

Reciprocal Subsidies: Phytodetrital Influence on the Reproduction of Methanotrophic Mussels,
and the Contribution of Mussel Larvae to Planktonic Food Webs in the Gulf of Mexico and
Western Atlantic

by

Caitlin Q. Plowman

A dissertation accepted and approved in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy
in Biology

Dissertation Committee:

Kelly Sutherland, Chair

Craig Young, Advisor

George von Dassow, Core Member

Kevin Eckelbarger, Core Member

Emilie Hooft, Institutional Representative

University of Oregon

Spring 2024

© 2024 Caitlin Q. Plowman

This work is openly licensed via [CC BY-NC-SA 4.0](https://creativecommons.org/licenses/by-nc-sa/4.0/).



DISSERTATION ABSTRACT

Caitlin Q. Plowman

Doctor of Philosophy in Biology

Title: Reciprocal Subsidies: Phytodetrital Influence on the Reproduction of Methanotrophic Mussels, and the Contribution of Mussel Larvae to Planktonic Food Webs in the Gulf of Mexico and Western Atlantic

There is growing evidence that chemosynthesis-based ecosystems in the deep sea influence the benthic and pelagic habitats around them, though the extent of these connections is unknown. Bathymodiolin mussels are among the dominant fauna at deep-sea chemosynthetic systems such as methane seeps and hydrothermal vents. Despite their symbiotic bacteria, many species in the subfamily have shown evidence of periodic reproduction driven by seasonal changes in surface productivity. In this dissertation, I explore the reproductive patterns of bathymodiolin mussels from methane seeps in the Gulf of Mexico and Northwestern Atlantic and how they contribute to connections between surface waters and the deep sea.

In Chapter II, I use gonad histology to examine how the reproductive phenology changes with the season, ocean basin, and depth for three species of bathymodiolin mussels, *Gigantidas childressi*, *Bathymodiolus brooksi*, and *Bathymodiolus heckerae*. I find inter- and intra-specific variations across a bathymetric gradient and between the two ocean basins. The results further support that surface productivity plays a role in bathymodiolin reproduction. In Chapter III, I use carbon and nitrogen stable isotopes to investigate phytodetritus as a potential source of supplemental nutrition for reproduction. I also compare the phenology to satellite-derived measurements of surface productivity. Many of the sampled populations of bathymodiolin

mussels incorporate phytodetrital nutrition, but it is not a necessary subsidy for reproduction. The onset of gametogenesis occurs near a peak in surface productivity, and spawning occurs during a change in surface productivity that is shortly followed by a peak. Thus, surface productivity may be an environmental cue that helps synchronize the gametogenic phenology of these mussels.

While Chapters II and III examine how the water column affects the reproduction of bathymodiolin mussels, Chapter IV examines how spawned mussel eggs influence the water column. For *Gigantidas childressi* from three seep sites, I used histology to estimate individual fecundity, bomb calorimetry to measure the energy contained in an egg, and video of the sites to determine the size of the mussel beds. All metrics vary among the sites; changes in fecundity correspond to changes in phenology, while the nutrient density of eggs increased with depth. Each factor affects the total energy released through spawned eggs, but the estimated values are on par with the photosynthetic production of oligotrophic surface waters. Most of the spawned eggs are eaten and thus may represent a critical trophic subsidy for the surrounding ecosystems.

The energy these mussels use to create gametes comes mostly from chemosynthetic sources. Seasonal pulses of phytodetritus may then trigger the spawning of these eggs, releasing much of that energy into the pelagic food web. This research highlights the intimate reciprocal connection between processes in the surface ocean and the deep seafloor. This dissertation includes previously unpublished co-authored material.

CURRICULUM VITAE

NAME OF AUTHOR: Caitlin Q. Plowman

GRADUATE AND UNDERGRADUATE SCHOOLS ATTENDED:

University of Oregon, Eugene

DEGREES AWARDED:

Doctor of Philosophy, Biology, 2024, University of Oregon

Master of Science, Biology, 2017, University of Oregon

Bachelor of Science, Marine Biology, 2014, University of Oregon

AREAS OF SPECIAL INTEREST:

Deep-Sea Biology

Reproductive Ecology

Larval Ecology

Invertebrate Zoology

PROFESSIONAL EXPERIENCE:

Graduate Research Assistant, Oregon Institute of Marine Biology, University of Oregon
2015-2024

Graduate Teaching Assistant, Department of Biology, University of Oregon 2016-2023

Instructor, Department of Biology, University of Oregon 2022-2023

Co-Director, Oregon Marine Students Association, University of Oregon 2016-2020

GRANTS, AWARDS, AND HONORS:

Hill Fund Outstanding GE Award for Teaching, University of Oregon, 2023

Oregon Society of Conchologist's Scholarship, 2018-2023

Travel Award, University of Oregon Department of Biology, 2017

Neil Richmond Memorial Scholarship, Oregon Sea Grant, 2015 and 2017

PUBLICATIONS:

Beinart, R. A., Arellano, S. M., Chaknova, M., Meagher, J., Davies, A. J., Lopresti, J., Cowell, E. J., Betters, M., Ladd, T. M., Plowman, C.Q., Rice, L., Davis, D., Heffernan, M., Jimenez, V., Beaver, T., Becker, J., Bergen, S., Brunner, L., Calhoun, A., Hauer, M., Taradash, A., Giachetti, T., & Young, C.M. (2024). Deep seafloor hydrothermal vent communities buried by volcanic ash from the 2022 Hunga eruption. *Communications Earth and Environment*, 5(254). <https://doi.org/10.1038/s43247-024-01411-w>

Ladd, T. M., Selci, M., Davis, D., Cannon, O., Plowman, C. Q., Schlegel, I., Inaba, A., Mills, S. W., Meneses, M., Vetriani, C., Mullineaux, L. S., & Arellano, S. M. (2024). Faunal colonists, including mussel settlers, respond to microbial biofilms at deep-sea hydrothermal vents. *Deep Sea Research Part I*, 208(2024), 104314. <https://doi.org/10.1016/j.dsr.2024.104314>

Rist, S., Rice, L. N., Plowman, C. Q., Fountain, C. T., Calhoun, A., Ellison, C., & Young, C. M. (2022). Reproductive biology of the bathyal asteroid *Ctenodiscus crispatus* in the northeastern Pacific. *Invertebrate Biology*, 141(4). <https://doi.org/10.1111/ivb.12384>

Meyer-Kaiser, K. S., Plowman, C. Q., & Soltwedel, T. (2021). Reproduction, recruitment, and growth of the Arctic deep-sea hydroid *Bouillonina cornucopia*. *Invertebrate Biology*, 140(2), 1–9. <https://doi.org/10.1111/ivb.12332>

Plowman, C. Q., Trowbridge, C. D., Davenport, J., Little, C., Harman, L., & McAllen, R. (2020). Stressed from above and stressed from below: dissolved oxygen fluctuations in Lough Hyne, a semi-enclosed marine lake. *ICES Journal of Marine Science*, 1–12. <https://doi.org/10.1093/icesjms/fsaa108>

Trowbridge, C. D., Little, C., Plowman, C. Q., Williams, G. A., Pilling, G. M., Morritt, D., Rivera Vázquez, Y., Dlouhy-Massengale, B., Cottrell, D. M., Stirling, P., Harman, L., & McAllen, R. (2019). No ‘silver bullet’: Multiple factors control population dynamics of European purple sea urchins in Lough Hyne Marine Reserve, Ireland. *Estuarine, Coastal and Shelf Science*, 226. <https://doi.org/10.1016/j.ecss.2019.106271>

Little, C., Trowbridge, C. D., Pilling, G. M., Cottrell, D. M., Plowman, C. Q., Stirling, P., Morritt, D., & Williams, G. A. (2018). Long-term fluctuations in epibiotic bryozoan and hydroid abundances in an Irish sea lough. *Estuarine, Coastal and Shelf Science*, 210(November 2017), 142–152. <https://doi.org/10.1016/j.ecss.2018.06.007>

Trowbridge, C. D., Little, C., Plowman, C. Q., Ferrenburg, L. S., Resk, H. M., Stirling, P., Davenport, J., & McAllen, R. (2018). Recent changes in shallow subtidal fauna with new invertebrate records in Europe’s first marine reserve, Lough Hyne. *Biology and Environment*, 118B(1). <https://doi.org/10.3318/BIOE.2018.03>

Trowbridge, C. D., Davenport, J., Cottrell, D. M., Harman, L., Plowman, C. Q., Little, C., & McAllen, R. (2017). Extreme oxygen dynamics in shallow water of a fully marine Irish sea lough. *Regional Studies in Marine Science*, 11(155), 9–16

Trowbridge, C. D., Kachmarik, K., Plowman, C. Q., Little, C., Stirling, P., & McAllen, R. (2017). Biodiversity of shallow subtidal, under-rock invertebrates in Europe's first marine reserve: Effects of physical factors and scientific sampling. *Estuarine, Coastal and Shelf Science*, 187, 43–52. <https://doi.org/10.1016/j.ecss.2016.12.022>

Trowbridge, C. D., Davenport, J., Plowman, C. Q., Harman, L., & McAllen, R. (2017). Marine aloricate ciliate red tides in a temperate Irish sea lough. *Marine Biodiversity*, 47(3), 869–878. <https://doi.org/10.1007/s12526-016-0520-3>

Trowbridge, C. D., Little, C., Ferrenburg, L. S., Resk, H. M., Kachmarik, K., Plowman, C. Q., Stirling, P., & McAllen, R. (2016). Shallow subtidal octocorals in an Irish marine reserve. *Marine Biodiversity*, 46(4), 879–887. <https://doi.org/10.1007/s12526-016-0450->

ACKNOWLEDGMENTS

I am deeply grateful for the support of many mentors, colleagues, friends, and family throughout this degree. I am incredibly thankful for my committee members, Drs. Craig Young, Kelly Sutherland, George von Dassow, Kevin Eckelbarger, and Emilie Hooft. We expected this degree to take less time, and I appreciate your continued guidance and support.

I sincerely appreciate my advisor, Dr. Craig Young, who has mentored me for the last decade. Without you, none of this would be possible. Thank you for taking a chance on me as an undergrad and for your continued support. I will forever be grateful for the multiple *Alvin* dives where I could visit the deep sea and see my study systems with my own eyes. I am honored to be a part of your academic family and thankful that you took the time to teach us about the history of our science and the scientists who came before us.

My lab sisters, Dr. Shawn Arellano, Dr. Maya Watts, Dr. Kirstin Meyer, Lauren Rice, and Avery Calhoun, also deserve a huge thank you and many hugs. I am incredibly grateful for your mentorship and friendship. Shawn, it was a pleasure to sail with you so many times, and I appreciate all your advice about this project and my career path. Thank you for sending me on my first research cruise to hydrothermal vents. Maya, I appreciate everything you have taught me, the many times you provided a shoulder to cry on, and everything you have done to help me find employment, both as a GE and after graduation. Kirstin, I am highly grateful for your scientific and emotional support, and I cannot thank you enough for your help in editing these chapters. Lauren and Avery, I am so thankful for the support you have both given me and the many fun adventures we have shared. Lauren, I am incredibly grateful we could do much of this degree process together; I would not have made it without you.

Thank you to the whole OIMB community, even those not named here. Trish Mace, Lisa Samuelson, Debbie Seabright, Laura Screen, Bradd Beckett, James Johnson, Mike Johnson, and Ian Washington, I am particularly thankful for your friendships and for making OIMB a fun workplace. I am also profoundly grateful for the other graduate students at OIMB, who provided a supportive community and many fun times: Nicole Nakata, Christina Ellison, Jessie Masterman, Erin Jezuit, Matt Depaolis, Samantha Persad, Reyn Yoshioka, Ross Whippo, Hilarie Sorenson, and Joanna Lyle.

This work would not have been possible without funding from the National Science Foundation (OCE 1851383 to Craig Young) for the collaborative Seafloor Animal Larval Transport (SALT) project by Drs. Craig Young, Dave Eggleston, Roy He, and Shawn Arellano. I am grateful to the captains and crews of the R/Vs *Atlantis* and *Thomas G. Thompson*, HOV *Alvin*, and ROV *Jason* for facilitating the sample collection and the numerous students and technicians who assisted with sample processing both at sea and on land. I also want to thank the Oregon Society of Conchologists, who awarded me multiple scholarships that funded most of the stable isotope analyses, and Dr. Aaron Galloway for allowing me to use his bomb calorimeter.

DEDICATION

To my husband, Jesse Borland, who has walked every step of this journey with me,
kept me going along the way, and provided valuable help in the field and lab.

TABLE OF CONTENTS

Chapter	Page
I. GENERAL INTRODUCTION.....	17
1. Background.....	17
2. Study System	18
3. Scope and Aims	19
II. GAMETOGENIC PHENOLOGY OF BATHYMODIOLIN MUSSELS FROM METHANE SEEPS IN THE GULF OF MEXICO AND WESTERN ATLANTIC MARGIN	23
1. Introduction.....	23
2. Methods.....	25
2.1. Sample collection and shipboard processing	25
2.2. Histological Processing.....	28
2.3. Histological Analysis.....	29
2.4. Data Analysis	31
3. Results.....	32
3.1. Sexual dimorphism	32
3.2. Gametogenesis	34
3.3. Maturity Stages	34
3.4. Oocyte Sizes.....	35
3.5. Gametogenic Phenology	36

4. Discussion	41
III. PHYTODETRITUS AS A REPRODUCTIVE CUE FOR CHEMOSYNTHETIC MUSSELS AT METHANE SEEPS	46
1. Introduction.....	46
2. Methods.....	49
2.1. Mussel Collection	49
2.2. Stable Isotope Analysis.....	51
2.3. Surface Productivity.....	51
3. Results.....	54
3.1. Stable Isotopes	54
3.2. Relationship to Surface Productivity	60
4. Discussion	65
4.1. Phytodetritus as supplemental nutrition.....	65
4.2. Surface productivity as a reproductive cue.....	69
5. Conclusions.....	70
IV. FIRST ESTIMATES OF GAMETE SUBSIDIES FROM A DEEP CHEMOSYNTHETIC ECOSYSTEM TO THE PELAGIC FOOD WEB	72
1. Introduction.....	72
2. Methods.....	75
2.1. Mussel Beds	75
2.2. Fecundity.....	78
2.3. Egg Energetics	80
2.4. Data Analysis	83
3. Results.....	85
3.1. Mussel Bed Characteristics.....	85

3.2. Fecundity.....	89
3.3. Egg Sizes and Energy	92
3.4. Mussel Bed Spawning Energy	93
4. Discussion	96
4.1. Fecundity.....	96
4.2. Egg Energy.....	98
4.3. Bed Spawning Energy.....	100
5. Conclusion	102
V. GENERAL CONCLUSION	103
VI. REFERENCES CITED.....	106
VII. SUPPLEMENTAL FILES.....	121
Chapter II	121
Chapter III.....	132
Chapter IV.....	135

LIST OF FIGURES

Figure	Page
2.1. Map of collection sites color-coded by study species. Photos of adult mussels are all shown at the same scale.	26
2.2. Histological photos showing the stages of gametogenesis in females and males of <i>Bathymodiolus brooksi</i>	30
2.3. Shell length distributions of all individuals collected from each site.	33
2.4. Stacked bar graphs showing the distribution of maturity stages for females (F) and males (M) in each season at each site.	37
2.5. Population-level oocyte size frequency distributions for seep sites in the Gulf of Mexico sampled in three seasons.	38
2.6. Population-level oocyte size frequency distributions for seep sites in the Western Atlantic Margin sampled in three seasons.....	39
3.1. Map of methane seep sites, color-coded by species of mussel present. Grey boxes denote the areas in which chlorophyll-a and particulate organic matter of the ocean surface were evaluated	50
3.2. Carbon and nitrogen isotopic ratios. A) All data from all sites. B) Mean values $\pm$ standard deviation for sites in the Gulf of Mexico. C) Mean values $\pm$ standard deviation for sites in the Western Atlantic Margin.....	56
3.3. Boxplots of nitrogen and carbon isotopic ratios for tissues of <i>G. childressi</i> over three seasons. A) the Gulf of Mexico, and B) the Atlantic.....	59
3.4. Boxplots of nitrogen and carbon isotopic ratios for tissues of <i>B. heckeriae</i> from three seasons. F = February, J = June, O = October.	60
3.5. Monthly average ($\pm$ standard deviation) of chlorophyll-a (top) and particulate organic carbon (bottom) from 2003–2021 for the Gulf of Mexico (left) and U.S. Western Atlantic Margin (right).	61
3.6. Monthly average particulate organic carbon values in the Gulf of Mexico and the U.S. Western Atlantic Margin.....	62
3.7. Monthly average particulate organic carbon values from three years for the Gulf of Mexico and U.S. Western Atlantic Margin.	64

4.1.	Map of study sites.	76
4.2.	Representative photos and scaled area polygons of each mussel bed.	86
4.3.	Estimated average size frequency distributions of mussels per m ² at each site.....	88
4.4.	Estimated individual fecundity against body length A) The total number of ripe and developing oocytes. B) Annual fecundity with linear regression lines.....	90
4.5.	Average fecundity at each site for A) per mm of body length for a single female mussel and B) per square meter of each mussel bed.	91
4.6.	Boxplots showing the distribution of measurements for A) volume in nanoliters and B) energy in millijoules of spawned eggs from each site.	93
4.7.	The natural log of energy content (mJ) against volume (mm ³) for spawned eggs at each site.....	94
4.8.	The energy released by each mussel bed through spawning. A) Total value in gigajoules. B) The average nutrient density in megajoules per square meter.	95

LIST OF TABLES

Table	Page
2.1. Collection summary.....	27
2.2. Shell length comparison of males and females within each site.....	32
2.3. Comparison of oocyte sizes among collections at each site by season.....	40
3.1. Collection information. A) Site information and sample sizes for adult mussel collections. B) Coordinates for which surface productivity metrics were averaged.	53
3.2. Aggregated means ($\pm$ standard error), minimum, and maximum for A) $\delta^{13}\text{C}$ and B) $\delta^{15}\text{N}$ for all tissue types in each collection.....	55
3.3. Results of either 2-way ANOVAs (F) or non-parametric Sheirer-Ray-Hare (H) tests on the effects of season, tissue type (gill, foot, or gonad), and their interaction on carbon and nitrogen isotopic ratios.....	58
4.1. Site locations with collection dates and sample sizes.	77
4.2. Summary of data and equations used to estimate the total fecundity of each mussel bed.	81
4.3. Summary of data and equations used to estimate the total energy released through spawning for each mussel bed.	84
4.4. Mussel bed characteristics for each site.	87

CHAPTER I

GENERAL INTRODUCTION

1. BACKGROUND

Reproduction is a fundamental aspect of biology that ensures the continuation of life across many ecological scales. Organisms employ many different reproductive strategies, but they can be broadly characterized as asexual or sexual reproduction, depending on whether genetic material is exchanged between individuals. Sexual reproduction is the ancestral trait for all eukaryotes (Speijer et al., 2015) and is common among marine invertebrate taxa (Wangensteen et al., 2016). Gametogenesis, the creation of sex cells, is energetically costly to individuals, and the trade-offs between growth and reproduction are a core component of life-history theory (Audzijonyte & Richards, 2018). The energetic cost is significantly higher for the production of eggs than sperm and can be upwards of 300% higher than a female's basal metabolic rate (Hayward & Gillooly, 2011).

Successful reproduction, or creating offspring, requires more than just fertilization of gametes; the embryo must develop and survive to a juvenile stage. Benthic marine invertebrates often have a planktonic larval phase between embryo and juvenile. Reproductive phenology is thus optimized to ensure larval survival, and planktotrophic larvae are frequently released when larval food is available (Crisp, 1959). The success of larvae and juveniles is also aided by maternal effects, which are non-genetic effects of a mother's phenotype or environment on her offspring's phenotype (reviewed by Marshall et al., 2008). One of the most apparent maternal effects across taxa is offspring provisioning, which includes an egg's size and energy content (Bernardo, 1996; Thorson, 1950). An increase in egg size and energy generally leads to an increase in offspring size and fitness (Moran et al., 2013; Moran & McAlister, 2009). While there are many interspecific

differences in offspring provisioning, intraspecific plasticity has also been seen as a response to different environmental factors (Marshall et al., 2008). Reproductive provisioning can thus be variable between individuals and populations (Allen et al., 2008).

Even with adaptive changes in maternal effects to aid larval survival, most planktonic larvae die as a result of predation or environmental stress (Morgan, 1995; Rumrill, 1990; Thorson, 1950; Young & Chia, 1987). That means that most of the energetic investment in reproduction actually goes to the food web. Eggs are generally high in essential fatty acids, and large spawning events create temporary nutrient subsidies for other organisms (Fuiman et al., 2015). Gametes of both corals and fish are known to be critical trophic pathways on coral reefs (Allgeier et al., 2018; Pratchett et al., 2001), and fish spawning aggregations benefit many pelagic organisms (Fuiman et al., 2015). However, the role of reproductive products in trophic networks needs to be studied more and may constitute a significant source of energy flux through marine systems. Levin et al. (2016) suggested gametes may be an important vector for transporting energy from chemosynthetic systems in the deep sea.

2. STUDY SYSTEM

In the deep sea, chemosynthesis occurs in specialized habitats where primary production is driven by microbial oxidation of naturally occurring inorganic chemicals (Corliss et al., 1979; Corliss & Ballard, 1977) rather than photosynthesis. Chemosynthetic habitats also support dense invertebrate communities at hydrocarbon seeps, hydrothermal vents, and organic falls (Gage & Tyler, 1991; Tunnicliffe et al., 2003), with biomasses orders of magnitude above those from the abyssal plain (Van Dover, 2000). Communities in these habitats are often dominated by a small number of invertebrate species with symbiotic chemoautotrophic bacteria, such as mussels, tubeworms, and shrimp (Tyler et al., 2002).

The mussels in deep-sea chemosynthetic communities belong to the subfamily Bathymodiolinae, a highly specialized group within the Mytilidae family (Gustafson et al., 1998; Kenk & Wilson, 1985). Members of this group house chemoautotrophic symbionts in their gills, commonly sulfur- or methane-oxidizing (Duperron, 2010). Some species have two to six different types of symbionts as a way to cope with highly variable chemosynthetic environments (Distel et al., 1995; Fisher et al., 1993), and some have also retained the ability to filter feed (Page et al., 1991; Pile & Young, 1999).

Despite the varied symbioses and nutrition sources within bathymodiolin mussels, their reproductive patterns are similar to those of their shallow-water relatives (reviewed in Laming et al., 2018). Most species have periodic reproduction and spawning, which is thought to be tied to surface productivity in at least two species (Dixon et al., 2006; Tyler et al., 2007) despite their constant chemosynthetic food source. Bathymodiolin mussels are also similar to intertidal mytilids in that they create large beds that provide habitats for many associated animals (Kennicutt et al., 1985; MacDonald, Reilly, et al., 1990). Spawned eggs from intertidal mussel beds act as trophic subsidies to zooplankton (Kautsky, 1982), but the potential for energetic input to the water column from bathymodiolin eggs remains unstudied. Their prevalence, abundance, and importance to ecosystem functioning at chemosynthetic systems make bathymodiolins a critical group to study.

3. SCOPE AND AIMS

My goals with this dissertation project were to build upon my previous work on the reproductive patterns of bathymodiolin mussels from methane seeps (Plowman, 2017) and address two fundamental questions: (1) to what extent is reproduction supported by constant chemosynthetic food sources versus seasonally-pulsed phytodetritus? (2) how do reproductive processes contribute to water column carbon flux, and how does flux vary across depth? In short,

(1) how does the water column influence reproduction? (2) how does reproduction influence the water column? Samples from multiple seep sites were used in each study to investigate the degree of reproductive plasticity in bathymodiolin mussels and its potential drivers. Chapters II-IV are currently unpublished but will include Dr. Craig Young as a co-author as he funded the research, provided conceptual support, and will aid in preparing the final manuscripts.

In Chapter II, I start by describing the gametogenic phenology of six populations of bathymodiolin mussels from seep sites in the Gulf of Mexico and the northwestern Atlantic. Shallow sites (400 – 1070 m) housed beds of *Gigantidas childressi*, while deep sites (2160 – 3300 m) had *Bathymodiolus heckeræ*. At one site in the Gulf of Mexico (1020 m), *Bathymodiolus brooksi* co-occurred with *G. childressi*. My previous work showed evidence of periodic reproduction in the same populations with changes among sites (Plowman, 2017). The population of *G. childressi* from one site in the Gulf of Mexico (Brine Pool) is known to spawn roughly from October to February (Tyler et al., 2007). I used histological examinations of gonad material from seasonal samples to examine how reproductive phenology varied among species and across a depth range. The results indicate that drivers of reproductive timing for these deep-sea mussels operate on regional scales and support the idea that surface productivity plays a role in bathymodiolin reproduction.

Chapter III then tests two hypotheses for how surface productivity may affect the reproduction of deep-sea mussels. First, I hypothesized that adult mussels need the seasonal pulse of phytodetrital nutrients to build gametes. This idea has been supported in the vent mussel, *Bathymodiolus azoricus*, which showed histological evidence of increased feeding during the seasonal bloom in surface productivity (Dixon et al., 2006). I used carbon and nitrogen stable isotope ratios from mussel tissues to look for a seasonal shift in the input of phytodetrital nutrients.

These isotopes are commonly used in trophic studies (Hügler & Sievert, 2011; Minagawa & Wada, 1984). Nutrients derived from chemosynthetic sources have much more depleted isotopic ratios than those derived from photosynthetic sources (Brooks et al., 1987). I expected an increased signal of photosynthetic nutrients in the form of enriched isotopic ratios, particularly in gonad tissues, following the seasonal phytoplankton bloom.

Second, I hypothesized that surface productivity is a reproductive cue in seep mussels. Marine invertebrates that reproduce periodically rely on environmental cues to keep gametogenesis and spawning synchronized within a population (Smart et al., 2012). Therefore, if surface productivity was a reproductive cue for bathymodiolins, I expected the gametogenic phenology from Chapter I to correlate with peaks in surface productivity or changes therein. Surface productivity in the waters overlying the seep sites was determined using satellite-derived data for chlorophyll-a concentration (a proxy for phytoplankton abundance) and particulate organic carbon. Temporal changes in productivity were determined by examining almost two decades' worth of monthly composite data. This chapter will be published with an additional co-author, Dr. Laura McGee, who collaborated with me by processing the surface productivity data and will aid in preparing the final manuscript.

The main goal of Chapter IV was to estimate how much energy is released when a bed of bathymodiolin mussels spawn. This was done by examining one species, *Gigantidas childressi*, from three seep sites with different physical attributes. To make these energy estimates, I first needed to determine the number of eggs released, the energy content of those eggs, and the area and density of the mussel bed. I used histological examinations of gonad material to obtain the first quantitative fecundity estimates for any large bathymodiolin mussel. Adult mussels were injected with serotonin to induce spawning, and then egg energy was measured with bomb calorimetry. The

areas of each mussel bed were estimated in ArcGIS by drawing a polygon around all coordinate points where mussels had been visually confirmed during benthic vehicle dives. My lab mate, Avery Calhoun, conducted the work in ArcGIS as part of her graduate research. This study is the first quantitative estimate of the energy flux from gametes in a deep-sea chemosynthetic system and one of only a handful looking at eggs as trophic subsidies. Along with Dr. Craig Young, this manuscript will also have Dr. Shawn Arellano as a co-author for her assistance in conceptualizing the study, determining methodology, and collecting mussel eggs.

These chapters provide insight into the plasticity of reproductive timing and provisioning in bathymodiolin mussels and the connection between processes on the surface and the seafloor. Chapter V summarizes the broader patterns and implications of these results.

CHAPTER II

GAMETOGENIC PHENOLOGY OF BATHYMODIOLIN MUSSELS FROM METHANE SEEPS IN THE GULF OF MEXICO AND WESTERN ATLANTIC MARGIN

This chapter contains unpublished material coauthored with Craig Young, who funded and facilitated the study. We conceived the project together, but I was responsible for all sample collection and processing. I also conducted all the data analyses and wrote this manuscript with editorial input from Craig.

1. INTRODUCTION

The deep sea is generally sparsely populated with epibenthic megafauna, but dense and complex communities are found at chemosynthetic ecosystems such as organic falls, hydrothermal vents, and methane seeps (Corliss et al., 1979; Van Dover et al., 2002). These reducing ecosystems provide ephemeral and relatively small habitats, often isolated from each other but with high biomass and productivity (Girguis & Childress, 2006; McNichol et al., 2018). Mytilid mussels in the subfamily Bathymodiolinae are often among the dominant fauna in reducing habitats and form dense beds that are home to many other organisms (Gage & Tyler, 1991; Kennicutt et al., 1985; Macdonald et al., 1990). Their role as ecosystem engineers and near-ubiquitous presence makes bathymodiolin mussels a crucial group for advancing our understanding of chemosynthesis-based ecosystems.

The Bathymodiolinae at vents and seeps include three clades, each represented by a single genus: *Gigantidas*, *Bathymodiolus*, and “*Bathymodiolus*” (Zhang et al., 2021). Members of all clades obtain the bulk of their nutrition through thiotrophic and methanotrophic bacterial

symbionts living in their gills, with some species using a combination of both bacterial types (Duperron et al., 2007; Fiala-Medioni et al., 1986; Ritt et al., 2012; Streams et al., 1997). Supplemental filter feeding is also known to occur in one species of *Gigantidas* (Pile & Young, 1999) and one species of *Bathymodiolus* (Page et al., 1991).

Despite living in ecosystems with constant sources of chemosynthetic primary production, most bathymodiolins studied to date show evidence of periodic reproduction (reviewed by Laming et al., 2018). Their reproductive patterns generally resemble those of shallow-water mytilids (Eckelbarger & Young, 1999), which typically have seasonal gametogenic cycles, undergo broadcast spawning and external fertilization, and disperse with planktotrophic veliger larvae (Seed, 1976). The most well-studied seep species is *Gigantidas childressi*, with most studies conducted at the Brine Pool NR-1 seep site (650 m deep) in the Gulf of Mexico. The gonad of this species starts in the visceral mass and then extends into the mantle during a seasonal, synchronous gametogenic cycle (Tyler et al., 2007). These mussels broadcast their gametes and have embryonic development similar to that of other mytilids (Arellano & Young, 2009). Embryos develop into planktotrophic, swimming veliger larvae that have been found in surface waters (Arellano et al., 2014). The closely related *G. platifrons* from the Formosa cold seep (1120 m) in the South China Sea also reproduces seasonally (Zhong et al., 2020).

Most of the studied species of bathymodiolins are from hydrothermal vents and also exhibit seasonal reproduction (Comtet & Desbruyeres, 1998; Dixon et al., 2006; Le Pennec & Beninger, 2000; Rossi & Tunnicliffe, 2017; Salerno et al., 2005; Tyler & Young, 1999). The one exception to this trend is *B. thermophilus*, which is thought to have continuous gametogenesis (Berg, 1985; Hessler et al., 1988). They are effectively gonochoric, though protandry has been

reported in *B. elongatus* (Le Pennec & Beninger, 2000) and protogyny in *B. septemderium* (Rossi & Tunnicliffe, 2017), both of which live at hydrothermal vents. No gametogenic information currently exists for species of the genus *Bathymodiolus* from methane seeps.

While there is strong evidence for seasonal reproduction across the bathymodiolins, the drivers of seasonality still need to be better understood. Evidence suggests it may be linked to surface productivity (Dixon et al., 2006), which is correlated with the seasonal cycles of many abyssal plain species (Gage & Tyler, 1991). To understand potential cues, the full gametogenic phenology needs to be understood.

Here, we examine three species of seep mussels from the Gulf of Mexico and Western Atlantic Margin: *Gigantidas childressi*, *Bathymodiolus brooksi*, and *B. heckerae*. In an earlier study of these same species from fourteen seep sites in the same areas, we found evidence of seasonal reproduction in the two *Bathymodiolus* species and differences among sites (Plowman, 2017). The present study aimed to build on those results by examining fewer populations in more seasons to estimate the gametogenic phenology for each population and how that timing changes among species and sites.

2. METHODS

2.1. Sample collection and shipboard processing

Beds of bathymodiolin mussels were examined at three methane seep sites in the Gulf of Mexico (GoM) and three on the Western Atlantic Margin (WAM) of the U.S. (Figure 2.1; Table 2.1). The sites were chosen to represent paired shallow (400 – 650 m), mid (1020 – 1070 m), and deep (2160 – 3300 m) depths in both regions. The shallow (Brine Pool and Baltimore Canyon) and mid-depth (Mississippi Canyon and Chincoteague) sites in both basins had beds of *Gigantidas childressi*. At the mid-depth site in the GoM (Mississippi Canyon), patches of

Bathymodiolus brooksi were among patches of *G. childressi*. The deepest sites (Florida Escarpment and Blake Ridge) had beds of *Bathymodiolus heckeræ*. Sites were examined using either HOV *Alvin* deployed from R/V *Atlantis II* (expeditions AT24-42 and AT50-04) or ROV *Jason* deployed from R/V *Thomas G. Thompson* (expedition TN391), in three different seasons (spring, summer, fall) in 2020 – 2022 (Table 2.1). Adult mussels were scooped into a sealed, insulated collection box (“biobox”) on the vehicles to keep specimens in their natural seawater and in-situ temperatures until recovery.

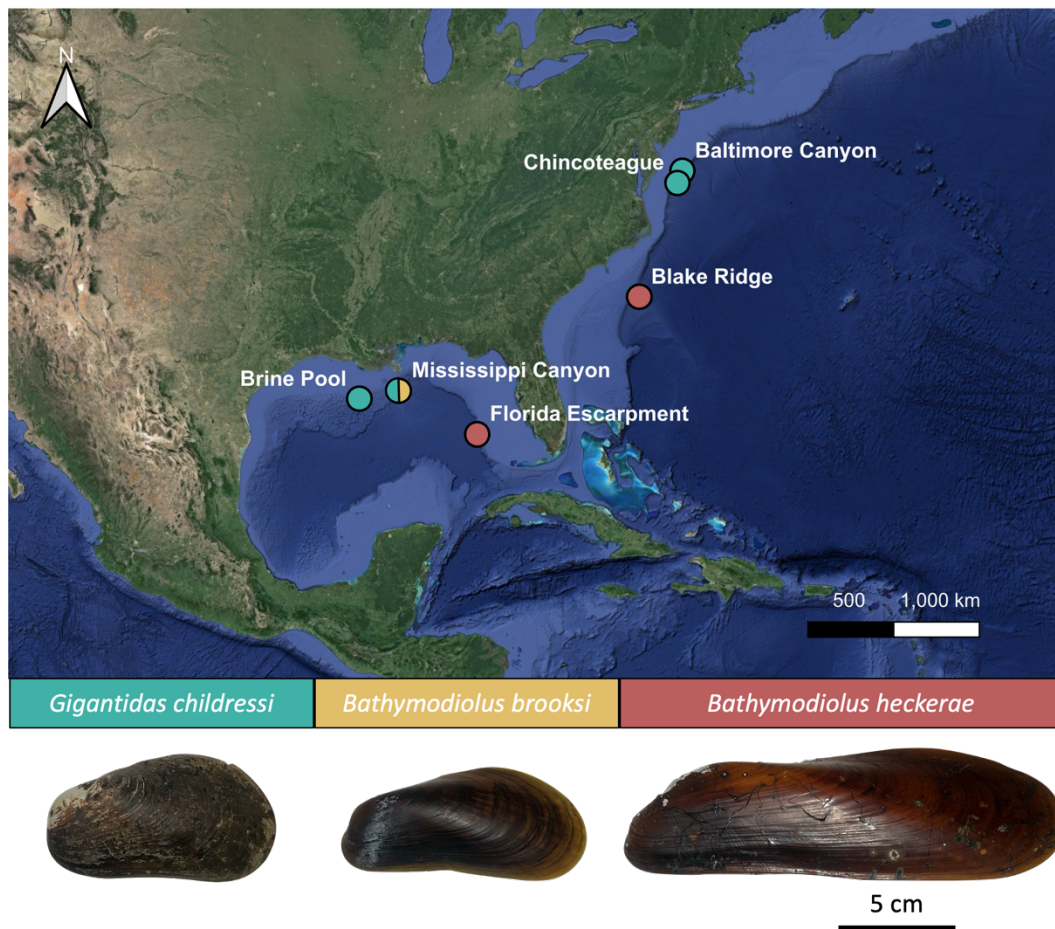


Figure 2.1. Map of collection sites color-coded by study species in the Gulf of Mexico and Western Atlantic Margin. Photos of adult mussels are all shown at the same scale.

Table 2.1. Collection summary.

Site Name	Depth (m)	Latitude (°N)	Longitude (°W)	Species	Spring (2020)		Summer (2021)		Fall (2022)		Total N	Male/ Female
					Date	N	Date	N	Date	N		
Brine Pool	650	27.72365	91.27955	<i>G. childressi</i>	25-Feb	50	13-Jun	50	16-Oct	60	160	0.65
Mississippi Canyon	1070	28.12204	89.13693	<i>G. childressi</i>	1-Mar	13	16-Jun	50	14-Oct	50	113	1.35
				<i>B. brooksi</i>	1-Mar	26	16-Jun	10	14-Oct	5	41	1.18
Florida Escarpment	3300	26.02884	84.91049	<i>B. heckerae</i>	3-Mar	85	10-Jun	50	22-Oct	50	185	1.57
Baltimore Canyon	400	38.04777	73.82258	<i>G. childressi</i>	11-Mar	51	28-May	36	27-Oct	60	147	0.97
Chincoteague	1020	37.54144	74.10192	<i>G. childressi</i>	11-Mar	50	29-May	50	27-Oct	48	148	1.36
Blake Ridge	2160	32.49424	76.19087	<i>B. heckerae</i>	8-Mar	51	4-Jun	50	30-Oct	50	151	1.39

Upon recovery, mussels were transferred into chilled, filtered seawater and stored in a cold room (4 °C or 7 °C) until processing. Ship-board processing occurred soon after collection (< 48 h). The maximum lengths (anterior to posterior), widths (left to right), and heights (dorsal to ventral) of closed mussel shells were measured. For Mississippi Canyon, the anterior length (distance between the umbo and the anterior edge) for each species was also recorded to aid in differentiating the two co-occurring species (Gustafson et al., 1998). The shells were removed, and individually labeled mussel bodies were preserved in 10% buffered formalin for later histological analysis.

2.2. Histological Processing

Samples of gonadal tissue from the visceral mass and the mantle were dissected and prepared for standard paraffin histology within 1-2 months of preservation. Samples were dehydrated in an ethanol series (70%, 80%, 90%, 95%) for 30 min in each concentration, then left overnight (~12 h) in 100% ethanol. They were then transferred into fresh 100% ethanol for 30 min to ensure no water was left before being transferred to toluene for 24 h of clearing. Samples were infiltrated in paraffin for three days in a 60 °C oven and then embedded in paraffin blocks. The blocks were trimmed by hand and then sliced at seven µm thickness on an AO Spencer 820 rotary microtome. Sections were allowed to expand in a warm water bath, then mounted on slides and left to dry and adhere overnight. Slides were stained with a modified version of Masson's Trichrome (Presnell & Schreibman, 1997) that used hematoxylin to stain nuclear material, Ponceau du xylin to stain cytoplasmic materials, and fast green to stain connective tissues. Coverslips were mounted using Permount® mounting medium. Two slides from each specimen, cut 150 µm apart, were examined.

2.3. Histological Analysis

Histological sections of both tissue types (gonad and mantle) from each female were photographed with a FLIR flea 3 camera attached to an Olympus BX50 compound microscope at 100x magnification. A stage micrometer was photographed at the same magnification as the slides and used for size calibration. Oocytes were measured using the program Fiji (Schindelin et al., 2012). Feret diameters were measured for 100 haphazardly selected oocytes from each female. Only those oocytes with clear nucleoli were measured to avoid measuring portions of the same oocyte from different sections and to standardize, as much as possible, the cross-sectional diameter of the oocyte (Grant & Tyler, 1983). Each individual (both male and female) was also assigned a subjective gametogenic maturity stage based on those described for *Gigantidas childressi* from the Brine Pool (Tyler et al., 2007):

Stage 1: Onset. Proliferation of oogonia or spermatogonia begins around the edge of the acinus (Figure 2.2).

Stage 2: Growth. Previtellogenic oocytes start to undergo vitellogenesis, or spermatids start appearing in the lumen (Figure 2.2).

Stage 3: Proliferation. Proliferation of vitellogenic oocytes on the periphery of the acinus or spermatids in the lumen (Figure 2.2).

Stage 4: Loading. Vitellogenic oocytes or spermatozoa start to fill the lumen (Figure 2.2).

Stage 5: Ripe. Lumen is packed with free oocytes or dense whorls of spermatozoa, and the individual is ready to spawn (Figure 2.2).

Stage 6: Post-spawning. Deflated acinus is surrounded by many somatic cells and contains a low number of un-spawned oocytes or peripheral spermatogonia (Figure 2.2).

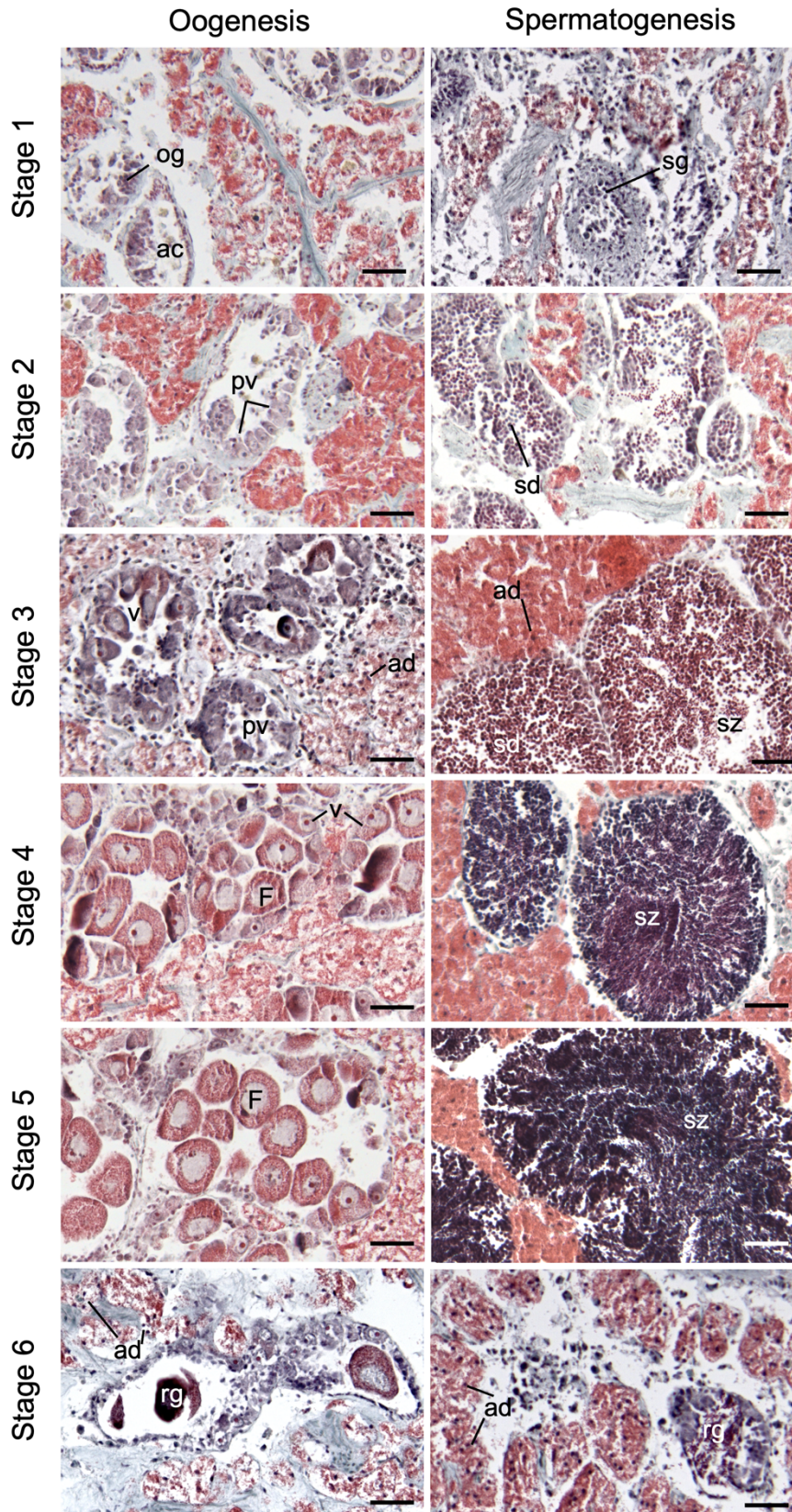


Figure 2.2. Histological photos showing the stages of gametogenesis in females and males of *Bathymodiolus brooksi*. Labels are: Acinus (ac), oogonia (og), spermatogonia (sg), previtellogenic oocyte (pv), spermatid (sd), vitellogenic oocyte (v), spermatozoa (sz), adipogranular cell (ad), free oocyte (f), residual gamete (rg). Scale bars are 50 μ m in all photos.

2.4. Data Analysis

A map of the study sites was created in QGIS version 3.34.3-Prizren (QGIS.org, 2024) using a Google Satellite base map. All statistical analyses and graphs were made in RStudio version 4.3.2 (R Core Team, 2023). All data were first tested for normality using the Shapiro-Wilk test and homogeneity of variances using Levene's test. Mussel shell sizes from all of the collection dates were pooled for each site. Two-sample Kolmogorov-Smirnov tests were then used to compare the sizes of males and females to test for sexual dimorphism. Oocyte sizes were used to generate oocyte-size-frequency distributions for each female mussel and averaged for each collection. For each site, a Kruskal-Wallis test was used to examine population-level differences among seasons and individual-level differences within each season. All were followed with a Dunn's posthoc test with Holm-adjusted p-values. In both cases, an effect size ($\eta^2[H]$) was calculated to compare the strength of seasonality between sites and the synchronicity of individuals within sites. Oocyte distributions from visceral mass and mantle tissues were compared within three sites with sufficient data (Brine Pool, Baltimore Canyon, and Chincoteague), using either a t-test or Wilcoxon rank-sum test, depending on normality and variance. Maturity stages for each sex were also compared among seasons within each site using chi-square contingency table analyses and stacked bar plots.

3. RESULTS

3.1. Sexual dimorphism

Males of *Gigantidas childressi* were significantly smaller than females for all sites except Brine Pool. Males were also significantly smaller than females for *B. heckerae* at both sites.

Bathymodiolus brooksi showed no evidence of sexual dimorphism, though the largest individuals collected were more often male than female. (Figure 2.3, Table 2.2)

Table 2.2. Shell length comparison of males and females within each site. Means $\pm$ standard deviation. KS Test = two-sample Kolmogorov-Smirnov tests. Significant p-values are bolded.

Site	Species	Mean Length (mm)		KS Test	
		Female	Male	D	p-value
Brine Pool	<i>G. childressi</i>	101 $\pm$ 12	96 $\pm$ 15	0.193	0.096
Mississippi Canyon	<i>G. childressi</i>	101 $\pm$ 17	89 $\pm$ 17	0.307	0.014
	<i>B. brooksi</i>	117 $\pm$ 51	164 $\pm$ 50	0.479	0.063
Florida Escarpment	<i>B. heckerae</i>	152 $\pm$ 33	121 $\pm$ 31	0.465	<0.001
Baltimore Canyon	<i>G. childressi</i>	88 $\pm$ 7	79 $\pm$ 10	0.423	<0.001
Chincoteague	<i>G. childressi</i>	105 $\pm$ 20	95 $\pm$ 18	0.316	0.001
Blake Ridge	<i>B. heckerae</i>	204 $\pm$ 72	150 $\pm$ 53	0.537	<0.001

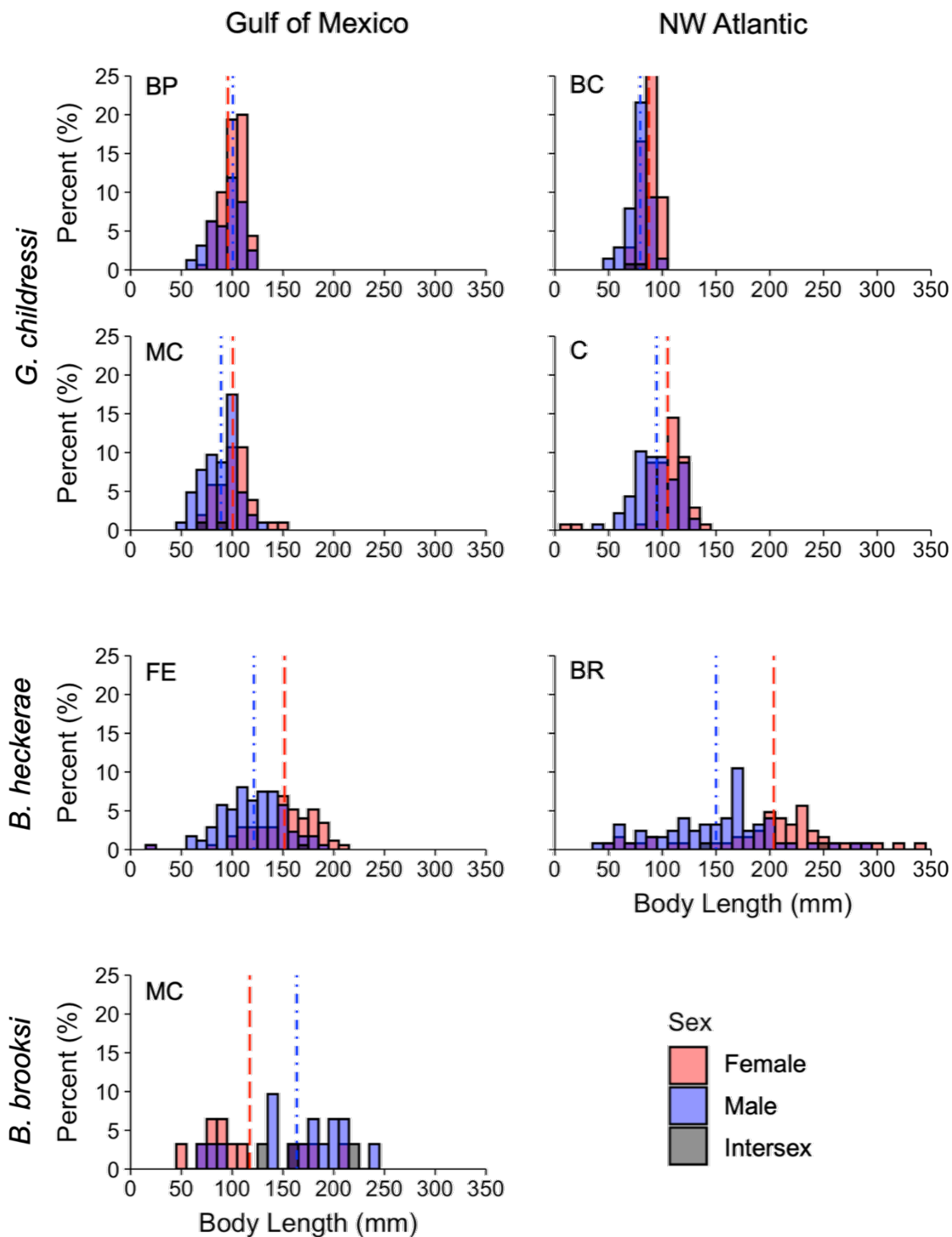


Figure 2.3. Shell length distributions of all individuals collected from each site. BP = Brine Pool; MC = Mississippi Canyon; FE = Florida Escarpment; BC = Baltimore Canyon; C = Chincoteague; BR = Blake Ridge.

3.2. Gametogenesis

All species examined were gonochoric, with a small number of intersexual individuals. Mississippi Canyon had the most, with 1.9% prevalence in *G. childressi* and 9.7% in *B. brooksi*. A single intersex individual of *B. heckerae* was found at Florida Escarpment (0.6%). The expression of intersexuality varied among observed individuals (Figure S2.1).

Based on histological sections, there were no obvious differences in the process of gametogenesis among the two genera and three species. Gonad material first appeared in the visceral mass and spread into the mantle. Gametogenesis often varied significantly among tissue types in *B. childressi* (Table S2.2). Acini, in which gametes were produced, started out fairly dispersed and became quite dense by the completion of gametogenesis. Oogonia and spermatogonia were found along the acinus walls, where they first divided into previtellogenic oocytes and spermatocytes. Spermatogenesis then proceeded with the proliferation of spermatids and spermatozoa that rapidly filled the lumen of the acinus, forming whorls (Figure 2.2). Oogenesis continued through vitellogenesis, where previtellogenic oocytes, still attached to the acinus wall, were packed with yolk material and increased in size. Oocytes undergoing vitellogenesis remained connected to the periphery by a stalk that thinned until releasing the primary oocytes into the lumen of the acinus (Figure 2.2). The spent stage of both sexes was characterized by thinning tissue and shrinking acini, often with leftover gametes in the lumen of some acini. In every species, some individuals were seen with both full (ready to spawn) and empty (already spawned) acini (Figure S2.2).

3.3. Maturity Stages

All species showed seasonal differences, indicating periodic gametogenesis, in the relative abundances of maturity stages of both sexes (Figure 2.4; Table S2.1). *Gigantidas*

childressi at the Brine Pool showed the highest gametogenic maturity in the fall. Males at the deeper Mississippi Canyon site followed a similar pattern, but the difference among females at Mississippi Canyon was not statistically significant over time (Table S2.1). The Atlantic populations of *G. childressi* were more mature in summer (Figure 2.4). Males of *B. brooksi* appeared to follow the same pattern as *G. childressi*, with advanced maturity in the fall (Figure 2.4). There was no statistical significance among the maturity stages of female *B. brooksi* over time (Table S2.1). Both the Gulf of Mexico and Atlantic populations of *B. heckerae* had the highest maturity in spring (Figure 2.4).

3.4. Oocyte Sizes

All species displayed a similar range of oocyte diameters ($\sim 10 - 80 \mu\text{m}$) and evidence of seasonal reproduction on the level of the population, with shifting, unimodal distributions (Figures 2.5 and 2.6). Oocyte sizes varied among seasons for all species, though the effect of season varied among sites (Table 2.3). The largest average oocyte sizes for *G. childressi* were seen in the fall for populations living in the Gulf of Mexico (Figure 2.5) and in the summer for populations in the Atlantic (Figure 2.6). Within each basin, the effect of seasonality on *G. childressi* oocyte sizes was strongest at the shallowest sites (Table 2.3). For *Bathymodiolus brooksi* and *B. heckerae*, all populations had the largest oocyte sizes in the fall (Figures 2.5 and 2.6). The populations of these species showed smaller effects of seasonality than *G. childressi*, except *B. heckerae* from Blake Ridge (2160 m) in the Atlantic.

The relatively narrow error in the population average oocyte size-frequency distributions indicates a synchronous population, which was confirmed by the individual oocyte size-frequency distributions (Figures S2.3–S2.8). There were significant differences among individuals in each collection, but the magnitude varied among seasons (Table S2.3). All of the

populations with the largest oocytes in fall (Brine Pool, Mississippi Canyon, Florida Escarpment, and Blake Ridge) had the least synchronicity among individuals in the spring (Table S2.3), when some individuals had residual ripe oocytes while undergoing early gametogenesis, and others were in a mid-development stage (e.g. Figure S2.7). Individuals at Baltimore Canyon showed the least synchronicity in summer, while the individuals at Chincoteague remained fairly synchronous in all seasons sampled.

3.5. Gametogenic Phenology

The combined data indicated different spawning seasons for each species, and within *Gigantidas childressi* from different regions. The Atlantic populations of *G. childressi* had the largest average oocyte sizes and highest maturity stages, indicative of the spawning season in the summer. The populations in the Gulf of Mexico showed a projected spawning season in the fall, with a lag in gametogenic development with depth. In every season sampled, the population average maturity stage values and oocyte sizes were smaller for *G. childressi* from Mississippi Canyon (1070 m) than those from Brine Pool (650 m). Similarly, the population of *Bathymodiolus brooksi* from Mississippi Canyon lagged in development compared to *G. childressi* from the same site.

Despite the regional differences, both populations of *B. heckeræ* showed evidence of an extended spawning period with the largest average oocyte sizes in fall and the highest maturity stages in spring. There was a relatively low effect of seasonality on the oocyte sizes of mussels from Florida Escarpment (3300 m, Gulf of Mexico) and no significant differences in the average maturity stages of females among the three seasons. The slightly shallower population at Blake Ridge (2160 m, US Western Atlantic Margin) showed a stronger effect of seasonality and more synchronicity among individuals than those from Florida Escarpment.

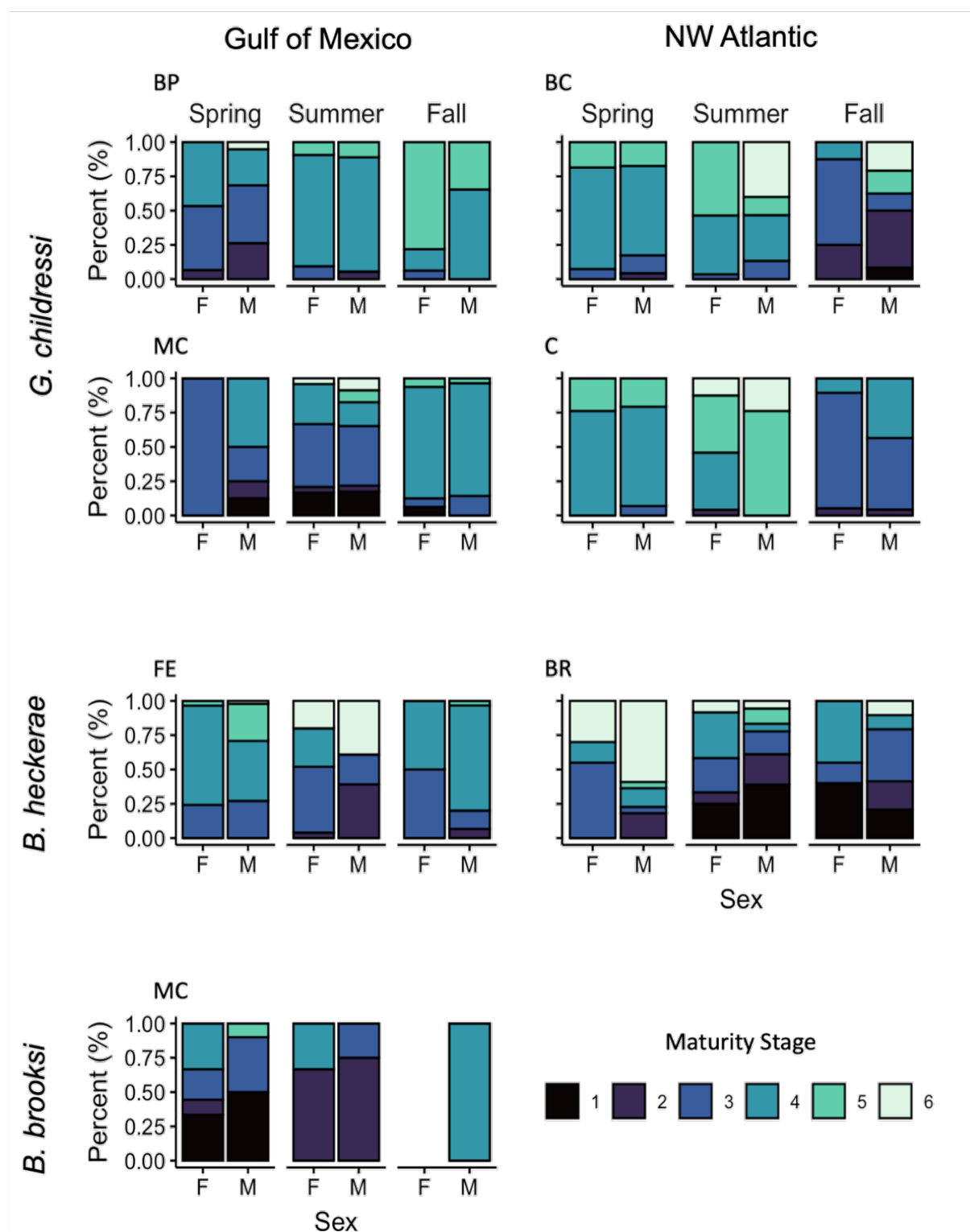


Figure 2.4. Stacked bar graphs showing the distribution of maturity stages for females (F) and males (M) in each season at each site. BP = Brine Pool; MC = Mississippi Canyon; FE = Florida Escarpment; BC = Baltimore Canyon; C = Chincoteague; BR = Blake Ridge.

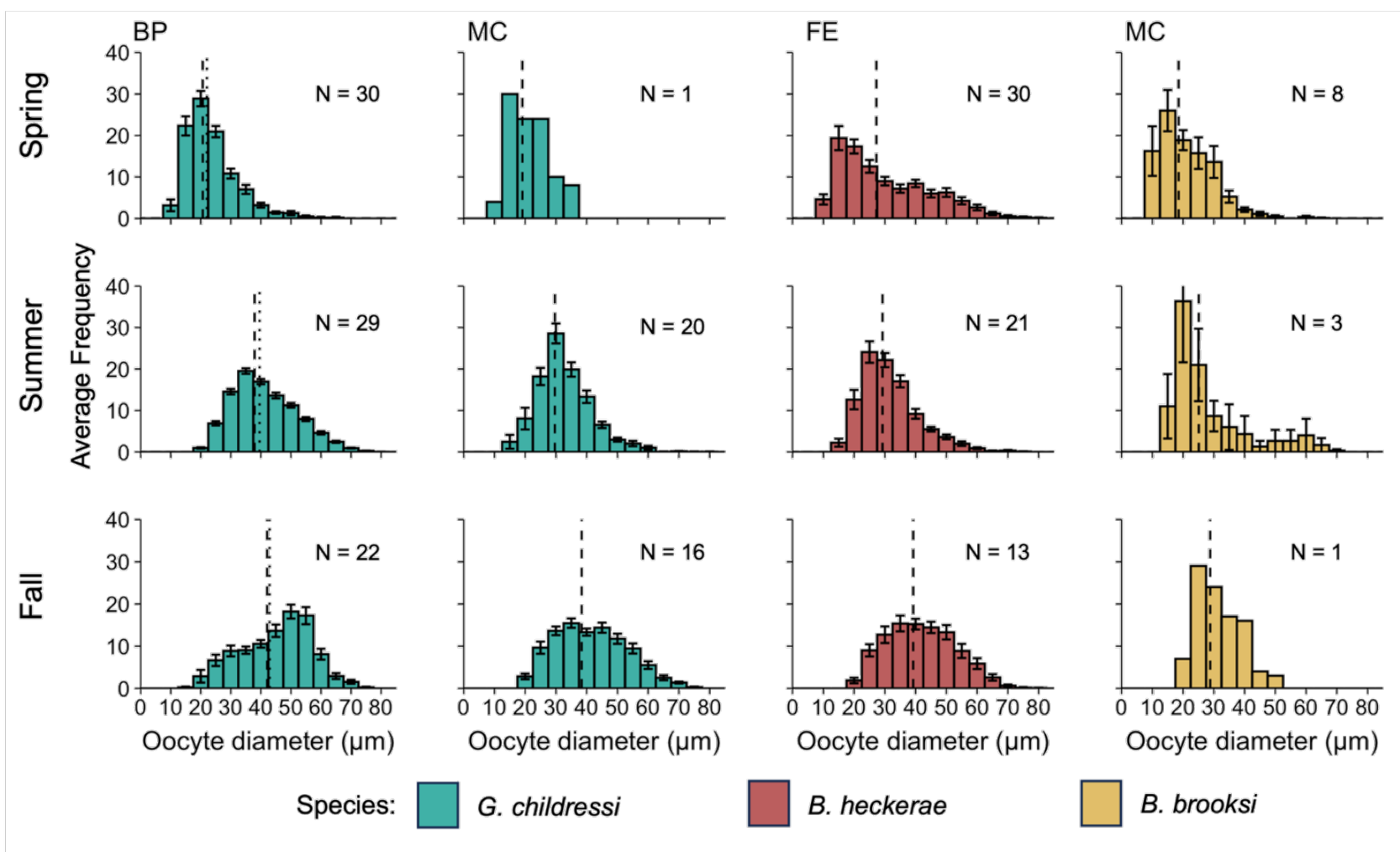


Figure 2.5. Population-level oocyte size frequency distributions for seep sites in the Gulf of Mexico sampled in three seasons. N = the number of females sampled. Error bars represent standard error. BP = Brine Pool; MC = Mississippi Canyon; FE = Florida Escarpment; BC = Baltimore Canyon; C = Chincoteague; BR = Blake Ridge.

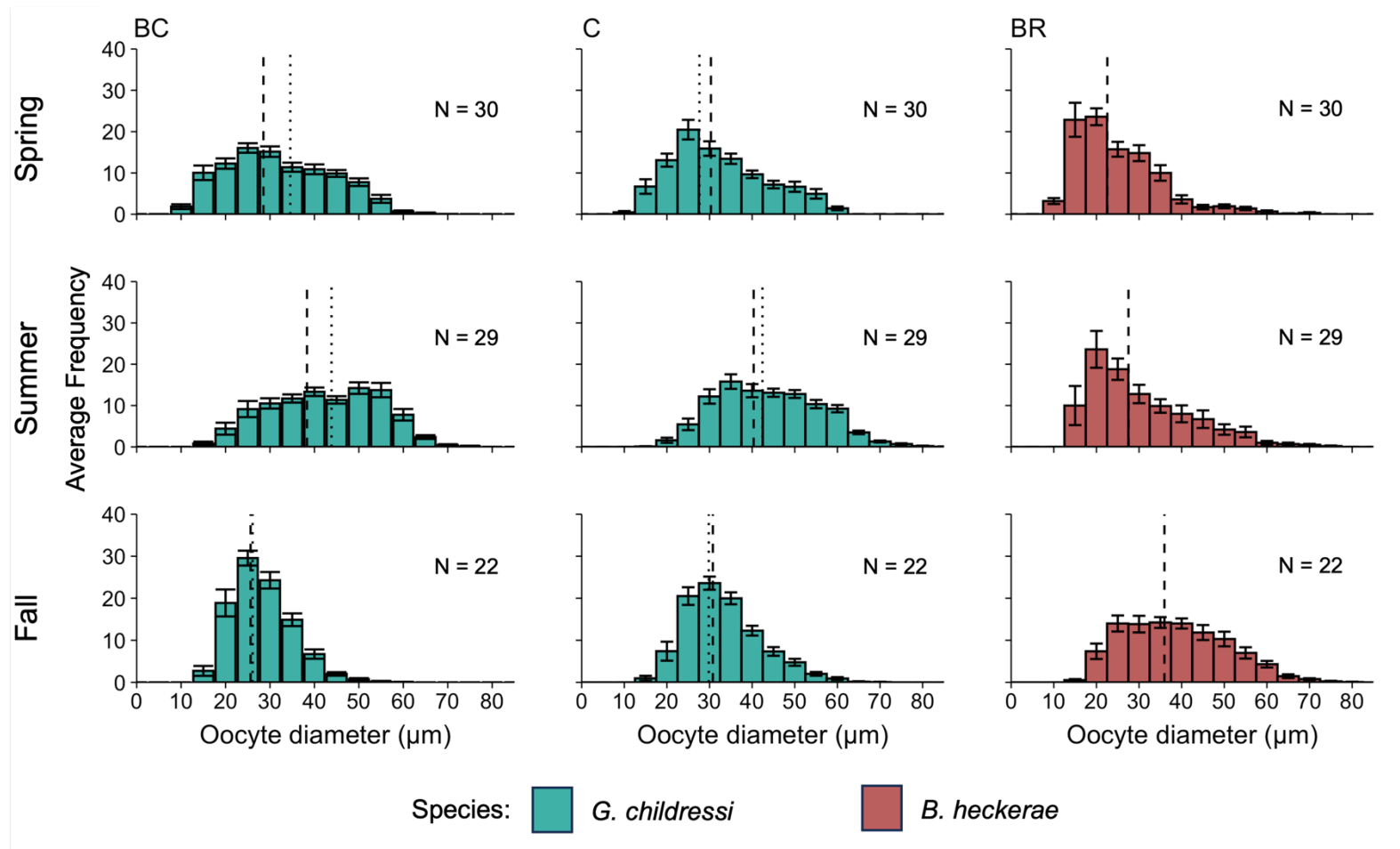


Figure 2.6. Population-level oocyte size frequency distributions for seep sites in the Western Atlantic Margin sampled in three seasons. N = the number of females sampled. Error bars represent standard error. BP = Brine Pool; MC = Mississippi Canyon; FE = Florida Escarpment; BC = Baltimore Canyon; C = Chincoteague; BR = Blake Ridge.

Table 2.3. Comparison of oocyte sizes among collections at each site by season. Medians are present with the interquartile range in parentheses, and means are $\pm$ standard deviation. KW Test = Kruskal-Wallis test; all p-values < 0.0001 , including results of Dunn's post-hoc tests. Effect sizes ($\eta^2[H]$) are an indication of the strength of seasonality at each site. Largest values for each site are bolded.

Site	Species	Median (IQR) Oocyte Diameter (μm)			KW Test		Mean $\pm$ SD Oocyte Diameter (μm)		
		Spring	Summer	Fall	H	$\eta^2[H]$	Spring	Summer	Fall
BP	<i>G. childressi</i>	19.24 (9.99)	37.45 (15.51)	44.22 (17.84)	3964.2	0.49	21.01 $\pm$ 8.66	38.74 $\pm$ 10.66	42.47 $\pm$ 11.77
MC	<i>G. childressi</i>	17.88 (10.36)	29.06 (10.66)	38.2 (17.77)	661.8	0.18	19.09 $\pm$ 6.82	30.17 $\pm$ 8.77	38.92 $\pm$ 11.69
MC	<i>B. brooksi</i>	16.89 (13.56)	20.51 (10.77)	27.5 (10.73)	165.1	0.14	18.56 $\pm$ 8.73	25.10 $\pm$ 12.27	28.82 $\pm$ 7.01
FE	<i>B. heckerae</i>	23.42 (22.14)	27.54 (11.94)	38.64 (16.56)	918.1	0.14	27.26 $\pm$ 14.31	29.22 $\pm$ 9.64	39.20 $\pm$ 11.09
BC	<i>G. childressi</i>	27.94 (18.60)	40.04 (19.73)	24.81 (9.25)	1499.4	0.21	29.34 $\pm$ 11.94	39.76 $\pm$ 12.22	25.67 $\pm$ 6.84
C	<i>G. childressi</i>	27.51 (15.87)	40.42 (18.22)	29.26 (11.64)	1077.3	0.18	29.75 $\pm$ 11.35	41.28 $\pm$ 11.89	30.73 $\pm$ 8.92
BR	<i>B. heckerae</i>	20.14 (13.12)	24.45 (17.24)	35.00 (18.25)	847.8	0.24	22.55 $\pm$ 10.19	27.52 $\pm$ 11.80	35.95 $\pm$ 11.97

4. DISCUSSION

In this study, we demonstrated that *Gigantidas childressi*, *Bathymodiolus brooksi*, and *B. heckarae* are all gonochoric with synchronous reproduction and sexual dimorphism. While the reproduction of *G. childressi* has been previously characterized, here we show that the process of gametogenesis is similar across the common bathymodiolins of the Gulf of Mexico. However, the degree of seasonality and the timing of the reproductive season vary across a bathymetric gradient and between populations in the Gulf of Mexico and Western Atlantic.

Sexual dimorphism with smaller males than females is a common pattern in species with protandric hermaphroditism, but all three species in this study are functionally gonochoric. Low levels of intersex individuals are fairly common in molluscs and other taxa and may be caused by environmental stress (Grilo & Rosa, 2017). Females reaching larger body sizes than males may be adaptive, given that fecundity tends to scale with body size. The well-studied intertidal mytilid *Mytilus edulis*, is also known to have sexual size dimorphism, with females growing faster per unit time and attaining a larger maximum body size (Mills & Côté, 2003). The lack of sexual size differences at the Brine Pool may be an artifact of sampling. The mussel bed at the Brine Pool has a known size distribution with smaller individuals closer to the brine and larger individuals at the edge of the bed (Arellano & Young, 2010; Macdonald et al., 1990; Smith et al., 2000). We consistently sampled from the middle of the bed (due to logistical constraints) and thus collected a narrow range of body sizes. The lack of sexual size dimorphism seen in *B. brooksi* at Mississippi Canyon may be an artifact of the small sample sizes.

The process of gametogenesis in all three species followed the published description for *G. childressi* (Tyler et al., 2007). These previous results were from the relatively shallow Brine Pool site, and we found that reproductive patterns for *G. childressi* hold true across its

bathymetric range. Our results also support previous limited evidence that the deeper living species *B. brooksi* and *B. heckerae* follow the same patterns as *G. childressi* (Plowman, 2017). The present study demonstrates that these species follow the predominant pattern of seasonal periodicity observed for most species in the *Bathymodiolus* group (Laming et al., 2018). The histological examination also provides some evidence for the possibility that individuals of these species can spawn multiple times during a reproductive season. Iterative spawning has also been observed in *Mytilus edulis* (Myint & Tyler, 1982) and has been suggested for *G. childressi* due to the roughly four-month spawning period at the Brine Pool seep (Tyler et al., 2007). However, *Bathymodiolus azoicus* from hydrothermal vents on the Mid-Atlantic Ridge has a single spawning event in January (Comtet et al., 1999; Comtet & Desbruyeres, 1998; Dixon et al., 2006). Here, all species showed periods where ovarian acini were both densely and sparsely filled with ripe oocytes during the proposed spawning seasons. A fully spent stage, with only a few remaining oocytes, has been seen in *G. childressi* in previous studies (Plowman, 2017; Tyler et al., 2007). Together, these observations suggest an iterative spawning pattern where a single female may spawn a portion of her total oocytes multiple times throughout the spawning season instead of releasing them all in the same spawning event.

The similarity in synchronous reproduction we observed across two genera and three species, as well as intertidal mytilids, suggests that reproductive periodicity may be phylogenetically constrained for the bathymodiolins (reviewed in Eckelbarger & Watling, 1995). For *G. childressi* populations, we observed a larger shift in phenology between the Gulf of Mexico and Western Atlantic than within, suggesting that a cue operating on large regional scales controls reproduction, with some effects of depth. The differences in estimated spawning seasons between the shallow seep sites in the two regions could thus be due to differences in the

seasonal cycles of surface productivity, while the slight differences between depths could be due to the increased sinking time for phytodetritus (Gooday & Turley, 1990). *Bathymodiolus heckerae*, the deepest species in this study, showed less pronounced seasonality than shallower *B. brooksi* and *G. childressi*. If seasonality in reproduction is indeed driven by phytodetrital input, there may be a weaker signal at depth due to less abundant and lower quality phytodetrital input.

These trends lend support to the idea that the gametogenic phenology is ultimately driven by phytodetrital input, which has been previously suggested for multiple bathymodiolin species (Dixon et al., 2006; Tyler et al., 2007) and is known to be the case for shallow water mussels like *Mytilus edulis* (Myint & Tyler, 1982), as well as other deep-sea animals (Young, 1994). Similar phenologic shifts in gametogenic timing have been seen among populations of *M. edulis* living in areas with different surface productivity patterns (Lowe et al., 1982; Newell et al., 1982). The observed phenotypic plasticity was later attributed to changes in cellular composition and nutrient storage (Lowe et al., 1994). Mytilid mussels use a variety of somatic cells to store nutrients, including adipogranular cells, which have been seen in high abundance in the inter-acinal tissue of *Gigantidas childressi* (Eckelbarger & Young, 1999). These storage cells allow mussels to store nutrients throughout the year and then use them quickly when conditions are favorable for gametogenesis (Eckelbarger & Hodgson, 2021; Lowe et al., 1994; Mathieu & Lubet, 1993). Some bivalves can also take advantage of increased food by transferring nutrients straight from the digestive system to developing gametes (Beninger et al., 2003).

For species that can grow and survive using chemosynthesis, it may seem counter-intuitive for the reproductive patterns to be set by surface productivity. However, the adults may require more nitrogen, or different essential amino or fatty acids, than what is available from methanotrophy alone to complete gametogenesis (Lee & Childress, 1996; Page et al., 1991; Pile

& Young, 1999). Alternatively, synchronizing reproduction to surface productivity could be adaptive to ensure there is enough food for planktotrophic larvae to develop, following the classical paradigm of Crisp's Rule (Qasim, 1956). Other potential cues for seasonality in the deep sea include temperature (Giese & Pearse, 1974; Orton, 1920), though seasonal changes in temperature at the depths in this study are smaller than those seen in shallow waters. There are also lunar changes in tides and currents in the deep sea that may impact animal behavior (Cuvelier et al., 2010; Girard et al., 2022), and more recent work has shown seasonal variations in deep-sea soundscapes (Lin & Kawagucci, 2024). Even if food is the driving factor, mussels may be responding to other changes in the environment that might forecast an increase in food availability the way light often does for shallow-water species.

Phenological studies such as this one are necessary to investigate the environmental factors driving biological patterns properly. Phenotypic data for deep-sea species, especially those from chemosynthetic systems, is generally lacking (Girard et al., 2022). While this study cannot elucidate definitively which cues are driving seasonal reproduction in cold-seep mussels, the estimated phenological patterns can now be tested against specific environmental factors, such as phytodetrital flux. Differences in phenology among species, sites, and depths observed here also have implications for understanding larval dispersal and site connectivity, as dispersal models on western Atlantic cold seeps typically only simulate a fall spawning (McVeigh et al., 2017; Young et al., 2012; Zhang et al., 2021). Understanding the environmental drivers of deep-sea reproduction will inform predicted impacts of environmental change and may become useful for effective conservation in the deep ocean.

BRIDGE

Chapter II showed different gametogenic phenologies between populations of seep mussels in the Gulf of Mexico and northwestern Atlantic, with a small effect of depth. Those results lend even more support to the idea that surface productivity plays a role in the reproduction of bathymodiolin mussels. Chapter III explores this connection further by looking for correlations between the observed phenologies and the patterns in productivity in the surface areas over the studied seep sites. The role of phytodetritus as a trophic subsidy to aid reproduction is also investigated using stable isotopes.

CHAPTER III

PHYTODETRITUS AS A REPRODUCTIVE CUE FOR CHEMOSYNTHETIC MUSSELS AT METHANE SEEPS

This chapter contains previously unpublished material with co-authors Laura McGee and Craig Young. I conducted all of the mussel sampling, processing, and analysis. Laura obtained the satellite-derived productivity data and condensed it to monthly averages. Craig acquired funding for the project and connected me with Laura. I wrote the manuscript with editorial support from both Craig and Laura.

1. INTRODUCTION

Cold seeps are areas of natural hydrocarbon seepage found along continental margins worldwide (Kiel & Tyler, 2010). Many seeps host dense, endemic communities (Gage & Tyler, 1991) supported by chemosynthetic bacteria that flourish year-round by oxidizing methane (methanotrophy) or hydrogen sulfide (thiotrophy) (MacAvoy et al., 2002). Due to the constant nutrition source, many animals that rely on symbiotic chemosynthetic bacteria display continuous reproduction. However, animals on the slope with planktotrophic larvae are notable exceptions (reviewed by Young, 2003). Bathymodiolin mussels, foundation species at many vents and seeps, display periodic reproduction over a wide depth range (reviewed by Laming et al., 2018).

The first cold-seep community was found on the Florida Escarpment (Paull et al., 1984), and the Gulf of Mexico seeps are the best-studied (Cordes et al., 2010; Levin et al., 2016). There are also hundreds of methane seeps off the U.S. Atlantic Margin (Skarke et al., 2014), and many

of them host dense communities of bathymodiolin mussels and associated fauna (Bourque et al., 2016; Mcveigh et al., 2018; Quattrini et al., 2015). The most common bathymodiolin mussels (subfamily Bathymodiolinae) in both regions are *Gigantidas childressi* and *Bathymodiolus heckerae*. A third species, *B. brooksi*, is also found in the Gulf of Mexico. All three species have seasonal reproduction, though seasonality appears less pronounced in the deepest species, *B. heckerae* (Chapter II). Reproductive periodicity in these mussels is thought to be driven by surface productivity and the resulting phytodetrital flux to the seafloor (Plowman, 2017; Tyler et al., 2007). The hydrothermal vent mussel, *B. azoricus*, has a seasonal cycle, where the onset of gametogenesis follows a period of apparently increased feeding that corresponds to the surface chlorophyll-a maximum (Dixon et al., 2006). That pattern would suggest that adult mussels rely on seasonal phytodetrital input to obtain energy for reproduction.

The shallowest seeps in the Gulf of Mexico (GoM) and along the Western Atlantic Margin (WAM) are dominated by *Gigantidas childressi*. Most populations of this species rely on a single species of methanotrophic symbiont (Duperron et al., 2007, 2013; Kellermann et al., 2012), though individuals in some populations may also host a thiotrophic symbiont (Assié et al., 2016; Coykendall et al., 2019). *Bathymodiolus brooksi* can co-occur with either *G. childressi* or *B. heckerae*. Thiotrophic symbionts are found in both *B. brooksi* and *B. heckerae*, though the former only hosts one phylotype while the latter hosts two, as well as a fourth methylotrophic symbiont capable of oxidizing many C1 compounds but not methane (Duperron et al., 2007, 2009). All three species have retained the capacity to filter feed, though filter feeding has only been experimentally demonstrated in *G. childressi* (Page, 1990; Pile & Young, 1999).

Isotopic studies have also shown evidence of mixotrophy for populations of *G. childressi* from the Gulf of Mexico (Riekenberg et al., 2016) and the Atlantic Margin (Demopoulos et al.,

2019). Carbon isotopic ratios ($\delta^{13}\text{C}$) vary with carbon source and can thus be used to identify sources of primary production and their relative contributions to a food web (Hügler & Sievert, 2011). Photosynthetic material from surface waters generally ranges in $\delta^{13}\text{C}$ from -24 to -22 ‰, while chemosynthetic material ranges from -75 to -28 ‰ (Brooks et al., 1987; Paull et al., 1985). The carbon isotope ratios for methane depend on how the organic material was initially degraded into methane, ranging from -80 to -60 ‰ for biogenic methane and -55 to -30 ‰ for thermogenic methane (Roberts & Aharon, 1994). Nitrogen isotopic ratios ($\delta^{15}\text{N}$) can be used to infer trophic positions (Minagawa & Wada, 1984) and identify inorganic nitrogen sources like ammonium (<0 ‰) and nitrate (5-7 ‰) (Lee & Childress, 1995, 1996; Riekenberg et al., 2016). Surface-derived nutrients have nitrogen ratios >6 ‰ (Saino & Hattori, 1987). Previous isotopic studies of *G. childressi* showed spatial differences that can be attributed to different methane sources (Becker et al., 2010, 2013; Cordes et al., 2005; Demopoulos et al., 2019). Riekenberg et al. (2016) also found temporal variation in the isotopic ratios of *G. childressi* populations in the Gulf of Mexico. They suggested using isotopes to look for the assimilation of detrital nutrients during surface blooms or gametogenesis. Such a seasonal increase in nutrition from phytodetritus has been demonstrated in the reproductive tissues of abyssal echinoids with seasonal reproduction using stable isotopes (Stevenson & Mitchell, 2016). Continuously reproducing echinoids in the same study did not have seasonal changes in stable isotope ratios.

The present study examines the role of phytodetrital nutrient input in the reproduction of the three common seep mussels in the Gulf of Mexico and the U.S. Western Atlantic Margin. We tested two hypotheses about the bathymodiolin mussels in these two regions: 1) they rely on phytodetritus as a supplementary nutrition source for reproduction, and 2) that surface primary production acts as a reproductive cue. To examine nutrition sources for gametogenesis, we

quantified the carbon and nitrogen isotopic ratios in gill, foot, and gonad tissues during spring, summer, and fall. Chemosynthetic symbionts reside in bathymodiolin gills, so this tissue was used as a control. Isotopic ratios in gonad tissue represent nutrient uptake and energy supporting reproduction, while those in the foot represent general somatic growth. We also examined surface chlorophyll-a and particulate organic carbon measurements in both regions to look for seasonal and interannual patterns that might correspond to seasonal changes in the isotopic ratios of the mussels. If the mussels rely on phytodetritus as supplemental nutrition, we expect to see an enrichment of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, especially in the gonad, following a surface phytoplankton bloom. If surface productivity and the resulting phytodetrital flux act as a reproductive cue, a peak or change in phytodetrital flux may trigger the onset of gametogenesis or spawning. Spawning when productivity is high ensures that planktotrophic larvae have access to abundant food, following the classic paradigm of Crisp's Rule (Qasim, 1956). By relating reproductive patterns to oceanographic factors, this study investigates the role that surface productivity plays in the reproductive patterns of deep-sea mussels.

2. METHODS

2.1. Mussel Collection

Adult mussels were collected from three methane seep sites in the Gulf of Mexico (GoM) and three in the Western Atlantic Margin (WAM) (Figure 3.1). While the specific depths varied, each region had a shallow and mid-depth site with *Gigantidas childressi* and a deep site with *Bathymodiolus heckeræ* (Table 3.1A). Collections were made in the spring of 2020 using HOV *Alvin* (deployed from R/V Atlantis II on expedition AT24-42), in the summer of 2021 with ROV *Jason* (deployed from R/V Thomas G. Thompson on expedition TN391), and in the fall of 2022 with *Alvin* (expedition AT50-04).

Mussels were collected into insulated “bioboxes” and transferred into chilled, filtered seawater upon recovery to the ship. They were then stored in a cold room (7 °C for shallow sites and 4 °C for mid- and deep-water sites). To investigate the sources of nutrition used for gametogenesis, samples of gill, gonad (from the visceral mass), and foot tissues were collected from 10 mussels at each site during each sampling expedition. These samples were flash-frozen to -80 °C.

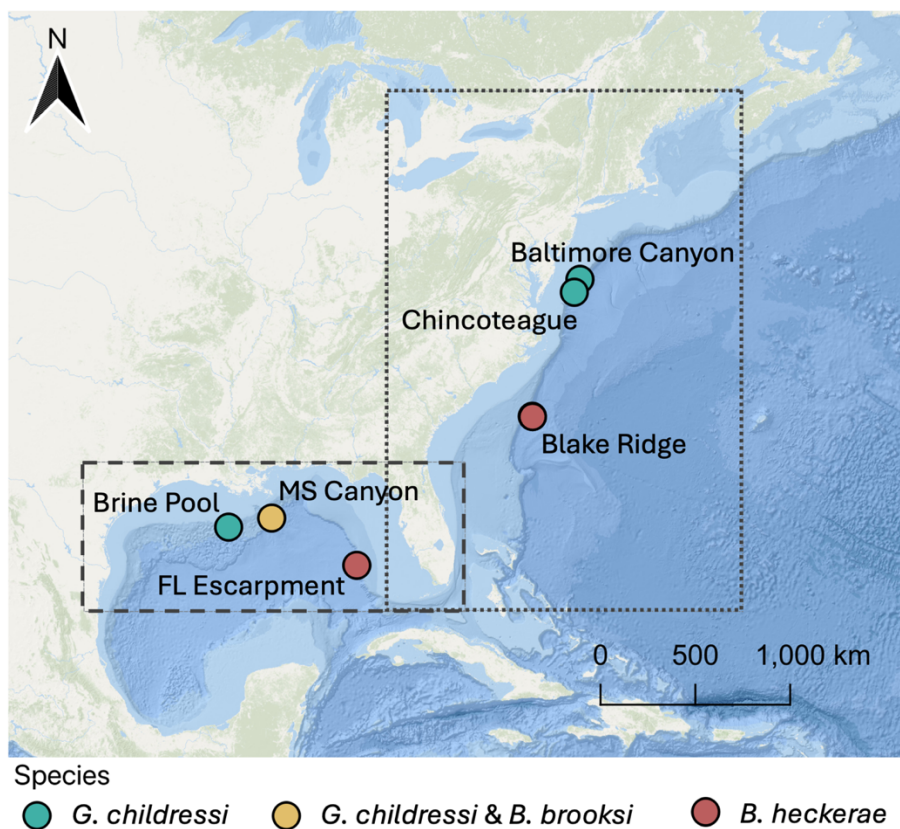


Figure 3.1. Map of methane seep sites sampled in this study, color-coded by species of bathymodiolin mussel present. Grey boxes denote the areas in the Gulf of Mexico (dashed) and Western Atlantic (dotted) in which chlorophyll-a and particulate organic matter of the ocean surface were evaluated. Map made in QGIS version 3.34.3-Prizren using the ESRI Ocean base map.

2.2. Stable Isotope Analysis

Tissue samples were dried for 48 h using a Labconco FreeZone lyophilizer, ground into a homogeneous powder, packaged into tin capsules, and sent to the Core Stable Isotope Laboratory at Washington State University for carbon and nitrogen isotopic measurements. Continuous flow–elemental analysis–isotopic ratio mass spectrometry with an ECS 4010 elemental analyzer (Costech Analytical, Valencia, CA) and a Delta PlusXP isotope ratio mass spectrometer (ThermoFinnigan, Bremen) was used to measure the isotopic ratios. Data were calibrated to NIST (National Institute of Standards and Technology) reference materials interspersed with sample materials during analysis. These ratios were reported in ‰, using the standard δ notation (Coplen, 2011), relative to the standards Vienna Pee Dee Belemnite for carbon ($\delta^{13}\text{C}$) and Atmospheric Air for nitrogen ($\delta^{15}\text{N}$).

Isotope values for each site were checked for normality using Shapiro-Wilks tests and variance with Levene's tests. Depending on those results, either a 2-way ANOVA or a nonparametric Sheirer-Ray-Hare test was used to look for effects of tissue type and season. Omnibus tests were followed by a Tukey HSD or Dunn's posthoc test to examine how isotopic ratios compared among tissue types within and across seasons. Isotopic analyses and figures were all made in RStudio version 4.3.2 (R Core Team, 2023).

2.3. Surface Productivity

Data for chlorophyll-a (CHL, mg m^{-3}) and particulate organic carbon (POC, mg m^{-3}) in surface waters were retrieved from the Moderate Resolution Imaging Spectroradiometer (MODIS) Aqua Level-3 Standard Mapped Image, monthly composite, 4 km products (NASA Ocean Biology Processing Group, 2018b, 2018a). The data were gathered from 1 Jan 2003 to 31 Dec 2021 for the Gulf of Mexico and Western Atlantic Margin overlying the studied seep sites

(Figure 3.1; Table 3.1B). CHL is a proxy for phytoplankton abundance and production, while POC measures biologically available carbon and accounts for zooplankton fecal pellets (Stukel et al., 2022; Turner, 2015).

Surface productivity data were visualized using Python's matplotlib package. Data was averaged spatially over each region (Table 3.1B). Since POC is more biologically relevant for deep-sea organisms, monthly averages were plotted for all 18 years to examine seasonal and inter-annual variation. Monthly averages from 2003, 2014/15, and 2020 were also compared to the estimated spawning seasons for the shallow-seep mussel populations in each region (Chapter II). These years were chosen to correspond with data from previous studies. Sample for the first gametogenic study of *G. childressi* was collected in 2003 (Tyler et al., 2007); preliminary gametogenic studies for all three species investigated here were conducted in the Gulf of Mexico in 2014, and the Western Atlantic Margin in 2015 (Plowman, 2017), and the first samples for this study were obtained in 2020.

Table 3.1. Collection information. A) Site information and sample sizes for adult mussel collections. B) Coordinates for which surface productivity metrics were generated and averaged. GoM = Gulf of Mexico, WAM = Western Atlantic Margin. S = shallow sites, M = mid-depth sites, D = deep sites. *G.c* = *Gigantidas childressi*, *B.b* = *Bathymodiolus brooksi*, *B.h* = *B. heckerae*.

A						Spring 2020		Summer 2021		Fall 2022		Total N
Site	Region	Depth (m)	Latitude (°N)	Longitude (°W)	Species	Date	N	Date	N	Date	N	
Brine Pool	GoM	650 - S	27.72365	91.27955	<i>G.c</i>	25-Feb	6	13-Jun	10	16-Oct	10	26
Mississippi Canyon	GoM	1070 - M	28.12204	89.13693	<i>G.c</i>	1-Mar	5	16-Jun	10	14-Oct	14	29
					<i>B.b</i>	1-Mar	6	16-Jun	9	14-Oct	0	15
Florida Escarpment	GoM	3300 - D	26.02884	84.91049	<i>B.h</i>	3-Mar	8	10-Jun	10	22-Oct	10	28
Baltimore Canyon	WAM	400 - S	38.04777	73.82258	<i>G.c</i>	11-Mar	10	28-May	9	27-Oct	9	28
Chincoteague	WAM	1020 - M	37.54144	74.10192	<i>G.c</i>	11-Mar	9	29-May	10	27-Oct	10	29
Blake Ridge	WAM	2160 - D	32.49424	76.19087	<i>B.h</i>	8-Mar	10	4-Jun	10	30-Oct	11	31

B				
Region	Latitude (°N)		Longitude (°W)	
	Min	Max	Min	Max
GoM	23.97	30.56	98.56	79.55
WAM	23.97	45.08	83.45	65.78

3. RESULTS

3.1. Stable Isotopes

Mussels belonging to all three species had isotopic ratios indicative of biogenic methanotrophy in gill, foot, and gonad tissues, though there were differences among sites (Figure 3.2). Isotope ratios were highly variable across sites; $\delta^{13}\text{C}$ ranged from -33 to -84 ‰ and $\delta^{15}\text{N}$ from -19 to 5 ‰ (Table 3.2). Mussels from the U. S. Western Atlantic Margin sites consistently had more enriched nitrogen values than those in the Gulf of Mexico (Figure 3.2A). In both regions, the shallow and mid-depth sites were more similar to each other than either was to the deep site. Nitrogen ratios were enriched in all tissues with increased depth in both regions. The two areas have opposite trends in carbon ratios with depth, where the Gulf of Mexico has more enriched carbon in the mid-depth site, while the deepest site in the Atlantic is the most enriched.

The effects of seasonality on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ also differed among sites, even within species. Populations at the Brine Pool (650 m, GoM, *G. childressi*), Chincoteague (1020 m, WAM, *G. childressi*), and Blake Ridge (2160 m, WAM, *B. heckeræ*) all showed significant seasonal changes in both carbon and nitrogen enrichment (Table 3.3; Figure 3.2B-C). Mussels at Mississippi Canyon (1070 m, GoM) had differing trends for the two co-occurring species, with some significant seasonal shifts in *G. childressi* but none in *B. brooksi* (Figure 3.2B; Table S3.1). No seasonal shifts in isotopic ratios were seen for mussels at Baltimore Canyon (500 m, WAM, *G. childressi*) or Florida Escarpment (3300 m, GoM, *B. heckeræ*) (Tables 3.3 and S3.1).

Table 3.2. Aggregated means ($\pm$ standard error), minimum, and maximum for A) $\delta^{13}\text{C}$ and B) $\delta^{15}\text{N}$ for all tissue types in each collection. *G.c* = *Gigantidas childressi*, *B.b* = *Bathymodiolus brooksi*, *B.h* = *B. heckeræ*. Sites above the dotted line are in the Gulf of Mexico, while those below are in the Western Atlantic Margin.

A		Spring			Summer			Fall		
Site	Species	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
Brine Pool	<i>G.c</i>	-65.89 $\pm$ 0.62	-71.15	-62.26	-61.98 $\pm$ 0.34	-65.67	-58.13	-60.47 $\pm$ 0.32	-64.02	-56.9
Mississippi Canyon	<i>G.c</i>	-54.44 $\pm$ 0.42	-57.21	-51.97	-60.39 $\pm$ 0.52	-65.33	-54.31	-55.87 $\pm$ 0.28	-59.45	-52.71
Mississippi Canyon	<i>B.b</i>	-54.02 $\pm$ 0.66	-57.59	-47.34	-55.99 $\pm$ 0.53	-62.87	-51.71	—	—	—
Florida Escarpment	<i>B.h</i>	-73.11 $\pm$ 0.54	-77.56	-69.31	-73.77 $\pm$ 0.86	-83.92	-65.52	-69.23 $\pm$ 0.79	-80.05	-64.74
Baltimore Canyon	<i>G.c</i>	-64.41 $\pm$ 0.30	-66.91	-58.96	-63.79 $\pm$ 0.42	-67.95	-59.06	-63.82 $\pm$ 0.24	-65.77	-61.25
Chincoteague	<i>G.c</i>	-70.81 $\pm$ 0.46	-76.19	-67.83	-69.04 $\pm$ 0.42	-73.29	-64.42	-65.23 $\pm$ 0.42	-67.37	-63.47
Blake Ridge	<i>B.h</i>	-41.47 $\pm$ 0.64	-52.09	-33.92	-49.68 $\pm$ 0.50	-56.78	-43.6	-48.70 $\pm$ 0.86	-58.12	-33.23

B		Spring			Summer			Fall		
Site	Species	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
Brine Pool	<i>G.c</i>	-17.66 $\pm$ 0.14	-18.97	-16.73	-17.24 $\pm$ 0.10	-18.44	-16.28	-16.82 $\pm$ 0.12	-17.98	-15.56
Mississippi Canyon	<i>G.c</i>	-16.37 $\pm$ 0.20	-17.45	-15	-14.13 $\pm$ 0.34	-17.35	-11.85	-16.65 $\pm$ 0.11	-18.06	15.5
Mississippi Canyon	<i>B.b</i>	-15.77 $\pm$ 0.24	-17.23	-13.86	-14.17 $\pm$ 0.38	-18.12	-11.54	—	—	—
Florida Escarpment	<i>B.h</i>	-9.54 $\pm$ 0.34	-12.85	-6.59	-9.35 $\pm$ 0.38	-14.25	-5.88	-9.05 $\pm$ 0.33	-12.06	-6.57
Baltimore Canyon	<i>G.c</i>	-0.84 $\pm$ 0.14	-2.24	0.51	-1.50 $\pm$ 0.09	-2.33	-0.15	-0.89 $\pm$ 0.10	-2.08	-0.19
Chincoteague	<i>G.c</i>	0.78 $\pm$ 0.13	-0.4	1.59	0.07 $\pm$ 0.19	-1.64	2.46	-0.52 $\pm$ 0.10	-1.65	0.37
Blake Ridge	<i>B.h</i>	2.60 $\pm$ 0.22	0	4.59	1.49 $\pm$ 0.24	-1.15	4.28	1.90 $\pm$ 0.17	-0.59	3.72

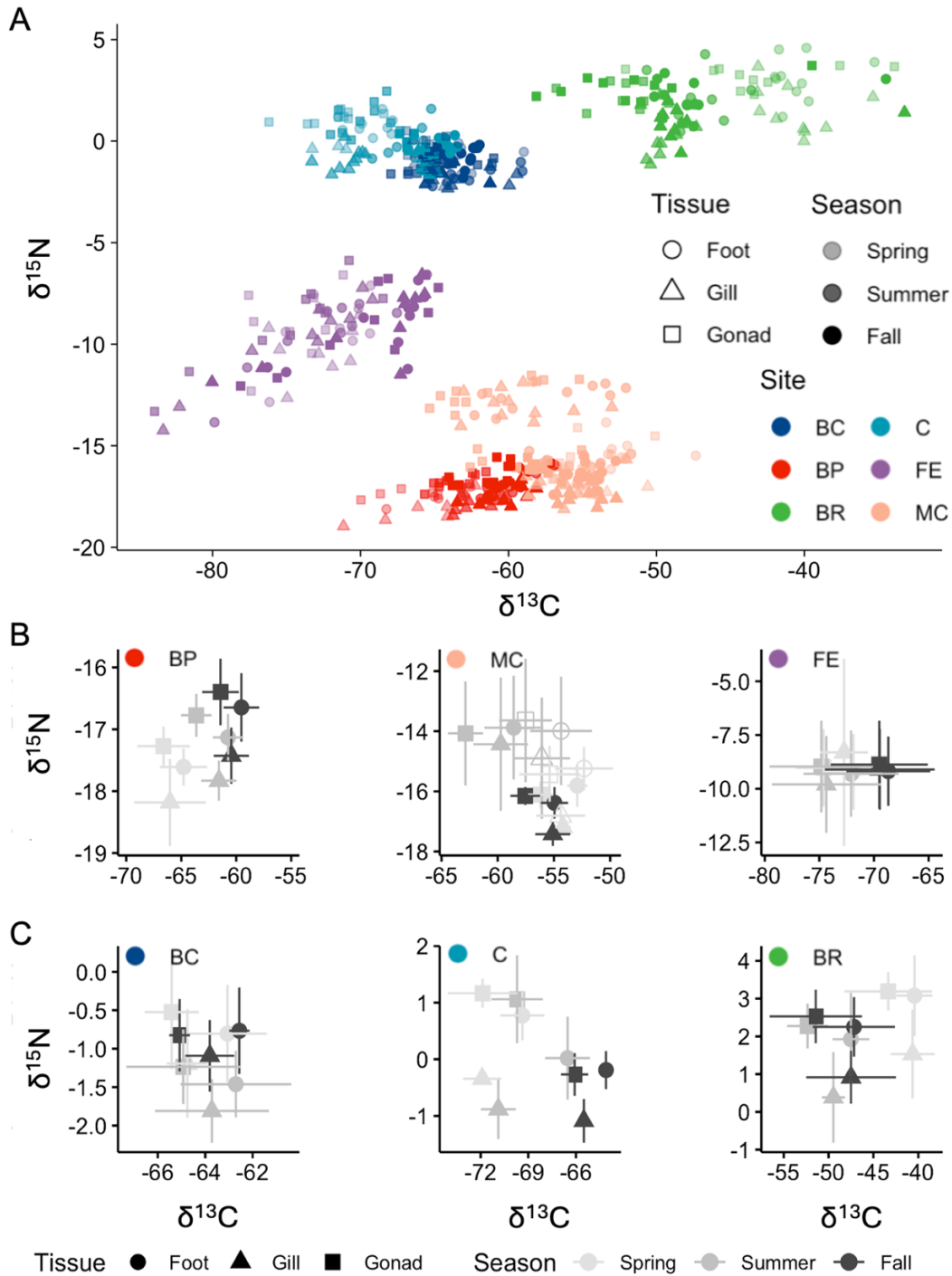


Figure 3.2. Carbon and nitrogen isotopic ratios. A) All data from all sites. B) Mean values $\pm$ standard deviation for sites in the Gulf of Mexico; BP = Brine Pool, MC = Mississippi Canyon, FE = Florida Escarpment. For MC, solid shapes represent *G. childressi*, and open shapes represent *B. brooksi*. C) Mean values $\pm$ standard deviation for sites in the Western Atlantic Margin; BC = Baltimore Canyon, C = Chincoteague, BR = Blake Ridge. Note differences among axis scales at each site in panels B and C.

In the populations with no seasonal changes, there were also no significant isotopic differences among the three tissue types within each season. One exception was that at Baltimore Canyon in the fall, gonad tissue was enriched in $\delta^{13}\text{C}$ compared to foot tissue (Tables 3.3 and S3.1). For populations with significant seasonal shifts in isotopes, gill tissue was significantly depleted in $\delta^{15}\text{N}$ compared to gonad and foot tissues in almost every season (Table S3.1).

At Brine Pool, carbon and nitrogen isotopes were steadily enriched in all tissue types throughout the year, though the difference was only significant between spring and fall (Figure 3.3A, Table S3.1). Within each season, ratios for each tissue type did not significantly vary, except in summer when the foot was more enriched in carbon than the gonad. In all tissue types, the isotopic ratios between *G. childressi* and *B. brooksi* at Mississippi Canyon were similar for $\delta^{15}\text{N}$ in all seasons, but $\delta^{13}\text{C}$ was more enriched in *B. brooksi* in the summer (Figure 3.3A). In *G. childressi*, gill was significantly enriched in nitrogen and depleted in carbon in summer compared to fall (Table S3.1). At Chincoteague, nitrogen ratios declined throughout the year while carbon ratios increased (Figure 3.3B). Those trends were significant in gonad tissue between spring and fall for both isotopes and in all tissues between spring and fall for carbon (Table S3.1). In summer, foot tissue was more enriched in $\delta^{13}\text{C}$ than gill. At Blake Ridge, gill tissue was significantly depleted in $\delta^{15}\text{N}$ compared to gonad and foot in all seasons (Table S3.1). None of the tissue types showed significant change in nitrogen ratios over time, but carbon was significantly enriched in all tissue types in spring compared to summer and fall (Figure 3.4).

Table 3.3. Results of either 2-way ANOVAs (F) or non-parametric Sheirer-Ray-Hare (H) tests on the effects of season, tissue type (gill, foot, or gonad), and their interaction on carbon and nitrogen isotopic ratios. Bolded p-values indicate where pairwise post-hoc tests revealed one or more tissue types that change over time or differences among tissue types in the same season.

Site	Species	Isotope	Season		Tissue Type		Interaction	
			Test Stat	p-value	Test Stat	p-value	Test Stat	p-value
Brine Pool	<i>G. childressi</i>	Carbon	F = 155.13	< 0.0001	F = 10.567	< 0.0001	F = 1.45	0.788
		Nitrogen	F = 19.411	< 0.0001	F = 34.232	< 0.0001	F = 0.088	0.986
Mississippi Canyon	<i>G. childressi</i>	Carbon	H = 44.726	< 0.0001	H = 17.336	< 0.001*	H = 0.806	0.938
		Nitrogen	H = 29.806	< 0.0001	H = 19.498	< 0.0001	H = 3.498	0.478
Mississippi Canyon	<i>B. brooksi</i>	Carbon	H = 3.641	0.056	H = 12.035	< 0.01*	H = 0.004	0.998
		Nitrogen	H = 7.050	< 0.01*	H = 6.192	< 0.05*	H = 1.231	0.541
Florida Escarpment	<i>B. heckerae</i>	Carbon	F = 11.905	< 0.001*	F = 1.811	0.171	F = 0.235	0.918
		Nitrogen	F = 0.575	0.565	F = 1.397	0.254	F = 0.312	0.869
Baltimore Canyon	<i>G. childressi</i>	Carbon	H = 2.031	0.362	H = 30.967	< 0.0001	H = 1.046	0.903
		Nitrogen	H = 20.032	< 0.0001*	H = 8.375	< 0.05*	H = 0.720	0.949
Chincoteague	<i>G. childressi</i>	Carbon	H = 52.970	< 0.0001	H = 15.509	< 0.001	H = 1.712	0.789
		Nitrogen	H = 17.486	< 0.001	H = 30.444	< 0.0001	H = 4.176	0.383
Blake Ridge	<i>B. heckerae</i>	Carbon	F = 47.56	< 0.0001	F = 10.70	< 0.0001*	F = 0.31	0.871
		Nitrogen	F = 11.086	< 0.0001*	F = 31.005	< 0.0001	F = 0.087	0.986

* - Significance was due to non-relevant pairwise comparisons (i.e. spring gonad and fall foot)

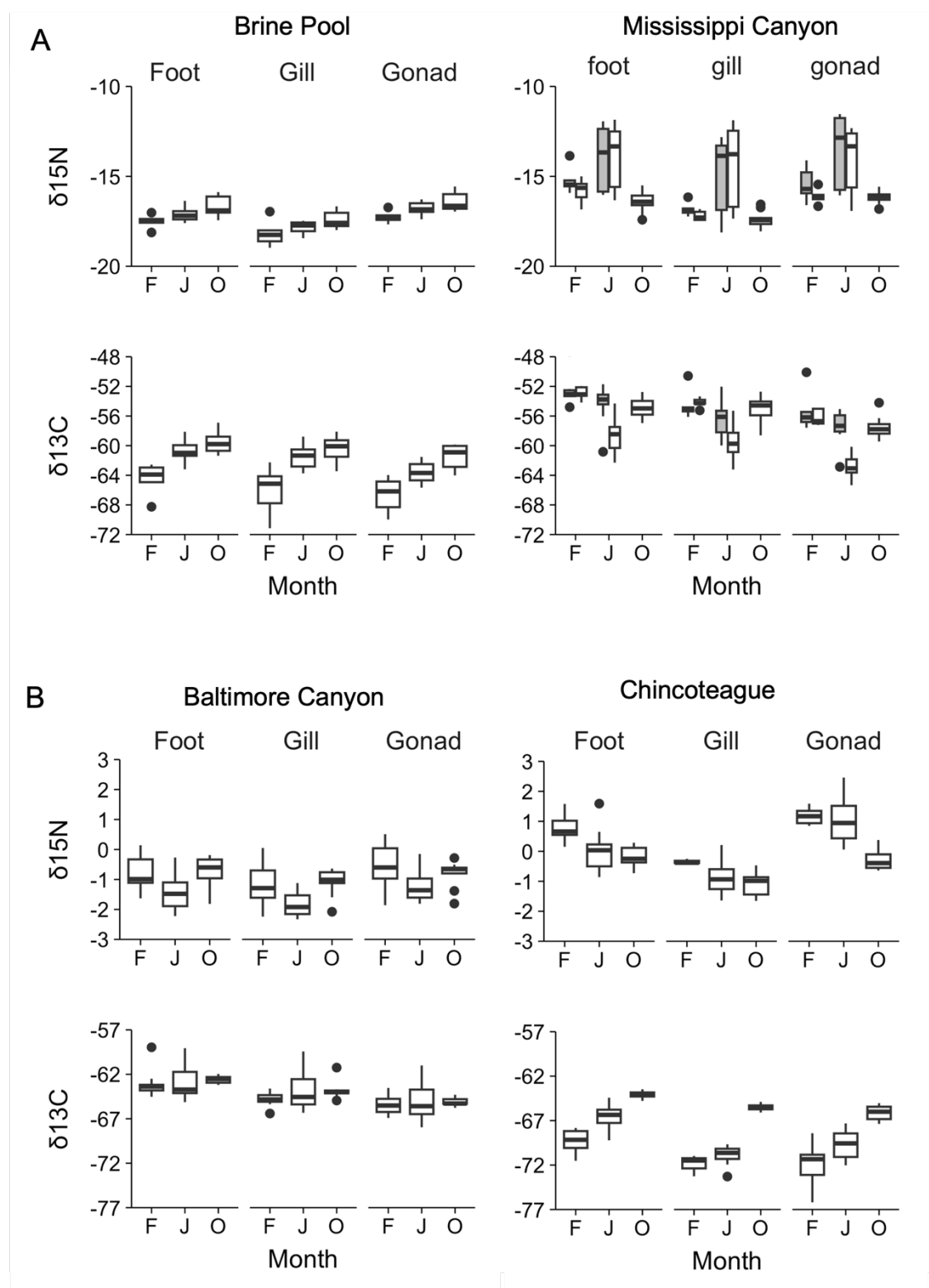


Figure 3.3. Boxplots of nitrogen and carbon isotopic ratios for tissues of *G. childressi* over three seasons. A) Values from populations in the Gulf of Mexico. Grey boxes show the values for co-occurring *B. brooksi*. B) Populations from the Atlantic. Note the change in y-axis scales between panels A and B. F = February, J = June, O = October

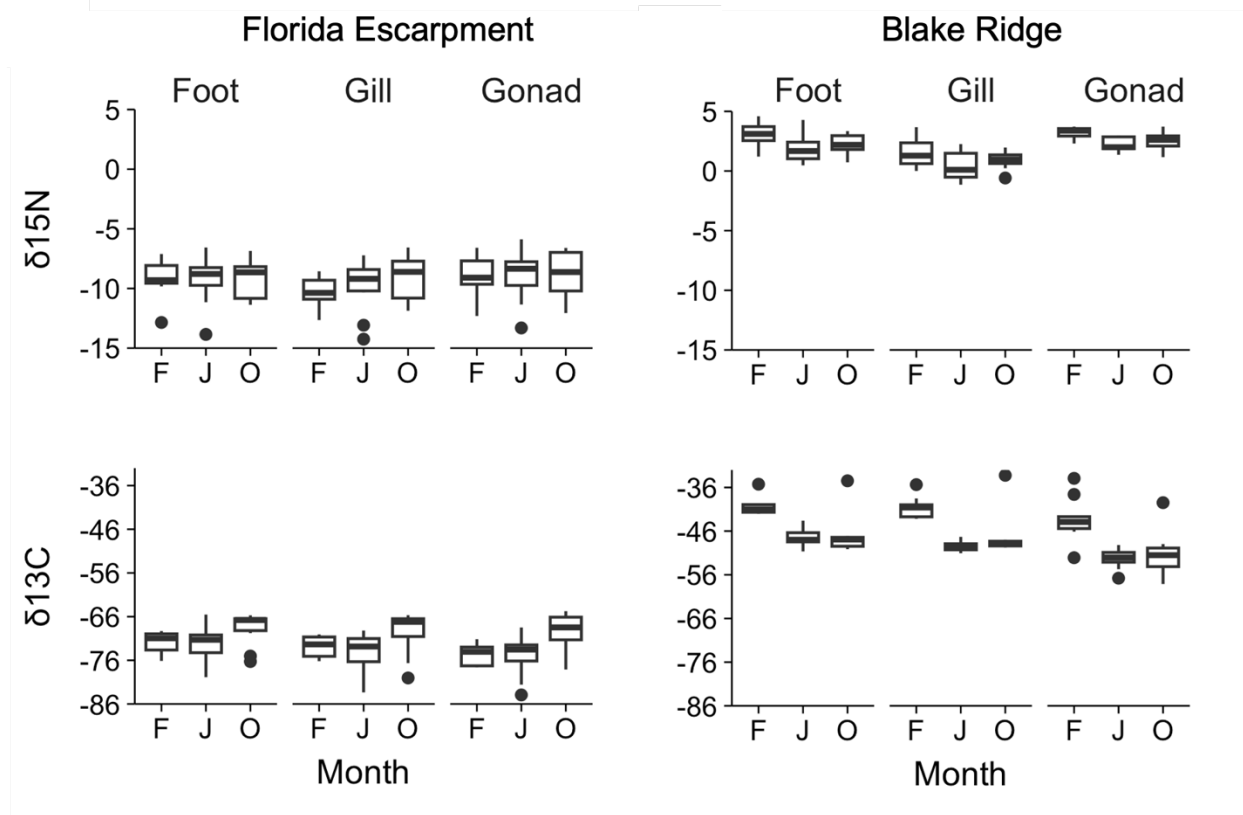


Figure 3.4. Boxplots of nitrogen and carbon isotopic ratios for tissues of *B. heckeriae* from three seasons. F = February, J = June, O = October.

3.2. Relationship to Surface Productivity

The Gulf of Mexico (GoM) had higher surface productivity year-round and more interannual variability than the U.S. Western Atlantic Margin (WAM), though the latter had more seasonal variation (Figure 3.5). There were two seasonal peaks in surface production for the WAM in spring and fall. The spring bloom was larger than the fall bloom, and the lowest production and available particulate organic carbon (POC) occurred in the summer. For the GoM, production was relatively constant throughout the year, and POC started increasing in late summer and peaked in late winter. Further examination of monthly averages across the entire 18-year time series confirmed that the seasonal patterns were relatively constant over the two decades examined (Figure 3.6). However, from late 2013 through early 2019, both regions

experienced a lower magnitude of interannual variation and disruption from the previous seasonality. Late 2020 had the highest values of the whole time series, and the next highest peak was in early 2010. Our spring collection occurred during a peak in surface productivity, while the summer collection was near the annual minimum for both regions. The fall collection was after the end of the time series data.

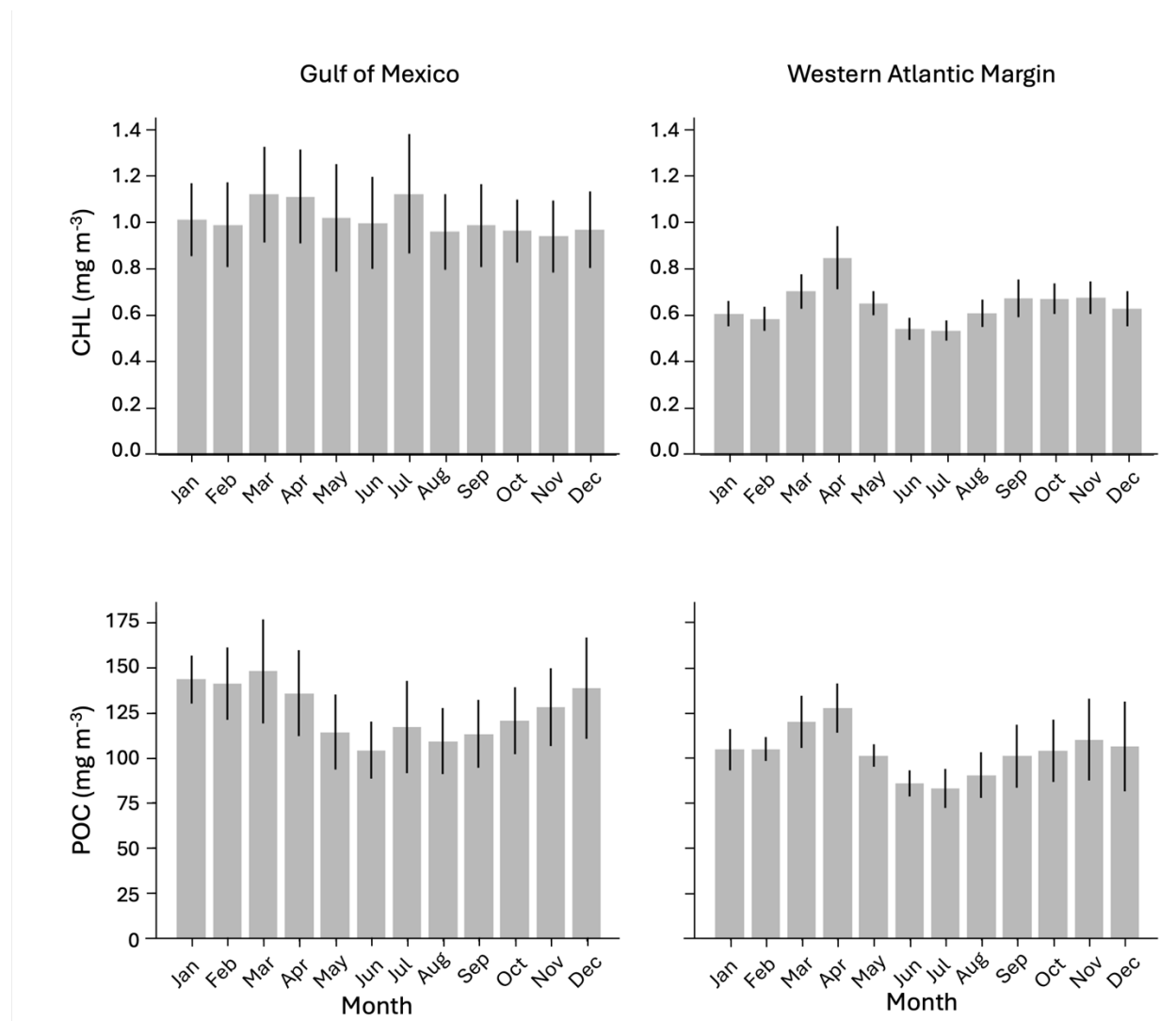


Figure 3.5. Monthly average ($\pm$ standard deviation) of chlorophyll-a (top) and particulate organic carbon (bottom) from 2003–2021 for the Gulf of Mexico (left) and U.S. Western Atlantic Margin (right).

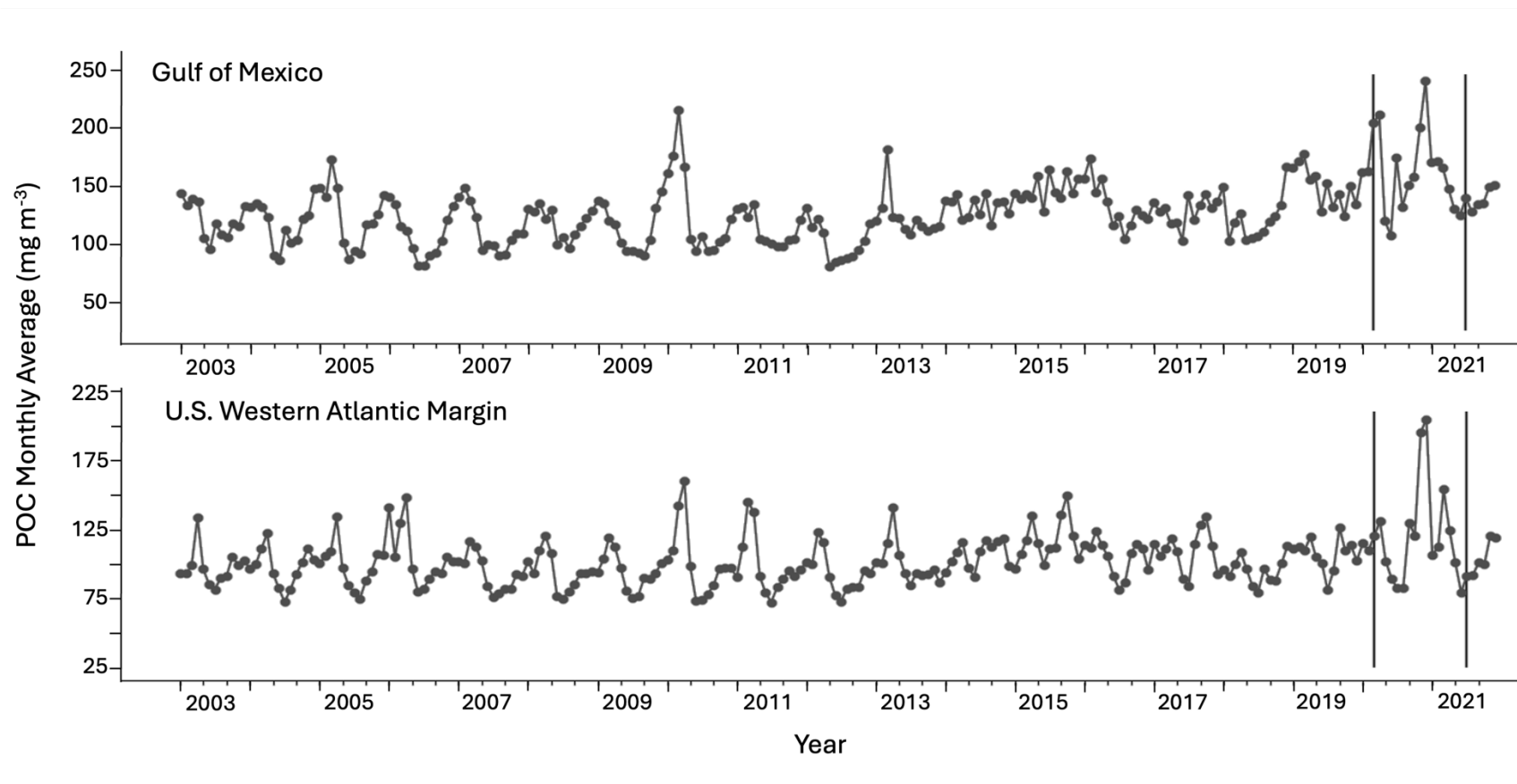


Figure 3.6. Monthly average particulate organic carbon values in the Gulf of Mexico and the U.S. Western Atlantic Margin from 2003 to 2021. Vertical lines denote the timing of the first two collections of this study. The last collection occurred in 2022, after the end of time series data. Note the change in the y-axis scale between panels.

There were no consistent patterns relating stable isotope ratios of mussel tissues and surface productivity among sites. For *G. childressi* at Brine Pool, the most depleted values in both isotopes occurred closest to the annual surface bloom. At Mississippi Canyon, the highest nitrogen and lowest carbon values occurred in the season with the lowest surface productivity. In the Atlantic, nitrogen was the most enriched and carbon the most depleted at Chincoteague during the largest annual peak in surface productivity. The pattern was flipped in the fall with the most depleted nitrogen values and most enriched carbon values. In *B. heckeriae* at Blake Ridge, the most enriched values in both isotopes were seen around the surface spring bloom.

The projected spawning periods for mussels at shallow seeps in both regions aligned with changing surface productivity. In the Gulf of Mexico, POC values increased during the suspected fall spawning season (Figure 3.7). In the Atlantic, POC values decreased after the spring bloom during the estimated summer spawning season before increasing again in the fall (Figure 3.7). Both regions experienced a peak in surface POC shortly after the spawning period, during early gametogenesis. Together, the data presented here did not support our hypothesis that these mussels rely on supplemental nutrition from phytodetritus for reproduction. The temporal correlation between the gametogenic phenology and surface seasonal productivity cycles provided some support for our second hypothesis that surface productivity acts as a reproductive cue.

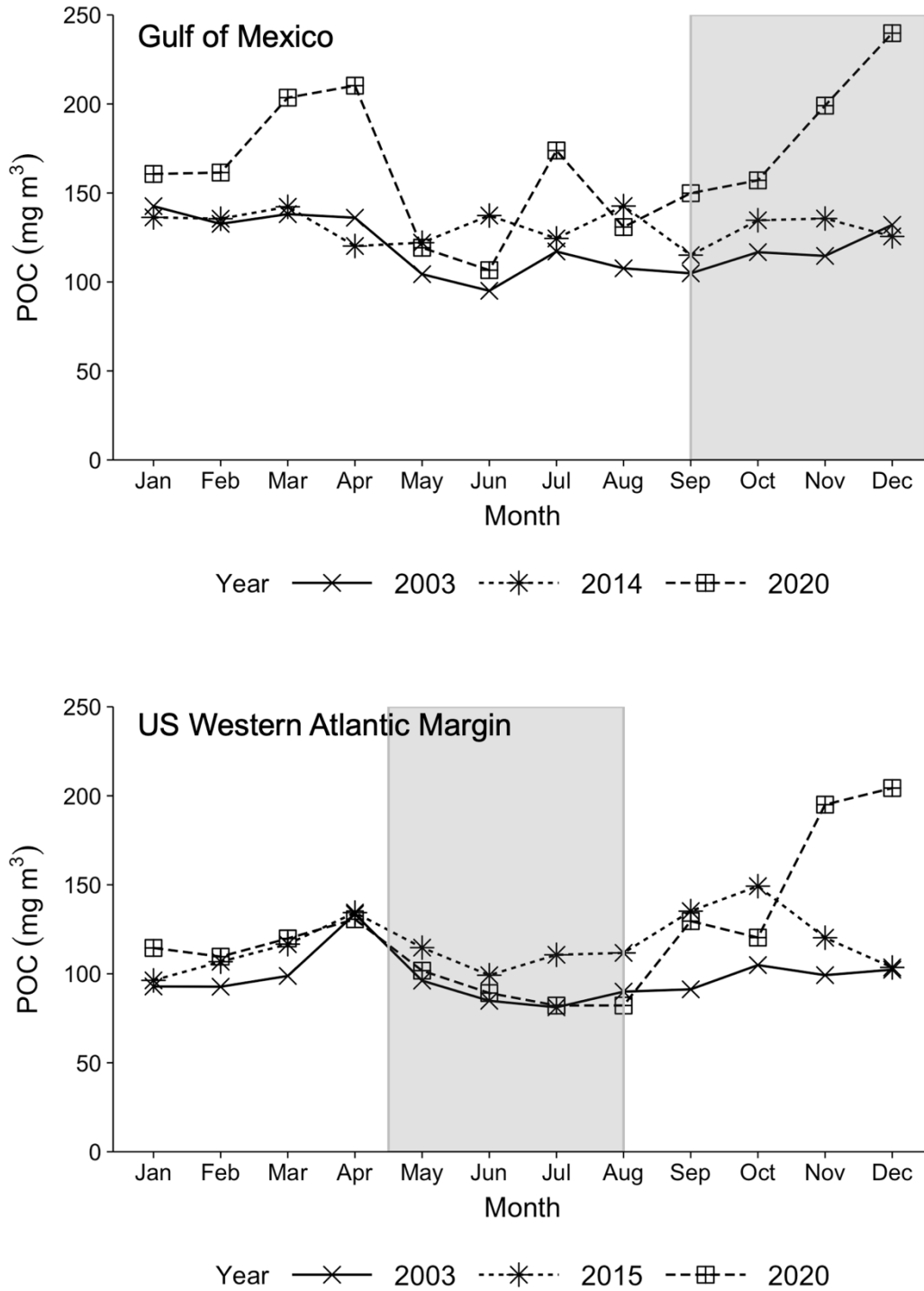


Figure 3.7. Monthly average particulate organic carbon values from three years for the Gulf of Mexico and U.S. Western Atlantic Margin. Grey bars denote the estimated spawning season of mussels from the Brine Pool in the Gulf of Mexico and Baltimore Canyon in the Atlantic. The three years were chosen to represent multiple points along the timeline and correspond to sampling efforts for reproductive studies of *Gigantidas childressi*.

4. DISCUSSION

This is the first study to look at seasonal changes in isotopic ratios in a deep-sea bivalve, though a similar analysis has been conducted in shallow-water mussels (Ezgeta-Balić et al., 2014). Unlike many deep-sea animals, for example, deposit-feeding echinoids on the abyssal plain (Stevenson & Mitchell, 2016), chemosynthetic mussels have a reliable, stable source of carbon and do not necessarily need phytodetrital input to supplement the energetically costly process of reproduction. However, they can feed on particulate organic matter (Pile & Young, 1999) and appear to use phytodetrital sources when available (Demopoulos et al., 2019; Riekenberg et al., 2016). Surface productivity maintains a robust seasonal signal and could provide a region-wide cue to synchronize the onset of gametogenesis or spawning. Methods for maintaining live *G. childressi* in laboratory settings are improving (Hiebenthal et al., 2024), so testing this hypothesis experimentally may soon be possible. Other drivers of seasonality in the deep sea include tidal cycles and currents, though particle flux tends to be the most important (Tyler, 1988).

4.1. Phytodetritus as supplemental nutrition

The isotopic ratios reported here are within the ranges previously reported for *G. childressi*, *B. brooksi*, and *B. heckeræ* at our study sites (Becker et al., 2010, 2013; Demopoulos et al., 2019; Paull et al., 1985; Van Dover et al., 2003). Differences in carbon isotope ratios in mussels among sites were expected due to differences in the methane pools that feed the sites (Becker et al., 2013; Demopoulos et al., 2019; Riekenberg et al., 2016). Within each region, the differing nitrogen ratios in mussel tissues are likely caused by different degrees of reliance on ammonium among sites (Becker et al., 2010; Lee & Childress, 1995, 1996). The higher nitrogen isotope ratios in mussels from Atlantic sites compared to the Gulf of Mexico sites may indicate a

higher reliance on phytodetrital nutrients for populations in the Atlantic. Mussels at Baltimore Canyon are thought to rely on phytodetrital nutrients for about 30% of their diet (Demopoulos et al., 2019). For populations in the Gulf of Mexico, Duperron et al. (2007) concluded from stable isotope ratios that the mussels did not obtain significant nitrogen resources from photosynthetic matter, which is supported by the nitrogen ratios reported here. However, Riekenberg et al. (2016) found different levels of reliance on phytodetritus between sites in the Gulf of Mexico, with mussels at Brine Pool estimated to obtain up to 20% of their bulk nutrition from phytodetrital sources. Spatial differences have also been reported within a seep site (Dattagupta et al., 2004), though our seasonal samples were taken within a few meters of each other and thus not expected to be affected by small spatial scales.

At Mississippi Canyon, the two co-occurring species had similar ranges of isotopic ratios, which had previously been reported for sympatric populations of these species in the northern Gulf of Mexico (Becker et al., 2010). Among tissue types, gill tissue had the lowest nitrogen values, consistent with previous results for these species (Demopoulos et al., 2019; Van Dover et al., 2003) and likely due to the presence of the chemosynthetic symbionts. While it was not always significant, gonad tissues generally had lower $\delta^{13}\text{C}$ values than other tissue types. Depleted carbon values are often associated with a higher contribution of lipids and carbohydrates (Post et al., 2007; Riou, Duperron, et al., 2010), which are important for the production of gametes.

The seasonal shifts in isotopes at some sites indicate the mussels can use varied nutrient sources at different times of the year. In contrast, the lack of seasonality at other sites implies this is a facultative rather than a fixed strategy. Even when sites showed a significant seasonal shift in isotopic ratios, the direction of those shifts lacked a consistent pattern among sites, indicating

that the cause of change may not be the same at each site. Phytodetritus is one possibility, but it could also be due to changes in fluid seepage leading to varied symbiont use (Trask & Van Dover, 1999; Riou et al., 2010). Recent work has shown that the $\delta^{15}\text{N}$ values of individual amino acids can link to surface productivity more strongly than bulk $\delta^{15}\text{N}$ (Vokhshoori et al., 2021). That type of analysis, conducted seasonally, along with measurements of fluid seepage and local isotopic ratios, would help pinpoint the exact cause(s) of the observed seasonal changes.

We expected isotopic ratios to change in relation to surface productivity, but that was not the case. Nor were there consistent trends between seasonal shifts in isotopic ratios and gametogenic staging or depth. At Brine Pool, the population of *G. childressi* experienced a slight yet significant increase in both carbon and nitrogen isotopic ratios during gametogenesis. The gametogenic phenology for mussels at Mississippi Canyon is less clear but appears to be like that of the mussels at Brine Pool, although delayed by a few months (Chapter II). However, the seasonal changes in the isotope ratios for *G. childressi* at the two sites differed. The lack of seasonal variability in the isotope ratios for *B. brooksi* could be because its thiotrophic symbiont provides the extra nutrients that *G. childressi* procures through filter feeding. The lack of seasonal changes in the isotopic ratios of mussels from Baltimore Canyon could be attributed to a relatively constant uptake of surface-derived nutrients throughout the year, as submarine canyons are known to accumulate macroalgal detritus (Beaulieu, 2002). The seasonal isotopic shifts seen at Chincoteague do not support the enrichment of both isotopes throughout gametogenesis. The population of *G. childressi* from the nearby Norfolk Canyon (~1600 m) was recently discovered to house an additional thiotrophic symbiont (Coykendall et al., 2019). If the population at Chincoteague also has this extra symbiont, variable symbiont reliance could mask any effects of phytodetritus.

The population of *B. heckeræ* at Florida Escarpment (3300 m) has the most asynchronous gametogenesis, with only a weak seasonal signal, which is stronger at Blake Ridge (2160 m) (Chapter II). Both sites showed a wide range of $\delta^{13}\text{C}$ values throughout the study, likely due to the increased symbiont diversity. If we consider spring to represent the end of the spawning season at Blake Ridge, then both isotopes were enriched throughout gametogenesis, like the pattern seen at Brine Pool. The depth of the site should mean a weaker signal of lower-quality phytodetritus. The mussels at this site are thought to obtain about 60% of their nutrients from their thiotrophic symbionts and the other 40% from the methanotrophic symbiont (Van Dover et al., 2003). However, phytodetrital input was assumed to be negligible and thus not included in their isotope mixing model. It is unclear if the variability seen here is caused by phytodetrital influence or other factors.

The isotopic turnover time for *G. childressi* is estimated to be a little over one year (Dattagupta et al., 2004), though turnover rates vary among tissue types (Deudero et al., 2009). However, variations in isotopic ratios within a year have been reported for *G. childressi* from WAM sites (Demopoulos et al., 2019). Here, the relationship among tissue types within a sample remained relatively consistent throughout the year, suggesting similar turnover rates for each tissue type. Turnover time may mean the isotope values measured here do not actually reflect the sampling time but instead reflect the year prior. However, differences are seen between Baltimore Canyon and Chincoteague, which have very similar gametogenic timing (Chapter II), suggesting seasonal changes in nutrition are not solely driven by reproductive needs. While our seasonal samples are confounded with inter-annual variation, the patterns seen in isotopic ratios from mussels at different sites still support plasticity in nutrient acquisition within *G. childressi* and *B. heckeræ*.

4.2. Surface productivity as a reproductive cue

While there are differences between the Gulf of Mexico and the U.S. Western Atlantic Margin, both regions show evidence of a link between mussel reproductive cycles and surface productivity. Phytodetrital aggregates can sink about 125 m per day (Gooday & Turley, 1990). At this rate, detritus would reach the shallower sites in this study within a week of leaving the surface, while the deeper sites would have to wait up to a month. This could explain the apparent lag in the spawning seasons of *Gigantidas childressi* at Mississippi Canyon compared to that of Brine Pool. In the Gulf of Mexico, the main driver of seasonality in surface productivity is riverine input from the Mississippi Atchafalaya River System (Biggs et al., 2008). Though most of those nutrients are entrained on the continental shelf (Howe et al., 2020), eddies created by the Loop Current can entrain that water offshore and disrupt the seasonal particle flux to deep waters (Biggs et al., 2008; Damien et al., 2021). The increasing surface productivity in winter may be triggering the onset of gametogenesis as there is no long rest phase between gametogenic cycles in this species (Chapter II). Conversely, spawning occurs as levels of surface POC start increasing, so this may be a classic case of Crisp's Rule (Qasim, 1956), as some larvae would feed in surface waters while productivity is high (Arellano et al., 2014; Arellano & Young, 2009).

In the U.S. Western Atlantic Margin, the spawning seasons for Baltimore Canyon and Chincoteague began as surface productivity decreased following a spring peak but before the larger fall peak in productivity. The Northwestern Atlantic experiences lateral transport of particulate organic carbon through intermediate nepheloid layers from the continental shelf and bottom nepheloid layers resulting from the south-flowing Deep Western Boundary Current (Hwang et al., 2009). The lateral flux peaks in April and October, similar to surface POC, at

shallower depths and maintains a seasonal signal down to 2900 m, but with only a single peak in October (Hwang et al., 2009). This lateral POC transport may be responsible for the seasonal signal seen in the population of *Bathymodiolus heckerae* at Blake Ridge, which could also explain why the gametogenic phenology was more similar to that of mussels from Gulf of Mexico sites, which were also responding to a single peak in particle flux in late fall to early winter. The population at Florida Escarpment showed the least synchronicity among individuals, suggesting the cue used to synchronize the population is not very strong. At over 3000 m deep, this site would receive very little, low quantity phytodetritus compared to shallower sites.

5. CONCLUSIONS

Our results do not support the hypothesis that phytodetritus is a necessary source of supplemental nutrition for reproduction in any of the studied species; however, we do show that flux of particulate organic carbon could serve as a reproductive cue to help synchronize gametogenesis and/or spawning. Given that these mussels have a constant source of nutrition from their chemosynthetic symbionts, it is not unexpected that they do not require surface nutrients. If neither the adults nor the larvae require photosynthetic nutrients to live, it may be that using surface productivity as a cue to synchronize periodic reproduction within a population is a phylogenetically constrained trait (Eckelbarger & Watling, 1995). Alternatively, phytodetrital flux may be the most reliable seasonal cue these mussels experience.

These results support the existence of an essential link between surface processes and deep-sea chemosynthetic communities. As seasonal patterns in shallow waters change with climatic forcing, bathymodiolin mussel populations could be affected. Bathymodiolin mussels

continue to serve as a model group for understanding deep-sea dynamics and pelagic-benthic coupling.

BRIDGE

Chapter III further supports the hypothesis that surface productivity and the resulting flux of particulate organic carbon influences the reproductive patterns of bathymodiolin mussels and that some populations obtain nutrition from phytodetrital sources. Chapter IV examines the flow of reproductive energy from the opposite direction and estimates the magnitude of the nutritional subsidy to pelagic systems created by the spawning of deep-sea mussel beds.

CHAPTER IV

FIRST ESTIMATES OF GAMETE SUBSIDIES FROM A DEEP CHEMOSYNTHETIC ECOSYSTEM TO THE PELAGIC FOOD WEB

This section includes unpublished work co-authored with Craig Young and Shawn Arellano, who were both principal investigators on the grant that funded the research. I designed the study with their input and based on Shawn's previous (unpublished) work. Shawn also assisted with spawning mussels and collecting eggs. I processed all the samples, analyzed the data, and wrote this manuscript with editorial support from Craig.

1. INTRODUCTION

Hydrocarbon seeps, hydrothermal vents, and organic falls are all habitats in the deep sea, where primary production is driven by microbial chemosynthesis (Tunnicliffe et al., 2003). These habitats host dense macrofaunal communities dominated by endemic invertebrates (e.g., tube worms, shrimp, snails, clams, and mussels) supported by chemosynthetic symbionts (Gage & Tyler, 1991; Kennicutt et al., 1985). Despite only covering a small fraction of the vast abyssal plain (Ramirez-Llodra et al., 2010), there is growing evidence that these reducing systems impact their surrounding habitats and play a key role in global biogeochemical cycling (reviewed by Levin et al., 2016). Their relatively recent discovery (~50 years), coupled with the need for limited and expensive equipment to observe or sample these habitats, means there is still a lot to learn about the biology and ecology of these communities (Van Dover, 2014). The deep sea, and chemosynthetic systems in particular, are facing increasing threats from anthropogenic impacts (reviewed by Ramirez-Llodra et al., 2011), which underscores the need to advance our understanding of these communities and their sphere of influence (Levin et al., 2016).

Mussels belonging to the subfamily Bathymodiolinae are often among the dominant fauna at vents, seeps, and organic falls around the world (Gustafson et al., 1998; Kenk & Wilson, 1985; Laming et al., 2018). There have been many studies on bacterial symbionts (reviewed in Duperron, 2010), evolutionary history (Miyazaki et al., 2010), phylogeny (Zhang et al., 2021), population connectivity (e.g., Portanier et al., 2023), and life cycle ecology (reviewed by Laming et al., 2018) of many bathymodiolin species from a variety of habitats. One of the most common species at methane seeps in the Western Atlantic and Gulf of Mexico is *Gigantidas childressi* (Gustafson et al., 1998; Portanier et al., 2023). While it is a large mussel, *G. childressi* has smaller body sizes than *Bathymodiolus brooksi*, with which it can co-occur, or *B. heckerae*, which has the deepest depth range of the three species (Gustafson et al., 1998). The two *Bathymodiolus* species can be found together, but *G. childressi* does not extend into the depths of *B. heckerae*.

Gigantidas childressi, like shallow-water Mytilids, tend to form dense, sometimes patchy beds at seeps (Cordes et al., 2010; MacDonald, Russell Callender, et al., 1990). Larval *G. childressi* acquire methanotrophic symbionts as they transition from a planktonic to a benthic lifestyle (Franke et al., 2021). Though the bulk of adult mussel nutrition comes from their symbionts, they retain the ability to filter feed (Pile & Young, 1999), and stable isotope mixing models show they rely on a mixotrophic diet that also incorporates phytodetritus (Demopoulos et al., 2019; Riekenberg et al., 2016). Surface productivity also serves as a reproductive cue for the vent mussel *Bathymodiolus azoricus* (Dixon et al., 2006) and likely plays a role in determining the gametogenic phenology of *G. childressi* (Chapter III). Gametogenesis in *G. childressi* is similar to shallow-water Mytilids (Tyler et al., 2007; Chapter II). The species is gonochoric and undergoes broadcast spawning with external fertilization and embryonic development, typical of

other mytilid mussels (Arellano & Young, 2009). Egg and larval sizes indicate that larvae of *G. childressi* are planktotrophic. While no feeding was observed in lab-cultured larvae (Arellano & Young, 2009), wild-caught larvae have been found in surface waters where planktonic food is abundant (Arellano et al., 2014). Larvae appear long-lived and capable of long-distance dispersal (Arellano & Young, 2009; Young et al., 2012).

Despite being well-studied, the reproductive investment for *G. childressi* is unknown. There are no reports of fecundity for any large bathymodiolin mussels, though it is considered high (Laming et al., 2018). Maternal investment includes not only the number of eggs produced but also the size and energy content of each egg. Fecundity generally increases with increased body size but decreases with increased egg size. An increase in egg size typically leads to more energy (Moran et al., 2013). Across taxa, maternal investment is adapted to optimize either offspring or maternal fitness in response to environmental conditions (Marshall et al., 2008). Asteroids, for example, exhibit many examples of interspecific changes from producing high numbers of relatively low-energy eggs and planktotrophic larvae to low numbers of energy-rich eggs and lecithotrophic larvae (Prowse et al., 2008). Intraspecific plasticity in maternal provisioning has also been observed in many species, with an increase in egg nutrients generally leading to improved larval and juvenile fitness (Allen et al., 2008; Marshall et al., 2008)

The high biomass and ubiquitous nature of bathymodiolin mussels, coupled with their high fecundity, suggest that the energy contained in their spawned eggs may be significant. In marine invertebrates with planktotrophic larvae, very few of the released eggs survive to metamorphose into benthic juveniles, as larval mortality in the plankton is high (Morgan, 1995; Rumrill, 1990; Thorson, 1950; Young & Chia, 1987). Some of the energy from bathymodiolin eggs must thus be lost to the surrounding benthic and pelagic systems. Marine animal eggs are

known to have high concentrations of essential fatty acids, and the “egg boons” following spawning events are a significant pathway for redistributing nutrients in the opposite direction of most trophic flow (Fuiman et al., 2015). Leven et al. (2016) also suggested that gametes could represent an essential and currently unquantified energy flux out of chemosynthetic systems.

Here, we sought to answer two questions: i) What is the maternal investment of *G. childressi*, and is there intraspecific variation? ii) How much energy is released in the form of eggs when beds of this species spawn? We used histology to measure individual fecundity and bomb calorimetry to measure the energy content of spawned eggs. We then used the observed area, density, and sex ratio of a mussel bed to scale fecundity and energy to the level of the whole bed. To examine how bed characteristics (location, depth, size, density) might affect these values, we sampled three different mussel beds: Brine Pool in the Gulf of Mexico (650 m deep), Baltimore Canyon (400 m), and Chincoteague (1020 m) along the U.S. Western Atlantic Margin. Our previous work has shown that populations in the two ocean basins have different reproductive phenologies (Chapter II), which may be driven by changes in the flux of particulate organic carbon (Chapter III). We expect these patterns will translate into differences in maternal investment between populations at Brine Pool and the Atlantic sites.

2. METHODS

2.1. Mussel Beds

Three methane seep sites, with known beds of *Gigantidas childressi* mussels, were studied: Baltimore Canyon (400 m), Brine Pool (650 m), and Chincoteague (1020 m). The seep sites were explored with ROV *Jason* in May-Jun 2021 and HOV *Alvin* in Feb-Mar 2020 and Oct 2022 (Figure 4.1, Table 4.1). Images with a visible mussel bed were plotted in ArcGIS Pro 2.8 using the associated coordinate data captured by the vehicles (Frame Grabber and Sea Log). A

polygon was fitted around the points from all expeditions and measured to obtain the estimated area (A) of each mussel bed (Figure S4.1). Standard errors (2.25%) for the area estimations were calculated using the 0.15 m uncertainty reported by ArcGIS.

Mussels were transported to the surface in insulated boxes, then transferred into chilled, filtered seawater and stored in a 4 °C cold room for <48 h. Counts of mussels collected were standardized to an area of one square meter to estimate the density of each mussel bed. The body length (anterior to posterior; L) was measured for all collected mussels. Mean counts per body size class were calculated for each site by averaging the counts m^{-2} from the three collections. Using the proportion of females from Chapter II, the average counts were converted into the average number of female mussels in each size class per m^2 (C , Table 4.2).

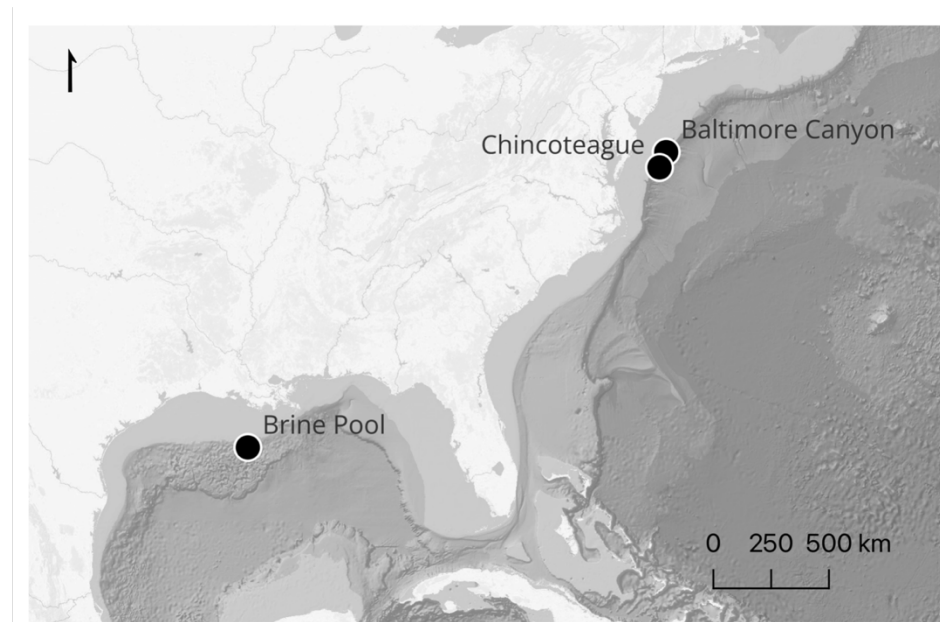


Figure 4.1. Map of study sites with scaled outlines and areas of the mussel beds at each site (see Figure S4.1).

Table 4.1. Site locations with collection dates and sample sizes. Expeditions beginning with AT were aboard the R/V *Atlantis II* using HOV *Alvin*. TN391 was aboard the R/V *Thomas G. Thompson* using ROV *Jason*. Values under “Measured” indicate the number of mussels from which body size data were obtained, and values in parentheses indicate the number of quadrats (0.25 m²) sampled. “Fecundity” is the number of individuals histologically analyzed for fecundity estimates. “Eggs” refers to the total number of spawned eggs combusted for calorimetry, and the number in parentheses lists the total number of combustions performed.

Site	Depth (m)	Latitude (°N)	Longitude (°W)	Metadata			Collection Numbers		
				Expedition	Date	Dive#	Measured	Fecundity	Eggs
Baltimore Canyon	400	38.04777	-73.82258	AT24-42	11-Mar-20	AL-5054	306 (2)	0	0
				TN391	28-May-21	J2-1323	335 (2)	3	27970 (1)
				AT50-04	27-Oct-22	AL-5119	72 (2)	0	0
				Total			713 (6)	3	27970 (1)
Brine Pool	650	27.72365	-91.27955	AT24-42	25-Feb-20	AL-5057	795 (4)	0	0
				TN391	13-Jun-21	J2-1334	211 (2)	0	0
				AT50-04	16-Oct-22	AL5112	196 (2)	3	338067 (6)
				Total			1202 (8)	3	338067 (6)
Chincoteague	1020	37.54144	-74.10192	AT24-42	11-Mar-20	AL-5065	282 (1)	0	0
				TN391	29-May-21	J2-1325	143 (1)	3	7148 (2)
				AT50-04	27-Oct-22	AL-5120	71 (1)	0	29000 (4)
				Total			496 (3)	3	36148 (6)

2.2. Fecundity

During the collections at Baltimore Canyon (May 2021), Chincoteague (May 2021, October 2022), and Brine Pool (October 2022), about 50 randomly selected mussels (> 10 mm body length) were removed from their shells. A small snip of gonad material from the visceral mass was examined via squash mount with an Olympus BX50 compound microscope. After a visual inspection, each individual was assigned a sex and preliminary reproductive stage (no gametes, developing, ripe). Three female mussels (70 to 130 mm body length) with ripe gonads were randomly chosen from each site for histological processing to measure fecundity. These mussels were preserved in 10% neutral buffered formalin for one to three months before being transferred into 70% ethanol and further dissected for analysis. A sample of gonad material from the visceral mass and the mantle was taken from each female using a 5 mm biopsy punch. The visceral mass was dissected, and all gonad material was removed from other tissue types, such as the digestive tract. The biopsied sample and all of the gonad material from the visceral mass were weighed on a BSM-120.4 electronic precision balance with a 0.033% uncertainty. Surface area was used to obtain the subsampled ratio of gonad tissue from the mantle. To do this, the mussel was laid flat, with the biopsy next to it, and photographed with a ruler. The surface areas of the biopsied sample and the total extent of gonads in the mantle were measured in the program Fiji (Schindelin et al., 2012).

Gonad subsamples were dehydrated using an ethanol series of 70%, 80%, 90%, and 95% for 30 min each, then left overnight (~12 h) in 100% ethanol. Dehydration was ensured by another 30 min soak in fresh 100% ethanol. Samples were then transferred to Toluene for 24 hours of clearing. Samples were infiltrated in paraffin for three days in a 60 °C oven and then embedded in paraffin blocks. The blocks were hand-trimmed, and the entirety of the sample was

serially sectioned at seven μm thickness using an AO Spencer 820 rotary microtome. Every four sections were isolated into a separate ribbon that was allowed to expand in a warm water bath, then mounted on a slide, and allowed to dry for at least 24 hours. Slides were stained using Masson's Trichrome (Presnell & Schreibman, 1997) modified with Mayer's hematoxylin. Slides were viewed using an Olympus BX50 compound microscope, and the total number of ripe and developing oocytes was counted in the first section of every other slide (every 56 μm).

To estimate the total number of oocytes from each block, the average values from two measured sections were applied to the missing seven sections in between. The values for each section were then summed to obtain values for the whole sample. The number of ripe oocytes was considered a metric of instantaneous fecundity, and the total fecundity of each subsample was calculated by including the number of developing oocytes that were likely to be released later in the same spawning season. The instantaneous and total fecundities of each subsample were then scaled based on the proportion of the subsample to total gonad tissue. The instantaneous fecundity ($F_{Instant}$) of each female was calculated by adding the total number of ripe oocytes from the visceral mass (R_V) and mantle tissues (R_M , Equation 1a). The total fecundity (F_{Total}) was the sum of ripe (R) and developing oocytes (D) from both tissue types (Equation 1b). Instant and total fecundities for each individual were standardized to values per mm of body length (IF , Equation 2) and then averaged for site-level fecundities ($\bar{IF}$). The average fecundity per site values were combined with the average size-frequency counts per m^2 (C) and scaled to the average instantaneous and total fecundity per m^2 for each site (BF, Table 4.2). Bed fecundity calculations excluded the counts for mussels with body lengths below 30 mm as they are unlikely to be reproductively mature (Plowman, 2017).

$$(1a) \quad F_{Instant} = R_V + R_M$$

$$(1b) \quad F_{Total} = R_V + R_M + D_V + D_M$$

$$(2) \quad lF = F/L$$

2.3. Egg Energetics

Mussels were injected with 1.0 ml of 2 mM serotonin hydrochloride into the anterior adductor mussels to induce spawning (Arellano & Young, 2009). Approximately 15 mussels from Baltimore Canyon and Chincoteague were injected in May 2021, and an additional 20 from Chincoteague and 60 from Brine Pool were injected in Oct 2022. Mussels were scrubbed clean before injection and left in bins of 0.2 μ m filtered seawater with two other individuals (three mussels per bin) after injection. The bins were kept in a 4 °C cold room and checked for spawning every hour. When observed, eggs were collected and gently passed into a new dish of filtered seawater through a 210 μ m sieve to remove mucus. Eggs were then manually pipetted into an isosmotic solution of 0.2 μ m filtered ammonium formate (3% w/v). Eggs went through three washes of ammonium formate to remove salt. All egg processing occurred in a 4 °C cold room or with the samples on ice.

Table 4.2. Summary of data and equations used to estimate the total fecundity of each mussel bed. Variables that were directly measured are bolded. Size classes started at 30 mm as mussels below that body size are unlikely to be reproductively mature (Plowman, 2017).

Description	Variable	Equation	Error Equation
Size classes (mm)	b	30–140; bins = 10	
2020 scaled counts	x	Raw counts / quadrats	
2021 scaled counts	y	Raw counts / quadrats	
2022 scaled counts	z	Raw counts / quadrats	
Mean counts per size class (m ⁻²)	$\bar{X}(b)$	$\bar{X}(b) = \frac{(x + y + z)}{3}$	$S_{\bar{X}(b)} = \sqrt{\frac{(x - \bar{X})^2 + (y - \bar{X})^2 + (z - \bar{X})^2}{3}} / \sqrt{3}$
Percent female	P	(Chapter II)	S_P
Mean counts of females per size class (m ⁻²)	$C(b)$	$C(b) = \bar{X} * P$	$S_{C(b)} = C(b) * \sqrt{\left(\frac{S_{\bar{X}}}{\bar{X}}\right)^2 + \left(\frac{S_P}{P}\right)^2}$
Mean fecundity (mm ⁻¹)*	$\bar{lF}$	$\bar{lF} = \frac{\sum_{i=1}^n \bar{lF}_i}{n}$	$S_{\bar{lF}} = \sqrt{\frac{\sum_{i=1}^n (lF_i - \bar{lF})^2}{n}} / \sqrt{n}$
Fecundity per size class	$F(b)$	$F(b) = \bar{lF} * b * C(b)$	$S_{F(b)} = F(b) * \sqrt{\left(\frac{S_{\bar{lF}}}{\bar{lF}}\right)^2 + \left(\frac{S_{\bar{lF}} * b}{C(b)}\right)^2}$
Total bed fecundity (m ⁻²)	BF	$BF = \sum F(b)$	$S_{BF} = \sqrt{\sum S_{F(b)}^2}$

* Sample sizes are given in Table 4.1. The general equation for the standard error of a mean was used since it resulted in a higher relative error than that from propagated errors.

Drops of the final suspension were added to a well plate and photographed under a Zeiss Stemi 508 dissecting microscope with an attached Axiocam 208 color camera. The number of eggs in each photo was counted in Fiji by manually adjusting the image thresholds and then using the Analyze Particles feature. Counts were confirmed by visually comparing the count masks to the original photos. Egg Feret diameters were also measured in Fiji from 100 spawned eggs per site (20 eggs each from five different images). For Chincoteague, 200 eggs were measured, 100 each from the summer 2021 and fall 2022 samples. Feret diameters were used to calculate egg volumes using the equation for a sphere ($1/6\pi d^3$).

For the Baltimore Canyon sample from summer 2021, all collected eggs went into a single 2 ml cryovial. For all other samples, collected eggs were spread into multiple cryovials, with about five to ten of the photographed wells going into each 2 ml cryovial. Excess ammonium formate was removed, and cryovials were flash-frozen and stored at -80 °C. Within one month of freezing, samples were dried for 48 hours using a Labconco FreeZone Lyophilizer. Once dry, samples were weighed on a BSM-120.4 electronic precision balance with a 0.033% uncertainty (m). Contents of multiple cryovials were combined and weighed, and benzoic acid was added as a spike material until the total weight was ~0.15 g. Samples were pressed into pellets and combusted using a Parr 6725 Semi-micro Oxygen Bomb Calorimeter, and results were calculated with a Parr 6772 Precision Thermometer using default and factory-recommended settings (Table S4.1). The sulfur content in eggs spawned by mussels from Brine Pool was less than 1% (Plowman and Young, unpublished data), so no sulfur correction was applied.

The results were reported for each combustion as cal/g with a relative error of 0.138 % (T). The reported values were divided by the total number of eggs combusted (O) to calculate the

nutrient density (cal/g) of an individual egg (D). Sample nutrient density was then divided by the mass of the combusted sample (m) to calculate the amount of energy (mJ) in a single egg (E , Equation 3). Energy units were converted from calories to millijoules using $1 \text{ cal} = 4186.8 \text{ mJ}$. Mean energy values per egg were calculated for each site ($\bar{E}$, Table 4.3).

$$(3) \quad E = \left(\frac{D}{m} \right) * 4186.8 = \left(\frac{T/O}{m} \right) * 4186.8$$

2.4. Data Analysis

Average fecundity values per m^2 for each site (BF) were multiplied by the corresponding value for energy contained in a single oocyte ($\bar{E}$) to estimate the total amount of energy (MJ) released per m^2 at each mussel bed (BE). Those values were then multiplied by the area (A) of each bed to calculate the total number of eggs released by each mussel bed (TE , Equation 4). Both bed (BE) and total energy (TE) were calculated using instantaneous ($\bar{IF}_{Instant}$) and total fecundity values ($\bar{IF}_{Total}$) to act as a minimum and maximum estimate, respectively. Measurement error was propagated according to standard estimation methods (Taylor & Thompson, 1982).

$$(4) \quad TE = A * BE = (BF * \bar{E}) * A$$

Table 4.3. Summary of data and equations used to estimate the total energy released through spawning for each mussel bed. Variables that were directly measured are bolded.

Description	Variable	Equation	Error Equation
Total eggs combusted	<i>O</i>		
Sample mass (g)	<i>m</i>		$S_m = 0.033\% * m$
Total combustion energy (cal/g)	<i>T</i>		$S_T = 0.138\% * T$
Nutrient density of sample (cal/g)	<i>D</i>	$D = T/O$	$S_D = S_T/O$
Energy per egg (mJ)	<i>E</i>	$E = \left(\frac{D}{m}\right) * 4186.8$	$S_E = \left(E * \sqrt{\left(\frac{S_D}{D}\right)^2 + \left(\frac{S_m}{m}\right)^2}\right) * 4186.8$
Mean energy per egg (mJ)*	$\bar{E}$	$\bar{E} = \frac{\sum_{i=1}^n E_i}{n}$	$S_{\bar{E}} = \sqrt{\frac{\sum_{i=1}^n (E_i - \bar{E})^2}{n}} / \sqrt{n}$
Energy released through spawning (MJ/m ²)	<i>BE</i>	$BE = (BF * \bar{E})/1e9$	$S_{BE} = \left(BE * \sqrt{\left(\frac{S_{BF}}{BF}\right)^2 + \left(\frac{S_{\bar{E}} * b}{\bar{E}}\right)^2}\right) / 1e$
Grand total energy released through spawning of each bed (GJ)	<i>TE</i>	$TE = (BE * A)/1000$	$S_{TE} = \left(TE * \sqrt{\left(\frac{S_{BE}}{BE}\right)^2 + \left(\frac{S_A}{A}\right)^2}\right) / 1000$

* Sample sizes are given in Table 4.1. The general equation for the standard error of a mean was used since it resulted in a higher relative error than that from propagated errors.

All statistical analyses and data visualization were conducted in RStudio version 4.3.2 (R Core Team, 2023). The underlying assumptions of normal distributions and homogenous variances were tested for all data types using the Shapiro-Wilk and Levene's tests, respectively. The effects of site and date on mussel body length were examined using a non-parametric Sheirer-Ray-Hare test, followed by Dunn's pairwise tests with p-values using the Holm method. The estimated body size frequency distributions were analyzed using a chi-square contingency table to compare the counts per size class. The estimated fecundity of each measured individual was plotted against its body length, with linear regression lines for each site. Standardized fecundities per mm of body length were compared among sites using an ANOVA followed by Tukey HSD pairwise tests. Volumes and Feret diameters of spawned eggs were compared between sites with a Kruskall-Wallis test. Linear regressions on log-log transformed data from all sites were used to investigate the relationship between egg energy and egg size for *G. childressi*. Energetic values were only statistically compared between Brine Pool and Chincoteague since only one combustion was used to obtain the values for Baltimore Canyon. Nutrient density (cal/g) and total energy (mJ) of an egg were compared using t-tests. Bed-level values (mussel density, fecundity/m², and spawning energy (GJ and MJ/m²) were compared using 95% confidence intervals (SE*1.96), with significance determined when there was no overlap in confidence intervals between values.

3. RESULTS

3.1. Mussel Bed Characteristics

The characteristics and estimated areas of mussel beds differed among sites (Table 4.4; Figures 4.2 and S4.1). Brine Pool had the smallest mussel bed, which formed a ring around the edge of the brine, while the bed at Chincoteague was almost 22 times larger. The mussel bed at

Baltimore Canyon occupies about six times more area than the bed at Brine Pool and about one-third of the bed at Chincoteague (Table 4.4). The density of mussels appeared to increase with depth, though the differences were not significant based on 95% confidence intervals. All sites contained about 500 mussels per square meter, with error rates of around 100 mussels (Table 4.4). The density of female mussels in each bed differed from the total density; Chincoteague had the lowest proportion of female mussels, while Brine Pool had the highest (Table 4.4). Baltimore Canyon was the closest to an equal ratio of male and female mussels in the bed.

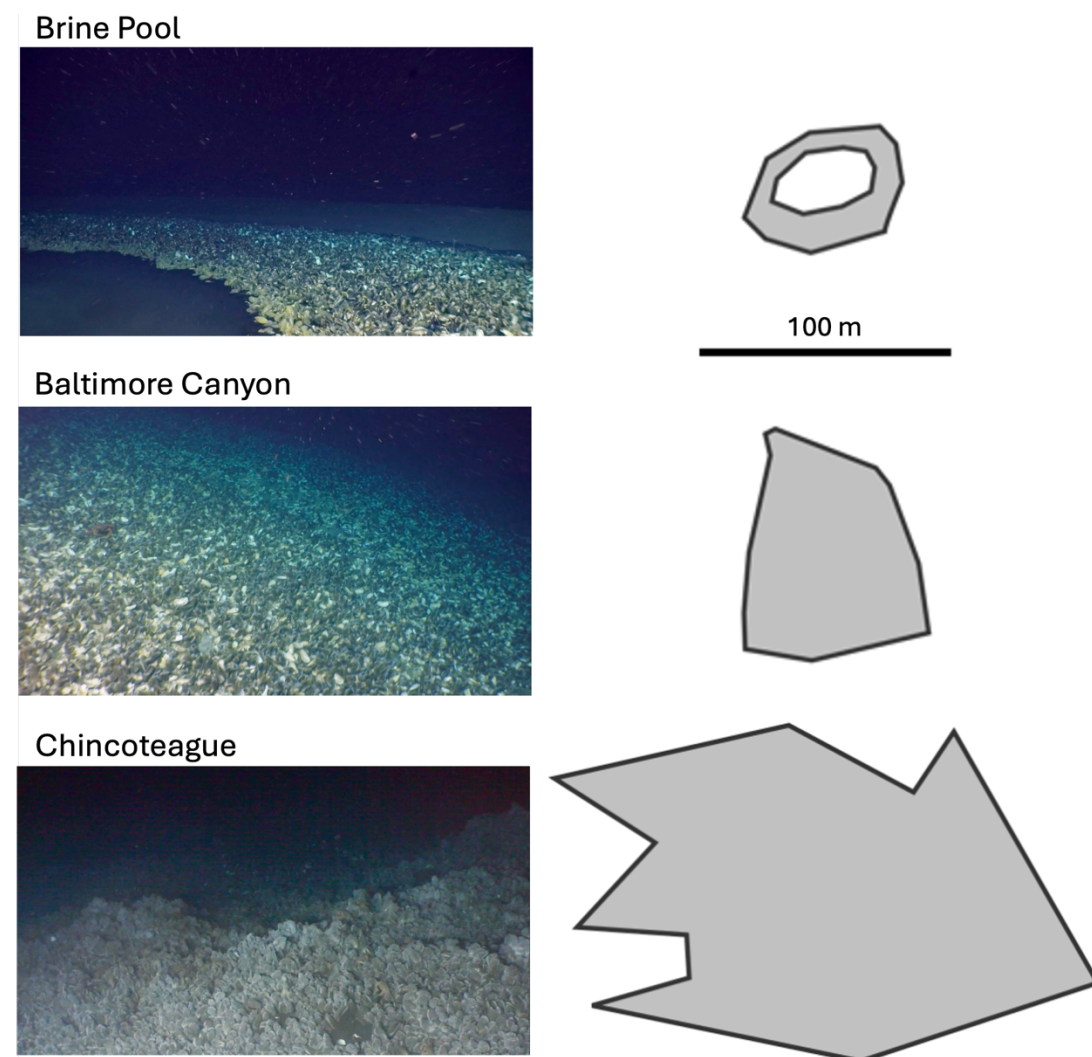


Figure 4.2. Representative photos and scaled area polygons of each mussel bed. Images taken by HOV *Alvin*. Polygons are from Figure S4.1.

Brine Pool had more estimated female mussels per quadrat, though all sites had female densities within the bounds of the standard error of the other sites (Figure 4.3). However, the size distribution of the estimated females per quadrat differed among sites ($\chi^2_{22} = 66.46$, $p < 0.01$; Figure 4.3). The bed at Baltimore Canyon had significantly more female mussels in the 80- and 90-mm size classes ($z > 2.54$, $p < 0.01$ for both) and fewer in the 110- ($z = -2.42$, $p < 0.05$) and 120-mm ($z = -2.16$, $p < 0.05$) size classes. Chincoteague had significantly more large females (120 mm size class: $z = 2.78$, $p < 0.01$; 130-mm size class: $z = 2.1$, $p < 0.05$), while the distribution at Brine Pool was not statistically different from the other sites. The variance in estimated counts (~20% relative error around the means) was driven by differences in the original collections. With all measured adults, mussel body sizes varied among sites ($H = 15.40$, $p < 0.001$), across collections ($H = 401.59$, $p < 0.0001$), and within sites across collections ($H = 163.69$, $p < 0.0001$). Overall, the mussels from the Brine Pool were significantly smaller than those at the other two sites, which had similar body sizes (Table 4.4). Mussels from Chincoteague showed the most variation among collections (Table S4.2).

Table 4.4. Mussel bed characteristics for each site. Values listed are means $\pm$ standard error.

Site	Area (m ²)	Density (#/m ²)	% Female*	Body length (mm)
Baltimore Canyon	4912 $\pm$ 110	475 $\pm$ 83	53.0 $\pm$ 7.6	61.3 $\pm$ 1.0
Brine Pool	786 $\pm$ 18	536 $\pm$ 104	60.9 $\pm$ 2.3	51.0 $\pm$ 0.9
Chincoteague	16942 $\pm$ 381	661 $\pm$ 136	44.0 $\pm$ 5.8	61.4 $\pm$ 1.8

* Data from Chapter II

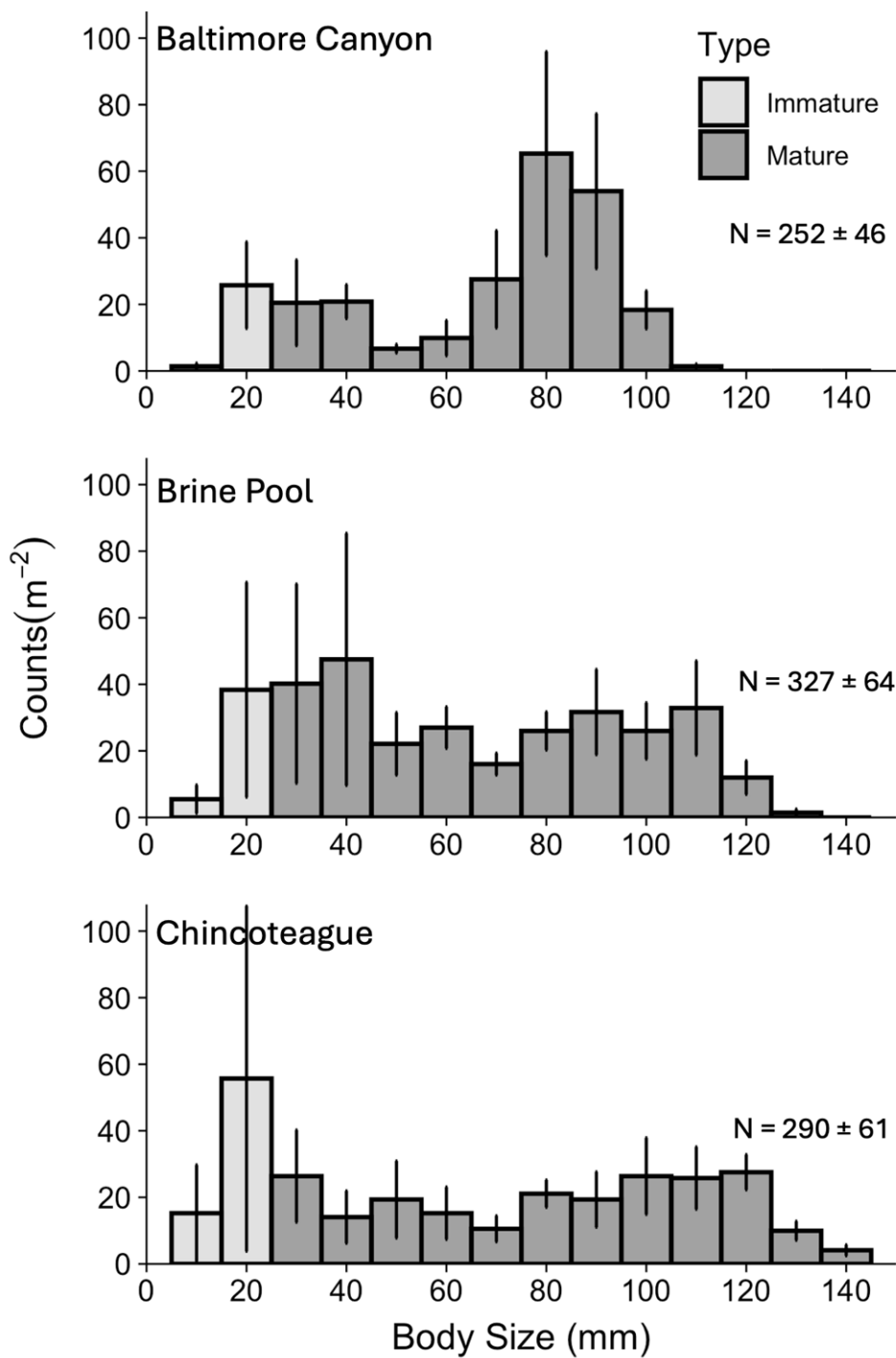


Figure 4.3. Estimated average size frequency distributions of mussels per m^2 at each site. N = total number of mussels (sum of counts) $\pm$ the propagated standard error. Only the mussels in sexually mature size classes were used in bed fecundity estimates.

3.2. Fecundity

Mussels from Brine Pool had higher fecundity than those from the other two sites, while those at Baltimore Canyon had the lowest (Figure 4.4). All individual fecundity counts had a relative uncertainty of 1-2%. The differences in the ratios of developing to ripe oocytes were significant among all sites ($F = 22.27$, $p < 0.01$, Figure 4.4A). Females from Brine Pool had the highest number of ripe oocytes, or instantaneous fecundity ($F = 24.39$, $p < 0.01$, mean $\pm$ SE = 9.7 ± 0.3 million eggs). In comparison, there was no statistical difference between the two Atlantic sites (Baltimore Canyon = 1.3 ± 0.2 million eggs, Chincoteague = 3.5 ± 1.5 million eggs). There were also significant differences in total fecundity (ripe plus developing oocytes) across the three sites ($F = 10.47$, $p < 0.05$, Figure 4.4B), which ranged from 2.7 ± 0.5 million eggs per individual at Baltimore Canyon to 11.6 ± 0.4 million eggs per individual at Brine Pool. All sites showed a generally positive relationship between fecundity and body size, though linear regressions of total fecundity against body size were not statistically significant (Table S4.3). Small sample sizes for each site ($n = 3$) preclude robust modeling. Still, the regression with the worst fit used the data from all three sites ($n = 9$, $R^2 = 0.16$ Table S4.3), indicating variation in how fecundity scales with body size across sites.

The mussels from Brine Pool had significantly higher instantaneous ($F = 19.78$, $p < 0.01$) and total ($F = 12.87$, $p < 0.01$) fecundity estimates than those from Baltimore Canyon or Chincoteague, while the latter two sites were similar (Figure 4.5A). The same patterns were seen in the fecundity estimates per square meter of each mussel bed, though the values were six orders of magnitude higher (Figure 4.5B). Based on 95% confidence intervals, there were no significant differences in the instant and total fecundity per square meter in the Brine Pool mussel bed. The

instantaneous bed fecundity from Brine Pool is more than double Chincoteague's total bed fecundity value.

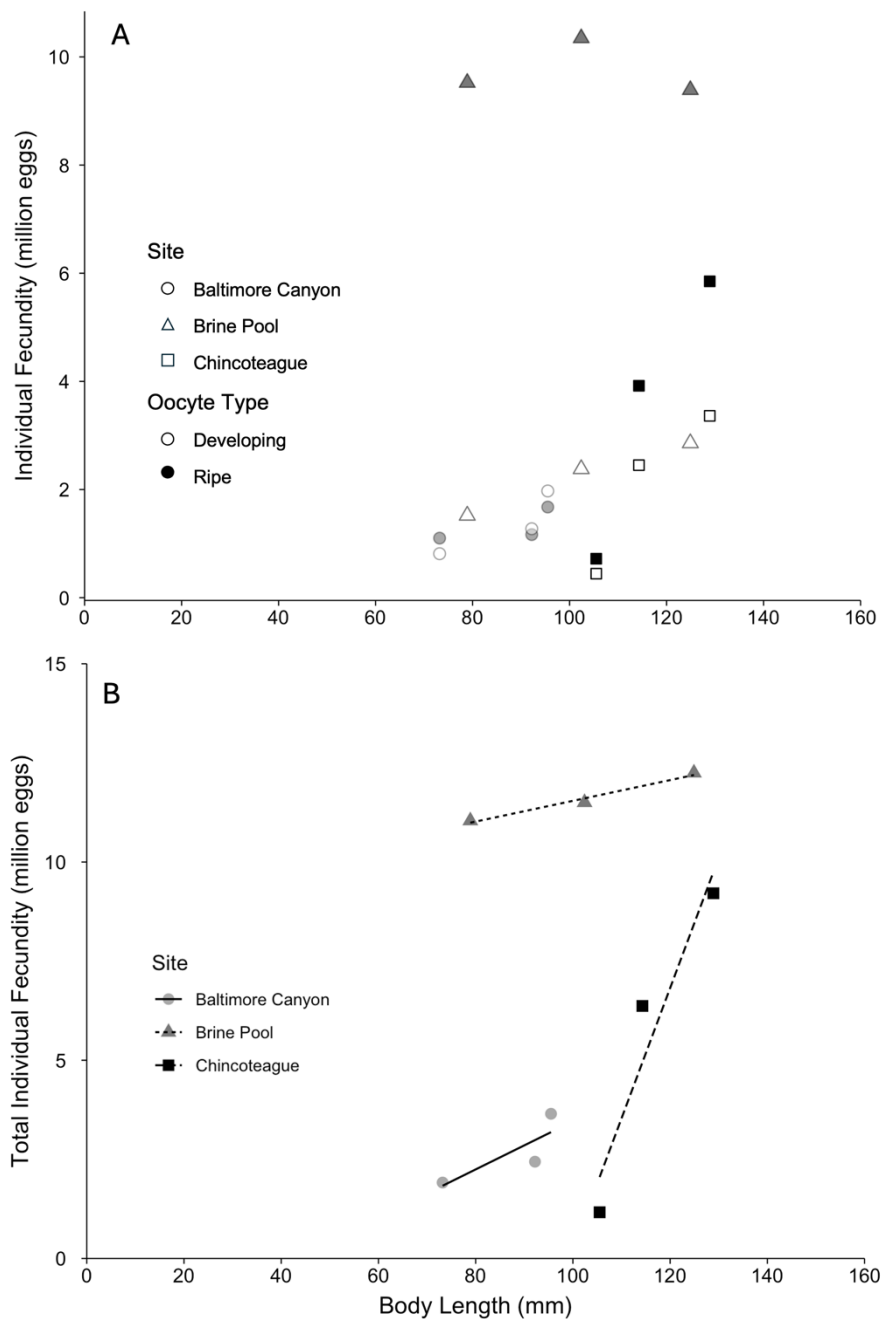


Figure 4.4. Estimated individual fecundity against body length. A) The total number of ripe and developing oocytes. B) Total fecundity with linear regression lines.

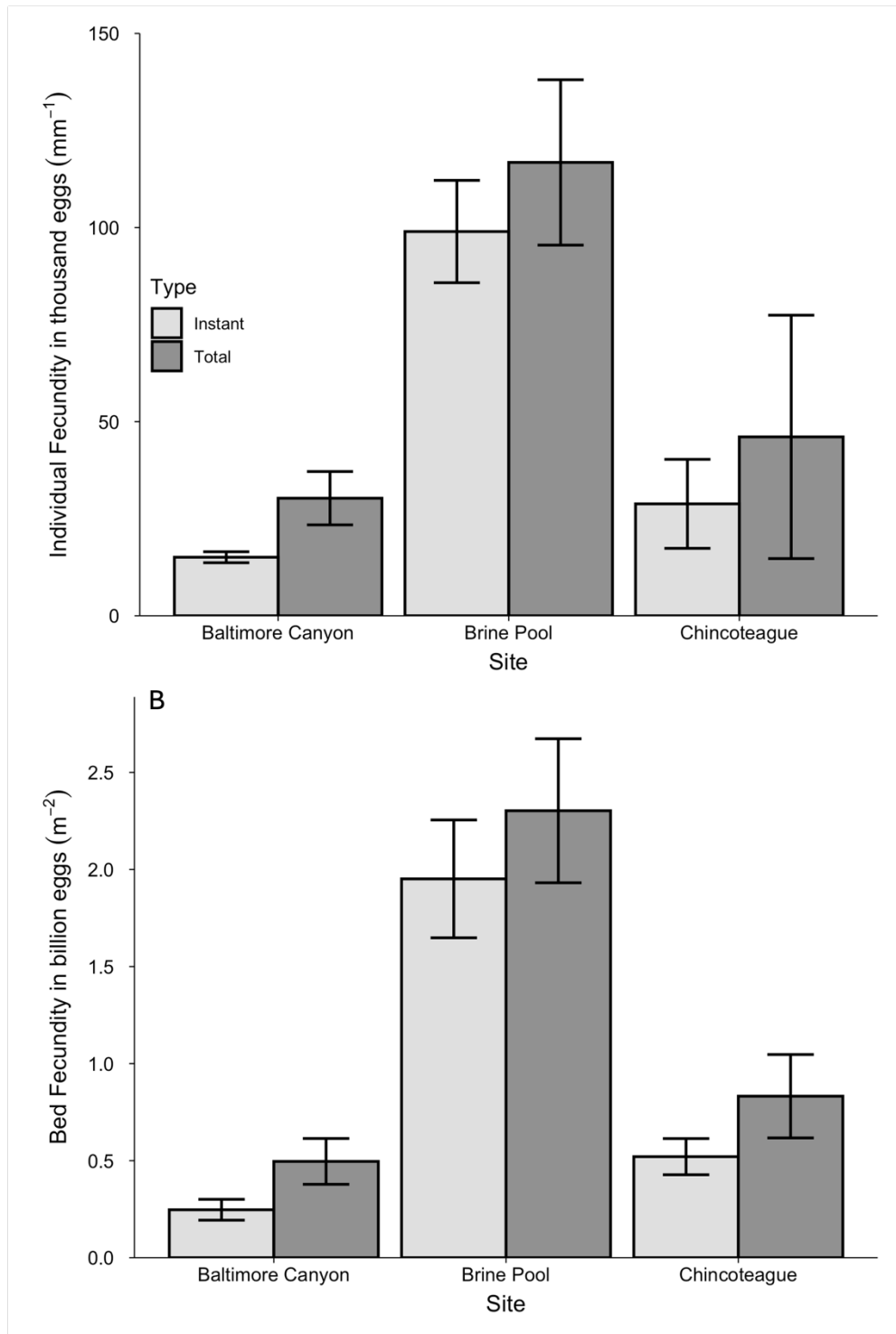


Figure 4.5. Average fecundity at each site for A) per mm of body length for a single female mussel and B) per square meter of each mussel bed. Error bars represent standard error. Note that the y-axis scale in B is six orders of magnitude higher than that of A.

3.3. Egg Sizes and Energy

Roughly 25% of the mussels we injected at sea and about 80% of the mussels that spawned were female ($n = 22$). The average Feret diameters of spawned eggs varied across sites ($H = 243.67$, $p < 0.0001$). The smallest diameters (mean $\pm$ SE) were spawned by mussels from Baltimore Canyon ($65.56 \pm 0.24 \mu\text{m}$). Spawned oocyte diameters were slightly larger from Brine Pool ($66.56 \pm 0.34 \mu\text{m}$), though the difference was not significant ($p > 0.05$). Mussels from Chincoteague spawned the largest eggs ($73.51 \pm 0.23 \mu\text{m}$), which were significantly larger than those from the other two sites ($p < 0.001$ for both). There was no significant difference in Feret diameters between eggs from Chincoteague spawned in May 2021 and October 2022 ($H = 0.20$, $p > 0.05$).

The patterns in egg diameters among sites were also seen in egg volumes ($H = 243.67$, $p < 0.001$), where eggs spawned by mussels from Chincoteague had about 35% more volume than those spawned by mussels from Brine Pool and Baltimore Canyon (Figure 4.6A). Changes in the volume of eggs among sites corresponded to a change in egg energy content (Figure 4.6B). Baltimore Canyon had the lowest egg energy (1.35 mJ, $n = 1$), markedly lower than that of eggs from Brine Pool (7.25 ± 1.8 mJ). Eggs from Chincoteague had significantly higher energy content (18.8 ± 3.5 mJ) than those from Brine Pool ($t = -2.89$, $p < 0.05$). Egg energy scaled with egg size (Figure 4.7, $F = 12.11$, $p < 0.01$), and the slope of the regression line was 4.4 ($R^2 = 0.48$). The nutrient density of eggs from Baltimore Canyon (9.14 mJ/nl) was about five times lower than that of eggs from Brine Pool (46.6 ± 11.8 mJ/nl). Eggs spawned by mussels from Chincoteague had by far the largest nutrient density (86.64 ± 16.9 mJ/nl), though the large errors mean it was not statistically different from that of eggs from Brine Pool ($t = -2.09$, $p > 0.05$).

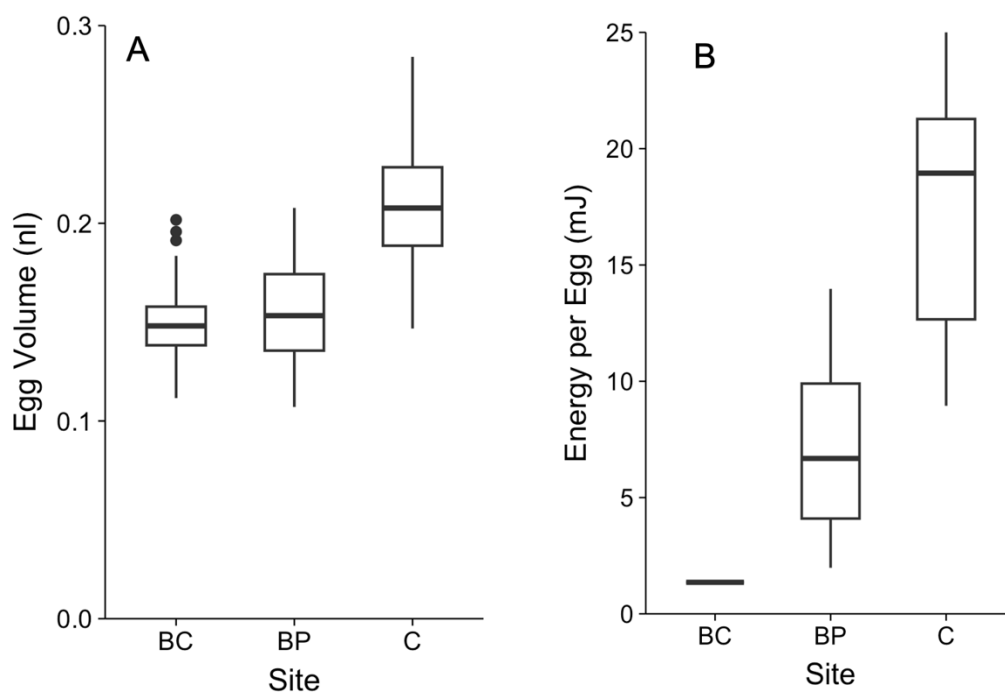


Figure 4.6. Boxplots showing the distribution of measurements for A) volume in nanoliters and B) energy in millijoules of spawned eggs from each site.

3.4. Mussel Bed Spawning Energy

When individual fecundity and the energy within an egg were scaled up based on the characteristics of each bed, the results were highly variable (Figure 4.8). The total amount of energy released through spawned oocytes, reported in gigajoules, increased by an order of magnitude with increasing depth among the seep sites examined (Figure 4.8A). The estimated total amount of energy released at Chincoteague (264.4 ± 84.7 GJ) was 80 times higher than that of Baltimore Canyon (3.3 ± 0.8 GJ) and 20 times higher than that of Brine Pool (13.1 ± 3.9 GJ). The minimum energy calculated using instantaneous fecundity at Chincoteague (165.5 ± 43.1 GJ) is still seven times higher than the maximum energy from Baltimore Canyon. The relative nutrient density of each bed, which standardized the energetic values per square meter, followed similar trends, though Brine Pool and Chincoteague were on the same order of magnitude

(Figure 4.8B). The total spawning of the mussel bed at Chincoteague ($15.61 \pm 5.0 \text{ MJ/m}^2$) was close to that of the Brine Pool bed ($16.71 \pm 5.0 \text{ MJ/m}^2$) and about 23 times more than the mussel bed at Baltimore Canyon ($0.67 \pm 0.2 \text{ MJ/m}^2$, Figure 4.8B).

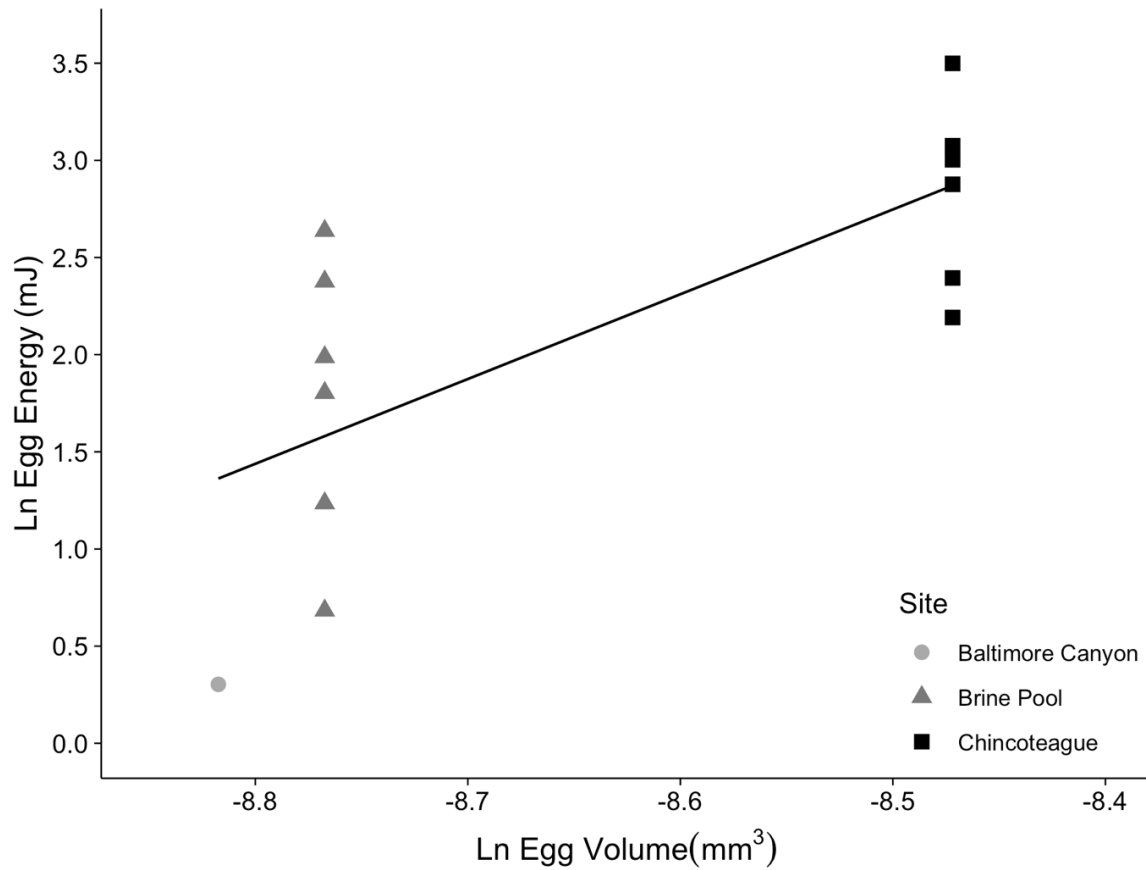


Figure 4.7. The natural log of energy content (mJ) against volume (mm^3) for spawned eggs at each site. Points represent the mJ/egg values from each calorimetry combustion against the average egg volume for that site. Scaling exponent (line) = 4.4, $R^2 = 0.48$.

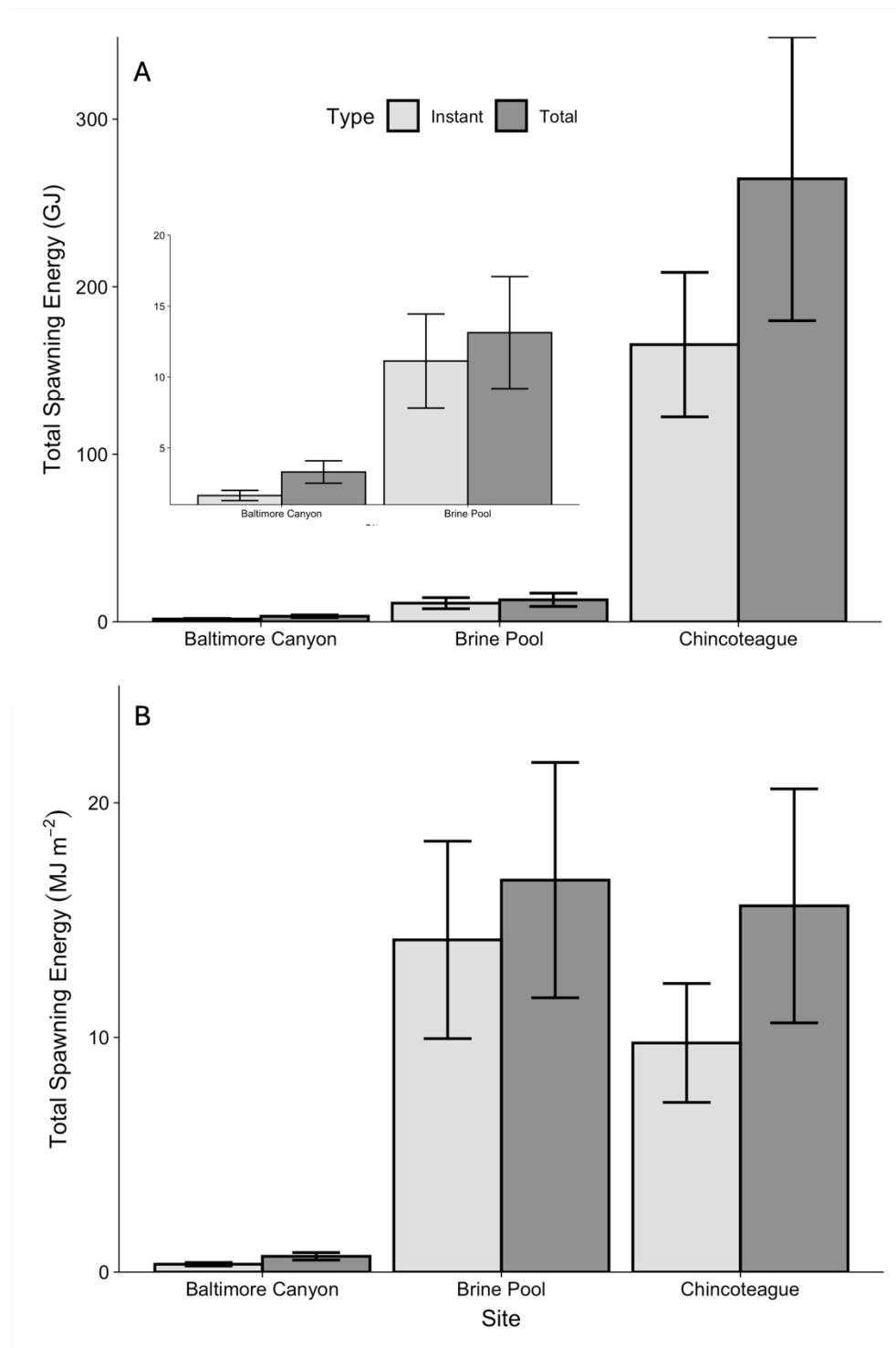


Figure 4.8. The energy released by each mussel bed through spawning. A) Total value in gigajoules, with an inset to better show the relationship between Baltimore Canyon and Brine Pool. B) The average nutrient density in megajoules per square meter. Error bars represent standard error.

4. DISCUSSION

This study provides the first estimates of energy flux through reproductive products for a chemosynthesis-based ecosystem while highlighting wide variations in reproductive parameters. We present the first quantitative estimate of fecundity and egg energy for a large bathymodiolin mussel. Our results indicate plasticity in maternal investment in eggs by *Gigantidas childressi* mussels living in different environments. Depth is an important factor, given the increased egg sizes and energy at the deepest seep site. Overall, *G. childressi* eggs appear to be more nutrient-rich than those of shallow-water mytilids. The high individual fecundity and egg energy, coupled with the large area covered by the mussel beds at these sites, lead to large amounts of energy released into the surrounding benthic and pelagic environments.

4.1. Fecundity

The fecundity of *Gigantidas childressi* is high yet variable ($\sim 5 - 11$ million eggs/female/year). Vesicomylid clams (*Calymene* spp.) from Sagami Bay in Japan are thought to have a continuous breeding cycle and spawn about 5.8×10^8 eggs m^{-2} each year (Fujikura et al., 2007). The estimates presented here for total fecundity (m^{-2}) of the bed at Baltimore Canyon are similar (5.0×10^8 eggs $\text{m}^{-2} \text{yr}^{-1}$), while all fecundity estimates for Chincoteague and Brine Pool exceed this value. The seasonal reproductive cycle of the bathymodiolins also means their spawned eggs would be present in higher concentrations for shorter timescales than those of *Calymene*. Populations of the related intertidal mussel, *Mytilus edulis*, living in the Baltic Sea produce an estimated 8×10^7 eggs $\text{m}^{-2} \text{yr}^{-1}$, and the resulting carbon flux (g C) accounts for about half of the total zooplankton production in the area (Kautsky, 1982). Increased age of an individual is known to decrease fecundity by up to 80% in *M. edulis* from the White Sea (Sukhotin & Flyachinskaya, 2009). The overall longevity and relationship of age to body size for *G. childressi*

are unknown (Laming et al., 2018), so we could not account for possible reproductive senescence.

We reported high intraspecific variability in individual fecundity across sites and within a single bed at Chincoteague. Intraspecific spatial variability in reproductive output has also been described for intertidal populations of *M. californianus* from California (Phillips, 2007a) and subtidal populations of *M. edulis* in the UK (Bayne et al., 1983). Phillips (2007a) also reported variability in the relationship of fecundity to body size in conspecifics from different beds, likely driven by changes in temperature among sites. The high instantaneous fecundities in mussels at Brine Pool compared to the Atlantic sites is likely a reflection of the single spawning period for Gulf of Mexico populations (Chapter II). This is further supported by the higher ratio of developing to ripe oocytes in mussels at the Atlantic sites since those populations spawn twice a year.

The larger egg size seen in mussels from Chincoteague would generally lead to lower fecundity values than those seen at Brine Pool with smaller eggs (Smith & Fretwell, 1974), but eggs from Baltimore Canyon were the same size as those from Brine Pool. Increased temperature is known to slow rates of gametogenesis in *Mytilus galloprovincialis* (Fearman et al., 2009). Within the genus *Mytilus*, the effects of temperature often have a complex interaction with nutrient availability (Oliveira et al., 2021; Zippay & Helmuth, 2012). No evidence exists that any seep sites studied here are nutrient-limited, though Baltimore Canyon would experience the highest temperatures as the shallowest site. The samples used to measure fecundity at Baltimore Canyon were collected in June 2021, but when we revisited the site in October 2022, many of the mussels were dead. The bed at that site may have been experiencing some environmental stress, which is known to lead to lower reproductive output in *M. edulis* (Myint & Tyler, 1982). Our

results for Baltimore Canyon could thus be impacted by environmental stress on mussels at this site.

4.2. Egg Energy

The variability in egg energy content between the shallower sites (Baltimore Canyon – 400 m and Brine Pool – 650 m) and the deepest site (Chincoteague – 1020 m) further supports the plasticity of maternal investment in *G. childressi*. The eggs at Chincoteague were not only larger but also more nutrient-dense. The energy content of an egg is generally thought to scale isometrically with egg size, though changes in egg composition can disrupt that pattern (McAlister & Moran, 2012; Moran et al., 2013). In Echinoderms with planktotrophic larvae, egg size is increased without any change in egg composition, which leads to lower nutrient density in larger eggs (Moran et al., 2013). (Moran et al., 2013) The increase in nutrient density with egg size seen here indicates that the composition of eggs from mussels of Chincoteague is likely different than that of eggs from the other sites. This could be confirmed by comparing the types and ratios of the biochemical components of eggs from mussels at various sites.

Further investigation into the causes of the change in egg energy among sites may also help elucidate why the mussels at Chincoteague create more nutrient-rich eggs. Given that mussels at all three of these sites have constant access to chemosynthetic nutrition and regular access to phytodetritus (Becker et al., 2010; Demopoulos et al., 2019; Chapter III), it is unlikely that adaptive pressure would favor conserving energy for the mother. Increasing egg size and energy with depth is, therefore, more likely an adaptive strategy to aid larvae. Studies of geminate species in multiple phyla show a trend of larger eggs in the low-food western Atlantic and smaller eggs in the comparatively food-rich eastern Pacific (Lessios, 1990; Moran, 2004). The deeper water around Chincoteague would have less phytodetritus available to larvae than at

Baltimore Canyon. Larvae from Chincoteague may thus require more energy to swim greater distances to shallow depths with planktotrophic food than larvae from shallower sites (Young et al., 2012). Studies on sea urchins have found that the effects of exogenous food availability are smaller for larvae from energy-rich eggs than those from low-energy eggs (McAlister & Moran, 2013). The plasticity seen in egg energy within *G. childressi* would thus allow for deeper populations to provision their larvae in such a way as to buffer them from the lower nutrients in the deep pelagic realm compared to near-surface waters. Increased egg nutrients have also been shown to increase the post-settlement survivorship in juvenile asteroids (Emlet & Hoegh-Guldberg, 1997), which could be advantageous for new *G. childressi* recruits as they gain their symbionts and transition to a reliance on chemosynthesis.

Like fecundity, our results for energy per egg were highly variable across and within sites. Female *Mytilus californianus* from California exhibit about a 57% difference in egg volume and 116% in egg energetic content ($\mu\text{g C}$) among individuals (Phillips, 2007b). The eggs from Baltimore Canyon that were combusted to measure energy content came from a single female. In contrast, the Brine Pool and Chincoteague data include eggs from multiple females, which may account for the within-site variability ($\sim 25\%$). Estimates of the energy content based on the biochemical composition of eggs of *M. galloprovincialis* range from 0.4 mJ egg^{-1} to 2.5 mJ egg^{-1} depending on environmental health and food availability (Bayne et al., 1979; Sánchez-Lazo & Martínez-Pita, 2012). Our values for eggs from mussels at Baltimore Canyon (1.35 mJ egg^{-1}) are within that range, while the values for Brine Pool (7.25 mJ egg^{-1}) and Chincoteague ($18.76 \text{ mJ egg}^{-1}$) are much higher. They are also much higher than those reported for echinoids with planktotrophic larvae (McAlister & Moran, 2012). However, they are much lower than the values reported for eggs of abalone *Haliotis rufescens* ($42.35\text{--}47.60 \text{ mJ egg}^{-1}$), which produces

lecithotrophic larvae (Jaekle & Manahan, 1989). Our results and previous knowledge of bathymodiolin reproduction (reviewed in Laming et al., 2018) align with a nutrient-rich egg to provision a planktotrophic larva.

4.3. Bed Spawning Energy

The mussel bed at Chincoteague dwarfs the other two examined here in terms of total energy output through spawning, which is driven by the larger area of mussels and higher nutrients per egg. However, per unit area, the smallest bed at Brine Pool releases just as much, if not more, energy than the other two sites. The increased density of female mussels and individual fecundity at Brine Pool is thus enough to match the large increase in egg energy seen at Chincoteague. The low fecundity and egg energy in mussels at Baltimore Canyon, compounded by low density, led to bed energy values that were orders of magnitude lower than those at the other two sites despite the moderate bed size. We suspect these low values result from greater environmental stress at Baltimore Canyon than at the other sites, supported by the mussel die-off between our second and third collections.

The estimates presented here for the mussel bed area and density do not account for patchiness, known to occur in beds of *Gigantidas childressi* at other seep sites (Macdonald et al., 1990). Brine Pool has previously been reported to have a uniform density over an area of 540 m² (MacDonald et al., 1990), which is smaller than our value of 786 m². The smaller bed area would decrease the estimate of the total energy released when the bed spawns to about 9 GJ annually but would not affect our reported MJ/m² values. The beds at Baltimore Canyon and Chincoteague have been reported as “extensive,” with rough area estimates larger than those reported here (Turner et al., 2020). Even with the uncertainty in bed size, the three sites studied represent a small fraction of the total number of seep communities in the Gulf of Mexico (Cordes

et al., 2009) and along the U.S. Atlantic Margin (Bourque et al., 2016) and are thus underestimates of the total area covered by bathymodiolin mussel beds. The differences seen among our collections show that both the density and body size of individual mussels change throughout the beds. More detailed descriptions of bed dynamics and individual fecundity at more body sizes would be needed to fully understand how mussel density and size affect total reproductive output.

The implications of the total energy flux from spawning at each site are hard to understand without detailed energy budgets and knowledge of how flows through these systems. However, there is growing evidence that trophic subsidies from seeps (and vents) influence fisheries, though the extent has yet to be quantified (Grupe et al., 2015). Baltimore Canyon and Chincoteague are known to be important feeding and reproductive grounds for the commercially important red crab, *Chaceon quinque-dens* (Turner et al., 2020). In the North Pacific Gyre, the largest pelagic habitat on Earth, the gross primary production from photosynthesis in surface waters has been estimated as $33 \text{ kJ m}^{-2} \text{ day}^{-1}$ (Grabowski et al., 2019). That value is equal to $\sim 12 \text{ MJ m}^{-2} \text{ yr}^{-1}$, which is less than our estimated values for energy released through spawning using the total fecundity estimates for the mussel beds at Chincoteague ($15.6 \text{ MJ m}^{-2} \text{ yr}^{-1}$) and Brine Pool ($16.7 \text{ MJ m}^{-2} \text{ yr}^{-1}$). The bed at Chincoteague still exceeds the surface production of the North Pacific Gyre if the lower egg energy value of 7.25 mJ egg^{-1} (measured in eggs from Brine Pool) is used in the calculations.

New seep sites are still being discovered, often in large numbers (Skarke, 2014). While there are likely more chemosynthetic communities than are currently known, phytoplankton production in the surface ocean undoubtedly covers orders of magnitude more surface area than bathymodiolin beds. However, typically $< 3\%$ of surface production reaches depths $> 1000 \text{ m}$

(Turner, 2015). Different types of microalgae (e.g., diatoms and dinoflagellates) have minimal energy stores and varying amounts of macromolecules and chemical energy (Finkel et al., 2016), and the quality of the resulting phytodetritus when it arrives on the seafloor is also variable (Beaulieu, 2002). Spawned eggs would constitute a high-quality source of chemosynthetically derived nutrients to higher trophic levels than the original photosynthetic nutrients (reviewed in Fuiman et al., 2015). While the chemosynthetic influence of seeps is thought to be mostly retained in the seep system, larval dispersal models show propagules can reach further into non-seep systems (reviewed by Levin et al., 2016). At cold seeps around 1000 m deep in the Gulf of Mexico, the planktonic microbial community appears to be influenced by chemosynthesis from the seabed to the euphotic zone (Rakowski et al., 2015). The Gulf of Mexico and the U.S. Western Atlantic Margin both experience particle resuspension events at small scales and in larger benthic nepheloid layers that transport material more than 100 m above the bottom (Diercks et al., 2018) and allow for lateral movement of organic matter in deep water (Hwang et al., 2009).

5. CONCLUSION

This study serves as a first step toward understanding the plasticity of maternal investment in a chemosynthetic species and how reproductive output may contribute to the export of nutrients from the seep system. While the estimates provided here could be more precise, the results support the idea that gametes are likely an important part of energy flux from chemosynthetic systems to deep pelagic food webs and should not be overlooked (Levin et al., 2016). Continued investigation of fundamental biological processes and their plasticity with response to environmental factors, especially for dominant fauna, are crucial for adequately understanding the complex ecology of these chemosynthetic systems, which will, in turn, be

necessary for effective management and conservation (Ardron et al., 2011; Van Dover, 2014). Our findings also support that chemosynthetic production may be more important for pelagic systems than previously thought (Ricci & Greening, 2024).

CHAPTER V

GENERAL CONCLUSION

This dissertation shows a strong bidirectional coupling between water column carbon and bathymodiolin reproduction, highlighting the intimate connection between processes in the surface ocean and the deep seafloor. I have also provided evidence of plasticity in many reproductive patterns in bathymodiolin mussels, particularly *Gigantidas childressi*, and the first estimates of spawned eggs as a trophic subsidy in the deep sea.

Chapter II described the reproductive phenology of multiple populations across three species (*G. childressi*, *Bathymodiolus brooksi*, and *B. heckeræ*) and found regional differences in spawning times and patterns. Shallower populations in the Gulf of Mexico experienced a spawning peak in fall, which followed what had previously been described for mussels at Brine Pool (Tyler et al., 2007). Populations at corresponding depths along the U.S. Western Atlantic Margin instead had a spawning peak in summer. There was also an effect with depth where the deeper populations seemed to experience a lag in development, less of a seasonal signal, and less synchronicity among individuals. These regional and depth-related differences provided further evidence in support of surface productivity as a reproductive cue in this mussel.

Chapter III examined this possibility with two lines of inquiry: phytodetritus as a nutritional subsidy and an environmental cue. Stable isotopes revealed that some sites experienced seasonal nutritional shifts, but there was no consistent pattern between those shifts

and the timing of surface blooms. Some adult mussels used phytodetrital nutrients, but that subsidy was not essential for successful reproduction as not all populations showed evidence of phytodetrital input. However, the patterns of surface productivity aligned well with the gametogenic phenology in both basins, which supports the hypothesis that the flux of particulate organic carbon acts as an environmental cue to synchronize reproduction in these seep mussels. Responding to phytodetritus may be a phylogenetically ingrained trait from their intertidal relatives (Eckelbarger & Watling, 1995), an adaptive trait to aid larval survival (Qasim, 1956), or it may be the strongest or most consistent seasonal signal available to them.

In Chapter IV, estimations for energy released through spawned eggs ($\text{m}^{-2} \text{yr}^{-1}$) were greater than those created by phytoplankton photosynthesis in the North Pacific Gyre (Grabowski et al., 2019). While phytoplankton cover significantly more surface area than bathymodiolin mussel beds, spawned eggs provide an input of similar levels of primary productivity, at higher trophic levels, to the deep-water systems around seeps. These results supported the idea that gametes are an overlooked source of energy flux out of chemosynthetic systems that may provide important subsidies to adjacent ecosystems. Chemosynthetic production may thus be more important for pelagic organisms than previously thought (Ricci & Greening, 2024). However, overall bed size, density, and egg energy content affected the energetic values of whole mussel beds. These results showed large intraspecific variances in egg size, nutrient density, and fecundity. The amount of energy within a single egg, especially at the deepest site, was greater than what is typically provisioned for planktotrophic eggs.

The plasticity seen in the reproductive traits of bathymodiolin mussels in this study is likely an adaptive response to environmental conditions. While high plasticity generally leads to better resiliency in the face of changing environments (reviewed in Ross et al., 2023). The

different responses of *G. childressi* from the two ocean basins suggest the species may be able to adapt their gametogenic phenology to anticipated changes in surface productivity (Hieronymus et al., 2024). Excessive offspring provisioning could aid in larval dispersal (Marshall et al., 2008) and thus help ensure species' longevity in changing environmental conditions.

This body of work serves as a step toward understanding the plasticity of maternal investment in a chemosynthetic species and a first look at how reproductive output may contribute to the export of nutrients from the seep system. The dynamic interplay of systems and nutrients seen here also highlights the need for more trophic studies to consider the energy contained in free-spawned gametes. These results also add to the growing body of evidence that chemosynthetic systems provide provisioning services to surrounding habitats. However, the magnitude of these services is different at each seep site. The data and findings herein may be useful for conservation and management efforts in the deep sea.

REFERENCES CITED

- Allen, R. M., Buckley, Y. M., & Marshall, D. J. (2008). Offspring size plasticity in response to intraspecific competition: An adaptive maternal effect across life-history stages. *American Naturalist*, 171(2), 225–237. <https://doi.org/10.1086/524952>
- Allgeier, J. E., Speare, K. E., & Burkepile, D. E. (2018). Estimates of fish and coral larvae as nutrient subsidies to coral reef ecosystems. *Ecosphere*, 9(6). <https://doi.org/10.1002/ecs2.2216>
- Ardron, J., Arnaud-Haond, S., Beaudoin, Y., Bezaury, J., Billet, D., Boland, G., Carr, M., Cherkashov, G., Cook, A., Deleo, F., & et al. (2011). *Environmental management of deep-sea chemosynthetic ecosystems: justification of and considerations for a spatially based approach*. <https://hal.science/hal-00667838>
- Arellano, S. M., Van Gaest, A. L., Johnson, S. B., Vrijenhoek, R. C., & Young, C. M. (2014). Larvae from deep-sea methane seeps disperse in surface waters. *Proceedings of the Royal Society B: Biological Sciences*, 281(1786), 1–8. <https://doi.org/10.1098/rspb.2013.3276>
- Arellano, S. M., & Young, C. M. (2009). Spawning, development, and the duration of larval life in a deep-sea cold-seep mussel. *The Biological Bulletin*, 216(2), 149–162. <https://doi.org/10.1086/bblv216n2p149>
- Arellano, S. M., & Young, C. M. (2010). Pre- and post-settlement factors controlling spatial variation in recruitment across a cold-seep mussel bed. *Marine Ecology Progress Series*, 414, 131–144. <https://doi.org/10.3354/meps08717>
- Assié, A., Borowski, C., van der Heijden, K., Raggi, L., Geier, B., Leisch, N., Schimak, M. P., Dubilier, N., & Petersen, J. M. (2016). A specific and widespread association between deep-sea *bathymodiolus* mussels and a novel family of Epsilonproteobacteria. *Environmental Microbiology Reports*, 8(5), 805–813. <https://doi.org/10.1111/1758-2229.12442>
- Audzijonyte, A., & Richards, S. A. (2018). The energetic cost of reproduction and its effect on optimal life-history strategies. *American Naturalist*, 192(4), E150–E162. <https://doi.org/10.1086/698655>
- Bayne, B. L., Moore, M. N., Widdows, J., Livingstone, D. R., Salkeld, P., Crisp, D. J., Morris, R. J., Gray, J. S., Holden, A. V., Newell, R. C., & McIntyre, A. D. (1979). Measurement of the responses of individuals to environmental stress and pollution: studies with bivalve molluscs [and discussion]. *Philosophical Transactions of the Royal Society of London. Series B., Biological*, 286(1015), 563–581.
- Bayne, B. L., Salkeld, P. N., & Worrall, C. M. (1983). Reproductive effort and value in different populations of the marine mussel, *Mytilus edulis* L. *Oecologia*, 59(1).

- Beaulieu, S. E. (2002). Accumulation and fate of phytodetritus on the sea floor. In R. N. Gibson, M. Barnes, & R. J. A. Atkinson (Eds.), *Oceanography and Marine Biology: an Annual Review* (Vol. 40, pp. 171–232). Taylor & Francis.
- Becker, E. L., Cordes, E. E., Macko, S. A., Lee, R. W., & Fisher, C. R. (2013). Using stable isotope compositions of animal tissues to infer trophic interactions in Gulf of Mexico lower slope seep communities. *PLoS ONE*, 8(12). <https://doi.org/10.1371/journal.pone.0074459>
- Becker, E. L., Lee, R. W., Macko, S. A., Faure, B. M., & Fisher, C. R. (2010). Stable carbon and nitrogen isotope compositions of hydrocarbon-seep bivalves on the Gulf of Mexico lower continental slope. *Deep-Sea Research Part II: Topical Studies in Oceanography*, 57(21–23), 1957–1964. <https://doi.org/10.1016/j.dsr2.2010.05.002>
- Beninger, P. G., Le Pennec, G., & Le Pennec, M. (2003). Demonstration of nutrient pathway from the digestive system to oocytes in the gonad intestinal loop of the scallop *Pecten maximus* L. *Biological Bulletin*, 205, 83–92.
- Berg, C. J. (1985). Reproductive strategies of mollusks from abyssal hydrothermal vent communities. *Bulletin of the Biological Society of Washington*, 6, 185–197.
- Bernardo, J. (1996). The particular maternal effect of propagule size, especially egg size: patterns, models, quality of evidence and interpretations. *American Zoologist*, 36, 216–236. <https://academic.oup.com/icb/article/36/2/216/147108>
- Biggs, D. C., Hu, C., & Müller-Karger, F. E. (2008). Remotely sensed sea-surface chlorophyll and POC flux at Deep Gulf of Mexico Benthos sampling stations. *Deep-Sea Research Part II: Topical Studies in Oceanography*, 55(24–26), 2555–2562. <https://doi.org/10.1016/j.dsr2.2008.07.013>
- Bourque, J. R., Robertson, C. M., Brooke, S., & Demopoulos, A. W. J. (2016). Macrofaunal communities associated with chemosynthetic habitats from the U.S. Atlantic margin: a comparison among depth and habitat types. *Deep Sea Research Part II: Topical Studies in Oceanography*, 137(2017), 42–55.
- Brooks, J. M., Kennicutt, M. C., Fisher, C. R., Macko, S. A., Cole, K., & Childress, J. J. (1987). Deep-sea hydrocarbon seep communities: evidence for energy and nutritional carbon sources. *Science*, 238(4830), 1138–1142.
- Comtet, T., & Desbruyeres, D. (1998). Population structure and recruitment in mytilid bivalves from the Lucky Strike and Menez Gwen on the Mid-Atlantic Ridge. *Ecology*, 163, 165–177.
- Comtet, T., Le Pennec, M., & Desbruyères, D. (1999). Evidence of a sexual pause in *Bathymodiolus azoricus* (Bivalvia: Mytilidae) from hydrothermal vents of the Mid-Atlantic Ridge. *Journal of the Marine Biological Association of the United Kingdom*, 79(6), 1149–1150. <https://doi.org/10.1017/S0025315499001514>

- Coplen, T. B. (2011). Guidelines and recommended terms for expression of stable-isotope-ratio and gas-ratio measurement results. *Rapid Communications in Mass Spectrometry*, 25(17), 2538–2560. <https://doi.org/10.1002/rcm.5129>
- Cordes, E. E., Becker, E. L., Hourdez, S., & Fisher, C. R. (2010). Influence of foundation species, depth, and location on diversity and community composition at Gulf of Mexico lower-slope cold seeps. *Deep-Sea Research Part II: Topical Studies in Oceanography*, 57(21–23), 1870–1881. <https://doi.org/10.1016/j.dsr2.2010.05.010>
- Cordes, E. E., Bergquist, D. C., & Fisher, C. R. (2009). Macro-ecology of Gulf of Mexico cold seeps. *Annual Review of Marine Science*, 1(1), 143–168. <https://doi.org/10.1146/annurev.marine.010908.163912>
- Cordes, E. E., Hourdez, S., Predmore, B. L., Redding, M. L., & Fisher, C. R. (2005). Succession of hydrocarbon seep communities associated with the long-lived foundation species *Lamellibrachia luymesii*. *Marine Ecology Progress Series*, 305, 17–29.
- Corliss, J. B., & Ballard, R. D. (1977). Oases of Life in the Cold Abyss. *National Geographic*, 441–453.
- Corliss, J. B., Dymond, J., Gordon, L. I., Edmond, J. M., Von Herzen, R. P., Ballard, R. d., Green, K., Williams, D., Bainbridge, A., Crane, K., & Van Andel, T. H. (1979). Submarine thermal springs on the Galapagos Rift. *Science*, 203(4385), 1073–1083.
- Coykendall, K. D., Cornman, R. S., Prouty, N. G., Brooke, S., Demopoulos, A. W. J., & Morrison, C. L. (2019). Molecular characterization of *Bathymodiolus* mussels and gill symbionts associated with chemosynthetic habitats from the U.S. Atlantic margin. *PLoS ONE*, 14(3). <https://doi.org/10.1371/journal.pone.0211616>
- Crisp, D. J. (1959). Factors influencing the time of breeding of *Balanus balanoides*. *Oikos*, 10(2), 275–289.
- Cuvelier, D., Legendre, P., Laes-Huon, A., Sarradin, P.-M., & Sarrazin, J. (2010). Biological and environmental rhythms in (dark) deep-sea hydrothermal ecosystems. *Marine Ecology Progress Series*, 418, 47–56. <http://www.oceannetworks.ca/>
- Damien, P., Sheinbaum, J., Pasqueron De Fommervault, O., Jouanno, J., Linacre, L., & Duteil, O. (2021). Do Loop Current eddies stimulate productivity in the Gulf of Mexico? *Biogeosciences*, 18(14), 4281–4303. <https://doi.org/10.5194/bg-18-4281-2021>
- Dattagupta, S., Bergquist, D. C., Szalai, E. B., Macko, S. A., & Fisher, C. R. (2004). Tissue carbon, nitrogen, and sulfur stable isotope turnover in transplanted *Bathymodiolus childressi* mussels: Relation to growth and physiological condition. *Limnology and Oceanography*, 49(4 I), 1144–1151. <https://doi.org/10.4319/lo.2004.49.4.1144>
- Demopoulos, A. W. J., McClain-Counts, J., Bourque, J., Prouty, N., Smith, B. J., Brooke, S., Ross, S., & Ruppel, C. D. (2019). Examination of *Bathymodiolus childressi* nutritional sources, isotopic niches, and food-web linkages at two seeps in the US Atlantic margin

- using stable isotope analysis and mixing models. *Deep Sea Research Part I: Oceanographic Research Papers*, 148, 53–66.
- Deudero, S., Cabanellas, M., Blanco, A., & Tejada, S. (2009). Stable isotope fractionation in the digestive gland, muscle and gills tissues of the marine mussel *Mytilus galloprovincialis*. *Journal of Experimental Marine Biology and Ecology*, 368(2), 181–188.
<https://doi.org/10.1016/j.jembe.2008.10.008>
- Distel, D. L., K-w Lee, H., Cavanaugh, C. M., & Somero, G. N. (1995). Intracellular coexistence of methano- and thioautotrophic bacteria in a hydrothermal vent mussel. *Proceedings of the National Academy of Science*, 92, 9598–9602. <https://www.pnas.org>
- Dixon, D. R., Lowe, D. M., Miller, P. I., Villemin, G. R., Colaço, A., Serrão-Santos, R., & Dixon, L. R. J. (2006). Evidence of seasonal reproduction in the Atlantic vent mussel *Bathymodiolus azoricus*, and an apparent link with the timing of photosynthetic primary production. *Journal of the Marine Biological Association of the United Kingdom*, 86(6), 1363–1371. <https://doi.org/10.1017/S0025315406014391>
- Duperron, S. (2010). The diversity of deep-sea mussels and their bacterial symbioses. In S. Kiel (Ed.), *The Vent and Seep Biota: Aspects from Microbes to Ecosystems* (pp. 137–167). Springer Science & Business Media. https://doi.org/10.1007/978-90-481-9572-5_6
- Duperron, S., Lorion, J., Samadi, S., Gros, O., & Gaill, F. (2009). Symbioses between deep-sea mussels (Mytilidae: Bathymodiolinae) and chemosynthetic bacteria: diversity, function and evolution. *Comptes Rendus Biologies*, 332, 298–310.
<https://doi.org/10.1016/j.crvi.2008.08.003>
- Duperron, S., Sibuet, M., MacGregor, B. J., Kuypers, M. M. M., Fisher, C. R., & Dubilier, N. (2007). Diversity, relative abundance and metabolic potential of bacterial endosymbionts in three *Bathymodiolus* mussel species from cold seeps in the Gulf of Mexico. *Environmental Microbiology*, 9(6), 1423–1438. <https://doi.org/10.1111/j.1462-2920.2007.01259.x>
- Eckelbarger, K. J., & Hodgson, A. N. (2021). Invertebrate oogenesis – a review and synthesis: comparative ovarian morphology, accessory cell function and the origins of yolk precursors. *Invertebrate Reproduction & Development*, 1–70.
<https://doi.org/10.1080/07924259.2021.1927861>
- Eckelbarger, K. J., & Watling, L. (1995). Role of phylogenetic constraints in determining reproductive patterns in deep-sea. In *Biology* (Vol. 114, Issue 3).
- Eckelbarger, K. J., & Young, C. M. (1999). Ultrastructure of gametogenesis in a chemosynthetic mytilid bivalve (*Bathymodiolus childressi*) from a bathyal, methane seep environment (northern Gulf of Mexico). *Marine Biology*, 135(4), 635–646.
<https://doi.org/10.1007/s002270050664>
- Emlet, R. B., & Hoegh-Guldberg, O. (1997). Effects of egg size on postlarval performance: Experimental evidence from a sea urchin. *Evolution*, 51(1), 141–152.
<https://doi.org/10.1111/j.1558-5646.1997.tb02395.x>

- Ezgeta-Balić, D., Lojen, S., Dolenec, T., Žvab Rožič, P., Dolenec, M., Najdek, M., & Peharda, M. (2014). Seasonal differences of stable isotope composition and lipid content in four bivalve species from the Adriatic Sea. *Marine Biology Research*, 10(6), 625–634. <https://doi.org/10.1080/17451000.2013.833338>
- Fearman, J. A., Bolch, C. J. S., & Moltschaniwskyj, N. A. (2009). Energy storage and reproduction in mussels, *Mytilus galloprovincialis*: The influence of diet quality. *Journal of Shellfish Research*, 28(2), 305–312. <https://doi.org/10.2983/035.028.0212>
- Fiala-Medioni, A., Alayse, A. M., & Cahet, G. (1986). Evidence of in situ uptake and incorporation of bicarbonate and amino acids by a hydrothermal vent mussel. *Journal of Experimental Marine Biology and Ecology*, 96, 191–198.
- Finkel, Z. V., Follows, M. J., & Irwin, A. J. (2016). Size-scaling of macromolecules and chemical energy content in the eukaryotic microalgae. *Journal of Plankton Research*, 38(5), 1151–1162. <https://doi.org/10.1093/plankt/fbw057>
- Fisher, C. R., Brooks, J. M., Vodenichar, J. S., Zande, J. M., Childress, J. J., & Jr., R. A. B. (1993). The Co-occurrence of methanotrophic and chemoautotrophic sulfur-oxidizing bacterial symbionts in a deep-sea mussel. *Marine Ecology*, 14(4), 277–289. <https://doi.org/10.1111/j.1439-0485.1993.tb00001.x>
- Franke, M., Geier, B., Hammel, J. U., Dubilier, N., & Leisch, N. (2021). Coming together - Symbiont acquisition and early development in deep-sea bathymodioline mussels. *Proceedings of the Royal Society B: Biological Sciences*, 288(1957). <https://doi.org/10.1098/rspb.2021.1044>
- Fuiman, L. A., Connelly, T. L., Lowerre-Barbieri, S. K., & McClelland, J. W. (2015). Egg boons: Central components of marine fatty acid food webs. *Ecology*, 96(2), 362–372. <https://doi.org/10.1890/14-0571.1>
- Fujikura, K., Amaki, K., James, P. B., Fujiwara, Y., Furushima, Y., Iwase, R., Yamamoto, H., & Maruyama, T. (2007). Long-term in situ monitoring of spawning behavior and fecundity in *Calymene* spp. *Marine Ecology Progress Series*, 333, 185–193.
- Gage, J. D., & Tyler, P. A. (1991). *Deep-sea biology: a natural history of organisms at the deep-sea floor*. Cambridge University Press.
- Giese, A. C., & Pearse, J. S. (1974). General Principles. In *Reproduction of marine invertebrates* (Vol. 1, pp. 2–38). Academic Press Inc.
- Girard, F., Litvin, S. Y., Sherman, A., McGill, P., Gannon, A., Lovera, C., Devogelaere, A., Burton, E., Graves, D., Schnittger, A., & Barry, J. (2022). Phenology in the deep sea: seasonal and tidal feeding rhythms in a keystone octocoral. *Proceedings of the Royal Society B: Biological Sciences*, 289(1985). <https://doi.org/10.1098/rspb.2022.1033>
- Girguis, P. R., & Childress, J. J. (2006). Metabolite uptake, stoichiometry and chemoautotrophic function of the hydrothermal vent tubeworm *Riftia pachytila*: Responses to environmental

- variations in substrate concentrations and temperature. *Journal of Experimental Biology*, 209(18), 3516–3528. <https://doi.org/10.1242/jeb.02404>
- Gooday, A. J., & Turley, C. M. (1990). Responses by benthic organisms to inputs of organic material to the ocean floor: A review. *Philosophical Transactions of the Royal Society of London. Series A, Mathematical and Physical Sciences*, 331, 119–138. <https://about.jstor.org/terms>
- Grabowski, E., Letelier, R. M., Laws, E. A., & Karl, D. M. (2019). Coupling carbon and energy fluxes in the North Pacific Subtropical Gyre. *Nature Communications*, 10(1). <https://doi.org/10.1038/s41467-019-09772-z>
- Grant, A., & Tyler, P. A. (1983). The analysis of data in studies of invertebrate reproduction. II. The analysis of oocyte size frequency data, and comparison of different types of data. *International Journal of Invertebrate Reproduction*, 6, 271–283.
- Grilo, T. F., & Rosa, R. (2017). Intersexuality in aquatic invertebrates: Prevalence and causes. In *Science of the Total Environment* (Vol. 592, pp. 714–728). Elsevier B.V. <https://doi.org/10.1016/j.scitotenv.2017.02.099>
- Grupe, B. M., Krach, M. L., Pasulka, A. L., Maloney, J. M., Levin, L. A., & Frieder, C. A. (2015). Methane seep ecosystem functions and services from a recently discovered southern California seep. *Marine Ecology*, 36(S1), 91–108. <https://doi.org/10.1111/maec.12243>
- Gustafson, R. G., Turner, R. D., Lutz, R. A., & Vrijenhoek, R. C. (1998). A new genus and five new species of mussels (Bivalvia, Mytilidae) from deep-sea sulfide/hydrocarbon seeps in the Gulf of Mexico. *Malacologia*, 40(1–2), 63–112. <https://doi.org/10.1007/s11307-006-0060-5>
- Hayward, A., & Gillooly, J. F. (2011). The cost of sex: Quantifying energetic investment in gamete production by males and females. *PLoS ONE*, 6(1). <https://doi.org/10.1371/journal.pone.0016557>
- Hessler, R. R., Smithey, W. M., Boudrias, M. A., Keller, C. H., Lutz, R. A., & Childress, J. J. (1988). Temporal change in megafauna at the Rose Garden hydrothermal vent (Galapagos Rift; eastern tropical Pacific). *Deep Sea Research Part A, Oceanographic Research Papers*, 35(10–11), 1681–1709. [https://doi.org/10.1016/0198-0149\(88\)90044-1](https://doi.org/10.1016/0198-0149(88)90044-1)
- Hiebenthal, C., Gehlert, F.-O., Schmidt, M., Reusch, T. B. H., & Melzner, F. (2024). Long-term culture system for deep-sea mussels *Gigantidas childressi*. *Limnology and Oceanography: Methods*, 1–19. <https://doi.org/10.1101/2021.11.24.469854>
- Hieronimus, J., Hieronimus, M., Gröger, M., Schwinger, J., Bernadello, R., Tourigny, E., Sicardi, V., Ruvalcaba Baroni, I., & Wyser, K. (2024). Net primary production annual maxima in the North Atlantic projected to shift in the 21st century. *Biogeosciences*, 21(9), 2189–2206. <https://doi.org/10.5194/bg-21-2189-2024>

- Howe, S., Miranda, C., Hayes, C. T., Letscher, R. T., & Knapp, A. N. (2020). The dual isotopic composition of nitrate in the Gulf of Mexico and Florida Straits. *Journal of Geophysical Research: Oceans*, 125(9). <https://doi.org/10.1029/2020JC016047>
- Hügler, M., & Sievert, S. M. (2011). Beyond the Calvin cycle: Autotrophic carbon fixation in the ocean. *Annual Review of Marine Science*, 3, 261–289. <https://doi.org/10.1146/annurev-marine-120709-142712>
- Hwang, J., Manganini, S. J., Montluçon, D. B., & Eglinton, T. I. (2009). Dynamics of particle export on the Northwest Atlantic margin. *Deep-Sea Research Part I: Oceanographic Research Papers*, 56(10), 1792–1803. <https://doi.org/10.1016/j.dsr.2009.05.007>
- Jaeckle, William. B., & Manahan, D. T. (1989). Growth and energy imbalance during the development of a lecithotrophic molluscan larva (*Haliotis rufescens*). *Biological Bulletin*, 117, 237–246.
- Kautsky, N. (1982). Studies on gonad cycle, fecundity, reproductive output and recruitment in a Baltic *Mytilus edulis* population. *Marine Biology*, 68, 143–160.
- Kenk, V. C., & Wilson, B. R. (1985). A new mussel (Bivalvia, Mytilidae) from hydrothermal vents in the Galapagos Rift Zone. *Malacologia*, 26(1–2), 253–271. <https://www.biodiversitylibrary.org/>
- Kennicutt, M. C., Brooks, J. M., Bidigare, R. R., Fay, R. R., Wade, T. L., & McDonald, T. J. (1985). Vent-type taxa in a hydrocarbon seep region on the Louisiana slope. *Nature*, 317(6035), 351–353. <https://doi.org/10.1038/317351a0>
- Kiel, S., & Tyler, P. A. (2010). Chemosynthetically-driven ecosystems in the deep sea. In S. Kiel (Ed.), *The Vent and Seep Biota* (pp. 1–14). <http://www.springer.com/series/6623>
- Laming, S. R., Gaudron, S. M., & Duperron, S. (2018). Lifecycle ecology of deep-sea chemosymbiotic mussels: A review. *Frontiers in Marine Science*, 5(282). <https://doi.org/10.3389/fmars.2018.00282>
- Le Pennec, M., & Beninger, P. G. (2000). Reproductive characteristics and strategies of reducing-system bivalves. *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology*, 126(1), 1–16. [https://doi.org/10.1016/S0742-8413\(00\)00100-6](https://doi.org/10.1016/S0742-8413(00)00100-6)
- Lee, R. W., & Childress, J. J. (1994). Assimilation of inorganic nitrogen by marine invertebrates and their chemoautotrophic and methanotrophic symbionts. *Applied and Environmental Microbiology*, 60(6), 1852–1858.
- Lee, R. W., & Childress, J. J. (1995). Assimilation of inorganic nitrogen by seep mytilid Ia, an undescribed deep-sea mussel containing methanotrophic endosymbionts: fate of assimilated nitrogen and the relation between methane and nitrogen assimilation. *Marine Ecology Progress Series*, 123, 137–148.

- Lee, R. W., & Childress, J. J. (1996). Inorganic N assimilation and ammonium pools in a deep-sea mussel containing methanotrophic endosymbionts. *Biological Bulletin*, 190(3), 373–384. <https://doi.org/10.2307/1543030>
- Lessios, H. A. (1990). Adaptation and phylogeny as determinants of egg size in echinoderms from the two sides of the isthmus of Panama. *The American Naturalist*, 135(1), 1–13.
- Levin, L. A., Baco, A. R., Bowden, D. A., Colaco, A., Cordes, E. E., Cunha, M. R., Demopoulos, A. W. J., Gobin, J., Grupe, B. M., Le, J., Metaxas, A., Netburn, A. N., Rouse, G. W., Thurber, A. R., Tunnicliffe, V., Van Dover, C. L., Vanreusel, A., & Watling, L. (2016). Hydrothermal vents and methane seeps: Rethinking the sphere of influence. *Frontiers in Marine Science*, 3(MAY). <https://doi.org/10.3389/fmars.2016.00072>
- Lin, T. H., & Kawagucci, S. (2024). Acoustic twilight: A year-long seafloor monitoring unveils phenological patterns in the abyssal soundscape. *Limnology and Oceanography Letters*, 9(1): 23–32. <https://doi.org/10.1002/lol2.10358>
- Lowe, D. M., Moore, M. N., & Bayne, B. L. (1982). Aspects of gametogenesis in the marine mussel *Mytilus edulis*. *Journal of the Marine Biological Association of the United Kingdom*, 62(1), 133–145.
- Lowe, D. M., Salkeld, P. N., & Carr, M. R. (1994). The effect of geographical location on the cellular composition of the mantle tissue of the mussel, *Mytilus edulis*. *Journal of the Marine Biological Association of the United Kingdom*, 74(1), 225–232.
- MacAvoy, S. E., Carney, R. S., Fisher, C. R., & Macko, S. A. (2002). Use of chemosynthetic biomass by large, mobile, benthic predators in the Gulf of Mexico. *Marine Ecology Progress Series*, 225, 65–78.
- Macdonald, I. R., Callender, W. R., Burke, R. A., McDonald, S. J., & Carney, R. S. (1990). Fine-scale distribution of methanotrophic mussels at a Louisiana cold seep. *Progress in Oceanography*, 24, 15–24.
- MacDonald, I. R., Reilly, J. F., Guinasso, N. L., Brooks, J. M., Carney, R. S., Bryant, W. A., & Bright, T. J. (1990). Chemosynthetic mussels at a brine-filled pockmark in the northern Gulf of Mexico. *Science*, 248(4959), 1096–1099. <https://doi.org/10.1126/science.248.4959.1096>
- Marshall, D. J., Allen, R. M., & Crean, A. J. (2008). The ecological and evolutionary importance of maternal effects in the sea. *Oceanography and Marine Biology: An Annual Review*, 46, 203–250.
- Mathieu, M., & Lubet, P. (1993). Storage tissue metabolism and reproduction in marine bivalves – a brief review. *Invertebrate Reproduction and Development*, 23(2–3), 123–129.
- McAlister, J. S., & Moran, A. L. (2012). Relationships among egg size, composition, and energy: A comparative study of geminate sea urchins. *PLoS ONE*, 7(7). <https://doi.org/10.1371/journal.pone.0041599>

- McAlister, J. S., & Moran, A. L. (2013). Effects of variation in egg energy and exogenous food on larval development in congeneric sea urchins. *Marine Ecology Progress Series*, 490, 155–167. <https://doi.org/10.3354/meps10420>
- McNichol, J., Stryhanyuk, H., Sylva, S. P., Thomas, F., Musat, N., Seewald, J. S., & Sievert, S. M. (2018). Primary productivity below the seafloor at deep-sea hot springs. *Proceedings of the National Academy of Sciences of the United States of America*, 115(26), 6756–6761. <https://doi.org/10.1073/pnas.1804351115>
- McVeigh, D. M., Eggleston, D. B., Todd, A. C., Young, C. M., & He, R. (2017). The influence of larval migration and dispersal depth on potential larval trajectories of a deep-sea bivalve. *Deep-Sea Research Part I: Oceanographic Research Papers*, 127, 57–64. <https://doi.org/10.1016/j.dsr.2017.08.002>
- Mcveigh, D., Skarke, A., Dekas, A. E., Borrelli, C., Hong, W.-L., Marlow, J., Pasulka, A., Jungbluth, S. P., Barco, R. A., & Djurhuus, A. (2018). Characterization of benthic biogeochemistry and ecology at three methane seep sites on the Northern U.S. Atlantic Margin 2. *Deep Sea Research Part II: Topical Studies in Oceanography*, 150, 41–56.
- Mills, S. C., & Côté, I. M. (2003). Sex-related differences in growth and morphology of blue mussels. *Journal of the Marine Biological Association of the United Kingdom*, 83(5), 1053–1057. <https://doi.org/10.1017/S0025315403008269h>
- Minagawa, M., & Wada, E. (1984). Stepwise enrichment of ^{15}N along food chains: Further evidence and the relation between $\delta^{15}\text{N}$ and animal age. *Geochimica et Cosmochimica Acta*, 48, 1135–1140.
- Miyazaki, J. I., de Oliveira Martins, L., Fujita, Y., Matsumoto, H., & Fujiwara, Y. (2010). Evolutionary process of deep-sea *Bathymodiolus* mussels. *PLoS ONE*, 5(4), 1–11. <https://doi.org/10.1371/journal.pone.0010363>
- Moran, A. L. (2004). Egg size evolution in tropical American arcid bivalves: The comparative method and the fossil record. *Evolution*, 58(12), 2718–2733. <https://doi.org/10.1111/j.0014-3820.2004.tb01624.x>
- Moran, A. L., & McAlister, J. S. (2009). Egg size as a life history character of marine invertebrates: is it all it's cracked up to be? *Biological Bulletin*, 216(3), 226–242. <https://doi.org/10.2307/25548157>
- Moran, A. L., McAlister, J. S., & Whitehill, E. A. G. (2013). Eggs as energy: Revisiting the scaling of egg size and energetic content among echinoderms. *Biological Bulletin*, 224(3), 184–191. <https://doi.org/10.1086/BBLv224n3p184>
- Morgan, S. G. (1995). Life and death in the plankton: larval mortality and adaptation. In *Ecology of marine invertebrate larvae* (pp. 279–321).
- Myint, U. M., & Tyler, P. A. (1982). Effects of temperature, nutritive and metal stressors on the reproductive biology of *Mytilus edulis*. *Marine Biology*, 67(2), 209–223.

- NASA Ocean Biology Processing Group. (2018a). *Moderate-Resolution Imaging Spectroradiometer (MODIS) Aqua Level-3 Standard Mapped Image Product Global 4 km Monthly Composite Particulate Organic Carbon Data, version 2018.1QL*. <https://doi.org/10.5067/AQUA/MODIS/L3M/POC/2018>
- NASA Ocean Biology Processing Group. (2018b). *Moderate-Resolution Imaging Spectroradiometer (MODIS) Aqua Level-3 Standard Mapped Image Product Global 4 km Science Quality Monthly Composite Chlorophyll-a Data, version 2018.1QL*. Accessed through NOAA Coastwatch. <https://doi.org/10.5067/AQUA/MODIS/L3M/CHL/2018>
- Newell, R. I. E., Hilbish, T. J., Koehn, R. K., & Newell, C. J. (1982). Temporal variation in the reproductive cycle of *Mytilus edulis* L. (bivalvia, mytilidae) from localities on the East Coast of the United States. *Biological Bulletin*, 162, 299–310.
- Oliveira, G. F., Siregar, H., Queiroga, H., & Peteiro, L. G. (2021). Main drivers of fecundity variability of mussels along a latitudinal gradient: Lessons to apply for future climate change scenarios. *Journal of Marine Science and Engineering*, 9(7). <https://doi.org/10.3390/jmse9070759>
- Orton, H. (1920). Sea-temperature, breeding and distribution in marine animals. *Journal of the Marine Biological Association of the UK*, 12(2), 339–366.
- Page, H. M., Fiala-Medioni, A., Fisher, C. R., & Childress, J. J. (1991). Experimental evidence for filter-feeding by the hydrothermal vent mussel, *Bathymodiolus thermophilus*. *Deep Sea Research Part A, Oceanographic Research Papers*, 38(12), 1455–1461. [https://doi.org/10.1016/0198-0149\(91\)90084-S](https://doi.org/10.1016/0198-0149(91)90084-S)
- Paull, C. K., Hecker, B., Commeau, R., Freeman-Lynde, R. P., Neumann, C., Corso, W. P., Golubic, S., Hook, J. E., Sikes, E., & Curray, J. (1984). Biological communities at the Florida Escarpment resemble hydrothermal vent taxa. *Science*, 226(4677), 965–967.
- Paull, C. K., Jull, A. J. T., Toolin, L. J., & Linick, T. (1985). Stable isotope evidence for chemosynthesis in an abyssal seep community. *Nature*, 317, 709–711.
- Phillips, N. E. (2007a). A spatial gradient in the potential reproductive output of the sea mussel *Mytilus californianus*. *Marine Biology*, 151(4), 1543–1550. <https://doi.org/10.1007/s00227-006-0592-x>
- Phillips, N. E. (2007b). High variability in egg size and energetic content among intertidal mussels. *Biological Bulletin*, 212, 12–19.
- Pile, A. J., & Young, C. M. (1999). Plankton availability and retention efficiencies of cold-seep symbiotic mussels. *Limnology and Oceanography*, 44(7), 1833–1839. <https://doi.org/10.4319/lo.1999.44.7.1833>
- Plowman, C. Q. (2017). *Reproductive patterns of cold-seep mussels in the Gulf of Mexico and Northwestern Atlantic* [Master's thesis]. University of Oregon.

- Portanier, E., Nicolle, A., Rath, W., Monnet, L., Le Goff, G., Le Port, A. S., Daguin-Thiébaud, C., Morrison, C. L., Cunha, M. R., Betters, M., Young, C. M., Van Dover, C. L., Biastoch, A., Thiébaud, E., & Jollivet, D. (2023). Coupling large-spatial scale larval dispersal modelling with barcoding to refine the amphi-Atlantic connectivity hypothesis in deep-sea seep mussels. *Frontiers in Marine Science*, 10. <https://doi.org/10.3389/fmars.2023.1122124>
- Post, D. M., Layman, C. A., Arrington, D. A., Takimoto, G., Quattrochi, J., & Montaña, C. G. (2007). Getting to the fat of the matter: Models, methods and assumptions for dealing with lipids in stable isotope analyses. *Oecologia*, 152(1), 179–189. <https://doi.org/10.1007/s00442-006-0630-x>
- Pratchett, M. S., Gust, N., Goby, G., & Klanten, S. O. (2001). Consumption of coral propagules represents a significant trophic link between corals and reef fish. *Coral Reefs*, 20, 13–17.
- Presnell, J. K., & Schreibman, M. P. (1997). *Humason's Animal Tissue Techniques* (5th ed.). The Johns Hopkins University Press.
- Prowse, T. A. A., Sewell, M. A., & Byrne, M. (2008). Fuels for development: Evolution of maternal provisioning in asterinid sea stars. *Marine Biology*, 153(3), 337–349. <https://doi.org/10.1007/s00227-007-0809-7>
- Qasim, S. Z. (1956). Time and duration of the spawning season in some marine teleosts in relation to their distribution. *Journal Du Conseil*, 21(2), 144–155.
- QGIS.org. (2024). *QGIS Geographic Information System*. QGIS Association.
- Quattrini, A. M., Nizinski, M. S., Chaytor, J. D., Demopoulos, A. W. J., Roark, E. B., France, S. C., Moore, J. A., Heyl, T., Auster, P. J., Kinlan, B., Ruppel, C., Elliott, K. P., Kennedy, B. R. C., Lobecker, E., Skarke, A., & Shank, T. M. (2015). Exploration of the canyon-incised continental margin of the northeastern United States reveals dynamic habitats and diverse communities. *PLoS ONE*, 10(10). <https://doi.org/10.1371/journal.pone.0139904>
- R Core Team. (2023). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing.
- Rakowski, C. V., Magen, C., Bosman, S., Rogers, K. L., Gillies, L. E., Chanton, J. P., & Mason, O. U. (2015). Methane and microbial dynamics in the Gulf of Mexico water column. *Frontiers in Marine Science*, 2(SEP). <https://doi.org/10.3389/fmars.2015.00069>
- Ramirez-Llodra, E., Brandt, A., Danovaro, R., De Mol, B., Escobar, E., German, C. R., Levin, L. A., Martinez Arbizu, P., Menot, L., Buhl-Mortensen, P., Narayanaswamy, B. E., Smith, C. R., Tittensor, D. P., Tyler, P. A., Vanreusel, A., & Vecchione, M. (2010). Deep, diverse and definitely different: Unique attributes of the world's largest ecosystem. *Biogeosciences*, 7(9), 2851–2899. <https://doi.org/10.5194/bg-7-2851-2010>
- Ramirez-Llodra, E., Tyler, P. A., Baker, M. C., Bergstad, O. A., Clark, M. R., Escobar, E., Levin, L. A., Menot, L., Rowden, A. A., Smith, C. R., & van Dover, C. L. (2011). Man and

- the last great wilderness: Human impact on the deep sea. *PLoS ONE*, 6(8).
<https://doi.org/10.1371/journal.pone.0022588>
- Ricci, F., & Greening, C. (2024). Chemosynthesis: a neglected foundation of marine ecology and biogeochemistry. *Trends in Microbiology*, S0966-842X.
<https://doi.org/10.1016/j.tim.2023.11.013>
- Riekenberg, P. M., Carney, R. S., & Fry, B. (2016). Trophic plasticity of the methanotrophic mussel *Bathymodiolus childressi* in the Gulf of Mexico. *Marine Ecology Progress Series*, 547, 91–106. <https://doi.org/10.3354/meps11645>
- Riou, V., Colaço, A., Bouillon, S., Khripounoff, A., Dando, P., Mangion, P., Chevalier, E., Korntheuer, M., Santos, R. S., & Dehairs, F. (2010). Mixotrophy in the deep sea: A dual endosymbiotic hydrothermal mytilid assimilates dissolved and particulate organic matter. *Marine Ecology Progress Series*, 405, 187–201. <https://doi.org/10.3354/meps08515>
- Riou, V., Duperron, S., Halary, S., Dehairs, F., Bouillon, S., Martins, I., Colaço, A., & Serraão Santos, R. (2010). Variation in physiological indicators in *Bathymodiolus azoricus* (Bivalvia: Mytilidae) at the Menez Gwen Mid-Atlantic Ridge deep-sea hydrothermal vent site within a year. *Marine Environmental Research*, 70(3–4), 264–271.
<https://doi.org/10.1016/j.marenvres.2010.05.008>
- Ritt, B., Duperron, S., Lorion, J., Sara Lazar, C., & Sarrazin, J. (2012). Integrative study of a new cold-seep mussel (Mollusca: Bivalvia) associated with chemosynthetic symbionts in the Marmara Sea. *Deep-Sea Research Part I: Oceanographic Research Papers*, 67, 121–132.
<https://doi.org/10.1016/j.dsr.2012.05.009>
- Roberts, H. H., & Aharon, P. (1994). Hydrocarbon-derived carbonate buildups of the northern Gulf of Mexico continental slope: A review of submersible investigations. *Geo-Marine Letters*, 14, 135–148.
- Ross, P. M., Scanes, E., Byrne, M., Ainsworth, T. D., Donelson, J. M., Foo, S. A., Hutchings, P., Thiagarajan, V., & Parker, L. M. (2023). Surviving the anthropocene: the resilience of marine animals to climate change. *Oceanography and Marine Biology: An Annual Review*, 61, 35–80. <https://doi.org/10.1201/9781003363873-3>
- Rossi, G. S., & Tunnicliffe, V. (2017). Trade-offs in a high CO₂ habitat on a subsea volcano: Condition and reproductive features of a bathymodioline mussel. *Marine Ecology Progress Series*, 574, 49–64. <https://doi.org/10.3354/meps12196>
- Rumrill, S. S. (1990). Natural mortality of marine invertebrate larvae. *Ophelia*, 32(1–2), 163–198.
- Saino, T., & Hattori, A. (1987). Geographical variation of the water column distribution of suspended particulate organic nitrogen and its ¹⁵N natural abundance in the Pacific and its marginal seas. *Deep-Sea Research*, 34(5/6), 807–827.

- Salerno, J. L., Macko, S. A., Hallam, S. J., Bright, M., Won, Y. J., McKiness, Z., & Van Dover, C. L. (2005). Characterization of symbiont populations in life-history stages of mussels from chemosynthetic environments. *Biological Bulletin*, 208(2), 145–155. <https://doi.org/10.2307/3593123>
- Sánchez-Lazo, C., & Martínez-Pita, I. (2012). Biochemical and energy dynamics during larval development of the mussel *Mytilus galloprovincialis* (Lamarck, 1819). *Aquaculture*, 358–359, 71–78. <https://doi.org/10.1016/j.aquaculture.2012.06.021>
- Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D. J., Hartenstein, V., Eliceiri, K., Tomancak, P., & Cardona, A. (2012). Fiji: an open-source platform for biological-image analysis. *Nature Methods*, 9(7), 676–682.
- Seed, R. (1976). Ecology. In B. L. Bayne (Ed.), *Marine Mussels: Their Ecology and Physiology* (pp. 13–66). Cambridge University Press.
- Skarke, A., Ruppel, C., Kodis, M., Brothers, D., & Lobecker, E. (2014). Widespread methane leakage from the sea floor on the northern US Atlantic margin. *Nature Geoscience*, 7(9), 657–661. <https://doi.org/10.1038/ngeo2232>
- Smart, T. I., Young, C. M., & Emlet, R. B. (2012). Environmental cues and seasonal reproduction in a temperate estuary: A case study of *Owenia collaris* (Annelida: Polychaeta, Oweniidae). *Marine Ecology*, 33(3), 290–301. <https://doi.org/10.1111/j.1439-0485.2011.00493.x>
- Smith, E. B., Scott, K. M., Nix, E. R., Korte, C., & Fisher, C. R. (2000). Growth and condition of seep mussels (*Bathymodiolus childressi*) at a Gulf of Mexico brine pool. *Ecology*, 81(9), 2392–2403.
- Speijer, D., Lukeš, J., & Eliáš, M. (2015). Sex is a ubiquitous, ancient, and inherent attribute of eukaryotic life. *Proceedings of the National Academy of Sciences of the United States of America*, 112(29), 8827–8834. <https://doi.org/10.1073/pnas.1501725112>
- Stevenson, A., & Mitchell, F. J. G. (2016). Evidence of nutrient partitioning in coexisting deep-sea echinoids, and seasonal dietary shifts in seasonal breeders: Perspectives from stable isotope analyses. *Progress in Oceanography*, 141, 44–59. <https://doi.org/10.1016/j.pocean.2015.12.004>
- Streams, M. E., Fisher, C. R., & Fiala-Médioni, A. (1997). Methanotrophic symbiont location and fate of carbon incorporated from methane in a hydrocarbon seep mussel. *Marine Biology*, 129, 465–476.
- Stukel, M. R., Kelly, T. B., Landry, M. R., Selph, K. E., Swalethorp, R., & Dolan, J. (2022). Sinking carbon, nitrogen, and pigment flux within and beneath the euphotic zone in the oligotrophic, open-ocean Gulf of Mexico. *Journal of Plankton Research*, 44(5), 711–727. <https://doi.org/10.1093/plankt/fbab001>

- Sukhotin, A. A., & Flyachinskaya, L. P. (2009). Aging reduces reproductive success in mussels *Mytilus edulis*. *Mechanisms of Ageing and Development*, 130(11–12), 754–761. <https://doi.org/10.1016/j.mad.2009.09.005>
- Taylor, J. R., & Thompson, W. (1982). *An introduction to error analysis: the study of uncertainties in physical measurements* (Vol. 2). University Science Books.
- Thorson, G. (1950). Reproductive and larval ecology of marine bottom invertebrates. *Biological Reviews*, 25(1), 1–45.
- Tunnicliffe, V., Kim, S., & Sibuet, M. (2003). Reducing environments of the deep-sea floor. In P. A. Tyler (Ed.), *Ecosystems of the Deep Oceans* (pp. 81–110). Elsevier.
- Turner, J. T. (2015). Zooplankton fecal pellets, marine snow, phytodetritus and the ocean's biological pump. *Progress in Oceanography*, 130, 205–248. <https://doi.org/10.1016/j.pocean.2014.08.005>
- Turner, P. J., Ball, B., Diana, Z., Fariñas-Bermejo, A., Grace, I., McVeigh, D., Powers, M. M., Van Audenhaege, L., Maslakova, S., Young, C. M., & Van Dover, C. L. (2020). Methane seeps on the US Atlantic Margin and their potential importance to populations of the commercially valuable deep-sea red crab, *Chaceon quinquedens*. *Frontiers in Marine Science*, 7. <https://doi.org/10.3389/fmars.2020.00075>
- Tyler, P. (1988). Seasonality in the Deep Sea. *Oceanography and Marine Biology Annual Reviews*, 26, 227–258.
- Tyler, P. A., German, C. R., Ramirez-Llodra, E., & Van Dover, C. L. (2002). Understanding the biogeography of chemosynthetic ecosystems. *Oceanologica Acta*, 25, 227–241. www.elsevier.com/locate/oceact
- Tyler, P. A., & Young, C. M. (1999). Reproduction and dispersal at vents and cold seeps. *Journal of the Marine Biological Association of the United Kingdom*, 79(2), 193–208. <https://doi.org/10.1017/S0025315499000235>
- Tyler, P. A., Young, C. M., Dolan, E., Arellano, S. M., Brooke, S. D., & Baker, M. (2007). Gametogenic periodicity in the chemosynthetic cold-seep mussel “*Bathymodiolus*” *childressi*. *Marine Biology*, 150(5), 829–840. <https://doi.org/10.1007/s00227-006-0362-9>
- Van Dover, C. L. (2000). *The ecology of deep-sea hydrothermal vents*. Princeton University Press.
- Van Dover, C. L. (2014). Impacts of anthropogenic disturbances at deep-sea hydrothermal vent ecosystems: A review. *Marine Environmental Research*, 102, 59–72. <https://doi.org/10.1016/j.marenvres.2014.03.008>
- Van Dover, C. L., Aharon, P., Bernhard, J. M., Caylor, E., Doerries, M., Flickinger, W., Gilhooly, W., Goffredi, S. K., Knick, K. E., Macko, S. A., Rapoport, S., Raulfs, E. C., Ruppel, C., Salerno, J. L., Seitz, R. D., Sen Gupta, B. K., Shank, T., Turnipseed, M., &

- Vrijenhoek, R. (2003). Blake Ridge methane seeps: Characterization of a soft-sediment, chemosynthetically based ecosystem. *Deep-Sea Research Part I: Oceanographic Research Papers*, 50(2), 281–300. [https://doi.org/10.1016/S0967-0637\(02\)00162-0](https://doi.org/10.1016/S0967-0637(02)00162-0)
- Van Dover, C. L., German, C. R., Speer, K. G., Parson, L. M., & Vrijenhoek, R. C. (2002). Evolution and biogeography of deep-sea vent and seep invertebrates. *Science*, 157, 1253–1257.
- Vokshoori, N. L., McCarthy, M. D., Close, H. G., Demopoulos, A. W. J., & Prouty, N. G. (2021). New geochemical tools for investigating resource and energy functions at deep-sea cold seeps using amino acid $\delta^{15}\text{N}$ in chemosymbiotic mussels (*Bathymodiolus childressi*). *Geobiology*, 19(6), 601–617. <https://doi.org/10.1111/gbi.12458>
- Wangenstein, O. S., Turon, X., & Palacín, C. (2016). Reproductive strategies in marine invertebrates and the structuring of marine animal forests. In *Marine Animal Forests* (pp. 1–24). Springer International Publishing. https://doi.org/10.1007/978-3-319-17001-5_52-1
- Young, C. M. (1994). A tale of two dogmas: the early history of deep-sea reproductive biology. In C. M. Young & K. J. Eckelbarger (Eds.), *Reproduction, larval biology, and recruitment in the deep-sea benthos* (pp. 1–25). Columbia University Press.
- Young, C. M. (2003). Reproduction, development and life-history traits. In P. A. Tyler (Ed.), *Ecosystems of the deep oceans* (pp. 381–426). Elsevier Science.
- Young, C. M., & Chia, F. S. (1987). Abundance and distribution of pelagic larvae as influenced by predation, behavior, and hydrographic factors. In A. C. Giese & J. S. Pearse (Eds.), *Reproduction of Marine Invertebrates* (Vol. 9, pp. 385–463). Academic Press.
- Young, C. M., He, R., Emlet, R. B., Li, Y., Qian, H., Arellano, S. M., Van Gaest, A., Bennett, K. C., Wolf, M., Smart, T. I., & Rice, M. E. (2012). Dispersal of deep-sea larvae from the intra-American seas: Simulations of trajectories using ocean models. *Integrative and Comparative Biology*, 52(4), 483–496. <https://doi.org/10.1093/icb/ics090>
- Zhang, K., Sun, J., Xu, T., Qiu, J. W., & Qian, P. Y. (2021). Phylogenetic relationships and adaptation in deep-sea mussels: Insights from mitochondrial genomes. *International Journal of Molecular Sciences*, 22(4), 1–13. <https://doi.org/10.3390/ijms22041900>
- Zhong, Z., Wang, M., Chen, H., Zheng, P., & Li, C. (2020). Gametogenesis and reproductive traits of the cold-seep mussel *Gigantidas platifrons* in the South China Sea. *Journal of Oceanology and Limnology*, 38(4), 1304–1318. <https://doi.org/10.1007/s00343-020-0027-4>
- Zippay, M. L., & Helmuth, B. (2012). Effects of temperature change on mussel, *Mytilus edulis*. *Integrative Zoology*, 7, 312–327. <https://doi.org/10.1111/j.1749-4877.2012.00310.x>

SUPPLEMENTAL FILES

CHAPTER II

Table S2.1. Summary of chi-square contingency table tests on maturity stages. Z-score interpretation lists where the differences are: ↑ indicates more of a given stage than expected and ↓ is less than expected. Ns indicates non-significate z-scores. Means are displayed ± standard deviation. Significant p-values and the highest means are bolded. Sites abbreviations: BP = Brine Pool, MC = Mississippi Canyon, FE = Florida Escarpment, BC = Baltimore Canyon, C = Chincoteague, BR = Blake Ridge.

Site	Species	Sex	Chi-Squared Test			Z-Score Interpretations			Population Means		
			χ^2 value	Df	p-value	Spring 2020	Summer 2021	Fall 2022	Spring	Summer	Fall
BP	<i>G. childressi</i>	F	72.52	6	<0.0001	↑ 3; ↓ 5	↑ 4; ↓ 5	↓ 4; ↑ 5	3.4 ± 0.6	4.0 ± 0.4	4.6 ± 0.7
		M	42.81	8	<0.0001	↑ 2; ↑ 3	NS	↑ 5	3.2 ± 1.0	4.0 ± 0.6	4.3 ± 0.5
MC	<i>G. childressi</i>	F	18.03	10	0.0544	-	-	-	3.0 ± 0.0	3.0 ± 1.2	3.8 ± 0.8
		M	25.99	10	0.0037	NS	↑ 4	↓ 4	3.1 ± 1.1	3.2 ± 1.4	3.9 ± 0.4
MC	<i>B. brooksi</i>	F	4.44	3	0.217	-	-	-	2.6 ± 1.3	2.7 ± 1.1	2.5 ± 0.7
		M	27.51	8	0.0006	NS	↑ 2	↑ 4	2.2 ± 1.4	2.3 ± 0.5	3.0 ± 1.1
FE	<i>B. heckeræ</i>	F	19.46	8	0.0126	NS	NS	NS	3.9 ± 0.6	3.8 ± 1.2	3.5 ± 0.5
		M	79.15	8	<0.0001	↓ 2	↑ 2; ↑ 6; ↓ 4	↑ 4	4.1 ± 0.8	3.8 ± 1.9	3.8 ± 0.6
BC	<i>G. childressi</i>	F	55.90	6	<0.0001	↑ 4	↑ 5	↑ 2-3; ↓ 4-	4.1 ± 0.5	4.5 ± 0.6	2.9 ± 0.6
		M	39.89	10	<0.0001	↑ 4; ↓ 6	↑ 6	↑ 2; ↓ 4	4.0 ± 0.7	4.8 ± 1.2	3.4 ± 1.8
C	<i>G. childressi</i>	F	61.64	8	<0.0001	↓ 3; ↑ 4	NS	NS	4.2 ± 0.4	4.6 ± 0.9	3.5 ± 0.5
		M	71.54	8	<0.0001	↓ 4	↓ 4; ↑ 5-6	↑ 3	4.1 ± 0.5	5.2 ± 0.4	3.4 ± 0.5
BR	<i>B. heckeræ</i>	F	26.13	8	0.001	↓ 1	NS	NS	4.0 ± 1.4	3.0 ± 1.5	2.8 ± 1.5
		M	34.283	10	0.0002	↓ 1; ↑ 6	NS	NS	4.8 ± 1.6	2.4 ± 1.6	2.8 ± 1.4

Table S2.2. Summary of statistical comparisons of *Gigantidas childressi* between oocyte sizes in the visceral mass and the mantle for each collection season. Either a t-test or a Wilcoxon rank-sum test was used, depending on the results of a prior Levene's test. Means are presented $\pm$ standard deviation. Significant p-values are bolded.

Site	Season	Visceral Mean	Mantle Mean	Test Stat	Df	p-value
Brine Pool	Spring 2020	20.65 $\pm$ 8.63	22.03 $\pm$ 8.68	t = -3.81	1409	0.0001
	Summer 2021	37.91 $\pm$ 10.39	39.57 $\pm$ 10.86	t = -4.21	2892	<0.0001
	Fall 2022	42.09 $\pm$ 11.28	42.93 $\pm$ 12.33	W = 568260		0.0324
Baltimore Canyon	Spring 2020	28.53 $\pm$ 11.77	34.56 $\pm$ 11.76	t = -8.94	465	<0.0001
	Summer 2021	38.32 $\pm$ 12.20	43.85 $\pm$ 11.32	W = 520322		< 0.0001
	Fall 2022	25.63 $\pm$ 6.85	26.09 $\pm$ 6.82	t = -0.79	179	0.4304
Chincoteague	Spring 2020	30.32 $\pm$ 11.78	27.63 $\pm$ 9.31	W = 417847		<0.0001
	Summer 2021	48.89 $\pm$ 14.26	51.40 $\pm$ 16.11	W = 448144		0.0003
	Fall 2022	37.57 $\pm$ 11.57	36.43 $\pm$ 14.20	W = 101070		0.0382

Table S2.3. Summary of Kruskal-Wallis tests used to compare the synchronicity between individual oocyte sizes for each collection. Results of all Dunn's post-hoc tests were $p < 0.0001$, and $\eta^2[H]$ measures effect size: 1 = fully asynchronous, 0 = fully synchronous. Test not performed when the sample size was smaller than 5 individuals.

Site	Species	Collection	H	Df	p-value	$\eta^2[H]$
Brine Pool	<i>G. childressi</i>	Spring 2020	3000	29	<0.0001	0.24
		Summer 2021	213	28	<0.0001	0.06
		Fall 2022	489	21	<0.0001	0.16
Mississippi Canyon	<i>G. childressi</i>	Spring 2020	-	-	-	-
		Summer 2021	454	19	<0.0001	0.22
		Fall 2022	149	15	<0.0001	0.08
Mississippi Canyon	<i>B. brooksi</i>	Spring 2020	229	7	<0.0001	0.34
		Summer 2021	-	-	-	-
		Fall 2022	-	-	-	-
Florida Escarpment	<i>B. heckerae</i>	Spring 2020	925	29	<0.0001	0.30
		Summer 2021	259	20	<0.0001	0.08
		Fall 2022	288	13	<0.0001	0.09
Baltimore Canyon	<i>G. childressi</i>	Spring 2020	230	25	<0.0001	0.08
		Summer 2021	778	26	<0.0001	0.28
		Fall 2022	294	16	<0.0001	0.10
Chincoteague	<i>G. childressi</i>	Spring 2020	229	20	<0.0001	0.10
		Summer 2021	201	19	<0.0001	0.09
		Fall 2022	317	18	<0.0001	0.14
Blake Ridge	<i>B. heckerae</i>	Spring 2020	298	14	<0.0001	0.19
		Summer 2021	297	7	<0.0001	0.19
		Fall 2022	226	12	<0.0001	0.14

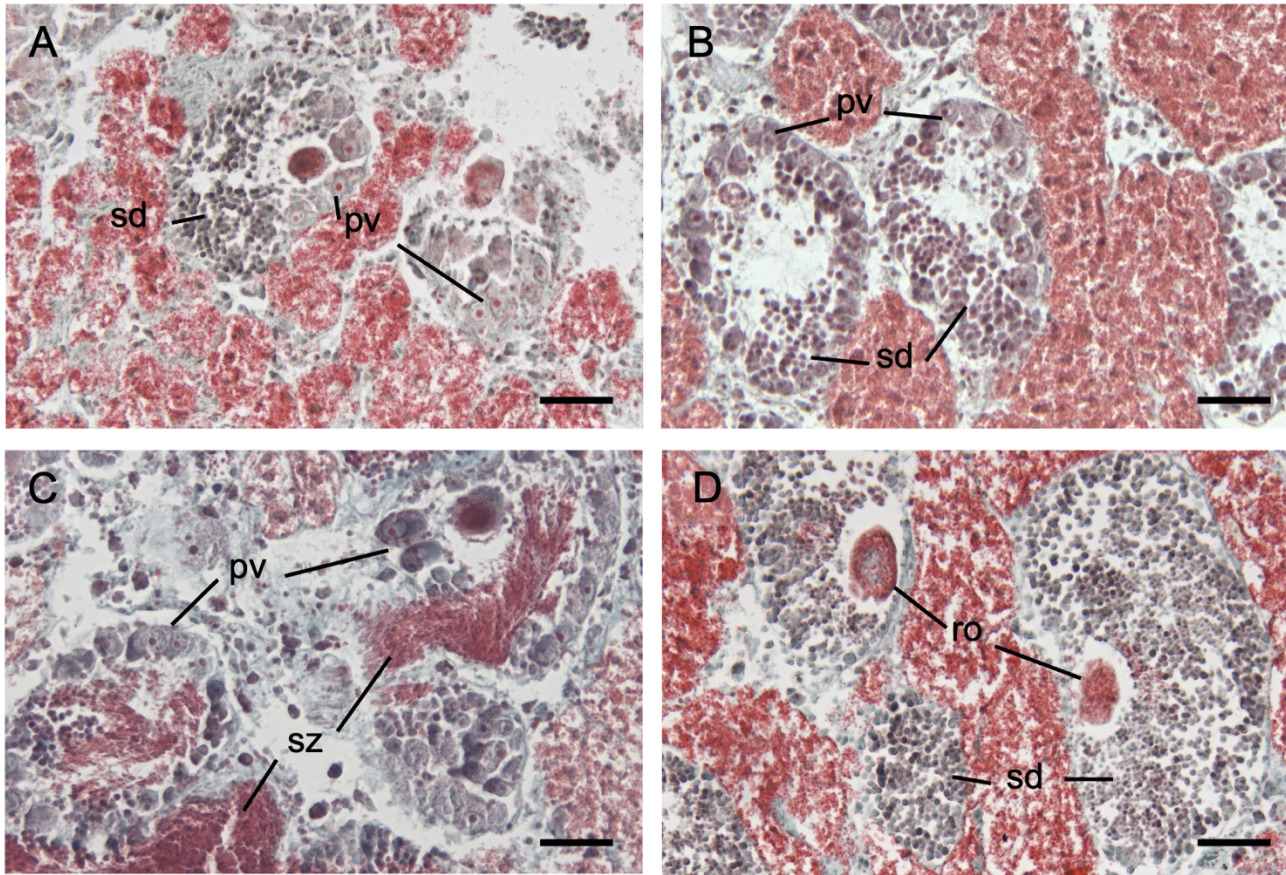


Figure S2.1. Histological sections from intersex individuals. Evidence of simultaneous hermaphroditism, oogenesis and spermatogenesis in the same stage, in both A) *G. childressi* and B) *B. brooksi*. C) Prodandry, advanced spermatogenesis, and early oogenesis, in *B. heckerae*. D) Protogyny, residual oocytes and early spermatogenesis, in *B. brooksi*. All scale bars are 50 µm. Labels are: spermatid (sd), previtellogenic oocyte (pv), spermatozoa (sz), and residual oocyte (ro).

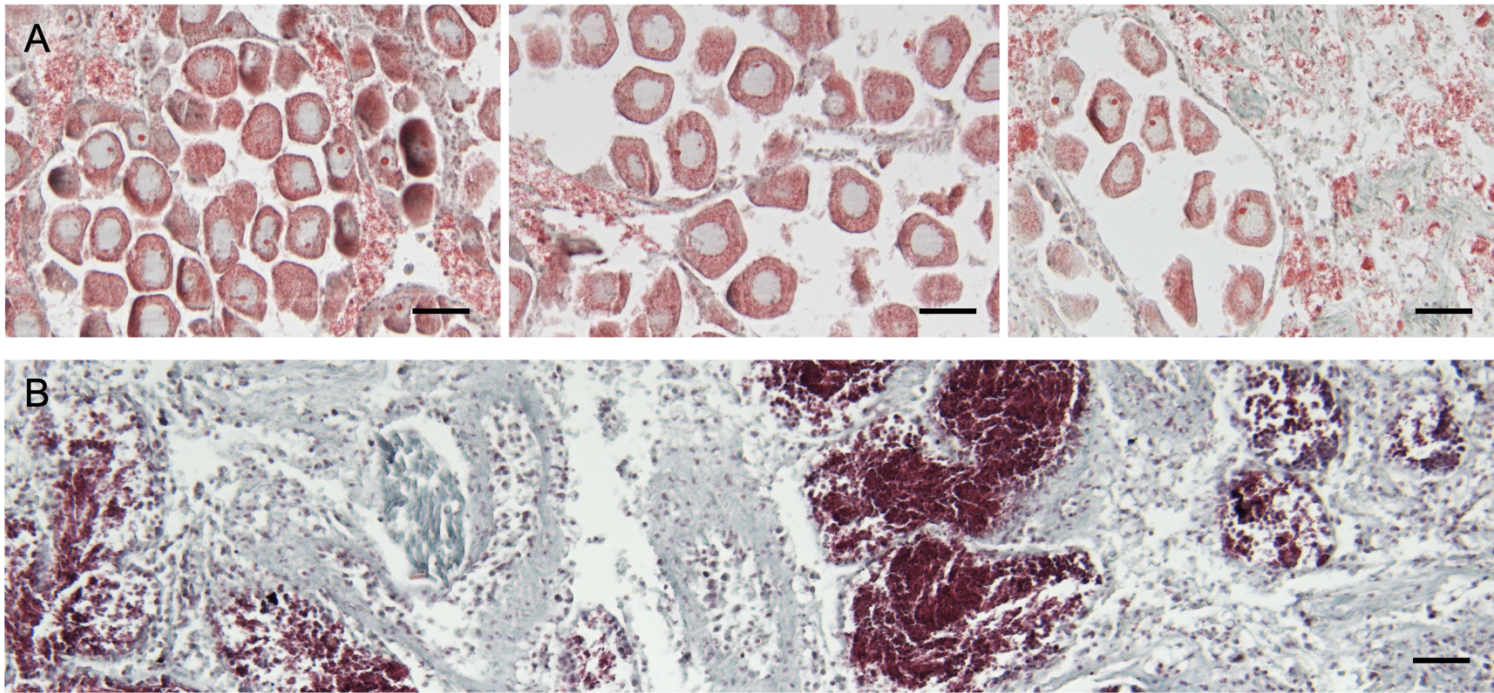


Figure S2.2. Histological photos showing evidence of iterative spawning. A) A series of micrographs from the same female *Gigantidas childressi* collected at Baltimore Canyon showing different acini in the same individual with decreasing densities of free oocytes. B) A single section of a male *B. heckeriae* from Florida Escarpment showing acini with different densities of spermatozoa. Both individuals were collected in the summer of 2021. All scale bars are 50 μm .

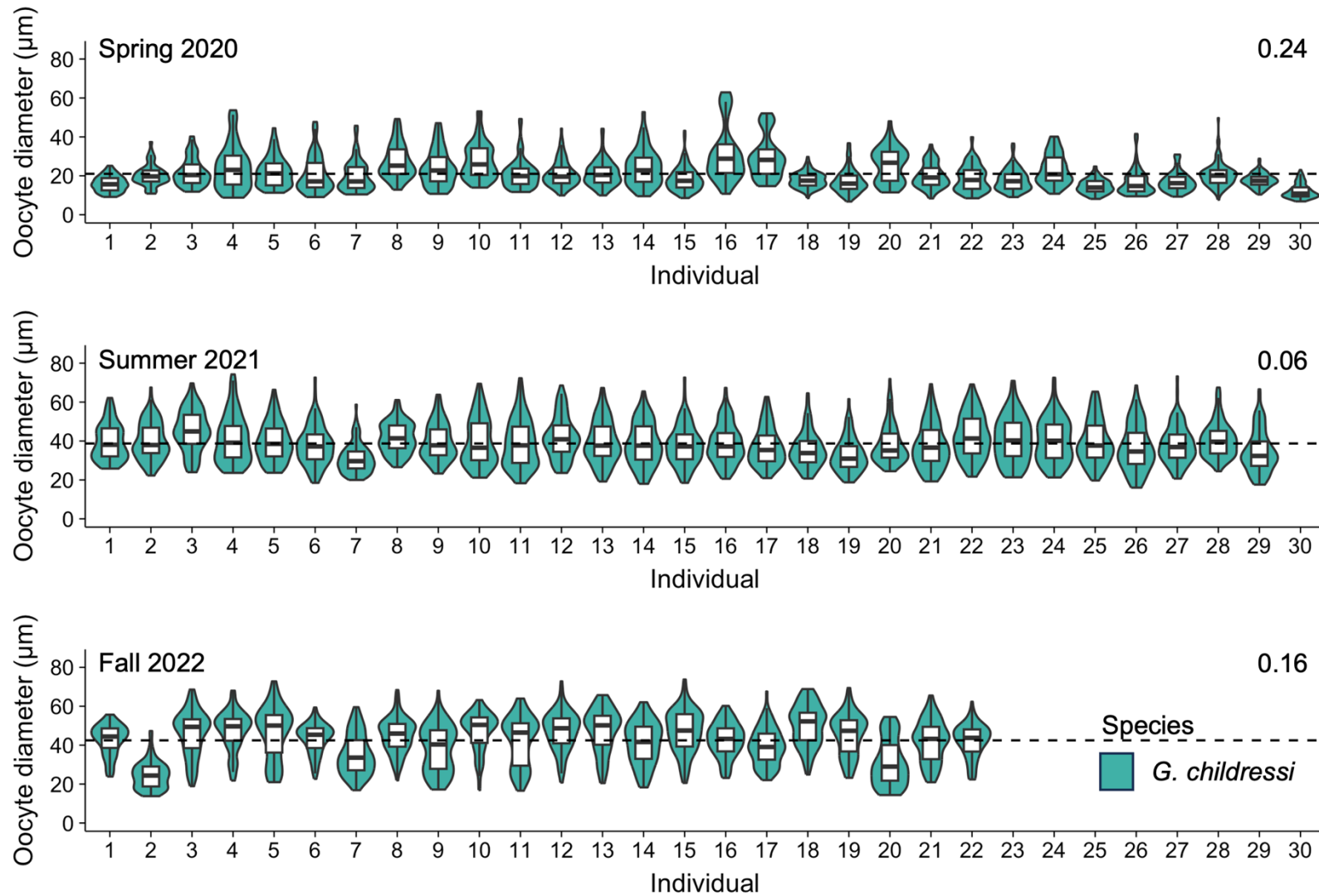


Figure S2.3. Oocyte diameter distributions for all sampled females from Brine Pool. Individuals are arranged from smallest to largest within each collection. Horizontal dashed lines denote the mean oocyte size. Numbers in the upper right are $\eta^2[H]$ effect sizes of a Kruskal Wallis test comparing synchronicity among individuals.

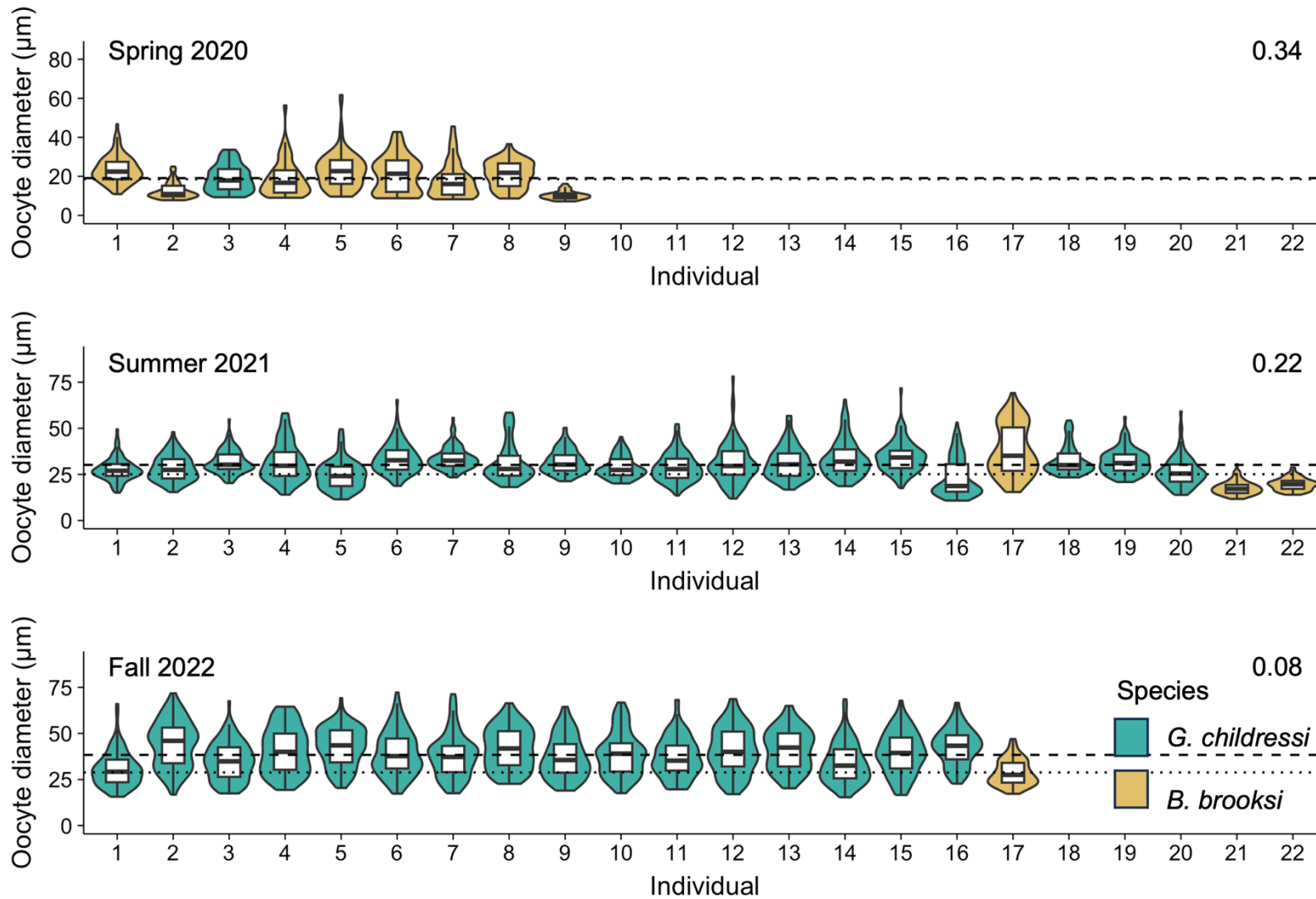


Figure S2.4. Oocyte diameter distributions for all sampled females from Mississippi Canyon. Individuals are arranged from smallest to largest within each collection. Horizontal dashed lines denote the mean oocyte size. Numbers in the upper right are $\eta^2[\text{H}]$ effect sizes of a Kruskal Wallis test comparing synchronicity among individuals.

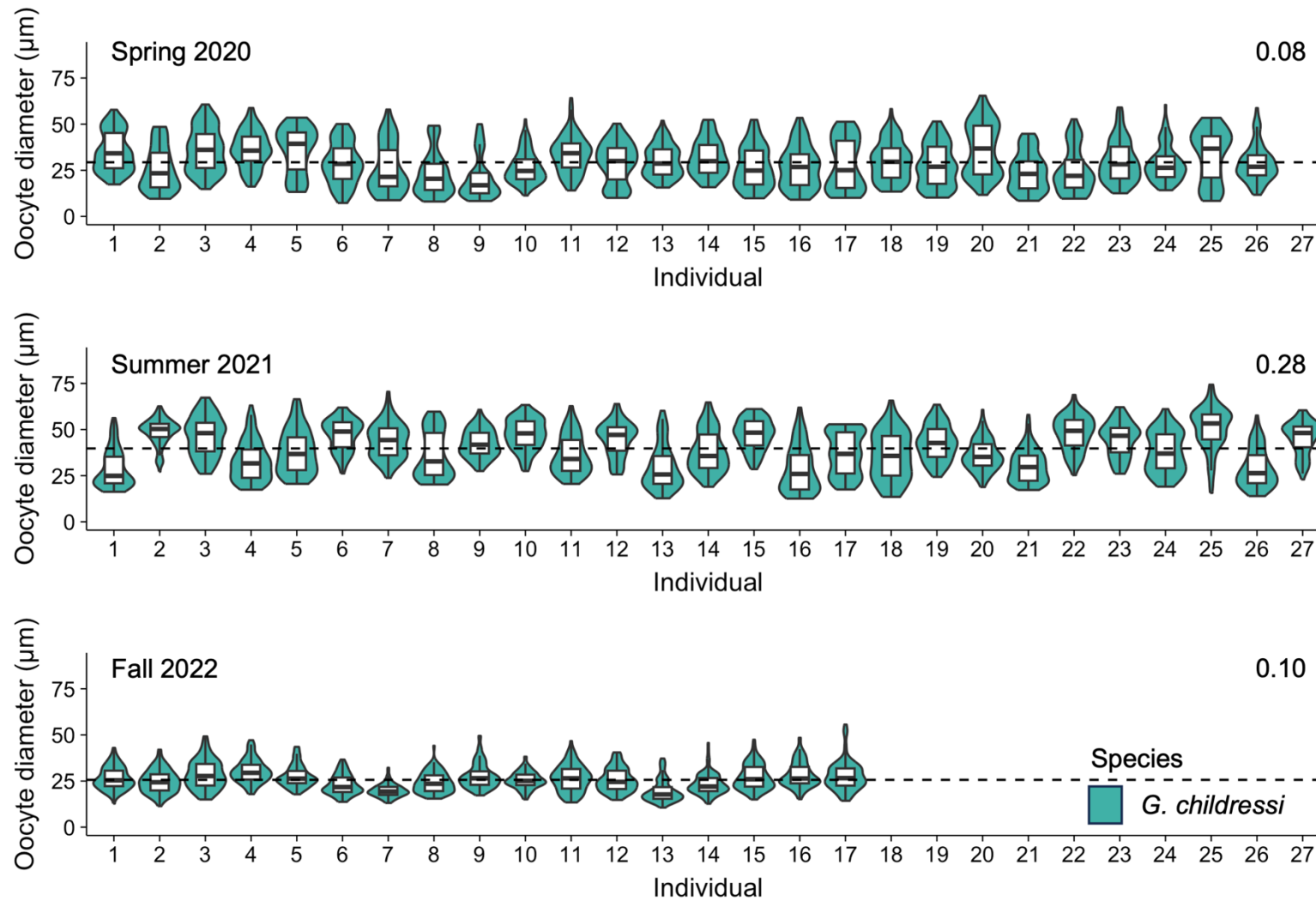


Figure S2.5. Oocyte diameter distributions for all sampled females from Baltimore Canyon. Individuals are arranged from smallest to largest within each collection. Horizontal dashed lines denote the mean oocyte size. Numbers in the upper right are $\eta^2[H]$ effect sizes of a Kruskal Wallis test comparing synchronicity among individuals.

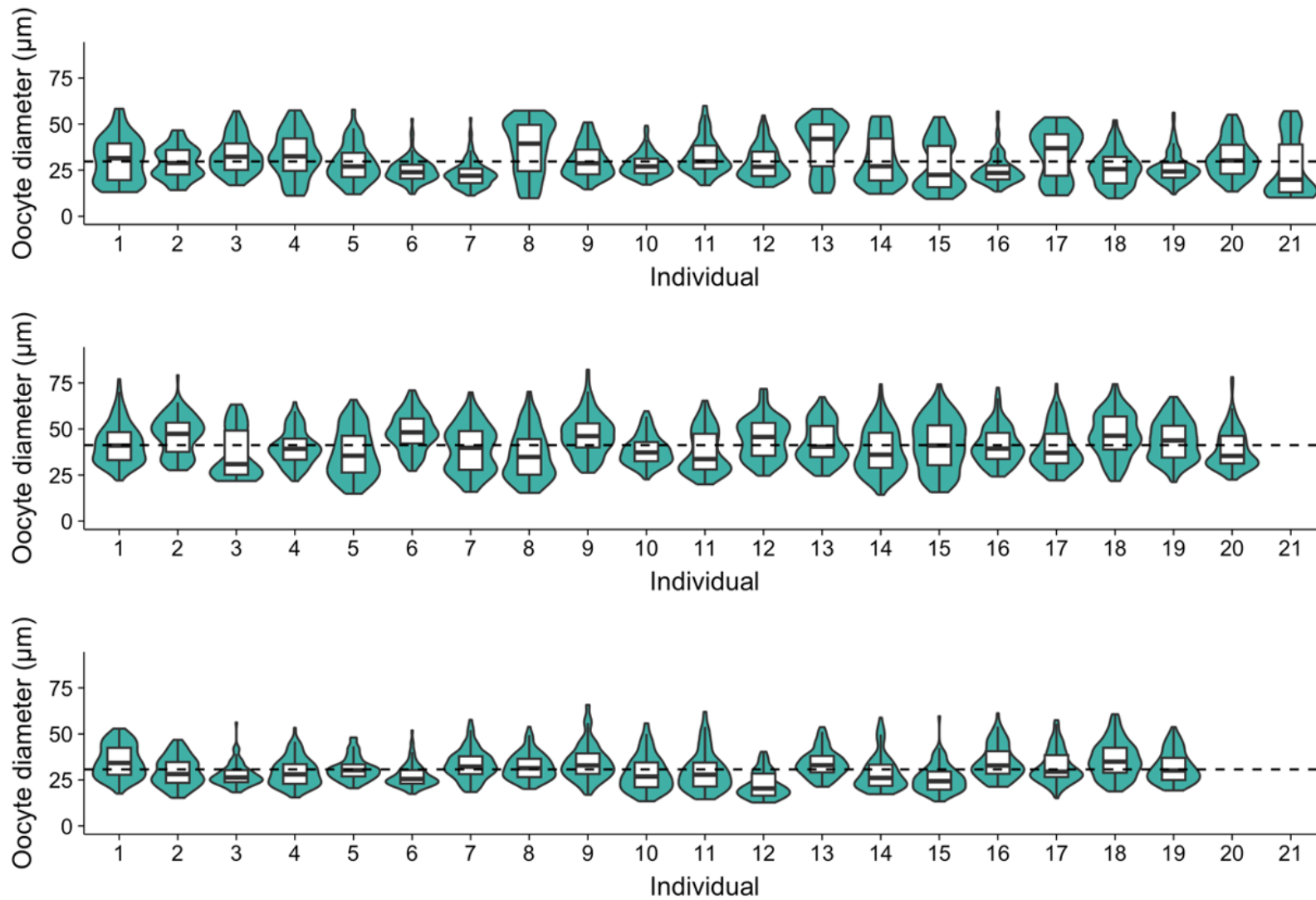


Figure S2.6. Oocyte diameter distributions for all sampled females from Chincoteague. Individuals are arranged from smallest to largest within each collection. Horizontal dashed lines denote the mean oocyte size. Numbers in the upper right are $\eta^2[H]$ effect sizes of a Kruskal Wallis test comparing synchronicity among individuals.

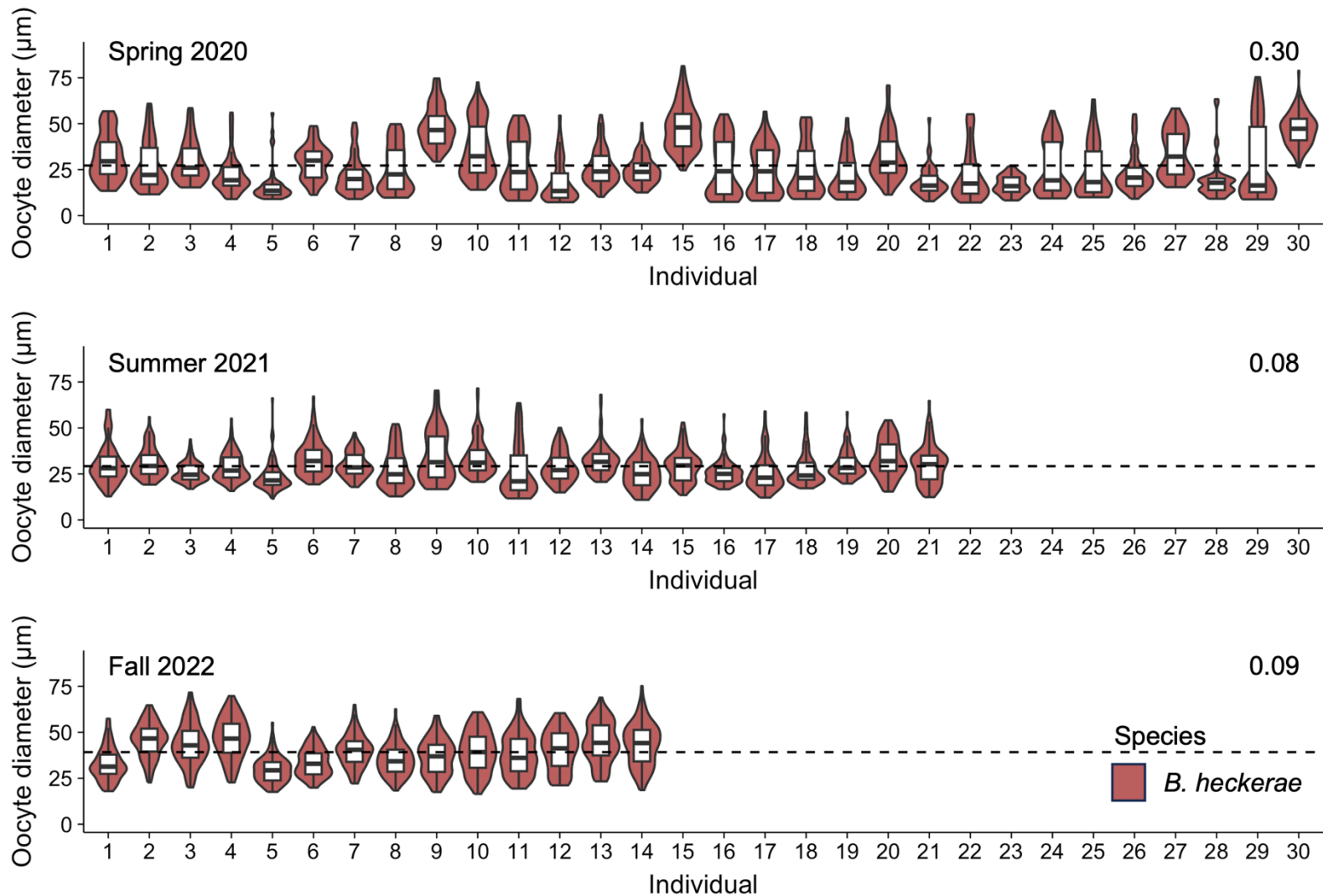


Figure S2.7. Oocyte diameter distributions for all sampled females from Florida Escarpment. Individuals are arranged from smallest to largest within each collection. Horizontal dashed lines denote the mean oocyte size. Numbers in the upper right are $\eta^2[H]$ effect sizes of a Kruskal Wallis test comparing synchronicity among individuals.

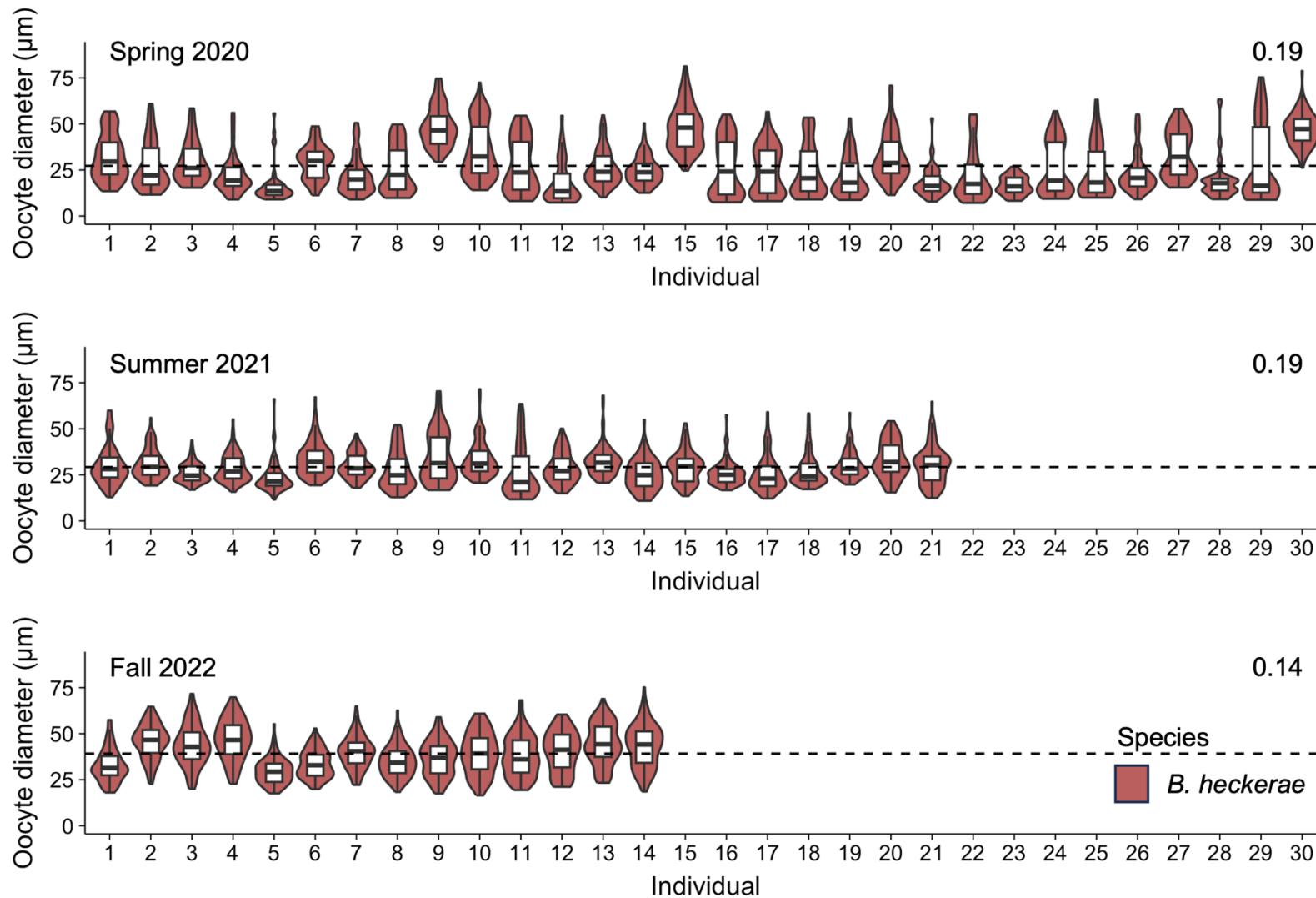


Figure S2.8. Oocyte diameter distributions for all sampled females from Blake Ridge. Individuals are arranged from smallest to largest within each collection. Horizontal dashed lines denote the mean oocyte size. Numbers in the upper right are $\eta^2[\text{H}]$ effect sizes of a Kruskal Wallis test comparing synchronicity among individuals.

CHAPTER III

Table S3.1. P-values from the pairwise post-hoc tests of the effects of season and tissue type (see Table 2) on $\delta^{13}\text{C}$ (below the grey diagonal) and $\delta^{15}\text{N}$ (above the grey diagonal). Tukey's HSD was used after a 2-way ANOVA and Dunn's test after a Sheirer-Ray-Hare. Significant p-values are bolded. The mussel species represented is listed in the upper left corner.

Brine Pool									
<i>G. childressi</i>	Spring_ foot	Spring_ gill	Spring_ gonad	Summer_ foot	Summer_ gill	Summer_ gonad	Fall foot	Fall gill	Fall gonad
Spring_foot		0.2251	0.9918	0.7890	0.9082	0.0588	0.0125	1.0000	0.0004
Spring_gill	0.8491		0.0243	0.0009	0.8541	0.0000	0.0000	0.0478	0.0000
Spring_gonad	0.5051	0.9997		0.9995	0.3127	0.4658	0.1776	0.9992	0.0109
Summer_foot	0.0073	0.0000	0.0000		0.0255	0.7122	0.3093	0.8718	0.0158
Summer_gill	0.0851	0.0005	0.0000	0.9843		0.0001	0.0000	0.5548	0.0000
Summer_gonad	0.9955	0.2440	0.0591	0.0217	0.2508		0.9994	0.0493	0.6439
Fall_foot	0.0001	0.0000	0.0000	0.8635	0.2610	0.0002		0.0078	0.9477
Fall_gill	0.0026	0.0000	0.0000	1.0000	0.9059	0.0072	0.9708		0.0001
Fall_gonad	0.0571	0.0003	0.0000	0.9958	1.0000	0.1739	0.3594	0.9570	

Mississippi Canyon									
<i>G. childressi</i>	Spring_ foot	Spring_ gill	Spring_ gonad	Summer_ foot	Summer_ gill	Summer_ gonad	Fall foot	Fall gill	Fall gonad
Spring_foot		0.4313	1.0000	1.0000	1.0000	1.0000	1.0000	0.0439	1.0000
Spring_gill	1.0000		1.0000	0.0022	0.0892	0.0064	1.0000	1.0000	0.4442
Spring_gonad	0.7209	1.0000		1.0000	1.0000	1.0000	1.0000	0.1746	0.9506
Summer_foot	0.0059	0.0945	1.0000		1.0000	1.0000	0.0702	0.0000	0.4361
Summer_gill	0.0005	0.0014	0.8296	1.0000		1.0000	1.0000	0.0006	1.0000
Summer_gonad	0.0000	0.0002	0.0692	0.7561	1.0000		0.1844	0.0000	0.9633
Fall_foot	1.0000	1.0000	1.0000	0.0619	0.0041	0.0000		0.1072	1.0000
Fall_gill	1.0000	1.0000	1.0000	0.0808	0.0059	0.0000	0.9077		0.0081
Fall_gonad	0.0133	0.1785	1.0000	1.0000	1.0000	0.1463	0.1313	0.1690	

Mississippi Canyon

<i>B. brooksi</i>	Spring_ foot	Spring_ gill	Spring_ gonad	Summer_ foot	Summmer_ gill	Summer_ gonad
Spring_foot		0.3258	1.0000	1.0000	0.8729	1.0000
Spring_gill	1.0000		0.8838	0.0250	0.3200	0.0089
Spring_gonad	0.3716	1.0000		1.0000	1.0000	1.0000
Summer_foot	1.0000	1.0000	1.0000		1.0000	1.0000
Summmer_gill	0.1266	1.0000	0.8246	0.9315		1.0000
Summer_gonad	0.0075	0.5596	1.0000	0.1033	1.0000	

Florida Escarpment

<i>B. heckeræ</i>	Spring_ foot	Spring_ gill	Spring_ gonad	Summer_ foot	Summmer_ gill	Summer_ gonad	Fall_foot	Fall_gill	Fall_gonad
Spring_foot									
Spring_gill	0.9999								
Spring_gonad	0.9172	0.9944							
Summer_foot	1.0000	0.9999	0.9035						
Summmer_gill	0.9594	0.9990	1.0000	0.9515					
Summer_gonad	0.8864	0.9913	1.0000	0.8646	1.0000				
Fall_foot	0.6554	0.3306	0.0432	0.5574	0.0495	0.0239			
Fall_gill	0.8726	0.5778	0.1151	0.8143	0.1364	0.0730	1.0000		
Fall_gonad	0.8948	0.6150	0.1307	0.8436	0.1554	0.0845	0.9999	1.0000	

Baltimore Canyon

<i>G. childressi</i>	Spring_ foot	Spring_ gill	Spring_ gonad	Summer_ foot	Summmer_ gill	Summer_ gonad	Fall_foot	Fall_gill	Fall_gonad
Spring_foot		1.0000	1.0000	0.7208	0.0275	1.0000	1.0000	1.0000	1.0000
Spring_gill	0.7855		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
Spring_gonad	0.0623	1.0000		0.0939	0.0015	0.7140	1.0000	1.0000	1.0000
Summer_foot	0.9872	0.6578	0.0404		1.0000	1.0000	0.3728	1.0000	0.4858
Summmer_gill	1.0000	1.0000	1.0000	1.0000		1.0000	0.0101	0.3451	0.0148
Summer_gonad	0.1666	1.0000	1.0000	0.1191	1.0000		1.0000	1.0000	1.0000
			0.002632			0.0089588			
Fall_foot	1.0000	0.0876	244	1.0000	0.3576	6		1.0000	0.9182
Fall_gill	1.0000	1.0000	0.5293	1.0000	1.0000	1.0000	1.0000		1.0000
Fall_gonad	0.1314	1.0000	1.0000	0.0955	1.0000	1.0000	0.00769	0.9223	

Chincoteague

<i>G. childressi</i>	Spring_ foot	Spring_ gill	Spring_ gonad	Summer_ foot	Summmer_g ill	Summer_ gonad	Fall foot	Fall gill	Fall gonad
Spring_foot		0.9764	1.0000	0.8453	0.0007	1.0000	0.3967	0.0001	0.2475
Spring_gill	1.0000		0.3991	1.0000	1.0000	0.6631	1.0000	1.0000	0.9631
Spring_gonad	1.0000	0.8539		0.1992	0.0000	1.0000	0.0575	0.0000	0.0280
Summer_foot	0.6464	0.1643	0.0121		0.0380	0.4126	1.0000	0.1011	1.0000
Summmer_gill	1.0000	1.0000	1.0000	0.0482		0.0001	0.8589	1.0000	1.0000
Summer_gonad	1.0000	1.0000	1.0000	0.3315	1.0000		0.1565	0.0000	0.0833
Fall_foot	0.0005	0.0007	0.0000	0.3211	0.0000	0.0001		0.3309	1.0000
Fall_gill	0.0844	0.0310	0.0004	1.0000	0.0022	0.0303	1.0000		0.4706
Fall_gonad	0.2989	0.0826	0.0030	1.0000	0.0143	0.1320	0.7287	1.0000	

	Blake Ridge								
<i>B. heckeræ</i>	Spring_ foot	Spring_ gill	Spring_ gonad	Summer_ foot	Summmer_g ill	Summer_ gonad	Fall foot	Fall gill	Fall gonad
Spring_foot		0.0084	1.0000	0.1251	0.0000	0.5375	0.4927	0.0000	0.8978
Spring_gill	1.0000		0.0036	0.9880	0.1367	0.7630	0.6865	0.8402	0.2555
Spring_gonad	0.6574	0.7412		0.0668	0.0000	0.3759	0.3294	0.0000	0.7724
Summer_foot	0.0009	0.0014	0.2000		0.0095	0.9982	0.9964	0.2407	0.8548
Summmer_gill	0.0000	0.0000	0.0073	0.9491		0.0011	0.0004	0.9222	0.0000
Summer_gonad	0.0000	0.0000	0.0000	0.0931	0.7118		1.0000	0.0494	0.9986
Fall_foot	0.0013	0.0021	0.2713	1.0000	0.8666	0.0455		0.0273	0.9986
Fall_gill	0.0006	0.0010	0.1791	1.0000	0.9397	0.0774	1.0000		0.0029
Fall_gonad	0.0000	0.0000	0.0001	0.2406	0.9393	0.9997	0.1317	0.2088	

CHAPTER IV

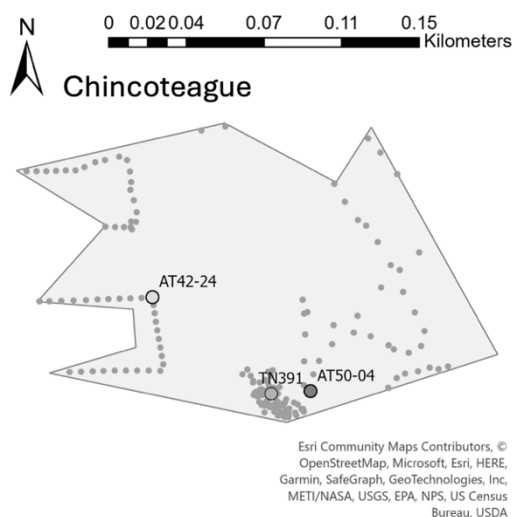
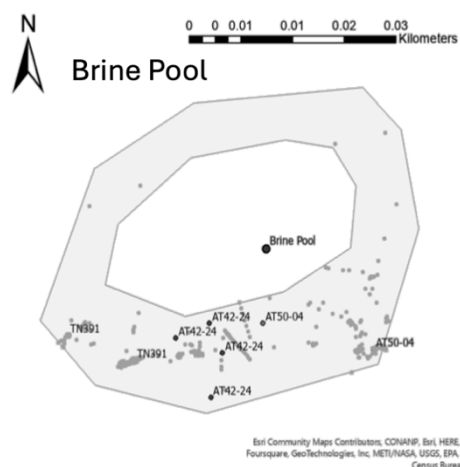
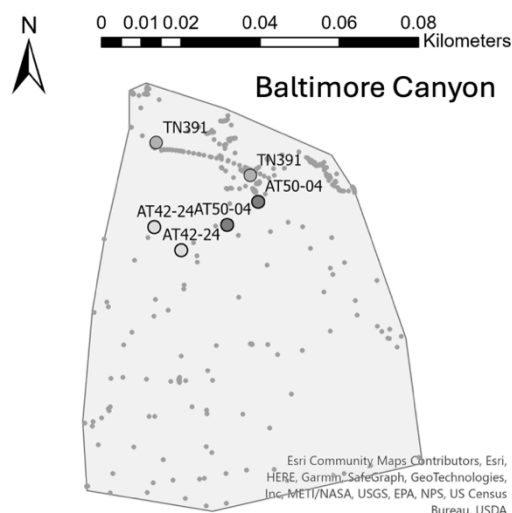


Figure S4.1. Polygons for each site which were used to measure the area of each mussel bed. Dark dots represent visual confirmation of mussels. Labels of cruise codes denote where adult mussels were collected. These figures were created in ArcGIS by Avery Calhoun.

Table S4.1. Details on the settings used for calorimetry with Parr 6772 Precision Thermometer and 6725 Semi-micro calorimeter.

Name	Value
Relative error	0.1383%
EE value	482.7445
Fuse correction	15
Acid correction	10
Sulfur correction	0
Bomb pressure (O ₂)	32 psi
Spike Material	Benzoid Acid

Table S4.2. P-value results from Dunn's post-hoc tests following a Sheirer-Ray-Hare test for the effects of site and date on mussel body length. BC = Baltimore Canyon, BP = Brine Pool, C = Chincoteague. Significant values are bolded.

	BC – 20	BC – 21	BC – 22	BP – 20	BP – 21	BP – 22	C – 20	C – 21	C – 22
BC – 20	-								
BC – 21	1	-							
BC – 22	1	1	-						
BP – 20	0.0001	0.0001	0.0001	-					
BP – 21	0.0001	0.0001	0.01	0.0001	-				
BP – 22	0.0001	0.0001	0.06	0.0001	0.9	-			
C – 20	0.0001	0.0001	0.001	0.001	0.0001	0.0001	-		
C – 21	1	0.0001	0.7	0.001	0.001	0.04	0.0001	-	
C – 22	0.0001	0.0001	0.0001	0.0001	0.01	0.0001	0.0001	0.0001	-

Table S4.3. Results of linear regression on annual fecundity with body size at each site. Atlantic represents a model run on the combined data for Baltimore Canyon and Chincoteague.

Site	Equation	F statistic	R squared	p-value
Baltimore Canyon	$y = 60591x - 2600415$	2.052	0.345	0.388
Brine Pool	$y = 26165x + 8926905$	44.87	0.956	0.094
Chincoteague	$y = 657611x - 65271510$	9.749	0.814	0.197
Atlantic	$y = 128009x - 8880031$	7.049	0.548	0.057
All	$y = 93383x - 2885919$	1.321	0.159	0.288