Biology Honors Thesis

Examining Parasite Diversity as an Indicator of Ecosystem Restoration Success

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Examining Parasite Diversity as an Indicator of Ecosystem Restoration Success

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I hereby declare I am the sole author of this thesis. It is the result of my own work and is not the outcome of work done in collaboration, nor has it been submitted elsewhere as coursework for this or another degree.

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ABSTRACT – In oceans across the world, oyster reefs, ecosystems generated by a foundational species of mollusks, are an essential source of habitat where fauna such as fishes, birds, and crustacea depend both indirectly and directly on the organism's habitat, food availability, and nursery capabilities for their offspring. Due to primarily anthropogenic effects, the Eastern oyster (*Crassostrea virginica*), found from the Gulf of St. Lawrence to the Gulf of Mexico, has been severely declining in numbers, resulting in impacts being felt across terrestrial and aquatic ecosystems. Because of their foundational role in providing habitat and protection for so many other species spanning multiple different taxa, the loss of these oyster reefs can have a ripple effect through communities. To accurately examine ecosystem health and biodiversity in oyster reef communities, researchers are now exploring an array of sampling schemes. One of these methods gaining in recognition is analyzing parasitic prevalence and diversity as a signal of ecosystem health post-restoration. In previous investigations, scientists have used parasites to examine predator and prey relationships, environmental issues, and coevolution. My study examined the abundance and diversity of crab hosts and the prevalence and diversity of parasites over time to determine whether there were changes in these biodiversity metrics post-restoration. Specifically, I investigated crab species that have been identified as known hosts for taxa-spanning endoparasites, including the Atlantic mud crab *Panopeus herbstii*, the white-fingered mud crab *Rhithropanopeus harisii*, the black-fingered mud crab *Dyspanopeus sayi*, the depressed mud crab *Eurypanopeus depressus*, and the stone crab *Menippe mercenaria.* These crabs were

collected along Taylor's Creek at Carrot Island, which is part of the Rachel Carson Reserve near Beaufort, NC. Oyster restoration occurred in late spring 2020, and host-parasite sampling began monthly in October 2020 until October 2021, as part of an ECU master's thesis, and then continued seasonally through Spring 2024. After collection, crabs were examined in the lab at ECU: crab hosts were identified to species level and then dissected to measure parasite diversity using standard techniques. This project holds significance because global biodiversity is currently facing an unparalleled threat, and with species already reduced to a fragment of the abundance they once were, these ecosystems could be nearing a point of no return without restoring vital habitats like oyster reefs. My data will prove valuable to the scientific community because it can serve as a model for how parasite data can be informative of community biodiversity changes post-restoration, which can be applied in other systems outside of oyster reefs and estuarine environments. From my research, I have acquired an understanding that parasitic abundance can be efficient and accurate when used as another metric for surveying for community biodiversity in ecosystems. There are many challenges and limitations to evaluating community biodiversity and ecosystem health after a restoration attempt has been made; so, having a novel solution like using a highly quantifiable and investigable method of biodiversity surveying (such as parasite surveys from abundant hosts) would allow for results with stronger confidence and evidentiary support.

Acknowledgements

I would like to thank everyone who has played a role in making this body of work possible: Dr. April M.H. Blakeslee, Carter Stancil, the members of the Blakeslee Lab, Dr. Rachel Gittman, Dr. Susan McRae, the professors and staff of the ECU Department of Biology, the Honors College, East Carolina University, and the State of North Carolina for their generous funding. Without these individuals I could not have done any of this work, let alone get into this world of science. The past few years working in a research lab have taught so much to me and broadened my horizons more than I could have ever imagined. I will always cherish my time conducting research.

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Introduction

The word biodiversity often comes to mind when considering Earth's ecosystems. This word represents biological diversity and refers to the extraordinary variety of life on Earth, from genes and species to ecosystems and their life-sustaining processes and functions. (Smithsonian, n.d.). Approximately nine million species are estimated to occupy our planet today (Wiens, 2023), with an interplay of species interactions representing a dynamic system that has changed vastly throughout the history of life on Earth. The biodiversity of Earth has resulted from processes that are evolutionarily and ecologically based, including speciation, extinction, and time being major agents of change (Sechrest & Brooks, 2002). Extinction has been a recurring phenomenon in the history of Earth, with five previous mass extinctions pruning branches off the tree of life, removing specific lineages and all possibilities of descendants that may evolve from them (American Museum of Natural History, 2019). Recently, some scientists have warned that we are entering what is being referred to as a 6th major extinction event that is entirely human caused and this decline of worldwide biodiversity is quickly becoming of the utmost importance to scientists (Cowie, Bouchet, & Fontaine, 2022). Experts in biology are now sounding an alarm, placing great emphasis on the need for a strong increase in current efforts towards fixing the main causes of biodiversity loss (Isbell et al., 2022). At current rates, nearly 37% of all species living on Earth are expected to be classified as threatened or driven to the brink of extinction by the year 2100 (Isbell et al., 2022).

The issue of biodiversity loss stands to negatively impact the lives of a lot more people than the world realizes. The loss of biodiversity is challenging because fixing the issue requires the knowledge of which factors, whether natural or anthropogenic, are direct and indirect impacts on biodiversity. Destruction of land and the ocean for human use has had the greatest detrimental effects on biodiversity, and instances such as direct exploitation of natural resources and pollution are some of the specific activities doing the most harm (Jaureguiberry et al., 2022). The World Conservation Union, IUCN, has estimated that the value of goods and services around the world provided by the environment and depended on by humanity amounts to nearly 33 trillion US Dollars annually (Bos, 2017). In the United States alone, more than 900 million people enter national parks, forests, and monuments to behold beauty and biodiversity unlike anywhere else on Earth (White et al., 2023).

Along the eastern seaboard of the United States, there have been numerous reported incidences of biodiversity loss in estuarine and coastal habitats including the Chesapeake Bay (Fisheries, 2020), the Albemarle River/Sound and the Pamlico Sound (Edwards, Sutherland, Wilson, & Beck, 2023), and Back Sound among others (Allen, 2024). Experts estimate that in North Carolina, some areas have suffered 85% decline in structurally complex oyster reefs, with some areas nearing >90% loss in specific places (Jackson, 2001) To support resilient communities that have faced stressors of ecological disturbances, living shorelines have become an important tool for protecting and stabilizing coastlines (Moore, Gittman, Puckett, Wellman, & Blakeslee, 2020). These living shorelines are made of natural materials like plants, sessile animals (like oysters), sand, and rocks (NOAA Fisheries, 2020). When naturally occurring, places such as this become teeming with life and create a more resilient area on the waterfront (Gittman et al. 2016; Fisk, 2021). With human activity causing ecosystem degradation at such unprecedented rates (United Nations, 2019), these estuarine nursey habitats could disappear possibly causing the loss of commercially valuable organisms (Gittman et al., 2016).

Besides searching for and utilizing natural methods to improve the health of coastal ecosystems and shorelines, scientists have also investigated more manmade fixes for the issue.

Scientists learned that bulkheads and seawalls offer lower biodiversity than natural shorelines (Scyphers, et al., 2016). Shorelines that have been reinforced with artificial structures have been found reflect more wave energy, which in turn causes scouring and sediment resuspensions (Scyphers, et al., 2016), a process that can cause a decrease in infaunal diversity as opposed to what you would find in natural marshes and estuaries with naturally intact “living” shorelines (Seitz et al., 2006). Additionally, scientists found living shorelines had a higher capability to enhance sustainability and bolster ecosystemic resilience to natural disasters (Smith, Puckett, Gittman, & Peterson, 2018).

Oyster reefs are one such living shoreline that have been shown to have a variety of beneficial ecosystem services, including on water quality (Piehler and Smyth, 2011), wave attenuation (Piazza et al., 2005), and improving biodiversity via habitat creation (Coen et al., 1999; Tolley and Volety, 2005; Humphries et al., 2011; Gittman et al. 2016). However, the state of North Carolina has experienced troubling fluctuations with its population of oysters for many years (Shaw, 2023). In the last 100 to 150 years, scientists have seen oyster harvest rates plummet to 75-80% less than what they once were historically (Garcia, 2023). Losses of these magnitudes severely impact the services the ecosystem can provide to the local area, so to help solve the compounding issue, oyster sanctuaries were made. The sole aim of these sanctuaries was to protect and preserve oyster populations to hopefully grow large, healthy oysters capable of being the brood stock that repopulates North Carolinian waters, so harvesting was strictly prohibited (“Oyster Sanctuaries | NC DEQ,” 2023).

To construct a new oyster reef, materials like natural oyster and clam shells, rip-rap marl, Reef Balls™, concrete, recycled crushed concrete, and rocks are used to create a habitat ideal for oyster settlement, survival, and growth (“Oyster Sanctuaries | NC DEQ,” 2023). As well as attracting oysters, these reefs also act as a home for fauna like clams, finfish, crustaceans, and large fish. This plethora of animals known for living around oyster reefs represents a restorative living laboratory for scientists to analyze and use for experimentation, with an area so large, experiments can be easily executed under natural conditions ("Research | NC DEQ," 2023). In marine systems, free-living taxa readily gravitate towards restored oyster reefs 1-2 years after reef construction is completed (Humphries et al., 2011; Moore et al., 2020). Nearly 10 years after original restoration, these habitats will hopefully have developed most of the attributes that make natural reefs functional biodiversity hotspots reefs (e.g., reef biomass, mesopredator abundance) (Smith et al., 2022; Moore et al. 2023). The Rachel Carson Reserve off the coast of Beaufort, North Carolina is a popular hotspot for restoration research and is the location of focus for my research. Encompassing 2,315 acres in total, the reserve has been transformed from an unproductive and desolate location into a functioning ecosystem (Allen, 2024).

With scientists actively performing restorative attempts to the oyster reefs of North Carolina, something importantly challenging should be noted: determining if restoration is effective. Biodiversity can be difficult to accurately track because of the inherent challenges associated with biodiversity surveying such as time, with some ecosystem restoration objectives taking up to 8 years to manifest (Moore, Baillie, Edmonds, Gittman, & Blakeslee, 2023), societal interference, evident in the sense of an inability to rally communities together to see the importance of providing assistance to restore to monitor the health of the ecosystem prior to, during, and after the attempt (Vander Naald, Sergeant, & Beaudreau, 2019), and sampling techniques that leave "holes" in samplings from the inability to document an ecosystem fully at any size (Caro, 2010). Therefore, oftentimes there is a need for a "shortcut" method in conservation and ecological monitoring (Caro, 2010). To work around this issue, scientists have started using surrogate species, which are group of organisms capable of serving as proxies for a bigger community, to reasonably quantify the effects of environmental change over time on community biodiversity (Wiens et al., 2008; Moore et al. 2020, 2023, 2024). To determine which species are best able to serve as effective surrogates, scientists aim to select organisms that can be easily collected via sampling and occupy a range of niches. These animals typically display varying sensitivities to pollution and environmental disturbances, so parasites have become recognized as a promising group of organisms for assessing the effectiveness of oyster reef rehabilitation (Moore et al. 2023). Parasites are animals or plants that derive their nutrients by living on the surface of, or inside the tissues of an organism of another species, being either completely or semi parasitic in nature. Specific parasites that are transmitted through trophic levels and therefore require several hosts, like trematodes, cestodes, and acanthocephalans, are appealingly suited to be used as surrogates of biodiversity. Many studies have shown these parasites require numerous invertebrate and vertebrate hosts to make it through their full life cycles (Fredensborg et al., 2006; Hechinger et al., 2007; Hechinger & Lafferty, 2005; Huspeni & Lafferty, 2004; Levakin et al., 2013; Moore et al., 2020). Sampling a population and observing a high prevalence of parasites has provided definitive evidence in prior studies that additional hosts necessary for parasites to live and complete their life cycles such as shorebirds, fishes, and crustaceans are present somewhere in the local ecosystem (Hechinger et al., 2007; Huspeni & Lafferty, 2004; Moore et al., 2023). Sampling in search of these parasites could also be an indication of how healthy the overall ecosystem is, too (Moore et al. 2023). By using the composition and diversity of parasites in a naturally occurring and functionally thriving reef, scientists can perform sample collections and determine the overall parasitic abundance and richness to see the effect of habitat quality on biodiversity (Moore et al. 2020, 2023). For my project, I used parasite data from panopeid mud crabs and stone crabs collected from Taylor's Creek in the Rachel Carson Reserve to determine whether parasites can be used as an effective metric of biodiversity changes post-restoration. Parasites in this type of ecosystem can serve as better indicators of trophic complexity for example, past work has shown that mean estimates of richness and diversity of crustacean and fish parasites better indicate successional changes in biodiversity following reef restoration than free-living taxa (Moore et al., 2023). By examining the overall infection abundance and richness of parasites, I tested my hypothesis that parasites are informative of biodiversity increases following oyster restoration along Taylor's Creek in Beaufort, North Carolina.

Methods

Study Site

The crabs gathered for this experiment were collected from the North Carolina Coastal Reserve and the National Oceanic and Atmospheric Association National Estuarine Research Reserve, in the Rachel Carson Reserve (RCR), located in the city of Beaufort, North Carolina, United States. Taylor's Creek is an estuarine tidal creek that flows predominantly eastward on the eastern side of the RCR parallel to the Beaufort waterfront, and it is the location where we performed our collection of specimens (Woodard 2020). During June of 2020, a shoreline restoration project was performed, and oyster reefs of varying sizes were created. There were two types of reefs created and between each reef was a spaced interval of 25 meters. Four high relief 15-meter x 1.5-meter x 0.3-meter and four low relief 15-meter x 1.5-meter x 0.2-meter were created. In addition, four control sites with no reef construction were established. For the purpose of my investigation, I only examined the restored oyster reefs and did not make comparisons of high relief or low relief, as (Woodard 2022) did not find many clear differences between the high or low relief reefs, and the control plots were not found to be informative. These living shorelines were aimed to both protect the shorelines from mechanical erosion and provide space for flora and fauna to colonize and increase the biodiversity of the local ecosystem (Woodard 2022). The choice of the reef location was organized by the Rachel Carson Reserve Site Manager in collaboration with the North Carolina Coastal Reserve Coordination (Woodard 2022). In total, the twelve sites created in a block design created an experiment with four replicates per level of treatment. These breakwaters were constructed by the Sandbar Oyster Company, popular for their innovative biodegradable man-made oyster habitats called "Oyster

Catchers.” These constructions were made of plant fiber cloth and a mineral-based hardening agent (Wellman 2022).

<u>*Reef Fauna Sampling*</u>

Samples collected from October 2020 - October 2021 were included in the master’s thesis of Nina Woodard (Woodard 2022). These samples were collected monthly (except January) during this time period. Samples after October 2021 were collected approximately every 3–4 months by various members of the Blakeslee and Gittman labs. The faunal samples for the experiment were collected using passive samplers. These samplers were made using plastic milk crates (19 x 22 x 16 cm) and were filled with roughly 3.5 pounds of dried out oyster shells. They were then zip tied shut with a mesh on top (Roche and Torchin, 2007; Blakeslee et al. 2021; Woodard 2002; Moore et al. 2020, 2023). A benefit of the passive samplers was that they provided fauna the ability to freely move in and out of the crates while still staying sessile and attached to the three dimensionally shaped habitat provided by the oyster shells (Moore et al., 2020; Blakeslee et al., 2021). To remain securely in place, each crate was zip-tied to a wooden stake roughly 5 meters from the median of the oyster reef. From 2020-2021. one crate was oriented with the face directly towards land, and the other crate was oppositely turned towards the ocean. In full, there were twenty-four crate deployments (Woodard 2022). After October 2021, one crate was removed from each site and the remaining crate was placed adjacent to the reef at the same parallel position as the reef.

The crates were left in the estuarine ecosystem between one and three months, and then once collected, the contents were sorted using a sieve (69 cm x 56 cm x 12 cm) with a 2mm mesh grid. Animals collected from the samplers included a diverse array of crustaceans including shrimp, crabs, and transient fishes too. After collection, the crab specimens were prepared for transport and brought back to East Carolina University for parasite processing.

For the parasitic dissections performed in this experiment, four species of mud crab and one species of stone crab were targeted. These were the Atlantic mud crab, *Panopeus herbstii*, the white-fingered mud crab, *Rhithropanopeus harisii*, the black-fingered mud crab, *Dyspanopeus sayi*, the flatback or depressed mud crab*, Eurypanopeus depressus*, and the stone crab, *Menippe mercenaria* (Woodard et al. 2022). These specific species are ones I have hypothesized will host parasites that serve as viable predictors of overall ecosystem biodiversity. I predicted that I should be able to make an accurate depiction of parasite intensity, species richness, and parasite diversity by quantifying these multiple endoparasites.

Abiotic Data

During the process of sampling, abiotic measurement such as water temperature (°C), dissolved oxygen (mg/L), and salinity (ppt) were recorded. These readings were taken using a hand-held YSI (Pro30 Model) at each oyster reef. In May of 2021, HOBO salinity loggers were deployed as well. During data and statistical analyses, the only data utilized was from the YSI hand-held devices. In my analyses, I used month as a predictor of seasonality, but future work should include analyses with these abiotic factors as predictors.

Host dissections and parasite identification

I identified and quantified the composition of species, their relative abundance, richness of taxa, and parasite diversity of all endoparasites that have multiple host life cycles including trematodes, entoniscid isopods, cestodes, nematodes, and acanthocephalans. Additionally, I recorded data for the occurrence of a rhizocephalan, *Loxothylacus panopaei*, a parasite with a direct life cycle known for infecting panopeid mud crabs such as *E. depressus*, and *R. harrisii* in

North Carolina waters (Blakeslee et al. 2021). The crabs were dissected using standardized protocols in the Blakeslee Research Lab (e.g., Moore et al. 2020, 2023). For all crabs dissected, the carapace width was measured in millimeters, and for a crab to be deemed eligible for dissection, it was required to be at least 4.0 millimeters wide (Tepolt et al. 2020). This limit also helped prevent impractical dissection of crabs too small to accurately analyze, or crabs too young to have developed the anatomical structures we are looking for. The guides and keys utilized for dissection and identification of crab species were provided by the lab (Williams 1984). Next, the mouthparts of the crab were examined depending on the characteristics of the claws. Panopeid crabs can be differentiated by species by looking at their mouthparts. *P. herbstii* and *E. depressus* both have an identifying red dot or swatch on them, and by pulling back the mouth part of the crab you can determine the species of crab. This red coloration on the mouthparts was especially helpful when differentiating between *E. depressus* and *D. sayi*. The telson of the crab was then retracted to determine the sex of the crab and whether it was infected by *L. panopaei*. By locating either claspers or pleopods, or in the instance of gravidity, eggs, could show the sex of the crab. After all the external characteristics had been noted, the gonadal, ganglia, and hepatopancreas tissue were removed and closely examined using a compound microscope at 4x scanning lens (Moore et al. 2020). If there was determination that a potential parasite was found, a standard protocol was used for identification. (Blakeslee et al. 2020; Moore et al. 2023).

Statistical Analyses

To analyze my data, I used boxplots to visualize changes in host-parasite biodiversity over time, with Kruskal-Wallis rank sum tests to examine statistical differences. This included parasite richness, parasite intensity, parasite prevalence, parasite diversity, crab abundance, stone crab abundance, *P. herbstii* abundance, and *D. sayi* abundance, and crab diversity. In addition, I also performed multivariate analyses. To do so, I used Primer 7.0.23 to import data, create a resemblance matrix of host species and abundance or parasite species and prevalence, and then the Bray-Curtis similarity index was used to construct non-metric multidimensional scaling (nMDS) plots to visualize sites and sampling events that were similar or different from one another. I also used a PERMANOVA to determine whether there were statistical differences in my crab and parasite communities with two major categorical variables (Year and Month). "Year" was examined to determine if there were changes in host and parasite communities over time from 2020 to 2024, and "Month" was used to examine potential seasonal effects on these communities.

Results

Changes in Parasite Prevalence, Richness, and Intensity over Time:

I examined three different metrics of parasite diversity over time: parasite prevalence, richness, and intensity. In terms of parasite prevalence by year, prevalence of infection has fluctuated, with two years (2021 and 2022) showing almost no parasites at the sites along Taylor's Creek (Figure 1, top left). Interestingly, 2020 demonstrated the highest prevalence. That year was only comprised of 3 surveyed months (October, November and December) which were sampled about 5 months after the restoration had occurred (May 2020). It could be that the limited sampling that year at a warmer time of the season when we have typically found more parasites (Moore et al. 2023; Blakeslee et al. 2021) played a role in the higher prevalence we detected in 2020. In addition, the parasite group that dominated the infections during this time point were entoniscid isopods which have been found in past work in our region to be more common in the fall (Williams et al. 2023; Moore et al. 2023). Following 2020, the years 2021 and 2022 both had lower prevalence than the remaining years, and there appeared to be a slight positive trend from 2022-2024. By month, there was also a wide fluctuation in the data in both distributions and averages (Figure 1, bottom left). Interestingly, the summer months, including July, August, and September, were noticeably lower in prevalence. Further research is warranted to determine the reason why the summer months were lower in infection prevalence, but temperature could play a role. For example, past work has shown that prolonged heat waves can disrupt parasite transmission because many parasite life cycles include an environmental stage as larvae, and thus, they may be more susceptible to temperature stress during their larval stages (Marcogliese 2016). Over the time period of the study, prevalence also fluctuated, with a negative trend in prevalence until the 7^{th} or 8^{th} event, and then a rising trend for the last two sampling events.

However, more data are needed to conclude whether there is a true trend r not (Figure 1, right).

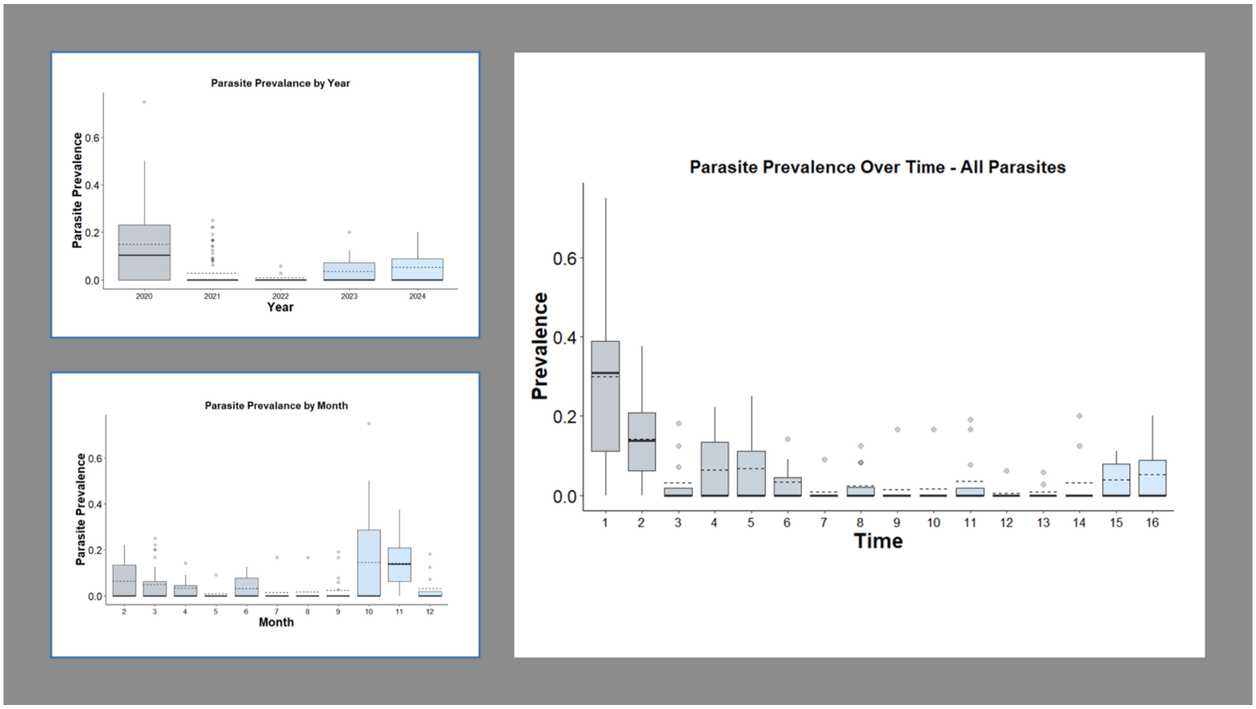

Figure 1. (Top Left) Examinations of parasitic prevalence by year (2020-2024). A Kruskall-Wallace test demonstrated significance overall (25.643, df = 4, p-value = 3.735e-05) with pairwise comparisons showing 2020 to be significantly different from all other years except 2024. **(Bottom Left)** Examination of parasitic prevalence by month. A Kruskall-Wallace test demonstrated significance overall (25.373, df = 10, p-value = 0.004681). **(Right)** Examinations of parasite prevalence over the entire surveyed time period (2020-2024). 1 = Oct 2020, 2 = Nov 2020, 3 = Dec 2020, 4 = Feb 2021, 5 = Mar 2021, 6 = Apr 2021, 7 = May 2021, 8 = June 2021, 9 = Jul 2021, 10 = Aug 2021, 11 = Sep 2021, 12 = Oct 2021, 13 = Sep 2022, 14 = Mar 2023, 15 = June 2023, 16 = Mar 2024. A Kruskall-Wallace test demonstrated significance overall (48.229, df = 15, p-value = 2.336e-05).

Parasite richness:

Richness is the measurement of how many parasite taxa were found during a sampling event (Figure 2). Parasites were grouped into taxa that represented the lowest taxonomic resolution we could achieve (see Methods). Overall, taxa richness was relatively low throughout the entirety of the survey time period (Figure 2, left). Similar to prevalence, the highest richness was in 2020, particularly October and November, and then was relatively low over the sampling period until 2023 and 2024 when it rose again. By Year, 2020 and 2023-2024 were similar in richness and 2021-2022 were very low in parasite richness (Figure 2, right).

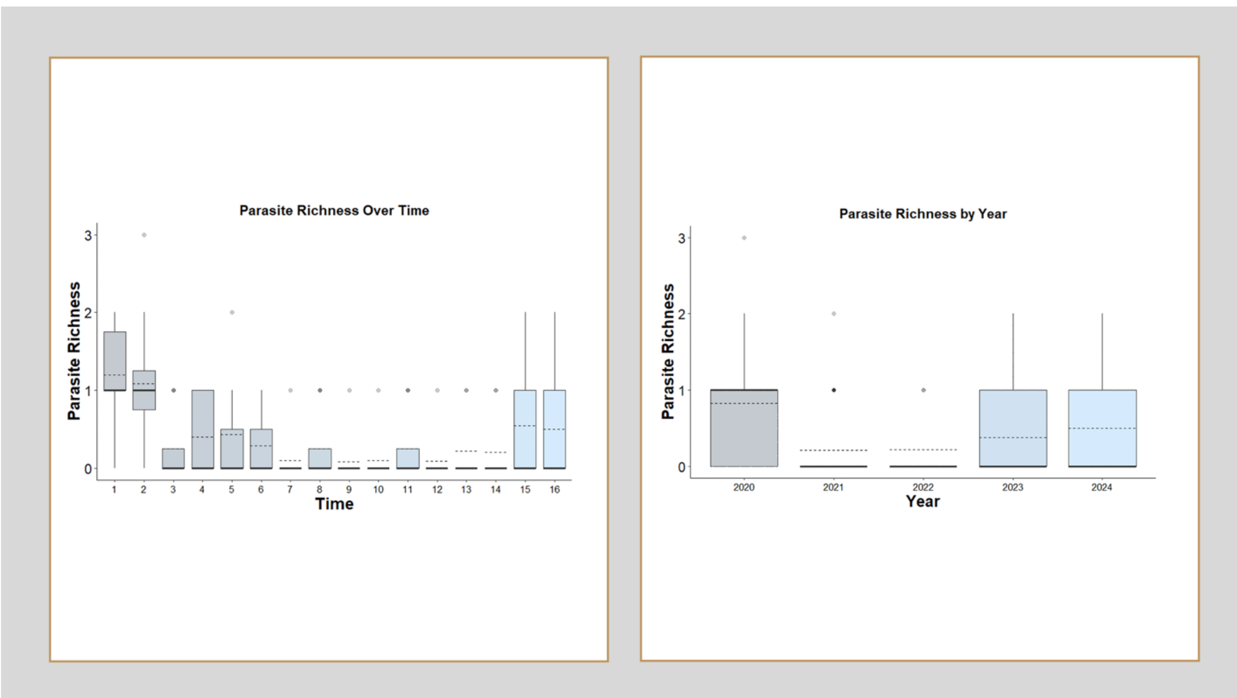

Figure 2 (Left) Parasite richness as a response to sampling point. 1 = Oct 2020, 2 = Nov 2020, 3 = Dec 2020, 4 = Feb 2021, 5 = Mar 2021, 6 = Apr 2021, 7 = May 2021, 8 = June 2021, 9 = Jul 2021, 10 = Aug 2021, 11 = Sep 2021, 12 = Oct 2021, 13 = Sep 2022, 14 = Mar 2023, 15 = June 2023, 16 = Mar 2024. A Kruskall-Wallace test demonstrated significance overall (43.781, df = 15, p-value = 0.000119). **(Right)** Parasite richness as a response to Year (2020-2024), showing the year of restorative attempt and 4 following years. A Kruskall-Wallace test demonstrated significance overall (23.292, df = 4, p-value = 0.0001107) with pairwise comparisons showing 2020 to be significantly different only from 2021.

Parasite Intensity:

Parasite intensity only includes infected hosts and examined the average number of parasites within hosts at a sampling event (Blakeslee et al. 2019). Parasite intensity demonstrated similar trends to prevalence, with the highest intensity in October and November 2020, with a decline in later months and then a slight trend towards increasing prevalence in the sampling events in 2023 and 2024. Intensity was dramatically higher in the first sample (October 2020), with a median intensity that was nearly 5 times the average intensity of most other samplings. As discussed above, this high intensity in October 2020 appears to be driven by entoniscid isopods which dominated parasite surveys in this time period. It is unclear why the entoniscids were so common at this particular survey point, but temperature could play a role, or other biotic and abiotic factors influencing entoniscid transmission in the system. For example, entonscids use copepods as first-intermediate hosts (Williams et al. 2023), so it may be that the presence and abundance of copepod hosts influenced the higher intensity of infection at this time; however, greater research would be needed to determine this.

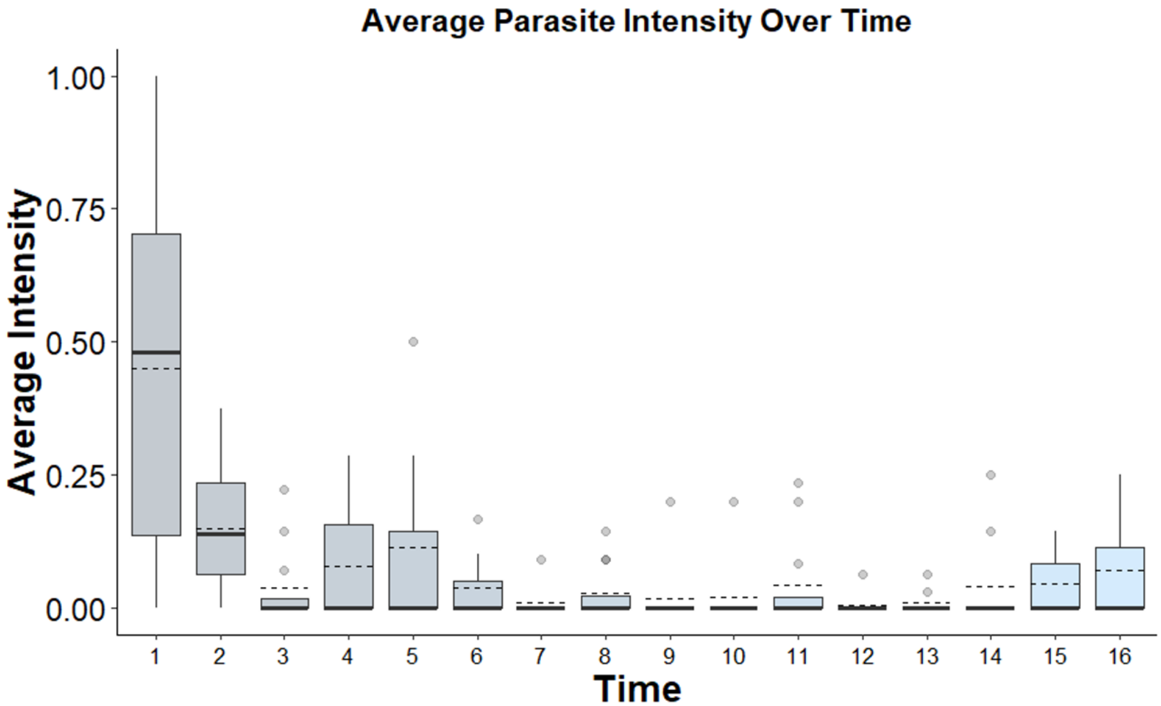

Figure 3. Average parasite intensity over time showing each individual sampling event. 1 = Oct 2020, 2 = Nov 2020, 3 = Dec 2020, 4 = Feb 2021, 5 = Mar 2021, 6 = Apr 2021, 7 = May 2021, 8 = June 2021, 9 = Jul 2021, 10 = Aug 2021, 11 = Sep 2021, 12 = Oct 2021, 13 = Sep 2022, 14 = Mar 2023, 15 = June 2023, 16 = Mar 2024. A Kruskall-Wallace test found significance overall in parasite intensity (47.301, df = 15, p-value = 3.295e-05).

Changes in Crab Host Abundance and Richness Over Time

I examined overall crab abundance and the abundance of three different species of crab that were most abundant during the survey period, including stone crabs (*Menippe* sp.), *Panopeus herbstii*, and *Dyspanopeus sayi*. When examining crab abundance by month (Figure 3, top left), abundance prove to be higher in the earlier and later part of the year, with the highest abundance being in the spring and fall, and the lowest abundance in the summer (July and August). This drop in abundance in the summer is interesting since we saw a similar trend in parasite prevalence for lower prevalence during summer months (Figure 1). Over the course of the sampling period, crab abundance fluctuated. By year, there was little change in crab abundance from 2020-2024, although the abundance was slightly higher in 2022-2024 compared to 2020-2021 (Figure 4, bottom left). Over time, there was a slight negative trend in abundance following the onset of the sampling, ending at the fifth event and then trending upwards afterwards especially in later time periods (2023 and 2024) (Figure 4, right). Parasite prevalence also trended upwards in later sampling events, and it would be informative to determine whether this upward trend in host and parasite abundance would continue through time.

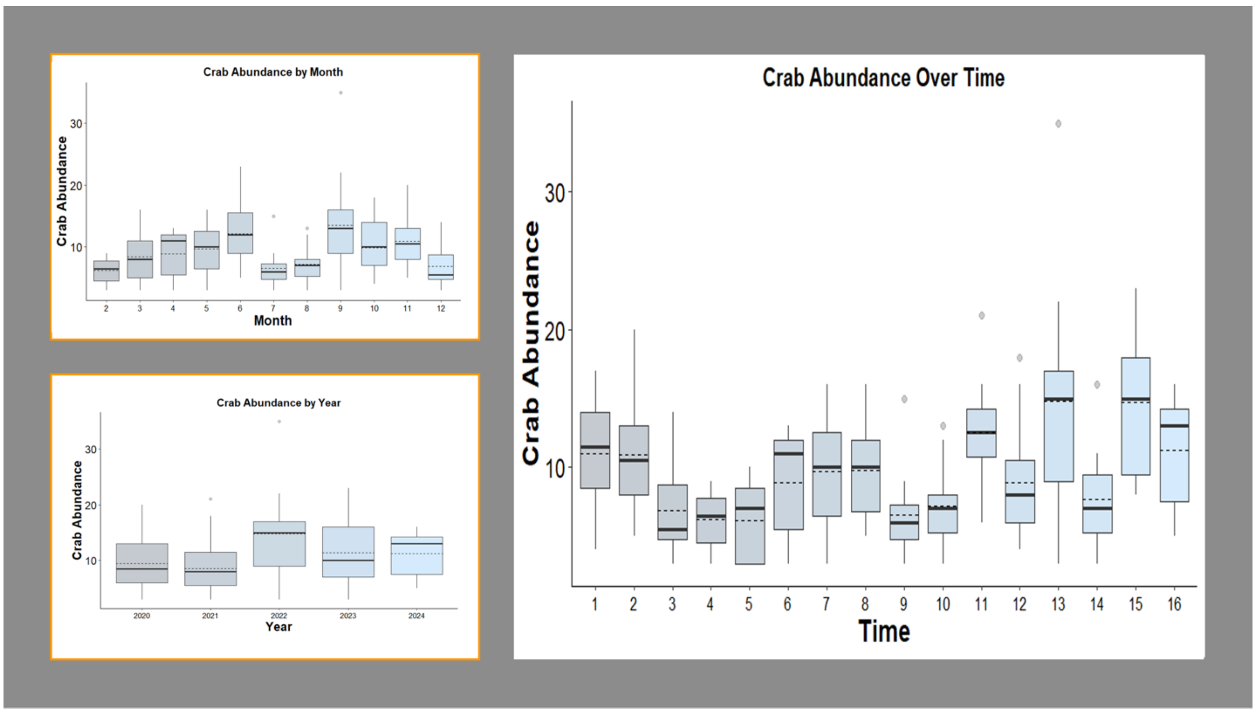

Figure 4. (Top Left) Examination of crab abundance over month (season). A Kruskall-Wallace test found significance overall (36.214, df = 10, p-value = 7.733e-05). **(Bottom Left)** Examination of crab abundance each year of sampling from 2020-2024. A Kruskall-Wallace test found significance overall (10.324, df = 4, p-value = 0.0353). However, there were no significant pairwise comparisons. **(Right)** Crab abundance throughout the entirety of sampling.
1 = Oct 2020, 2 = Nov 2020, 3 = Dec 2020, 4 = Feb 2021, 5 = Mar 2021, 6 = Apr 2021, 7 = May 2021, 8 = June 2021, 9 = Jul 2021, 10 = Aug 2021, 11 = Sep 2021, 12 = Oct 2021, 13 = Sep 2022, 14 = Mar 2023, 15 = June 2023, 16 = Mar 2024. A Kruskall-Wallace test found significance overall (47.045, df = 15, p-value = 3.622e-05).

Stone Crabs:

Over the course of the sampling period, the abundance of stone crabs fluctuated. It decreased from the initial time period until the 8th event when the abundance of stone crabs began to rise, coming to a peak at the 11th event, then facing a slight decline in abundance again until the end of sampling (Figure 5, left). On the whole, there does appear to be a trend for greater stone crab abundance in later sampling events (2022-2024) than earlier (2020-2021). On an annual basis, there was also considerable fluctuation in abundance with the highest abundance in 2022 (Figure 5, right).

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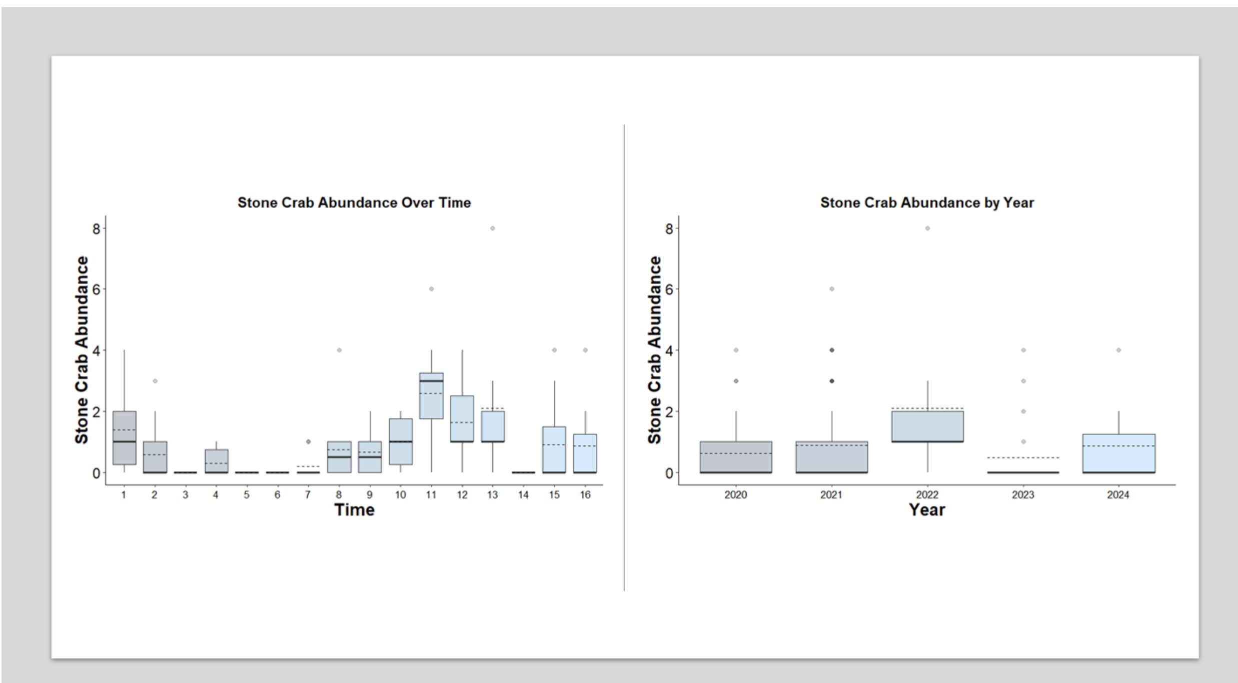

Figure 5. (Left) Stone crab abundance over the entirety of the survey period. 1 = Oct 2020, 2 = Nov 2020, 3 = Dec 2020, 4 = Feb 2021, 5 = Mar 2021, 6 = Apr 2021, 7 = May 2021, 8 = June 2021, 9 = Jul 2021, 10 = Aug 2021, 11 = Sep 2021, 12 = Oct 2021, 13 = Sep 2022, 14 = Mar 2023, 15 = June 2023, 16 = Mar 2024. A Kruskall-Wallace test found significance overall (63.05, df = 15, p-value = 7.486e-08). **(Right)** Stone crab abundance each year from 2020-2024. A Kruskall-Wallace test found significance overall (12.516, df = 4, p-value = 0.0139) with pairwise comparisons showing significant differences between 2020 and 2022 and 2022 and 2023.

Panopeus herbstii:

Abundance of *P. herbstii* was higher than stone crab abundance. By year, abundance averaged around 5 crabs per replicate and remained fairly constant between the years of 2020 and 2023, with a lower abundance in 2024 (Figure 6, left). One possible explanation is that 2024 only represented one sampling event in March 2024. Over the sampling period, there was considerable fluctuations in abundance of *P. herbstii* (Figure 6, right).

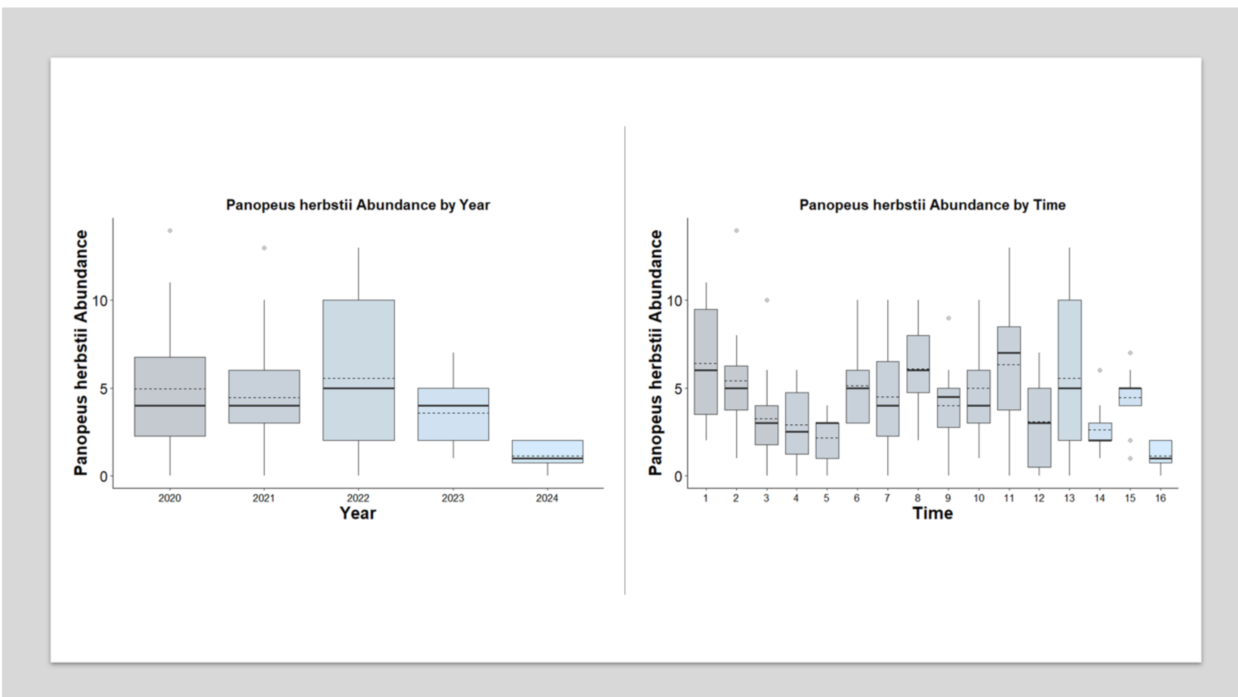

Figure 6. (Left) *P. herbstii* crab abundance each year 2020-2024. A Kruskall-Wallace test found significance overall (14.489, df = 4, p-value = 0.005887) with pairwise comparisons showing significant differences between 2020 and 2024, 2021 and 2024, and 2023 and 2024. **(Right)** *P. herbstii* abundance over the entirety of the survey period. 1 = Oct 2020, 2 = Nov 2020, 3 = Dec 2020, 4 = Feb 2021, 5 = Mar 2021, 6 = Apr 2021, 7 = May 2021, 8 = June 2021, 9 = Jul 2021, 10 = Aug 2021, 11 = Sep 2021, 12 = Oct 2021, 13 = Sep 2022, 14 = Mar 2023, 15 = June 2023, 16 = Mar 2024. A Kruskall-Wallace test found significance overall (42.293, df = 15, p-value = 0.0002026).

Dyspanopeus sayi:

Similar to *P. herbstii*, *D. sayi* also demonstrated fluctuating abundance between the years 2020 and 2024 (Figure 7, left). The abundance of these crabs peaking in the year 2022 having both the highest mean and widest variability. This is interesting considering that stone crabs and *P. herbstii* also demonstrated the highest abundances in 2022. It is unclear what may have led to the increase in crab abundance in this year and further investigation is required. Over the sampling time period, abundance also fluctuated. From the second sampling, there was a downward trend that bottomed out in the 10th sampling event and then appeared to rise in later sampling events in 2022 and 2024 particularly (Figure 7, right).

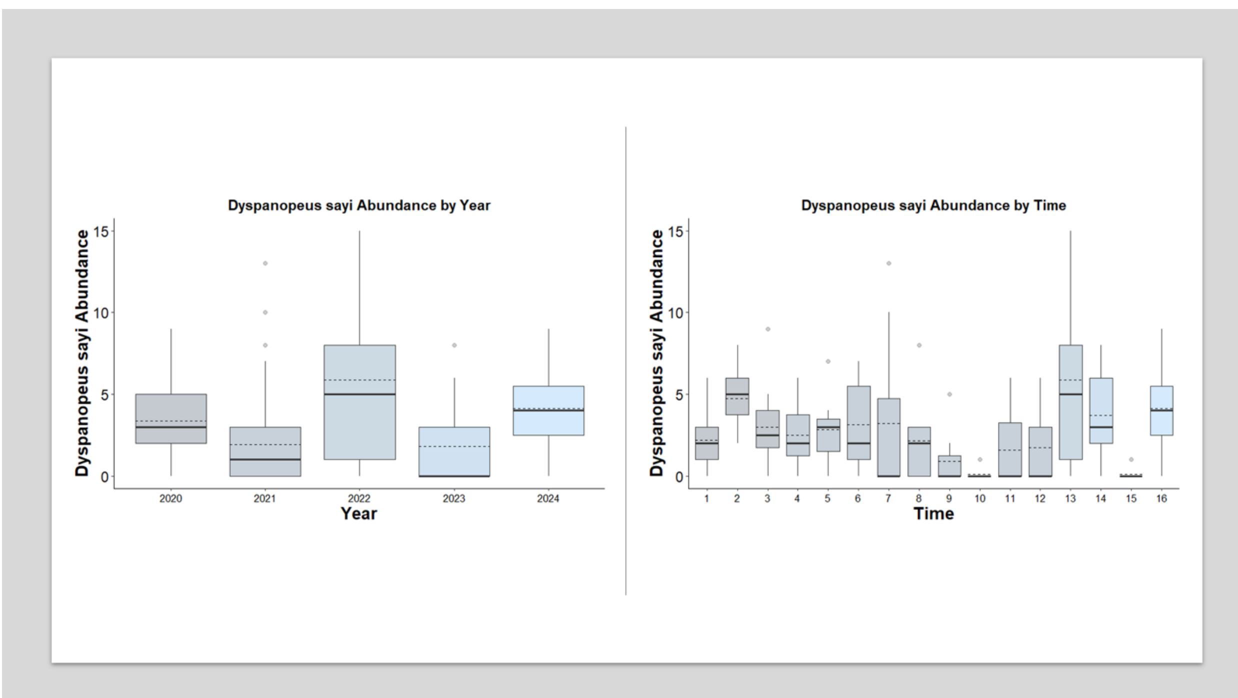

Figure 7. (Left) *D. sayi* crab abundance from 2020-2024. A Kruskall-Wallace test found significance overall (53.304, df = 15, p-value = 3.432e-06) with pairwise comparisons showing significant differences between 2020-2021 and between 2020-2023. **(Right)** *D. Sayi* abundance over the entirety of the study

period. 1 = Oct 2020, 2 = Nov 2020, 3 = Dec 2020, 4 = Feb 2021, 5 = Mar 2021, 6 = Apr 2021, 7 = May 2021, 8 = June 2021, 9 = Jul 2021, 10 = Aug 2021, 11 = Sep 2021, 12 = Oct 2021, 13 = Sep 2022, 14 = Mar 2023, 15 = June 2023, 16 = Mar 2024. A Kruskall-Wallace test found significance overall (53.304, df = 15, p-value = 3.432e-06).

Multivariate Analyses of Parasite Diversity

I also analyzed parasite diversity (parasite prevalence and richness) by both year and month using my data where I excluded uninfected crab samples. Uninfected crabs had to be removed in order to create the resemblance matrix for the nMDS plot. In the nMDS, the sample points closest together were most similar in parasite diversity. By year, there appeared to be distinct clusters of samples for 2020 and 2021 compared to 2023 and 2024 (Figure 8, left). A PERMANOVA showed significant differences in parasite diversity due to Year ($F = 3.7637$, $p = 0.001$). Overlaid vectors suggested nematodes (NEMA) and isopods were more associated with 2020 and 2021, while cestodes and *L. panopaei* (LOXO) were more associated with 2023 and 2024. By month, parasite diversity also showed some evidence of grouping by seasons. For example, the fall and winter samples were clumped together. A PERMANOVA also showed significant differences in parasite diversity due to Month ($F = 2.1639$, $p = 0.003$).

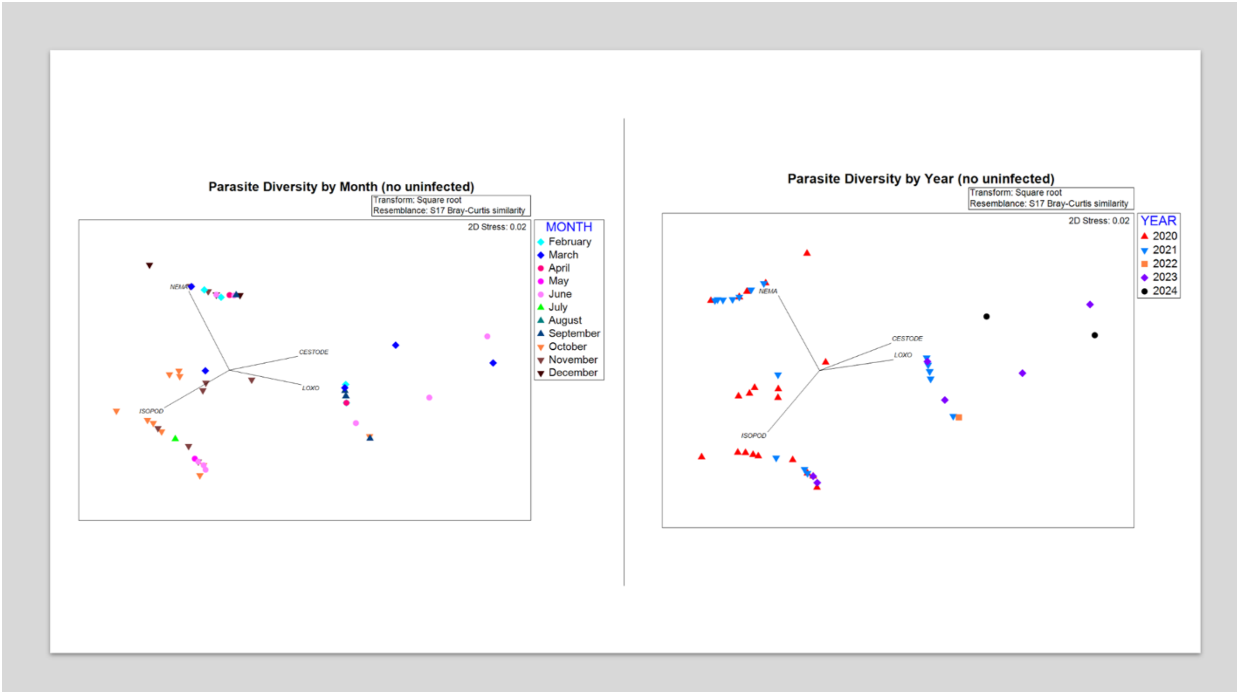

Figure 8. (Top Right) This nMDS plot demonstrates parasite diversity as a function of the year. (**Top Left**) This nMDS plot demonstrates parasite diversity as a function of month.

Multivariate Analyses of Crab Diversity

Multivariate analyses of crab diversity included crab abundance and species richness. When examining crab diversity by Month or Year, there is little evidence of groupings by season or time points. There appears to be some separation by season with winter months and spring months showing some separation, but it is difficult to see clear patterns (Figure 9, Left). However, a PERMANOVA by month is significant, suggesting there is some differentiation in crab diversity by season ($F = 5.8967$, $p = 0.001$). For Year, there are few clear patterns; however, once again a PERMANOVA did demonstrate year was significant ($F = 6.8436$, $p = 0.001$).

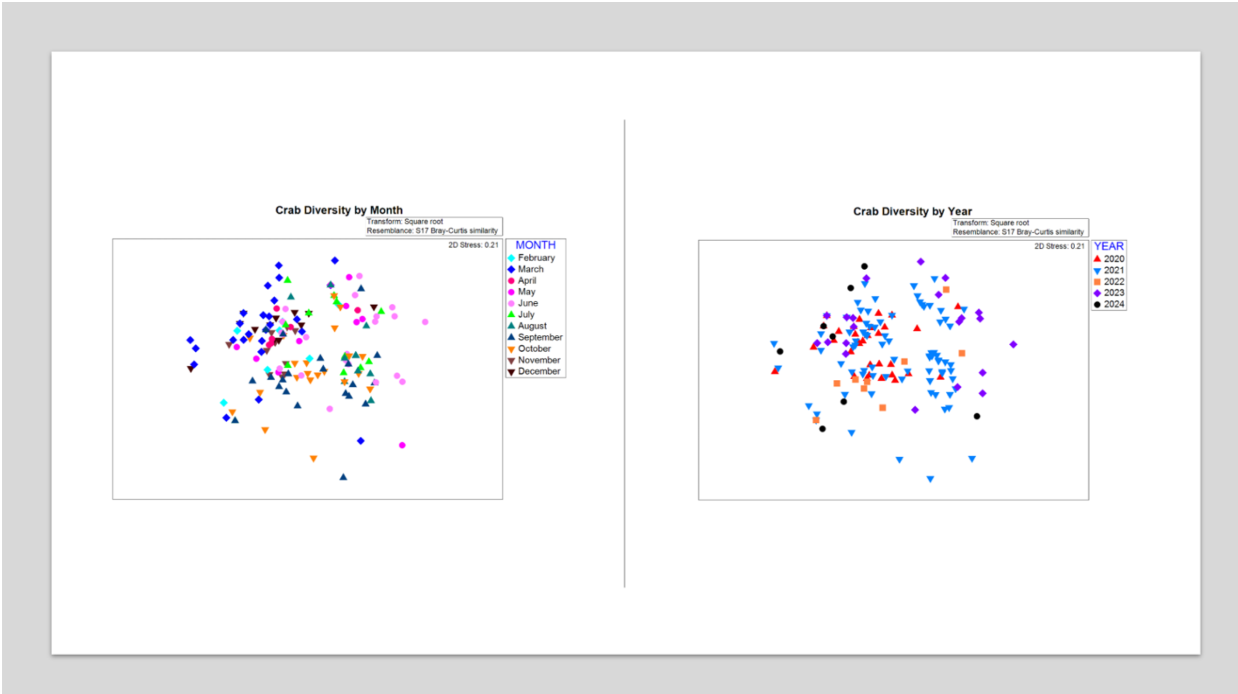

Figure 9. **(Left)** nMDS plot of crab diversity as a function of month. **(Right)** nMDS plot of crab diversity as a function of year (2020-2024).

Discussion and Conclusions

Past studies have recognized the challenges scientists and managers have with measuring whether their efforts in ecosystem restoration are successful or unsuccessful (Lindenmayer, 2018). To address this issue, recent work has shown that parasites can be a valuable tool to determine if an ecosystem is successfully reestablishing the necessary foundational interactions and processes to sustain and proliferate life (Moore, Baillie, Edmonds, Gittman, & Blakeslee, 2023). For my research, I examined host and parasite diversity to determine whether oyster reef restoration at the RCR had a positive impact on host-parasite biodiversity, using a common host group, crabs, and their endoparasites as my focal host-parasite fauna. I expected to find a positive change in host and parasite biodiversity over time (2020-2024). This would indicate that all the hosts necessary to complete parasite life cycles, like fish and shorebirds, were also present at the sites, indicating that the shoreline was on the right track towards restoration (Moore et al. 2020). After obtaining data showing time elapsed since the restorative attempt and counts of parasitic and crab abundance, I was able to perform a few different analyses to determine whether my data provided helpful insight into this current issue. Overall, my analyses suggested that while not being directly conclusive, my data did depict a trend towards increasing biodiversity with time (particularly 2021-2024), and that there could be a myriad of possibilities influencing the oyster reef restoration project at Taylor's Creek. However, to exactly pinpoint the mechanisms behind these changes require further research, as well as more time to determine if trends are truly directional and longstanding.

Development of oyster reefs has proven to be a beneficial method of providing ecological assistance to struggling estuarine ecosystems lacking biogenic habitat by enhancing community biodiversity and host-parasite interactions (Moore et al., 2020) through the creation of threedimensional reef structures (Coen et al., 1999; Coen & Luckenbach, 2000; Tolley et al., 2005; Luckenbach et al., 2005; Moore et al., 2020). I quantified mobile species and parasite abundance, prevalence, richness, diversity, and intensity of restored oyster reefs along Taylor's Creek, Beaufort, NC to determine whether the parasitic diversity of these reefs could be an accurate predictor for overall ecosystem biodiversity in an area that has recently undergone ecosystem restoration. My predictions that parasitic diversity had the potential to be an accurate indicator were not fully upheld, because there were too many unknown and uncontrollable variables that could have influenced the outcome of my survey, and an inability to measure and assess these unknown variables influences my ability to make clear conclusions. In the following paragraphs, I will discuss in detail the impacts that potentially affected abundance of free-living crab species and the parasite community structure throughout my research study.

I found that parasite prevalence was widely variable throughout nearly the entirety of my sampling, with the only real incidence of strong parasitic infection occurring initially in sampling as a result of heavy isopod infection. Overall, the lower parasite diversity detected throughout the time period suggests that habitat function may not yet be fully restored in the system (Smith et al., 2022; Moore et al. 2023). As previously discovered by researchers, it can take oyster reefs numerous years (>10 years) to emulate diversity and function of a natural reef (Moore, Baillie, Edmonds, Gittman, & Blakeslee, 2023), and though free-living species often quickly reinhabit oyster reefs 1-2 years after the restoration (Humphries et al., 2011; Moore et al., 2020), abundance may not match natural and older reef populations until the 6^{th}-10^{th} year (Smith et al., 2022, Moore et al. 2023). Detecting changes in host-parasite communities require time (Burt et al., 2011; Hill et al. 2021), and as a result, long term monitoring of these projects must be done to fully deem an oyster reef to be rehabilitated (Baggett et al., 2015; Smith et al., 2022). In my study, we observed some trends towards increasing diversity and abundance later in the survey time period, but this requires further investigation into the future to determine if this trend continues.

For sampling of reef fauna, there were a few noteworthy results. In my study, there were differences in the abundance of stone crabs versus *P. herbstii* and *D. sayi*. Stone crabs have been found to prefer habitat complexity, being an important requirement for benthic crustaceans because of the multitude of different sized refuges organisms use over their lifetimes as refuges from predators (Shervette, Perry, Rakocinski, & Biesiot, 2004). Structure in a given community is a driving factor of species diversity, and all sites in the survey utilized passive samplers filled with oyster cultch (Gittman et al., 2016; Moore et al., 2020). Woodard (2022) found in her study that these passive samplers provided essentially the only structure available within control plots able to recruit fauna. However, in the high and low relief restored reefs, there was much more structure available including the restored oyster reefs, a sampler facing towards land, and a sampler facing towards the sea (Woodard 2022).

For my free-living target crab species, the most abundant species sampled was the Atlantic mud crab *P. herbstii*. Unsurprisingly, this species is frequently found amongst loose oyster shell at the base of oyster reefs (McDonald, 1977, 1982; Meyer 1994), providing support for why it was the most abundant species found in our passive samplers. The second most abundant crab was *D. sayi*, which is typically found in elevated sections of oyster reefs (Gregalis et al., 2009), may be why we saw fluctuations over time, recruiting specific species of crabs in specific locations. It could also be hypothesized that differing environmental and abiotic and biotic factors could be influencing species abundance, with crab species having species-specific preferences for salinity, temperature, and willingness to endure competition, predation, and the search for habitat.

The location of Taylor's Creek and the positioning of the restoration project could have also influenced my data. With the oyster reefs positioned parallel to the shore, zones of flow are created: a seaward side and a landward side (Chowdhurry et al., 2019). Naturally, oyster reefs orient perpendicularly to the shore, but parallel reefs have become the standard as they are built to also assist with the provision of shoreline protection (Lenihan, 1999; Colden et al., 2016). Because of this, there are instances of tradeoff between shore stabilization and oyster and sessile fauna productivity. My fieldwork provided some new insights into the importance of parasitic diversity and long-term monitoring post-restoration as an indicator of ecosystem health and overall success of a restoration attempt. My results show that crab abundance and parasite abundance after four years of sampling and experimentation do not yet appear to be matching levels we would see in a natural reef (Moore et al. 2023). Abundance and prevalence of both crabs and parasites were highly variable, with no clear trends over time. Also, parasite abundance was much higher at the onset of the sampling than we were expecting, further complicating what can be concluded from data collection without really understanding the cause of that high prevalence and diversity at the beginning of the survey period. In the final sampling events, there appears to be some trends forming, and the data supports the need for longer standing periods of monitoring and ecosystem surveillance. Looking forward, projects in the future analyzing the effectiveness of ecosystem restoration should continue to use time as a prediction for restoration success. Failure to consider time and long-term monitoring will continue to be increasingly problematic and will result in substandard oyster reef rehabilitation and overall community restoration (Moore et al. 2023).

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