Examining Population Genetics and Disease Incidence in Local Grass Shrimp Species

Charles Brooks III “Trip”

A thesis submitted to the Department of Biology, East Carolina University, in partial fulfillment of the requirements of Biology Honors Thesis and Graduate with Honors

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East Carolina University

Department of Biology

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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.

Signed:_____Charles M. Brooks III_____ Date: 4/22/24

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ABSTRACT -

In coastal ecosystems, *Palaemon spp.* (*P. pugio, P. vulgaris, and P. intermedius*) are ecologically significant species that serve as food sources and bioindicators. They may also be potential reservoir species for an emerging pathogen that infects commercially valuable penaeid shrimp species in coastal North Carolina (NC). Shrimp Black Gill (sBG) is a pathology caused by the ciliate parasite *Hyalophysa lynni*. This disease has been shown to negatively impact commercial shrimp populations in Georgia and South Carolina, but it represents a recent climate-induced spread into temperate waters, including North Carolina. By analyzing grass shrimp species collected from NC estuarine populations in the Pamlico Sound, my investigation aimed to (1) determine the species composition of grass shrimp and gene flow among populations, and (2) use microscopy and PCR-based tools to determine the proportion of shrimp infected (i.e., infection prevalence) by *Hyalophysa lynni*. Altogether, genetic tools allowed me to identify the three species of grass shrimp across five sites in the Pamlico Sound, and I found *P. pugio* to be in the greatest proportion among all grass shrimp at these sites. I also found all three grass shrimp harbored the ciliate that causes sBG. Because grass shrimp inhabit Pamlico Sound year-round, they may serve as reservoir hosts for commercially valuable species, like Penaeid shrimp and blue crabs, which migrate into the Sound at certain times of the year. My research helps to provide a better understanding of the species' composition, migration, and disease incidence of grass shrimp in our local estuaries. By understanding disease prevalence and species movements in these systems, we can better understand the impact of emerging diseases and use these baseline data to investigate transmission, infection, and long-term impacts of sBG in the Pamlico Sound in the future.

Keywords: marine ecosystems, climate change, parasite, ciliate, *Palaemon pugio, Palaemon vulgaris, Palaemon intermedius*

Acknowledgements

I have so many people I want to thank for their continuous support and encouragement. Firstly, Dr. April Blakeslee, Dr. Rachel Gittman, and Dr. Jim Morley, all of whom have had an incredible impact on me and my academic journey. I would not be pursuing the passions I am or conducting the research I am without their support, patience, and guidance. Both the Blakeslee and Gittman Labs' graduate students for their devotion to helping and their never ceasing support of me. I cannot thank these labs enough and everyone in them for the zeal they have shown for the topic and for me. I want to thank my family, friends, and fiancée for their support and for putting up with my absence and long hours. I want to thank my funding sources: ECU's URCA Award, ECU Water Resource Center, and North Carolina Biotechnology Center.

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Background:

According to the National Oceanic and Atmospheric Association (NOAA), nearly 1.2 million people live in the coastal areas of North Carolina (NC). This region is also shared with a multitude of marine organisms (NC Wildlife Commission 2024). Estuarine habitats provide valuable nurseries and structure for a variety of fish and crustaceans year-round (Ross, 2003). These estuarine habitats support organisms that are commercially valuable to people and industries in North Carolina, including red drum, white shrimp, brown shrimp, and blue crab (NC Wildlife Commission 2024). These organisms require specific water parameters, such as salinity and temperature, to maintain healthy populations. When these parameters shift because of anthropogenic global change, populations and communities can become disrupted and may present opportunities for other organisms to colonize (Sorte et. al., 2010). These colonizers may be other free-living organisms or can be parasitic and infectious. Not only may these range expanding species impact local populations, but they may persist and establish in unique and unprecedented ways.

Grass Shrimp (Palaemon spp.)

One group of organisms that are native in NC are shrimp within the genus *Palaemon spp.*, commonly called grass shrimp. These species serve as crucial components of the coastal ecosystem due to their ability to serve as food sources for commercially valuable organisms (He et.al., 2022). These shrimps also have ecological implications as they transfer energy and nutrients among trophic levels of the coastal food web (Williams, 1984). Grass shrimps are also reported to be food sources for many species of crustaceans and fish, including the commercially valuable blue crab (*Callinectes sapidus*) and Red Drum (*Sciaenops ocellatus*) (Welsh, 1976;

Westmoreland, 2017). Beyond serving as a food source, these species also have an important ecological role by serving as detritivores in the ecosystem and general omnivores in the food web, including predating on smaller invertebrates (Reinsel et. al., 1992). Of this group, three distinct species, *P. pugio, P. vulgaris, P. mundusnovas* (formally known as *P. intermedius)* can be found in North Carolina estuaries (Williams, 1984).

Palaemon pugio is one of the three species of grass shrimp found on North Carolina's coastline. While this shrimp's range extends from Nova Scotia to the Gulf of Mexico, *P. pugio* is predominately found along the Atlantic coastline of the United States (Cházaro-Olvera, 2009). *Palaemon pugio* is not restrained to estuarine or brackish waters; however, it has been found to be more abundant in brackish water systems compared to other similar species such as *P. vulgaris* (Gallin, 2002). *Palaemon pugio* has a wide range in temperature and salinity tolerances, with temperature tolerances ranging from 10-30 ^{O}C and salinity tolerances ranging from 0 ppt to 35 ppt (Gallin, 2002).

Studies indicate that *P. pugio* breeding is temperature-related along with possibly being triggered by photoperiods (Little 1968; Gallin, 2002). Moreover, its breeding range depends on seasonal and geographical factors with northern populations having shorter breeding periods while southern populations have longer periods of reproduction (Gallin, 2002; Pinto, 2019). While breeding periods vary, female length and maturity seem to be relatively consistent along the coast (Little, 1968).

Like many of their relatives, *P. pugio* are considered omnivores or detritivores. Their diets vary based on location; however, dietary components include loose organic material in both benthic and marsh grass habitats (Reinsel et. al., 2001). They are also known to predate smaller crustaceans and invertebrates, though compared to relatives, *P. pugio* have smaller chela, or

primary claws, making them more inclined to scavenge than predate (Welsh, 1975; Gallin, 2002).

Palaemon vulgaris is seemingly a less researched species of grass shrimp but also ranges in similar geographic spaces as other North American Atlantic *Palaemon* species. Research from New Jersey indicates that the species demonstrates a drop in activity levels during August and September with fewer individuals captured or identified during this time period (Schreiber et. al., 2019). Moreover, the species has a greater salinity tolerance than *P. pugio* (Williams, 1984). The chela is also more developed in this species, possibly indicating the greater propensity for predation of smaller organisms in the ecosystem (Welsh, 1975; Gallin, 2002).

Palaemon mundusnovas appears to be the most cryptic, as research on this grass shrimp species is sparse with general points of information stating that it holds similar geographic, behavioral, and habitat preferences as the two other species (Pennington et. al., 2024).

All three species' ranges overlap geographically alongside relatively similar breeding periods ranging from April to September on North Carolina's coastal waters (Williams, 1984). However, the three grass shrimp species often do not outcompete or exclude one another due to slight variations in substrate and cover preferences, salinity tolerance, and energy expenditure (Thorp, 1976). Because of this, North Carolina along with other coastal ecosystems can support all three species within estuarine habitats.

Beyond serving as trophic staples, these organisms may also serve as bioindicators for coastal parameters, water quality, and disease prevalence along the coastline (Pennington et. al., 2024). Moreover, viral and parasitic diseases have also been examined within grass shrimp species. For example, the yellow-head virus was found within *P. pugio* in the Gulf of Mexico,

and it was theorized that the shrimp passed the infection to both crab and other shrimp species (Ma et. al., 2009).

Shrimp Black Gill Disease

Similar to the yellow-head virus, Black Gill Disease seems to be another infectious pathogen capable of moving among estuarine species. Black Gill Disease is an infection that impacts the gill tissues of multiple taxa of crustaceans. This syndrome has been documented to impact prawns, shrimp, and crab species with specific catalysts including fungi, bacteria, and protozoa. Shrimp Black Gill Disease (sBGD) specifically is a disease that impacts both *Penaeid spp.* and *Palaemon spp.* shrimp species along the Southeast United States coastline. This disease is brought about by the apostome ciliate *Hyalophysa lynni* which causes melanization and necrosis of shrimp gill tissue, leading to lower metabolic functions, poorer oxygen uptake in the host, and possibly higher predation rates (Frischer et. al., 2018). Originally described in the Gulf of Mexico, the range in which *H. lynni* is impacting shrimp populations has increased, expanding quickly along the Southeastern U.S. coastline, reaching North Carolina waters in 2015 and continuing onward into the Chesapeake Bay (Gambill et. al., 2015).

The factors influencing the parasite's range expansion are believed to be tied to rising ocean temperatures associated with climate change (Byers et al. 2021). Prior research has shown a positive correlation between sBGD disease prevalence with warmer temperatures and low dissolved oxygen (Frisher et. al., 2018); however, the influence of salinity on infection status is not well known. Moreover, sBGD has been predominantly observed in the commercially and ecologically valuable *Penaeid spp*. of shrimp (Frischer et. al., 2018). These shrimps are highly valued as a crucial consumer product for coastal communities. It is believed that other non-target

crustacean species may be serving as reservoir species for sBG (Frischer et. al., 2022). For example, it is hypothesized that *Palaemon spp.* of shrimp can carry sBG asymptomatically, i.e., they do not outwardly indicate infection through melanization and necrosis of gill tissue as observed in *Penaeid* spp. shrimp (Frischer et. al., 2022).

I hypothesized that, just as grass shrimp can serve as reservoir host species for sBG further south in the parasite's range, these shrimps could also be reservoir hosts in NC waters where the parasite is an emerging pathogen in Penaeid shrimp. Furthermore, by better understanding how grass shrimp move and interact in NC estuaries, we can establish their potential impact on commercially valuable penaeid shrimp (e.g., Brown and White shrimp), which co-occur with grass shrimp and can also contract the disease (North Carolina Division of Marine Fisheries). My research used molecular tools to better understand grass shrimp species composition, gene flow, and sBG infection in the Pamlico Sound, a highly important estuary for ecologically and commercially valuable crustaceans, and potentially the most productive penaeid shrimp nursery in the southeast USA (NCDMF 2022).

Methods:

To build my molecular dataset to examine both the genetic structure of grass shrimp species and to explore whether the damaging ciliate parasite, *Hyalophysa lynni*, was infecting grass shrimp species, samples were collected from North Carolina estuarine populations by the Blakeslee and Morley labs in 2022 (described below). The collection and examination of samples were used to determine the species composition of grass shrimp and gene flow among populations and to determine the proportion of shrimp infected (prevalence) by *H. lynni*. While the ranges and movements of *Palaemon spp*. have been investigated in the Gulf of Mexico

(Westmoreland, 2017), an examination of grass shrimp gene flow and movement has yet to be explored (to my knowledge) in the Pamlico Sound. In addition, to date, there is no clear understanding of whether grass shrimp are serving as hosts to *H. lynni* in the Pamlico Sound, where sBG represents an emerging disease in penaeid shrimp species.

Sampling and Collection

Along the North Carolina coastline, five regions were selected for collecting grass shrimp samples. These included: Englehard, Stumpy Point, Swan Quarter, Rodanthe, and Oregon Inlet (Figure 1). The latter two were classified as Outer Banks and the three formers were Inner Banks. Within each region, we selected two locations with three sites within them. Within each site, we aimed to collect 30 grass shrimp (mixed species). However, we did not meet that quota at all sites due to either lack of samples or poor collection conditions. Grass shrimp for this project were collected in the spring, summer, and fall of 2022 by the Blakeslee and Morley labs. With each collection of grass shrimp, approximately 300 individuals were taken. This sampling method was also broken into four temporal components with samples being collected in March, May, June, July, September, and October. These sampling months were classified into four groupings: (1) March, (2) May and June, (3) July and September, and (4) October. The field collections consisted of using a seine net or dip nets along marsh beds and in seagrass beds to collect ~30 samples of grass shrimp per site for a total of approximately 90 shrimp per location (x 2 locations = 180 shrimp per region) (Figure 2). However, the availability of shrimp for

collection varied so these numbers were not always obtained.

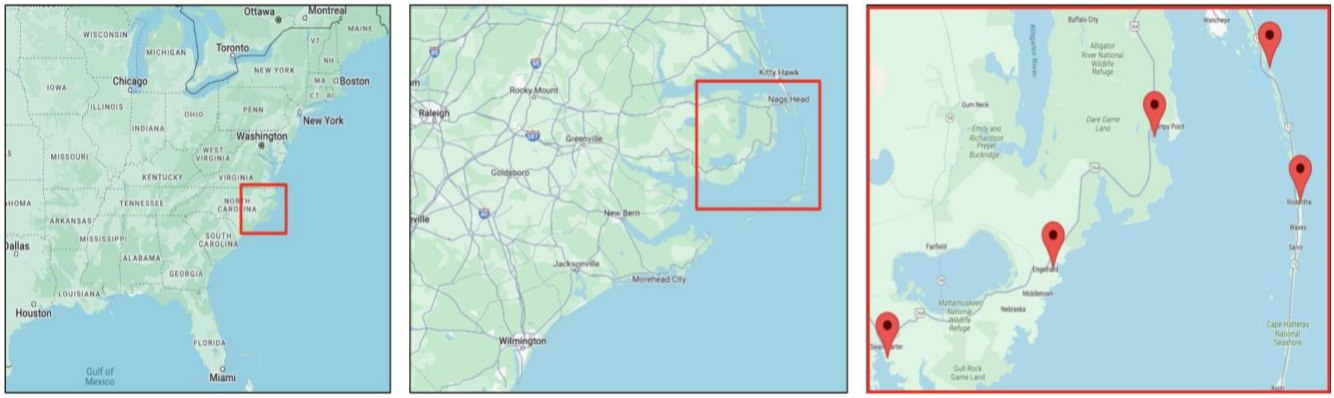

Figure 1: *A visual representation of the locations in which sampling occurred for the 2022 grass shrimp collection. Moving left to right, the map demonstrates focus on the Atlantic coastline and specifically North Carolina's Pamlico Sound is the area where the five regions of investigation occur.*

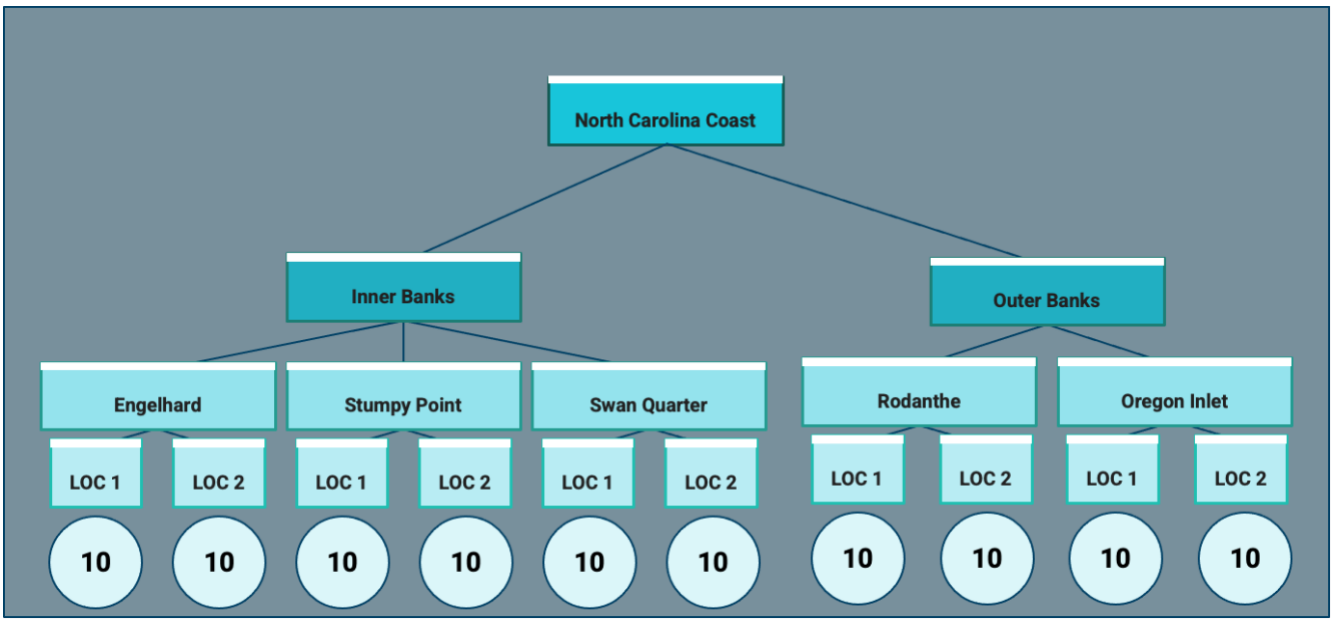

Figure 2: *A visual model of the sites is broken down with "LOC" meaning location within each region. The three sites within each location are not represented; however, across all 3 sites, we selected 10 subsamples from the 90 shrimp per location.*

Dissections

While the morphology of grass shrimp can be used as a means of identification (Figure 3), it can be challenging due to the size of the organism and possible damage during collection and examination. During lab dissections, shrimp were measured from tail to head in mm and a visual check for sBG was conducted by looking in gill tissues under a stereomicroscope. Following the visual examinations, gill tissues were collected and preserved for DNA extraction.

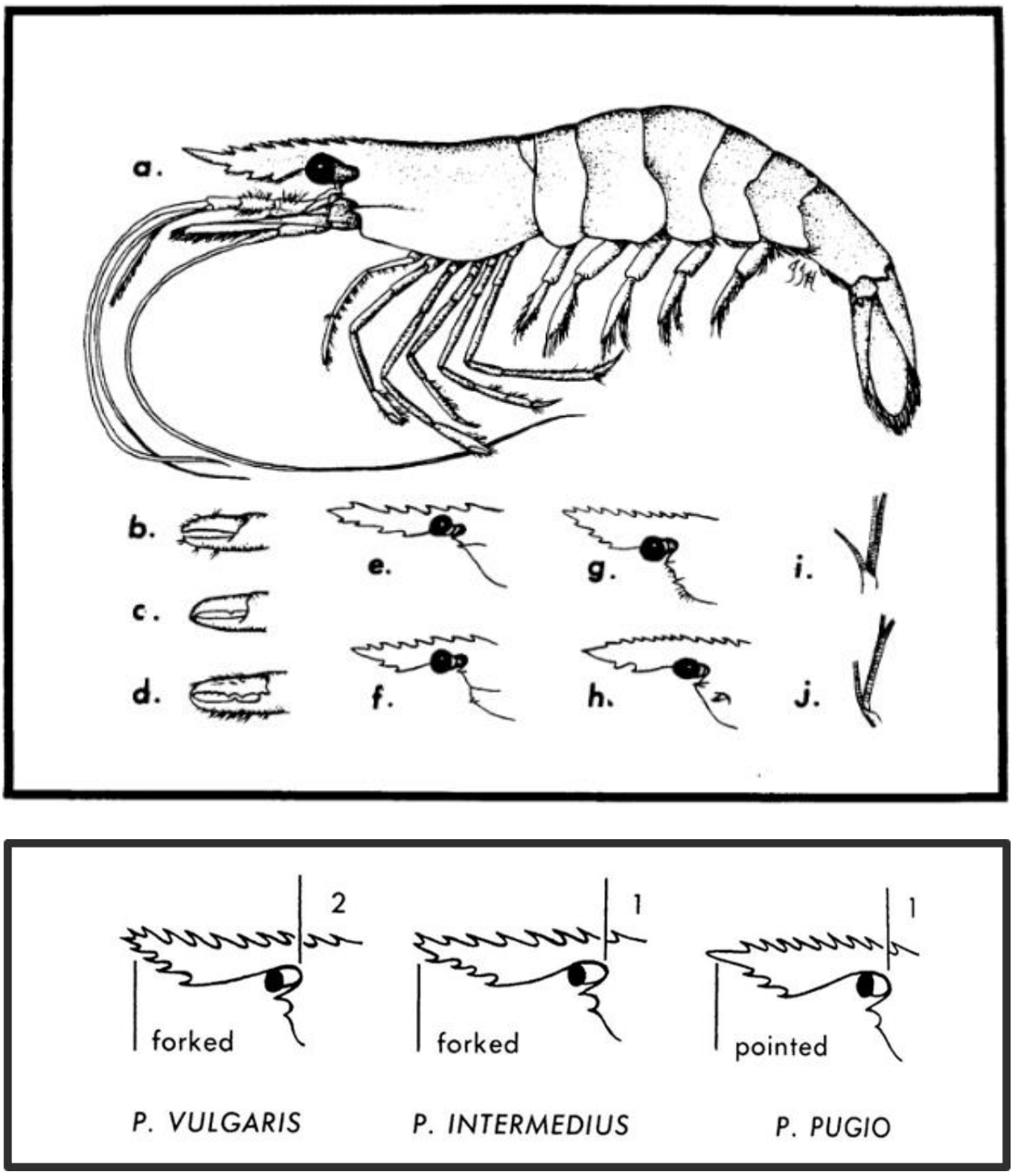

Figure 3: *Images provided by (Westmoreland, 2017) demonstrate the nuanced differences between different species of Palaemon spp. Notice chela and rostra of: b) P. pugio, c) P. intermedius, d) P. vulgaris.*

DNA Extractions

For DNA extraction, 1022 samples were collected, and DNA extractions were conducted from the spring of 2023 to the fall of 2024. Gill tissue removed from shrimp samples was stored in 1.5 mL tubes at -20 ºC before DNA Extraction. DNA Extraction used a standard CTAB DNA Extraction protocol that was modified from Scott C. France's and Loretta L. Hoover's procedure (France and Hoover, 1996). Extraction began by adding 600 µl of CTAB buffer to the 1.5 mL tube containing gill tissue. CTAB buffer was made with 6.05 g of Tris (hydroxymethyl aminomethane), 40.90 g of NaCl, 3.72 g of EDTA, 10 g of CTAB (Cetyl trimethyl ammonium bromide), and 1000 µl of 2-mercaptoethanol. Once CTAB was added, the samples were homogenized using a sterilized plastic pestle. 20 µl of Proteinase K was added to digest proteins in the samples and begin the process of removing contaminants. The tubes were then placed in a TheraHerm incubator for 2 hrs at 65 º C at 15 rpm. 600 µl chloroform was added, shaken, and suspended using a centrifuge at 10,000 rpm at 20 º C. Clear DNA-rich fluids at the top of the suspension were removed and placed in new 1.5 µl tubes. This DNA-rich fluid was then mixed with 1000 µl of cold 100% absolute molecular-grade ethanol and centrifuged at 10,000 rpm at 4 º C. The genetic material appears as a "pellet" and the ethanol pipetted off. This step of adding ethanol was repeated with 70% molecular-grade ethanol twice more before drying off the ethanol. The dried pellets were then centrifuged with 50 µL molecular-grade water and allowed to reconstitute in a 4 º C refrigerator overnight.

Post reconstitution, the purity and concentrations of samples were examined using the Nanodrop 6000 spectrophotometer. Purities ideally ranged between from 1.8-2.0 ng/µL and concentrations ranged from ~200-2000 ng/µL. Samples above 100 ng/uL were diluted to 100 ng/uL prior to PCR.

PCR

Samples were thawed and centrifuged to room temperature for 1.5 minutes. During this time, the PCR master mix was made using the following proportions:

Mixing Agent	Amount
Master Mix	12.5 μL
Forward primer	1.5 μL
Reverse Primer	1.5 μL
Nuclease free Water	8.5 μL

Table 1: *Breakdown of PCR master mix solutions used in DNA extraction methods.*

In a 96 well PCR plate, 1 μL of diluted DNA solution and 24 μL of the master mix (Table 1) were combined for each sample. The plate was then centrifuged for an additional 1.5 minutes and checked for uniformity. Once centrifuged, the plate was placed in a Master Cycler thermal cycler for 1 hour and 45 minutes, using the following PCR profile: an initial denaturation phase at 95°C for 2 min; then 30 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 60 s; and a final extension phase of 72°C for 5 min (Steinberg et al. 2008).

Agar gels were made with 1.5g Agar to 150 mL of TAE solution. The mixture was microwaved until homogenized. Once cooled, 4.5 μL of Ethidium bromide was added and stirred into the solution. The final solution was poured into a leveled tray with gel combs in place.

After both the Agar gel and DNA solution were set and incubated respectively, 10 μL of DNA solution was combined with 1.5 μL of gel loading buffer dye. 12 μlLof solution were then placed into the agar wells with the ends of the rows receiving 4 μL of DNA ladder. The gel was run at 90V for approximately 30-40 minutes. Once completed, photographs were taken using a

transilluminator and gel documentation system. These photographs were examined in comparison to the DNA ladder, which produces a band every 100 base pairs (bp), to determine if bands appeared at the right fragment length size on the gel. This protocol was followed for investigating grass shrimp species and also for investigating the presence of sBGD in grass shrimp. Primers used for investigating grass shrimp were the Universal Folmer primers (LCO 1490 and HCO 2198) (Folmer et al. 1994), which amplify a ~600 bp fragment of the Cytochrome Oxidase I (COI) gene. Primers for sBG were designed by Frischer et al. (2017) and amplify a ~200 bp fragment of the 18S rRNA gene.

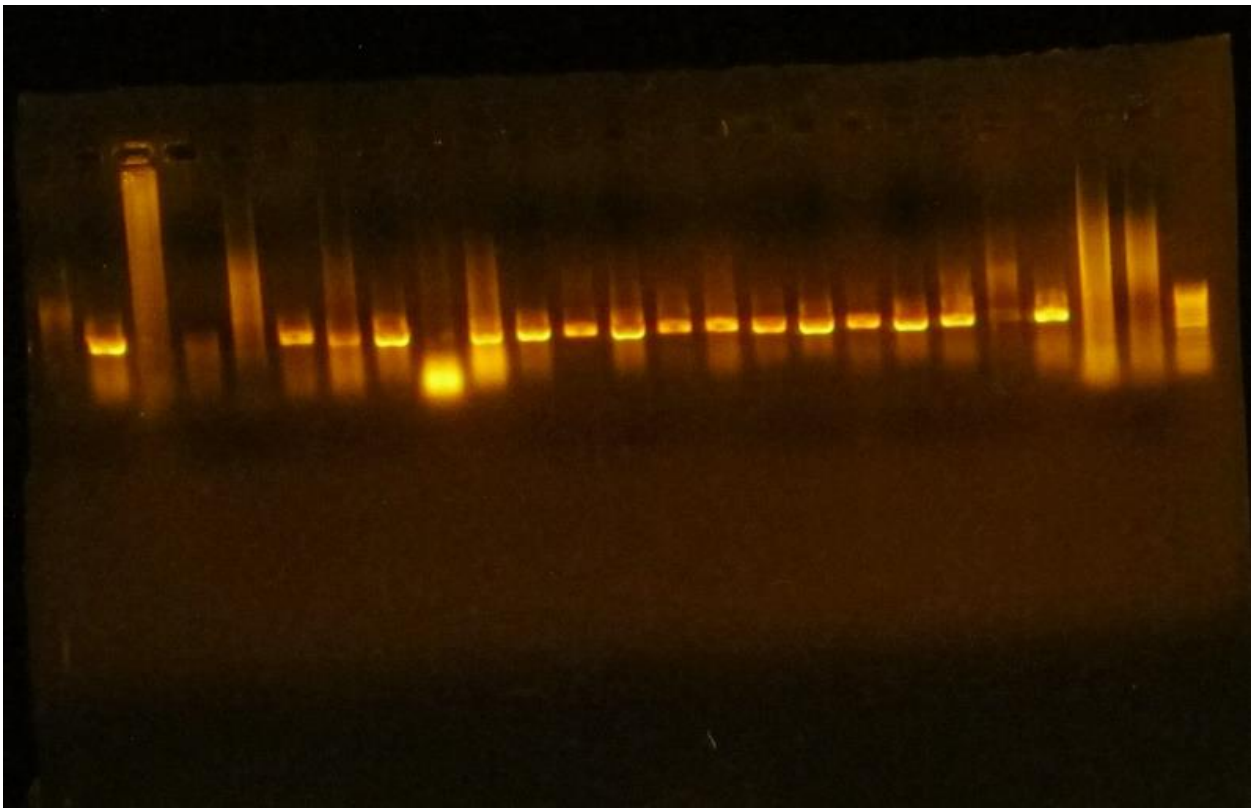

Figure 4: *Example photograph of confirmation of grass shrimp DNA using standard PCR. The bright bands mid-well indicate an identification of grass shrimp DNA and were sent out for sequencing.*

Sequencing

Potential positives for sBGD and grass shrimp PCR (Figure 4) were sent out to Psomagen, Inc. (Maryland) for DNA sequencing. Once confirmation of sBGD positivity was given along with the sequences for the grass shrimp samples, sequences were compared and aligned using the Geneious: Bioinformatics software for Sequence Data Analysis. Alignments were made for *P. pugio*, *P. vulgaris*, and *P. mundusnovas* separately along with a fourth alignment for the individuals confirmed positive with sBGD. Sequences were assembled and manually inspected for ambiguities using Geneious 2024.0.2 (Biomatters Ltd). Sequences were aligned without gaps using the ClustalW algorithm (Larkin et al., 2007) and collapsed into haplotypes using TCS v.1.21 (Clement et al. 2000). The optimal nucleotide substitution model was selected based on the Akaike information criterion in jModelTest in Geneious 2024.0.2 (Darriba et al. 2012). The selected model was then used in Bayesian phylogeny reconstructions using MrBayes (Huelsenbeck & Ronquist, 2001) in Geneious 2024.0.2. We calculated fixation indices for population pairs based on pairwise differences between haplotypes (φST) in Arlequin. Pairwise φST results were visualized with a nonmetric multidimensional scaling (nMDS) analysis (using Primer 6; Plymouth Marine Laboratory) to look for spatial patterns between and among populations. The spatially closest populations are most genetically similar.

Results/Discussion:

Through the PCR testing and subsequent DNA sequencing, 104 grass shrimp samples were successfully sequenced, along with 27 positive sBGD cases in grass shrimp species. Below, I first discuss my results for grass shrimp species composition and gene flow, and then follow it with a discussion of my sBG results in grass shrimp.

Grass Shrimp Population Genetics

When investigating the genetic distribution and prevalence of *Palaemon spp.* in North Carolina, I hypothesized that the three species (*P. pugio*, *P. vulgaris*, and *P. mundusnovus*) would be present and distributed throughout our study sites. The mobility of the organisms was a factor that could impact whether different sites experienced genetic divergence in a significant capacity.

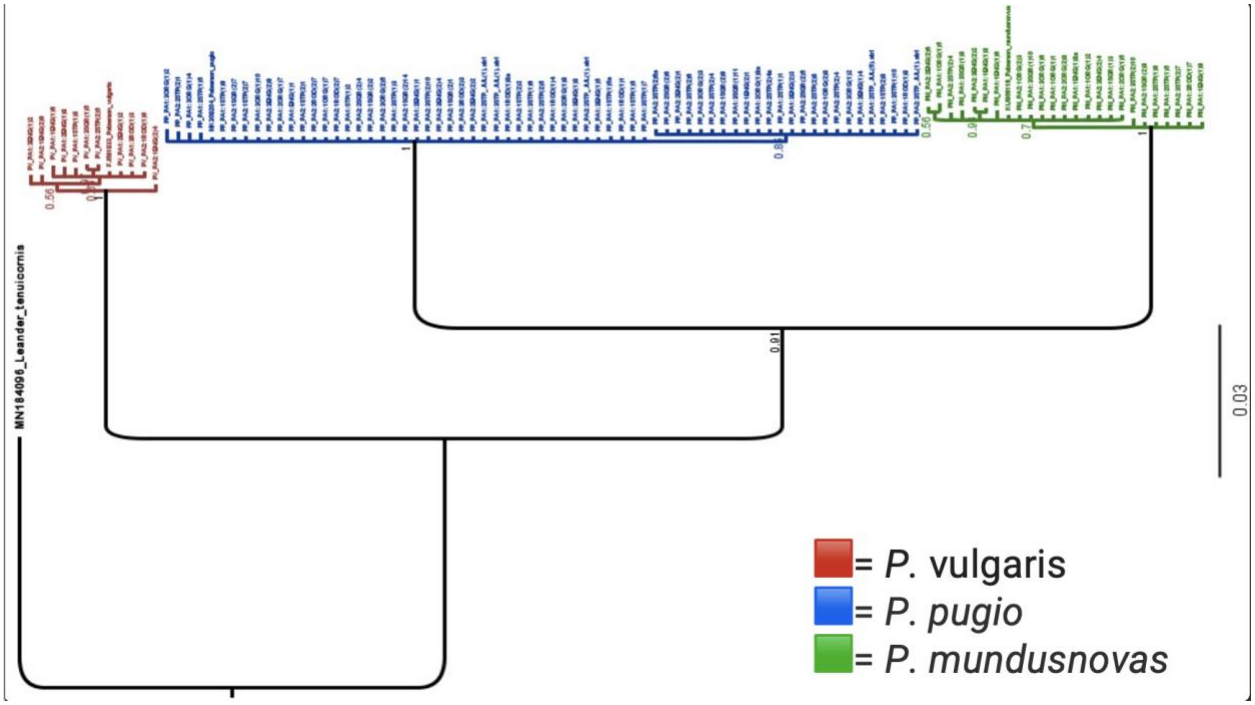

Figure 5: *Bayesian phylogenetic tree of a fragment of the COI gene (645 bp) of Palaemon shrimp sequenced for this study. There were 104 samples confirmed and organized into the three Palaemon species.*

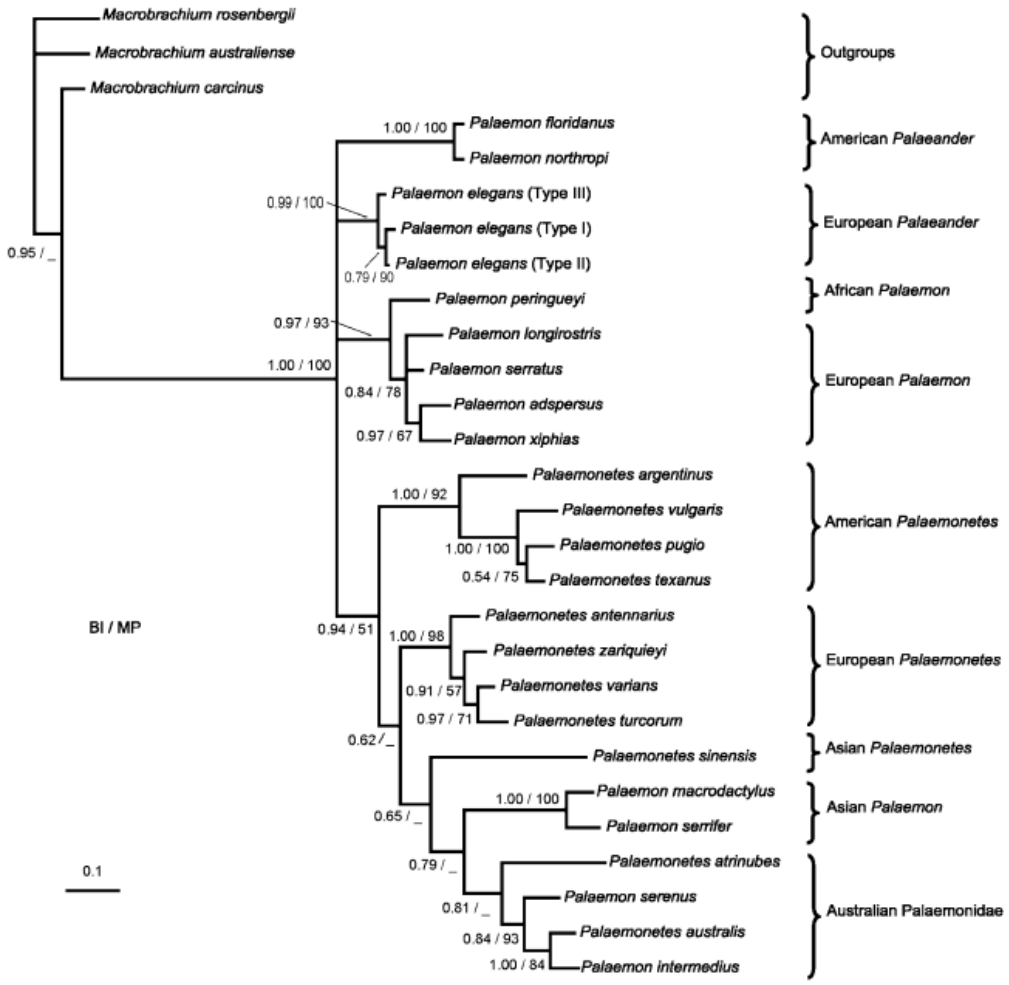

Figure 6: *The phylogeny of the greater Palaemon spp. family created using molecular comparison (Cuestra et. al., 2012) and highlighting the clades and regional location of each species.*

The two phylogenies above represent the genetic breakdown of the 104 samples that were sent out due to expected positive confirmation from PCR for grass shrimp DNA (Figure 5) and the greater tree of the *Palaemon spp* (Figure 6) (Cuestra et. al., 2012). The Cuestra et al. (2012) phylogenetic tree highlight the common species found in the "American Palaemonetes" group, including the expected *P. pugio* and *P. vulgaris. Palaemon mundusnovas* is absent from the

"American Palaemonetes" group as the name has been debated in the literature and was eventually clarified because at the time there was two *P. intermedius* titled species one in the Americas (what is now *P. mundusnovus*) and the other in Australia (which took precedence). The comparison of the phylogeny generated by Cuestra et al. (2012) and the one which I produced through DNA sequencing provide further evidence that I detected all three species of *Palaemon* (formerly *Palaemonetes*) that have previously been reported in the Americas. Of the 104 sequences in my phylogeny (Figure 5), 11.42% were *P. vulgaris*, 64.42% were *P. pugio*, and 24.04% were *P. mundusnovus*. All three species were found at all our sampling regions except *P. mundusnovas* found at Oregon Inlet.

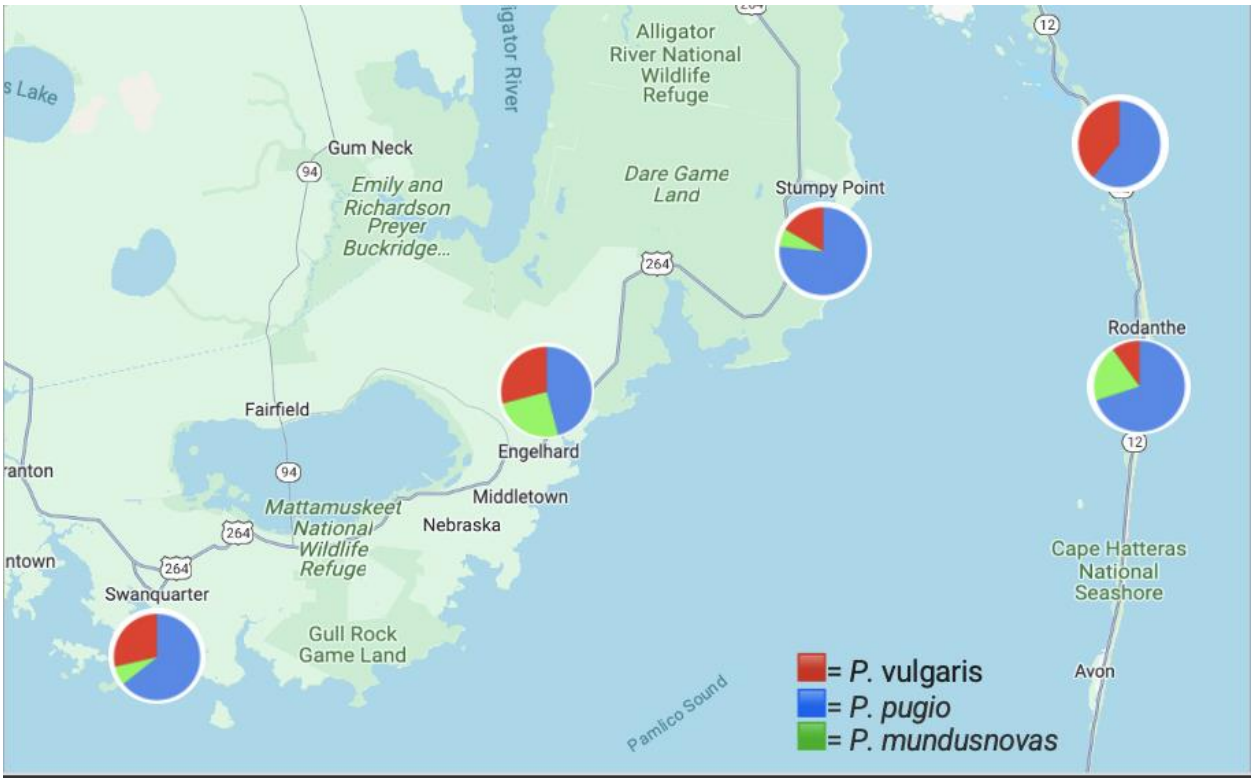

Figure 7: *A map of the sites and the grass shrimp species composition at each site represented*

as pie charts. Palaemon pugio had the highest proportion among the three grass shrimp species at each site, and P. mundusnovas was absent from Oregon Inlet.

Figure 7 demonstrates the prevalence of *P. pugio* in the Pamlico Sound system, which was highest among all three grass shrimp species. This was consistent with other studies that have shown *P. pugio* to be a very prevalent species throughout its range (Cházaro-Olvera, 2009). Comparatively, *P. vulgaris* and *P. mundusnovas* were less prevalent in our study, which could suggest they are more sparsely dispersed throughout their range, possibly due to the salinity preferences (Williams 1984). Our sample size for *P. vulgaris* totaled 24 samples across all five locations; thus including more sequences across our different sampling points would help us understand if these proportions are consistent across sites and seasons.

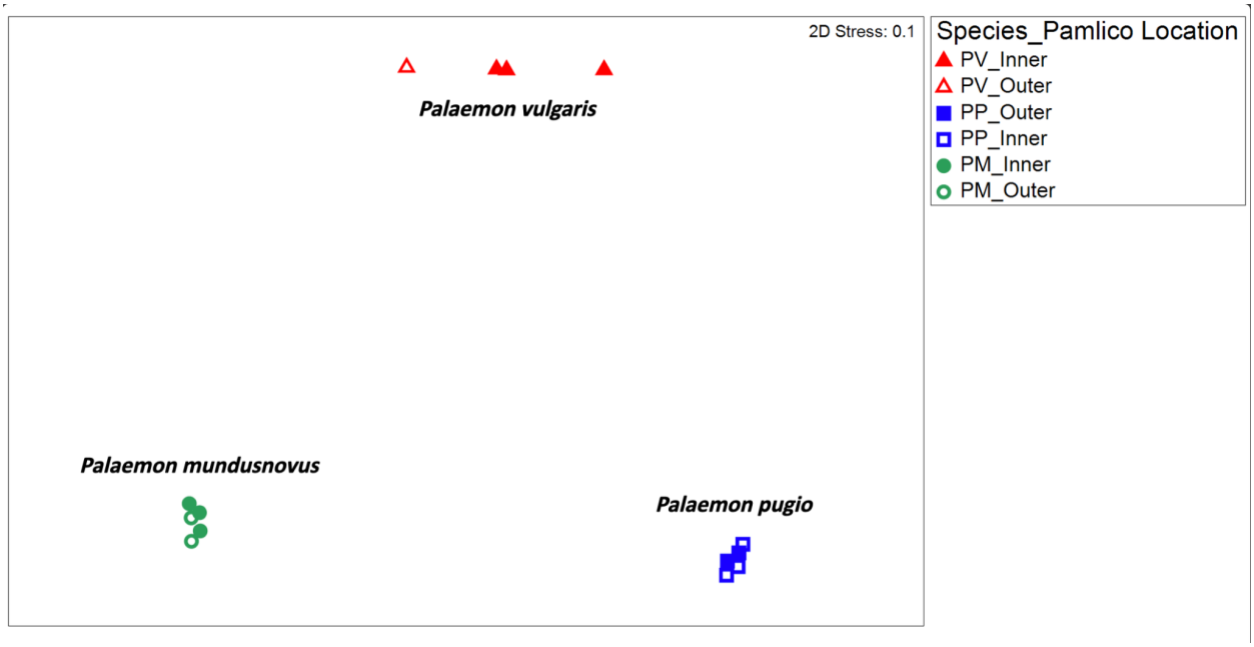

Figure 8: *A pairwise F_{ST} Value demonstrating the three distinct genetic make-up of the species found in North Carolina.*

In Figure 8, pairwise F_{ST} values (i.e., pairwise differentiation) were visualized in an nMDS plot to compare the genetic similarities among populations in the inner versus outer banks and among the three species. The closer the shapes are to each other, the more similar they are genetically (in terms of their haplotype frequencies) (e.g., Blakeslee et al. 2020). Altogether, the figure shows that the three species are distinctively different from one another, as expected from the results of the phylogenetic tree.

Within each grass shrimp species, additional pairwise F_{ST} values were calculated and nMDS plots were used to visualize the results per species, with a specific aim of comparing the similarities/differences between shrimp populations in the inner versus outer banks to look for possible population differentiation.

When examining the pairwise F_{ST} values for *P. vulgaris*, there appears to be some separation among the populations (Figure 9). The isolation of the Rodanthe population may suggest that there is some genetic differentiation in the outer banks versus inner banks for *P. vulgaris.* There is much less literature on *P. vulgaris,* and this species is often used as a control group to compare to *P. pugio*; as a result, I could not identify literature on differing genetic structures for *P. vulgaris* in different habitats across its range. This could serve as a future point of investigation to determine if there is greater range in genetic diversity for *P. vulgaris* in different regions. However, of note is that *P. vulgaris* was the least sampled, so a greater examination of the species would need to occur along with strong statistical analyses to determine whether this separation is significant.

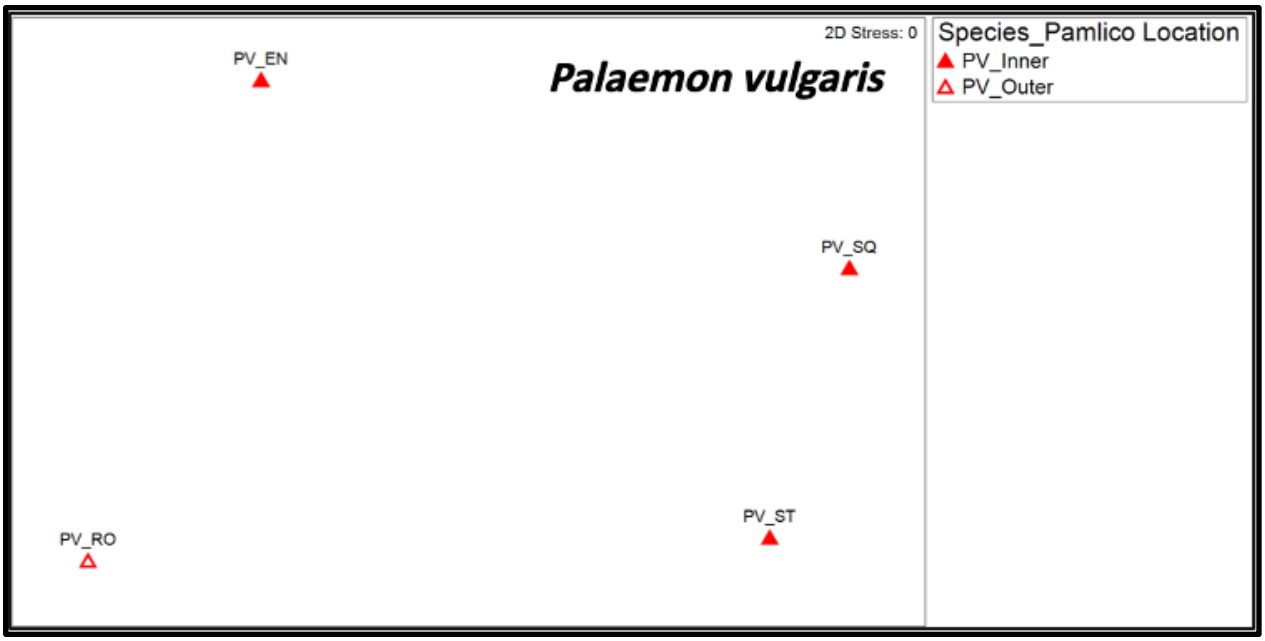

Figure 9: *Palaemon vulgaris' genetic structure visualized in an nMDS plot, examining potential differentiation based on inner and outer banks locations.*

Palaemon pugio had the greatest number of samples positively identified and showed what appeared to be evidence of genetic differentiation between the inner and outer banks populations (Figure 10). Rodanthe was also isolated in *P. pugio*; however, the inner banks seem to be more cohesive by clustering together in the nMDS plot.

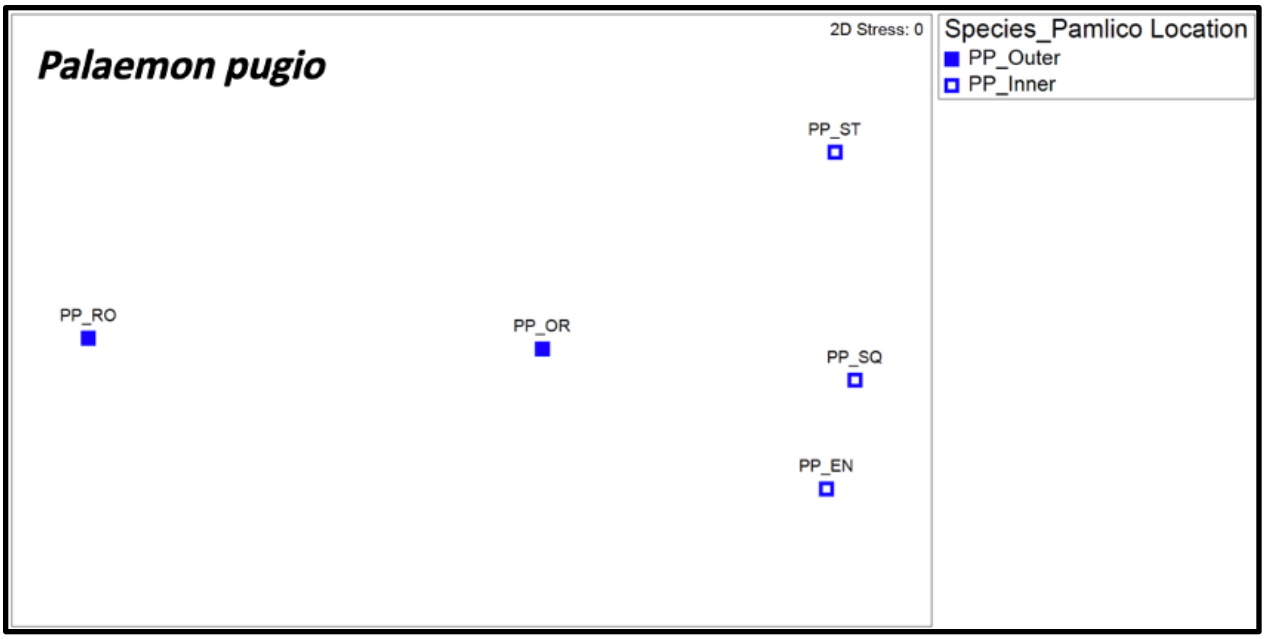

Figure 10: *Palaemon pugio's genetic diversity is based on inner and outer banks locations.*

The nMDS plot for *P. mundusnovas* appeared to have three separations (Figure 11). There was clumping with Swan Quarter (SQ) and Engelhard (EN), which were in close proximity, and between Stumpy Point (ST) and Rodanthe (RO), which also were in close proximity. However, Oregon Inlet was separated out by itself, which may suggest it is a more isolated population. However, greater research is needed to determine if the population is distinctive in a significant capacity.

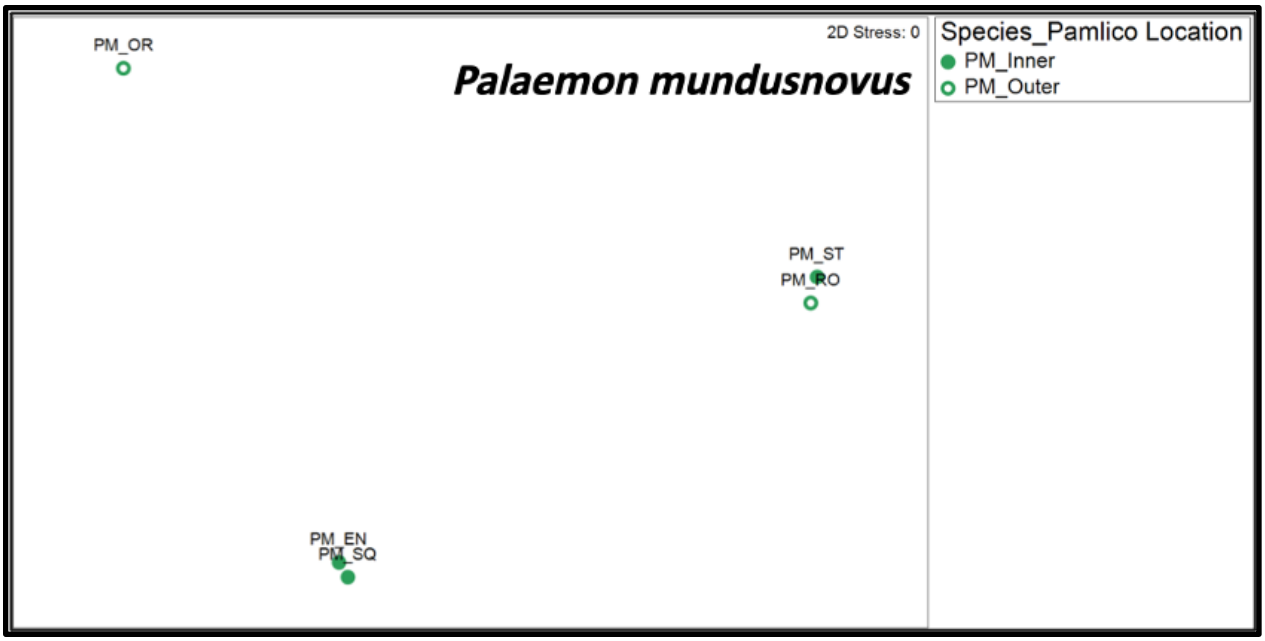

Figure 11: *Palaemon mundusnovus' genetic diversity based on inner and outer bank locations.*

Altogether, the three species showed some levels of differentiation between the inner and outer banks, but there was no clear and consistent pattern. These results appear to support the idea that all three species are relatively mobile within their habitat (Bauer et. Al., 2001). Compared to the *Penaeid spp.* Which have a life stage involving leaving the saltmarshes and estuarine systems on the NC coastline (Figure 12), grass shrimp species maintain the habitat year-round (Bauer, 2018).

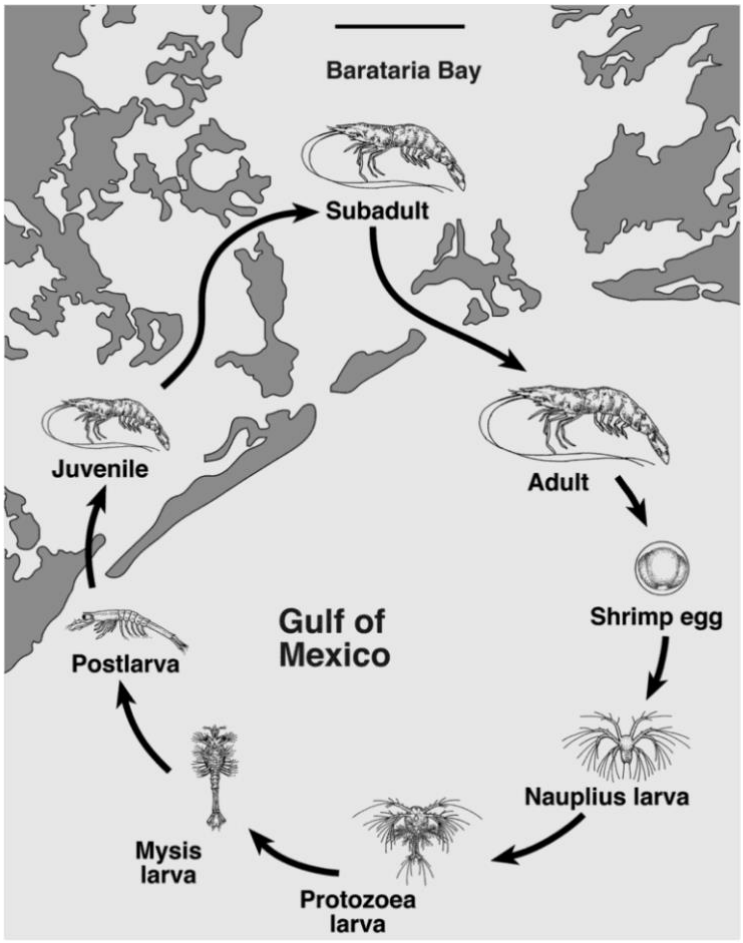

Figure 12: (Bauer, R. T. 2018) *proposed lifestyle of Penaeid shrimp species including inner and outer bank*s locations.

Shrimp Black Gill Disease

Of the 96 samples sent out for sequencing, 27 came back positive for sBGD (representing 28.13% prevalence in the examined populations at the Englehard and Swan Quarter locations). The sequences we received matched 100% to an *H. lynni* sequence (OL467313.1) on Genbank (https://blast.ncbi.nlm.nih.gov/Blast.cgi#alnHdr_2147542128). This demonstrates that grass shrimp can be hosts for *H. lynni* in North Carolina waters, and thus have the potential to serve as potential reservoir hosts of *H. lynni* throughout the year.

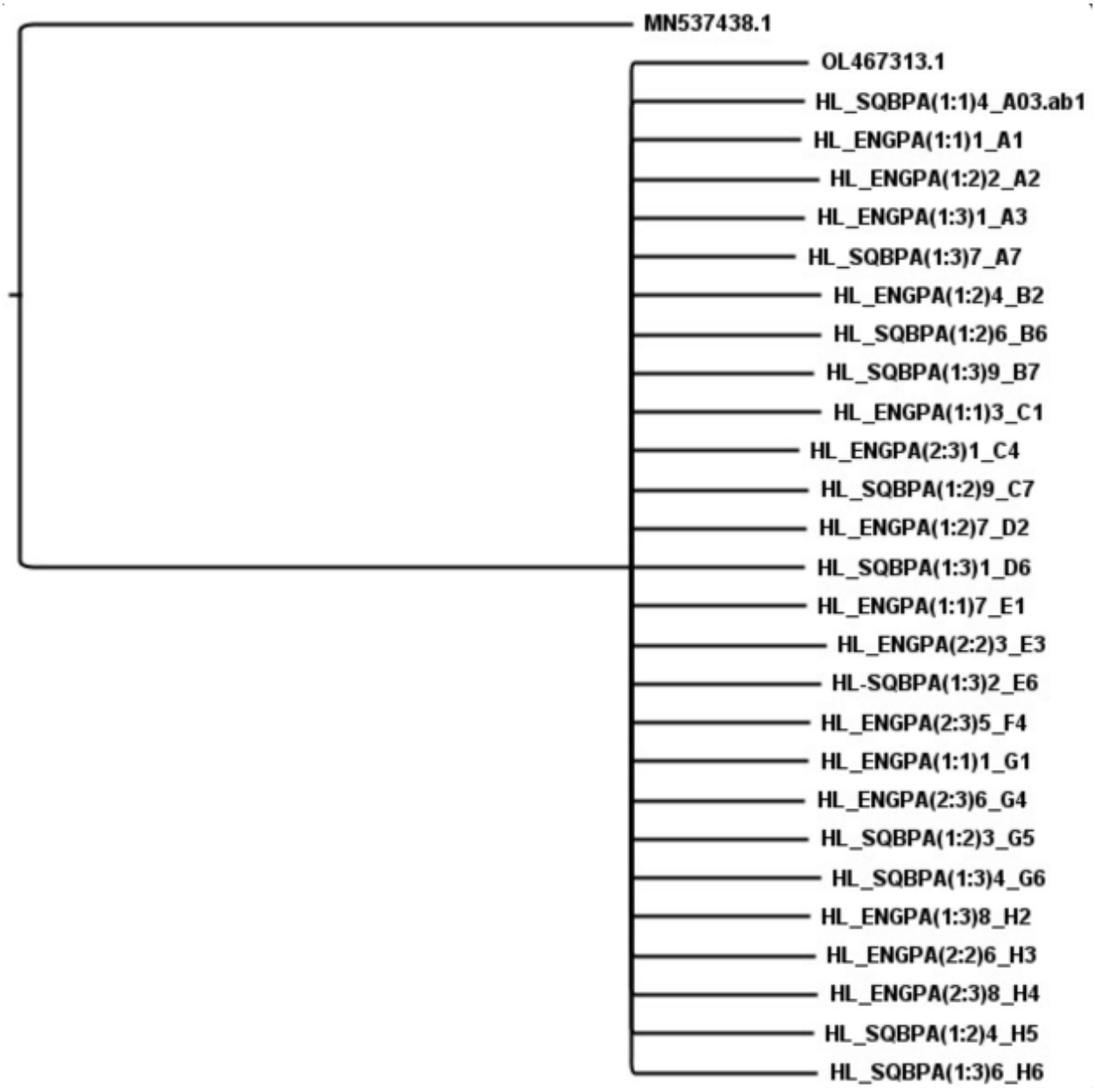

Figure 13: *The 27 positive confirmation of sBGD samples from both Engelhard and Swan Quarter locations.* There were very few substitutions among the samples.

Interestingly, none of our grass shrimp showed visual signs of sBG, suggesting that these shrimps may be asymptomatic when hosting the ciliate. Literature shows that the response of darkening gill tissue in grass shrimp occurs when they are introduced to pollutants and chemicals

which caused the black gill response, but this has not been observed when in the presence of ciliates (Doughie & Rao, 1983). sBGD has a salinity tolerance that is found along estuarine systems but not in freshwater or open waters. Because of this, sBGD may be able to persist in grass shrimp throughout the year while diminishing in *Penaied spp.* as they return to deeper coastal waters.

The samples that were examined for each species of grass shrimp and those that were positively marked for the parasitic ciliate were not completely aligned. Because of this, the proportion of infection in each species needs further investigation. Greater research should be conducted to determine if there are specific proportions of species with sBGD infections. One thought may be that *P. pugio* may show higher rates of infection due to its great presence in the region and the species' lower salinity tolerance (Williams, 1984).

Future Directions:

With the data discovered in this preliminary investigation, there are opportunities for greater research. Investigating samples collected in the 2023-2024 sampling year would help us determine grass shrimp composition and gene flow across years. In addition, these examinations could enhance our understanding of the infection prevalence per Palaemon species, and not just infection confirmation. Furthermore, experiments could be designed to determine how North Carolina populations of grass shrimp may present sBGD infections. Since grass shrimp do not manifest visual symptoms of Black Gill Disease, it is difficult to determine infection without genetic sampling. However, an experimental design that purposefully exposed grass shrimp to *Hyalophysa lynni* in the laboratory, would allow for a closer examination of potential symptoms, including behavioral changes that are not easily observable in the field.

My data is significant because it is important that we determine if sBGD can persist and spread in grass shrimp not only for scientific research but also for the maintenance and preservation of commercially valuable *Penaeid* shrimp populations. Altogether, this investigation helped to create novel data set that focused on *Palaemon spp.*, and their potential as reservoir hosts for the parasitic ciliate *Hyalophysa lynni*.

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