Biodiversity and habitat characteristics as indicators of community change following oyster reef restoration

By

Grace Loonam

July, 2025

Directors of Thesis: April M.H. Blakeslee, Ph.D. and Rachel Gittman, Ph.D.

Major Department: Biology

ABSTRACT

As coastal habitats, oyster reefs are often on the frontlines of direct and indirect sources of anthropogenic ecological degradation. This impacts both the structure and function of these foundational habitats, which offer ecosystem services to the environment as well as nearby coastal communities. Restoration of these habitat-forming species offers a way to study the resilience and recovery of communities following human disturbance and over time while restoring lost ecosystem function and services following disturbances. To this end, two oyster restoration approaches were implemented in 2018 in the Rachel Carson Reserve in Beaufort, NC: traditional shell bags, and a novel biodegradable substrate for the time, Oyster Catcher™. These sites underwent initial post-restoration monitoring (habitat: 2018-2020 and biodiversity: 2018-2019). My project analyzed changes in habitat and biodiversity characteristics of the two restoration approaches and nearby natural reefs five years post-restoration relative to these earlier monitoring points. My results show how substrate and time can influence the efficacy of oyster reef restoration as well as the how restoration can be a tool for studying ecological succession.

For habitat characteristics, I compared previous monitoring data to 2023 samples to assess how oyster densities and elevation varied by restoration treatment. While oyster densities increased from 2018-2020 in the previous monitoring effort, my results showed a decrease in oyster densities from 2020-2023. Moreover, in 2020, the restored oyster reefs were within the "Optimal Growth Zone", while in 2023, varying water levels resulted in all live oysters on the reefs residing above this ideal elevation range. In all cases, however, Oyster Catcher reefs outperformed shell bag reefs in terms of oyster densities, offering some insight into the potential application of novel breakwater substrates to the issue of oyster reef restoration.

For community composition, I used free-living and parasite communities and compared samples from 2023 to previous data to understand how these metrics have changed with time since restoration. While free-living fish communities peaked in biodiversity one-year post restoration, the species documented are more transient species; by contrast, the species more strongly affiliated with the 2023 samples (e.g., the oyster toadfish, *Opsanus tau*) are recognized as true reef resident species. Free-living crab communities showed a different trend, with biodiversity increasing with time post-restoration. In both cases, treatment was not a significant driver of community composition, although mud crab abundances appeared to be higher at restored reefs than loose cultch reefs. For parasite communities, samples collected from fish and snail hosts showed increasing prevalence and biodiversity with time, consistent with previous literature. However, crab parasites peaked in prevalence one-year post-restoration, with lower prevalence across most taxa in 2023. These results show how surrogate taxa, such as parasites, can augment biodiversity surveys and further assess community composition and function. These results stress the importance of monitoring oyster reef restoration projects over time, as early post-restoration trends do not consistently reflect the long-term condition of these efforts.

Biodiversity and habitat characteristics as indicators of community change following oyster reef restoration

A Thesis
Presented to the Faculty of the Department of Biology
East Carolina University

In Partial Fulfillment of the Requirements for the Degree
Master of Science in Biology

By
Grace Loonam
July, 2025

Director of Thesis: April M.H. Blakeslee, Ph.D. and Rachel Gittman, Ph.D.

Thesis Committee Members:

Hannah Sirianni, Ph.D.
Brandon Puckett, Ph.D.

Acknowledgements

I would like to thank my advisors, Dr. April Blakeslee and Dr. Rachel Gittman for their constant support and guidance throughout the completion of degree. You went above and beyond on many occasions, and I am incredibly grateful to have you as mentors to learn from and look up to. I deeply appreciate you sharing your knowledge and expertise with me while helping build me into a better scientist and more thoughtful researcher, and cannot thank you enough for your enthusiasm and encouragement. I feel privileged to have been co-advised by you both.

I would also like to thank the rest of my committee, Dr. Hannah Sirianni and Dr. Brandon Puckett, for their support throughout my thesis and for always being available to me throughout this process. Dr. Sirianni, thank you for your excellent geospatial analyses courses and for teaching me the basics of the affiliated software; Dr. Puckett, thank you for all of the field assistance and for the additional work you put in towards the geospatial component of this work. I'm incredibly grateful to have met and worked with you both throughout my time at ECU.

The Biology Department at ECU has also been a tremendous source of support and opportunity since I arrived, and I am beyond glad I came here to complete my Master's degree. I found not only an enthusiastic and driven faculty, but an amazing graduate student community that has truly made my experience here phenomenal and unforgettable. I would like to specifically shout out the members of the Blakeslee and Gittman labs not only for their help in the lab and in the field, but also for being such amazing people and friends that inspire and uplift me all the time.

Finally, I want to thank my family (chosen family too!) for their love and endless support of all of my passions and pursuits. I love you all so much, and you have been such a vital component of my growth here and everywhere.

Table of Contents

LIST OF TABLES vi
LIST OF FIGURES vii
LIST OF ABBREVIATIONS x
INTRODUCTION 1
Project Background and Significance 7
Research Questions and Hypotheses 9
MATERIALS AND METHODS 11
Study Site 11
Experimental Design 12
Publications and Datasets 12
Contemporary Surveys 13
Lab Processing for Parasites 20
Statistical Analyses 23
RESULTS 25
Habitat Characteristics 25
Biodiversity of reef-affiliated communities: Free-living Organisms 27
Free-living Fish 27
Free-living Crabs 30
Biodiversity of reef-affiliated communities: Parasitic Organisms 36
Fish parasites 36
Crab parasites 39
Snail parasites 42
DISCUSSION 49
Habitat Characteristics 49
Free-living communities 52
Parasite communities – Fish, crabs, and snails 55

CONCLUSIONS AND SIGNIFICANCE 58
FUTURE DIRECTIONS 59
REFERENCES 64

LIST OF TABLES

Table 1: Research questions and data sources and type.

Table 2: Study sites.

Table 3: A visual summary of the biodiversity data collected by year.

LIST OF FIGURES

Figure 1: Restoration treatments at Carrot Island in 2018, 2020, and 2023.

Figure 2: Map of study sites.

Figure 3: OC and SB samples taken for habitat data, with diagrams to illustrate general recruitment area shape and affiliated surface area formulas.

Figure 4: Oyster densities (#/m^2) by restoration treatment by year, 2018-2023.

Figure 5: Average oyster density (#/m^2) by average elevation (m) at which sample was taken for 2018, 2020, and 2023.

Figure 6: A non-metric multidimensional scaling plot made in Primer 7 using the Bray-Curtis similarity index to assess the changes in free-living fish communities at Carrot Island sites by treatment (LC, OC, and SB) and time relative to the restoration effort (Pre-restoration, "Pre"; one-year post-restoration, "1YR"; and five-years post-restoration, "5YR").

Figure 7: Average Shannon-Wiener Diversity Index scores of free-living fish communities by time (relative to restoration) and by treatment.

Figure 8: Average abundance of oyster toadfish (Opsanus tau) by time (relative to restoration) and by treatment.

Figure 9: A non-metric multidimensional scaling plot made in Primer 7 using the Bray-Curtis similarity index to assess the changes in free-living crab communities at Carrot Island sites by treatment (LC, OC, and SB) and time relative to the restoration effort (Pre-restoration, one-year post-restoration, and five-years post-restoration).

Figure 10: Average Shannon-Wiener Diversity Index scores of free-living crab communities by time (relative to restoration) and by treatment.

Figure 11: 2023 Mud crab abundances by treatment.

Figure 12: Average Shannon-Wiener Diversity Index scores of free-living crab communities in 2023 by treatment.

Figure 13: Total abundances of porcelain crabs in 2023 by time (months) and by treatment.

Figure 14: Average biomass (g) of fouling communities collected from plastic tiles by treatment.

Figure 15: A non-metric multidimensional scaling plot made in Primer 7 using the Bray-Curtis similarity index to assess the changes in fish parasite communities at Carrot Island sites by site type (loose cultch, Oyster Catcher, and Shell Bag) and time relative to the restoration effort (Pre-restoration, one-year post-restoration, and five-years post-restoration).

Figure 16: Parasite prevalence by taxa within various fish hosts by time relative to restoration (pre-restoration, 1-year post-restoration, and 5-years post-restoration.

Figure 17: A non-metric multidimensional scaling plot made in Primer 7 using the Bray-Curtis similarity index and a cluster analysis to assess the changes in crab parasite communities at Carrot Island sites by site type (loose cultch, shell bag, and Oyster Catcher) and year (pre-restoration in 2018, one-year post-restoration in 2019, and five-years post-restoration in 2023).

Figure 18: Parasite prevalence by taxa within various crab hosts by time relative to restoration (pre-restoration, 1-year post-restoration, and 5-years post-restoration.

Figure 19: Rank-abundance curves depicting the trematode communities affiliated with the eastern mud snail (I. obsoleta) at Carrot Island in restored (SB and OC) and LC plots.

Figure 20: A non-metric multidimensional scaling plot made in Primer 7 using the Bray-Curtis similarity index and a cluster analysis to assess the changes in snail trematode communities (in terms of prevalence and richness) at Carrot Island sites by site type (LC and restored, which includes SB and OC) and year (pre-restoration in 2018, one-year post-restoration in 2019, and five-years post-restoration in 2023).

Figure 21: Parasite diversity (measured using the Shannon-Wiener Index) within I. obsoleta hosts by time relative to restoration (pre-restoration, 1-year post-restoration, and 5-years post-restoration.

Figure 22: Parasite richness within I. obsoleta hosts by time relative to restoration (pre-restoration, 1-year post-restoration, and 5-years post-restoration.

Figure 23: Parasite prevalence within I. obsoleta hosts by time relative to restoration (pre-restoration, 1-year post-restoration, and 5-years post-restoration.

Figure 24: A non-metric multidimensional scaling plot made in Primer 7 using the Bray-Curtis similarity index and a cluster analysis to assess the changes in free-living communities at Carrot Island sites by site type (as shape; LC=loose cultch SB=shell bag, and OC=Oyster Catcher), shoreline (as shading; C=creek, R=ramp), and year (as color; 2023 and 2024).

Figure 25: Oyster densities at Carrot Island through 2024.

Figure 26: Net shoreline movement (in meters) at Carrot Island along the ramp shoreline and one side of the creek shoreline.

LIST OF ABBREVIATIONS

EPA: Environmental Protection Agency

RCR: Rachel Carson National Estuarine Research Reserve

NERR: National Estuarine Research Reserve

NC DEQ: North Carolina Department of Environmental Quality

NOAA: National Oceanic and Atmospheric Administration

OC: Oyster Catcher^{TM}

SB: Shell Bag

LC: Loose Cultch

NAVD88: National American Vertical Datum of 1988

OGZ: Optimal Growth Zone

RMS: Root mean square

IACUC: Institutional Animal Care and Use Committee

PERMANOVA: Permutational Analysis of Variance

GLM: Generalized Linear Model

nMDS: non-metric multidimensional scaling

AICc: Akaike information criterion

S-W: Shannon-Wiener Diversity Index

INTRODUCTION

Anthropogenic ecological degradation has impacted ecosystem community composition and function on a global scale. As coastlines are an integral point of human activity (transportation, recreation, and other social and economic endeavors), it is understandable why habitats in this setting have been particularly affected. In the United States, where almost 40% of the population resides in counties along the coasts (US Dept. of Commerce, 2023), declines across habitat types have been observed for decades, commonly attributed to dredging (Beck et al., 2011), overharvesting (Kirby, 2004), and deliberate alteration of habitat for alternative land uses (Goldberg et al., 2020; Kennish, 2001), among other harmful practices (Kennish, 2001; Beck et al., 2011). However, human-induced changes to habitats and communities, including restoration, provide opportunities to not only investigate the resilience and recovery of communities following disturbance (Moore et al. 2020, 2023; Young et al., 2001), but also how they assemble and shift over time (Audino et al., 2017; Gong et al., 2023). While the aforementioned actions are a clear driver of this initially negative process, habitat restoration offers a way to study changes in community assembly and biodiversity over time, while also providing a mechanism for restoring lost ecosystem function and services following human disturbance (Gittman et al., 2018, 2024). One such habitat – that provides a bevy of ecosystem services and is thus a priority for restoration – is oyster reefs (Gittman et al., 2018, 2024).

Oysters are bivalve mollusks that have long been recognized as fundamental residents of estuarine communities located all over the world (Galtsoff 1964; Beck et al. 2011). As filter feeders, these organisms play an incredibly important role in improving and maintaining water quality (Grabowski and Peterson 2007; zu Ermgassen et al. 2013). Specifically, oysters can help reduce eutrophication and improve the degree to which light passes through the water column,

thereby supporting the growth of submerged aquatic vegetation, by filtering out phytoplankton, sediment particles, and excess nutrients from the water column (Grabowski and Peterson 2007; zu Ermgassen et al. 2013). Further, the formation of reefs provides the foundation for numerous estuarine organisms to settle within the complex habitat that reefs provide, allowing for predator evasion and sources of food (Michaelis et al., 2020), while simultaneously stabilizing coastal areas by buffering against erosion, wave energy, and extreme weather events (Grabowski and Peterson 2007). With the extensive ecosystem services provided by oysters, the need for their presence is undeniable, and thus restoration efforts have strived to restore reef ecosystem function, and by extension, the benefits that accompany this foundational habitat.

However, although some restoration methods have produced promising results, there are also concerns and documented shortcomings that affirm the need to continue to improve upon existing techniques (Buckley & Crone, 2008; Kondolf, 1998). One demonstrated issue is the type of substrate and materials selected for restoration, the significance of which has been particularly emphasized after inadvertent issues have arisen from previous efforts (Comba et al., 2023; Hennebert et al., 2014). For example, the convenient use of old tires as a base for reestablishing oyster reefs was praised in a report to the federal solid waste management programs under the EPA in 1970s, detailing a project that had begun to do so in 1968 (Stone et al., 1974). The approach was implemented in Chesapeake Bay as a part of a study in 1983 (Feigenbaum et al., 1989), but later work scrutinized this method based on concerns about chemical leaching (Hartwell et al., 1998). Although water quality investigations for this effort did not immediately reveal any significant impacts at the time of sampling, the authors noted that there could be long-term effects (Hartwell et al., 1998). Another demonstrated issue is the prevalence of plastic use in other approaches, which is a point of contention given our burgeoning understanding of

microplastics and their abundance in the environment (Comba et al., 2023; Walters et al., 2022). A key example is the traditional shell bag method of placing oyster cultch in plastic mesh bags in oyster restoration efforts (Dunn et al., 2014; Comba et al., 2023). To date, we lack consensus on the most cost-effective and environmentally friendly alternatives for artificial materials (Walters et al., 2022). In addition to cost, these materials can be susceptible to other risks as well, as seen in a Pamlico Sound study in which *Cliona*, a boring sponge, was generally more prevalent on oyster reefs built with calcium carbonate material (some with limestone marl and others with oyster shells) when compared to other substrates (Dunn et al., 2014). Though the sponge alone was not found to significantly influence the survivorship of the oysters, it could still be a notable threat to their survival in conjunction with other factors that can be difficult to control for, and therefore the durability of different substrates in variable settings is important to consider as well (Dunn et al., 2014).

Further restoration concerns stem from the potential for facilitated recruitment of invasive species to restored regions (Abella & Chiquoine, 2019; Jude & DeBoe, 1996), as nonindigenous species have already demonstrated an elevated rate of occurrence on artificial structures (of various composition, including seawalls and pilings in aquatic systems) when compared to natural locations (Glasby et al., 2007), suggesting notable implications for the use of structures that employ materials/species extrinsic to system being restored (Abella & Chiquoine, 2019). The introduction or accidental support of invasive species in this manner could have significant repercussions for the ecosystem at large, as a greater prevalence of invasive species may cause native organisms to experience heightened competition with the invaders but no abatement in their usual predation risk, and in some cases, may potentially augment it (Noonburg & Byers, 2005). Further, facilitation of invasive species could be the

starting point for a subsequent cascade of changes in the structure of the ecosystem and the food web dynamics therein (David et al., 2017), as seen in the case of the invasive snail *Euglandina rosea* introduced in Moorea (Clarke et al., 1984). This snail decimated a native snail population in Moorea rather than inhibiting the spread of another species that had also been introduced to the area and for which *E. rosea* was introduced as an idea for controlling the population (Clarke et al., 1984); as a result, the ecosystem not only lost a native species, but gained two demonstrably effective invasive species that could go on to further outcompete other significant players in the ecosystem (David et al., 2017, Clarke et al., 1984).

Even if the challenges presented above are avoided, restoration efforts, to be considered effective, must still withstand the test of time and function comparably to natural sites typical for the region. Despite general understanding that restoration efforts are needed to reinstate lost ecosystem services, there is often only short-term (1-2 years) post-project monitoring, consistent with time constraints imposed by grant-funded project timelines (often less than 3 years) (Lindenmayer & Likens, 2018). This is a trend within living shoreline monitoring specifically as well (Baker & Gittman, 2025). Thus, limited monitoring post-restoration typically results from insufficient time and funding typically allotted by grant funding cycles or federal/state agencies (Lindenmayer & Likens, 2018). Without monitoring, the success and evolution (i.e., changes in habitat quality and biodiversity through time) of a restoration project is uncertain. Some examples with short-term monitoring (around one-year post-restoration) provide positive insights regarding biodiversity support and habitat provision, but do not monitor the progression of these sites further, as seen in a 2018 restoration effort at Carrot Island, located off the coast of Beaufort, North Carolina (Moore et al., 2020). Additionally, space-for-time approaches have been implemented to begin to address this gap and have been praised for their ability to evaluate

the effects of restoration over a longer time scale without one data set in the same location (Moore et al., 2023; Wogan & Wang, 2018; Damgaard, 2019). However, longer-term monitoring of a project initially monitored for only a short period post-implementation may reveal that potentially optimistic outcomes at the beginning do not later lead to functional equivalency between restored habitat and its natural or un-restored counterpart. This can be indicated by the absence of certain key ecological qualities or species, as seen in a joint eelgrass/oyster reef restoration effort in the San Francisco estuary (Pinnell et al., 2021). In this system, although initial observation of the sites indicated the presence of invertebrate communities distinct from the controls and previous assemblages associated with restored locations (before the remediation effort took place), additional monitoring three years later revealed that notable species in nearby natural sites (*Phyllaplysia taylori* and *Pentidotea resecata*) were not representatively reflected in the restored areas (Pinnell et al., 2021). Some of this long-term inadequacy may be attributed to the restoration design. Different approaches to oyster reef restoration vary in their structure, with some designed to promote oyster growth overall while others target the provision of a specific ecosystem service (Morris et al., 2019), such as wave attenuation (Tso et al., 2023). While these structures may result in higher wave attenuation, shortly after construction, the effectiveness of this design deteriorates with time as the design is not suitable for oyster survivorship (Morris et al., 2019). By contrast, restored reefs configured with the biological needs of the oysters in mind are more sustainable over time and accrue wave attenuation benefits as the habitat develops naturally (Morris et al., 2019). This result illustrates both the added need to consider the design of restored structures but also further emphasizes the importance of continuing to evaluate the progression of these efforts well beyond the initial construction period. This can reveal the shortcomings of short-term monitoring that may suggest early success, potential design flaws

that do not yield long-term results, as well as highlight the benefits of employing a more sustainable technique.

The need to engage in long-term monitoring of restoration efforts is further compounded by climate change. Broadly, modeled climate change-related impacts can illustrate the projected impacts of this phenomena on coastal systems, and greater storm activity predicts higher wave heights (Ruggiero et al., 2008). This impact, in conjunction with expected sea level rise, has the potential to lead to notable erosion of coastal habitat as well as elevated flooding in these areas (Ruggiero et al., 2008). These elements further complicate restoration efforts, as the ability of employed approaches are already uncertain, and now the added stress of surviving in suboptimal conditions must be considered in an ongoing era of global environmental change.

As such, there is still a prominent and pressing need to a) ascertain the most effective approaches to restoring oyster reefs as well as b) address the lack of long-term monitoring of restoration to determine the sustainability of specific restoration techniques and improve our ability to predict future effects of restoration. Evaluating these methods through an ecological lens is a logical approach, as these results would reflect the long-term products of the restoration better than defining their success by the metrics affiliated with less ecological functions (e.g., exclusively focusing on shoreline stabilization). However, one metric of ecological assessment is biodiversity, and comprehensive biodiversity surveys can be difficult to achieve given the constraints described above. Thus, alternative approaches have been proposed, including utilizing surrogate taxa to capture the broader community biodiversity of a restored system (Lindenmeyer et al., 2015). Although seemingly counterintuitive, recent work has shown that parasites can be strong indicators of healthy ecosystems and intact communities, and in some cases, may better signify positive changes in biodiversity following restoration than their free-

living counterparts (Moore et al., 2023). As parasites require the presence of hosts to survive, multi-host parasites (e.g., digenean trematodes, cestodes, acanthocephalans, and nematodes) can be excellent surrogates for the multiple links that exist among species in a community (Byers et al., 2011: Huspeni et al. 2024; Hechinger & Lafferty 2005; Moore et al. 2024). As a result, parasite diversity has been used in numerous past studies as indicative of ecological complexity and a measure of restoration success (Moore et al., 2020; Moore et al., 2023; Huspeni & Lafferty, 2004).

Project Background and Significance

North Carolina ecosystems are currently being impacted by a variety of climate change affiliated phenomena that accentuate the need for effective restoration efforts while simultaneously impeding their ability to succeed. Coastal erosion spurred by sea level rise has already changed the coastal landscape, and is projected to drive additional losses of shoreline habitat by 2100, with between ~40 and ~130 centimeters of sea level rise predicted in some areas along North Carolina's coasts (Kopp et al., 2015). This trend could pose a significant threat to oyster reefs (restored or otherwise); although growth rates for oysters in the area (~2.7cm per year) are expected to keep up with regional sea level rise (Rodriguez et al., 2014), it is possible that their survivorship could be affected by other factors affiliated with climate change that are projected to prove devastating to the coasts already (Kopp et al., 2015). This region is also susceptible to organisms that are actively creeping up the Atlantic coastline. Dubbed "the Caribbean Creep" by JT Carlton, numerous tropical or subtropical species have been detected expanding out of their historic ranges in the Caribbean to higher latitudes along the Atlantic coast in a move spurred by climate change (Canning-Clode et al., 2011; Fowler et al., in review). One species representative of this shift is *Petrolisthes armatus*, the green porcelain crab, which

has recently expanded its range up to Beaufort, NC (Blakeslee et al., 2024). The combination of these location-specific factors, in conjunction with the inherent challenges to restoration described above, highlights the importance of following up on oyster reef restoration efforts in this region.

In 2018, oyster habitat was restored in the Rachel Carson National Estuarine Research Reserve (RCR) off the coast of Beaufort, North Carolina to assess the efficacy of two restoration techniques on habitat (Wellman et al., 2022) and associated biodiversity (Moore et al., 2020). The first of these restoration treatments was a novel substrate, Oyster Catcher^{TM} (OC), which is composed of biodegradable components like steel wire and burlap, while the other method is the more traditional tactic of oyster shell bags (SB), in which plastic mesh bags are filled with old oyster shell (Wellman et al., 2022). Both interventions, intended to provide substrate for juvenile oysters in the water column to settle on and grow, demonstrated initial recruitment of oysters despite ongoing erosion in the region (Wellman et al., 2022). However, prior research has suggested that the loose cultch in shell bags do not always yield long-term results, and overall, the long-term performance of novel oyster breakwater substrates is still uncertain (Keller et al., 2019). Initial monitoring of the restored sites in the RCR only continued two years post-restoration, and thus the persistence of the restored reefs themselves is uncertain. Additionally, the green porcelain crab was not recorded in initial monitoring, and thus the impact that this invasion has had in the restored regions is also unknown. As a result, it is important to understand the restoration approaches employed in this area and ensure that they have remained effective over time and do not have any unintended consequences on the surrounding ecosystem.

Research Questions and Hypotheses

My project investigated changes in habitat complexity and successive community assembly in restored versus natural oyster reefs within the RCR 5 years post-restoration by including pre-restoration (2017) and short-term post-restoration (2018-2019) data from previously published work (Moore et al., 2020; Wellman et al., 2022) and added new seasonal and annual data collected in 2023. My project addressed the following research questions:

Research Question 1: **A)** How do habitat characteristics change over time? **B)** How does free-living and parasitic biodiversity change over time?

I hypothesized that habitat characteristics and biodiversity increased over time in the restored plots and approached levels supported by natural reefs in 2023 compared to 2018-2020 (Moore et al., 2023; Wellman et al., 2022). This is consistent with the results of the original habitat characteristics monitoring at these sites, where oyster densities increased substantially with time post-restoration (Wellman et al., 2022). Further, the trends in previous investigations revealed that the community assemblages associated with mature restored reefs have more in common with natural reefs than their newly restored counterparts, indicated by elevated parasite diversity (Moore et al., 2023). Further, I hypothesized that parasites would show stronger signals of community succession than hosts, as previous investigations of comparative restoration success over time did not reveal notable differences between restoration ages using free-living taxa, but were able to make these distinctions using parasite diversity (Moore et al., 2023).

Research Question 2: **A)** How does biodiversity vary by reef type (loose cultch reef, reef restored with OC and reef restored with SB)?

I hypothesized that supported biodiversity would be highest in loose cultch, then OC, and lastly SB reefs. It is important to note that this is somewhat contradictory to the initial results

observed in the short-term monitoring of this restoration effort, in which SB reefs displayed a statistically insignificant but generally higher average positive change in biodiversity than OC reefs (Moore et al. 2020). However, this hypothesis takes into account previous literature highlighting the lack of sustained results with the use of SB substrate (Keller et al., 2019). Further, given the nature of the two reef constructions (OC in a more spacious framework, SB in a relatively compact layout) I hypothesized that the spacious OC design would facilitate the recruitment of both oysters and mobile reef-resident fauna due to the importance of vertical relief in the establishment and growth of oyster reef ecosystems (Soniat et al., 2004).

> ***Research Question 3:*** How does the presence and abundance of the non-native porcelain crab (*Petrolisthes armatus*) differ among loose cultch reefs and constructed reefs with differing substrates?

I hypothesized that non-native porcelain crabs would have higher abundances in the constructed reefs when compared to loose cultch reefs. This result would follow the previously illustrated trend of greater recruitment of nonindigenous organisms to artificial structures (Glasby et al., 2007). Additionally, prior work has suggested that more biodiverse ecosystems exhibit stronger biotic resistance to non-native and invasive species, and thus it is hypothesized that the loose cultch reefs (broadly older with more established ecosystems than restored reefs of either type from 2018) would have fewer *P. armatus* than these younger restored sites (Kimbro et al., 2013).

MATERIALS AND METHODS

Study Site

The Rachel Carson Reserve is one of ten sites that fall under the North Carolina Coastal Reserve and National Estuarine Research Reserve (NERR) program; it consists of several islands, one of which being Carrot Island (34°42’12.23” N, 76°37’34.29” W), where this work is based (NC DEQ, n.d.). The reef sites described in this study fall along a protected creek shoreline at Carrot Island (Wellman et al., 2022), which primarily experiences only small boat wakes due to the narrow/shallow channel (Theuerkauf et al., 2015). This setting provided us with a relatively “low” wave energy shoreline as an environmental variable to consider when evaluating the durability of the restoration designs and community composition. All sites are located adjacent to the shoreline, which are salt marshes primarily comprised of *Spartina alterniflora.* All restoration plots were originally restored in 2018 using one of two restoration treatments: Oyster Catcher^{TM} (OC), a novel substrate composed of biodegradable components including steel wire and burlap, and oyster shell bags (SB), a more traditional treatment in which plastic mesh bags are filled with oyster cultch (Fig. 1; Wellman et al., 2022).

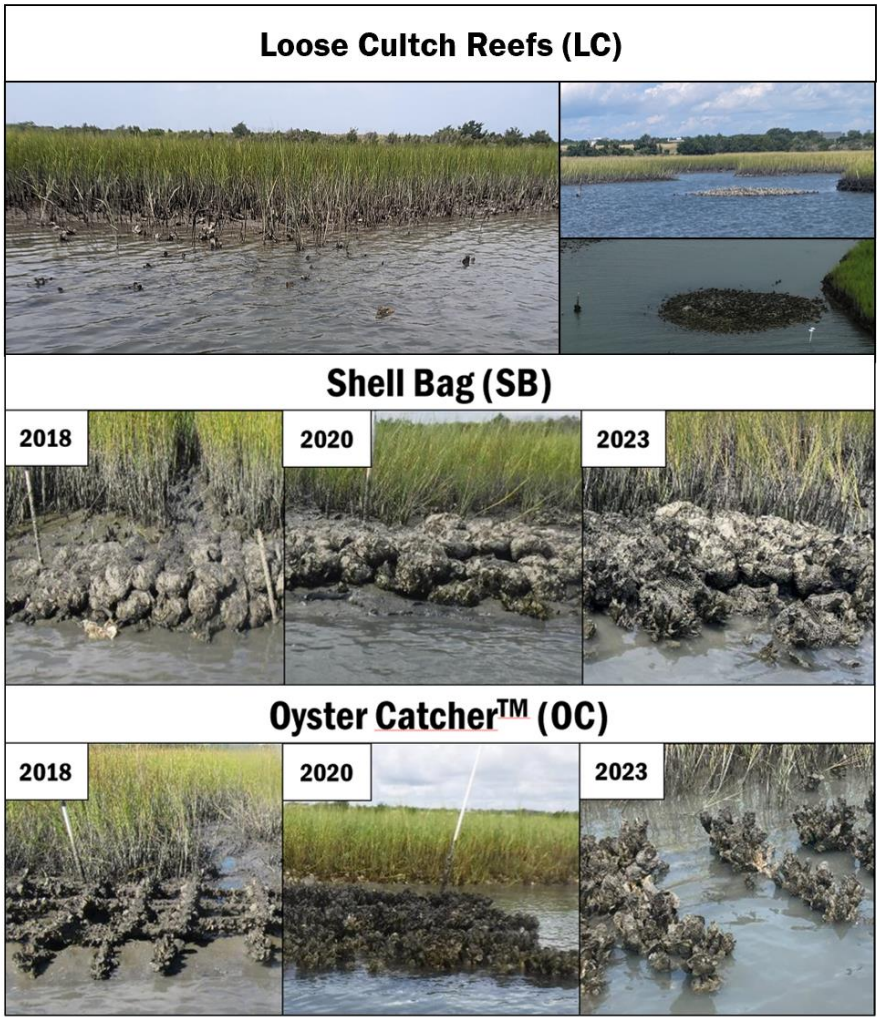

Figure 1. Restoration treatments at Carrot Island in 2018, 2020, and 2023. The top three images show two of the loose cultch reefs as photographed in 2023; loose cultch reefs varied widely in their appearance. The middle three images depict shell bag (SB) reefs, while the bottom three images show Oyster Catcher™ (OC) reefs.

Experimental Design

Publications and Datasets

This study incorporates data from multiple sampling years, some of which were collected between 2018-2020 and have been described and analyzed in previous publications (Moore et al., 2020; Wellman et al., 2022). These prior data are integrated into my study and analyzed alongside data newly collected in 2023 to more fully quantify changes in the system over time

(Table 1). The methods used to collect the 2018-2020 data can be found in their respective publications (Table 1; Moore et al. 2020; Wellman et al. 2022) and the 2023 methods will be described further below.

Table 1. Research questions and data sources and type. This table highlights the type of data that will be used to answer each research question, the year it was collected, and the publication it was published in (if applicable).

Research Questions and Data Sources		Survey Years (Prior Publication, if applicable)			
		2018-2019 Biodiversity (Moore et al. 2020)	2018-2020 Habitat (Wellman et al. 2022)	2023 Biodiversity	2023 Habitat
RQ1.	A. How do habitat characteristics change over time?		X		X
	B. How does free-living and parasitic biodiversity change over time?	X		X	
RQ2.	How does biodiversity vary by reef type (loose cultch reef, reef restored with OC and reef restored with SB)?	X		X	
RQ3.	How does the presence and abundance of the non-native porcelain crab differ among loose cultch reefs and constructed reefs with differing substrates?			X	

Contemporary Surveys

My study assesses a total of 13 oyster reef sites in 2023 for community composition (biodiversity) and includes both recently restored sites and natural reef sites or older restored cultch reefs; these latter reefs were originally restored in 2012 but now more closely resemble natural reefs in structure (Figs. 1, 2). Reef habitat structure data were also collected from one additional reef plot for each restoration treatment (denoted by the H in Fig. 2), for a total of two

additional sites at this location. In the Carrot Island creek, I surveyed a total of four SB reefs, four OC reefs, and five loose cultch reefs – including the natural sites or older restored cultch reef sites (Fig. 2).

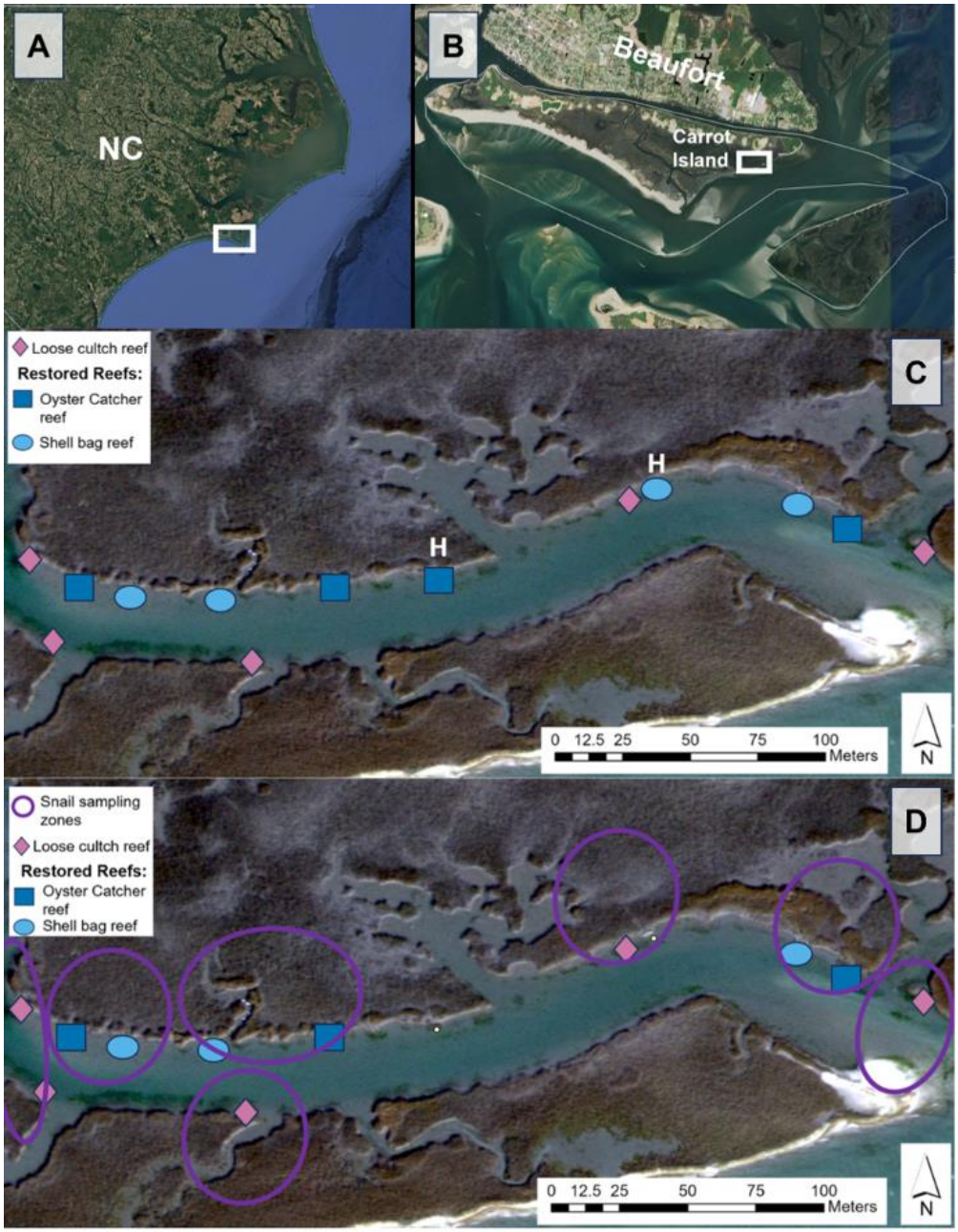

Figure 2. Map of study sites. (A) The general location of Beaufort, NC and the study sites. (B) The broader thin white border depicts the extent of the Rachel Carson Reserve, with the interior white box around the location of the Carrot Island sites. (C) Specific study sites are depicted with markers, color-coded and shape specific by site type; (n=4 OC, n=4 SB, n=5 LC; see inset

map key). Sites that were only used for habitat data collection and not biodiversity surveys have a small white "H" above the sample marker. (D) Snail (Ilyanassa obsoleta) sampling zones were located adjacent to reef sites and are denoted with purple circles.

Field Sampling: Habitat characteristics

As I was interested in how the communities affiliated with the reefs in the study have changed with time and by substrate, I used previously established protocols (Wellman et al., 2022) to quantify oyster lengths and densities to use as predictors for the biodiversity metrics (Moore et al., 2020). The reefs were surveyed in August 2023, as this falls within the previously determined recruitment season for oysters (May – October) and after the first peak (August) (Ortega & Sutherland, 1992), allowing for ample recruitment prior to sampling. For loose cultch reefs (*n=5 LC*), I used a 0.0625-m^2 quadrat to record the lengths of ten adult and juvenile oysters (n=20 oysters from each quadrat, across n=5 quadrats, one quadrat per LC site) and enumerate all live oysters within the quadrat. Oysters were given the designation of "adult" if they were >24mm, while "juveniles" were those <24mm, as defined in the initial monitoring effort (Wellman et al., 2022) and previous literature (La Peyre et al., 2014). For restored (SB and OC) sites in 2023 (*n=4 OC, n=4 SB*), I removed representative portions of each reef (one shell bag from each SB reef and one 10cm section of each OC reef's substrate frame) to process back in the lab for oyster length (mm), live/dead oysters, the presence of associated organisms (in addition to the abundance and diversity of panopeid mud crab species and porcelain crabs found within each habitat sample), and dimensions of the sample. SB samples were simply lifted off the existing reef (extricating where interwoven with other bags without much destruction of the remaining reef) whereas the OC samples were more destructively removed by sawing through the substrate frame with a hacksaw at each end of the 10cm sample. At all sites, I used a Trimble R10 Global Navigation Satellite System Receiver (with a horizontal accuracy of +/- 8mm + 0.5ppm RMS using the North Carolina 3200 US/State Plane 1983 reference system, and a

vertical accuracy of +/- 15mm + 0.5ppm RMS using NAVD88 with Geoid 18A to record the position and elevation of the sample location (*Trimble R10 MODEL 2 GNSS System Datasheet*, 2021). For lab processing the SB samples, dimensions were taken by using a ruler to measure up to three lengths, widths, and heights of the shell bag across three different portions of the sample (as the bags were often not uniformly shaped and this approach allowed an average value for each measurement to be obtained). For OC samples, dimensions were taken by using a ruler to measure up to three lengths and diameters of the sample. The number of oysters and these dimensions were used to calculate oyster densities for each sample. To do so, I divided the total number of live oysters by the calculated surface area of each sample, following the formulas for rectangular prisms and right cylinders for SB and OC samples, respectively, as these shapes best reflected the area oysters could originally recruit to at the time of initial restoration (Fig. 3; Wellman et al., 2022). While this may not reflect the actual surface area available to oysters based on the change in morphology of the samples over time and with previous growth to consider, this is the metric that was used in earlier monitoring at this site and thus provides the best metric for comparison over time based on the availability of this prior data (Fig. 3; Wellman et al., 2022)

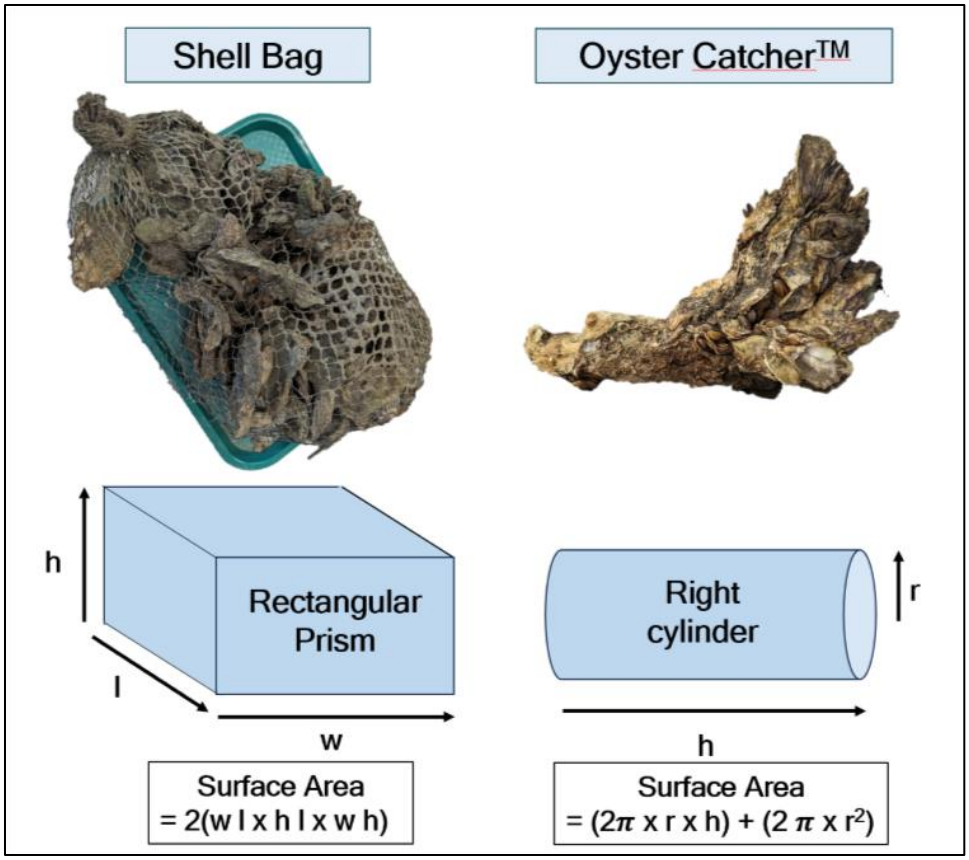

Figure 3. OC and SB samples taken for habitat data, with diagrams to illustrate general recruitment area shape and affiliated surface area formulas. The recruitment area for SB oysters most closely resembled rectangular prisms, and so this formula was used to calculate surface area for SB samples; the recruitment area for OC oysters most closely resembled right cylinders, and so this formula was used to calculate surface area for OC samples.

Field Sampling: Mobile faunal surveys

I deployed passive samplers ("crab condos") at all sites sampled for biodiversity about one meter seaward of the reef; crab condos were constructed by filling plastic milk crates (19 x 22 x 16cm) with oyster cultch and covering the open top with a mesh cover that was zip-tied to the crate (to be lifted and replaced during sampling events) (Moore et al. 2020). The crate was then zip-tied to either a wooden stake or PVC pole that was driven into the sediment with a mallet, located ~1m in front of each treatment reef. These passive samplers were then left to recruit organisms for ~6-8 weeks. This follows the same design and methods of other long-term monitoring efforts conducted by the Smithsonian Environmental Research Center (e.g., Tepolt et

al. 2020) and in the Blakeslee/Gittman labs (e.g., Moore et al., 2020, Blakeslee et al., 2021). During sampler processing, I removed the mesh top and emptied the contents of the condo into a 2mm mesh sieve (56 x 56 x 13cm), sorting through the loose cultch to record and/or gather the contents. Between sampling dates, I returned the surveyed cultch to the condo and re-zip-tied the mesh to redeploy the crate and allow it to recruit for the following field date.

The passive samplers were retrieved and surveyed for recruited organisms three times in June, September, and October, 2023, following deployment in April 2023. All panopeid mud crabs, stone crabs, porcelain crabs, and fish (including oyster toadfish, blennies, pinfish, belted sandfish, and skilletfish) were brought back to the lab to be identified to the genus or species level and dissected for metazoan macroparasites using protocols from Moore et al. (2020) and Moore et al. (2023). Species identifications of panopeid mud crabs (*Eurypanopeus depressus*, *Panopeus herbstii*, and *Dyspanopeus sayii*) were determined using Williams (1984) for morphological indicators and confirmed using standard barcoding techniques (e.g., Blakeslee et al. 2024) to assign any species that were morphologically ambiguous (unpublished data supplied upon request). Stone crabs were identified to genus (*Menippe spp.*). All other mobile fauna (shrimp, brittle stars, oyster drills, urchins, flatworms, etc.) were identified to the lowest possible taxonomic designation and recorded in the field and released. All unknown individuals were photographed and identified later in the lab using online field guides and/or referral to faculty and alum of East Carolina University that are familiar with local fauna.

Field Sampling: Snail surveys

In addition to organisms collected and recorded via the crab condos, I also used active sampling protocols (hand-collecting and dip-netting) to sample eastern mudsnails (*Ilyanassa obsoleta*) adjacent to the sample sites in April, June, September, and October 2023 (N=4

sampling events). As organisms were collected haphazardly in the field, I followed previously established protocols for this site (Moore et al. 2020) and grouped the samples of the specimens at a block scale by site type for the restored sites for a total of seven snail sampling zones (n=3 restored sites, with COC1 and CSB1 snails collectively Block 1 snails, COC3 and CSB3 snails collectively Block 3 snails, and COC4 and CSB4 snails collectively Block 4 snails; and n=4 loose cultch sites, with CNR1, CNR2, CNR3, and a combined collection from CRR2 and CRR3). At each zone, I aimed to collect at least 100 mudsnails from all sites where they were present on sample days. These samples were brought back to the lab and dissected for parasites (see parasite dissection protocol below).

A full list of site names, their general locations, and the sampling strategies (habitat, biodiversity, etc.) employed at each location are shown in Table 2.

Table 2. Study sites. This table lists the restoration treatment, the type of data that was collected there (biodiversity and/or habitat), and the group it was placed in for snail sampling blocks (if applicable).

Site Name	Restoration Treatment	Surveyed for Biodiversity?	Block Group for Snail Sampling (if applicable)	Surveyed for Habitat?
CNR1	Loose Cultch	Yes	NA	Yes
COC1	Oyster Catcher	Yes	Block 1	Yes
CSB1	Shell Bag	Yes	Block 1	Yes
CNR2	Loose Cultch	Yes	NA	Yes
COC2	Oyster Catcher	No	NA	Yes
CSB2	Shell Bag	No	NA	Yes
CNR3	Loose Cultch	Yes	NA	Yes
COC3	Oyster Catcher	Yes	Block 3	Yes
CSB3	Shell Bag	Yes	Block 3	Yes
COC4	Oyster Catcher	Yes	Block 4	Yes
CSB4	Shell Bag	Yes	Block 4	Yes
CRR2	Loose Cultch	Yes	RR2/RR3	Yes
CRR3	Loose Cultch	Yes	RR2/RR3	Yes

Sessile faunal surveys

In order to obtain a coarse understanding of the differences in the sessile communities present across the restoration sites, I also attached 13.5 cm^2 plastic plates (Piedmont Plastics, Atlanta, GA) to the crab condos to recruit sessile invertebrates, per the same techniques in the previous faunal study at Carrot Island (Moore et al., 2020). These plates were collected in June, September, and October of 2023, and post-collection processing (described as follows) followed previously established methods (Moore et al., 2020). First, the accumulated biomass was removed and contained in a foil package. To remove moisture from the samples and obtain their dry weights, the foils were then dried in a drying oven for ~72 hours at 72°C. The dry weights were then obtained using a scale (Mettler Toledo model ME103E) to be compared to biomasses obtained from the initial monitoring effort (Moore et al., 2020), as depicted in Table 3.

Lab Processing for Parasites

For parasite data, I selected organisms (eastern mudsnails, panopeid crabs, stone crabs, porcelain crabs, and oyster toadfish) known from previous studies to be important hosts of trophically transmitting macroparasites in this system (trematodes, cestodes, acanthocephalans, nematodes, entoniscids; Moore et al. 2020, 2023, 2024). These hosts were dissected using previously established protocols as described below (Moore et al., 2020). Dissections and husbandry proceedings were approved by East Carolina University's IACUC (D346b) and applied to vertebrate organisms collected for parasite dissections as indicated in the summary table below (Table 3). The abundance and richness of parasites was recorded for each host species alongside several demographic characteristics of the host species, the exact details of which varied by host type, as described below.

For mudsnails, prior to dissection, I recorded the size (mm) of the snail measured as the distance from the bottom of the aperture to the apex of the snail's spire using digital calipers. Following this, the snail was destructively dissected using a hammer, with forceps to remove the gonadal region from within the snail's spire, which is where trematodes of *I. obsoleta* concentrate (Blakeslee et al. 2012). The gonads were pulled apart and subdivided further using forceps and studied under a light microscope (1-5X) for parasites, with positive cases recorded along with the species identification. Species identifications were made by analyzing the cercariae (larval stage) and sporocysts/rediae of the parasite under the aforementioned magnification and using morphological traits to reach an identification by referencing several sources, including Blakeslee et al. (2012), Phelan et al. (2016), Stunkard (1983), McDermott (1951), and Curtis (2007).

For all crabs, the demographic factors recorded included species, size, and sex (Moore et al. 2020, 2023). The species included all mud crabs (*Dyspanopeus sayii, Eurypanopeus depressus,* and *Panopeus herbstii*), porcelain crabs *(Petrolisthes armatus*), and stone crabs (*Menippe spp.*). Size was measured using digital calipers and recorded as carapace width (mm), taken as the widest length across the carapace for all crabs. An additional carapace length measurement for porcelain crabs only, taken from the middle of the base of the carapace to the rostrum. In addition to sex, female crabs carrying a brood were noted as ovigerous. Following the recording of these characteristics, dissections occurred by separating the upper from the lower carapace with a sterilized scalpel. All hepatopancreas and gonad tissues were removed from the crab's viscera and placed onto glass slides, where they were systematically examined under a compound microscope (4X,10X) to search for parasites. The identification and abundance (if applicable; e.g., trematode cysts) of parasites present were recorded, with

identifications of parasite taxa determined using morphological indicators from several sources, including Williams et al. (2023), Yamaguti (1971), Anderson et al. (1975), Schmidt (1986), Khalil & Bray (1994), Blakeslee et al. (2015), Moore et al. (2020), and Moore et al. (2023).

For oyster toadfish (*Opsanus tau*), the total length of the fish was measured (in mm) from the mouth to the tip of the tail prior to dissection using digital calipers. Each fish was externally scanned for ectoparasites, and then additional tissues and structures surveyed for parasites included the mouth, the brain, the gills, all fins (pectoral, pelvic, dorsal, ventral, caudal, and tail), heart, liver, stomach, intestines, and gonads. External examination and assessment of the mouth cavity was completed under a light microscope (1-5X), while the evaluation of all other structures was completed on slide or a compressorium under a compound microscope (4X,10X). Located parasites were recorded and identified using morphological characteristics and referencing prior publications and keys, including Yamaguti (1971), Anderson et al. (1975), Schmidt (1986), Khalil & Bray (1994), Moore et al. (2020), and Moore et al. (2023).

Table 3. A visual summary of the biodiversity data collected by year. Citations for data affiliated with prior publications (if applicable) are included. P = organisms were only dissected for parasites; FL + P = free-living diversity was recorded as well as affiliated parasite diversity.

	2018 (Moore et al., 2020)	**2019** (Moore et al., 2020)	**2023**
Snails	P	P	P
Crabs	FL + P	FL + P	FL + P
Fish	FL + P	FL + P	FL + P
Mobile Fauna	Crustaceans, Fish	Crustaceans, Fish	All
Sessile Fauna	No	Yes	Yes

Statistical Analyses

Habitat complexity changes were quantified using generalized linear models to investigate changes in oyster densities by time and treatment, as well as the interaction between these two variables. I also plotted average oyster density by average elevation over time. I also used multiple community diversity analyses and visualizations in Primer 7 to examine the effects of time, treatment, and the interaction of these two variables on free-living and parasite community assembly for passive sampler and snail trematode data; these included nMDS using the Bray-Curtis similarity index and PERMANOVAs (Moore et al., 2020; Moore et al., 2023). Community data was either fourth- or square-root transformed based on overall model visuals in the nMDS plots. Following analyses in Primer 7, further refinement of the trends was completed through Kruskal-Wallis tests and generalized linear models in R (*stats* package version 4.4.1). In all relevant analyses, the best model was selected using AICc model performance tests (Moore et al., 2020; Wellman et al., 2022). Both habitat complexity and biodiversity data were compared to the results of the monitoring efforts that took place shortly after the restoration efforts were implemented (Moore et al. 2020; Wellman et al. 2022).

For the habitat data, when assessing oyster densities by elevation over time, I had to average the available elevation data due to the absence of sample-specific elevation points from earlier monitoring efforts (in the prior monitoring effort, SB samples were taken from near the midpoint of the reef, while OC samples were removed from one corner of the reef) (Wellman et al., 2022). Thus, for these calculations, I plotted the previously calculated oyster densities for specific sites by the average elevations available to me (all four corners of each reef) and compared these results to the average densities by elevation I recorded in 2023 at the point each sample was taken. In terms of the biodiversity data, the free-living fish and crab data included all

organisms brought back to the lab for dissection as well as those with species identified in the field and recorded, but released back to the field during sampling. Each dataset was analyzed using the abundance of recorded species (numbers of individuals recorded). For all parasite data (crab parasites, fish parasites, and snail parasites), the data were analyzed in terms of prevalence. More specifically, the number of individuals infected by a certain parasite species or taxa was divided by the total number of that host species dissected in that sampling effort. In all data analyses (across free-living and parasites), the data for each individual site was averaged across the entire sampling period into which it was binned (pre-restoration, one-year post-restoration, and five-years post-restoration). To clarify, seasonality was not taken into account when analyzing trends over time relative to restoration. The only exception to this is a subset of analyses specific to the 2023 crab data in order to analyze specific trends concerning porcelain crabs (which were not present in earlier sampling efforts) and their potential connection to 2023 mud crab trends. Otherwise, seasonal data across all sampling years was lumped together to avoid losing statistical power in analyses. Intensity data was collected, but not analyzed here, for crab and fish parasites within select taxa (trematodes, nematodes, and monogeneans only) where applicable – to clarify, number of trematode cysts or adults, and number of adult nematode worms or adult monogeneans – as intensity was not quantifiable for other parasite taxa in these organisms, nor for number of larval trematode cercariae in the snails.

RESULTS

Habitat Characteristics

Restored oyster reefs of both treatments changed markedly over the study period and relative to early monitoring efforts. Early monitoring efforts (2018-2020) noted significant outperformance of the OC reefs relative to SB reefs, with OC reefs increasing in density by >400% over the study period and ending with >800% higher densities than SB reefs (Wellman et al., 2022). Our surveys (2023) revealed a significant decrease in oyster densities across both treatments from 2020 – 2023 (GLM, family = poisson, $p < 0.001$) (Fig. 4), and revealed a significant time by treatment interaction as well (GLM, family = Poisson, $p < 0.001$).

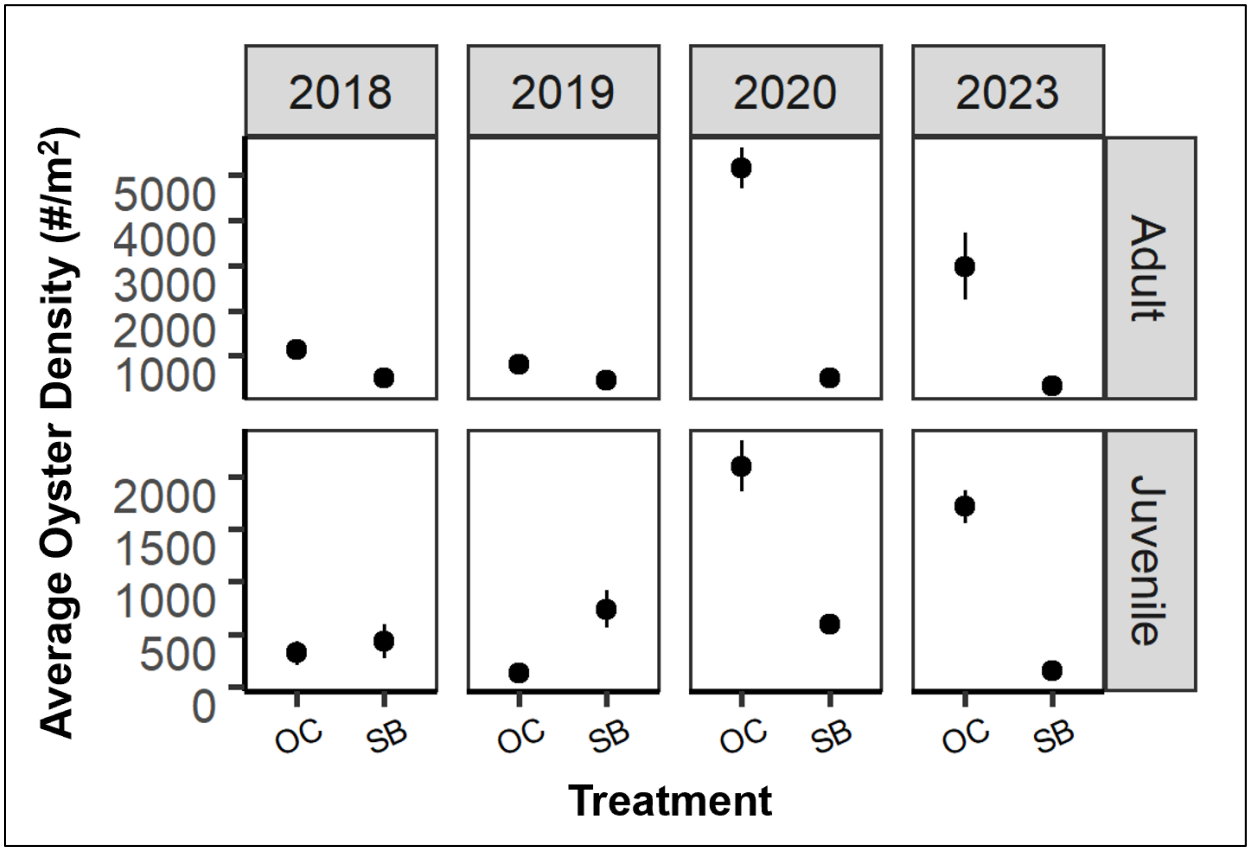

Figure 4. Oyster densities (#/m^2) by restoration treatment by year, 2018-2023. Results are also separated into size classes, with juvenile oysters (24mm or less) below adult oysters (25mm or greater). 2018-2020 data from Wellman et al. 2022.

The highest oyster densities were recorded in 2020 (Fig. 4). At the time of the 2020 sampling, reef elevations fell within the hypothesized "Optimal Growth Zone" (OGZ; -0.3m – -0.1m NAVD88) for intertidal oysters in central coast NC (Ridge et al., 2015). In 2023, the highest oyster densities recorded were also for OC reefs (as in 2020), but the live oysters surveyed on these reefs were primarily located above the OGZ (Fig. 5). Notably, SB reefs were outperformed by OC reefs regardless of elevation relative to the OGZ in 2023 (Fig. 5).

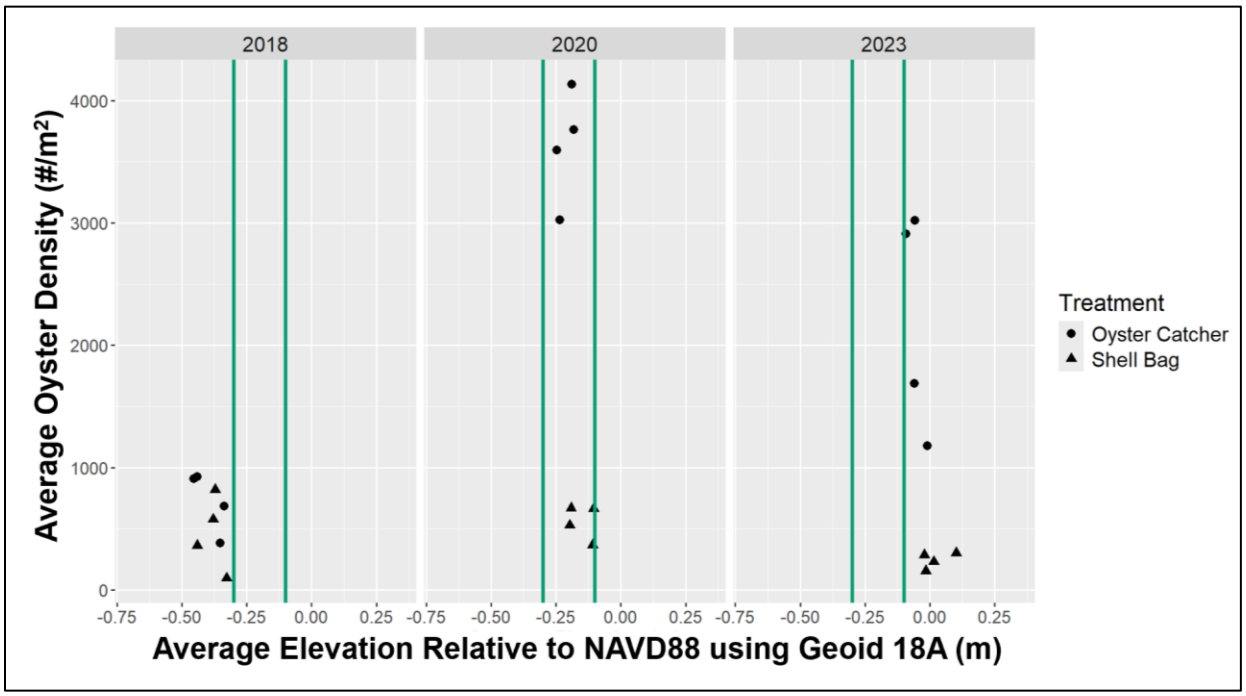

Figure 5. Average oyster density (#/m^2) by average elevation (m) at which sample was taken for 2018, 2020, and 2023. The optimal growth zone for oysters is plotted in green (-0.3m – -0.1m NAVD88; Ridge et al., 2015). Treatment is differentiated by shape (OC reefs by circles, SB reefs by triangles).

Biodiversity of reef-affiliated communities: Free-living Organisms

Free-living Fish

Free-living fish communities varied significantly with time relative to restoration (PERMANOVA, p=0.001), visible both in the early monitoring effort (with higher fish diversity represented in the one-year post-restoration sampling than in the pre-restoration fish communities; p=0.02) and with the addition of five-year post-restoration data (Fig. 6). The composition of the fish communities sampled five-years post-restoration varied more significantly from the one-year post-restoration data than the pre-restoration data (p=0.001 and p=0.003, respectively), with lower overall fish diversity affiliated with the pre- and five years post-restoration sampling. These results suggest that oyster reef restoration does not always retain the initially recruited fish community that follows restoration. Notably, species such as striped blennies, sheepshead, grey snapper, and gobies, were more abundant pre-restoration and one-year post-restoration, whereas oyster toadfish, for example, were more strongly affiliated with the five-year post-restoration fish assemblages (Fig. 6). However, given the distribution of the loose cultch reef points as well (broadly separated by year), these results may suggest a broader change in fish communities at the creek level (Fig. 6). In terms of treatment, a PERMANOVA considering time with post-hoc pair-wise tests assessing treatment revealed that none of the treatment levels (LC, SB, OC) varied significantly from one another (p>0.05). Considering this relationship with a time by treatment interaction term in the PERMANOVA was also found to be insignificant (p=0.093).

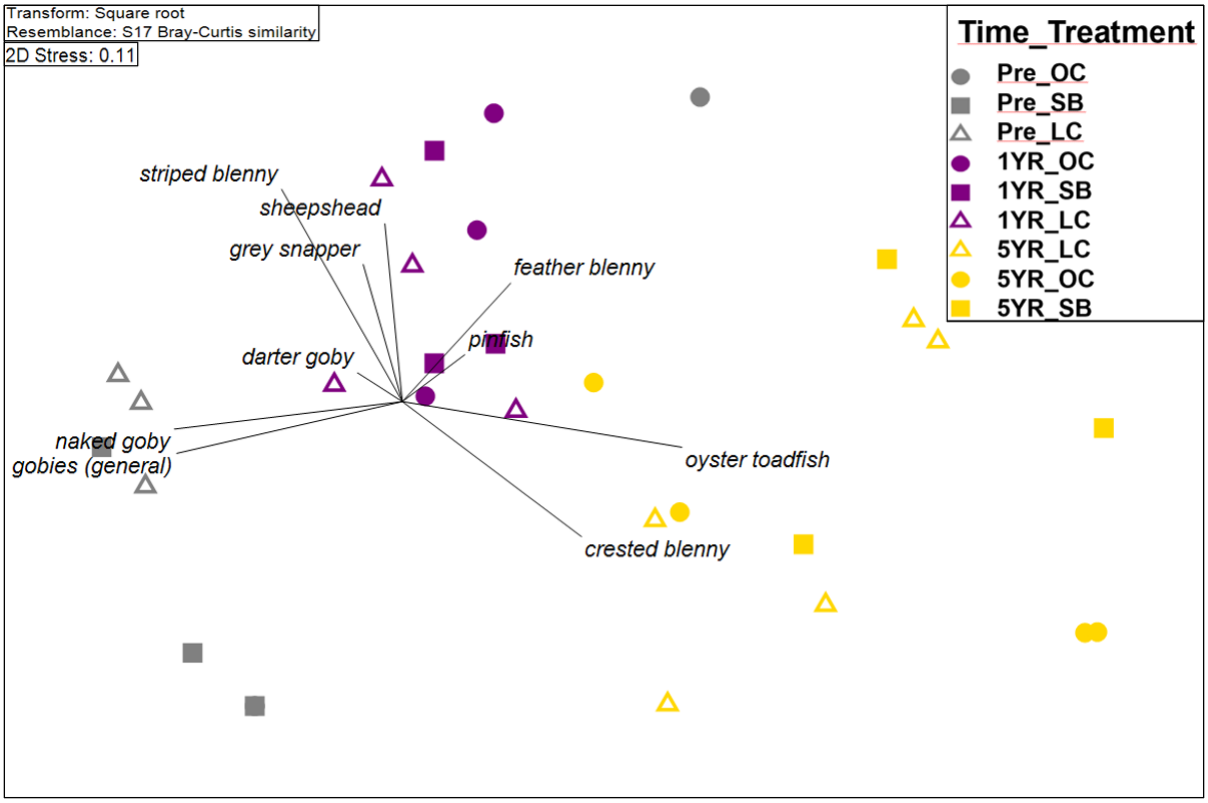

Figure 6. A non-metric multidimensional scaling plot made in Primer 7 using the Bray-Curtis similarity index to assess the changes in free-living fish communities at Carrot Island sites by treatment (LC, OC, and SB) and time relative to the restoration effort (Pre-restoration, "Pre"; one-year post-restoration, "1YR"; and five-years post-restoration, "5YR"). Time is differentiated by color (pre-restoration in gray, one-year post-restoration in purple, and five-years post-restoration in gold), treatment by the shape (open triangles reflect LC sites, shaded circles reflect OC sites, and shaded squares represent SB sites). The overlaid vectors represent the species that drive the distribution of the plotted samples.

The average Shannon-Wiener diversity score revealed that the one-year post-restoration community had the greatest average diversity score across all three treatments (Fig. 7). Using Kruskal Wallis tests with a Dunn test (with a Bonferroni correction) on these average index scores, I found that these differences were significant over time overall ($p = 7.5015e-05$), but not between pre- and 5-years post-restoration ($p>0.05$). Treatment (LC, SB, OC), however, was not significant overall when using the same test ($p=0.74$).

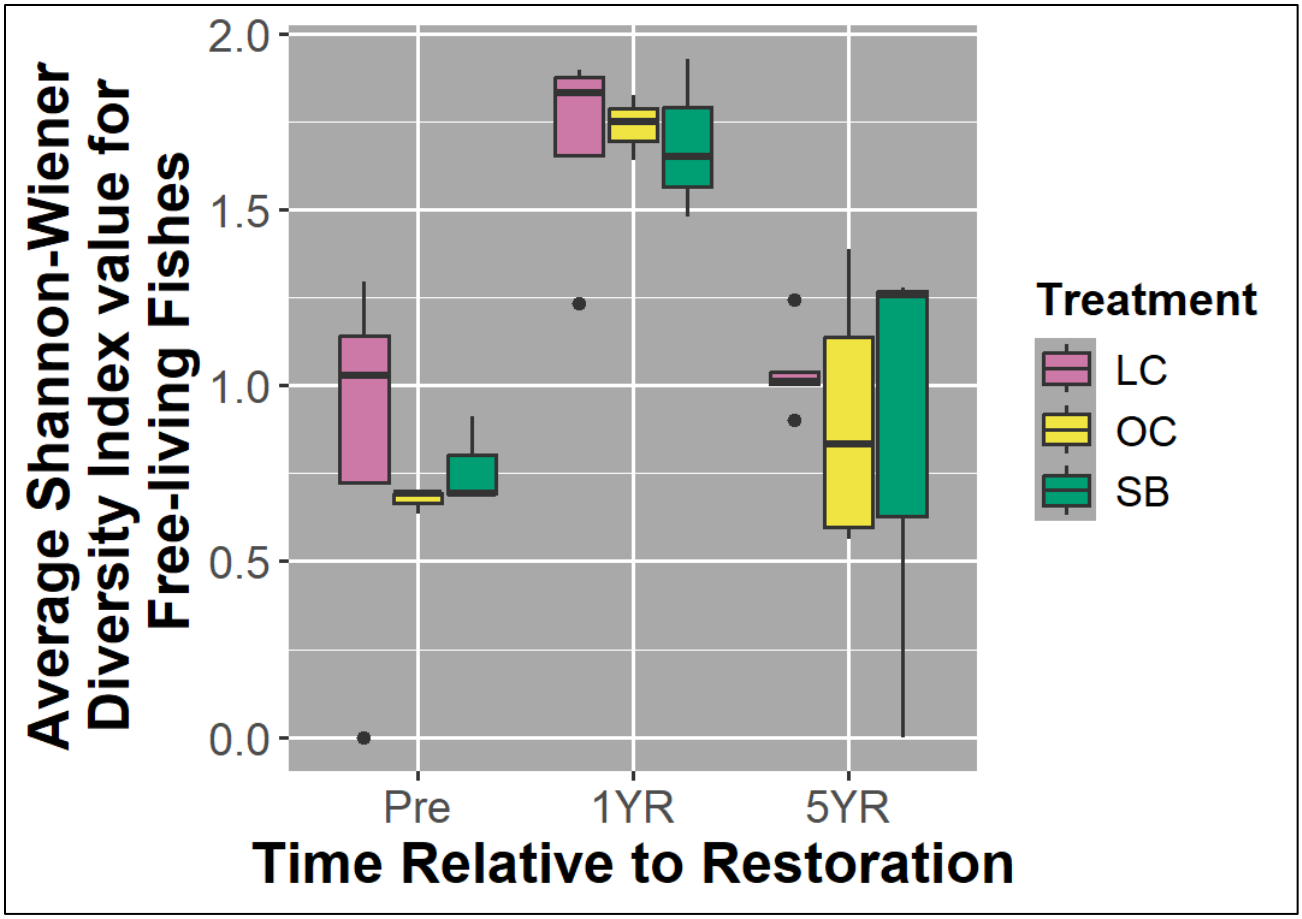

Figure 7. Average Shannon-Wiener Diversity Index scores of free-living fish communities by time (relative to restoration) and by treatment.

The average abundance of oyster toadfish significantly increased over time, with the highest average abundances of this species five years post-restoration (Kruskal-Wallis, p=1.2234e-05). Pair-wise tests revealed all sampling events were significantly different from one another, with the greatest difference between five-years post-restoration and pre-restoration (Kruskal-Wallis with Dunn Test and Bonferroni Correction, p=6.1404638e-06). Treatment was not found to be significant (Kruskal-Wallis, p=0.78661).

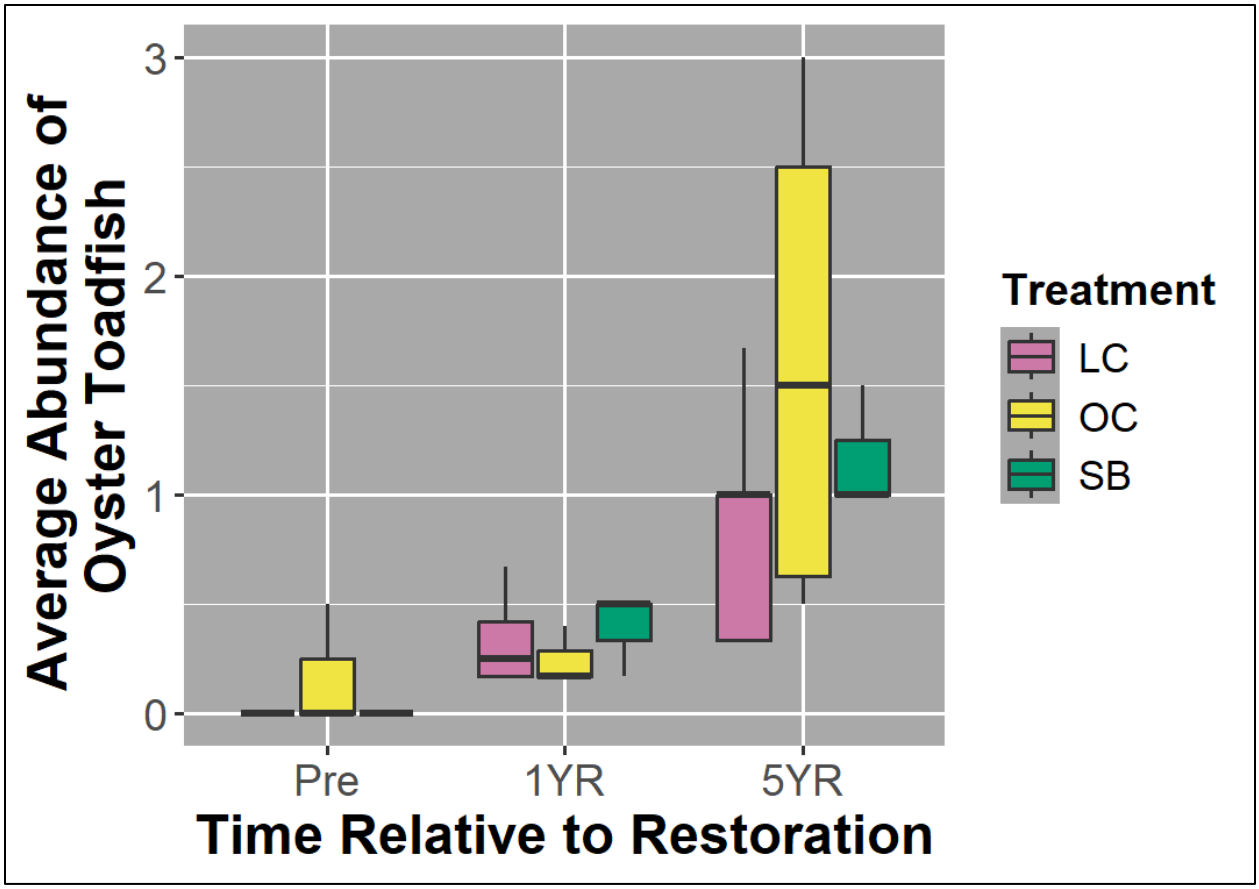

*Figure 8. Average abundance of oyster toadfish (*Opsanus tau*) by time (relative to restoration) and by treatment.*

Free-living Crabs

The composition of free-living crab communities at Carrot Island increased in biodiversity over time; a PERMANOVA integrating the effects of time and treatment (as well as the interaction of these two variables) on the crab community composition and found time to be a significant factor (Fig. 9; p=0.003). Notably, further post-hoc pair-wise comparisons of time periods revealed five-year post-restoration crab communities to be significantly different from pre- and one-year post-restoration communities (p=0.003 and 0.006, respectively), while the earlier two sampling efforts did not differ significantly from one another (p=0.192). It is likely that porcelain crabs, as well as *P. herbstii*, *D. sayii*, and stone crabs are driving the differences marked in the 2023 sampling (Fig. 9). The interaction of time and treatment was not significant

(p=0.842). While treatment was also found to have no statistically significant effect in the PERMANOVA (p>0.05), as with the free-living fish communities, points representing the free-living crab communities at loose cultch reefs seem to also separate by sampling year (Fig. 9). This may once again indicate that there is a broader creek level shift in community composition at play (Fig. 9).

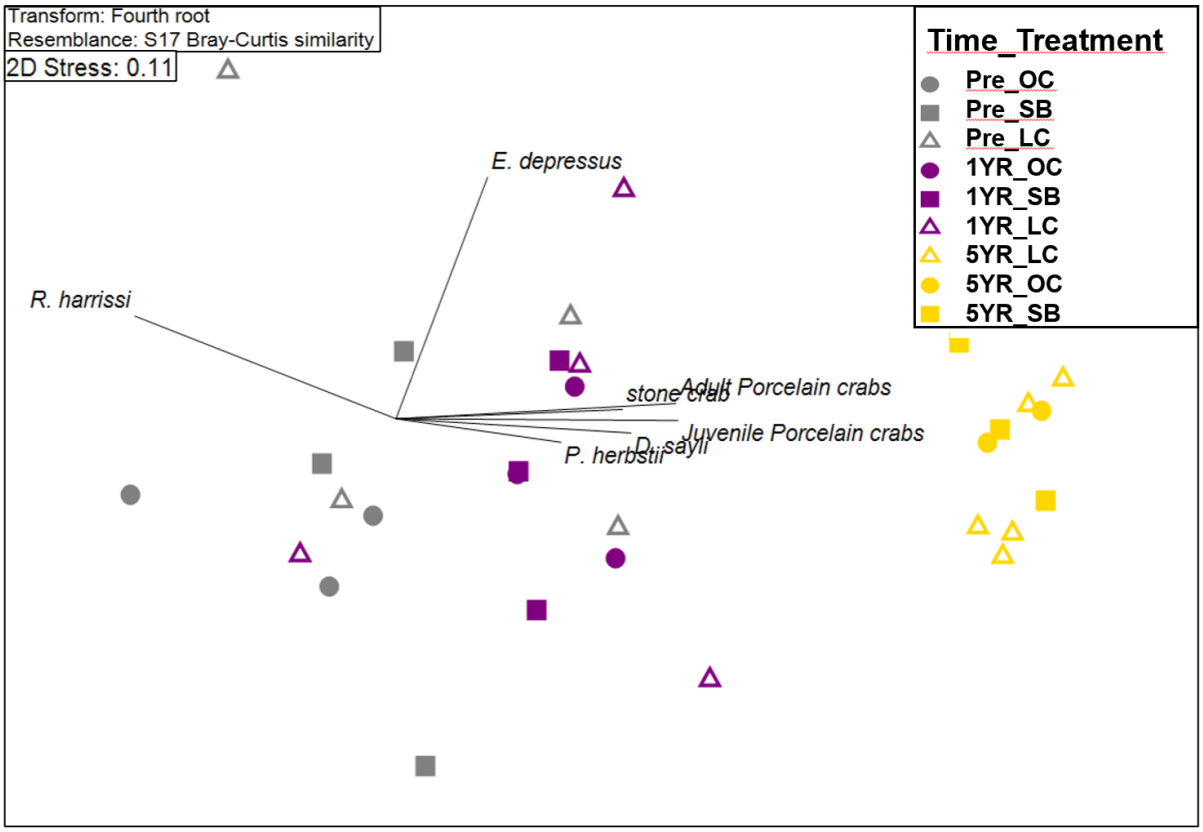

Figure 9. A non-metric multidimensional scaling plot made in Primer 7 using the Bray-Curtis similarity index to assess the changes in free-living crab communities at Carrot Island sites by treatment (LC, OC, and SB) and time relative to the restoration effort (Pre-restoration, one-year post-restoration, and five-years post-restoration). Time is differentiated by color (pre-restoration in gray, one-year post-restoration in purple, and five-years post-restoration in gold), treatment by the shape (open triangles reflect LC sites, shaded circles reflect OC sites, and shaded squares represent SB sites). The overlaid vectors represent the species that drive the distribution of the plotted samples.

The average Shannon-Wiener diversity score values revealed that the five-year post-restoration free-living crab community had the greatest average diversity scores across all three

treatments (Fig. 10). A PERMANOVA confirmed the significance of this trends, with Shannon-Wiener diversity scores for crab communities present five-years post-restoration significantly higher than earlier communities sampled (p=0.0002 for pre-restoration and 0.0123 for one-year post-restoration). The two earlier monitoring points did not significantly vary from one another (p=0.6977). However, as seen previously, S-W scores did not vary significantly by treatment (p=0.7935).

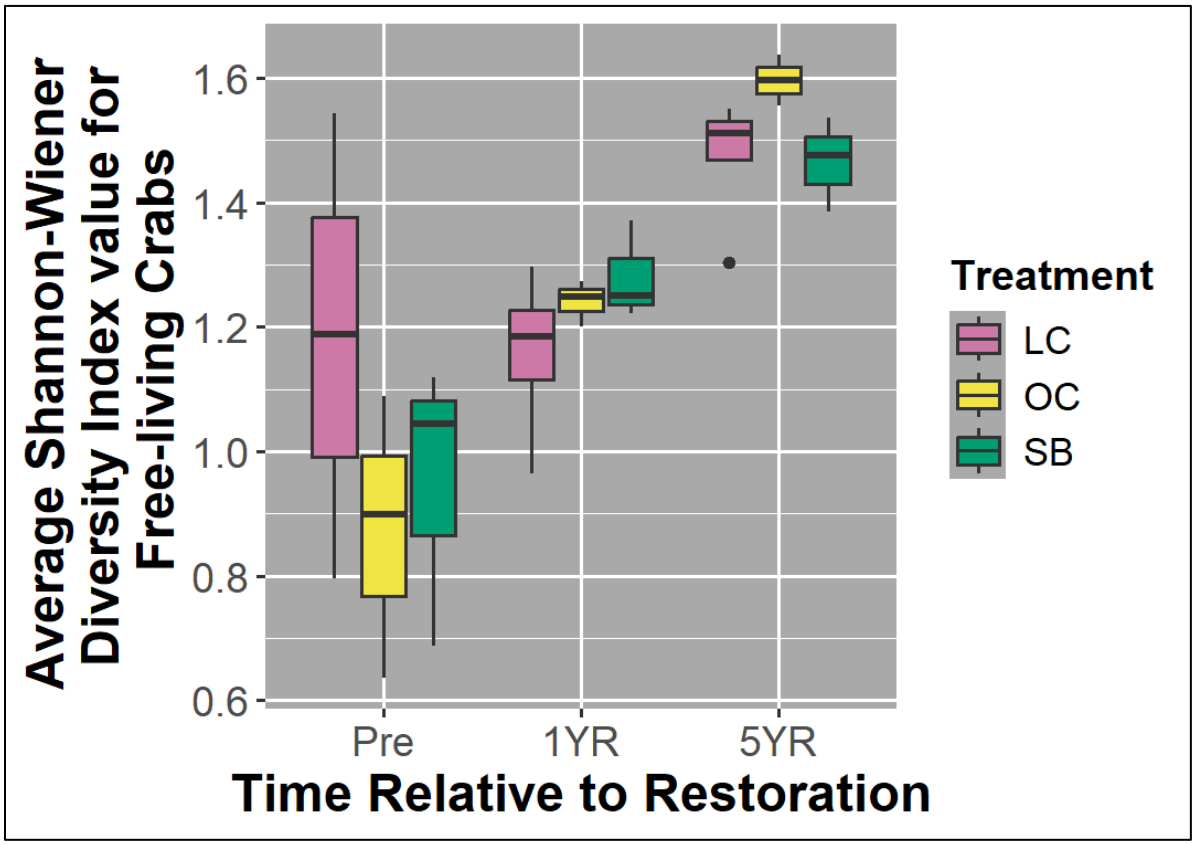

Figure 10. Average Shannon-Wiener Diversity Index scores of free-living crab communities by time (relative to restoration) and by treatment.

Mud crab abundance varied significantly by treatment in 2023 (Fig. 11; Kruskal-Wallis, p=0.02), with OC boasting significantly greater abundances than loose-cultch reefs (Kruskal-Wallis and Dunn-test with Bonferroni correction, p=0.0176). However, OC abundances were not

significantly greater than SB reefs (Kruskal-Wallis and Dunn-test with Bonferroni correction, p=0.1231), nor did the latter vary significantly from loose cultch reefs (p=1.0). Mud crab abundances did not vary significantly by month (Kruskal-Wallis, p=0.2717).

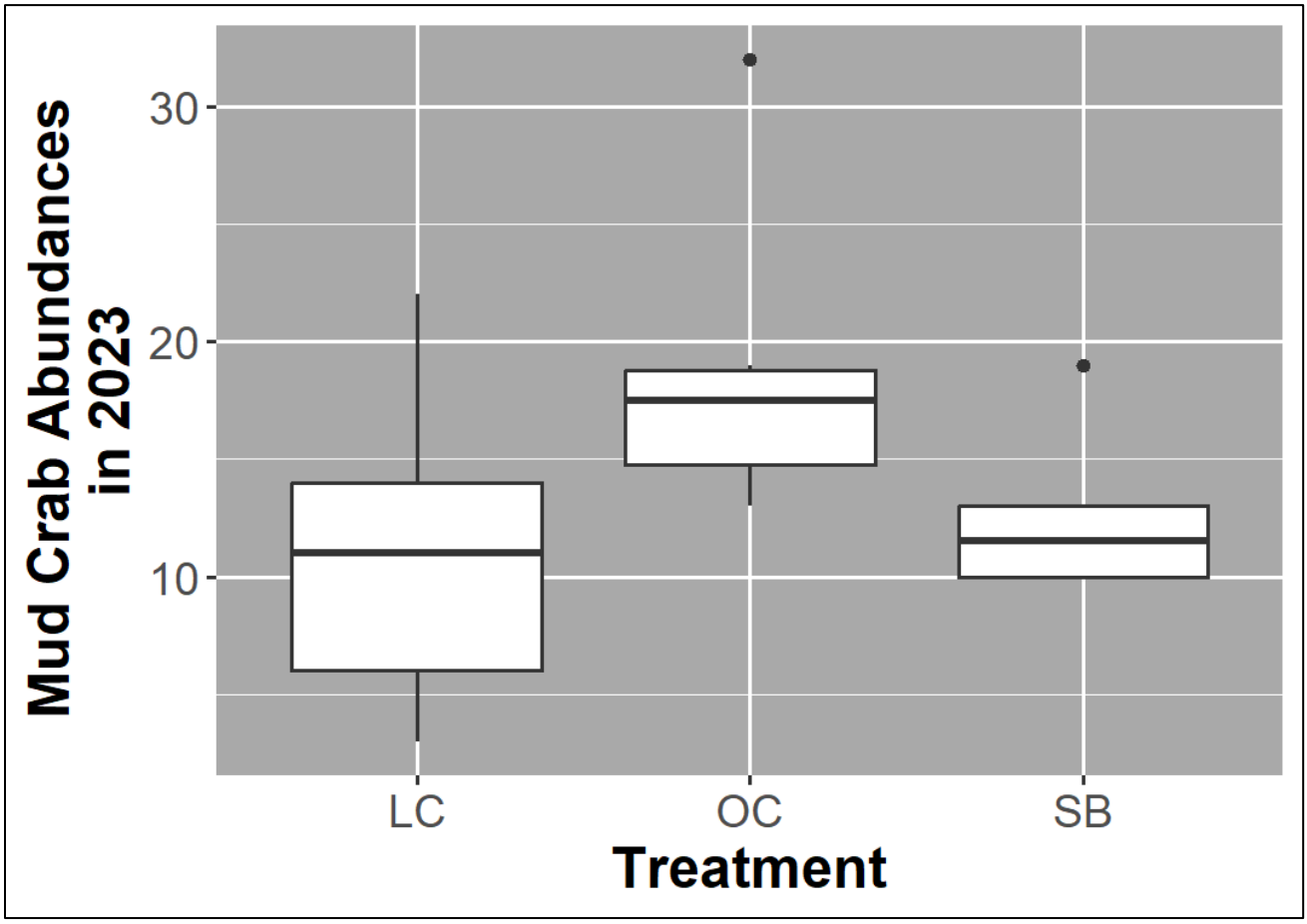

Figure 11. 2023 Mud crab abundances by treatment.

Green Porcelain Crabs

Porcelain crabs (*P. armatus*) were not noted in the early biodiversity monitoring efforts (Moore et al., 2020; Blakeslee et al. 2024) and likely drove some of the differences seen between the 5-year post-restoration communities and those of earlier monitoring (Fig. 9-10). The average Shannon-Wiener diversity index scores affiliated with crab communities across each treatment in 2023 were higher at SB and OC reefs relative to LC reefs, though these differences were not

statistically significant (Fig. 12; Kruskal-Wallis, p=0.9556). The average Shannon-Wiener diversity index score also did not vary significantly by sampling month in 2023 (p=0.5115).

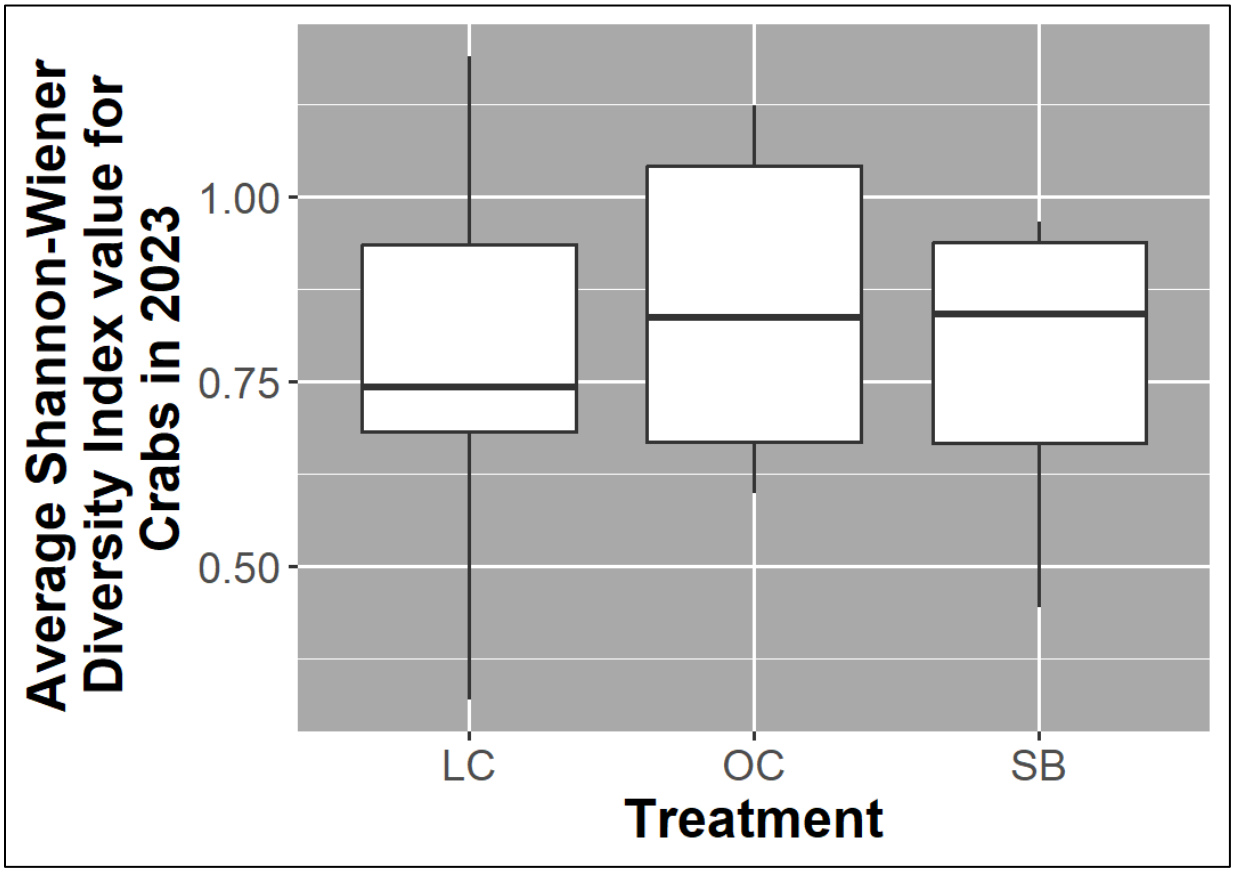

Figure 12. Average Shannon-Wiener Diversity Index scores of free-living crab communities in 2023 by treatment.

Total porcelain crab abundances varied significantly by sampling month in 2023, with the highest abundances in October (Fig. 13; Kruskal-Wallis, p=6.6004e-05). A GLM considering the interactive effects of sampling month and treatment on total porcelain crab abundance revealed that OC was significantly lower than LC reefs, while LC reefs did not significantly differ from SB reefs (p=0.0005 and 0.87, respectively).

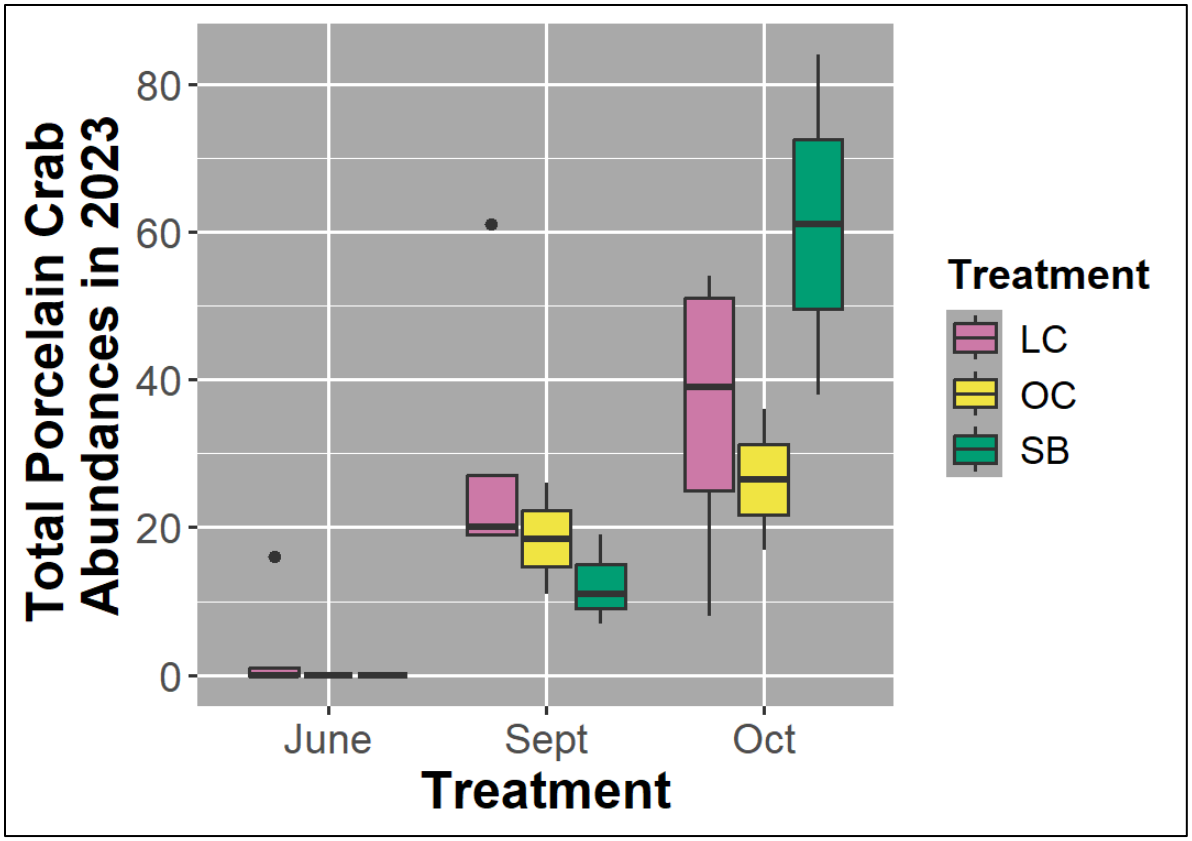

Figure 13. Total abundances of porcelain crabs in 2023 by time (months) and by treatment.

Sessile Faunal Biomass

In terms of the comparative sessile communities over time, the average biomass of fouling organisms was significantly different over time (Kruskal-Wallis, p=0.03297) but not by treatment (Kruskal-Wallis, p=0.3955). On average, fouling community biomass was greater in 2019 (one-year post-restoration) than in 2023 (five-years post-restoration) (Fig. 14).

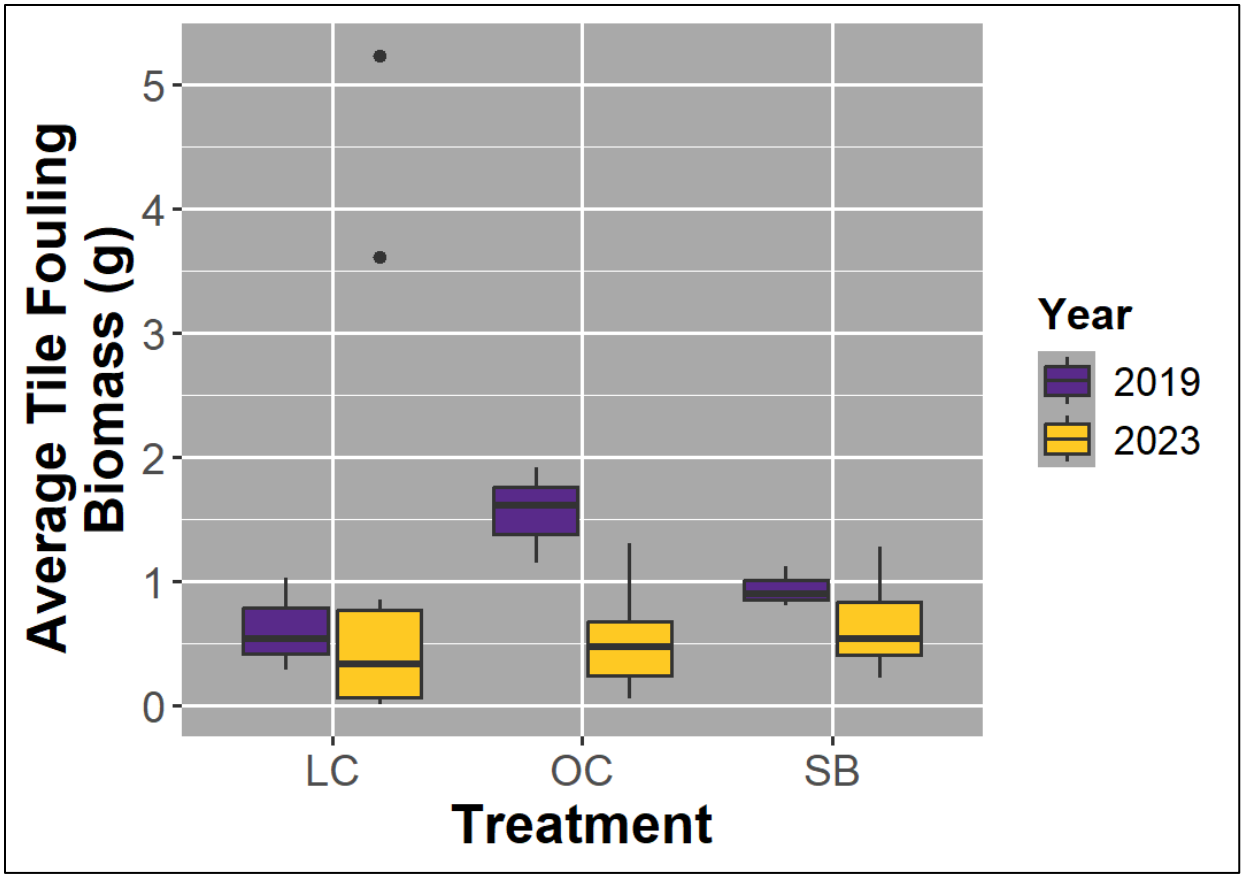

Figure 14. Average biomass (g) of fouling communities collected from plastic tiles by treatment. Boxplots are color-coded by collection year: 2019, one-year post-restoration, and 2023, five years post-restoration.

Biodiversity of reef-affiliated communities: Parasitic Organisms

Fish parasites

Initial assessment via a non-metric multidimensional scaling plot suggests markedly different fish parasite communities relative to time since restoration, with many of the "1YR" points clustered near the lower portion of the figure while the "5YR" and "Pre" points are located primarily in the top half of the plot (Fig. 15). Nematodes and cestodes appear to be driving much of the distribution of the five-year post-restoration points, and trematodes and monogeneans seem to be closely affiliated with this time point as well (in addition to the pre-restoration data) (Fig. 15). Many of the restored sites across all years (depicted as the shaded

circles and squares for Oyster Catcher and Shell Bag, respectively) are also primarily clustered in the middle of Fig. 15, while the loose cultch sites (shown as unshaded triangles) are less tightly affiliated. However, PERMANOVA results integrating the effects of time and treatment suggest no significant influences of any of these factors ($p>0.05$) nor the interaction between time and treatment ($p=0.162$). Further study of these factors in PERMANOVA runs where each lone factor was included revealed a significant effect of time only ($p=0.014$), with post-hoc pair-wise tests finding significant differences between one-year post-restoration and both pre-restoration and five-years post-restoration ($p=0.001$ and 0.044, respectively).

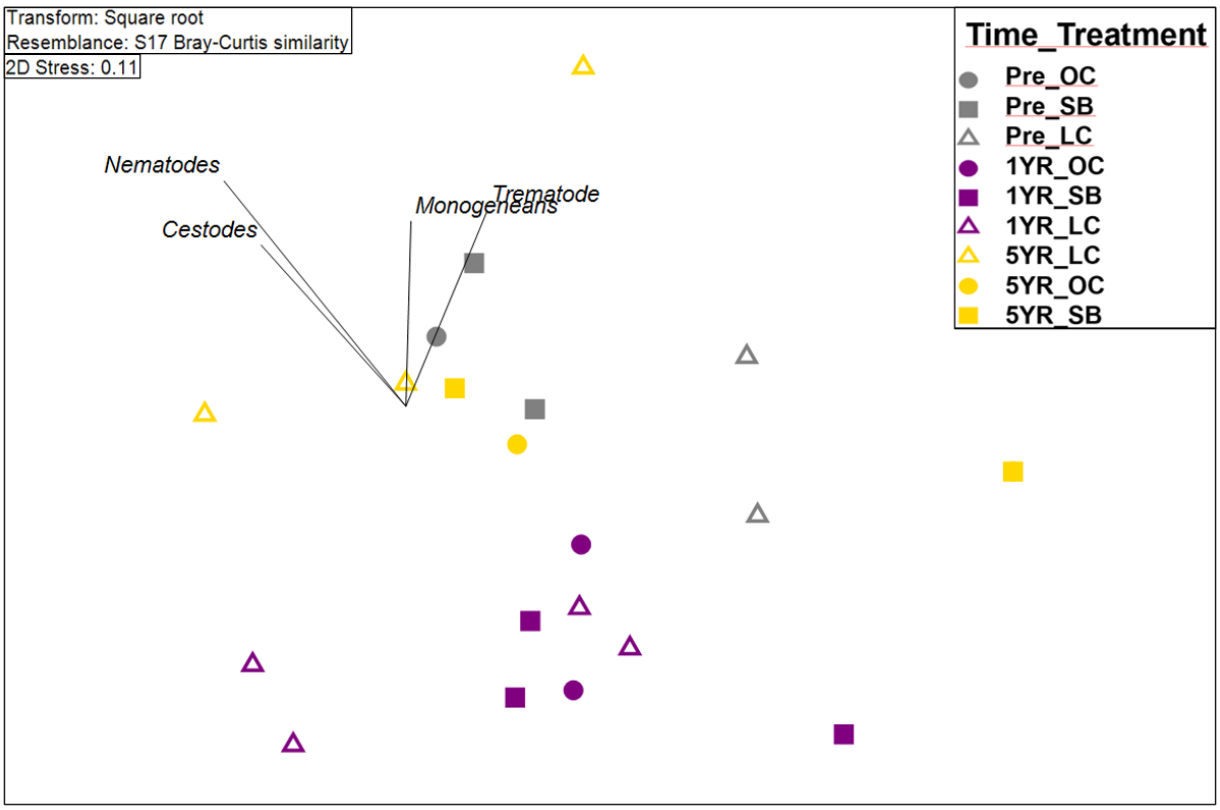

Figure 15. A non-metric multidimensional scaling plot made in Primer 7 using the Bray-Curtis similarity index to assess the changes in fish parasite communities at Carrot Island sites by site type (loose cultch, Oyster Catcher, and Shell Bag) and time relative to the restoration effort (Pre-restoration, one-year post-restoration, and five-years post-restoration). Time is differentiated by color (pre-restoration in gray, one-year post-restoration in purple, and five-years post-restoration in gold), treatment by the shape (open triangles reflect loose cultch sites,

shaded circles reflect Oyster Catcher sites, and shaded squares represent Shell Bag sites). The overlaid vectors represent the species that drive the distribution of the plotted samples.

Further analyses revealed that two of the four parasite taxa found in fish were individually significantly different over time: trematodes (Kruskal-Wallis, p=0.013755; Fig. 16, top left) and cestodes (Kruskal-Wallis, p=0.030575; Fig. 16, top right). Monogeneans were only found in 2023 and thus not further evaluated, while nematodes were not significantly affected by time (Kruskal-Wallis, p=0.2505; Fig. 16, bottom left). Average trematode prevalence was highest in the pre-restoration sampling period and was followed by the five-year post-restoration sampling period, from which it did not significantly differ (Kruskal-Wallis and Dunn-test with Bonferroni correction, p=0.752672089; Fig. 16, top left). As such, the lowest trematode prevalence was documented one-year post-restoration, though this only differed significantly from the pre-restoration sampling period (Kruskal-Wallis and Dunn-test with Bonferroni correction, p=0.014337667; Fig. 16, top left). Cestode prevalence followed the same trend, with the highest average pre-restoration, then five-years post-restoration, and then one-year post-restoration, with the only significant difference between pre-restoration and one-year post-restoration (Kruskal-Wallis and Dunn-test with Bonferroni correction, p=0.024812465; Fig. 16, top right).

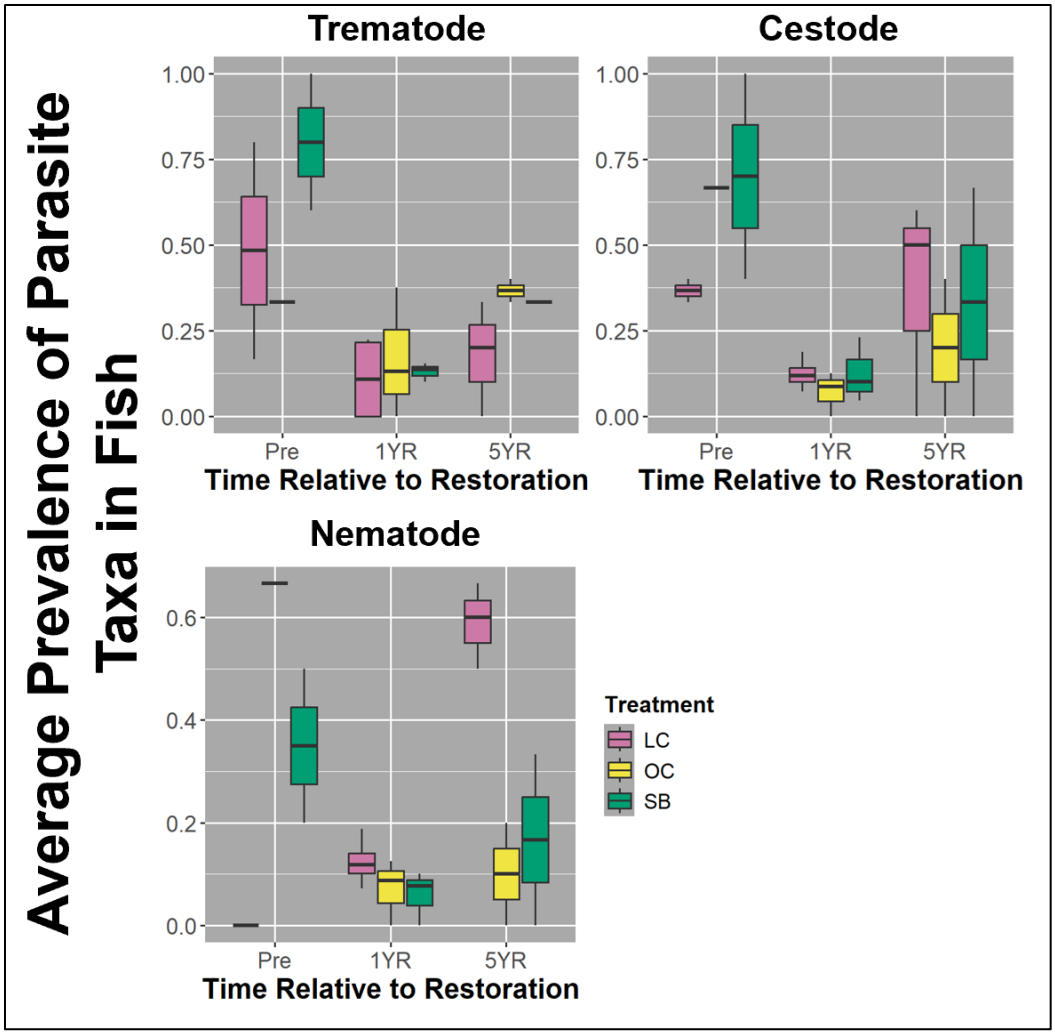

Figure 16. Parasite prevalence by taxa within various fish hosts by time relative to restoration (pre-restoration, 1-year post-restoration, and 5-years post-restoration. The top left figure reflects the changes in trematode prevalence across all fish hosts. The top right figure depicts the changes in cestode prevalence across all fish hosts. The bottom left figure shows the changes in nematode prevalence across all fish hosts. Seasonal differences were not analyzed to preserve statistical power.

Crab parasites

A cluster analysis largely supported temporal separation of the data, as the majority of sample points clustered with points that shared a sampling year; that is, 2023 points in one cluster, 2020 in another, and 2018 in a third (Fig. 17). While there are some points mixed in with the pre-restoration cluster that are from later sampling efforts, the majority are LC reefs, and there is more overlap between the 1-year and 5-year post restoration clusters (Fig. 17). A

PERMANOVA also highlighted time alone as a significant influence (p=0.001), but not treatment significant (p=0.253), nor the interaction of these two variables (p=0.463). Post-hoc pair-wise tests further indicated that each time period of sampling significantly differed from the rest (p=0.003 for all), further emphasizing the visual distinctions represented in Fig. 17. However, treatment appears to be more distinct when assessed via Fig. 17, particularly when studying the 1-year post-restoration sampling points, as shell bags points are closest to one another, and similar patterns are seen with the Oyster Catcher and loose cultch samples from this time period (Fig. 17).

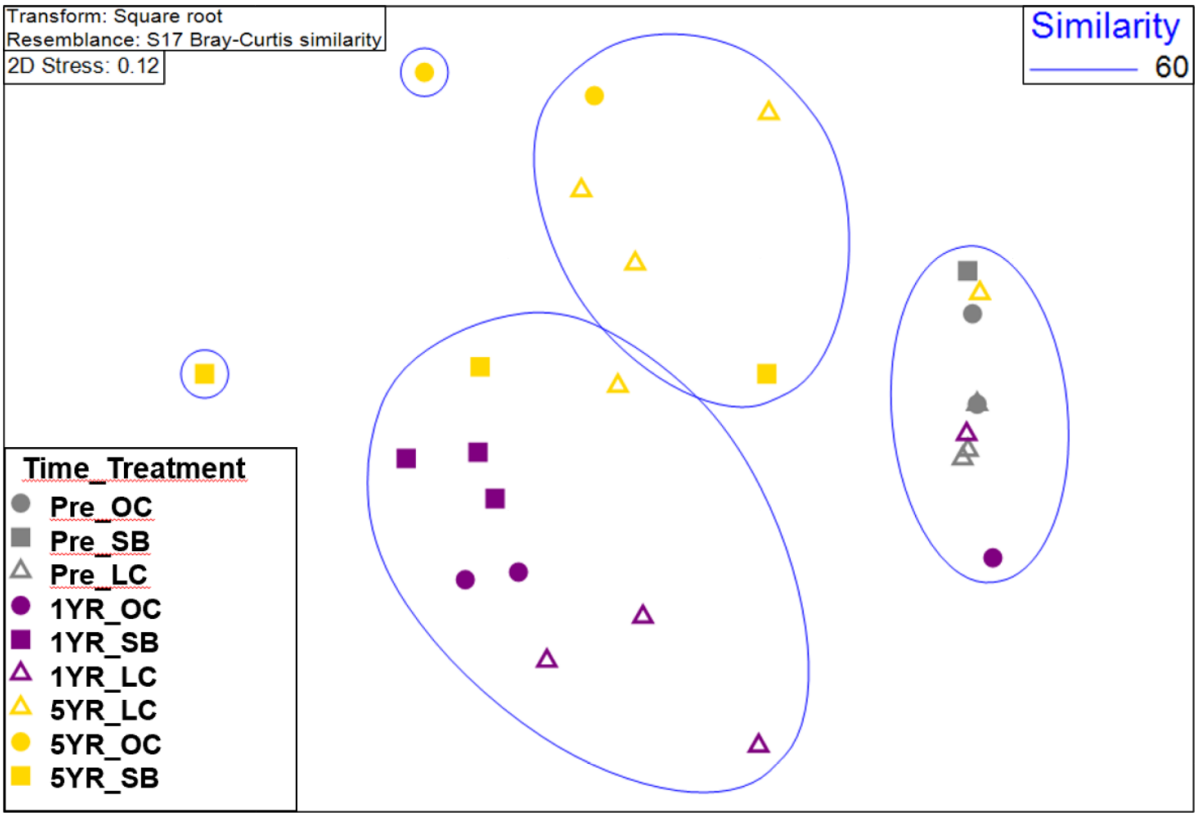

Figure 17. A non-metric multidimensional scaling plot made in Primer 7 using the Bray-Curtis similarity index and a cluster analysis to assess the changes in crab parasite communities at Carrot Island sites by site type (loose cultch, shell bag, and Oyster Catcher) and year (pre-restoration in 2018, one-year post-restoration in 2019, and five-years post-restoration in 2023). The cluster analysis reveals groupings of communities that are more than 60% similar to one another (shown in light blue circles). Years are differentiated by color (2018 in gray, 2019 in

purple, and 2023 in gold), site type by the shading of the shape (open shape reflects loose cultch sites and restored sites are depicted by solid shapes, with Oyster Catcher depicted as circles while shell bag sites are squares).

Trematode parasite prevalence varied significantly with time for crabs (Kruskal-Wallis, p=0.029553; Fig. 18, top left), in addition to cestodes (Kruskal-Wallis, p=0.0022851; Fig. 18, top right), nematodes (Kruskal-Wallis, p=0.012366; Fig. 18, bottom left), and entoniscids (Kruskal-Wallis, p=0.00040056; Fig. 18, bottom right). Rhizocephalans were not further analyzed due to low overall prevalence/absence from multiple time points. For trematodes, cestodes, and entoniscids, prevalences were highest one-year post-restoration, but these margins were only occasionally significant over time. For trematodes, the only significant difference was in comparison to the five-year post-restoration sampling (Kruskal-Wallis, p=0.040519171; Fig. 18, top left). For cestodes and entoniscids, the difference was significant relative to both pre-restoration (Kruskal-Wallis, p=0.0020840311, Fig. 18, top right for cestodes; Kruskal-Wallis, p= 0.00039704444, Fig. 18, bottom right for entoniscids) and five-years post-restoration (Kruskal-Wallis, p=0.0493214857, Fig. 18, top right for cestodes; Kruskal-Wallis, p= 0.01566482376, Fig. 18, bottom right for entoniscids). For nematodes, the average prevalence was highest five-years post-restoration, then one-year post restoration, but only the former was significantly greater than the pre-restoration (Kruskal-Wallis, p=0.010820811, Fig. 18, bottom left) sampling period.

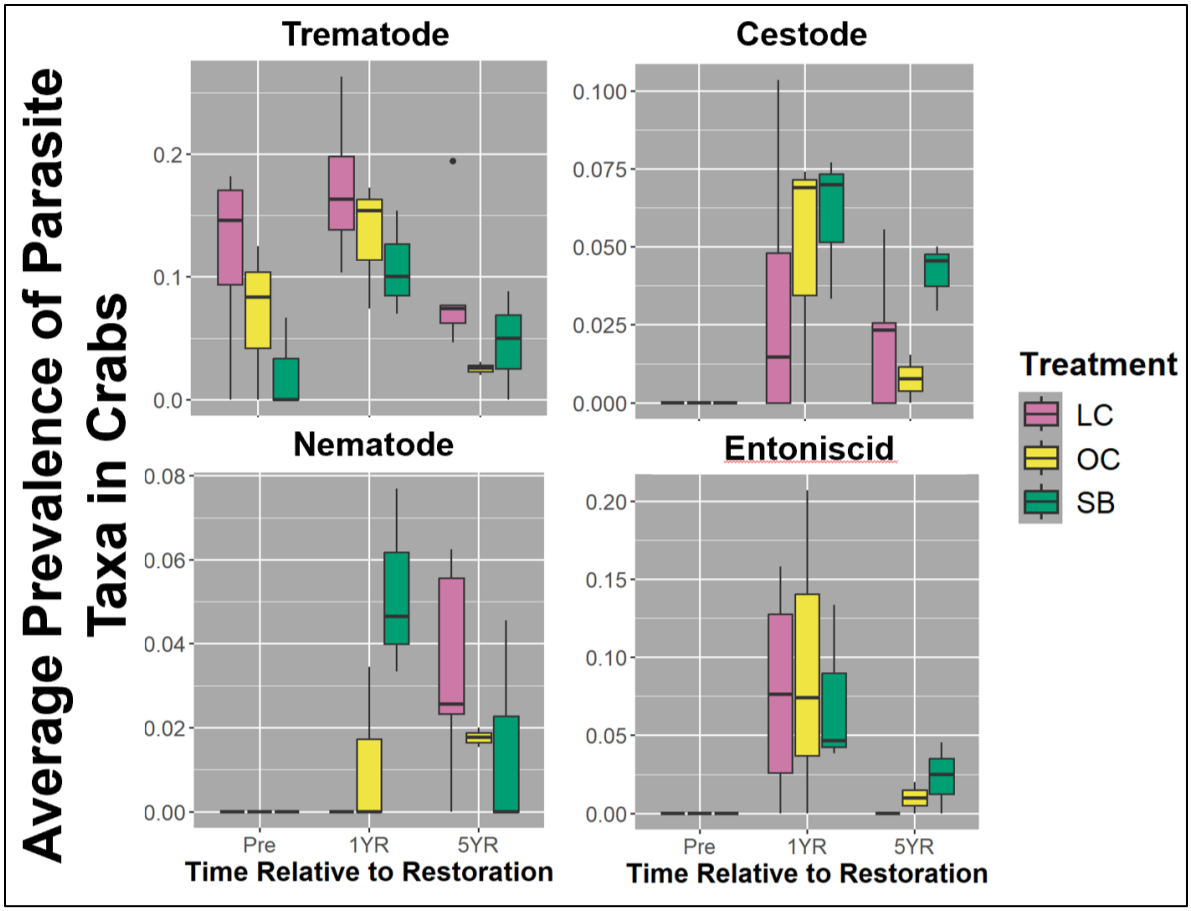

Figure 18. Parasite prevalence by taxa within various crab hosts by time relative to restoration (pre-restoration, 1-year post-restoration, and 5-years post-restoration. The top left figure reflects the changes in trematode prevalence across all crab hosts, while the top right figure depicts the changes in cestode prevalence across all crab hosts. The bottom left figure shows the changes in nematode prevalence across all crab hosts, while the bottom right figure shows the changes in entoniscid prevalence across all crab hosts.

Snail parasites

Snail trematode communities were first visualized through rank-abundance plots and nMDS plots (Figs. 19). With rank-abundance plots, steeper slopes reflect less evenly diverse communities, while shallower slopes indicate the abundances are more equal across all species in the community. Pre-restoration, loose cultch communities displayed a shallower slope than the restored plots, and this remains true in the one-year post-restoration plot, although there are more species – six compared to four in the pre-restoration time chunk – present in the system as a

whole (Fig. 19). While there were only five species located across 2023 surveys, this is the first time point at which snail trematode communities affiliated with restored reefs have a shallower slope than loose cultch reef communities (Fig. 19).

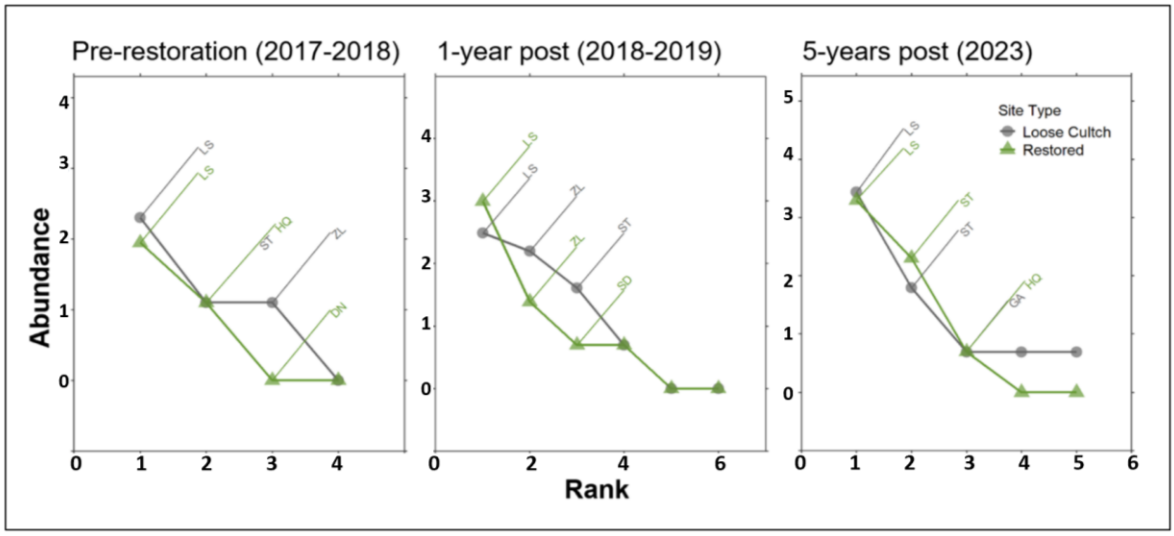

*Figure 19. Rank-abundance curves depicting the trematode communities affiliated with the eastern mud snail (*I. obsoleta*) at Carrot Island in restored (SB and OC) and LC plots. Letters within the figure represent abbreviations of the scientific name of the parasite species represented at that point; LS* = Lepocreadium setiferoides, *ST* = Stephanostomum tenue, *HQ* = Himasthla quissetensis, *ZL* = Zoogonus lasius, *DN* = Diplostomum nassa, *SD* = Stephanostomum dentatum, *GA* = Gynaecotyla adunca *(not pictured: PM* = Pleurogonius malaclemys*). Only the top three species found at each site type for each sampling year are listed by abbreviation.*

Further interpretation of the differences in parasite communities between the pre-restoration, one-year post, and five-year post-restoration communities is possible through a nMDS plot (Fig. 20). Closer points in these figures reflect sampling events/categories that are more similar to each other based on the index score, while more disparate points are less similar by this index. A cluster analysis grouping communities with >50% similarity showed restored reefs well-mixed with loose cultch reefs across all time points, and placed 5 year restored reefs quite close to the loose cultch reefs of the same time point, indicating that the restored reefs may

now be more closely resembling their more-natural counterparts in terms of their parasite richness and abundances (Fig. 20).

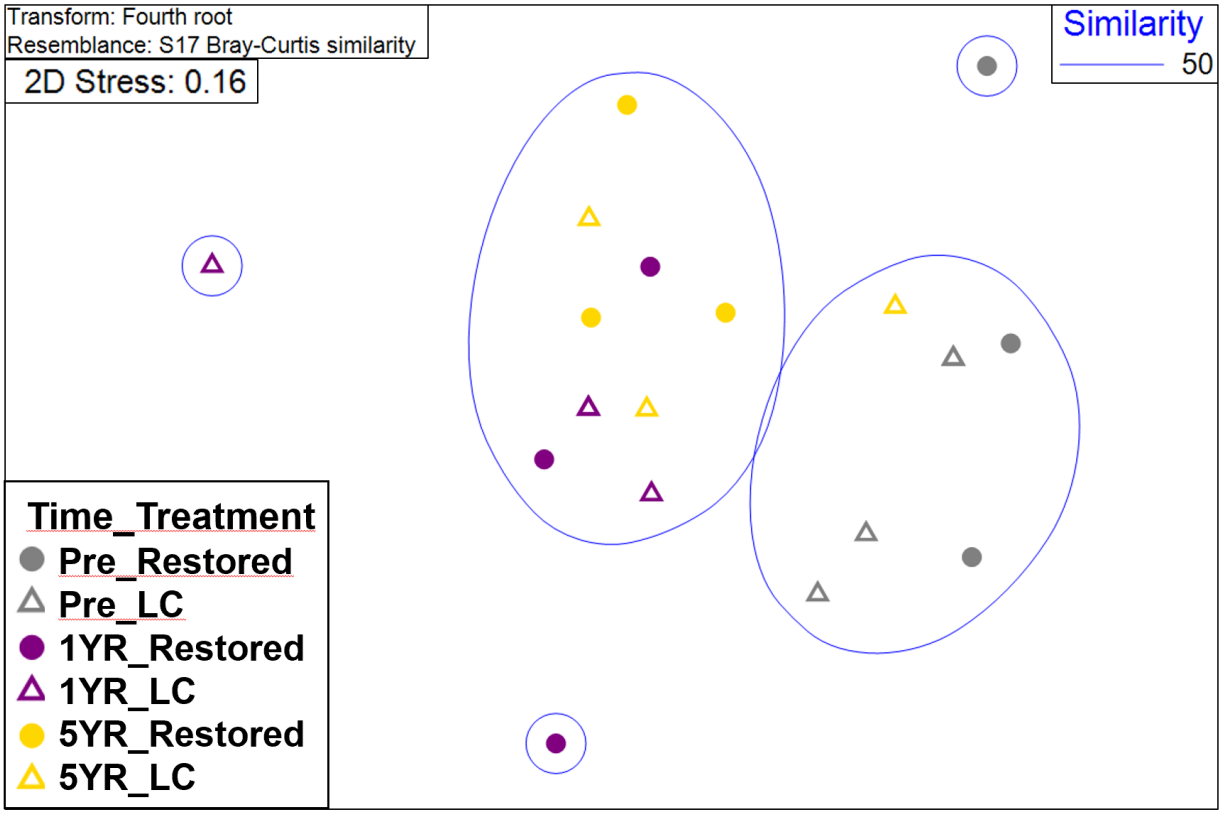

Figure 20. A non-metric multidimensional scaling plot made in Primer 7 using the Bray-Curtis similarity index and a cluster analysis to assess the changes in snail trematode communities (in terms of prevalence and richness) at Carrot Island sites by site type (LC and restored, which includes SB and OC) and year (pre-restoration in 2018, one-year post-restoration in 2019, and five-years post-restoration in 2023). The cluster analysis reveals groupings of communities that are more than 50% similar to one another (shown in blue circles). Years are differentiated by color (2018 in gray, 2019 in purple, and 2023 in gold), site type by the shading of the shape (open shape reflects loose cultch sites and restored sites are depicted by solid shapes).

To more critically quantify some of these visually described trends, I assessed the data using a PERMANOVA (permutational analysis of variance) on the Bray-Curtis similarity index scores that integrated the abundances and richness of parasites found at each reef type across each time point. When considering both time and treatment, only time was isolated as a significant influence on the snail trematode communities (treatment alone was not significant,

p=0.738, nor was the interaction between time and treatment, p=0.907), with post-hoc pair-wise tests revealed that there were significant differences particularly between five-years post-restoration and pre-restoration (p=0.009) and 1-year post-restoration and pre-restoration (p=0.002), but not between the two post-restoration monitoring points (p=0.215). I additionally ran a Kruskal-Wallace test with a Dunn test (with a Bonferroni correction) on the Shannon-Wiener Index scores for the snail parasite communities by restored and loose cultch reefs across the same time periods and did not find any significant differences between the scores over time or by site type (p>0.05) (Fig. 21).

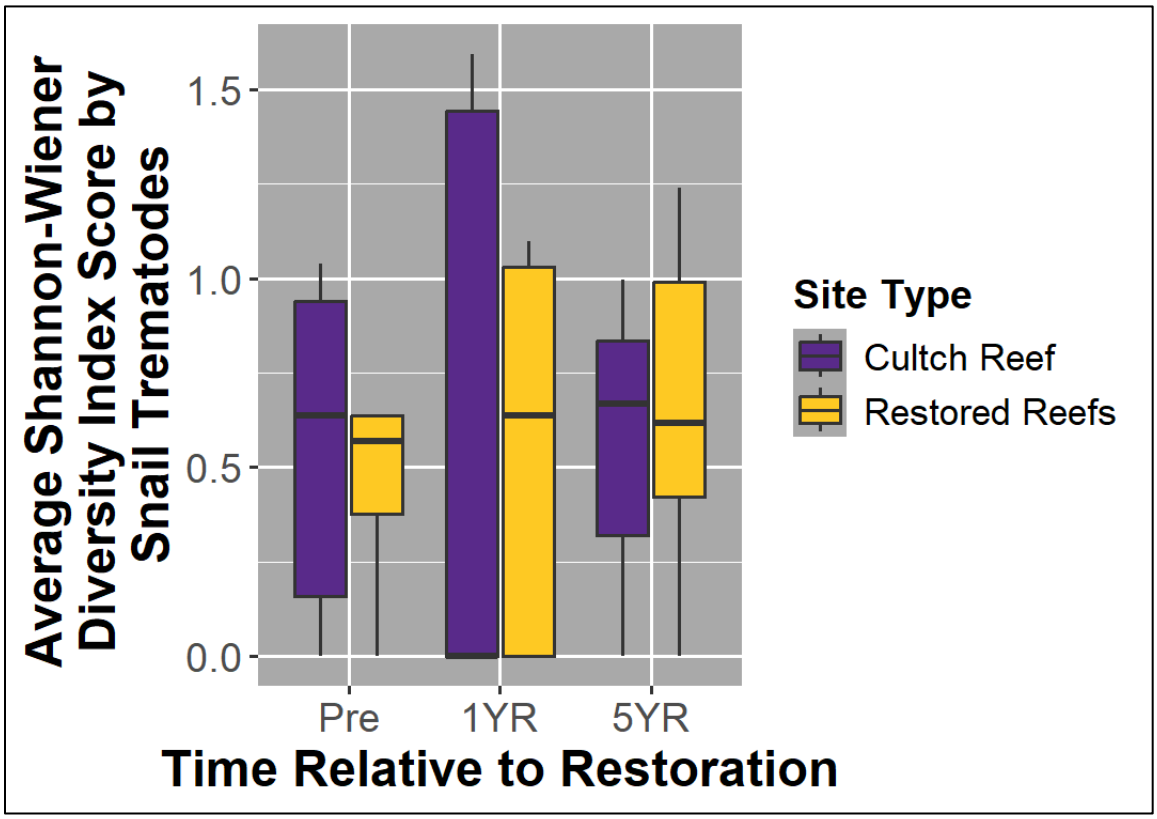

Figure 21. Parasite diversity (measured using the Shannon-Wiener Index) within I. obsoleta *hosts by time relative to restoration (pre-restoration, 1-year post-restoration, and 5-years post-restoration. Insignificant differences were found between the parasite diversity of snails sampled across these three time periods when using the Shannon-Wiener Index as an indicator of the community composition.*

To assess whether these factors may be significant in terms of either parasite abundance or richness (rather than the two collectively via the PERMANOVAs and Shannon-Wiener approaches), I ran separate Kruskal-Wallace tests on the snail trematode richness and prevalence over time (Figs 22-23). I again used the Dunn tests (and a Bonferroni correction) and visualized the results as boxplots (Figs 22-23). Richness followed a similar trend of differences that lacked statistical significance across time (Fig. 22).

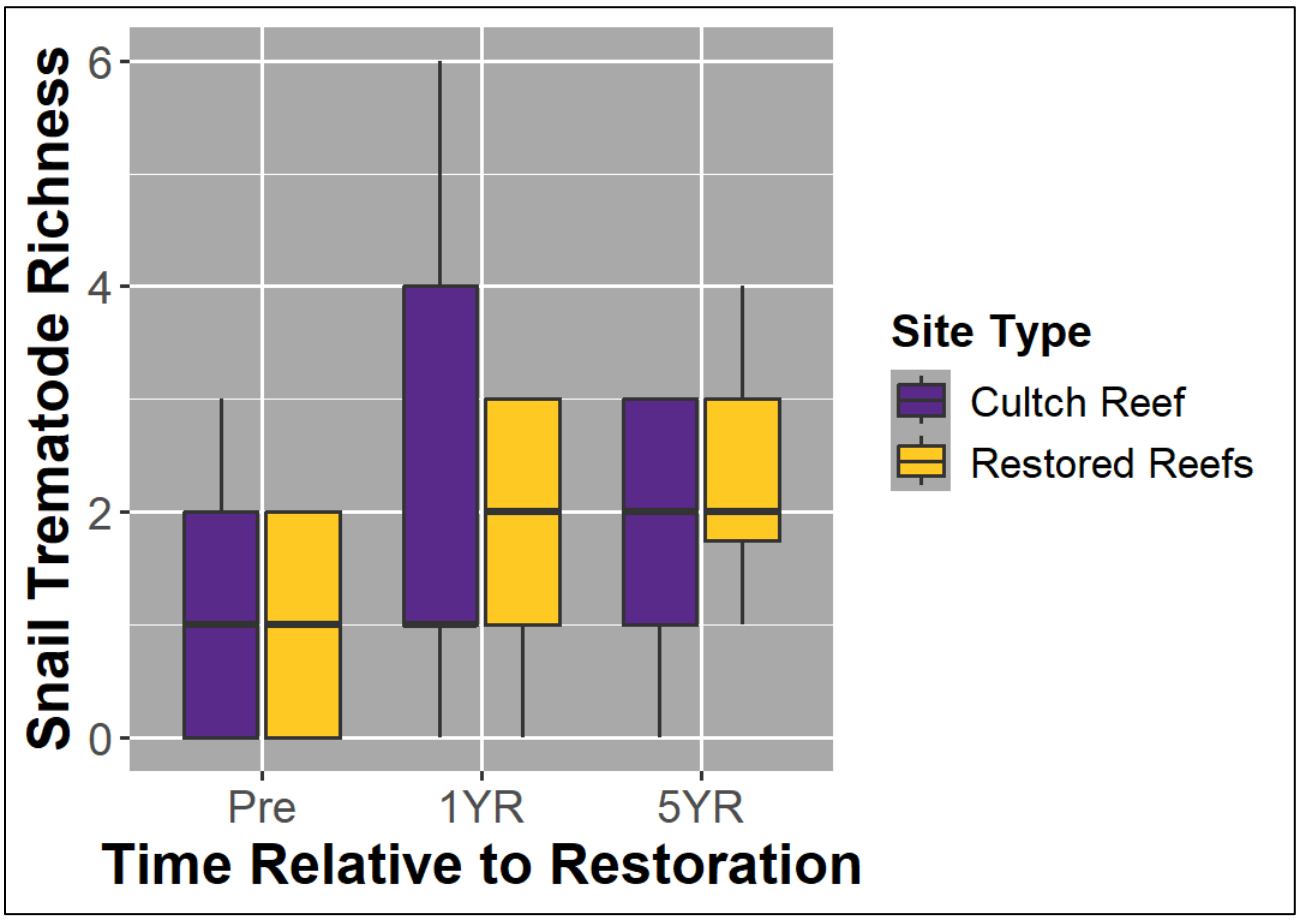

Figure 22. Parasite richness within I. obsoleta *hosts by time relative to restoration (pre-restoration, 1-year post-restoration, and 5-years post-restoration. Insignificant differences were found between the parasite richness of snails sampled across these three time periods.*

However, analyzing snail parasite prevalence over time revealed that when considering all snail parasite species in the system, there were significant differences by time period

(p=0.0008077), with both post-restoration sampling periods varying significantly from the pre-restoration sampling period (p=0.0169519694 and 0.0017362389 for 1-year post and 5-years post-restoration, respectively) (Fig. 23, top left). With all species considered together, however, the snail parasite prevalences from the 1-year post and 5-year post-restoration sampling events were not significantly different (p>0.05). To refine these differences further, I separated the data by final host species that the parasites use, and ran the analysis again while separately assessing the comparative prevalences of parasites that use birds as a final host and of parasites that use fish as a final host (Fig. 23, bottom left and top right, respectively). I did not have many snail parasites that use birds as a final host overall across all sampling years, and this may partially explain why parasite prevalence did not significantly differ over time in this subset (Fig. 23, top right). The snail parasites that use fish as a final host provided more definitive results, as the prevalence varied significantly over time overall (p=0.023059), and multiple comparisons revealed that the only statistically significant difference between a set of years was present between the pre-restoration and 5-years post restoration time frame (p=0.021557911) (Fig. 23, bottom left). Thus, notably, the 1-year post-restoration monitoring did not yet see statistically significantly different prevalences in fish-using parasites that also infect *I. obsoleta.*

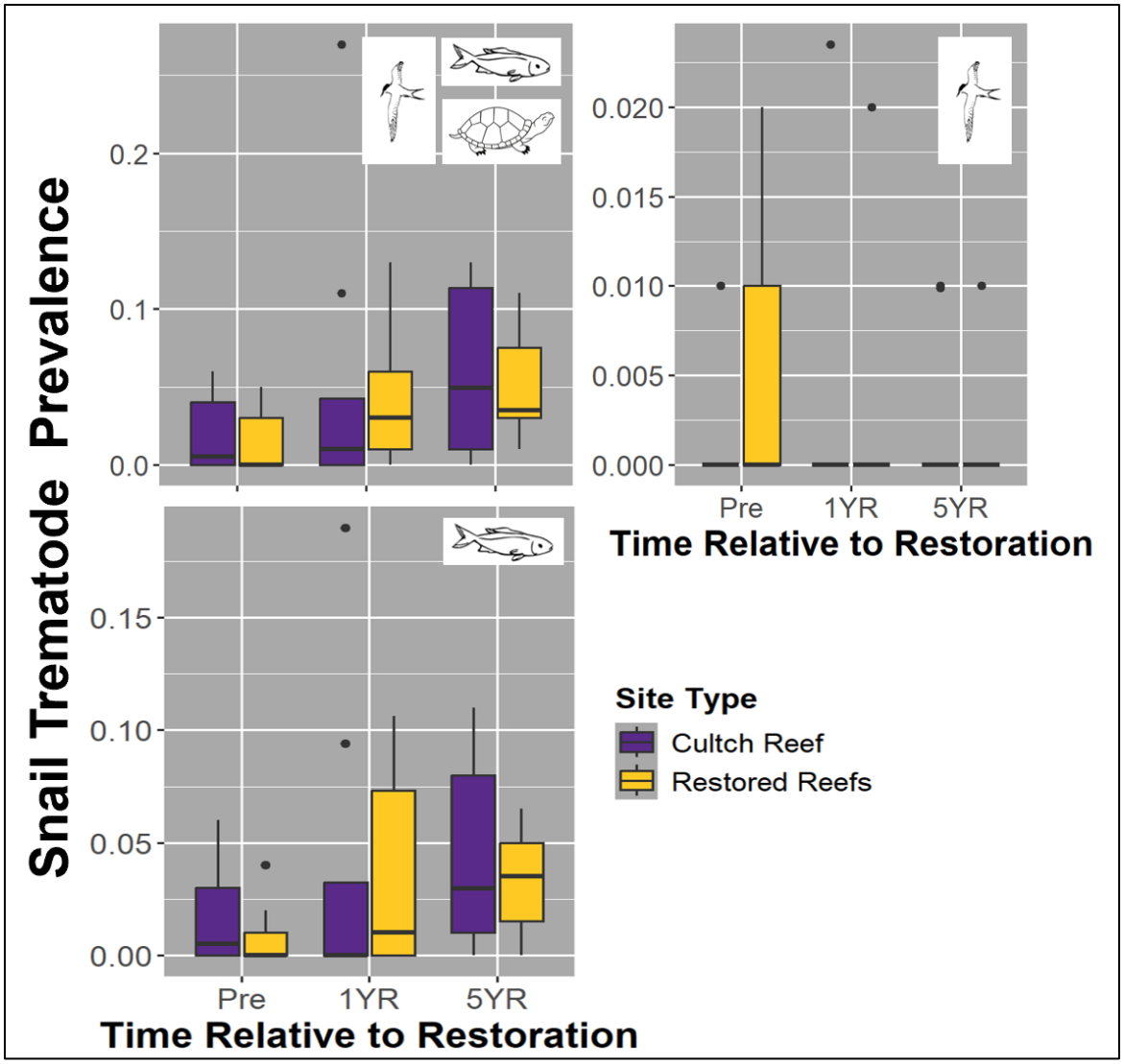

Figure 23. Parasite prevalence within I. obsoleta *hosts by time relative to restoration (pre-restoration, 1-year post-restoration, and 5-years post-restoration. The top left figure reflects the overall changes in parasite prevalence across all parasite species. The top right figure depicts the changes in parasite prevalence only of parasite species that use a bird as a final host. The bottom left figure shows the changes in parasite prevalence only of parasite species that use a fish as a final host.*

DISCUSSION

With ongoing threats to oyster populations and coastal regions at large (Beck et al., 2011, Kirby, 2004), there is substantial interest in and potential for restoration efforts to be implemented and innovated to ensure the successful recovery of oyster reefs and their affiliated ecosystem services (Sutton-Grier et al., 2015, Moore et al., 2020; Wellman et al., 2022, Morris et al., 2021). However, even within this prolific period of restoration work, there are still limitations to the implementation of restoration projects, with funding being a major driver (zu Ermgassen & Löfqvist, 2024; Lindenmayer & Likens, 2018). As such, it is important to ensure that the various approaches taken are effective over time, as the projects that are actually able to be put into place are utilizing limited funding that has little room for error by insufficient restoration methods. My thesis addresses these needs by providing a thorough analysis of the efficacy of two oyster reef restoration treatments five years post restoration, comparing their results to one another and to nearby loose cultch reefs. In doing so, my results provide some guidance on the most effective treatments (by different metrics) used in this restoration effort that may be beneficial to other restoration practitioners; additionally, they highlight the importance of monitoring over time, as the efficacy of the treatments did change over time when compared to earlier monitoring results (Moore et al., 2020; Wellman et al., 2022).

Habitat Characteristics

My results broadly reveal that the habitat and biodiversity affiliated with oyster reef restoration plots can vary markedly with time following restoration, consistent with previous literature describing variation in biodiversity and habitat metrics of restoration assessment over both short- (Moore et al., 2020; Wellman et al., 2022; Humphries et al., 2011; Overton et al., 2024;) and long-term (Huspeni & Lafferty, 2004; Moore et al., 2023; Grizzle et al., 2021)

periods of monitoring efforts. In terms of changes in habitat characteristics, previous monitoring efforts at Carrot Island initially suggested increasing oyster densities with time following restoration (Fig. 4; Wellman et al., 2022). This was particularly true for the novel breakwater substrate Oyster Catcher (especially in terms of adult oyster densities) by 2020, although recruitment of juvenile oysters to shell bag reefs was also growing over time up through 2020 (Fig. 4; Wellman et al., 2022). While Oyster Catcher continued to outperform shell bag reefs in 2023, our results also show significantly lower oyster densities relative to those recorded at the conclusion of the initial monitoring effort of 1-2 years, suggesting that what had initially looked promising may not withstand the test of time (Fig. 4; Wellman et al., 2022). These results are consistent with previous work assessing oyster reef restoration (using oyster density as an evaluation metric) over time, with a study assessing these efforts in New Hampshire recording peak oyster densities one-year post-restoration, with notable decline by the sampling period three-years post-restoration (Overton et al., 2021). Similar results were seen in subtidal oyster reefs in North Carolina as well, with high oyster densities earlier on in the restoration monitoring period followed by declines after only a few years (Puckett et al., 2018). Densities of target restoration species have served as an effective long-term indicator of restoration success in other systems, such as wetlands (Adam et al., 2024). However, this study particularly highlighted how certain characteristics that are noted in monitoring efforts are not universally effective long-term indicators. Ultimately, Adam et al. surmised that stress-affiliated metrics were the most effective initial indicators of long-term restoration success, but the exact metric varied across the different wetland communities assessed; only one was based on shoot density (Adam et al., 2024). As such, it is possible that there are other signals (potentially stress-related, as suggested by Adam et al., 2024) that should be assessed early on post-restoration to more definitively gauge the long-

term effectiveness of oyster reef restoration efforts; or, at least be evaluated in conjunction with oyster densities to guide predictions. Other oyster reef restoration assessments have evaluated restoration results using a wide variety of criteria and made a determination on the "success" of the effort by these characteristics, including the provision of various ecosystem services (water quality filtering, shoreline stabilization, habitat provision) (La Peyre et al., 2014). With the last option, however, comes the potential for oyster predation depending on the recruited species, which has been cited as a potential cause for a lack of restoration success in the past (La Peyre et al., 2013).

Comparison of the average water levels in the area in August 2020 and 2023 reveal averages of 2.33 ± 1.2 ft (full range: -0.33 – 4.96) and 2.25 ± 1.16 ft (full range: 0.04 – 5.25) respectively (*NOAA Tides & Currents*). These varying water levels may be contributing to the results at Carrot Island; in 2020, as peak oyster densities were recorded, the average elevations of the live oysters on the reefs were located within the optimal oyster growth zone for this area based on previous literature (-0.3m − -0.1m NAVD88) (Fig. 5; Wellman et al., 2022; Ridge et al., 2015). While oysters will still certainly grow outside of this optimal range, as the term suggests, it is not in the most ideal conditions that facilitate further growth (Ridge et al., 2015). Thus, the position of many of the 2023 live oysters above the OGZ, and with lower overall densities, may be attributed to the more variable water levels in 2023 that could have shifted the OGZ for this area (Fig. 5; Ridge et al., 2015). However, it is important to consider this result within the context of ongoing sea level rise (Poulter et al., 2009), as some locations within this general area are projected to see increases in sea level of up to a meter (Kopp et al., 2015), which could further impact oyster settlement and survival.

In addition to the consideration of the changes to the reef structures themselves, the free-living (La Peyre et al., 2014; Moore et al., 2023) and parasite habitat (Moore et al., 2023; Huspeni & Lafferty, 2004) communities affiliated with these reefs are also vital indicators of restoration effectiveness, in terms of improving community biodiversity with the addition of foundational habitat. Initial monitoring efforts of these sites demonstrated significant differences in free-living fish and crab communities as well as their affiliated parasite communities over time, but not by treatment, although there were trends towards greater crab richness one-year post-restoration in the shell bag treatment (Moore et al., 2020). Similarly significant differences with time, but not treatment, were observed when examining biodiversity from 2023, as time continued to display a significant effect on the community composition of free-living fish, free-living crabs, and parasites of fish, crabs, and snails, as well as fouling communities, though to varying degrees (Figs. 6-23).

Free-living communities

In terms of free-living fish communities, the significant differences between both post-restoration sampling periods and the pre-restoration sampling suggest an overall positive impact of the addition of oyster habitat to the system on fish communities, and potentially a broader pattern of change in these communities over time without a specific treatment-driven effect (Figs. 6-7). However, it is interesting to see the changes between the two post-restoration sampling events, as the one-year post-restoration sampling period yielded the fish communities with the highest average Shannon-Wiener diversity index scores across all three treatments (Fig. 7). Other systems have similarly used mobile fauna to highlight restoration success, such as the use of arthropods to effectively assess changes in grassland communities following restoration (Déri et al., 2010). These results are also consistent with previous research regarding oyster reef

restoration, which has noted quick recruitment of mobile nekton to restored habitat (La Peyre et al., 2014; Moore et al., 2020; Humphries et al., 2011), and thus suggest that oyster reef restoration does not always retain the initially recruited fish community that follows restoration.

Some of our results particularly suggests that some of the species driving the results of the 1-year sampling point include striped blennies, various gobies, sheepshead, and snapper, while one of the main species affiliated with the 5-year sampling point is oyster toadfish (Figs. 6-7). It is worth noting that oyster toadfish, which were present at significantly higher average abundances in 2023 relative to previous sampling events (Fig. 8), have not only been highlighted as an ecologically significant fish species in previous research (Moore et al., 2023), but also a known of several other small reef-resident fish (Philips & Swears, 1979) and crustaceans (Grabowski, 2004). Additionally, several of the species that distinguished the one-year post-restoration sampling event (including sheepshead, gray snapper, among others) are more broadly recognized as transient fishes in regards to reefs (Coen et al., 1999; Gittman et al., 2016), indicating that the higher abundance of toadfish, for example, may be reflective of a stronger reef-resident community five-years post-restoration than earlier monitoring efforts documented. The 1-year post-restoration reefs may potentially have been earlier in their development and not yet supporting higher numbers of these reef-residents (Moore et al., 2020), but still ecologically significant in their support of transient fishes (Coen et al., 1999; Smee & Stunz, 2017).

For free-living crab species, the recorded differences over time appear to be largely driven by the introduction of the green porcelain crab (*P. armatus*) to the system in between the initial monitoring effort and the five-year post-restoration sampling, but three other xanthid crab species – *D. sayii, P. herbstii,* and *Menippe spp.* – also appear to be more broadly driving the visible distinction of the five-year post-restoration communities (Fig. 9; Blakeslee et al., 2024).

While treatment again showed no significant differences through time, more specific analysis considering sampling month (in 2023 only) and treatment revealed higher average porcelain crab abundances in September and October (relative to June) and in shell bag and loose cultch reefs relative to Oyster Catcher reefs (Fig. 13). The prominence of *P. armatus* in this sampling period is not without precedent, as this crab was the most abundant crab species recorded at various sampling locations in its expanded range in earlier work (Mack et al., 2019). The only exception was a North Carolina site; however, the sampling period was several years prior to the 2023 surveys described here, and thus the expansion may not yet have reached its current extent (Mack et al., 2019). Seasonal trends observed here differ slightly from those previously described, as porcelain crab abundances are typically lower in the colder seasons (Hartman, 2003), but it is possible that the 2023 October sampling was more reflective of Fall rather than Winter conditions, explaining the relatively high densities (Fig. 13).

A closer examination of variation in crab abundances by treatment in 2023 reveals interesting patterns that have evolved. Oyster Catcher reefs boasted the greatest abundances of xanthid crabs in 2023, though only significantly differentiated from loose cultch reefs (Fig. 11). Taken in conjunction with the previously described oyster density results, it is interesting that the more intact, oyster-dense reefs (Oyster Catcher) are supporting greater densities of the native xanthid crabs while the less successful shell bag reefs appear to be dominated the porcelain crabs. There are multiple potential explanations for these differences; there is notable variation in the design and layout of the reef types, and the more spacious Oyster Catcher design (more vertical relief, lattice-like design) could serve as more desirable habitat, as described in previous work (Fig. 4; Wellman et al., 2022; Soniat et al., 2004). It is possible that the xanthid and porcelain crabs are competing for this space, and that these results illustrate niche partitioning

due to competition, which has been described in previous research across a variety of systems and taxa (Di Bitetti et al., 2010; Giménez et al., 2018; Aguilera et al., 2013). Prior literature has also documented consumption of porcelain crabs by mud crabs in introduced regions (Hollebone & Hay, 2008), and thus the discrepancies in average abundances of each by treatment type could reflect predation of (or predator avoidance by) the porcelain crabs (Kinney et al., 2019).

Without further classification and analysis of the fouling organisms collected in 2019 and 2023, it is difficult to characterize the degree of change in the sessile communities with regard to time and restoration. However, it is worth noting that the fouling community biomass was higher in 2019 relative to 2023, and that this measure was taken prior to the true growth in terms of oyster densities at the restored reefs (Figs. 4, 14). As oyster and fouling organisms have been previously described as competitors, it is possible that the elevated oyster densities could be impacting the successful establishment and persistence of fouling organisms (Arakawa, 1990). This would coincide with the higher oyster densities present in 2023 than in 2019 (Fig. 4; Wellman et al., 2022) but further study is needed to ascertain whether this is the dynamic at play in Carrot Island or if there are other potentially influential factors.

Parasite communities – Fish, crabs, and snails

Parasites of a variety of taxa have previously been studied for their potential to serve as indicators of ecosystem health and restoration success (Moore et al., 2020, 2023, 2024; Morales-Serna et al., 2019; Huspeni & Lafferty, 2004). Previous research has demonstrated that parasites often better indicate biodiversity changes following disturbances (including changes like restoration) than their free-living counterparts (Moore et al., 2023). However, this is not a universal trend, as there are recorded instances of parasites associated with a certain host species (or multiple host species) that do not change in tandem with their hosts nor in response to

changes with their surrounding environment (Moore et al., 2020; Morales-Serna et al., 2019). In one case, mangrove restoration efforts in the Gulf of Mexico were assessed using parasites of an affiliated fish (*Poecilia velifera*) five years post-restoration, and parasite communities did not substantially differ between restored sites and nearby conserved sites (Morales-Serna et al., 2019). However, I will note that there was no earlier or pre-restoration monitoring to compare this data to, and thus the lack of differentiation by site type may be more important than the authors initially concluded and, instead representing larger scale conditions at that time point rather than the restoration effort (Morales-Serna et al., 2019).

The variation in fish parasite communities at Carrot Island is particularly notable with time, with one-year post-restoration communities significantly different than those affiliated with the other two sampling periods (pre- and five-years post-restoration, with $p=0.001$ and 0.044 via PERMANOVA, respectively) (Fig. 15). The initial monitoring effort demonstrated significantly lower parasite prevalence across taxa one-year post restoration relative to pre-restoration data, and it is intriguing to see that certain species, while still not reaching their pre-restoration prevalence, are now beginning to more closely resemble these data overall in the five-year post-restoration data (Fig. 15; Moore et al., 2020). Thus, the comparison of the fish parasite data over time as well as to the free-living fish data is intriguing, as the cumulative effect may suggest a more ecologically complex and interconnected oyster reef resident community at both the host and parasite levels (Moore et al., 2023).

The crab parasite data, however, does not generally resemble the same trend as the fish parasite data, with many parasite taxa reaching their highest average prevalences one-year post restoration rather than increasing with time (with the exception of nematodes) (Fig. 17). Paradoxically, some of the parasite taxa that have increased with time since 2019 (the one-year

post-restoration sampling time period) are some of the same taxa that appear to have peaked in prevalence in 2019. As one of those taxa is trematodes, the snail parasites assessed in this study offer an additional angle in support of the broader trend indicated by the fish parasite data and previous literature (Moore et al., 2023). The utility of snail trematode parasites as an indicator of restoration success has been established in previous work, with one study finding significant increases in trematode prevalence at restored saltmarsh sites relative to control sites after six years (Huspeni & Lafferty, 2004). Similarly, as described when discussing Fig. 19, the five-year post-restoration time period is the first time that the slope of the restored curve is less steep than the loose cultch curve, suggesting that the restored communities may now have slightly more evenly distributed abundances across the parasite species represented therein than those of the loose cultch reefs from the same time period (Fig. 19). Additionally, it is intriguing to see that the relatively higher prevalence of trematode parasites in fishes is mirrored in the significantly greater prevalence of fish-using trematode parasites in snails. This result highlights a key benefit of parasite surveys, as they have the ability to provide detail beyond the biodiversity of a site: the connections among host species within a system, which can otherwise be difficult to observe (Huspeni & Lafferty, 2004).

CONCLUSIONS AND SIGNIFICANCE

These results largely reveal that there are many ways in which the overall "success" and progressive development of a restored oyster reef system can be evaluated, and that different methods of assessment offer unique insight into the overall development of the system in response to the surrounding conditions and dynamic communities within. The habitat data described here (oyster densities over time and by elevation) offer a quantitative summary of the overall status of the reef structures themselves, and provide evidence of significant variation in this metric with time and by restoration method. As such, these results can be more informative of the potential future of the reef with ongoing changes in the system, such as sea level rise. By contrast, the biodiversity results reveal how effectively these restoration sites serve as habitat for reef organisms, with changes at the parasite level reflecting longer-term, broad-scale changes underway in the system that could be missed (or misunderstood) by exclusively host-level assessments. As such, it is important to use methods that will assess the metric that is most relevant to the objective of restoration managers; those targeting shoreline stabilization will want to select a method that is going to guarantee sustained live oyster density over time, while those desiring reef habitat may not need to be as particular about their restoration treatment selection, depending on the target mobile species.

Furthermore, my work reveals that the results of short-term monitoring do not necessarily indicate the long-term results of restored oyster reefs, for better or worse (Moore et al., 2020; Wellman et al., 2022). As such, regardless of the intent of the restoration effort and the method(s) used to assess it, it is critical to invest the time and resources to evaluate its condition over time rather than reaching an ultimate determination of restoration success or failure after short-term initial monitoring.

FUTURE DIRECTIONS

Additional components of this broader research project are not described above, but will be further analyzed and included in future publications. Specifically, additional habitat and biodiversity data was collected from another shoreline (hereafter “ramp”) with restoration plots located at Carrot Island, and these data are going to be compared to the data collected above in the creek. In addition, I conducted the same surveys in 2024, with some modifications: most notably, I did not sample for parasites of fish in 2024 due to a significant alteration in reef condition between 2023 and 2024, especially along the ramp shoreline. Furthermore, geospatial analyses will be used to further contextualize the overall changes happening at the landscape scale at Carrot Island, as this may highlight many of the broader drivers of change inducing the variation I see in the habitat data at the smaller scale.

In terms of my progress on this aspect of my work, I have thus far completed a preliminary assessment of the variation in the free-living communities at Carrot Island between 2023 and 2024 (Fig. 24). This plot not only highlights a clear distinction in these communities across this year, but the creek shoreline points are also more tightly clustered together in the center of the plot while the ramp plots are much more distributed around the periphery of the plot.

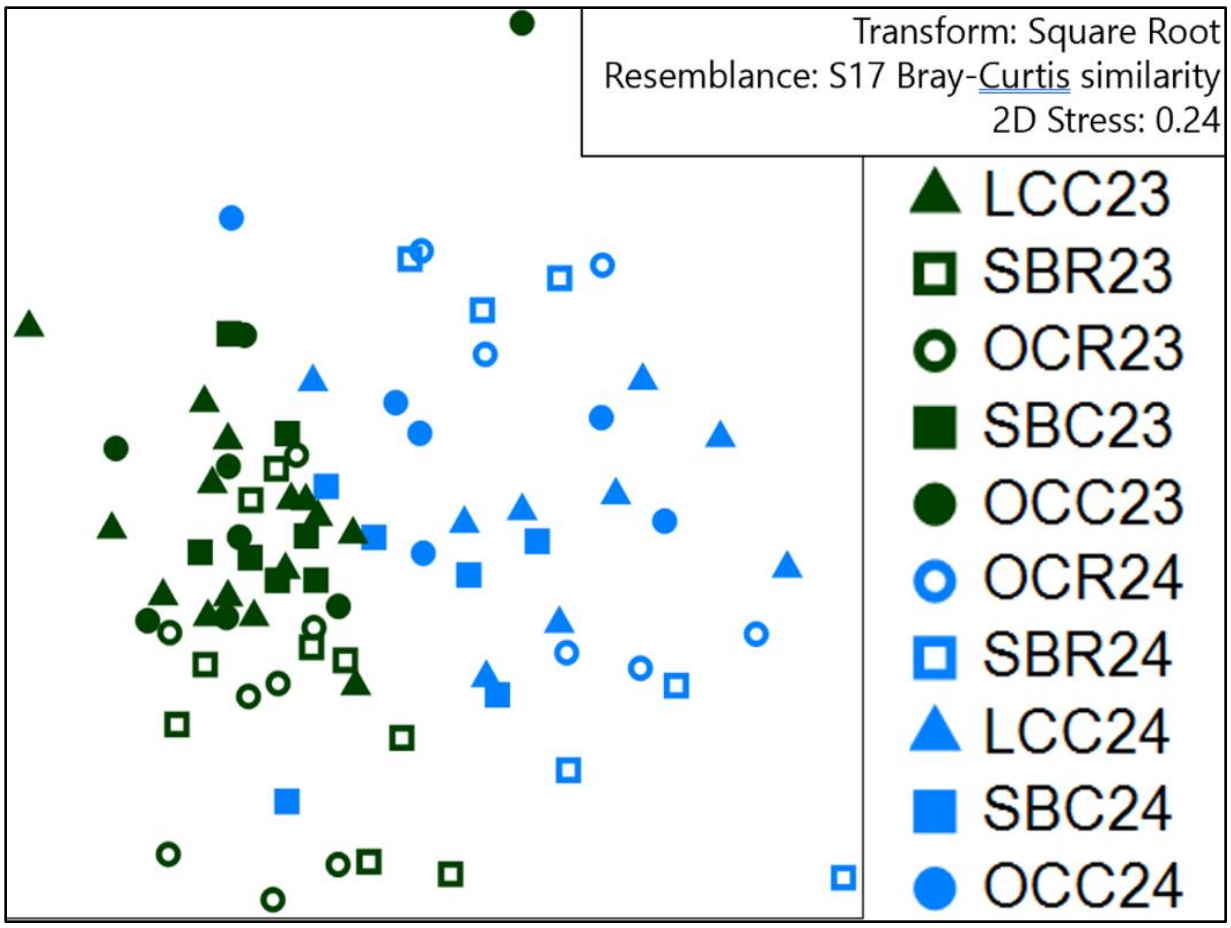

Figure 24. A non-metric multidimensional scaling plot made in Primer 7 using the Bray-Curtis similarity index and a cluster analysis to assess the changes in free-living communities at Carrot Island sites by site type (as shape; LC=loose cultch SB=shell bag, and OC=Oyster Catcher), shoreline (as shading; C=creek, R=ramp), and year (as color; 2023 and 2024).

In terms of habitat data, significant decreases have already been documented across reef types and shorelines between 2023 and 2024 (Fig. 25). Notably, some of the steepest declines have occurred in locations where reefs, particularly shell bag reefs, have fallen below the optimal growth zone for oysters.

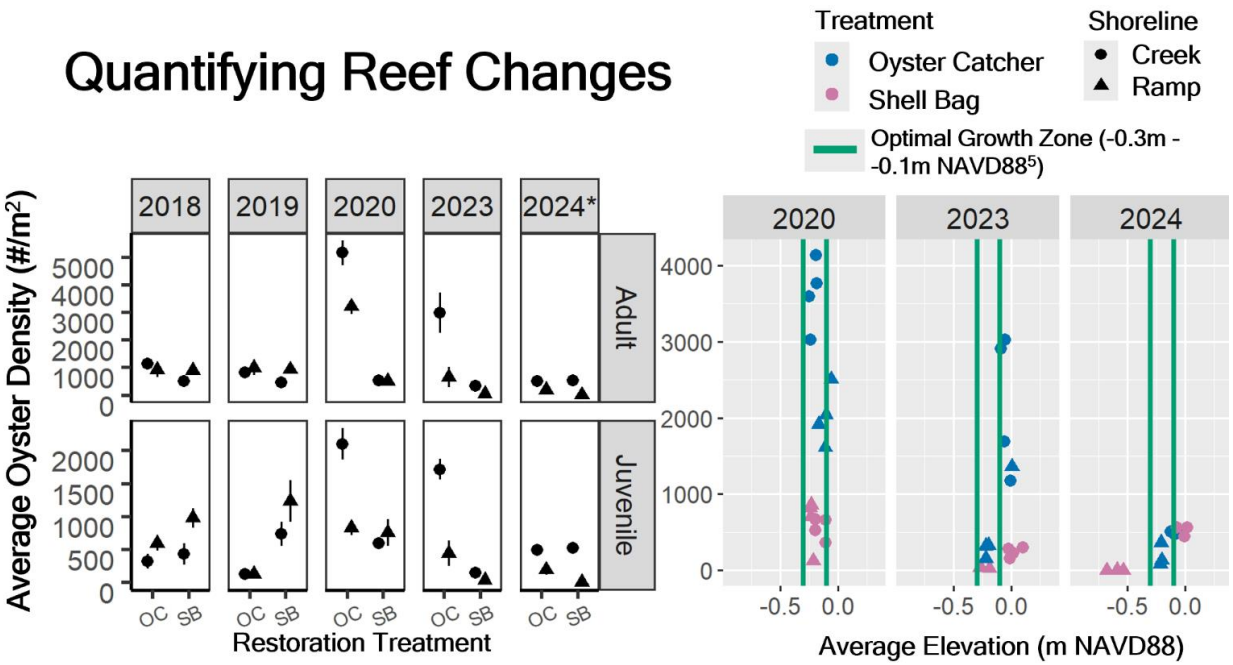

Figure 25. Oyster densities at Carrot Island through 2024. Left: Oyster densities by year from 2018-2024, separated by shoreline and treatment. Right: Oyster densities by year and elevation from 2020-2024, separated by shoreline and treatment. The optimal growth zone for oysters is shown in green.

Finally, an initial horizontal shoreline change analysis at Carrot Island reveals notable shoreline movement along the ramp shoreline, but not as much on one side of the creek shoreline (Fig. 26). This also provides further justification for assessing changes in habitat and biodiversity metrics at the shoreline level.

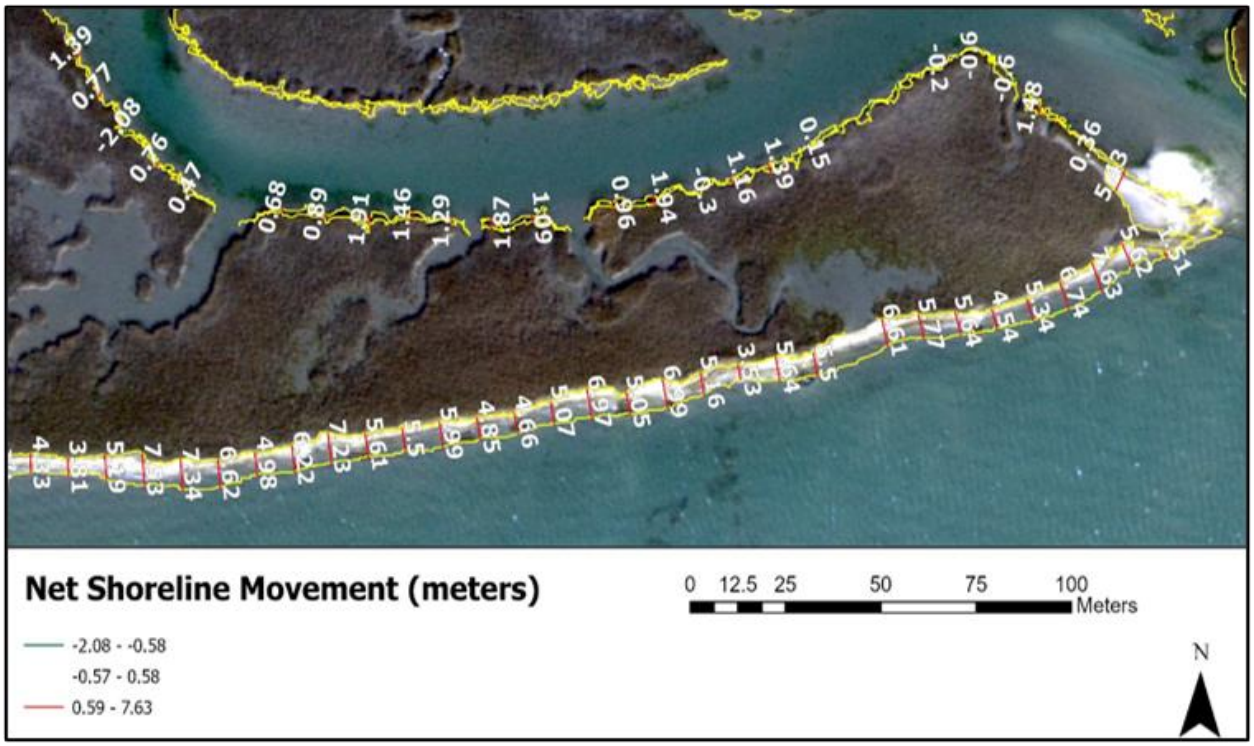

Figure 26. Net shoreline movement (in meters) at Carrot Island along the ramp shoreline and one side of the creek shoreline. This change was assessed using pre-restoration imagery (from 2014) and post-restoration imagery (2020).

By continuing to process and analyze these data, in conjunction with the above work already described in my thesis, I will be able to effectively contextualize the changes that have occurred at the restoration sites and broader landscape scene over time at Carrot Island. Completing the comparison between the biodiversity and habitat data collected in 2023 and 2024 will allow me to gain additional insight to the effects that disturbance has on these restoration sites as well as their affiliated communities (with the substantial loss of oysters on the ramp shoreline between 2023 and 2024). The overarching geospatial analysis will allow me to understand the different conditions at play not only between the ramp and the creek, but in terms of time relative to restoration, as I have access to data encompassing a ten-year period (2014-2024) that allows for an analysis of the change in shoreline position across this time scale. From

these data, I hope to adequately assess the effectiveness of the restoration sites at Carrot Island using multiple perspectives and at multiple scales, with the context of larger-scale changes at the location taken into account.

REFERENCES

Abella, S. R., & Chiquoine, L. P. (2019). The good with the bad: When ecological restoration facilitates native and non-native species. *Restoration Ecology*, 27(2), 343–351. https://doi.org/10.1111/rec.12874

Aguilera, M., Valdivia, N., & Broitman, B. (2013). Spatial niche differentiation and coexistence at the edge: Co-occurrence distribution patterns in Scurria limpets. *Marine Ecology Progress Series*, 483, 185–198. https://doi.org/10.3354/meps10293

Anderson, R.C., Chabaud, A.G., Willmot, S. (Eds.), 1975. Keys to the Nematode Parasites of Vertebrates. CAB Farnham Royal Bucks, Slough, UK, pp. 1–27.

Arakawa, Kohman Y. (1990) Competitors and fouling organisms in the hanging culture of the pacific oyster, *Crassostrea gigas* (thunberg), *Marine & Freshwater Behaviour & Phy*, 17:2, 67-94, DOI: 10.1080/10236249009378759

Audino, Lívia D., Stephen J. Murphy, Ludimila Zambaldi, Julio Louzada, and Liza S. Comita. 2017. "Drivers of Community Assembly in Tropical Forest Restoration Sites: Role of Local Environment, Landscape, and Space." *Ecological Applications* 27(6):1731–45. doi: 10.1002/eap.1562.

Baker, R., Gittman, R.K. Co-Funding Robust Monitoring with Living Shoreline Construction is Critical for Maximizing Beneficial Outcomes. Estuaries and Coasts 48, 5 (2025). https://doi.org/10.1007/s12237-024-01433-9

Beck, M. W., R. D. Brumbaugh, L. Airoldi, A. Carranza, L. D. Coen, C. Crawford, O. Defeo, G. J. Edgar, B. Hancock, M. C. Kay, H. S. Lenihan, M. W. Luckenbach, C. L. Toropova, G. Zhang, and X. Guo. 2011. Oyster Reefs at Risk and Recommendations for Conservation, Restoration, and Management. BioScience 61:107–116.

Blakeslee, A.M., Altman, I., Miller, A.W., Byers, J.E., Hamer, C.E. and Ruiz, G.M. 2012. Parasites and invasions: a biogeographic examination of parasites and hosts in native and introduced ranges. *Journal of Biogeography*, 39(3), pp.609-622

Blakeslee, A. M. H., Keogh, C. L., Fowler, A. E., & Griffen, B. D. (2015). Assessing the effects of trematode infection on invasive green crabs in eastern north america. *PLOS ONE*, 10(6), e0128674. https://doi.org/10.1371/journal.pone.0128674

Blakeslee, A., Moore, C., Stancil, C., Woodard, N., Geesin, M., Manning-Moore, C., Aguilar, R., Smith, S., & Gittman, R. 2024. Caribbean Creeping Crabs: Northward expansion of the green

porcelain crab in North Carolina, USA. *BioInvasions Records.* 13(1): 109-120, https://doi_org/10. 3391/bir.2024.13.1.10

Buckley, M. C., & Crone, E. E. (2008). Negative off-site impacts of ecological restoration: Understanding and addressing the conflict. *Conservation Biology,* 22(5), 1118–1124. https://doi.org/10.1111/j.1523-1739.2008.01027.x

Byers, J. E., Altman, I., Grosse, A. M., Huspeni, T. C., & Maerz, J. C. 2011. Using parasitic trematode larvae to quantify an elusive vertebrate host: Trematode and host abundance. *Conservation Biology,* 25(1), 85–93.

Canning-Clode, J., Fowler, A. E., Byers, J. E., Carlton, J. T., & Ruiz, G. M. 2011. 'Caribbean creep' chills out: Climate change and marine invasive species. *PloS ONE,* 6(12), e29657.

Clarke, B., Murray, J., & Johnson, M. S. 1984. The Extinction of Endemic Species by a Program of Biological Control!

Coen, Loren D., Mark W. Luckenbach, and Denise L. Breitburg. 1999. "The Role of Oyster Reefs as Essential Fish Habitat: A Review of Current Knowledge and Some New Perspectives." *American Fisheries Society Symposium* 22:438–454. https://fisheries.org/docs/books/x54022xm/26.pdf

Comba, D., Palmer, T. A., Breaux, N. J., & Pollack, J. B. (2023). Evaluating biodegradable alternatives to plastic mesh for small-scale oyster reef restoration. *Restoration Ecology*, 31(3), e13762. https://doi.org/10.1111/rec.13762

Curtis, L. (2002). Ecology of larval trematodes in three marine gastropods. *Parasitology, 124*(7), 43-56. doi:10.1017/S0031182002001452

Curtis, L. A. (2007). Larval trematode infections and spatial distributions of snails. *Invertebrate Biology*, 126(3), 235–246. https://doi.org/10.1111/j.1744-7410.2007.00093.x

Damgaard, C. (2019). A critique of the space-for-time substitution practice in community ecology. *Trends in Ecology & Evolution,* 34(5), 416–421. https://doi.org/10.1016/j.tree.2019.01.013

David, P., Thébault, E., Anneville, O., Duyck, P.-F., Chapuis, E., & Loeuille, N. 2017. Impacts of invasive species on food webs. In *Advances in Ecological Research* (Vol. 56, pp. 1–60). Elsevier.

Déri, E., Magura, T., Horváth, R., Kisfali, M., Ruff, G., Lengyel, S., & Tóthmérész, B. (2011). Measuring the short-term success of grassland restoration: The use of habitat affinity indices in

ecological restoration. *Restoration Ecology,* 19(4), 520–528. https://doi.org/10.1111/j.1526-100X.2009.00631.x

Di Bitetti, M. S., De Angelo, C. D., Di Blanco, Y. E., & Paviolo, A. (2010). Niche partitioning and species coexistence in a Neotropical felid assemblage. *Acta Oecologica*, 36(4), 403–412. https://doi.org/10.1016/j.actao.2010.04.001

Dunn, R. P., Eggleston, D. B., & Lindquist, N. 2014. Oyster-Sponge Interactions and Bioerosion of Reef-Building Substrate Materials: Implications for Oyster Restoration. *Journal of Shellfish Research*, *33*(3), 727–738. https://doi.org/10.2983/035.033.0307

Feigenbaum, David, Mark Bushing, Jay Woodward, and Alan Friedlander. 1989. "Artificial Reefs in Chesapeake Bay and Nearby Coastal Waters." *Bulletin of Marine Science,* 44(2): 734-742.

Fowler AE, Blakeslee AMH, Davinack A, Aguilar R, Andersen M, Benadon C, Choong H, Green-Gavrielidis L, Greenberg SR, Hartshorn E, Hobbs NV, Labbe S, Larson K, McCuller M, Moloney DM, Parsons SK, Stancil C, Thornber C, Pederson J, Carlton JT. (in review). "Caribbean Creep meets Chesapeake Creep: 2023 Rapid Assessment Survey of Marine Bioinvasions of the Mid-Atlantic Coast, USA." *Biological Invasions.*

Galtsoff, P. S. 1964. *The American Oyster, Crassostrea virginica Gmelin*. U.S. Government Printing Office.

Geraldi, N., Simpson, M., Fegley, S., Holmlund, P., & Peterson, C. 2013. Addition of juvenile oysters fails to enhance oyster reef development in Pamlico Sound. *Marine Ecology Progress Series*, *480*, 119–129. https://doi.org/10.3354/meps10188

Giménez, J., Cañadas, A., Ramírez, F., Afán, I., García-Tiscar, S., Fernández-Maldonado, C., Castillo, J. J., & De Stephanis, R. (2018). Living apart together: Niche partitioning among Alboran Sea cetaceans. *Ecological Indicators*, 95, 32–40. https://doi.org/10.1016/j.ecolind.2018.07.020

Gittman, Rachel K., Charles H. Peterson, Carolyn A. Currin, F. Joel Fodrie, Michael F. Piehler, and John F. Bruno. 2016. Living Shorelines Can Enhance the Nursery Role of Threatened Estuarine Habitats. *Ecological Applications* 26(1):249–63. doi: 10.1890/14-0716.

Gittman, Rachel K., F. Joel Fodrie, Christopher J. Baillie, Michelle C. Brodeur, Carolyn A. Currin, Danielle A. Keller, Matthew D. Kenworthy, Joseph P. Morton, Justin T. Ridge, and Y. Stacy Zhang. 2018. Living on the Edge: Increasing Patch Size Enhances the Resilience and Community Development of a Restored Salt Marsh. *Estuaries and Coasts* 41(3):884–95. doi: 10.1007/s12237-017-0302-6.

Gittman RK, Baillie CJ, Cros A, Grabowski JH, McKinney MM, Saccomanno VR, Smith CS, DeAngelis B. Assessing how restoration can facilitate 30× 30 goals for climate-resilient coastal ecosystems in the United States. *Conservation Biology.* 2024 Dec 31:e14429.

Glasby, T. M., Connell, S. D., Holloway, M. G., & Hewitt, C. L. 2007. Nonindigenous biota on artificial structures: Could habitat creation facilitate biological invasions? *Marine Biology,* 151(3), 887–895.

Goldberg, L., Lagomasino, D., Thomas, N., Fatoyinbo, T. 2020. Global declines in human-driven mangrove loss. Global Change Biology 26: 5844-5855.

Gong, Xiaoqian, Scott Jarvie, Qing Zhang, Qingfu Liu, Yongzhi Yan, Nier Su, Peng Han, and Fengshi Li. 2023. "Community Assembly of Plant, Soil Bacteria, and Fungi Vary during the Restoration of an Ecosystem Threatened by Desertification." *Journal of Soils and Sediments* 23(1):459–72. doi: 10.1007/s11368-022-03329-2.

Grabowski, J. H. (2004). Habitat complexity disrupts predator–prey interactions but not the trophic cascade on oyster reefs. *Ecology*, 85(4), 995-1004.

Grabowski, J. H., & Peterson, C. H. 2007. Restoring Oyster Reefs to Recover Ecosystem Services. In *Ecosystem Engineers* (pp. 281–298). Elsevier Inc. https://www.fws.gov/doiddata/dwh-ar-documents/1187/DWH-AR0005495.pdf

Grizzle, Raymond, Krystin Ward, Ray Konisky, Jennifer Greene, Holly Abeels, and Robert Atwood. n.d. "Oyster Reef Restoration in New Hampshire, USA: Lessons Learned During Two Decades of Practice." *Ecological Restoration* 39(4):260–73.

Hartman, M.J. 2003. Population dynamics and trophic interactions of *Petrolisthes armatus*, an invasive decapod crustacean. Dissertation, University of South Carolina, Columbia, SC. 143 pp.

Hartwell, S. I., Jordahl, D. M., Dawson, C. E. O., & Ives, A. S. 1998. Toxicity of scrap tire leachates in estuarine salinities: Are tires acceptable for artificial reefs*? Transactions of the American Fisheries Society*, 127(5), 796–806.

Hechinger, R.F. and Lafferty, K.D., 2005. Host diversity begets parasite diversity: bird final hosts and trematodes in snail intermediate hosts. *Proceedings of the Royal Society B: Biological Sciences*, 272(1567), pp.1059-1066.

Hennebert, P., S. Lambert, F. Fouillen, and B. Charrasse. 2014. "Assessing the Environmental Impact of Shredded Tires as Embankment Fill Material." *Canadian Geotechnical Journal* 51(5):469–78. doi: 10.1139/cgj-2013-0194.

Hollebone, Amanda L., and Mark E. Hay. 2008. "An Invasive Crab Alters Interaction Webs in a Marine Community." *Biological Invasions,* 10:347–358. https://doi.org/10.1007/s10530-007-9134-9

Humphries, A. T., La Peyre, M. K., Kimball, M. E., & Rozas, L. P. 2011. Testing the effect of habitat structure and complexity on nekton assemblages using experimental oyster reefs. *Journal of Experimental Marine Biology and Ecology,* 409(1–2), 172–179. https://doi.org/10.1016/j.jembe.2011.08.017

Huspeni, T.C. and Lafferty, K.D. 2004. Using larval trematodes that parasitize snails to evaluate a saltmarsh restoration project. *Ecological Applications*, 14(3), pp.795-804.

Huspeni, T. C., Hechinger, R. F., & Lafferty, K. D. 2004. Trematode parasites as estuarine indicators: opportunities, applications, and comparisons with conventional community approaches. *Estuarine indicators* (pp. 319-336). CRC Press.

Jude, D. J., & DeBoe, S. F. 1996. Possible impact of gobies and other introduced species on habitat restoration efforts. Canadian Journal of Fisheries and Aquatic Sciences, 53(S1), 136–141. https://doi.org/10.1139/f96-001

Keller, D. A., Gittman, R. K., Brodeur, M. C., Kenworthy, M. D., Ridge, J. T., Yeager, L. A., Rodriguez, A. B., & Fodrie, F. J. 2019. Salt marsh shoreline geomorphology influences the success of restored oyster reefs and use by associated fauna. *Restoration Ecology*, 27(6), 1429–1441.

Kennish, M. J. 2001. Anthropogenic Impacts. Pages 29–35 *in* M. J. Kennish, editor. Encyclopedia of Estuaries. Springer Netherlands, Dordrecht.

Khalil, A.J., Bray, R.A., 1994. Keys to the Cestodes Parasites of Vertebrates. Wallingford Oxon (768 pp).

Kimbro, D. L., Cheng, B. S., & Grosholz, E. D. 2013. Biotic resistance in marine environments. *Ecology Letters*, 16(6), 821–833. https://doi.org/10.1111/ele.12106

Kinney, K.A., Pintor, L.M. & Byers, J.E. 2019. Does predator-driven, biotic resistance limit the northward spread of the non-native green porcelain crab, *Petrolisthes armatus*? *Biol Invasions* 21, 245–260. https://doi.org/10.1007/s10530-018-1821-1

Kirby, M. X. 2004. Fishing down the coast: Historical expansion and collapse of oyster fisheries along continental margins. *Proceedings of the National Academy of Sciences* 101:13096–13099.

Kondolf, G. M. 1998. Lessons learned from river restoration projects in California. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 8(1), 39–52. https://doi.org/10.1002/(SICI)1099-0755(199801/02)8:1<39::AID-AQC250>3.0.CO;2-9

Kopp, R. E., Horton, B. P., Kemp, A. C., & Tebaldi, C. 2015. Past and future sea-level rise along the coast of North Carolina, USA. Climatic Change, 132(4), 693–707. https://doi.org/10.1007/s10584-015-1451-x

La Peyre, M.K., Schwarting, Lindsay, and Miller, Shea. 2013. Preliminary assessment of bioengineered fringing shoreline reefs in Grand Isle and Breton Sound, Louisiana: U.S. Geological Survey Open-File Report 2013–1040.

La Peyre, M. K., Humphries, A. T., Casas, S. M., & La Peyre, J. F. 2014. Temporal variation in development of ecosystem services from oyster reef restoration. *Ecological Engineering*, 63, 34–44. https://doi.org/10.1016/j.ecoleng.2013.12.001

Lindenmeyer, D., Barton, P., Pierson, J. (Eds.). 2015. Indicators and Surrogates of Biodiversity and Environmental Change. CRC Press.

Lindenmayer, D.B., Likens, G.E. 2018. Effective ecological monitoring. 2nd ed. CRC Press

Mack, K.J., Podolsky, R.D., Shervette, V. et al. 2019. Spatial and Temporal Associations Between Native Crabs and the Invading Green Porcelain Crab, *Petrolisthes armatus*, Throughout Its Northernmost Invaded Range. *Estuaries and Coasts* 42, 537–547. https://doi.org/10.1007/s12237-018-0472-x

McDermott, J.J. 1951. Larval trematode infection in *Nassa obsoleta* (Say), from New Jersey waters. Master's Thesis, Rutgers University, New Brunswick

Michaelis, A. K., Walton, W. C., Webster, D. W., & Shaffer, L. J. 2020. The role of ecosystem services in the decision to grow oysters: A Maryland case study. *Aquaculture, 529,* 735633. Doi: 10.1016/j.aquaculture.2020.735633

Moore, C. S., Gittman, R. K., Puckett, B. J., Wellman, E. H., & Blakeslee, A. M. H. 2020. If you build it, they will come: Restoration positively influences free-living and parasite diversity in a restored tidal marsh. *Food Webs, 25,* e00167. https://doi.org/10.1016/j.fooweb.2020.e00167

Moore, C. S., Baillie, C. J., Edmonds, E. A., Gittman, R. K., & Blakeslee, A. M. H. 2023. Parasites indicate trophic complexity and faunal succession in restored oyster reefs over a 22-year period. *Ecological Applications,* 33(4), e2825. https://doi.org/10.1002/eap.2825

Moore, C. S., Gittman, R. K., & Blakeslee, A. M. H. 2024. Parasites as indicators of biodiversity and habitat complexity in coastal ecosystems. *Ecosphere*, 15(7), e4928. https://doi.org/10.1002/ecs2.4928

Morales-Serna, F. N., Rodríguez-Santiago, M. A., Gelabert, R., & Flores-Morales, L. M. 2019. Parasites of fish Poecilia velifera and their potential as bioindicators of wetland restoration progress. *Helgoland Marine Research,* 73(1), 1. https://doi.org/10.1186/s10152-019-0522-1

Morris, R. L., Bilkovic, D. M., Boswell, M. K., Bushek, D., Cebrian, J., Goff, J., Kibler, K. M., La Peyre, M. K., McClenachan, G., Moody, J., Sacks, P., Shinn, J. P., Sparks, E. L., Temple, N. A., Walters, L. J., Webb, B. M., & Swearer, S. E. 2019. The application of oyster reefs in shoreline protection: Are we over-engineering for an ecosystem engineer? *Journal of Applied Ecology*, *56*(7), 1703–1711. https://doi.org/10.1111/1365-2664.13390

Morris, R. L., La Peyre, M. K., Webb, B. M., Marshall, D. A., Bilkovic, D. M., Cebrian, J., McClenachan, G., Kibler, K. M., Walters, L. J., Bushek, D., Sparks, E. L., Temple, N. A., Moody, J., Angstadt, K., Goff, J., Boswell, M., Sacks, P., & Swearer, S. E. 2021. Large-scale variation in wave attenuation of oyster reef living shorelines and the influence of inundation duration. *Ecological Applications*, 31(6), e02382. https://doi.org/10.1002/eap.2382

N. C. Coastal reserve and national estuarine research reserve | nc deq. n.d. Retrieved from https://www.deq.nc.gov/about/divisions/coastal-management/nc-coastal-reserve

Noonburg, Erik G., and James E. Byers. 2005. “More harm than good: when invader vulnerability to predators enhances impact on native species.” *Ecology* 86(10):2555–60. doi: 10.1890/05-0143.

Overton, K., Dempster, T., Swearer, S. E., Morris, R. L., & Barrett, L. T. 2024. Predictors of outplanted marine bivalve survival in restoration: A review and synthesis. *Journal of Applied Ecology*, 61(12), 2884–2896. https://doi.org/10.1111/1365-2664.14795

Phelan, K., Blakeslee, A.M., Krause, M. and Williams, J.D. 2016. First documentation and molecular confirmation of three trematode species (Platyhelminthes: Trematoda) infecting the polychaete *Marenzelleria viridis* (Annelida: Spionidae). Parasitology Research, 115, pp.183-194.

Phillips, Robert R., and Stanley B. Swears. 1979. Social hierarchy, shelter use, and avoidance of the predatory toadfish (*Opsanus tau*) by the striped blenny (*Chasmodes bosquianus*). *Animal Behavior*, 27, 1113-1121. https://doi.org/10.1016/0003-3472(79)90059-9

Pinnell, C. M., Ayala, G. S., Patten, M. V., & Boyer, K. E. 2021. Seagrass and Oyster Reef Restoration in Living Shorelines: Effects of Habitat Configuration on Invertebrate Community Assembly. *Diversity*, *13*(6), 246. https://doi.org/10.3390/d13060246

Poulter, B., Feldman, R. L., Brinson, M. M., Horton, B. P., Orbach, M. K., Pearsall, S. H., Reyes, E., Riggs, S. R., & Whitehead, J. C. 2009. Sea-level rise research and dialogue in North Carolina: Creating windows for policy change. *Ocean & Coastal Management*, *52*(3–4), 147–153. https://doi.org/10.1016/j.ocecoaman.2008.09.010

Puckett, B. J.; Theuerkauf, S. J.; Eggleston, D. B.; Guajardo, R.; Hardy, C.; Gao, J., and Luettich, R. A., 2018. Integrating Larval Dispersal, Permitting, and Logistical Factors Within a Validated Habitat Suitability Index for Oyster Restoration. Frontiers in Marine Science, 5, 76. doi: 10.3389/fmars.2018.00076.

Rachel carson reserve | nc deq. n.d. Retrieved from https://www.deq.nc.gov/about/divisions/coastal-management/nc-coastal-reserve/reserve-sites/rachel-carson-reserve

Riggs, S. R., & Ames, D. v d P. 2003. *Drowning the North Carolina coast: Sea-level rise and estuarine dynamics*. North Carolina Sea Grant.

Rodriguez, A. B., Fodrie, F. J., Ridge, J. T., Lindquist, N. L., Theuerkauf, E. J., Coleman, S. E., Grabowski, J. H., Brodeur, M. C., Gittman, R. K., Keller, D. A., & Kenworthy, M. D. 2014. Oyster reefs can outpace sea-level rise. *Nature Climate Change*, 4(6), 493–497. https://doi.org/10.1038/nclimate2216

Ruggiero, P. 2008. Impacts of Climate Change on Coastal Erosion and Flood Probability in the US Pacific Northwest. *Solutions to Coastal Disasters* 2008, 158–169. https://doi.org/10.1061/40968(312)15

Schmidt, G.D., 1986. CRC Handbook of Tapeworm Identification. CRC Press, Boca Raton (675 pp).

Smee, Delbert L., and Gregory W. Stunz. 2017. "Macrofauna Using Intertidal Oyster Reef Varies in Relation to Position within the Estuarine Habitat Mosaic." *Marine Biology*, 164(8). https://doi.org/10.1007/s00227-016-3033-5

Soniat, T. M., Finelli, C. M., & Ruiz, J. T. 2004. Vertical structure and predator refuge mediate oyster reef development and community dynamics. *Journal of Experimental Marine Biology and Ecology*, 310(2), 163–182. https://doi.org/10.1016/j.jembe.2004.04.007

"Station Home Page - Beaufort, Duke Marine Lab, NC - Station ID: 8656483 ." *NOAA Tides & Currents, Center for Operational Oceanographic Products and Services,* https://tidesandcurrents.noaa.gov/stationhome.html?id=8656483#info.

Stone, R. B., C. C. Buchanan, and F. W. Steimle. 1974. Scrap tires as artificial reefs. U.S. Environmental Protection Agency, Publication SW-119, Washington, D.C. https://nepis.epa.gov/Exe/ZyNET.exe/2000QPKT.txt?ZyActionD=ZyDocument&Client=EPA&Index=Prior%20to%201976&Docs=&Query=&Time=&EndTime=&SearchMethod=1&TocRestrict=n&Toc=&TocEntry=&QField=&QFieldYear=&QFieldMonth=&QFieldDay=&UseQField=&IntQFieldOp=0&ExtQFieldOp=0&XmlQuery=&File=D%3A%5CZYFILES%5CINDEX%20DATA%5C70THRU75%5CTXT%5C00000002%5C2000QPKT.txt&User=ANONYMOUS&Password=anonymous&SortMethod=h%7C-&MaximumDocuments=1&FuzzyDegree=0&ImageQuality=r75g8/r75g8/x150y150g16/i425&Display=hpfr&DefSeekPage=&SearchBack=ZyActionL&Back=ZyActionS&BackDesc=Results%20page&MaximumPages=1&ZyEntry=5

Stunkard, H. 1983. The marine cercariae of the Woods Hole, Massachusetts region, a review and a revision. *Biological Bulletin,* 164, 143–162.

Sutton-Grier, A. E., Wowk, K., & Bamford, H. 2015. Future of our coasts: The potential for natural and hybrid infrastructure to enhance the resilience of our coastal communities, economies and ecosystems. *Environmental Science & Policy*, 51, 137–148. https://doi.org/10.1016/j.envsci.2015.04.006

Theuerkauf, E. J., Stephens, J. D., Ridge, J. T., Fodrie, F. J., & Rodriguez, A. B. 2015. Carbon export from fringing saltmarsh shoreline erosion overwhelms carbon storage across a critical width threshold. *Estuarine, Coastal and Shelf Science*, *164*, 367–378. https://doi.org/10.1016/j.ecss.2015.08.001

Trimble R10 MODEL 2 GNSS System Datasheet. 2021. Trimble, Inc. https://dev-geospatial.trimble.com/sites/geospatial.trimble.com/files/2021-07/022516-332B_TrimbleR10-2_DS_USL_0721_LR.pdf

Tso, G. L., Narayan, S., & Gittman, R. K. 2023. Wave attenuation performance of emergent reef-type breakwaters and oyster shell bags. *ASCE Inspire* 2023, 564–573. https://doi.org/10.1061/9780784485163.066

US Department of Commerce, N. O. and A. A. 2023. *What percentage of the American population lives near the coast?* Retrieved from https://oceanservice.noaa.gov/facts/population.html

Walters, L. J., Roddenberry, A., Crandall, C., Wayles, J., Donnelly, M., Barry, S. C., Clark, M. W., Escandell, O., Hansen, J. C., Laakkonen, K., & Sacks, P. E. 2022. The use of non-plastic materials for oyster reef and shoreline restoration: Understanding what is needed and where the field is headed. *Sustainability*, 14(13), 8055.

Wellman, E. H., Baillie, C. J., Puckett, B. J., Donaher, S. E., Trackenberg, S. N., & Gittman, R. K. 2022. Reef design and site hydrodynamics mediate oyster restoration and marsh stabilization outcomes. *Ecological Applications*, *32*(2), e2506. https://doi.org/10.1002/eap.2506

Williams, J. D., Boyko, C. B., Tepolt, C. K., & Blakeslee, A. M. H. 2023. Cryptic diversity in endoparasitic isopods (Bopyroidea: Entoniscidae) from mud crabs (Panopeidae) along the Atlantic coast of North America, with the description of a new genus and new species as revealed by molecular and larval characters: the long and the short of it. *Journal of Crustacean Biology*, 43(1), ruac065. https://doi.org/10.1093/jcbiol/ruac065

Wogan, G. O. U., & Wang, I. J. 2018. The value of space-for-time substitution for studying fine-scale microevolutionary processes. *Ecography*, 41(9), 1456–1468. https://doi.org/10.1111/ecog.03235

Yamaguti, S., 1971. Synopsis of Digenetic Trematodes of Vertebrates. Keigaku Publishing Co, Tokyo, Japan.

Young, Truman P., Jonathan M. Chase, and Russell T. Huddleston. 2001. "Comparing, Contrasting and Combining Paradigms in the Context of EcoloNcal Restoration." *Ecological Restoration,* 19(1), 5–13. https://citeseerx.ist.psu.edu/document?repid=rep1&type=pdf&doi=6c4d6056239d28b261d5402fbac35ab6d7ba033c

zu Ermgassen, P. S. E., Spalding, M. D., Grizzle, R. E., & Brumbaugh, R. D. 2013. Quantifying the loss of a marine ecosystem service: Filtration by the eastern oyster in US estuaries. *Estuaries and Coasts,* 36(1), 36–43. Doi: 10.1007/s12237-012-9559-y

zu Ermgassen, S. O. S. E., & Löfqvist, S. 2024. Financing ecosystem restoration. *Current Biology*, *34*(9), R412–R417. https://doi.org/10.1016/j.cub.2024.02.031