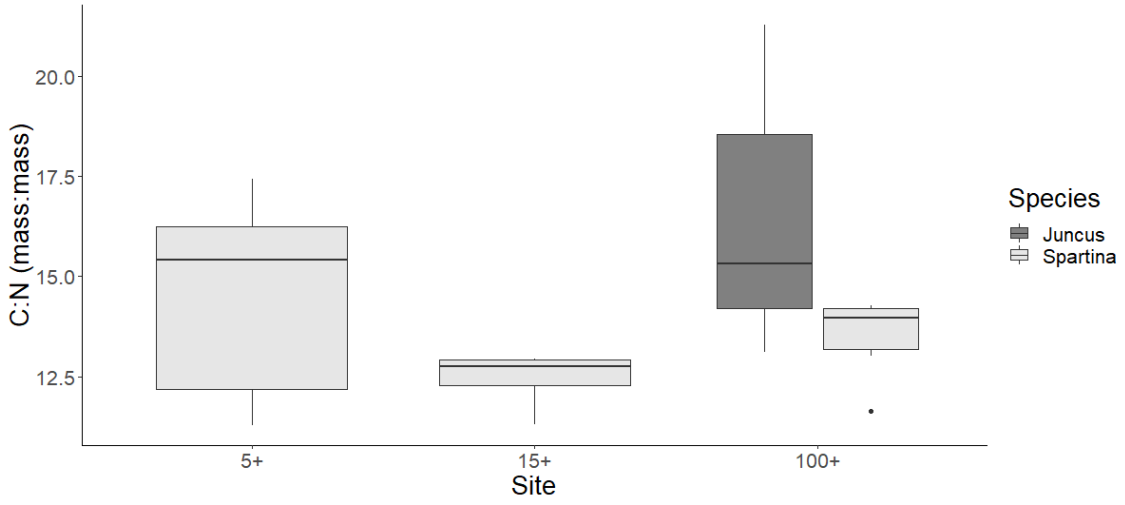


Figure 3.11 *C:N ratio in sediment samples by site.*

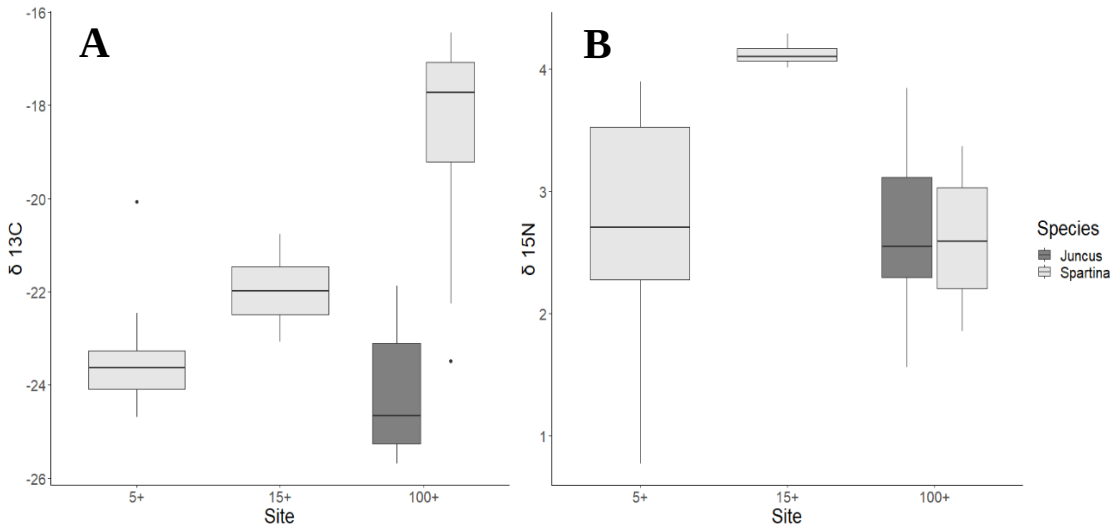


Figure 3.12 *Range of sediment isotopes values $\delta^{13}C$ (A) and $\delta^{15}N$ (B).*

Separated by site and vegetation species present.

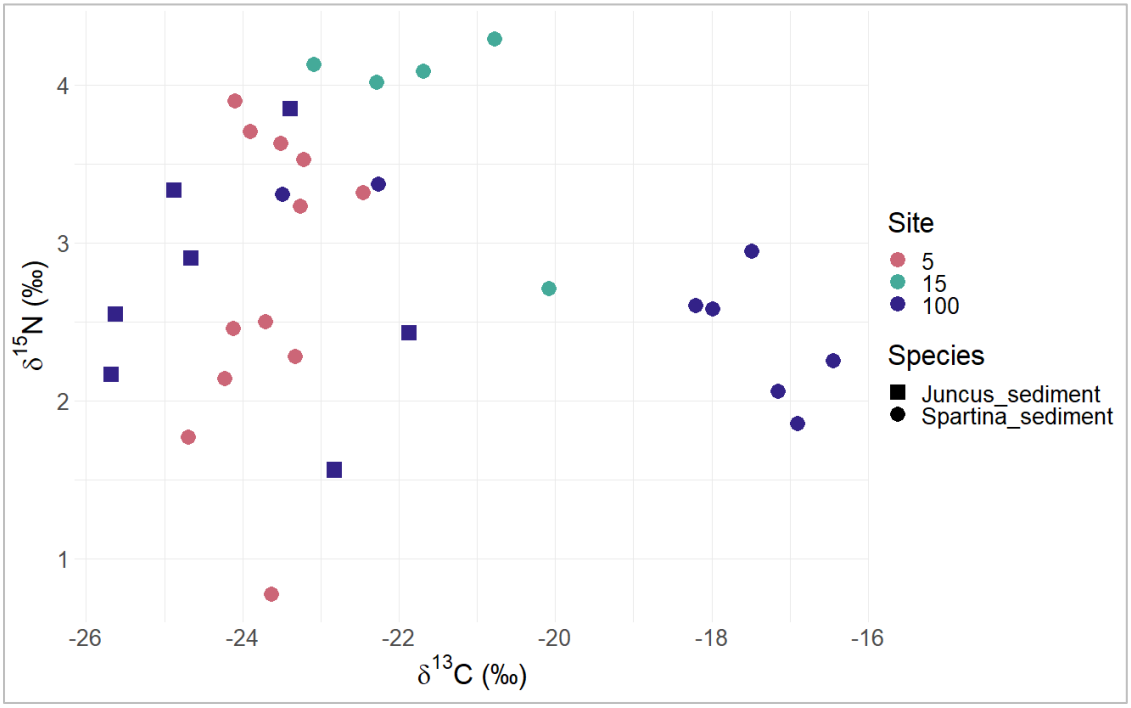


Figure 3.13 *Plot of sediment stable isotopes.*

Separated by site (color) and primary vegetation species present in sample (shape)

Table 3.16 *Summary ANOVA table for S. alterniflora sediment isotopes by site*

Stable Isotope	Source	Df	F value	*P*
$\delta^{13}C$	Site	2	17.7	<0.0001
	Residuals	22		
$\delta^{15}N$	Site	2	6.3	0.007
	Residuals	22		

Table 3.17 *Post hoc Tukey test of S. alterniflora sediment.*

Contrast	$\delta^{13}C$ Estimate	$\delta^{13}C$ *P*	$\delta^{15}N$ Estimate	$\delta^{15}N$ *P*
(15+) – (5+)	1.44	0.33	1.36	0.01*
(100+) – (5+)	4.66	<0.001*	-0.14	0.9
(100+) – (15+)	3.22	0.02*	-1.5	0.008*

Summary of Multiple Comparisons of Means with Tukey contrast separating S. alterniflora sediment δ13C and δ15N isotope values by site.

3.3.2 Primary Producers

Mean $\delta^{13}C$ and $\delta^{15}N$ stable isotopes of *S. alterniflora*, *J. roemerianus* and BMA differed significantly by site (Table 3.19). There was no significant difference in *S. alterniflora* $\delta^{13}C$ values between sites however there was a significant different in *Spartina* $\delta^{15}N$ between sites. *S. alterniflora* at the 5+ yr and 15+ yr sites were significantly more enriched in $\delta^{15}N$ than the 100+ yr site ($P = 0.008$ and $P = 0.001$, respectively), with no difference between the 5+ yr and 15+ yr sites ($P = 0.23$) (Table 3.20).

Similarly, there was no significant difference in *J. roemerianus* $\delta^{13}C$ values between sites however there was a significant difference in $\delta^{15}N$ between sites. *J. roemerianus* at the 15+ yr site was significantly more depleted in $\delta^{15}N$ than the 100+ yr site ($P < 0.001$)

Finally, there was no significant difference in benthic microalgae (BMA) $\delta^{15}N$ values between the sites, however there was significant difference in $\delta^{13}C$. BMA at the 15+ yr site was significantly more enriched in $\delta^{13}C$ than the 5+ yr and 100+ yr sites (P = 0.001 and P = 0.004, respectively).

Table 3.18 *Summary table for primary producer stable isotopes.*

Species Present	N	‰ $\delta^{13}C \pm SD$	Range $\delta^{13}C$	‰ $\delta^{15}N \pm SD$	Range $\delta^{15}N$
5+ yr					
Juncus	3	-26.8 ± 0.2	0.5	1.8 ± 0.9	1.6
Spartina	25	-13.9 ± 1.1	4.1	4.8 ± 2.0	9.9
BMA	11	-23.8 ± 2.0	7.4	2.64 ± 1.2	4.4
15+ yr					
Juncus	3	-26.3 ± 1.8	3.5	-0.5 ± 0.4	0.8
Spartina	6	-13.6 ± 1.1	2.7	5.8 ± 1.8	5.6
BMA	10	-18.5 ± 2.6	9.1	1.1 ± 1.5	5.9
100+ yr					
Juncus	27	-25.6 ± 2.2	9.4	3.2 ± 1.2	4.7
Spartina	21	-14.2 ± 1.03	3.6	3.66 ± 1.11	4.2
BMA	12	-22.1 ± 1.9	6.1	1.1 ± 1.4	5.2

Mean values of δ13C and δ15N by site and vegetation species.

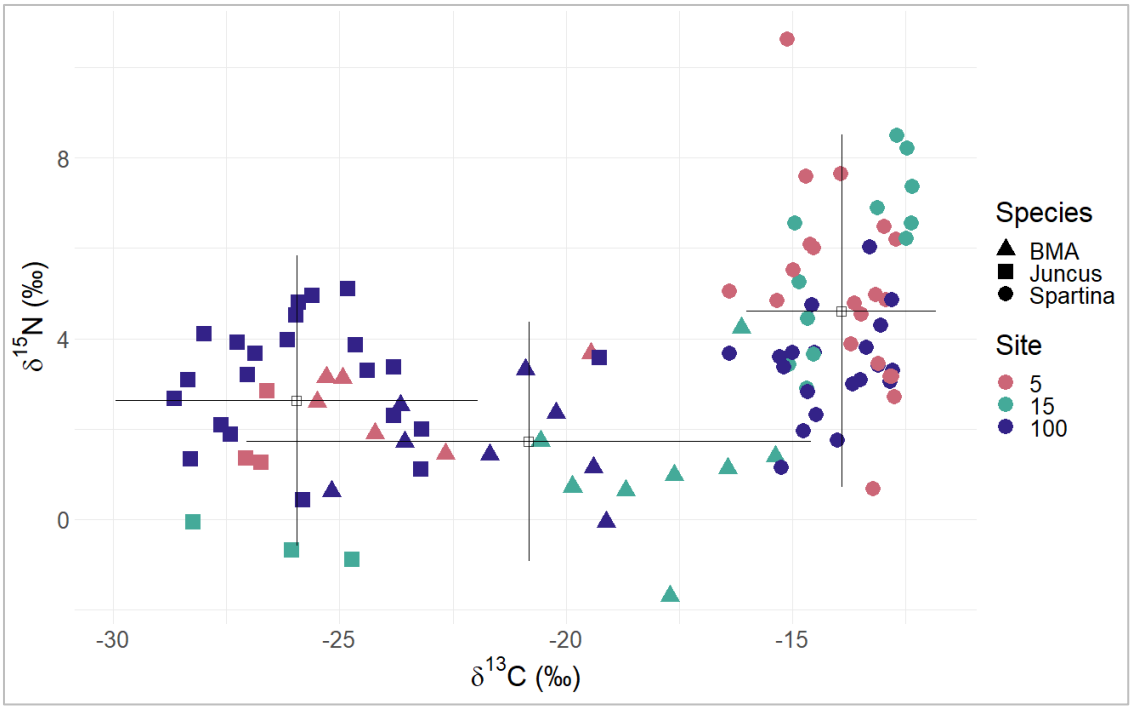


Figure 3.14 *Plot of vegetation stable isotopes.*

Separated by site (color) and vegetation species (shape)

Table 3.19 *Summary ANOVA table of vegetation isotopes by site*

Vegetation	Stable Isotope	Source	Df	F value	*P*
Spartina	$\delta^{13}C$	Site	2	0.69	0.5
		Residuals	49		
	$\delta^{15}N$	Site	2	8.7	<0.0005
		Residuals	49		
Juncus	$\delta^{13}C$	Site	2	0.38	0.69
		Residuals	26		
	$\delta^{15}N$	Site	2	13.6	<0.001*
		Residuals	26		
BMA	$\delta^{13}C$	Site	2	14.5	<0.0001*
		Residuals	19		
	$\delta^{15}N$	Site	2	2.5	0.11
		Residuals	19		

Table 3.20 *Tukey post hoc test of vegetation isotopes by site.*

Vegetation	Contrast	$\delta^{13}C$ Estimate	$\delta^{13}C$ *P*	$\delta^{15}N$ Estimate	$\delta^{15}N$ *P*
Spartina	(15+) – (5+)	0.16	0.91	0.93	0.23
	(100+) – (5+)	-0.28	0.69	-1.7	0.008*
	(100+) – (15+)	-0.44	0.509	-2.45	0.001*
Juncus	(15+) – (5+)	0.47	0.96	-2.36	0.05
	(100+) – (5+)	1.02	0.70	1.33	0.18
	(100+) – (15+)	0.55	0.90	3.70	<0.001*
BMA	(15+) – (5+)	5.9	0.0001*	-1.5	0.10
	(100+) – (5+)	1.9	0.22	-1.0	0.32
	(100+) – (15+)	-3.9	0.004*	0.5	0.72

Pairwise comparison of means with Tukey adjustment for S. alterniflora and BMA vegetation stable isotope δ13C and δ15N by site.

3.3.3 Consumers

Mean $\delta^{13}C$ values did not differ between acid washed samples and non-acid washed samples (Estimate = 0.98, $R^2 = 0.96$ and Estimate = 1.02, $R^2 = 0.85$, respectively). Therefore, we used isotope values from the non-acid washed samples for the rest of the analysis.

Mean $\delta^{13}C$ and $\delta^{15}N$ of consumers differed significantly by site ($\delta^{13}C$ $P < 0.001$, $\delta^{15}N$ $P < 0.001$) and feeding strategy ($\delta^{13}C$ $P = 0.03$, $\delta^{15}N$ $P < 0.001$) (Table 3.23). Consumer isotope values at the 15+ yr site were significantly more enriched in $\delta^{13}C$ compared to the 100+ yr site ($\delta^{13}C$ $P = 0.03$, $\delta^{15}N$ $P = 0.02$). Similarly, consumer isotopes at the 15+ yr site were more depleted in $\delta^{15}N$ compared to the 5+ yr site ($P < 0.001$) and the 100+ yr site ($P = 0.02$). In terms of feeding strategies, omnivores consistently had more enriched $\delta^{13}C$ and $\delta^{15}N$ than grazers and filter feeders in each site. Filter feeders were only collected at the 100+ yr site and could not be compared between sites.

There was also an interaction effect of feeding strategy × lifestyle, site feeding × strategy, and site × lifestyle.

Ellipse overlap was also used to show isotopic similarity between groups (Fig 3.15). Ellipse overlap is defined as the proportion of non-overlapping area to the area of the two ellipses and was drawn around 40% of consumer isotope data. Consumer isotopes were-grouped by feeding strategy at each site and ellipses were compared between sites. Omnivores at the 5+ yr site and 100+ yr site had high ellipse overlap of 51.2% (Table 3.25). Omnivores at the 15+ yr site, on the other hand, had lower ellipse overlap with omnivores from the 5+ yr and 100+ yr sites (3.37% and 0%, respectively). Grazers at the 5+ yr site and the 100+ yr site had ellipse overlap of 6.24%. There was no overlap with grazers at the 15+ yr site.

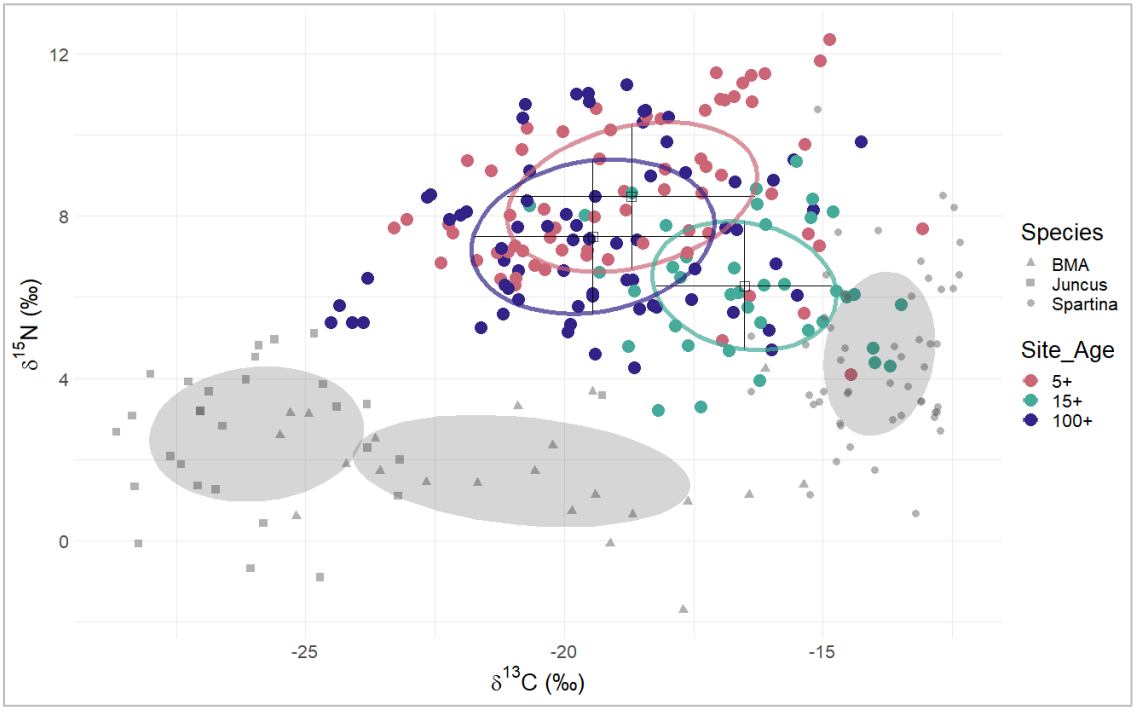


Figure 3.15 *Plot of consumer stable isotopes*. *Separated by site (color). Ellipses are drawn at 40% with a bivariate normal distribution. Error bars display mean with standard deviation. Shaded grey regions indicate 40% ellipses drawn around stable isotopes of vegetation species J. roemerianus, S. alterniflora, and BMA.*

Table 3.21 *Summary table for consumer stable isotopes by site.*

Site	N	‰ $\delta^{13}C \pm SD$	Range $\delta^{13}C$	‰ $\delta^{15}N \pm SD$	Range $\delta^{15}N$
5+	66	-18.7 ± 2.4	10.2	8.5 ± 1.8	8.3
15+	39	-16.5 ± 1.7	7.2	6.3 ± 1.6	6.1
100+	69	-19.5 ± 2.3	10.24	7.5 ± 1.9	7.0

Table 3.22 *Summary table for consumer stable isotopes by site, feeding strategy and lifestyle.*

	Sample Type	N	‰ $\delta^{13}C$ mean ± SD	Range $\delta^{13}C$	‰ $\delta^{15}N$ mean ± SD	Range $\delta^{15}N$
5+						
	Filter Feeders	–	–	–	–	–
	Grazers	9	-19.4 ± 2.8	9.31	7.0 ± 0.4	1.2
	Omnivore	57	-18.6 ± 2.3	8.83	8.7 ± 1.8	8.3
	Resident Species	60	-18.8 ± 2.5	10.2	8.5 ± 1.8	8.3
	Transient Species	6	-17.9 ± 1.1	2.4	8.4 ± 2.0	5.7
15+						
	Filter Feeders	–	–	–	–	–
	Grazers	4	-17.6 ± 1.1	2.54	3.8 ± 0.7	1.6
	Omnivore	35	-16.4 ± 1.8	7.19	6.6 ± 1.4	5.0
	Resident Species	31	-16.4 ± 1.8	7.2	6.5 ± 1.7	6.1
	Transient Species	8	-16.9 ± 1.4	4.6	5.6 ± 0.7	2.0
100+						
	Filter Feeders	10	-20.8 ± 1.6	4.05	7.6 ± 0.7	2.1
	Grazers	32	-19.7 ± 2.7	10.24	6.0 ± 1.0	5.6
	Omnivore	27	-18.7 ± 1.8	6.0	9.2 ± 1.4	4.4
	Resident Species	66	-19.5 ± 2.3	9.3	7.4 ± 1.9	7.0
	Transient Species	3	-18.1 ± 3.4	6.4	9.2 ± 0.7	1.4

Table 3.23 *Summary ANOVA table for consumer stable isotopes.*

Stable Isotope	Df	$\delta^{13}C$ F value	$\delta^{13}C$ *P*	$\delta^{15}N$ F value	$\delta^{15}N$ *P*
Site	2	13.1	<0.0001*	33.2	<0.0001*
Feeding Strategy	1	4.77	0.03*	93.9	<0.0001*
Lifestyle	1	0.08	0.77	1.47	0.23
Site × Feeding Strategy	2	0.09	0.85	3.43	0.03*
Site × Lifestyle	2	0.78	0.39	0.56	0.57*
Feeding Strategy × Lifestyle	1	6.61	0.01*	6.13	0.01*
Residuals	154				

Mean δ13C and δ15N, by site, sample type, and feeding strategy.

Table 3.24 *Tukey post hoc test of consumer stable isotopes.*

Contrast	$\delta^{13}C$ Estimate	$\delta^{13}C$ *P*	$\delta^{15}N$ Estimate	$\delta^{15}N$ *P*
(15+) – (5+)	1.40	0.31	-3.2	<0.001*
(100+) – (5+)	-1.26	0.49	-1.2	0.08
(100+) – (15+)	-2.66	0.03*	2.1	0.02*

Pairwise comparison of means with Tukey adjustment for consumer δ13C and δ15N isotopes by site.

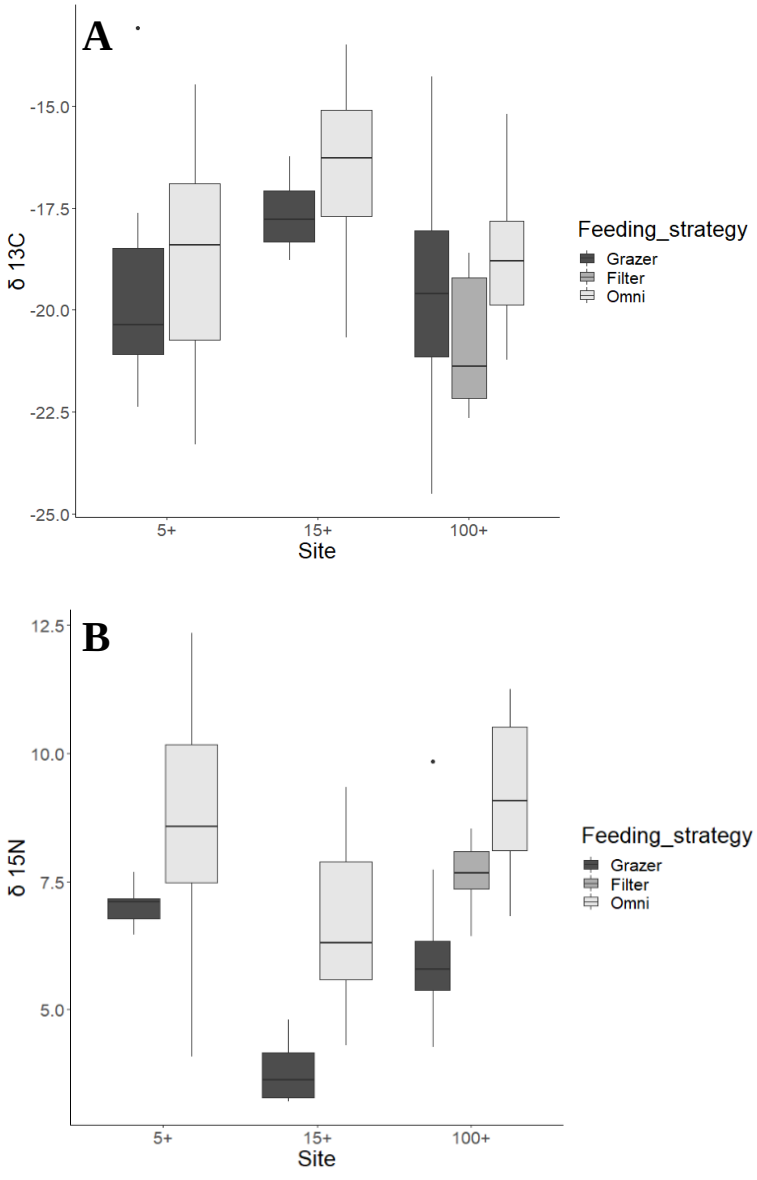


Figure 3.16 *Boxplot of consumer isotope values*

(A) $\delta^{13}C$ and (B) $\delta^{15}N$ by site and feeding strategy.

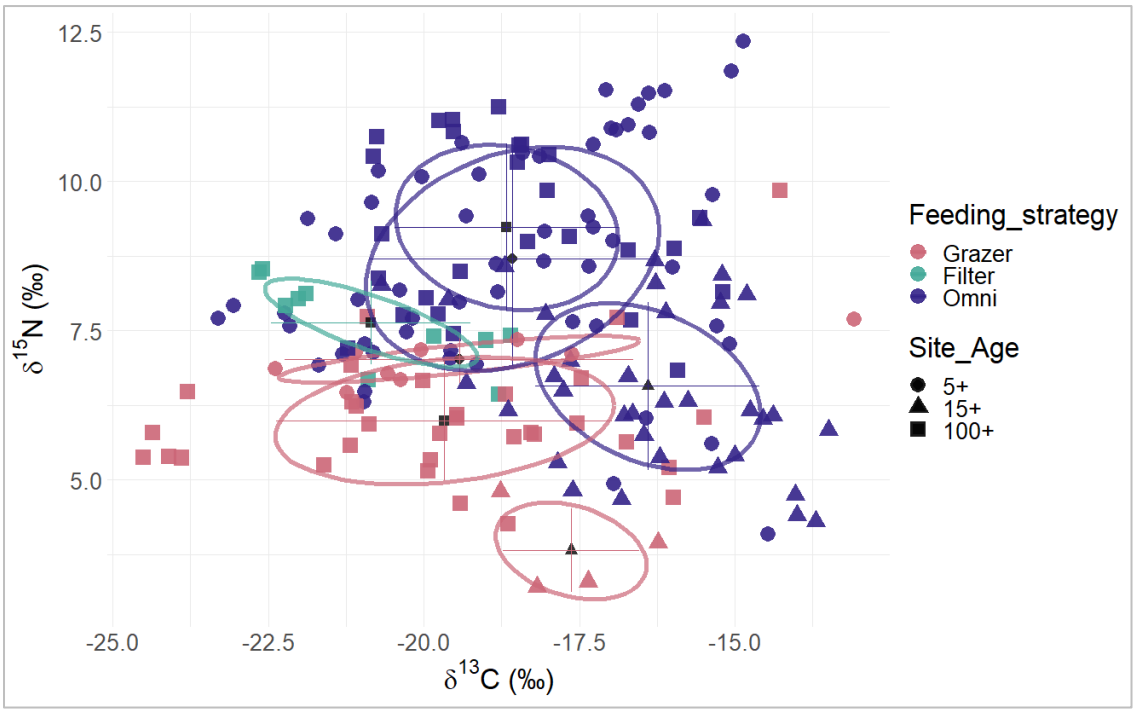
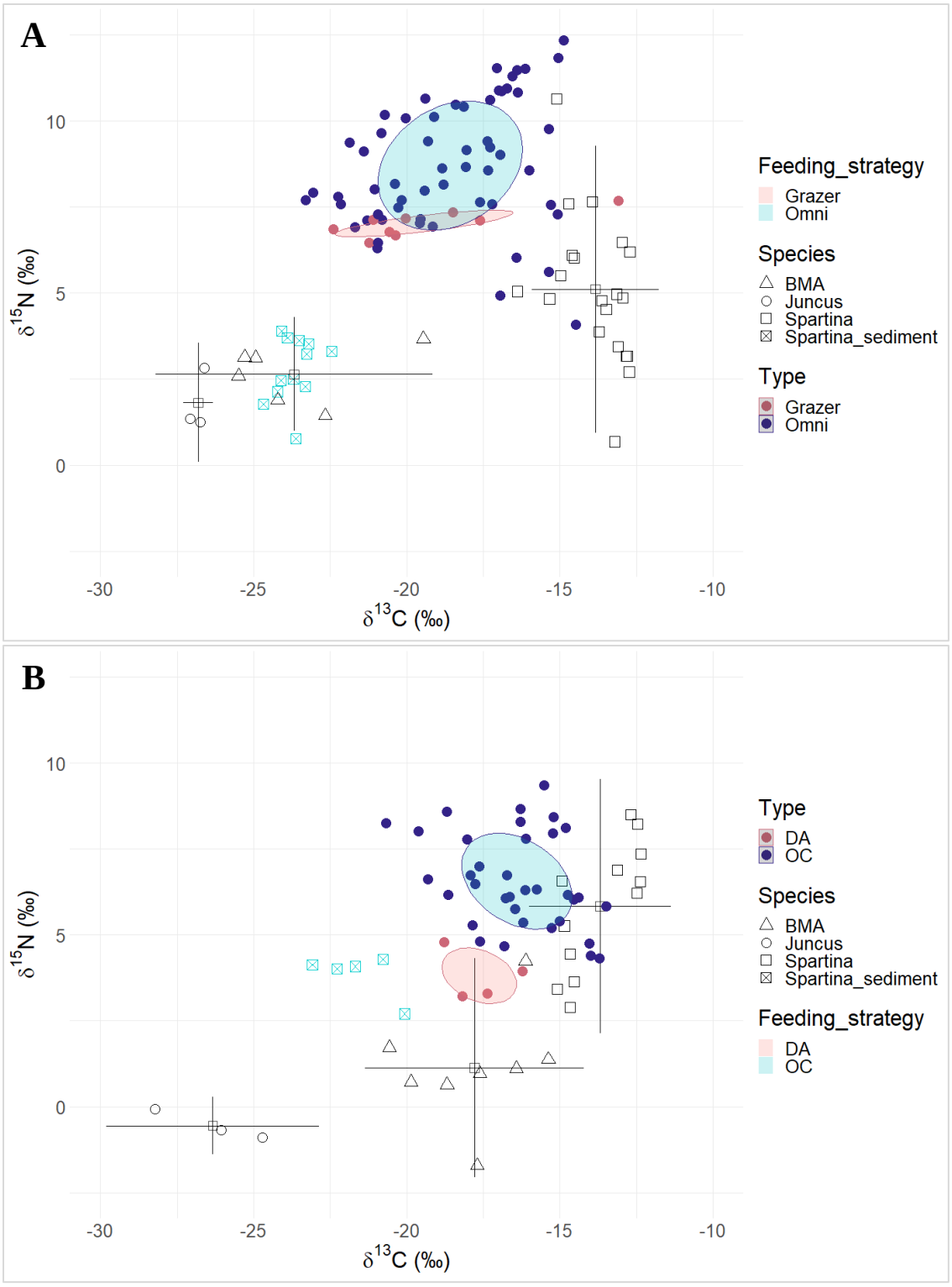


Figure 3.17 *Plot of consumer stable isotopes separated by feeding strategy.* *Separated by site (shape) and feeding strategy (color), with 40% ellipses drawn around each site × feeding strategy combination. Black shapes and lines represent the mean and standard deviations of each site × feeding strategy combination.*

Table 3.25 *Percent ellipses overlap by site and feeding group using SIBER.*

	Grazer	Omnivore
(5+) – (15+)	0%	3.37%
(5+) – (100+)	6.24%	51.2%
(15+) – (100+)	0%	0%

Ellipse overlap is defined as the proportion of non-overlapping area to the area of the two ellipses.


A
10
5
0
-30
-25
-20
-15
-10
δ15N (‰)
δ13C (‰)
Feeding_strategy
Grazer
Omni
Species
BMA
Juncus
Spartina
Spartina_sediment
Type
Grazer
Omni
B
10
5
0
-30
-25
-20
-15
-10
δ15N (‰)
δ13C (‰)
Type
DA
OC
Species
BMA
Juncus
Spartina
Spartina_sediment
Feeding_strategy
DA
OC

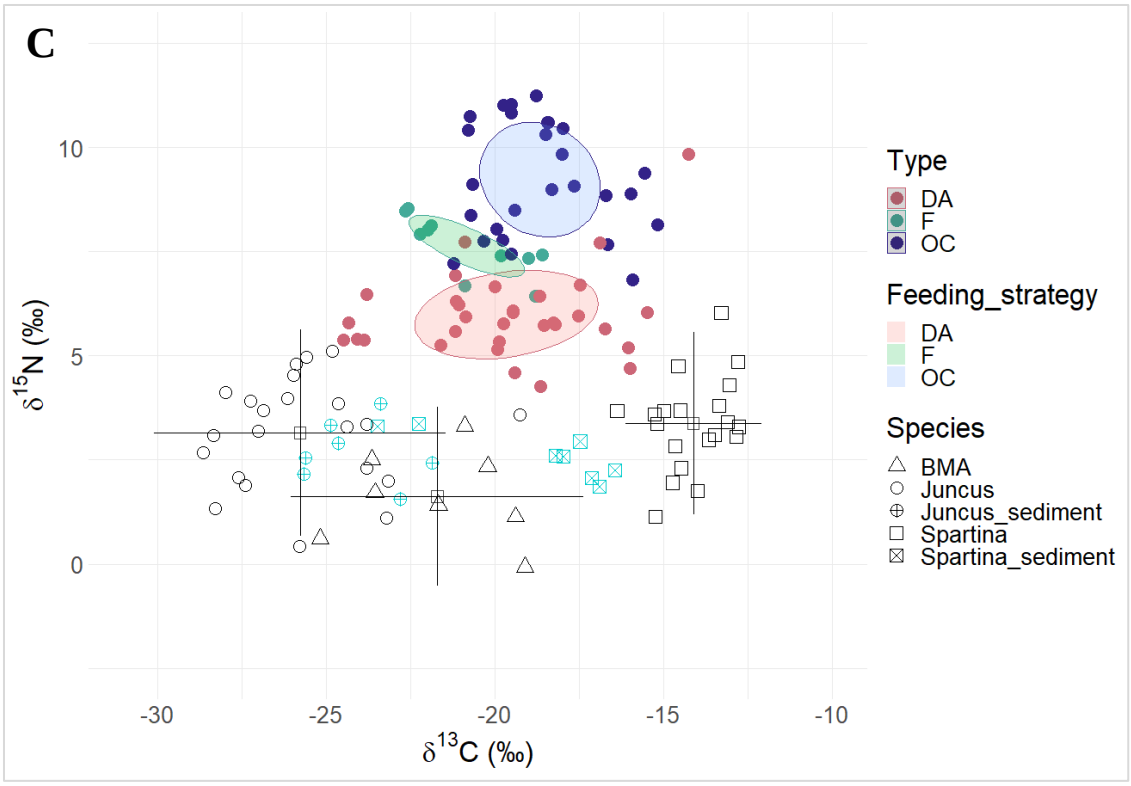


Figure 3.18 *Plot of stable isotopes at each site.*

Separated by sediment, vegetation, and consumers separated by feeding strategy. (A) Constructed 5+ yr site, (B) constructed 15+ yr site, and (C) natural 100+ yr site.

CHAPTER IV – DISCUSSION

A major goal of coastal marsh restoration is to establish landscape, soil, and vegetation characteristics that support ecological functions that are equivalent to a natural system with the intention of supporting similar consumer communities and trophic interactions (Llewellyn and La Peyre 2011; Hollweg et al. 2019). In this study, we used structural and trophic metrics of vegetation and consumers to compare two created marshes of different ages with a natural reference marsh on Deer Island, MS.

We found major differences in community structures between sites. Marsh-surface invertebrates and nekton showed opposite trends in abundance, whereas nekton diversity showed very little difference among sites. Stable isotopes of consumers showed the older restored site had significantly different nitrogen and carbon values than the natural site. In many ways, the younger 5+ yr restored site more resembled the natural marsh than the older 15+ yr restored site. Possible explanations can be found in landscape, soil and vegetation differences between sites, such as significantly higher elevations and lower SOC levels at the constructed sites (Murphy 2020). Results from this project demonstrate that created marshes using Beneficial Use techniques on Deer Island have not yet created a "natural" system that supports crucial invertebrate and nekton communities, but there is still potential for the 5+ yr site to reach natural levels over time.

4.1 Marsh-surface invertebrates

We used the presence and abundance of organisms to compare the sites' ability to support consumers with the idea that higher abundance indicates higher quality of marsh

(La Peyre et al. 2007). At the natural site, almost all quadrats (97.95%) contained at least one organism while only a small percentage of the quadrats at the 5+ yr and 15+ yr sites contained organisms (17.37% and 13.53% respectively). The abundant presence of invertebrates at the natural site versus their scarce presence at the two constructed sites indicates that conditions were likely more favorable for invertebrate feeding and growth at the natural marsh. In addition, mean abundance per quadrat was significantly higher at the natural site compared to the two constructed marshes for all invertebrate species sampled.

Fiddler crab burrow abundance was significantly higher in the natural site across all sampling events, except during Fall 2019 when natural abundances dropped below that of the constructed sites. Similarly, olive snail abundance was highest at the natural site across all sampling events, except during Fall 2019 when natural abundances dropped. This decline of fiddler crab and olive snail in abundance in Fall 2019 could be caused by the Bonne Carré Spillway opening along the Mississippi River in Summer 2019. The excessive freshwater output from the spillway resulted in nutrient loading and change in salinity within the Mississippi Sound, causing major die-offs of oysters, crab and shrimp (Byrd 2019). Shrimp landings dropped 33% and oyster landings dropped to nearly zero in 2019 (Byrd 2019). Nutrient loading and freshwater input also likely caused the cyanobacteria algal bloom that affected the area during that summer. The adverse effects of the Bonne Carré Spillway opening negatively affected the inhabitants within the Mississippi Sound, and could have affected invertebrates and nekton consumers on Deer Island, MS.

Olive snail abundance was evaluated using both quadrat and minnow trap sampling. This was because olive snails are known to submerged themselves underwater for long periods of time to feed on the marsh floor (Heard 1982). We found that olive snails collected with minnow traps showed an opposite trend as those collected with quadrats, with the highest abundance per minnow trap being at the 5+ yr constructed site. This suggests that olive snails are utilizing the submerged areas more at the 5+ yr site, while utilizing the marsh surface more at the natural site. A potential explanation to these differences between sampling methods could be the bowl-like landscape at the 5+ yr site. The high berm prohibits marine predators from foraging on olive snails, potentially allowing them to thrive and reproduce in this favorable habitat. However, this landscape would not prevent marsh birds, a main predator of olive snails, from feeding within the site.

Periwinkles and ribbed mussels were absent from the constructed sites all together, possibly due to unfavorable environmental conditions and the sites' hydrology. Ribbed mussels rely on low elevation for regular tidal flooding to facilitate filter feeding (Moody and Kreeger 2021). The 15+ yr site does not experience flooding due to high elevation while the 5+ yr site experiences irregular flooding with minimal water flow due to the high berm, suggesting these sites may be unfavorable habitats for ribbed mussels. Periwinkle recruitment is more associated with the recovery of above ground marsh vegetation as they use the stalks to avoid predators at high tide (Baumann et al. 2018). Live aboveground vegetation biomass at the 15+ yr site was significantly lower than at the natural site, while vegetation biomass at the 5+ yr site was comparable to the natural site. For that reason, we can assume that vegetation is not the controlling factor for

periwinkle abundance at the 5+ yr site, and another environmental variable is affecting this. The constant flooding within the site is a likely explanation. Despite having gills, periwinkles do not often stay submerged for long periods of time, therefore the 5+ yr site would not provide a favorable habitat for them (Heard 1982). The hydrology of both constructed sites could also limit the recruitment and dispersal of our sampled marsh-surface invertebrates. The constructed sites have very little connectivity to open water due high berms and lack of rivers or channels that would allow movement between sites. Overall, the constructed sites are physically more isolated than the natural marsh, which may negatively affect the abundance of marsh surface invertebrates.

Similar invertebrate recovery at constructed marshes has been recorded in other restoration projects along the northern Gulf of Mexico. Restored marshes (age 5 and 15) in Galveston Bay, TX had fiddler crab and periwinkles densities that were substantially higher in reference areas compared to their constructed counterparts (Staszak and Armitage 2013), which is similar to the results seen in this study. However, other studies showed a quicker recovery of benthic invertebrates than seen at our restored marshes on Deer Island. A meta-analysis of periwinkle recovery in the northern Gulf of Mexico documented periwinkle density recovered to natural levels in ~4 years (Baumann et al. 2018). The variable results in invertebrate recovery seen across the Gulf of Mexico may be in part due to differences in landscape and soil development. The accumulation of near surface organic matter and detritus is crucial for reestablishing benthic communities in created marshes because it serves as a major food source (Moy and Levin 1991b; Craft 2000; Craft and Sacco 2003). Soil organic matter at our constructed marshes were consistently lower than at the natural 100+ site (Murphy 2020). A study conducted by

Craft et al. (2007) in created *S. alterniflora* marshes in North Carolina found that constructed marshes may take at least 28 years to reestablish soil characteristics (Craft et al. 2007). Elevation, bulk density, and grain size also serve as good predictors of constructed site health because they can promote the retention of organic matter (Levin et al. 1996; Thomas 2004). Created marshes with lower elevation, like our 5+ yr restored site, were found to have a more rapid infauna recovery, followed by epifaunal colonization with fiddler crabs being one of the first colonists (Levin et al. 1996). Murphy (2020) found that the natural site had significantly lower elevation than the 15+ yr and 5+ yr restored site. Although there are still major environmental differences between the constructed and natural sites, the 5+ yr site was more similar to the natural site than the 15+ yr site in numerous ways, suggesting it can support invertebrate and nekton growth better than its older counterpart. This highlights the importance of site construction, particularly site elevation and source material, in determining what invertebrate community will reestablish in the site.

4.2 Nekton

Minnow traps were used to examine the nekton community residing in submerged portions of the marsh. We observed that the likelihood of catching at least one organism in a trap was the opposite to that observed in quadrats. The 5+ yr constructed site had the highest percentage (93.1%) of minnow traps containing at least one organism, followed by the 15+ yr site (66.7%), and lastly the 100+ yr site with less than half of the traps (46.4%) containing one or more organisms. While conditions at the natural site may be more favorable for marsh surface invertebrates, conditions at the 5+ yr site may be more favorable for nekton.

Mean nekton abundance and species richness in the traps differed significantly among sites and had significant site × season interactions. The 5+ yr site had the most individuals per trap during Spring and Fall. The 5+ yr site also had the highest species richness across all seasons. However, in Summer, there was no difference in nekton abundance or species richness across sites. The high nekton abundance and richness at the 5+ yr site compared to the natural site is surprising, and is inconsistent with other marsh restoration studies which found nekton abundance highest in reference marshes (Hollweg et al. 2019). Elevation and resident species' life history may explain these unexpected results. The bowl-like landscape at the 5+ yr site allows water and nekton to enter during high tides or storm events and traps nekton during low tides. Some marsh residents, such as *Fundulus spp*., have adapted to survive in small ponds under stressful conditions for long periods of time, which may have allowed these marsh residents to flourish (Griffith 1974; Virani and Rees 2000; Kneib 2003). In addition, the high berm prevents marine predators, which could normally control nekton populations from entering the site with daily tidal flooding. However, this would not prevent marsh birds from foraging within the site. Nekton breeding during the spring and summer could have also contributed to the high abundance seen at all sites during the summer.

We caught fewer nekton at the natural site compared to the 5+ yr site, despite regular flooding and movement of water through created tidal channels (mosquito ditches). This is inconsistent with results seen in other paired restoration studies in the northern Gulf of Mexico. In a meta-analysis of nekton recovery, Hollweg et al (2019) found that nekton in a created marsh took ~14 years to reach natural levels (Hollweg et al. 2019).

A potential explanation to why our results differs from other studies may be due to more predator interactions at the natural site, which potentially prevented prey nekton species from becoming overly abundant (Hirzel and Le Lay 2008). Another explanation could be a sampling bias in the minnow traps that prevented certain species from being captured while targeting others. Minnow traps are a passive, size-selective sampling gear, and have been shown to vary in efficiency (Rozas and Minello 1997; Layman and Smith 2001). Results from Layman & Smith (2001) found that minnow traps characterized significantly different fish assemblages than exhaustive seining, with almost all minnow trap species being the shallow-water, marsh resident *Fundulus spp.* (Layman and Smith 2001). Species most often excluded from minnow traps include larger, transient species or pelagic feeding fish that do not frequent the bottom which are more often found in open water habitats similar our natural site. Layman & Smith (2001) also found that in a controlled lab setting, *F. heteroclitus* were observed moving in and out the traps with ease, depending on their size (Layman and Smith 2001). This suggests small organisms could be caught in the field and exit the trap before it is collected, and the trap contents are recorded. Minnow traps are known to selectively capture certain resident species, such as *F. heteroclitus*, and may be a poor representation of the entire community assemblage. Instead, enclosure samplers such as throw traps have been shown to have a higher catch efficiency and lower variability for small organisms in shallow water because they are less size restrictive and are less likely for animals to escape from (Rozas and Minello 1997).

For this reason, community assemblage results from the nMDS and ANOSIM should be interpreted with caution. Results from the ANOSIM indicate there is a

significant difference in community assemblage between sites, but this difference is small. To visualize results from the ANOSIM, minnow trap samples were plotted in ordination space with nMDS based on a Bray Curtis dissimilarity matrix of the consumer assemblage collected using minnow traps. In terms of position on the nMDS plot, it is apparent that minnow trap assemblage at the 5+ yr and 15+ yr constructed sites are more different from each other (more separated along the first axis) than they are from the natural site. Minnow traps from the natural site exhibit wide variability compared to the constructed sites. Species that had a significant correlation along the first 2 axes were also plotted with vector length representing magnitude of significance.

An addition, the three axes of the nMDS plot only explained 45.3% of the estimated variance, suggesting that site may not be the best indicator of community assemblage in the minnow traps. Other environmental variables that may describe the nekton community assemblage better include area of marsh edge, connectivity to open water, dissolved oxygen, water temperature, or salinity.

4.3 Stable Isotope Analyses of Trophic Interactions

When assessing the functional equivalence of created marshes, it is important to pair structural metrics such as those described above with stable isotopes to understand how resources are being allocated and to characterize trophic support (Boecklen et al. 2011). Studies have compared structural indicators with stable isotopes and found that while nekton abundance and vegetation may be structurally equivalent, trophic support to predator species may be lacking (Llewellyn and La Peyre 2011). In this study, we measured $\delta^{13}C$ and $\delta^{15}N$ values of sediment, vegetation, and consumers at each site to compare trophic support between the constructed and natural sites.

Stable isotopes of sediment with *S. alterniflora* and *J. roemerianus* present in the sample varied in $\delta^{13}C$ across sites. *S. alterniflora* sediment at the constructed sites had significantly more depleted $\delta^{13}C$ values than *S. alterniflora* sediment samples at the natural site. This was surprising, as the constructed sites are dominated primarily by *S. alterniflora,* with almost no *J. roemerianus* present. A potential explanation to this depleted $\delta^{13}C$ signal could be the source dredge material used to fill the constructed sites. The source material from the Biloxi shipping channel was of marine origin, with phytoplankton likely being the main contributor to the organic carbon pool in the marine sediment. Dillon et al (2015) found that phytoplankton collected in Grand Bay NEER reflected a depleted $\delta^{13}C$ signal, ranging from -21.6‰ to -24.8 ‰, which very closely matches *S. alterniflora* sediment seen in our constructed sites. Therefore, the depleted $\delta^{13}C$ signal observed in sediment at the constructed sites could be a remnant from the marine source material.

S. alterniflora sediment also varied in $\delta^{15}N$ across sites, with the 5+ yr site having a large N range. Dredged material could have been placed in patchy areas across the site, resulting in heterogeneity of sediment origins with varying $\delta^{13}C$ and $\delta^{15}N$ values. We did not see a large variability in sediment at the 15+ yr site, but the sample size was extremely low. If we collected and ran more sediment samples from the 15+ yr site, we could possibly observe a large variability in $\delta^{15}N$ similar to what we observed in the 5+ yr site.

Vegetation stable isotopes of *S. alterniflora, J. roemerianus,* and benthic microalgae (BMA) varied across the sites. *S. alterniflora* $\delta^{13}C$ values at all sites were tightly grouped between -12‰ to -16‰ and reflect C values typical of a C4

photosynthetic pathway, with no difference between sites. Similarly, *J. roemerianus* at all sites reflected $\delta^{13}C$ values typical of a C3 photosynthetic pathway, with no difference between sites. *S. alterniflora* and *J. roemerianus* $\delta^{15}N$ values, on the other hand, differed between sites. The 5+ yr and 15+ yr constructed sites had significantly more enriched $\delta^{15}N$ values of *S. alterniflora,* which may reflect the enriched $\delta^{15}N$ values in the sediment. The 15+ yr site also had significantly more depleted $\delta^{15}N$ values of *J. roemerianus*. Sample size of *J. roemerianus* taken at the constructed sites was extremely low due to the scarce presence of *J. roemerianus* and may not represent the entire range of $\delta^{15}N$. BMA differed significantly in $\delta^{13}C$ across sites with the 15+ yr site having more enriched $\delta^{13}C$ values and the 5+ yr site having more depleted $\delta^{13}C$ values compared to the natural site. BMA at the 100+ yr site had similar $\delta^{13}C$ values as prior reports from local natural marshes (Sullivan and Moncreiff 1990). Overall, $\delta^{13}C$ stable isotopes found in *J. roemerianus* and *S. alterniflora* reflected values measured in other studies along coastal Mississippi, with $\delta^{15}N$ values varying (Hackney and Haines 1980; Sullivan and Moncreiff 1988; Dillon et al. 2015).

Consumer stable isotopes of $\delta^{13}C$ and $\delta^{15}N$ also varied significantly across sites. Consumers at the 15+ yr site had more enriched $\delta^{13}C$ and more depleted $\delta^{15}N$ values compared to the 5+ yr site a 100+ yr site, whereas the consumer isotopes at the 5+ yr and 100+ yr sites showed minimal differences. Depleted $\delta^{15}N$ values in consumers at the 15+ yr site is unexpected given that *S. alterniflora* $\delta^{15}N$ values were enriched at the site. Therefore, it is unlikely that consumers acquired their N source from *S. alterniflora*, meaning that there is another N pool at the 15+ yr site that we did not capture in our

sampling design. The $\delta^{13}C$ values in consumers at the 15+ yr site is expected as it reflects a *Spartina*-dominated (C4) system.

Consumers at the 100+ yr site and 5+ yr site reflect similar $\delta^{13}C$ values, although this does not mean they acquired their C signature from the same plant species. The vegetation assemblage at the natural and 5+ yr site were very different, with the natural site being dominated by both *J. roemerianus* and *S. alterniflora.* Consumers at the natural site likely have a mixed diet of *J. roemerianus, S. alterniflora*, and BMA. The 5+ yr site, on the other hand, is primarily dominated by *S. alterniflora* and we would expect to see consumers reflecting a strong C4 signal, but this was not the case. There are multiple explanations for this unexpectedly depleted $\delta^{13}C$ signal observed in consumers from the 5+ yr site. One explanation could be a mixed diet of C3 and C4 plants, but this is unlikely as there is hardly any *J. roemerianus* present in the site. Another explanation could be that the organisms are moving in and out of the site, or external C sources are entering the site. This is also unlikely because the high berm prevents daily tidal input and inhibits nekton from moving between sites. A third explanation could be that consumers are feeding primarily on benthic microalgae. BMA collected at our 5+ yr site is more depleted in $\delta^{13}C$ (around -23.8‰) than the other sites and could be driving the consumer $\delta^{13}C$ values down. The first 2 explanations are unlikely given the vegetation assemblage and landscape; therefore, we can assume the $\delta^{13}C$ values of consumers at the 5+ yr site are likely a reflection of a BMA diet, or at least a mixture of BMA and *S. alterniflora*, within the 5+ yr site.

We also compared feeding strategies separated by filter feeders, grazers, and omnivores between each site to investigate how resources move through trophic levels at

each site. Omnivores were found to have higher $\delta^{15}N$ values compared to grazers and filter feeders, which is expected given that $\delta^{15}N$ becomes more enriched with trophic position. We also used ellipse overlap to compare feeding strategies between sites. Grazers from the 5+ yr and 100+ yr site overlapped 6.24%, while neither site overlapped with grazers from the 15+ yr site. Similarly, omnivores from the 5+ yr and 100+ yr site showed high ellipse overlap of 51%, with minimal to no overlap with the 15+ yr site. Similar to our structural metric results, stable isotopes of consumers showed that the older restored site more dissimilar to the natural site while the younger restored site, which is already exhibiting characteristics similar to that of the natural site.

CHAPTER V – CONCLUSION

The goal of this study was to evaluate consumer trophic support at restored sites on Deer Island, MS. Based on structural (diversity and abundance) and isotopic metrics of invertebrates and nekton species, it appears that the constructed sites may not be progressing along a similar succession trajectory toward conditions at the natural site. If created marsh age was the driving factor in restoring the consumer community, we would expect to see abundances approaching natural levels over time, with the older restored site being more similar to the natural marsh than the younger restored site (Fig 5.1). However, we observed the younger 5+ yr restored site exceeding its older counterpart in many ways, such as nekton abundance and species richness, suggesting that other environmental factors are likely influencing consumer recolonization.

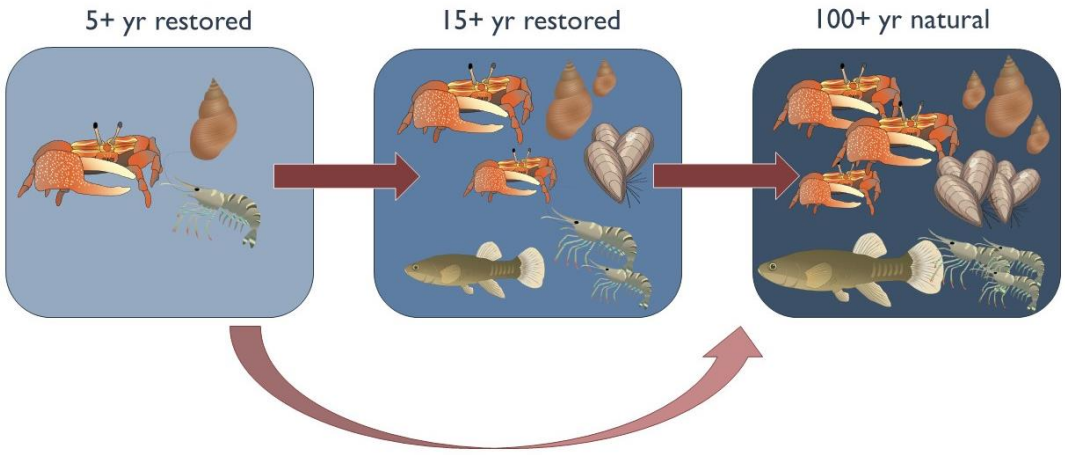


Figure 5.1 *Conceptual diagram of invertebrate and nekton trajectory over time.*

Red arrows indicate the restored sites progressing toward the natural site condition.

In our study, slow succession of foundational characteristics, such as elevation and vegetation assemblage, may have prevented the constructed sites from progressing along this expected successional trajectory. For instance, *J. roemerianus* largely failed to reestablish in either of our constructed sites, which resulted in a system that does not yet reflect a natural Mississippi marsh. There is potential for the younger restored site to increase in elevation over time and achieve more natural vegetation and tidal inundation, as accretion rates are higher in newly created marshes (Nyman et al. 2006). In addition, projected sea level rise may lower the relative elevation at the 15+ yr site and alter ecosystem processes to support more natural vegetation and marsh inhabitants in the future.

After a restored marsh achieves reference levels of landscape, soil, and vegetation characteristics over time, then we may also expect the stable isotopes of consumers to change in response. For instance, if a created site were dominated by *S. alterniflora* (C4) initially but recolonized with *J. roemerianus* (C3) over time, then we would expect C isotopes of consumers to become more depleted as the system shifts from a C4-dominated system to a mixed system of C3 and C4 primary producer signals (Fig. 5.2). We would also expect C and N levels in the soil to change. A site filled with dredged material may reflect a wide range of N isotope values initially because of heterogeneity in sediment origins, but over time N values could level off to a more consistent range with establishment of natural microbial processes in the sediments and plant rhizosphere.

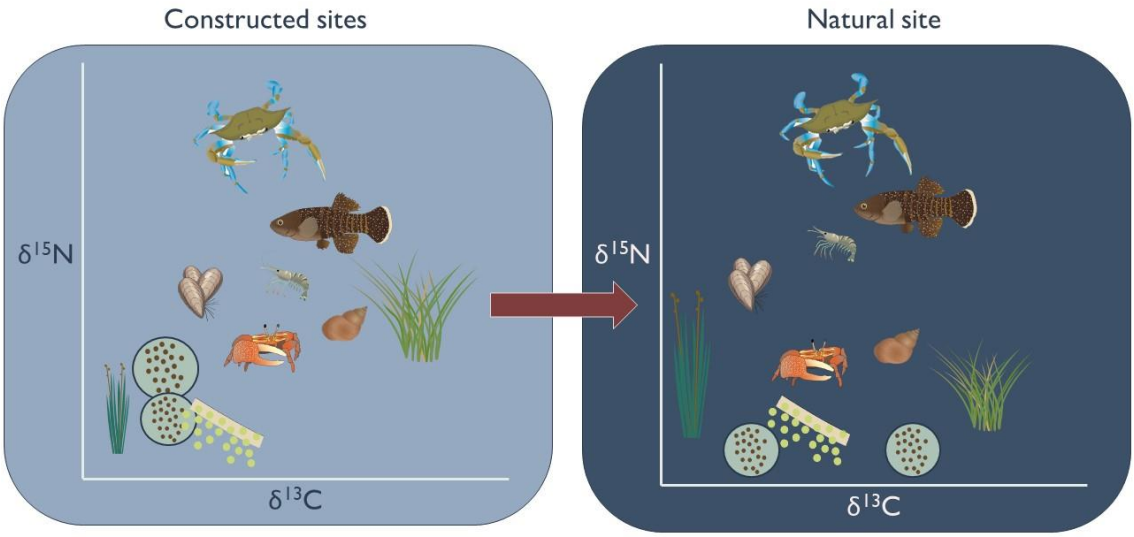


Figure 5.2 *Conceptual diagram of stable isotope trajectory over time. Red arrow indicates the restored sites progressing toward the natural site*

Although the two constructed sites are still lagging in many ways, the 5+ yr site appeared to reflect more similar consumer characteristics to the natural marsh compared to the 15+ yr site, suggesting that it may be approaching functional equivalence faster. If the goal of marsh creation includes natural levels of consumer trophic support, it is imperative to consider construction methods that create natural landscape, hydrology, soil, and vegetation.

APPENDIX A – List of Nekton Species by Sampling event

Table A.1 *Spring 2018 nekton species.*

Site	Species	Abundance	n
5+ yr site	*Mugil curema*	3	5
	Fundulus spp.	5	
	Poecilia latipinna	5	
	Adinia xenica	1	
	Cyprinodon variegatus	16	
15+ yr site	*Neritina usnea*	2	2
	Palaemonetes spp.	1	
100+ yr site	*Neritina usnea*		3
	Fartantepenaeus aztecus	2	
	Palamonetes spp.	2	

Nekton species collected using minnow traps at each site, from the Spring 2018 sampling season.

Table A.2 *Fall 2018 nekton species.*

Site	Species	Abundance	n
5+ yr site	*Fundulus spp.*	67	5
	Poecilia latipinna	53	
	Neritina usnea	2	
	Callinectes sapidus	3	
	Cyprinodon variegatus	18	
15+ yr site	*Callinectes sapidus*	3	3
	Fundulus spp.	1	
	Palaemonetes spp.	24	
100+ yr site			0

Nekton species collected using minnow traps at each site, from the Fall 2018 sampling season.

Table A.3 *Spring 2019 nekton species.*

Site	Species	Abundance	n
5+ yr site	*Gambusia holbrooki*	2	5
	Fundulus spp.	2	
	Poecilia latipinna	10	
	Neritina usnea	9	
	Cyprinodon variegatus	6	
15+ yr site	*Neritina usnea*	2	2
	Cyprinodon variegatus	1	

Table A.3 (continued)

100+ yr site	*Mugil cephalus*	1	5
	Palaemonetes spp.	5	
	Cyprinodon variegatus	1	
	Uca virens	3	
	Neritina usnea	5	

Nekton species collected using minnow traps at each site, from the Spring 2019 sampling season.

Table A.4 *Summer 2019 nekton species.*

Site	Species	Abundance	n
5+ yr site	*Uca virens*	1	8
	Fundulus spp.	1	
	Poecilia latipinna	7	
	Neritina usnea	11	
	Gambusia holbrooki	1	
	Cyprinodon variegatus	1	
	Callinectes sapidus	3	
	Adinia xenica	1	
15+ yr site	*Gambusia holbrooki*	1	7
	Neritina usnea	1	
	Palaemonetes spp.	5	
	Cyprinodon variegatus	9	
	Poecilia latipinna	7	
	Callinectes sapidus	5	
	Fundulus spp.	7	
100+ yr site	*Uca virens*	4	9
	Cyprinodon variegatus	3	
	Poecilia latipinna	17	
	Gambusia holbrooki	2	
	Fundulus spp.	62	
	Adinia xenica	7	
	Neritina usnea	5	
	Palaemonetes spp.	5	
	Mugil cephalus	1	

Nekton species collected using minnow traps at each site, from the Summer 2019 sampling season.

Table A.5 *Fall 2019 nekton species.*

Site	Species	Abundance	n
5+ yr site	*Gambusia affinis*	1	6
	Callinectes sapidus	1	
	Palaemonetes spp.	1	
	Poecilia latipinna	1	
	Neritina usnea	6	
	Fundulus spp.	1	
15+ yr site	*Farfantepenaeus aztecus*	3	6
	Litopenaeus setiferus	3	
	Poecilia latipinna	1	
	Neritina usnea	2	
	Callinectes sapidus	2	
	Palaemonetes spp.	5	
100+ yr site	*Cyprinodon variegatus*	1	3
	Fundulus spp.	28	
	Litopenaeus setiferus	2	

Nekton species collected using minnow traps at each site, from the Fall 2019 sampling season.

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