GENOME-TRACKED BIOGEOGRAPHY AND PHYSIOLOGY OF GEUKENSIA DEMISSA

by

PEYTON WHITE

(Under the Direction of John Wares)

ABSTRACT

Within the Mid-Atlantic Bight, there are interesting latitudinal patterns of physiological change. Physiological variation in oxygen consumption for *Geukensia demissa* has been identified between the northernmost and southernmost locations in this range, where Erlenbach & Wares (2023) reveal Massachusetts displayed a higher oxygen consumption than Georgia. However, this fails to assess any pattern or transition between these geographic limits. Using oxygen consumption data, we seek to contribute to the evidence of physiological variation through a more fine-scale evaluation of this latitudinal gradient and hypothesize that this physiological pattern will vary spatially. We collected oxygen consumption data from 158 *G. demissa* individuals across 8 sample sites, calculated VO_2, and analyzed those against latitude, sea surface temperature, and genetic background, as well as possible interactions. Evaluating this data at a higher spatial resolution is meaningful to enhance our understanding of latitudinal variation in environmental response, in a marsh-essential marine organism.

INDEX WORDS: Biogeography, Ecophysiology, Evolutionary Ecology, *Geukensia*

GENOME-TRACKED BIOGEOGRAPHY AND PHYSIOLOGY OF GEUKENSIA DEMISSA

by

PEYTON WHITE

B.S., The University of Georgia, 2024

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2025

GENOME-TRACKED BIOGEOGRAPHY AND PHYSIOLOGY OF GEUKENSIA DEMISSA

by

PEYTON WHITE

Major Professor:	John Wares
Committee:	Jill Anderson
	Jeb Byers

Electronic Version Approved:

Ron Walcott
Vice Provost for Graduate Education and Dean of the Graduate School
The University of Georgia
May 2025

DEDICATION

This thesis is dedicated to everyone in my life who provided me with knowledge, support, and encouragement throughout my education. I couldn't have done it without this support system.

ACKNOWLEDGEMENTS

I want to express my sincerest gratitude to Dr. John Wares for his guidance, mentorship, encouragement throughout my studies at the University of Georgia and this project. Throughout this time, I not only gained plentiful knowledge, but developed into a better scientist and learned to step outside of my comfort zone with confidence.

Thanks also to my committee members Dr. Jill Anderson and Dr. Jeb Byers, for providing the insightful suggestions that brought new perspectives and thoroughness to my research.

I would also like to recognize my family and those close to me for providing continuous enthusiasm and support throughout not just my work on this project, but throughout my academic career.

Finally, I want to express my appreciation to the Katelyn Chandler Memorial Undergraduate Research Award and Elaine Lutz Foundation for Aquatic Biodiversity for providing funding for this project and making my work possible.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION

Background

Oftentimes, as ecologists, we become fascinated by some observable phenomena. Either a species is found where it shouldn't be, an organism displays a ‘cool’ behavior, or maybe there are interesting color morphs of a species. However, understanding or researching a species at genetic level is something often overlooked or simply unachievable if you don't possess the skills or equipment to do it. Molecular ecology can often be used to contextualize or contribute a deeper understanding to observable phenomena like those just mentioned. Evolutionary ecology, consisting of understanding adaptation, gene flow, evolution, etc. can help paint a picture of a meaningful story behind why we might observe things the way they are in the present.

Genetic diversity often leads to implications on the environment, which is the principle of the ‘genes to ecosystems’ idea (Wymore et al., 2011). Fragments of DNA composed of nucleotide bases give rise to amino acid sequences, and the way in which genetic code is structured dictates how physical features and physiological functions turn out. These features and functions are what respond or adapt (or don’t) in the face of environmental pressures. One such physiological function, essential for life, is metabolism.

Metabolism is said to be a fundamental measure of ecosystem function (Odum & Odum, 1976), and the discipline that addresses the link between physiology and environment is ecophysiology. Ecophysiology seeks to understand both how environmental factors can impact

an organism's physiological functioning, as well as how an organism's physiology may influence how it behaves in and contributes to its environment.

Both molecular/evolutionary ecology and ecophysiology can be studied within the context of biogeography – the understanding of species diversity and richness regarding geographic distribution. Genetic patterns and physiological processes can be observed both across space and over time which can ring warning bells if a species is ever found to be in need of restorative or rescue efforts. Humans have historically had and currently have a significant impact on the reduction of global biodiversity, and it is often biogeographic data that informs us of this fact (Whittaker et al., 2005).

Life History

Not all interesting species worth studying happen to be charismatic megafauna. The Atlantic Ribbed Mussel *Geukensia demissa* begins life before settling down. Gametes are released into the water via broadcast spawning where fertilization occurs, and larvae free float in coastal waters for up to 2 months (Virgin et al., 2019). These spawning events typically start in the early summer with gamete release in June-August, and don't conclude until early fall, where recruitment is temperature-dependent but generally 2-3 weeks (Virgin et al., 2019; Thompson et al., 2012). Once settled in the intertidal zone of salt marshes, they can accumulate in densities of 2,000-3,000 individuals per square meter in New England, and in some locations such as New York, up to 10,000 individuals per square meter (Cohen, 2011). *G. demissa* is also a fairly tolerant species, able to persist in temperatures up to $45\,°C$ (Jost & Helmuth, 2007), salinities from 5% to 75% (Bertness, 1980), and are able to withstand moderate levels of pollution. They make great study organisms due to their limited mobility, how densely they occur in marsh habitats and ease to find, and tolerance to laboratory environments.

Purpose of Study

Research Plan and Hypotheses

Between Chesapeake Bay and Massachusetts, the *Geukensia demissa* populations diverge in mitochondrial diversity, but more recent work has shown additional gene regions with intriguing ecological associations. Amylase, a metabolic gene that aids in metabolism and digestion of starches, exhibits distinct amino acid sequences in more northern populations – but the geographic transition in type is not clear yet and needs to be sampled further. Using genome sequencing and bioinformatics followed by experimental approaches, we sought to detect whether collected mussels have the northern or southern allelic types of amylase following Erlenbach & Wares (2023), or are heterozygous at this locus. Once detected, we will experimentally evaluate how individual mussels respond to different environmental conditions. Knowing that in general, *G. demissa* exhibits a mitochondrial transition as well as a transition in amylase diversity in this broad geographic region, my goal is to identify how sharp the genetic cline is in this region and use that information to design experiments that also capture how discrete the metabolic transition in *G. demissa* is. Overall, this work evaluates molecular, spatial, and physiological components.

We hypothesize that this divergence is primarily temperature driven, and that the resulting genetic differentiation is a sharp transition. At the southern end of the Mid-Atlantic Bight, at Cape Hatteras, is where the northward-moving Gulfstream Current begins to cleave away from the coastline, leaving the Mid-Atlantic Bight suddenly with a pocket of colder water. Since this change in temperature is so defined, we also predict that the northern type will have a distinct metabolic rate from the southern type when looking at differences in oxygen consumption (Erlenbach & Wares, 2023).

Purpose

This research can advance knowledge and understanding within science from a genomic, physiological, and spatial point of view for a species that is critical for Atlantic coastal salt marsh health. Meshing the disciplines of genetics and ecology together could reveal interesting gene-to-ecosystem patterns, where detected genetic variations may affect entire ecosystems. Answering these questions will help to understand how historical and evolutionary processes are affecting the ecology of these mussels in a more integrative way. Furthermore, determining what spatial factors might be causing this divergence within their population could help predict how their species will respond to Earth's warming climate, assist in making predictions about how other species may respond, and emphasize the importance of thermal tolerance.

CHAPTER 2

MOLECULAR MATERIAL FOR A MARVELOUS MARINE MUSSEL

Introduction

In organisms with high dispersal potential, adaptation is important but often counterbalanced by high gene flow. An element of identifying the potential for adaptation is identifying or measuring limits to gene flow (Sanford et al., 2003). Terrestrially, these limits can consist of physical barriers such as mountains or deserts, but in marine environments other boundaries such as currents or environmental gradients are of particular interest. Even organisms with broad dispersal capabilities, and consequently high gene flow, may be limited in range due to the direction of larval transport (Pringle et al., 2017).

Selection and adaptation are mechanisms that generate spatial patterns in genomic data, whether via traits observed or hidden to the naked eye, but it is also important to separate how abiotic forces can establish patterns in coastal organisms via isolation or drift (Hice et al., 2012). The Atlantic coast of North America stretches ~3,000 km, and temperature and chlorophyll-a concentration, among other factors, vary greatly along this range. These baseline environmental gradients are steeper along this coast than most, globally, and changing faster than most regions of the coastal world (Baumann & Doherty, 2013). The combination of spatial breadth, strong environmental gradients, and rapid environmental change has guided many recent efforts to understand patterns of biodiversity along this coast. Exploring these questions of organismal diversity can benefit from molecular analyses to help identify how species are responding to

these gradients (Erlenbach & Wares, 2023). These patterns of diversity are also of value in the understanding of biogeographic transitions (Wares, 2002; Altman et al., 2013).

For example, marine researchers have often used genomic diversity to evaluate spatial patterns of connectivity (Riginos & Liggins, 2013). As an example that spans a community of marine organisms, Diaz-Ferguson et al. (2009) used such divergence patterns across 6 salt marsh ecosystem species on the Atlantic coast and found that many exhibit only slight divergence from Florida to Massachusetts, but two species showed stronger divergence between sample locations in Virginia and Massachusetts. This region in the northern Mid-Atlantic Bight appears to be an important transitional region for coastal genomic biodiversity, distinct from canonical biogeographic transitions (Wares, 2002; Altman et al., 2013). Recognition of shared community spatial patterns of transition in diversity may be of value for habitat management and restoration (Brinton, 2021).

One of the species found by Diaz-Ferguson et al. (2009) to have strong divergence between Virginia and Massachusetts – apparently moreso than between Virginia and populations to the south – was *Geukensia demissa*. The ribbed mussel *G. demissa* (Family Mytilidae) has a distribution that spans the distance from Nova Scotia, Canada to southern Florida, USA and is crucially important to marsh habitats along the Eastern coast of North America. As bivalves, they filter feed on starch-heavy phytoplankton and carry an important role in nutrient cycling and marsh health. They contribute to their ecosystem by burrowing into the mud at the basal portion of the essential cordgrass, *Spartina alterniflora* (Bortolus et al., 2019), providing root stabilization (Bertness, 1984) while also excreting nitrogen and phosphorus-rich fecal waste back into the marsh system (Angelini et al., 2016) which fertilize and nutrify the grasses. This mussel aggregation stimulates the grass' growth (Anderson & Padilla 2025), which in turn provides

habitat for other marsh organisms. The mussels also reap benefits as they gain relief from the harsh sun during low tides, resulting in a facultative mutualistic relationship (Bertness, 1984). Given that they span such a broad geographic range, they also, as a species, endure various environmental conditions such as differences in temperature, salinity, and phytoplankton availability. Mussels also reproduce via broadcast spawning, where their larvae can free-float for approximately two months before settling, leading to high dispersal capability and gene flow.

In trying to identify if northern and southern populations of *Geukensia* are divergent in functional ways, Erlenbach & Wares (2023) explored expression variation as well as sequence variation of populations in Georgia and Massachusetts. They found that amylase, an enzyme that aids in digestion of starches, was the most highly divergent metabolic gene identified between southern and northern populations of *Geukensia* (Erlenbach & Wares, 2023), supporting a further exploration into amylase as an ecologically valuable genomic marker. As the Erlenbach & Wares (2023) preliminary study suggested a strong spatial pattern in amylase genotypes, this study is a renewed exploration that expands and refines the spatial analysis of that diversity.

Amylase diversity has also been shown to be important in the functioning of other marine invertebrates, such as the crustacean *Gammarus palustris* and the oyster *Crassostrea gigas.* Guarna & Borowsky (1993) identified a genetic-linked feeding preference in *G. palustris* based on amylase alleles, where one homozygote showed preference for the alga *Enteromorpha intestinalis*, and the other homozygote showed preference for *Ulva lactuca*, tying feeding preference and starch concentration to this digestive enzyme. Distinct growth of oysters (*C. gigas*) was also observed based on amylase diversity, where Prudence et al. (2006) suggest that amylase polymorphism is likely not neutral.

The specific region that we seek to amplify in amylase appears to be highly divergent between Georgia and Massachusetts (Erlenbach & Wares, 2023) – more than even the mitochondrial gene divergence in earlier studies (Diaz-Ferguson et al., 2009). To know if amylase can be considered as an ecologically or environmentally sensitive molecular marker in *Geukensia*, we want to ensure that the sequences inferred by Erlenbach & Wares represent a single copy gene region. Our predictions for this diversity would be that distinct alleles should be in Hardy-Weinberg equilibrium at local scale, but if responding to environmental and ecological variation, will vary geographically.

Methods

Through a variety of collection methods, the sites that underwent both Amylase and COI sequencing/genotyping range from Antigonish, Nova Scotia (*45.668° N, - 61.878° W*) to Savannah, GA (*32.038° N, -81.048° W*). This totaled to 11 sample sites and 183 individual mussels providing genomic data for this study. Specimens were collected from sites in Table 2.1, and mantle-edge tissue was taken from all 183 mussels for DNA extraction following protocol from Wares (2023).

Primers and Amplification/PCR

Based on the sequence data reconstructed in Erlenbach & Wares (2023), we aligned the amylase sequence region from Massachusetts and Georgia individuals and developed 2 sets of PCR primers. While one primer pair generated inconsistent PCR results – later examination showed that targeted region spans an intron-exon boundary – the second pair GdAmy239F (CCATCTTATTGACATTGGTGTAG) and GdAmy593R (TCCTCTCTGGTTGTCATGGTT) amplify a 354bp fragment when using an annealing temperature of 50°C.

Table 2.1: Master table showing sample sites listed in order of decreasing latitude. A "Data Collected" of 'Both' refers to application in both this chapter and the following, and 'Sequencing only' only appears in this chapter. Mean VO_2 was rounded to four digits. * The two NJ collection sites (Boat Basin and Bivalve) were pooled together resulting in the same "Observed Northern Allele frequency" and "Chi Test p-value of Amylase".

Site	ID	Coordinates	Date Collected	Data Collected	Observed Northern Allele Frequency	Chi Test p-value of Amylase	Mean VO_2
Antigonish, Nova Scotia	GDAG	45.668° N, - 61.878° W	12/6/2023	Sequencing only (n=11)	0.72727	0.932	NA
Plum Island Ecosystem, MA	GDPIE	42.759° N, -70.891° W	3/6/2024	Both (n=14)	0.78571	0.989	0.1633
Woods Hole, MA	GDWH	41.520° N, -70.668° W	3/11/2024	Both (n=24)	0.70883	0.982	0.1836
Goldstar Battalion Beach, NY	GDGB	40.897° N, -73.436° W	6/1/2024	Both (n=20)	0.85	0.905	0.1775
Green Island, NY	GDGI	40.617° N, -73.504° W	6/2/2024	Both (n=20)	0.625	0.968	0.1343
Boat Basin, NJ	GDRUb	39.555° N, -74.363° W	12/10/2024	Sequencing only (n=6)	0.78571 *	0.964 *	NA
Bivalve, NJ	GDBV	39.234° N, -75.031° W	12/11/2024	Sequencing only (n=8)	0.78571 *	0.964 *	NA
George Island Landing, MD	GDMD	38.041° N, -75.363° W	7/25/2024	Both (n=20)	0.45	0.982	0.1393
Gloucester Point, VA	GDVA	37.307° N, -76.417° W	7/24/2024	Both (n=20)	0.8	0.969	0.1408
Charleston, SC	GDSC	32.762° N, -79.967° W	10/1/2024	Both (n=20)	0.075	0.997	0.1476
Savannah, GA	GDGA	32.038° N, -81.048° W	9/1/2024	Both (n=20)	0.02778	0.999	0.1242

COI primers from Francis & Wares (2022) were designed to improve amplification efficiency in *Geukensia*. PCR with these primers also used a 50°C annealing temperature. Sequencing COI gives a strong baseline to display the mitochondrial divergence within *G. demissa* of the Atlantic coast, based on prior studies showing COI to have statistically interesting differentiation within this range (Diaz-Ferguson et al., 2009).

Sequencing

Each individual was sequenced in both the forward and reverse direction for amylase and forwards only for CO1 using Sanger sequencing (Psomagen services). The sequence data received from Psomagen was analyzed first through Codon-Code Aligner software for quality assurance in base calling. Sequences from the "amylase" fragment exhibit clear Sanger chromatograph polymorphisms, repeatedly across individuals, that were called using IUPAC ambiguity codes. Amylase is a diploid gene, so we then used DNASP (Rozas et al., 2017) and an algorithm that phases haplotypes from polymorphic sites in the sequence data to help see where along the coast the transition in types with greater resolution is occurring (as in Sotka et al., 2004).

One key aspect of determining whether or not this represents a single-copy gene region requires that the data meet base assumptions, e.g. Hardy-Weinberg allele and genotype frequencies should align within spatial samples or regional samples. This assessment prevents the misinterpretation of paralogous genomic regions, which could co-amplify but should not do so in such a predictable pattern.

Given the divergence of 2 primary allele types in Erlenbach & Wares (2023), with almost complete divergence in frequency of these types between Georgia and Massachusetts and very low linked SNP diversity, we developed a restriction enzyme assay using *Eco*RV (New England

Biolabs) to genotype individuals. We believe we are capturing the most important distinction across the range of *G. demissa* since the restriction site for *Eco*RV (GAT|ATC) is located at an amino acid difference between the two allele types. Though there are a few other nucleotide polymorphisms in these sequences, the most dominant and spatially informative variants define two primary allele types reflected in the original data from Erlenbach & Wares (2023); as in Schmidt & Rand (1999), we are binning the rarer variants and only focusing on the two primary allele groups.

Once amylase sequences were returned from Psomagen and bases called in Aligner, for Amylase only, we could use the information from Codon Code Aligner to compare our called results with the *Eco*RV restriction enzyme. With post-PCR product made from the AMY primers and the *Eco*RV restriction enzyme kit, genetic variation could be determined within an hour by running the samples on an agarose gel. This enzyme finds binding sites on bases at the motif sequence of GAT/ATC (New England Biolabs), and performs a blunt end cut everywhere this sequence occurs. After 40 minutes on a 1.5% agarose gel, enough separation has occurred to reveal banding patterns appearing at varying distances, indicating different allele pairings. One band on the gel indicates the individual is homozygous (one genotype with a band at 355nt, the other at 175-180nt), and because the marker is codominant, we can see two bands representing the heterozygous individuals. Upon discovery that this enzyme was consistently and accurately displaying the allele pairings seen in our sequence data for amylase, we proceeded with this method for genotyping amylase for the remaining sites in this study.

Analyses

The COI data were analyzed among our regional samples using pairwise PhiST calculations in R using packages poppr (Kamvar et al., 2014) and ade4 (Dray & Dufour, 2007).

We expect our results to recapitulate those of Diaz-Ferguson et al. (2009) but with improved spatial resolution to test for isolation-by-distance.

After generating genotypic and sequence diversity for amylase, we assess allele type and genotype frequency within regional samples to address Hardy-Weinberg expectations, i.e. that genotype frequencies are predicted by allele frequencies within samples. With some locations having small sample sizes, we pooled diversity within 1° latitudinal bins for this analysis. We also estimate Hudson's Snn (Hudson, 2000) among regional samples, and assess the sequence data for estimation of nucleotide diversity, divergence, and Tajima's D.

Results

Amylase sequence data

Sequence data from amylase are accessible at Genbank (accession numbers PQ672190-PQ672279 and PV013581-PV013594) from 84 individuals collected throughout the species range. End trimming of sequence data used default settings in CodonCode Aligner. All nucleotide calls with PHRED scores <15 were visually assessed and called as unknown, an IUPAC ambiguity, or as a single base. Two individuals from Georgia exhibited poor amplification and were unable to be genotyped for amylase (GDGA 04 & GDGA 05), though they did amplify for COI.

The amylase sequences certainly seem to be extraordinary in terms of diversity. Genome-wide nucleotide diversity π was estimated using Illumina sequence data from Wares (2023). The all-sites VCF files from those data were analyzed using pixy (Samuk & Korunes 2021) using a window size of 100nt, resulting in an average $\pi = 0.00094$ for a subset of mussels from northeastern Florida and the Georgia coast. We note this is not a random sample of the genome as it only includes those regions captured by RAD-seq. In contrast, estimates of π for the amylase

sequences in this study are similar within allele groups (0.00048, 0.00124) but significantly higher when all sequences analyzed simultaneously ($\pi = 0.01436$). Further evidence of admixture of distinctly evolved sequences comes from Tajima's D which is negative within allele groups (-1.488, -1.148) but positive when all sequences analyzed simultaneously (D = 0.378); Wares & Ewers (2012) note this effect when evolutionarily independent sequences are combined in similar proportions.

However, at multiple scales the allele groups meet Hardy-Weinberg genotype ratio expectations; the observed allele frequencies accurately predict the observed genotype frequencies. With the restriction digest genotype calls, our results for chi-square tests of Hardy Weinberg are presented in Table 1 by location; individual COI and/or amylase accession numbers or genotypes are in a supplemental table.

COI sequence data

Sequence data from COI are accessible at Genbank from 183 individuals, using similar protocol for end trimming in Aligner and visual assessment protocol. A broader analysis of this mitochondrial diversity for the entire North American coast distribution of *G. demissa* is separately in preparation (Smith, White, et al.) and shows that these data exhibit statistically significant ($p<0.001$) isolation by distance (Wright, 1943).

Spatial variation statistics (Snn and/or PhiST)

For amylase, we only have sequence data from individuals in Florida/Georgia, New Jersey, Connecticut/Massachusetts, and Nova Scotia. Overall, these data exhibit Hudson's Snn of 0.394 ($p<0.001$). For the paired data of FL/GA versus NJ Snn is 0.838 ($p<0.001$); for NJ versus CT/MA Snn is 0.5995 (ns); and for CT/MA versus Nova Scotia Snn is 0.661 (ns).

Overall, we find that within regions there are no deviations from expected genotype frequencies given observed allele frequencies, suggesting the behavior of a single locus with two primary allele types. We do see a strong deviation in overall pattern of diversity for amylase from the pattern exhibited by the mitochondrial sequence data along the Atlantic coast.

Discussion

The key finding from the sequencing of amylase is that amylase does appear to behave as a single copy locus, reinforcing this same observed behavior of variation indicated by Erlenbach & Wares (2023). However, this evaluation should be extended as genomic resources improve. Further clarification of amylase organization in the *G. demissa* genome (Wares & Pirro, 2023) will require better scaffolding of existing genomic data, though so far BLAST results of the fragment we have genotyped suggest only a single high-identity region in the available genome scaffolds (J. P. Wares, unpub.). Should the data have suggested amylase is a multi-copy genomic region, determining and understanding amylase variants would be more difficult, and could consequently hinder our ability to interpret its spatial patterns in allele and genotype frequency. Nevertheless, the apparent spatial pattern of recovered amplicon/sequence data is interpreted here as representing a single-locus genotype because we find it hard to identify any other mechanism that would lead to such a consistent fit between allele and genotype frequencies in a spatially congruent manner.

The overall frequency change between southern and northern populations is more complex than appeared in Erlenbach & Wares, and suggests a likely hierarchical pattern perhaps driven by environmental variation. The amylase type frequencies are substantially different between sample locations in SC, GA, FL and other sites, evidenced by the steep change over our latitudinal sample range (Figure 2.1). Although earlier work (Diaz-Ferguson et al., 2009)

suggested that mitochondrial diversity exhibited the greatest change over geographic distance between Virginia and Massachusetts, more recent and extensive mitochondrial analysis shows that mitochondrial diversity largely follows a pattern of isolation by distance (Smith, White, et al., in prep). Thus, the sharp transition in frequency of amylase allele frequencies is quite distinct from that of the mitochondrial data, as well as spatial allele frequencies in other metabolic loci studied in *G. demissa* (Sarver et al., 1992), and may instead be in accord with coastal temperature transitions across Cape Hatteras.

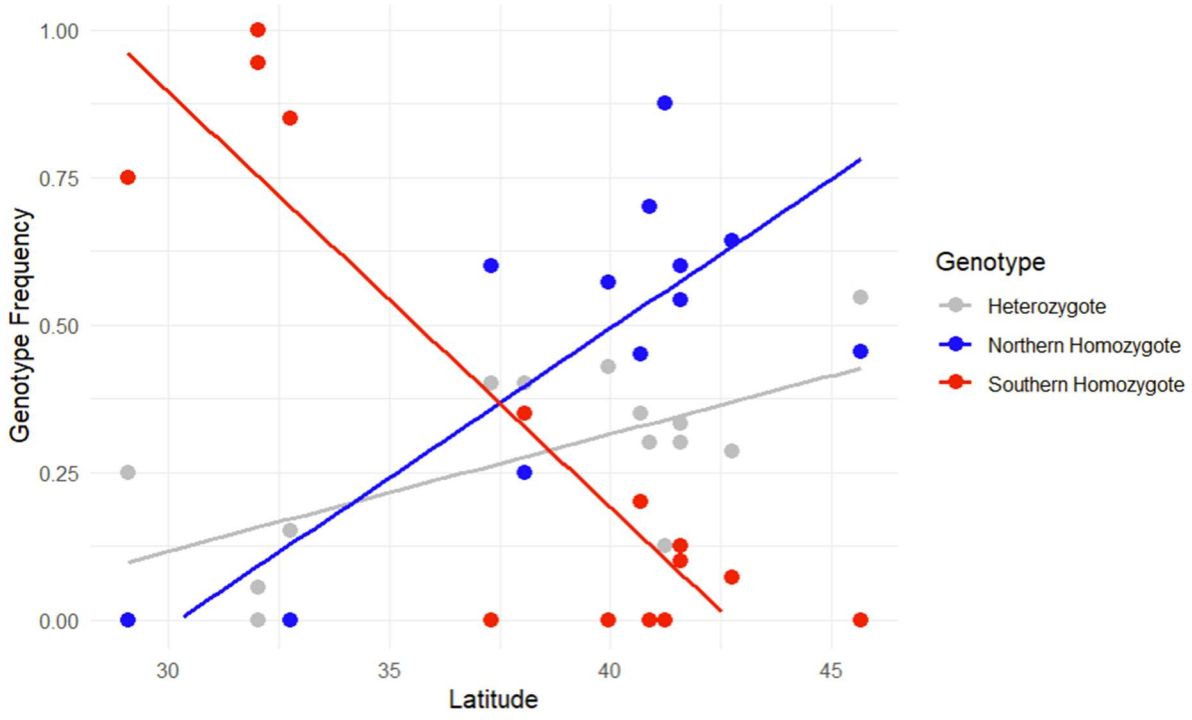

Figure 2.1: Amylase Genotype Frequency across Latitude. Includes three linear trend lines for each of the three respective genotypes.

One possible reason for this sudden change in amylase frequency around Cape Hatteras could be due to a potentially hindered ability for free floating larvae from the south to reach north of Cape Hatteras, due to the direction of the Gulfstream current and its flow away from the coast at Hatteras. Mussels broadcast spawn, which typically leads to the assumption of high gene flow, but this could be complex along the Atlantic coast due to current direction or nearshore geography (Pringle et al., 2017). Similar coastal patterns continue to be found along this range with other species, making interesting comparisons with the pattern observed in amylase. In the polychaete *Diopatra cuprea*, significant genomic transitions along the Atlantic coast occur in the upper Virginian province (Ziegler et al., 2025) as in Diaz-Ferguson et al. (2009); Dennis & Hellberg (2010) identify cryptic endemic diversity in the snail *Melampus* associated with the transition between the South Atlantic Bight and the Mid Atlantic Bight. And more broadly, coastal diversity tends to exhibit intraspecific transitions in the northern Mid Atlantic Bight (Wares, 2002). How these patterns of 'neutral' diversity map against functional traits like amylase can be complex (Hice et al., 2012).

The overall diversity of amylase is unusual with low diversity within allele classes. Our data agrees with recent work suggesting balancing selection as being more common than once believed (Nunez et al., 2021), similar to how the acorn barnacle *Semibalanus balanoides* was found to have adapted to heterogeneous environments of thermal stress as a result of where their larvae settled on the rocky intertidal (Schmidt & Rand, 1999). Across an environmental gradient, balancing selection of *Carcinus maenas* is also suspected to be the pattern explaining cold-tolerance adaptations (Tepolt et al., 2022), displaying that balancing selection can be detected on the intertidal across gradients. Although balancing selection seems like the most obvious explanation for the pattern we observed, a history of introgression may also be at play. *G.*

granosissima was once more established on the Atlantic coast (Sarver et al., 1992; Wares, 2023), and *G. granosissima* mitotypes were recovered in Smith, White et al. (in prep) in Virginia. This could support a history of much broader introgression than ever previously known, and this introgression may be more important to the evolutionary ecology of *G. demissa* than we can presently detect.

Similar to the important role amylase has shown for growth and diet (Guarna & Borowsky, 1993; Prudence et al., 2006), amylase in our research (Chapter 3) has shown to be associated with rates of oxygen consumption, a general proxy for metabolic rate. Our work contributes to the overall value of amylase as an ecologically valuable genetic marker, meaning this could be applied to other marine organisms or systems in the future. Understanding this genetic diversity can also be important for habitat restoration purposes. Should *G. demissa* ever require restorative efforts this work suggests that, depending on the location, some *G. demissa* individuals may be more fit to be transplanted to a particular area than those taken from elsewhere on the coast. Paying attention to these findings can increase assurance of optimal viability for their species.

CHAPTER 3

GASPING FOR ANSWERS: DISSOLVED OXYGEN AND MUSSEL METABOLISM

Introduction

Traits in any species vary in space, partly driven by adaptation and partly by interaction with the environment. This is important for recognizing that populations from distinct parts of a geographic range will have different responses to a changing climate. A single species can overlap multiple distinct ecoregions, which may exhibit unique environmental conditions (Spalding et al., 2007). This phenomenon of a species adapting to be most fit for its environment can be attributed to natural selection, a mechanism of evolutionary change leading to trait variation. However, with the threat of a rapidly warming climate (Baumann & Doherty, 2013) it is questioned whether climate change will outpace species' capacity to adapt (Pershing et al., 2015). Common responses to climate change include latitudinal or elevational range shifts (Poloczanska et al., 2016), however with marine intertidal species, they generally do not have the capability to do the latter.

Physiology represents a set of traits that can vary greatly within a species depending on geographic distribution. Because adaptation is a mechanism to increase fitness, these traits are most often beneficial for their survival in terms of growth, reproduction, or physiological capabilities. Terrestrially, increased shifts poleward or towards higher elevations provide colder temperatures and relief (Poloczanska et al., 2016), and often pair with physiological differences that can make these colder-adapted organisms distinct from their warmer-adapted, same-species counterparts. For intertidal species, they rely primarily on gradient adaptation or latitudinal

shifts. When populations from their respective climates are evaluated at the same temperature, the colder originating population is likely to have greater trait performance than the warmer originating population revealing countergradient variation (Villeneuve et al., 2021). This has been observed in marshes, where in a thermal performance study on the intertidal snail species *Littorina saxatilis*, cold-adapted populations reflected a higher maximum thermal performance when compared to their warmer-adapted, low latitude counterparts, resulting in this 'countergradient' response (Dwane et al., 2022).

While some such spatial patterns reflect adaptation – an evolutionary outcome – environmental gradients may also lead to trait variation that is primarily driven by plastic response to the environment. Understanding whether this variation is due to plasticity or heritable diversity influences how we might predict responses or manage these species. Teasing these two types of responses apart can be quite difficult. If a trait seems to follow an environmental gradient, experiments are necessary to assess whether the same variation is expressed in a common garden trial. By evaluating how abruptly or gradually a change in phenotype occurs along a latitudinal gradient may help us understand what mechanisms underlie environmentally driven trait variation.

Intertidal species make great study organisms to evaluate the impact of trait variation along latitudinal gradients, as they are confined to their narrow coastal ecosystems. The Atlantic Coast stretches ~3,000 km, and the known range of *G. demissa* spans nearly all of that. The Gulf of Maine, Virginian, and Carolinian marine ecoregions (Spalding et al., 2007) of the United States meet in the coastal area known as the Mid-Atlantic Bight, where numerous environmental conditions vary drastically across this space, most notably water temperature and primary production, both of which sharply shift with the colder waters north of Cape Hatteras. This begs

the question of how a species like *G. demissa* persists across such a large range, and whether any trait variation in *G. demissa* could be an important indicator of the salt marsh community they are obligately associated with (Anderson & Padilla, 2025).

Previous research has pointed towards the idea that the Atlantic coast distribution of *G. demissa* exhibits interesting intraspecific variation. In the case of *G. demissa* and other intertidal species, physiological differences have been observed along latitudinal gradients of environmental change. Interestingly, *G. demissa* was found to be the most divergent among the intertidal species investigated in Díaz-Ferguson et al. (2009), and subsequent work by Erlenbach & Wares (2023) identified a strong and apparently heritable shift in oxygen consumption between northern and southern populations. This temperature performance work showed what we expect – increased oxygen consumption at higher temperatures – but also a surprising increase in oxygen consumption of the northern samples (Massachusetts) relative to those in the south (Georgia). However, this study failed to assess any spatial pattern or transition trend between these distant subsets of the species' range.

Using oxygen consumption data from a larger number of latitudinally-sampled locations, we seek to contribute to the evidence of physiological variation through a more fine-scale evaluation of this latitudinal gradient, and hypothesize that this physiological pattern will vary spatially in ways that guide our recognition of how adaptation may be important in the diversity of these coastal mussels. Additionally, we wanted to determine what functional diversity related to latitudinal shifts in phytoplankton availability may be present within *G. demissa.* Since the digestive enzyme amylase has also been shown to have a strong divergent genomic signature across the range of *G. demissa* (Erlenbach & Wares 2023), and it has a history of important fitness-related variation in other marine invertebrate species, we wanted to see if this genomic

marker changes from south to north and possibly correlates with observed variation in respiration rate in ribbed mussels.

Methods

Study area and Sampling

To evaluate latitudinal variation in both physiological and genomic traits, we sampled from 2 sites in each of 4 spatial regions in the range of *G. demissa* (Figure 3.1). Based on the scant genomic data available to date (Díaz-Ferguson et al., 2009), we emphasized sampling in the Mid-Atlantic Bight region to possibly bracket divergent evolutionary lineages of *G. demissa*. At each sampled location, we collected up to 30-35 live *G. demissa* and transported them to Athens, GA for maintenance in a common environment. The samples from Georgia and South Carolina ensured that we also sampled diversity from the warmer SST environment south of Cape Hatteras, and ensured we can replicate the work of Erlenbach & Wares (2023).

For the mussels to be used in the oxygen consumption experiments, it was imperative that they were collected and maintained alive. Mussels were transported overnight with ice packs, either by shipment or in coolers of seawater with aeration. Tide cycles played a factor in determining collection times as we timed for maximum low tides of the particular sampling areas, and the total time span of collection, whether mailed or collected ourselves, occurred between the months of March and October of 2024. All specimens were collected under appropriate state and local scientific collecting permits.

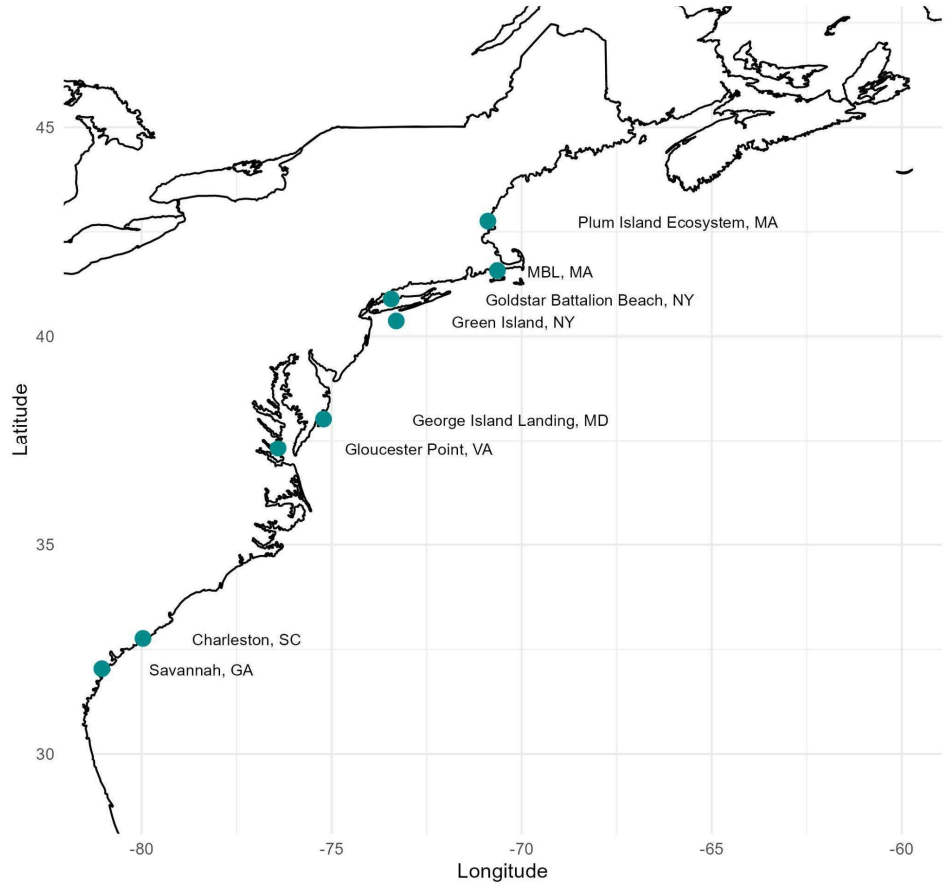

Figure 3.1: Map of Collected Sample Sites. Map of eight collection sites used for common garden oxygen consumption.

Common Garden

Two 70L tanks were established to maintain the same environmental conditions in the laboratory, with approximately 40L of artificial seawater in each, mixed from deionized water and Instant Ocean salt. Having two established tanks ensured too many mussels weren't crowded into one tank, which could cause waste to increase and pH to decrease too rapidly for survival. Conditions were maintained at 26-28 ppt salinity, a pH of 8.0-8.2, and a temperature of 20℃ by the use of Teco automatic heater/chiller units. We completed partial water changes on both tanks

daily, and mussels received phytoplankton (Seachem 1-20µm phytoplankton mix) as a daily food source.

Oxygen consumption

Mussels were allowed to acclimatize in their tanks under the stated conditions for 14 days, starting immediately after their entry to the lab, before oxygen logging was performed (Erlenbach & Wares, 2023). This allows the mussels to come down from any physiological stress that could have been inflicted from collection or shipping, and ensures all mussels are evaluated after adjusting to the same conditions. Two days prior to the start of the experiment, or 12 days after they began their acclimation period, we stopped feeding the mussels phytoplankton to ensure they were in the same, low-nutrient state. This ensures that individuals are no longer metabolizing any phytoplankton, as there could be variation in their clearance rates. Mussels varied in size/mass, and mussels with epibionts such as barnacles were not chosen for the experiment as barnacles respiring with the mussel could alter the logged oxygen consumption of the mussel.

An individual mussel was placed into a uniquely labeled mesh bag and sealed in a container of tank water along with a stir bar and HOBO U-26 temperature/oxygen logger inside, which was then placed into the temperature-controlled tank over a stir plate. Two of these apparatuses were established so that two mussels could be logged simultaneously. Having the stir plates external to the tank allowed the water in each chamber to be circulated without altering the temperature, as happens when small pumps are used (Wares and Duffin 2019). With all air expelled from the containers to ensure no air pockets and with the stir plates at ~100rpm, the loggers were allowed to read dissolved oxygen concentration and temperature for 75 minutes. Because the heater/chiller unit operates on a negative feedback loop, logging data for 75 minutes

ensures that even if the unit kicks on there is a long enough period of uninterrupted data collection for analysis. After 75 minutes the mussel was removed and the volume of remaining water in the container was measured to log the total chamber volume minus that displaced by the mussel. This was repeated for all 158 mussels.

After all mussels had undergone this procedure, they were given unique identifying tags and placed in a -20°C freezer for euthanasia. After a minimum of 24 hours in the freezer, they were stored in 95% undenatured ethanol for a minimum of 24 hours, then again for another 24 hours after a change of ethanol. This process dehydrates the muscle tissue by drawing water out of the individual cells through osmosis, which is important for weighing the true mass of the mussels in addition to the tissue sample taken for genetic analysis. Once the mussels were dehydrated they were taken out of the ethanol and allowed to dry further, until visibly matte, for weighing. Scraping all meat from inside the shells, the total mass, mass of only the tissue, and mass of only the shell was measured.

VO_2 estimation

One way to test the physiological capability of an organism is to look at their rate of oxygen consumption (VO_2) as a proxy for metabolism (Speakman, 2005). A 'good' oxygen series from the loggers, in our case, was a decreasing series of oxygen values for 50 minutes. Following the mass-specific aquatic formula from Fly et al. (2012), VO_2 was calculated by factoring in the change in dissolved oxygen ($\Delta[O_2]$) with other parameters such as body mass (m), volume of water in the chamber (V), and elapsed time of which the data was collected (t) (Fly et al., 2012).

$$VO_2 = \frac{(\Delta[O_2] \times V)}{(t \times m)}$$

The resulting VO_2 is in units of μmol O_2 g^{-1} h^{-1}. Having measured the masses of the individual mussels allows us to eliminate body size as a confounding variable, as larger individuals generally consume more oxygen than smaller counterparts should all conditions be equal. Our protocol was identical to that of Erlenbach & Wares (2023).

Linear model

VO_2 data alone provides insight into physiological differentiation between *G. demissa* to the north and south of Cape Hatteras, but of the latitude, SST (Sea Surface Temperature) and genotype data we have, we needed to evaluate which predictors are more influential to explain this difference.

To determine whether to use a generalized linear model or a general linear model, we first tested for normality using a Shapiro-Wilk test on both the raw, and later log transformed VO_2 data. These tests indicated our data was non-normal under both scenarios, however evaluating histograms indicated this question needed further consideration. Ultimately a linear model on the raw VO_2 data provided the best analysis given that the VO_2 data is 0 truncated, since a VO_2 of 0 would indicate the mussel is deceased, and could have made numerically testing for normality inaccurate.

Terms for this model include amylase genotype frequency, SST, and VO_2. We had recorded the month in which the oxygen trials were conducted and sought to incorporate month as an error term in our model to account for seasonal variation, but the month was inversely correlated with the latitude of sampling sites, as we collected from north to south. Due to this inverse correlation, we drop out the month term from our modeling. We cannot definitively say that VO_2 variation isn't caused by month, but we wanted to look at spatial factors as explanatory variables. Mean annual SST data was downloaded from the NOAA (2024) World Ocean Atlas

products in 1-degree grids, and SST values were selected at a depth of 0 meters at the closest latitude and longitude to our individual sample sites.

When evaluating what factors are most important in explaining the observed spatial variation in VO_2, we used four general linear models to evaluate the impact of SST, amylase genotype, an additive model of these factors, and an interaction model. These four linear models were run on the full set of eight sample sites, as well as the data subset of data with GA and SC excluded, totaling to 8 linear models evaluated. Akaike Information Criterion (AIC) weighting was then used to determine which model is most explanatory of VO_2 variation for the full data set, and for the subset excluding GA and SC.

We had originally sought to use 'latitude' as a way to evaluate biogeography, but after consideration we deemed SST to be more relevant. The aim with using SST is to look at something potentially more predictive than a mere set of consistently changing values, such as latitude. This was done primarily to draw a more meaningful physiological-ecological comparison, but also in part because values of SST change more abruptly (given what is known about the Gulf stream and the cold pool) than a consistent change in latitude and could reveal a stronger response. Essentially, if we had only looked at latitude and observed variation the next question would have been; what environmental factors are changing that could drive this change? In addition to this process of evaluating amylase genotype frequency and SST on VO_2, we also confirmed a latitudinal significance via this same 8-model structure to confirm change across the range. SST was replaced with our values for latitude merely to confirm a spatial change, but we proceed with reporting SST due to this ecological relevance.

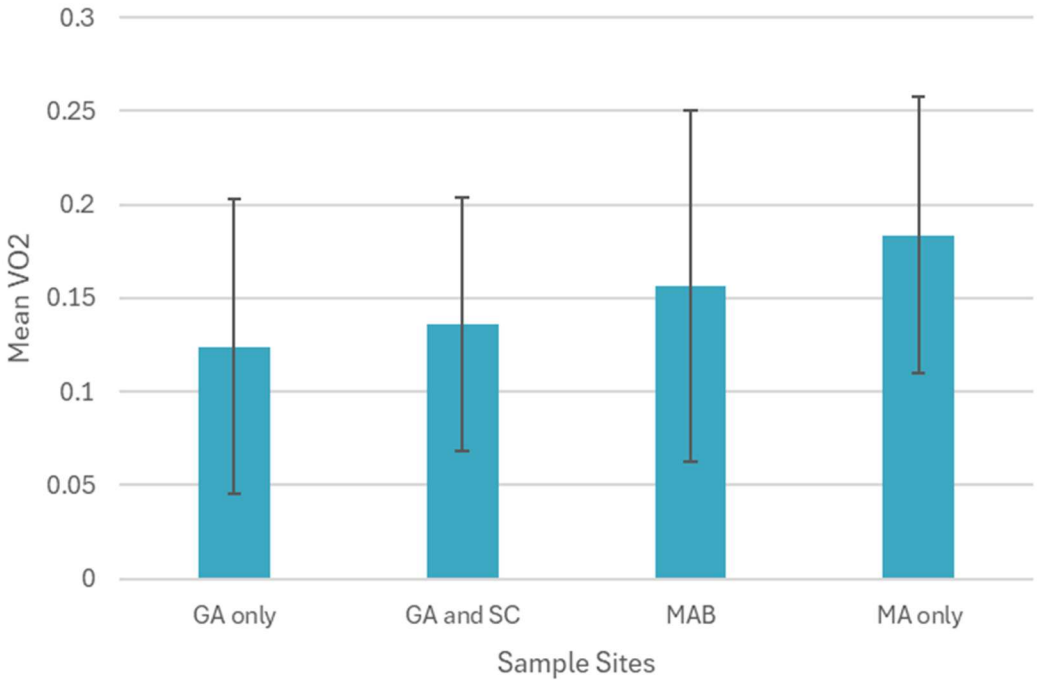

Figure 3.2: Mean VO_2 at Sample Sites. Mean population VO_2s of the southernmost ('GA only') and northernmost ('MA only') limits of our sampling range, and grouped regional mean VO_2s to the south ('GA and SC') and north ('MAB') of Cape Hatteras, NC. Error bars are standard deviation.

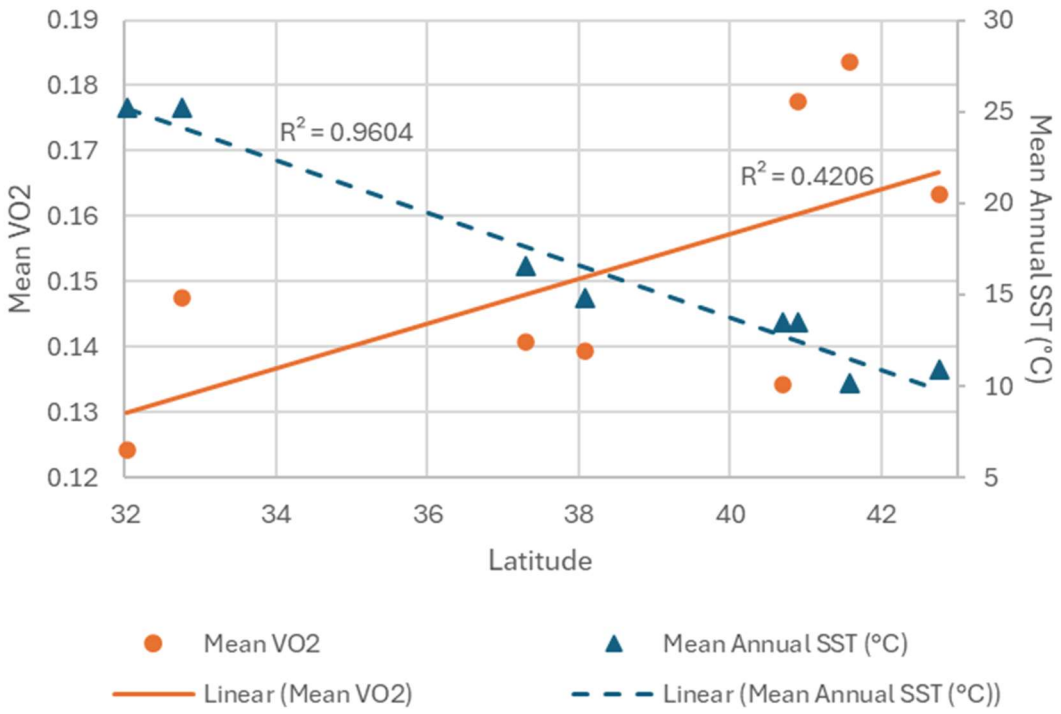

Figure 3.3: Mean VO_2 with Mean Annual SST. Mean VO_2 (y1) and Mean Annual SST (y2) for all eight sampling locations (Latitude). Lines are respective linear trend lines, and y1 and y2 axis bounds have been adjusted to aid visualization.

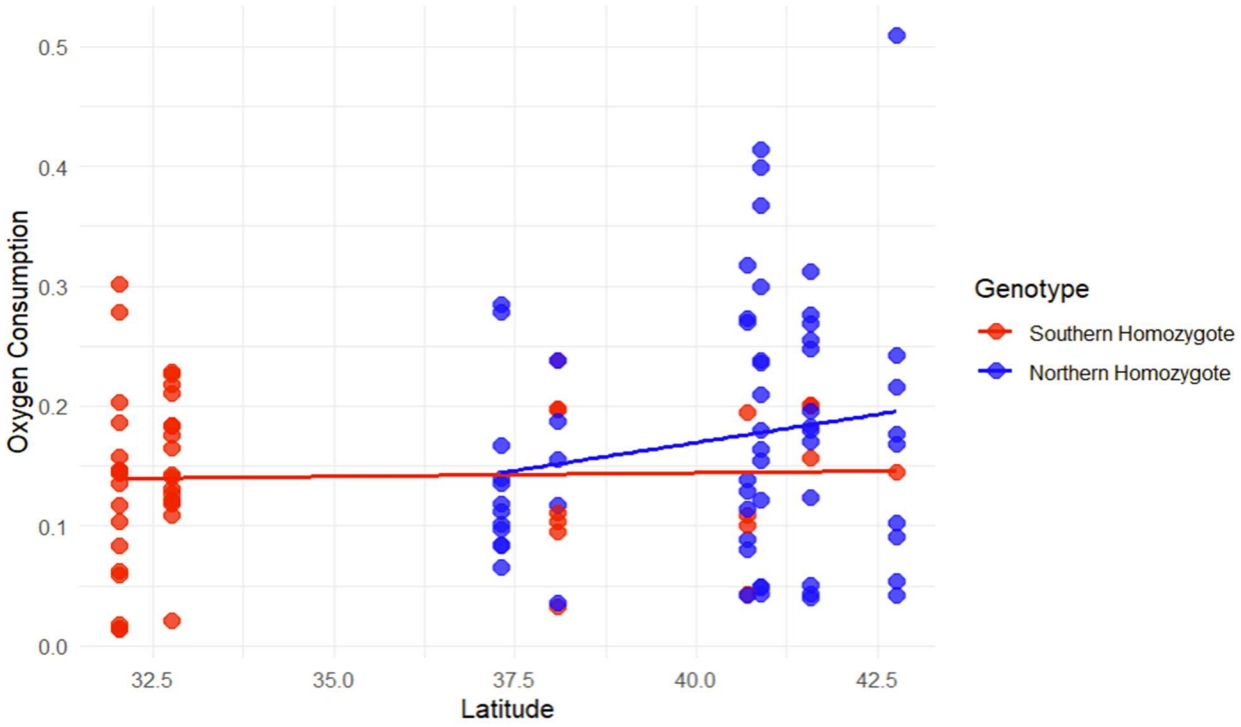

Figure 3.4: Full Range VO_2 and Genotype. Oxygen consumption for all eight sites (GA-MA) at respective latitudes. Oxygen consumption is colored by individuals (dots) to indicate either Southern Homozygote (red) or Northern Homozygote (blue). Lines are linear trend lines of oxygen consumption for both genotypes.

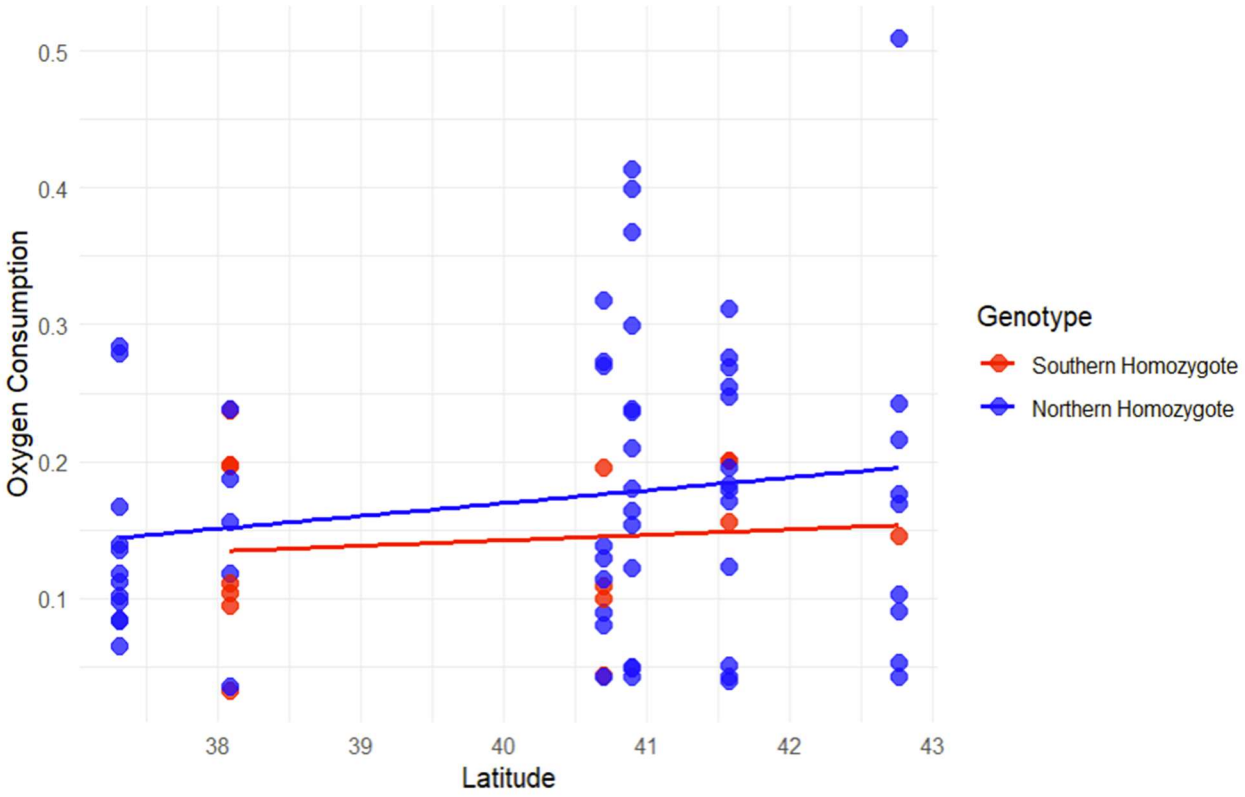

Figure 3.5: Mid-Atlantic Bight VO_2 and Genotype. Oxygen consumption for the six Mid-Atlantic Bight sites (VA-MA) at respective latitudes. Oxygen consumption is colored by individuals (dots) to indicate either Southern Homozygote (red) or Northern Homozygote (blue). Lines are linear trend lines of oxygen consumption for both genotypes.

Table 3.1: VO_2 Linear Models. P-values and AIC results from general linear models evaluating the impact of various predictors on VO_2. Both results from the full eight sample sites ("Full") and the six sites of the Mid-Atlantic Bight ("GA/SC Eliminated") are included. AICw is rounded to three digits, and highest AICw is bolded for each data scenario.

Data	**Model Predictor**	**p-value**	**AIC fit**	**AIC Δ**	**AIC w**
Full	Null	NA	-316.793	1.513	0.282
Full	Genotype	0.048	-313.862	4.444	0.0652
Full	SST	0.063	-318.306	0.000	**0.602**
Full	SST:Genotype	0.183	-311.696	6.610	0.0221
Full	SST + Genotype	0.119	-312.219	6.086	0.0287
GA/SC Eliminated	Null	NA	-221.376	1.736	0.137
GA/SC Eliminated	Genotype	0.088	-222.358	0.754	0.224
GA/SC Eliminated	SST	0.089	-222.323	0.789	0.220
GA/SC Eliminated	SST:Genotype	0.281	-220.565	2.547	0.0915
GA/SC Eliminated	SST + Genotype	0.061	-223.112	0.000	**0.327**

Results from just VO_2 data show a 15.15% increase in the mean VO_2 from the south to north of Cape Hatteras (GA and SC mean $VO_2 = 0.136$, MAB mean $VO_2 = 0.157$). When comparing the southernmost and northernmost geographic limits of our sampling range, Georgia and Massachusetts, as done in Erlenbach & Wares (2023), there is a 47.90% increase in VO_2 (GA mean $VO_2 = 0.124$, MA mean $VO_2 = 0.184$) (Table 2.1, Figure 3.2).

AIC weighting shows that the model of 'SST' is the strongest, simplest, and most explanatory model for the observed trend of VO_2 (w=0.602) when running the model on our dataset containing all eight sample sites (Table 3.1).

Because GA and SC were distinct in terms of VO_2 and genotype frequency from those found north of Cape Hatteras, being composed of only Southern individuals and a few heterozygotes, zooming into the area of the Mid-Atlantic Bight allows us to look deeper into potential interactions between VO_2 and genotype. Running the four linear models again using VO_2 as the response and the same predictors as previously done, but this time on the dataset excluding GA and SC, reveals the additive model of SST and genotype is the best fit (p= 0.061) and the strongest model based on AIC weighting (w=0.327) (Table 3.1). This inclusion of genotype as an important factor when narrowing the geographic region essentially trims down the sole importance of SST.

Discussion

Overall findings reveal that we have repeated the observation of reduced respiration in southern latitudes in *G. demissa*. This research was influenced by Erlenbach & Wares (2023), and upon evaluating the results we can claim that aspects of their study, particularly the aspect of oxygen consumption, have been successfully reproduced. Looking at strictly the VO_2 data from the north and south of Cape Hatteras and comparing these mean VO_2s, we find a 15.15%

increase in oxygen consumption from south to north of this location, and a 47.90% increase in oxygen consumption from the sampling range limits of Georgia to Massachusetts (Figure 3.2). This is comparable to Erlenbach & Wares (2023) where they found Massachusetts was higher than Georgia by a 55% difference at some evaluated temperatures. This supports the general idea that populations of the same species from different regions can display different metabolic rates and reinforces the main physiological result from Erlenbach & Wares (2023). Our data supports that respiration rate from more northern populations (eg. MA) are higher than those in the south (eg. GA).

Typically with ectothermic species such as mussels, whose body temperature is regulated by their environment, as ambient environmental temperature increases their rate of oxygen consumption and overall metabolic rate follows. However, this more so refers to daily or seasonal temperature fluctuations an ectotherm may experience, and does not account for intraspecific variation across ranges where adaptation or acclimatization occurred. Numerically speaking we see the opposite of this fact, where the warmer location has lower mean VO_2 than cooler locations, since our study was conducted at one single temperature (20 °C) but these mussels came from sites with differing seasonal temperatures and different genotypic diversity. The general concept that drives our inference is countergradient variation, where when different populations are evaluated at the same temperature the colder-adapted population is likely to reflect a greater trait performance than the warmer-adapted population (Dwane et al., 2022; Villeneuve et al., 2021). Here, this was observed with our data as we show that VO_2 is positively correlated with latitude, and interestingly shows a good inversely correlated fit with annual mean SST (Figure 3.3). Despite this, it does not appear as a “step function” of a sudden, steep change as we might have initially hypothesized given the Diaz-Ferguson et al. (2009) gene flow data.

We have shown that overall mitochondrial diversity along the coast, with a much higher sample size and denser spatial sampling than previous studies, exhibits a pattern of isolation by distance reflecting an equilibrium between larval movement and local genetic drift (Smith, White, et al. in prep). Yet, the amylase genotype frequencies were not at all linear across our range. Our data shows a distinct frequency in GA and SC from those in the Mid Atlantic Bight (Figure 3.4), and mean VO_2 from a pooled GA and SC was 15.15% lower than the 6 sites of the Mid-Atlantic Bight (Figure 3.2).

Partly explained in the methods, we made a switch to use SST in lieu of latitude in the linear models to find what best predicts VO_2. Although SST is what we report for reasons detailed previously (see 'Linear Model' section of 'Methods'), we went into this research searching for a biogeographic explanation and still did evaluate VO_2 with respect to latitude. Díaz-Ferguson et al. (2009) and Wares (2002) both previously pinpointed the Upper Virginian as being a location of transition, and interestingly VO_2 appears to be higher north of Long Island, NY (Table 2.1). Our modeling did not focus on isolating this area (as we did by cutting out GA and SC for analysis), but additional analysis could help identify whether this Upper Virginian region is a separate physiological transition.

Although mean annual SST is a valuable predictor, of course there are numerous ways to isolate temperature effect – e.g. is it the winter temperature or summer, or is it subaerial exposure that is important (Jost & Helmuth, 2007)? It can be difficult and time-intensive to pinpoint which of these many metrics is *most* important to explaining variation in VO_2, and goes beyond the scope and ability of this study, but finding that SST is of value is a great starting point and emphasizes ecological relevance.

Overall, it is interesting that VO_2 changes in a relatively linear sense in our samples. The genetic control of metabolism (as suggested by Erlenbach & Wares) is likely to involve many contributing gene regions, and so the gradual change corresponding to isolation by distance is not surprising – but it makes it harder to understand the mechanism driving this apparent physiological adaptation. Amylase alone is unlikely to make an enormous contribution to this trait, as there are many other genes that contribute to metabolic functions, but for now we can only conclude it is a genetic marker that displays an interesting correlational pattern.

That being said, amylase has been shown to be a predictive marker for other significant performance-related traits in other marine organisms (Chapter 2) and may itself be more distributed by the large change in SST at or near Cape Hatteras. Additional sampling and possible experiments could help resolve that mechanism. Also, a more complex sampling or experimental approach could help sort out the many correlated components of this work, as latitude, temperature, metabolism, mitotype, and amylase genotype all change across this gradient.

In regard to study design, it is possible that "maternal" or source effects may not be eliminated by the 2-week acclimatization period, though it would be substantially harder to perform this with lab-raised generations given the life history of *Geukensia*. Overall the idea that there is genetic control over the metabolism and performance of these mussels, part of a "keystone interaction" (Angelini et al., 2016), has broad implications for marsh restoration (Bilkovic et al., 2021). The repeated inference of a component of local adaptation affecting metabolism (Erlenbach & Wares, 2023) is an important step in establishing both models for how locally mussels can be transferred for restoration, as well as suggesting future research to more specifically characterize the fitness landscape of ecophysiological variation in these mussels.

The integrative assessment of performance/physiology, genetic factors, and biogeographic transitions is an important step for understanding what elements of diversity are important for salt marsh ecosystems. Developing this work more broadly across the marsh community would be of value to (1) gain a better and broader understanding of genomic and physiological transitions across this range and be able to generate correlations with other species, and (2) understanding necessary information to ensure optimal viability should these, or other marsh-essential marine organisms ever be in need of restorative efforts via transplant or other means.

CHAPTER 4

CONCLUSION

Main Results

Main takeaways from the molecular data and analysis are that mtDNA displays isolation by distance and amylase sequencing displays distinct genotype frequencies to the north and south of Cape Hatteras. Mean VO_2 is higher in the north, consistent with countergradient variation, and shows an inverse correlation with mean annual SST.

It is important to note that correlations like this, while important for ecological relevance, can make this work difficult. On a linear coastline such as the Atlantic, there will be many correlated environmental gradients. Temperature itself, whether subaerial or sea temperature, drives other factors such as primary productivity and growing season. Nevertheless, even just one connection in a web of correlations can enhance our understanding of biodiversity.

Looking at the bigger picture, we were able to replicate the work by Erlenbach & Wares (2023) and confirm the instance of countergradient variation with metabolic rate, supporting the general idea that organisms from different regions have different metabolic rates. We have also continued exploration of amylase as a useful indicator of metabolic function in *Geukensia demissa* and were able to make comparisons between the latitudinal patterns of this with oxygen consumption data. These two handfuls of data were explored against the context of biogeographic change along the Atlantic coast and ultimately displayed intraspecific patterns that vary over space. Ultimately, metabolic rate ties to functional importance, and consequently ecosystem impact and productivity.

Applications

Marine ecosystems can be relatively understudied in relation to the immense size of our world's oceans, especially in terms of research that encompasses work from multiple disciplines of ecology. Skill sets of biogeography, physiology, animal care, and genetics have all been utilized in this work, and can be applied broadly to our needs for marine ecosystems.

Biogeography, the study of species diversity and distribution, can be telling of the impact and severity of environmental changes such as changing temperatures and human impacts (e.g. overfishing, ecotourism, and habitat destruction). A glaring example would be in coral reef ecosystems where corals face all these threats. This biogeographic data in marine ecosystems often leads to fisheries management strategies and the designation of marine protected areas (MPAs), and in some extreme cases habitat restoration (e.g. coral outplanting on reefs).

Studying physiology can also help indicate numerous environmental stressors and determine the severity of their impact, which can consequently draw attention to the need for change. Physiological stressors such as noise pollution (e.g. boat motors, sonar pings, offshore wind turbines), toxins and chemicals (e.g. non-reef safe sunscreen, oil spills, fertilizer runoff), etc., can all inflict physiological stress or impede an organism's functioning. Understanding how these stressors impact the health of a species or general area, marine or estuarian, can inspire changes such as specific conservation strategies or mitigation efforts.

Additionally, animal care and husbandry allow for up-close and personal, hands-on observations and study that may be difficult in the field. This is a large and often necessary component of research in biogeographic, physiological, and genetic understandings, as done here in our research. In-laboratory animal care is also a large component of recovery efforts such as

captive breeding programs and rehabilitation, and requires plentiful knowledge of maintaining life support systems, water quality parameters, and meeting species-specific diet and care needs.

Genetics provides more informed restorative efforts, such as breeding programs to ensure genomic diversity and prevent inbreeding, as well as restoration by transplant where it should be taken into consideration that a species from one location may be more genetically fit for an area than the same species from a different location, when the goal of a transplant is to maximize viability. Genetics also helps to identify the severity of environmental impacts through the lens of genotypic diversity, like bottleneck effects where the reduction in population size and consequent reduction in genetic diversity could harm a species' ability to combat future ecosystem changes, ultimately increasing their vulnerability.

All of these skills can be put to use individually, or together in combination to help draw meaningful past, present, and future conclusions about general marine ecosystems or specific ecosystem components. No one *Guekensia* is the same as another *Geukensia*, and despite visual similarity, molecular and physiological data coupled with a biogeographic understanding supports this fact.

REFERENCES

Chapter 1 Literature Cited

Bertness, M. D. 1980. GROWTH AND MORTALITY IN THE RIBBED MUSSEL GEUKENSIA-DEMISSA (BIVALVIA, DREISSENACEA). The veliger 23:62--69.

Cohen, A. N. 2011. The Exotics Guide: Non-native Marine Species of the North American Pacific Coast. Center for Research on Aquatic Bioinvasions, Richmond, CA, and San Francisco Estuary Institute, Oakland, CA. Revised September 2011.

Erlenbach, T. R., and J. P. Wares. 2023. Latitudinal variation and plasticity in response to temperature in Geukensia demissa. Ecology and Evolution 13:e9856.

Jost, J., and B. Helmuth. 2007. Morphological and Ecological Determinants of Body Temperature of Geukensia demissa, the Atlantic Ribbed Mussel, and Their Effects on Mussel Mortality. Biological Bulletin 213:141–151.

Odum, H. T., and E. C. Odum. 1976. Energy basis for man and nature.

Thompson, C., R. York, and S. Gallager. 2012. Species-specific abundance of bivalve larvae in relation to biological and physical conditions in a Cape Cod estuary. Marine Ecology Progress Series 469:53–69.

Virgin, S. D. S., K. A. Sorochan, A. Metaxas, and M. A. Barbeau. 2019. Effect of temperature on the larval biology of ribbed mussels (*Geukensia demissa*) and insights on their northern range limit. Journal of Experimental Marine Biology and Ecology 512:31–41.

Whittaker, R. J., M. B. Araújo, P. Jepson, R. J. Ladle, J. E. M. Watson, and K. J. Willis. 2005. Conservation Biogeography: assessment and prospect. Diversity and Distributions 11:3–23.

Wymore, A. S., A. T. H. Keeley, K. M. Yturralde, M. L. Schroer, C. R. Propper, and T. G. Whitham. 2011. Genes to ecosystems: exploring the frontiers of ecology with one of the smallest biological units. New Phytologist 191:19–36.

Chapter 2 Literature Cited

Altman, S., J. Robinson, J. Pringle, J. Byers, and J. Wares. 2013. Edges and Overlaps in Northwest Atlantic Phylogeography. Diversity (Basel) 5:263–275.

Anderson, N. S., and D. K. Padilla. 2025. Settlement and Metamorphosis of the Ribbed Mussel, Geukensia demissa, in Response to Environmental Cues. The Biological Bulletin:000–000.

Angelini, C., J. N. Griffin, J. van de Koppel, L. P. M. Lamers, A. J. P. Smolders, M. Derksen-Hooijberg, T. van der Heide, and B. R. Silliman. 2016. A keystone mutualism underpins resilience of a coastal ecosystem to drought. Nature Communications 7:12473.

Baumann, H., and O. Doherty. 2013. Decadal Changes in the World's Coastal Latitudinal Temperature Gradients. PLOS ONE 8:e67596.

Bertness, M. D. 1984. Ribbed Mussels and Spartina Alterniflora Production in a New England Salt Marsh. Ecology 65:1794–1807.

Bortolus, A., P. Adam, J. B. Adams, M. L. Ainouche, D. Ayres, M. D. Bertness, T. J. Bouma, J. F. Bruno, I. Caçador, J. T. Carlton, J. M. Castillo, C. S. B. Costa, A. J. Davy, L. Deegan, B. Duarte, E. Figueroa, J. Gerwein, A. J. Gray, E. D. Grosholz, S. D. Hacker, A. R. Hughes, E. Mateos-Naranjo, I. A. Mendelssohn, J. T. Morris, A. F. Muñoz-Rodríguez, F.

J. J. Nieva, L. A. Levin, B. Li, W. Liu, S. C. Pennings, A. Pickart, S. Redondo-Gómez, D. M. Richardson, A. Salmon, E. Schwindt, B. R. Silliman, E. E. Sotka, C. Stace, M. Sytsma, S. Temmerman, R. E. Turner, I. Valiela, M. P. Weinstein, and J. S. Weis. 2019. Supporting Spartina: Interdisciplinary perspective shows Spartina as a distinct solid genus. Ecology 100:e02863.

Brinton, A. J. 2021, August 2. Marsh Restoration: Ribbed Mussels (Geukensia demissa) as a Revival Mechanism to Rebuild the Coastal Salt Marshes of Long Island, New York. Research Square.

Dennis, A. B., and M. E. Hellberg. 2010. Ecological partitioning among parapatric cryptic species. Molecular Ecology 19:3206–3225.

Díaz-Ferguson, E., J. D. Robinson, B. Silliman, and J. P. Wares. 2010. Comparative Phylogeography of North American Atlantic Salt Marsh Communities. Estuaries and Coasts 33:828–839.

Dray, S., and A.-B. Dufour. 2007. The ade4 Package: Implementing the Duality Diagram for Ecologists. Journal of Statistical Software 22:1–20.

Erlenbach, T. R., and J. P. Wares. 2023. Latitudinal variation and plasticity in response to temperature in Geukensia demissa. Ecology and Evolution 13:e9856.

Ewers, C., and J. P. Wares. 2012. Examining an Outlier: Molecular Diversity in the Cirripedia. Integrative and Comparative Biology 52:410–417.

Francis, I. G., and J. P. Wares. 2022, January 20. Mitochondrial diversity in the ribbed mussel, Geukensia demissa, relative to high marsh plant diversity at the southern edge of the distribution. bioRxiv.

Guarna, M. M., and R. L. Borowsky. 1993. Genetically controlled food preference: biochemical mechanisms. Proceedings of the National Academy of Sciences of the United States of America 90:5257–5261.

Gerrodette, T. 2011. Inference without significance: measuring support for hypotheses rather than rejecting them. Marine Ecology 32:404–418.

Hice, L. A., T. A. Duffy, S. B. Munch, and D. O. Conover. 2012. Spatial scale and divergent patterns of variation in adapted traits in the ocean. Ecology Letters 15:568–575.

Hudson, R. R. 2000. A New Statistic for Detecting Genetic Differentiation. Genetics 155:2011–2014.

Kamvar, Z. N., J. F. Tabima, and N. J. Grünwald. 2014. Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. PeerJ 2:e281.

Korunes, K. L., and K. Samuk. 2021. pixy: Unbiased estimation of nucleotide diversity and divergence in the presence of missing data. Molecular Ecology Resources 21:1359–1368.

Nunez, J. C. B., S. Rong, D. A. Ferranti, A. Damian-Serrano, K. B. Neil, H. Glenner, R. G. Elyanow, B. R. P. Brown, M. Alm Rosenblad, A. Blomberg, K. Johannesson, and D. M. Rand. 2021. From tides to nucleotides: Genomic signatures of adaptation to environmental heterogeneity in barnacles. Molecular Ecology 30:6417–6433.

Pringle, J. M., J. E. Byers, R. He, P. Pappalardo, and J. Wares. 2017. Ocean currents and competitive strength interact to cluster benthic species range boundaries in the coastal ocean. Marine Ecology Progress Series 567:29–40.

Prudence, M., J. Moal, P. Boudry, J. Y. Daniel, C. Quéré, F. Jeffroy, C. Mingant, M. Ropert, E. Bédier, A. Van Wormhoudt, J. F. Samain, and A. Huvet. 2006. An amylase gene

polymorphism is associated with growth differences in the Pacific cupped oyster Crassostrea gigas. Animal Genetics 37:348–351.

Riginos, C., and L. Liggins. 2013. Seascape Genetics: Populations, Individuals, and Genes Marooned and Adrift. Geography Compass 7:197–216.

Rozas, J., A. Ferrer-Mata, J. C. Sánchez-DelBarrio, S. Guirao-Rico, P. Librado, S. E. Ramos-Onsins, and A. Sánchez-Gracia. 2017. DnaSP 6: DNA Sequence Polymorphism Analysis of Large Data Sets. Molecular Biology and Evolution 34:3299–3302.

Sanford, E., M. S. Roth, G. C. Johns, J. P. Wares, and G. N. Somero. 2003. Local selection and latitudinal variation in a marine predator-prey interaction. Science (New York, N.Y.) 300:1135–1137.

Sarver, S. K., M. C. Landrum, and D. W. Foltz. 1992. Genetics and taxonomy of ribbed mussels (Geukensia spp.). Marine Biology 113:385–390.

Schmidt, P. S., and D. M. Rand. 1999. INTERTIDAL MICROHABITAT AND SELECTION AT MPI: INTERLOCUS CONTRASTS IN THE NORTHERN ACORN BARNACLE, SEMIBALANUS BALANOIDES. Evolution; International Journal of Organic Evolution 53:135–146.

Sotka, E. E., J. P. Wares, J. A. Barth, R. K. Grosberg, and S. R. Palumbi. 2004. Strong genetic clines and geographical variation in gene flow in the rocky intertidal barnacle Balanus glandula. Molecular Ecology 13:2143–2156.

Tepolt, C. K., E. D. Grosholz, C. E. de Rivera, and G. M. Ruiz. 2022. Balanced polymorphism fuels rapid selection in an invasive crab despite high gene flow and low genetic diversity. Molecular Ecology 31:55–69.

Wares, J. P. 2002. Community genetics in the Northwestern Atlantic intertidal. Molecular Ecology 11:1131–1144.

Wares, J. P. 2024. The Genomic Ghosts of Geukensia granosissima. Estuaries and Coasts 47:494–503.

Wares, J. P., and S. Pirro. 2023. The Complete Genome Sequences of 2 Species of Geukensia (Brachidontinae, Mytilidae). Biodiversity Genomes.

Wright, S. 1943. ISOLATION BY DISTANCE. Genetics 28:114–138.

Ziegler, A. J., T. M. Bell, S. K. Berke, A. E. Strand, and E. E. Sotka. 2025. Multiple cryptic lineages and restricted gene flow in the decorator worm Diopatra Cuprea. Marine Biology 172:48.

Chapter 3 Literature Cited

Anderson, N. S., and D. K. Padilla. 2025. Settlement and Metamorphosis of the Ribbed Mussel, Geukensia demissa, in Response to Environmental Cues. The Biological Bulletin:000–000.

Angelini, C., J. N. Griffin, J. van de Koppel, L. P. M. Lamers, A. J. P. Smolders, M. Derksen-Hooijberg, T. van der Heide, and B. R. Silliman. 2016. A keystone mutualism underpins resilience of a coastal ecosystem to drought. Nature Communications 7:12473.

Baumann, H., and O. Doherty. 2013. Decadal Changes in the World's Coastal Latitudinal Temperature Gradients. PLOS ONE 8:e67596.

Bilkovic, D. M., R. E. Isdell, A. G. Guthrie, M. M. Mitchell, and R. M. Chambers. 2021. Ribbed mussel Geukensia demissa population response to living shoreline design and ecosystem development. Ecosphere 12:e03402.

Díaz-Ferguson, E., J. D. Robinson, B. Silliman, and J. P. Wares. 2010. Comparative Phylogeography of North American Atlantic Salt Marsh Communities. Estuaries and Coasts 33:828–839.

Dwane, C., E. L. Rezende, O. Tills, J. Galindo, E. Rolán-Alvarez, S. Rundle, and M. Truebano. 2023. Thermodynamic effects drive countergradient responses in the thermal performance of *Littorina saxatilis* across latitude. Science of The Total Environment 863:160877.

Erlenbach, T. R., and J. P. Wares. 2023. Latitudinal variation and plasticity in response to temperature in Geukensia demissa. Ecology and Evolution 13:e9856.

Fly, E. K., C. J. Monaco, S. Pincebourde, and A. Tullis. 2012. The influence of intertidal location and temperature on the metabolic cost of emersion in *Pisaster ochraceus*. Journal of Experimental Marine Biology and Ecology 422–423:20–28.

Jost, J., and B. Helmuth. 2007. Morphological and Ecological Determinants of Body Temperature of Geukensia demissa, the Atlantic Ribbed Mussel, and Their Effects on Mussel Mortality. Biological Bulletin 213:141–151.

NOAA 2024. URL: https://www.google.com/url?q=https://www.ncei.noaa.gov/access/world-ocean-atlas-2023/bin/woa23.pl?parameterOption%3Dt&sa=D&source=docs&ust=1742226763112894&usg=AOvVaw27c4oExppaOfjRL9PpdIK-. Date accessed: November, 2024.

Pershing, A. J., M. A. Alexander, C. M. Hernandez, L. A. Kerr, A. Le Bris, K. E. Mills, J. A. Nye, N. R. Record, H. A. Scannell, J. D. Scott, G. D. Sherwood, and A. C. Thomas. 2015. Slow adaptation in the face of rapid warming leads to collapse of the Gulf of Maine cod fishery. Science 350:809–812.

Poloczanska, E. S., M. T. Burrows, C. J. Brown, J. García Molinos, B. S. Halpern, O. Hoegh-Guldberg, C. V. Kappel, P. J. Moore, A. J. Richardson, D. S. Schoeman, and W. J. Sydeman. 2016. Responses of Marine Organisms to Climate Change across Oceans. Frontiers in Marine Science 3.

Spalding, M. D., H. E. Fox, G. R. Allen, N. Davidson, Z. A. Ferdaña, M. Finlayson, B. S. Halpern, M. A. Jorge, A. Lombana, S. A. Lourie, K. D. Martin, E. McManus, J. Molnar, C. A. Recchia, and J. Robertson. 2007. Marine Ecoregions of the World: A Bioregionalization of Coastal and Shelf Areas. BioScience 57:573–583.

Speakman, J. R. 2005. Body size, energy metabolism and lifespan. Journal of Experimental Biology 208:1717–1730.

Villeneuve, A. R., L. M. Komoroske, and B. S. Cheng. 2021. Environment and phenology shape local adaptation in thermal performance. Proceedings of the Royal Society B: Biological Sciences 288:20210741.

Wares, J. P. 2002. Community genetics in the Northwestern Atlantic intertidal. Molecular Ecology 11:1131–1144.

Wares, J. P., and P. J. Duffin. 2019, March 21. Re-evaluation of homozygous lethality for EF1A indel polymorphism in Pisaster ochraceus. bioRxiv.