

Winter 2006

A test of the nutrient-productivity model in the Gulf of Maine using the intertidal mussel *Mytilus edulis*

Meredith A. Bailey

University of New Hampshire, Durham

Follow this and additional works at: <https://scholars.unh.edu/thesis>

Recommended Citation

Bailey, Meredith A., "A test of the nutrient-productivity model in the Gulf of Maine using the intertidal mussel *Mytilus edulis*" (2006).
Master's Theses and Capstones. 216.
<https://scholars.unh.edu/thesis/216>

This Thesis is brought to you for free and open access by the Student Scholarship at University of New Hampshire Scholars' Repository. It has been accepted for inclusion in Master's Theses and Capstones by an authorized administrator of University of New Hampshire Scholars' Repository. For more information, please contact nicole.hentz@unh.edu.

A TEST OF THE NUTRIENT-PRODUCTIVITY MODEL IN THE GULF OF MAINE
USING THE INTERTIDAL MUSSEL MYTILUS EDULIS

BY

MEREDITH A. BAILEY
B.S. University of New Hampshire, 2004

THESIS

Submitted to the University of New Hampshire

in Partial Fulfillment of

the Requirements for the Degree of

Master of Science

in

Zoology

December, 2006

UMI Number: 1439259

INFORMATION TO USERS

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleed-through, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

UMI[®]

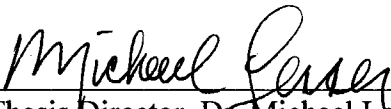
UMI Microform 1439259

Copyright 2007 by ProQuest Information and Learning Company.

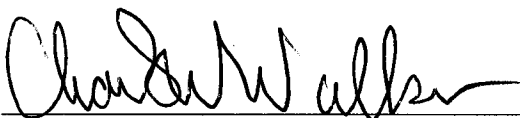
All rights reserved. This microform edition is protected against unauthorized copying under Title 17, United States Code.

ProQuest Information and Learning Company
300 North Zeeb Road
P.O. Box 1346
Ann Arbor, MI 48106-1346

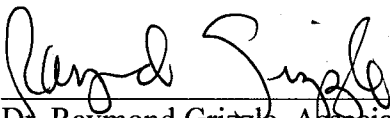
This thesis has been examined and approved.



Thesis Director, Dr. Michael Lesser, Professor
of Zoology



Dr. Charles W. Walker, Professor of Zoology



Dr. Raymond Grizzle, Associate Professor of
Zoology

15 Dec 2006
Date

ACKNOWLEDGEMENTS

First, I would like to extend my gratitude to my committee members; Dr. Michael Lesser, my primary advisor, who provided me with extremely valuable support and guidance throughout the process of this work; Dr. Charles Walker, an excellent teacher and resource; and Dr. Ray Grizzle, whose knowledge and experience gave me new views on the project. I am sincerely appreciative their input and assistance throughout the course of this work.

I would also like to thank Dr. Brian Beal of the University of Maine, Machias, for mussel sample collection in 2004, Dr. Otto Marshall of Marine Biological Laboratory, Woods Hole, MA, for collection of the stable isotope data, and the Dr. David L. Berlinksy lab for performing RNA/DNA ratios. Thank you to the following: to Dr. Thomas D. Kocher for guidance on population genetics, to Dr. James M. Pringle for providing insight on physical oceanography and for use of his map, to Dr. Ann Bucklin for first introducing me to marine molecular biology, to UNH Center for Coastal Ocean Observing and Analysis (COOA) for SeaWifs data, and to NOAA Ocean Exploration, UNH Center for Marine Biology (CMB), the Cooperative Institute of New England Mariculture and Fisheries (CINEMAR), and UNH College of Life Sciences and Agriculture for funding.

Thank you as well to April Blakeslee, my fellow graduate student, who helped me with several issues with computer programs, and to Judy Jacques, who helped me troubleshoot problems with my molecular techniques. Finally, I extend my love and appreciation to my parents and sister for their support and for pushing me onward, and gratitude and affection to my friends, Jen, Jim, Judy, Jon, and Joe: You've all helped me more than I can say.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS	iii
LIST OF TABLES	vii
LIST OF FIGURES	viii
ABSTRACT	x
I. INTRODUCTION	1
<i>The Gulf of Maine</i>	1
<i>GOM Intertidal Habitats</i>	10
<i>Biology and Ecology of Mytilus edulis</i>	21
II. MATERIALS AND METHODS	30
<i>Specimen Collection</i>	30
<i>Condition Indices</i>	32
<i>Stable Isotopes</i>	32
<i>RNA/DNA Ratios</i>	33
<i>Statistical Analysis</i>	34
<i>Remote Sensing</i>	34
<i>DNA Extraction, Amplification, and Sequencing</i>	35
<i>Phylogenetic Analysis</i>	36

III. RESULTS	38
<i>2004 Sampling Data</i>	38
<i>2006 Sampling Data</i>	49
<i>Interannual Variability in the GOM</i>	58
IV. DISCUSSION	62
<i>2004 Sampling Data</i>	62
<i>2006 Sampling Data</i>	73
<i>Recent Trends and Projections for the Future</i>	78
REFERENCES	82

LIST OF TABLES

Table 1. Collection data by location and date	30
---	----

LIST OF FIGURES

Figure 1. General circulation patterns of the Gulf of Maine	4
Figure 2. Collection sites on the Gulf of Maine coast	31
Figure 3. <i>Mytilus edulis</i> samples by size class for 2004	39
Figure 4. Mean (\pm SE) shell lengths and condition indices for 2004	41
Figure 5. Mean (\pm SE) isotope values for 2004	42
Figure 6. Mean (\pm SE) RNA/DNA ratio values for 2004	43
Figure 7. SeaWiFS data for dissolve chlorophyll <i>a</i> for 2004	45
Figure 8. SeaWiFS data for sea surface temperature for 2004	45
Figure 9. Maximum parsimony tree for <i>Mytilus edulis</i> collected in 2004	47
Figure 10. Maximum likelihood tree for <i>Mytilus edulis</i> collected in 2004	48
Figure 11. <i>Mytilus edulis</i> samples by class size for 2006	50
Figure 12. Mean (\pm SE) shell lengths and condition indices for 2006	51
Figure 13. Mean (\pm SE) isotope values for 2006	52
Figure 14. Mean (\pm SE) RNA/DNA ratio values for 2006	53
Figure 15. SeaWiFS data for dissolve chlorophyll <i>a</i> for 2006	55
Figure 16. SeaWiFS data for sea surface temperature for 2006	55
Figure 17. Maximum parsimony tree for <i>Mytilus edulis</i> collected in 2006	56
Figure 18. Maximum likelihood tree for <i>Mytilus edulis</i> collected in 2006	57
Figure 19. SeaWiFS long-term climatology data	60

Figure 20. Maximum parsimony analysis of *M. edulis* 61

ABSTRACT

A TEST OF THE NUTRIENT-PRODUCTIVITY MODEL IN THE GULF OF MAINE USING THE INTERTIDAL MUSSEL *MYTILUS EDULIS*

By

Meredith A. Bailey

University of New Hampshire, December, 2006

Historical concepts of top-down control (predator-prey interactions) on rocky intertidal community structure have transitioned to studies on bottom-up effects (nutrient supply and larval transport) as significant factors affecting rocky intertidal community structure. Studies performed on rocky intertidal locations along the Gulf of Maine (GOM) at multiple sites and seasons in 2004 and 2006 examined the ecology of *Mytilus edulis* populations by measuring size frequency distributions, diet quality (stable isotope composition) and physiological performance of individuals using condition indices and RNA/DNA ratios. Data were correlated to satellite imagery for sea surface temperature and chlorophyll *a* concentration and individuals were genetically tested to look for lineage sorting. Populations of *M. edulis* in the GOM were found to be genetically homogenous, consuming a mixed diet of phytoplankton and detritus, with shell size and physiological performance tied to chlorophyll *a* concentration and temperature, providing strong evidence for community structure being linked to environmental variability.

CHAPTER I

INTRODUCTION

The Gulf of Maine

The Gulf of Maine (GOM) in the Northwestern Atlantic is a broad body of water some 600 km in length, partially enclosed to the west and north by the American states of Massachusetts, New Hampshire and Maine and the Canadian provinces of New Brunswick and Nova Scotia, and is characterized by a set of unique physical, climatological and biological features. Flanked by the Scotian Shelf on the east and Georges Bank to the south, the waters of the GOM are situated entirely over the continental shelf, which is over 200 km wide in this region. Georges Bank, at roughly 300 km long and 150 km wide, crests at a depth of 60 m, with several ridges extending to less than 5 m below the surface, forming a significant division between the open Atlantic over the North American continental slope and the interior gulf. The GOM contains three distinct basins, isolated from each other beneath the 200 m isobath, which are Wilkinson Basin to the west, Jordan Basin in the northeast, and Georges Basin in the southeast. The Northeast Channel, a glacially-carved channel over 250 m deep, cuts through the shelf at this point, giving the GOM a steep bathymetric profile and providing the only connection between the Northwest Atlantic Slope Water and the GOM, permitting deeper, nutrient-rich waters to mix closer to shore. Large volumes of

terrestrial freshwater runoff, as well as freshwater delivered by the Labrador Current, are important factors in water chemistry and vertical mixing in the GOM (Townsend et al. 2004). The cold waters brought by the Labrador Current, flowing south, meet the much warmer tropical Gulf Stream, generating a strong seasonal thermocline and pycnocline throughout the region of the Northwest Atlantic continental shelf although these layers generally diminish or disappear within shallower coastal regions, such as the GOM, due to tide- and wind-driven mixing (Townsend et al. 2004). Temperature changes, freshwater runoff, and Scotian Shelf influx all have a strong influence on the surface waters of the GOM, while the characteristics of the water in the basins are determined largely by Northwestern Atlantic Slope Water of two types, a cold mass from the Labrador Sea and a relatively warmer mass from the central slope (Brown and Beardsley 1978, Ramp et al. 1985).

Circulation and vertical mixing in the GOM is primarily cyclonic and driven by tides and density contrasts. There are a number of internal gyres in the GOM, the strength and appearance of which are seasonally variable (Xue et al. 2000). Hydrographic observations and drifter data collections in the spring of 2004 showed a strong cyclonic (counterclockwise) gyre present over Jordan Basin, a secondary cyclonic gyre over Georges Basin, a much lesser cyclonic circulation pattern in the western Gulf, and a stronger anticyclonic gyre on Georges Bank itself (Butman and Beardsley 1987, Xue et al. 2000). The two major surface general gyral patterns (Figure 1), counterclockwise in the Gulf and counterclockwise over the Bank, are present year round, and are strongest in spring (Butman et al. 1982). These gyres, in particular those situated over Jordan Basin

and Georges Basin, are the product of, it has been suggested, density-driven gradients as well as coastal and shelf water currents. The major current dominating the eastern GOM in warmer months is the eastern Maine coastal current (EMCC) system, beginning as a pocket of cold (ca. 10°C), tidally-mixed and nutrient-rich Scotian Shelf water and which is driven across the mouth of the Bay of Fundy, along the coast of eastern Maine, and is pushed back into the central Gulf by the outflow of freshwater from the Penobscot River (Townsend et al. 1987, Townsend et al. 2004). Satellite imagery of sea surface temperature shows a distinct plume of colder water spreading into the central Gulf along the eastern side of the Penobscot Bay outflow. The EMCC tends to break down in winter months as vertical stratification lessens, and wind-driven surface currents are a significant factor in re-forming the gyre in the eastern Gulf during the spring (Xue et al. 2000). On the western side of the Penobscot outflow, this river combined with the discharges of the Kennebec, Androscoggin, and Merrimack drives the western Maine coastal current (WMCC), a current that flows along the southwestern perimeter of the GOM, in accordance with the general cyclonic gyre of the western Gulf and the Gulf as a whole (Geyer et al. 2004, Xue et al. 2000). These coastal currents are all significantly impacted by wind-driven circulation, barotropic motions and baroclinic shears (Geyer et al. 2004).

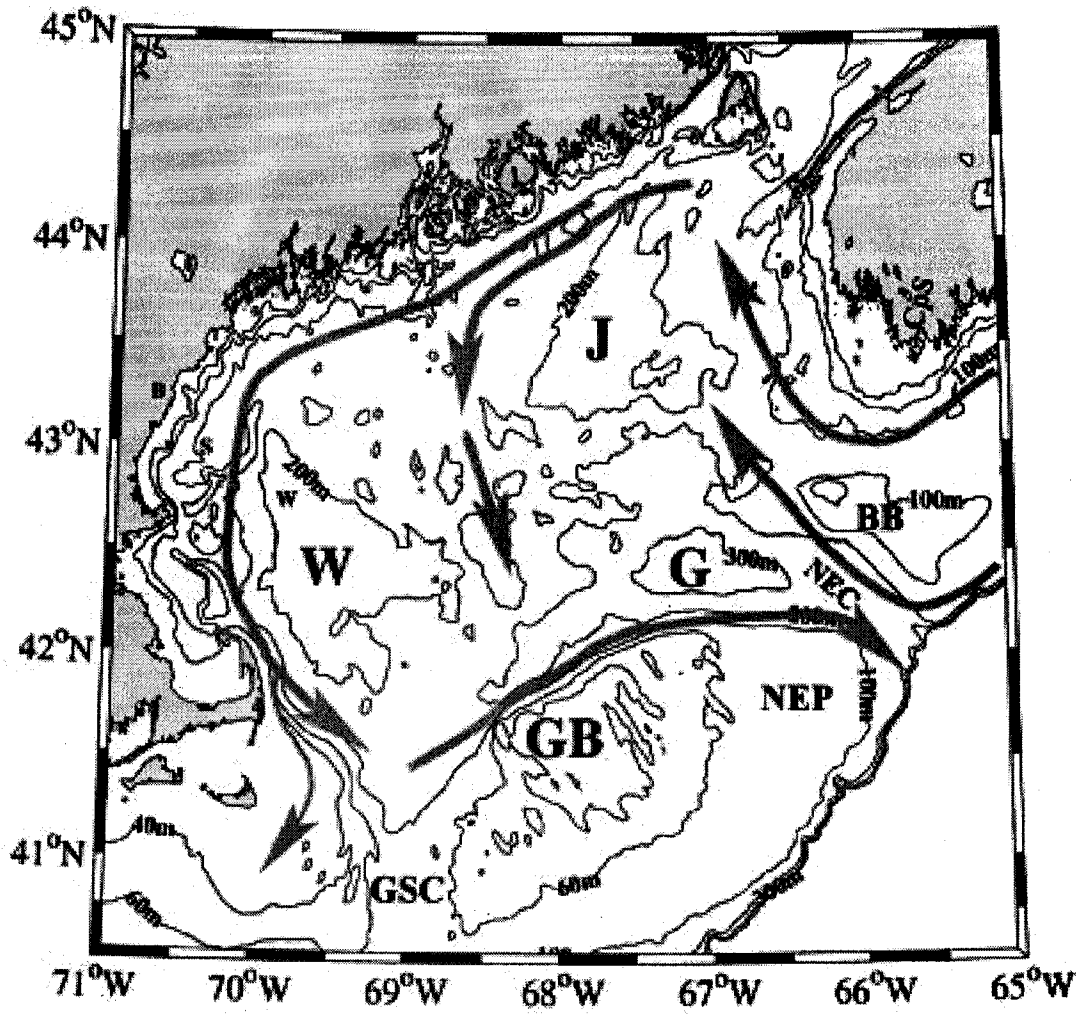


Figure 1. General surface circulation pattern for the Gulf of Maine. BB – Browns Bank, CpS – Cape Sable, G – Georges Basin, GB – Georges Bank, GSC – Great South Channel, J – Jordan Basin, NEC – Northeast Channel, NEP – Northeast Peak, W – Wilkinson Basin. Figure redrawn with permission from Johnson et al.

Studies in the Northeast Channel by Ramp et al. (1985) also support the importance of winter/spring wind in driving surface currents, but indicate that there is insufficient atmospheric force in summer to greatly affect currents during warmer months; this may apply to the GOM as a whole as well. Slope Water influx via the Northeast Channel is likewise seasonally variable, both in volume as well as in the degree of constancy of flow. Northeast Channel inflow to the GOM has two seasonal maxima, the primary occurring in August and a secondary event in the winter, which had a less consistent rate of flow in comparison to the summer; the lowest rate was found to occur in April and May, during the height of the vernal runoff period (Ramp et al. 1985). Flow rates tended to be less variable and more steady in summer, and stronger, although far more incidental, in winter. Seasonal heating and cooling cycles play an important role in summer vertical stratification and winter convection; in addition, Gulf-wide coastal circulation during winter months tends to be further offshore than during the summer (Xue et al. 2004). Coastal currents are weakest in mid- and late winter, when the gyre system is in general at its most incoherent. Butman and Beardsley (1987) found this to be true, as well, for the gyre over Georges Bank, which experienced strongest southwestward flow in September and weakest in March.

Seasonal cycling, coupled with tidal forces and the cold, dense fresher water brought by the Labrador Current, produces three distinct water masses and controls the majority of the hydrographic structure, nutrient circulation, and, subsequently, biological productivity of the GOM. These three water masses are known as Maine Surface Water, Maine Intermediate Water, and Maine Bottom Water, and are produced by seasonal heating;

Intermediate Water is formed during winter (Hopkins and Garfield 1979). Maine Surface Water is a layer of warm, low-density water, extending through the upper surface layers with salinity between 31.6-33.2‰ and which may be even less saline due to local runoff (Hopkins and Garfield 1979). Beneath the thermocline generated by MSW layer is the Maine Intermediate Water, a cold layer of less saline water residing between approximately 50 m and 150 m, formed coastally. The MIW and the MSW blend to one mass during the winter months, and the two diverge as the result of spring warming, with the MIW being the remains of the winter water mass (Hopkins and Garfield 1979). Maine Bottom Water, although warmer than the Intermediate Water, remains benthic due to an increased density from higher salinity and resides in the basins, flowing benthically through the Northeast Channel to mix with Slope Water (Hopkins and Garfield 1979). While distinct over the basins, these water masses tend to be mixed along the coast and the division between them grows less pronounced throughout summer and autumn as the result of tidal mixing. Ramp et al (1985) found that water volume entering the GOM through the Northeast Channel tended to be a mix of both Warm Slope Water and Labrador Slope Water, while that which exited the Gulf through that same passage was mostly Maine Intermediate Water. The GOM has typically swift tidal currents as the result of its large tidal ranges; the southwestern Gulf has the most moderate range at roughly 2 to 3 m, which increases heading the northeast to 6 m, and finally to one of the most extreme tidal ranges in the world located in the Bay of Fundy at more than 10 m (Townsend et al. 1987, Townsend et al. 2004). Tidal currents in the Northeast Channel have been found to be accountable for over 50% of the variance in current velocity (Ramp et al. 1985). Tidal forces strongly affect circulation as well as mixing within the

100 m isobath, particularly over the shallows of Georges Bank, within the Bay of Fundy, and in coastal waters (Xue et al. 2000). Seasonal circulation, particularly coastal, is also slightly affected by freshwater discharge from the local watersheds; although an estimated $8 \times 10^{11} \text{ m}^3$ of freshwater is emptied into the GOM yearly from the four largest rivers, the Merrimack, the Kennebec, the Penobscot, and the St. John, most of the influence of this influx appears to be largely on the surface salinity (Xue et al. 2000). The overall cyclonic motion of GOM circulation as a whole produces a salinity gradient over the GOM that is the most pronounced in the late spring and early summer as the gyre causes the accumulation of spring freshwater runoff from these rivers in the western portion of the Gulf. Brown and Beardsley (1978) observe that explaining observed salinity variance on the continental shelf requires a significant amount of tidal mixing, a quality that has been recorded several times, as by Ramp et al. (1985). The Great South Channel, although a great deal shallower than the Northeast Channel at approximately 70 m, and carrying a much lower volume of water, has been identified as a source of egress of intermediate salinity shelf water from the GOM to the Nantucket Shoals, possibly the product of the tidal mixing that occurs in the anticyclonic gyre on Georges Bank (Brown and Beardsley 1978). Seasonal mixing and winter convection, however, is most likely the mechanism that permits this vertical mixing to extend down to the depths of the Maine Intermediate Water.

The success of the commercial fisheries in the GOM is heavily dependent on primary productivity, in particular the annual plankton bloom that occurs every spring; the eastern Gulf has been identified as an important spawning ground for commercial fish stocks

such as herring (Townsend et al. 1987). This primary productivity is, in turn, the result of nutrient cycling, upwelling and mixing; the same tidal mixing that is responsible for degrading the distinction between the three water masses in the GOM is also responsible for driving nutrient transport, particularly in the northeastern Gulf. As the EMCC moves southwest, stratification increases and nutrient concentrations decrease, with a corresponding increase in phytoplankton and zooplankton biomass. A noticeable offshore region of productivity coincides with the EMCC plume in the central Gulf, indicating the importance of the EMCC, and the cold, nutrient-rich Scotian Shelf water, in the ecology of the eastern GOM. Nutrients and nitrogen that originate in surface Scotian Shelf Water are as important to primary productivity in the GOM as those which have their source in the deep Slope Water and enter through the Northeast Channel (Townsend 1998). An estimated 44% of the inorganic nutrients used in primary productivity over Jordan Basin originate on the Scotian Shelf and are transported by EMCC-generated upwelling in the eastern Gulf (Townsend et al. 1987). Nutrient upwelling and fluxes driven by the EMCC and tidal mixing have combined to cause the GOM to be both historically and currently a region of high biological productivity; the least productive, offshore areas of the Gulf average some $270 \text{ gC m}^{-2} \text{ yr}^{-1}$ (O'Reilly et al. 1987). Thus, In winter, when the stratification produced by the thermocline, pycnocline, and the EMCC breaks down due to decreased temperature and increased storm activity, winter convection continues to draw these nutrients to the surface, making them available for the annual bloom each spring (Townsend et al. 2004). A second source of nutrients is from deep Slope Water flowing through the Northeast Channel; however, although the nutrient load in the deep Slope Water is much greater than that of the Scotian Shelf, less

than an estimated quarter of that load is brought to the surface layers via upwelling, mixing and convection for use in primary productivity (Townsend 1998, Townsend et al. 2004). Studies suggest that much of the deep Slope Water nutrient load exits the GOM through the Northeast Channel with the outflow of Maine Intermediate Water, without ever having reached the surface layer, a model in accordance with the circulation patterns observed in the Northeast Channel (Townsend et al. 2004, Ramp et al. 1985). Regardless of its upstream source either on the Scotian Shelf or from deep Slope Water, this nutrient-rich cold water is an important source of upwelling and nutrient turnover in the GOM, particularly over Jordan Basin, and ultimately is the cornerstone to biological productivity in the GOM (Townsend 1998). A study by Townsend et al (1987) found that dissolved nitrogen is significantly higher along coastal Maine from the mouth of the Bay of Fundy to the Penobscot Estuary, between 0.1 and 6.0 μM , while in the western Gulf, surface NO_3 levels are frequently below 0.1 μM during summer months. Surface chlorophyll concentrations are similarly elevated in the eastern Gulf, as much as six times higher off western Nova Scotia than off the coast of New Hampshire, however, primary consumers, such as adult copepods and macrozooplankton, had a much greater summer biomass in the western Gulf (Townsend et al. 1987). These circulation and mixing patterns ultimately produce two distinct surface water regions in the GOM as characterized during the summer. To the east of the Penobscot outflow is a cyclonic gyre of colder ($10 - 13^\circ\text{C}$), high-nutrient water with surface salinity measurements of 31.0 – 32.0 psu, while west of the Penobscot the waters are warmer ($12 - 18^\circ\text{C}$), contain less dissolved nitrogen, and are slightly less saline, particularly along the coast, ranging from 30.0 – 31.5 psu (Townsend et al 1987).

GOM Intertidal Habitats

Historically, the GOM was subject to the Pleistocene glacial period of approximately 20,000 years ago. The lack of a habitat refuge beyond the extent of glaciation, on account of the general lack of hard substrate south of the glacial limit in Long Island Sound, indicates that this period of time was particularly difficult for obligate rocky intertidal organisms. The disturbance effects of ice scour provided new substrate following the retreat of the glaciers for the recolonization of the intertidal species of the northwestern Atlantic coast, a consequence of migration from Iceland and Europe (Wares and Cunningham 2001).

Nearly the entirety of the coastal GOM is characterized by granitic rock that provides the substrate for a distinctive intertidal community structure throughout most of the GOM. Many of the organisms found in the GOM intertidal are sessile and dependent on the ambient water currents for their nutrients and food as well as for distributing their propagules. Additionally, the community structure lends itself to population and abundance estimates that can be easily made, a feature that is fairly unique among habitats (Connell 1972). A key characteristic of the intertidal is vertical zonation, a minute-scale regionalism in the presence and distribution of organisms throughout the intertidal region that is determined by number of varied factors, including air exposure, temperature changes, resistance to desiccation, nutrient availability, vulnerability to predation, and competition with surrounding organisms.

These factors and patterns of vertical zonation, are largely a product of, and are

controlled directly by, tidal range and force (Connell 1972). Upper zone limits indicate that physical factors, such as desiccation, solar radiation, and temperature, highlighted in many studies as a critical abiotic influence affecting distribution and survival success of organisms of the rocky intertidal, are the limiting factors for species vertical distributions, and these are all largely a function of exposure during low tide (Helmuth and Hofmann 2001). However, these limits can be extended in conditions that offset those physical factors: tide pools, moisture from spray, north-facing or shading rocks, or thick algal cover have all demonstrated an effect on vertical zonation patterns. Organisms may possess, as well, a certain degree of flexibility in their responses to stressors; that is, some may be capable of successfully handling an isolated instance of acute stress, such as a temperature extreme, by acclimating to a series of similar but less intense stressors for a period of time beforehand (Helmuth and Hofmann 2001). There exists a significant and strong correlation between tolerance to temperature extremes and tidal height, indicating that those species which are more frequently or regularly exposed to elevated temperatures may stand a better likelihood of surviving sudden temperature extremes (Somero 2002). Physiological adaptations of intertidal organisms and their tolerance limits, in conjunction with the various physical factors themselves, are critical in determining the vertical zonation and distribution of organisms throughout the intertidal zone. Some intertidal organisms have been shown to have altered plasma membranes as a result of acclimatization to temperature fluxes, and a few are even capable of regulating changes in response to tidal and temperature cycles (Somero 2002).

Lower limits for species habitat zones seem to be influenced more by biological factors,

such as predation and competition, than direct physical factors. In competition studies, the barnacle *Semibalanus balanoides* consistently out-competed the barnacle *Chthamalus stellatus* by both higher growth rates and direct mortality by smothering or crushing the second species (Connell 1972). Thus, although *C. stellatus* could survive at a wide range of depths throughout the intertidal, it was restricted to high shore levels by interaction with *S. balanoides*. Similarly, zonation bands throughout the intertidal can provide refuges for organisms who would otherwise be consumed by predators at higher or lower regions. Vertical zonation may also be subject to organismal age; population distribution of the barnacle *S. balanoides* in Scotland showed 3-year-old individuals at the uppermost portion of its range with younger individuals living lower in this zone, and studies in California revealed a similar age-correlated distribution for the limpet *Acmaea scabra*, most likely due to an increased susceptibility to desiccation in younger individuals, a condition that may as well be correlated to organism size (Connell 1972). These biological limitations on species distribution and vertical zonation become increasingly more effective as environments become physically less demanding and less harsh. Horizontal distribution is largely a function of exposure, wave activity, and availability and angle of shelter.

Although this typifies the general habitat to be found in community structure on the coast of the GOM, the rocky intertidal frequently displays fine-scale variation as a result of the variability of exposure to physical and biological factors that is the result of the fractured surfaces and orientation presented by a rocky granite shore. Rather than presenting a coherent single surface, the rocky intertidal displays minute pockets of wide

heterogeneity as the result of degree of substrate vertical position, positioning in relation to solar radiation, tide pools, and seclusion from wave activity. Range limits can be expanded through north-facing rock slopes, crevices, or wave spray. Experiments by Menge (1976) at Pemaquid Point, Maine, demonstrated that mean mussel cover at a site within 15 cm of a crevice was over 10% greater than a replicate at a site with no crevice refuges within a meter. Larger organisms may provide shelter for organisms beneath them; the vertical range of many intertidal sessile organisms is extended by the presence of heavy overhanging carpets of canopy algae such as *Ascophyllum nodosum* and *Fucus vesiculosus*, thus showing that frequently the range and distribution of some organisms is closely tied to the corresponding characteristics of another. The mussel *Mytilus edulis* is another such organism whose presence alters the community structure; these mussels trap detritus, reduce water flow, and create refuges for cryptic organisms (Connell 1972). Both intraspecific competition and interspecific competition produce small-scale variation in organism distribution and cover, as can predation, however, Menge (1976) has found that predation and interspecific competition has a only low and unpredictable affect on the high intertidal community structure in the GOM.

The GOM, with its consistent granitic substrate throughout the region, and low species diversity, exhibits a simple trophic system which makes it an excellent choice for studying mechanisms driving the organization of intertidal community structure. The general structure of the GOM rocky intertidal, as reported by Menge (1976), is dominated by barnacles in the high intertidal, and a mid-intertidal zone inhabited mostly by mussels and some barnacles. The degree of unused space throughout the intertidal appears to be a

function of wave activity and disturbance, with exposed mid-intertidal areas showing significantly more coverage by mussels and barnacles in comparison to areas subjected to less disturbance; this indicates that in the areas of less disturbance, biological factors such as predation and competition are exerting a greater pressure on organism survival, structure, and distribution (Menge 1976). Furoid algae in the high intertidal are scarce at exposed shores, but can grow at this zone when protected from wave disturbance; they are dominate the mid-intertidal in general, but tend to be reduced in exposed areas and still non-existent in areas of the mid-intertidal that are severely exposed (Menge 1976). In the high and mid-intertidal, the dominant grazer is the common periwinkle, *Littorina* spp. and the dominant predator is the gastropod *Thais lapillus*, which primarily consumes barnacles, namely *Semibalanus balanoides* (Menge 1976). In the mid-tidal, interspecific competition between mussels and barnacles governs much of the distribution of both species throughout this zone, with mussels generally outcompeting and replacing barnacles in horizontal or inclined substrata; on vertical surfaces, however, barnacles routinely out-compete mussels for space (Menge 1976). This ability of barnacles to generally outcompete mussels on vertical substrata may be a function not only of a general deficiency of mussels to be effective competitors on a vertical surface, either as a result of inefficient settlement, feeding, or the necessity of devoting more energy to support by byssal thread attachment, but also a product of the nearly total lack of predation on organisms on vertical surfaces at exposed areas (Menge 1976). Predation occurring on vertical as well as horizontal surfaces is, however, far more significant in protected areas, and has some impact on percent of available space and thus the interspecific competition for this space in the mid-intertidal. This predation effect,

however, is a fairly low influence, and Menge (1976) still identifies competition over predation as the primary biological factor driving intertidal community structure in the GOM.

Many studies of community ecology of the rocky intertidal habitat have been performed on the western coast of North America or in Europe, leaving the east coast and the GOM largely understudied, although there are significant differences between the environments. One such change, aside from the actual physical and structural differences, is the more complicated food web and broader trophic level system. On the Pacific coast of North America, the presence and density of limpets such as *Acmaea* spp and *Patella* spp, which are significant predators on barnacles, is vastly elevated over the coast of New England. Whereas the abundance of limpets on the west coast can be as high as over 300 individuals per m², throughout the majority of the GOM, frequently less than 10 limpets per m² are typically found; according to Menge (1976) the highest density of limpets in the GOM was at Grindstone Neck, located in Maine roughly 40 km east of Penobscot Bay. The furoid canopy also has a tendency to be thicker in New England in comparison to those found on the west coast of North America which, although it protects both *Mytilus* and *Balanus* from desiccation, it seems to also inhibit juvenile settlement on the rock substrate as well as increase the effect of predation by *Thais* by increasing the foraging period for that gastropod (Menge 1976). These two conclusions are entirely contrary to results found in similar studies in the northeastern Pacific coast (Dayton 1971).

General models of community ecological structure and organization set forth a number of major predictions (Connell 1975, Menge and Sutherland 1976). First, these models predict that in environments which are generally sheltered, predation is the primary force which forms community structure. Second, that as living conditions in a given habitat become increasingly more harsh, the effectiveness of predators, and their importance in community structure and organization, diminishes. With the decrease in the importance of predation, interspecific competition becomes the more critical biological factor influencing community structure. Finally, small-scale spatial heterogeneity of community structure is the product of isolated instances of escape from predation and disturbance. Older models, such as the Hairston, Smith and Slobodkin model, focused on trophic relationships and population regulators, tended to assume a terrestrial, trilevel trophic system of primary producers, herbivores, and carnivores, and failed to take into account spatial distribution, organization, and diversity (Menge and Sutherland 1987). Connell (1972) criticizes older models of community structure as being based primarily in hypotheticals and indirect evidence, and, as such, may be based on a number of incorrect assumptions, such as the importance of the idea of the ecological niche and its effects on competition. This would result in competition between similar species where their ranges overlap; Connell is, however, dubious concerning the importance of competition determining species abundance and distribution, with the exception of "a few examples, some taken from the rocky intertidal" (1972). Two new models emerged, suggesting two different methods of environmental influence on trophic structure. In the first, it was suggested that the physical environment affects primary productivity, while the second argued that primary productivity levels remained largely constant, and that the

environment had a more significant impact on higher trophic levels, resulting in a simple, curtailed trophic system; trophic system interactions should decrease in complexity with increasing environmental stress (Menge and Sutherland 1976; Menge and Sutherland 1987).

Menge and Sutherland (1976) argue that in systems with fewer trophic levels, as is typified by the GOM, the importance of competition is more important than predation, as there are fewer trophic levels. Competitively inferior species may be given an refuge by the results of combined predation and the environmental extremes found in the GOM rocky intertidal – so much so that there have been assertions that the GOM rocky intertidal system is more directly under the control of physical factors than biological factors (Sanders 1968, Menge and Sutherland 1987). While Menge (1976) maintains the importance of biological factors over physical, it still remains that the physical stressors of the rocky intertidal environment in the GOM is a critical part of determining species diversity and abundance. The ultimate conclusion of Menge (1976) in regards to community structure patterns is that predation is of primary importance, except on exposed headlands, where interspecific competition is the foremost factor; biological and physical disturbance are considered important secondary mechanisms. Paine and Levin's (1981) model applied to the northern Pacific coast of the United States places a fair amount of importance on physical disturbance and assumes a general consistency of other factors over the northern Pacific rocky intertidal. Consideration of this assumption in the context of Menge's (1976) findings intimates that Pacific coast models are inappropriate to apply to the GOM, not only because of the added competition and trophic complexity,

but also as a function of the inherent supposition of the model itself. The GOM displays a range of environmental stress gradients, including exposure to wave action, wind, desiccation, and temperature. As a result, the GOM is somewhat unique in the apparent importance of both competition as well as predation in determining and governing its community structure. Community structure determination in the GOM is surprisingly complex for a system that is, on the surface, seemingly quite simple and straightforward.

The concepts of top-down and bottom-up processes, their interrelationship, and how these affect rocky intertidal community structure have received increasing attention over the past 15 years. Bottom-up ecological models focus on models of nutrient supply and larval recruitment, both of which are largely a function of circulation patterns, particularly upwelling. Upwelling is responsible not only for delivering cold, nutrient-rich water to the surface layers for use by primary producers, but is also a major factor in larval transport, thus playing a key role in determining locations for settlement and the distribution and abundance of adult organisms. The presence of an abundance of or diverse population of herbivores indicates bottom-up control (Menge 1992). Top-down processes encompass the historical focus of how trophic interactions, such as predation and competition, determine abundance, distribution, and diversity of populations. Keystone predators controlling populations of sessile organisms, such as limpets feeding on barnacles or sea stars on mussels on the Pacific Coast, demonstrates top-down control (Menge 1976, Menge 1992). Communities which might otherwise be virtually identical in structure may vary greatly as a result of near-shore oceanographic processes. This was shown to be true in a study conducted on the Oregon coast; at one study site, there was a

high abundance of macrophytes and a low abundance of invertebrates, filter feeders, and predators, whereas at a site 83 km southward, there were few macrophytic algal species and a high abundance of barnacles, mussels, limpets, chitons, sea stars, and whelks (Menge et al. 1997). With respect to top-down controls, the southern site was found to have higher predation by sea stars and higher grazing by limpets and chitons than the northern site; mussel recruitment and growth of invertebrate prey, both bottom-up effects, were both found to be greater at the southern site as well. Although there was a higher flow rate at the northern site, the southern site showed a higher concentration of phytoplankton, an important food source of the invertebrate filter feeders found in higher abundance at that location. This generated larger and healthier larval recruits better suited for survival, in turn generating more prey for the southern site predators (Menge et al. 1997). The higher levels of chlorophyll *a* and phytoplankton at the southern site were correlated with a band of nutrient-rich, cold-water upwelling.

More recently, focus on community ecology of the rocky intertidal has shifted from biological factors or environmental factors to the concept of benthic-pelagic coupling, a more holistic method which incorporates climate, physical oceanography, cycling, seasonality, coastal topography, environmental stress, species composition, diversity, and distribution, trophic interactions, and larval settlement and recruitment altogether (Schiel 2004). This novel approach is an attempt to construct a more encompassing view of how multiple biological, physical, and climatological factors function as a whole. The tendency of many community ecologists to fail to incorporate literature concerning wave-induced erosion and similar geophysical environmental influences in their predictions is

sited by Thompson et al. (2002) as a distinct problem in intertidal community ecology. It is only within recent years that attention has been given to these effects and, furthermore, placed within a context that includes long-term or historical observations; a recent study monitoring beds of *M. californianus* on the wave-exposed Pacific Coast found marked declines in biomass and cover in respect to the past thirty years (Smith et al. 2006). With global climate predictions forecasting a warming trend and oceanic surface rise, understanding the climate and physical oceanography of coastal regions is critical to comprehend community dynamics. Currently, there is increasing focus on interrelationships between populations, important particularly in light of the necessity of establishing quota limits and stock reserves on commercially valuable or ecologically critical species, but equally vital for understanding community succession following disturbance, gene flow, and predicting systemic shifts caused by the introduction of an invasive species (Schiel 2004).

Another recent development in intertidal community ecology study is a more philosophical one: the consideration that ecological models that may be applicable for one locale may be entirely unfit for use in other regions, even when the species composition, general spatial characteristics, and temporal cycles are similar. While there is an incentive to join observations from many regional studies in an attempt to construct a more global image of intertidal ecology as a whole, there is likewise the caveat that every study site is subject to inherent, local variation which is, perhaps more likely than not, entirely unique to those circumstances and conditions; models developed from the data at one given site may require careful scrutiny or revision before they can be used to

predict or explain the relationships of another. This is particularly true in view of the fact that most models, even those reviewed recently, are based on a small subset of the full complement of organisms found in a rocky intertidal habitat. The recent consideration now being given to benthic-pelagic coupling has had a secondary effect of highlighting areas of marine ecological study that show significant gaps in scrutiny and understanding; for example, the complete life histories of the majority of intertidal organisms have not been fully described, and in some cases, information concerning larval stages, critical for understanding settlement and recruitment, is all but nonexistent. Research frequently focuses on perceived keystone species, leaving other organisms underrepresented in research. Schiel (2004), for example, points out that although the rocky intertidal displays over 1000 described species, those that are the most studied are nearly entirely barnacles, mussels, and plankton: as many as 17 barnacle and mussel species out of 20 observed organisms in one study, while the importance of many macroalgal species goes understudied. Until these data have been more comprehensively established, ecological models, particularly in respect to predictions of commercially-valuable stocks, effects of pollution or coastal development, adjustments following the introduction of a foreign species, and comparisons between spatially separated populations will continue to be highly tentative.

Biology and Ecology of Mytilus edulis

The blue mussel, *Mytilus edulis*, a cold-temperate habitat generalist, is known to have worldwide distribution, along with the morphologically-similar species *M. galloprovincialis* and *M. trossulus*; along the coastline of the western Atlantic, studies

have shown that *M. edulis* has a range extending from Cape Hatteras, NC, north well into Canada. It was assumed for many years that the range of *M. trossulus*, stretching from southeastern Nova Scotia north to eastern Newfoundland, did not extend into the GOM, however work by Hennigar et al (1996) used allozyme electrophoresis of the mannose phosphate isomerase (*Mpi*) locus to demonstrate the presence of *M. trossulus* into the GOM and Bay of Fundy. A continuation and expansion of this study using mt16S-F, Glu-5', and ITS markers indicated the presence of *M. trossulus* in significant abundance in the eastern GOM as far west as Little Machias Bay, Maine, and minorly present from Machiasport, Maine, to Penobscot Bay, and even in the Damariscotta River estuary (Rawson et al 2001). Rawson et al. (2001) also show some limited hybridization between the two species based on the three alleles studied. In contrast, in the Baltic Sea, where *M. edulis* and *M. trossulus* also co-exist, there appears to be much more extensive, enough to consider the two as semispecies (Vainola and Hvilson 1991). Likewise, the European coastline from England to Spain demonstrates a similar story of extensive hybridization between *M. edulis* and *M. galloprovincialis*, and significant hybridization is known to occur between *M. galloprovincialis* and *M. trossulus* species on the coast of Southern California (Innes and Bates 1999, Kijewski et al 2006). Hybridization between worldwide-distributed *Mytilus* species seems to be fairly common, more so than the limited amount found in the GOM by Rawson et al. (2001). Morphologically, *M. edulis* tends to be larger than *M. trossulus*; Innes and Bates (1999) found that *M. edulis* had a significantly greater mean shell length than *M. trossulus* in 16 populations collected off the coast of eastern Newfoundland. However, based on analysis of eight morphometric characteristics, a fifth of each species had been misclassified, indicating that

identification of *M. edulis* and *M. trossulus* based on morphological characters is somewhat unreliable.

Mytilus is the dominant competitor on substrata of all angles throughout the mid-intertidal, and overshadow barnacles significantly from midsummer to early winter (Menge 1976). Since, as a sessile filter feeder, *Mytilus* is dependant on surrounding water conditions and ambient nutrient concentrations, environmental conditions and bottom-up affects play as critical a role in the population structure of *Mytilus* as trophic interactions do. Food availability, upwelling, and primary productivity are important factors affecting mussel growth rates; in studies off of California, *M. edulis* growth rates were positively correlated with concentrations of chlorophyll *a* during spring and summer months, and that growth rate was most likely a function of food quality over food quantity (Page and Ricard 1990). The same study found that, in terms of the Pacific coast of North America, water temperature is not a significant influence on *Mytilus* growth, however, this same conclusion has not been conclusively demonstrated to be valid for the GOM (Page and Ricard 1990). As a sessile organism, *M. edulis* may be more likely to face mortality from physical environmental stress, making refuges and adaptations in response to stress more critical (Menge and Sutherland 1987). Studies on mussels in the northern Pacific coast of the United States show that the lower limit of mussel ranges is very distinct and is determined by predation by the starfish *Pisaster ochraceus*, however, the GOM has no such predatory echinoderms on *Mytilus* species (Paine and Levin 1981). Likewise, the western coast has the presence of a second *Mytilus* species, *M. californianus*, creating another level of interspecific competition, an interaction that is not

present in the GOM.

Like most members of the class Bivalvia, *Mytilus* is a filter feeder, using ciliated gills to retain particulate matter from the surrounding environment for nutrient accumulation. Suspension-feeding bivalves use laterofrontal cirri or cilia to produce currents that redirect particles laterally onto ctenidial filaments, greatly increasing efficiency of particle capture (Ward and Shumway 2004). Although there has been found to be significant variation from individual to individual, *M. edulis* is capable of filtering and retaining particles as small as 7-8 μm (Griffiths and Griffiths 1987). Some of this filtered material is rejected and expelled as pseudofeces; however, during conditions of low particle concentrations, bivalves will ingest all filtered material. With increasing particle density, an increasing amount of filtered material is ejected as pseudofeces, suggesting that bivalves have the capacity to ascertain the value of in-siphoned particles and reject inorganic matter in favor of particles with more nutritive value. Kiørboe et al. (1980) demonstrated this, using *M. edulis* to show its ability to select algal cells from a mixture of organic and inorganic particles, and further, that as concentration of inorganic particulate matter increased, so did the efficiency of particle selection. Filtration rate for *M. edulis* remains largely constant for particle sizes up to 600 μm , but to compensate for the increased mass of total amount taken in, it rapidly increases pseudofeces production (Foster-Smith 1975). While there is a general tendency for some bivalves to increase filtration with temperature until a maximum efficiency around 20°C, in *M. edulis*, as temperature increases from 5° to 15°C, the efficiency of absorption declines (Griffiths and Griffiths 1987). Obtaining maximum efficiency in siphoning, filtration, digestion, and

absorption, while beneficial to any organism, takes on a higher importance in a sessile intertidal organism such as *M. edulis*, where tidal cycles force several hours of non-feeding behavior. It appears that *M. edulis* may have turned this apparent limitation around, for laboratory studies have shown higher growth rates for individuals grown in a discontinuous particle supply in comparison to those permitted to feed constantly (Winter 1976). Several studies highlight the ability of a number of bivalves to adjust capture efficiency in response to surrounding particle concentration, particularly in response to tidal cycles, but *M. edulis*, in both laboratory and field experiments, demonstrated constantly high capture efficiency regardless of ambient particle concentration (Ward and Shumway 2004). *M. edulis* also shows some degree of phenotypic plasticity; mussels respond to increasing turbidity by enlarging palps and producing small ctenidia, but it is uncertain as to whether this plays a role in observed seasonal changes in capture efficiency (Ward and Shumway 2004, Bayne et al. 1977).

Another limitation due to the periodic exposure caused by tidal cycles is the effect it has on respiration. While intertidal bivalves are capable of extracting oxygen directly from the air, *M. edulis* oxygen consumption in the open air drops to 4-17% of the rate when submerged (Widdows et al 1979). *M. edulis*, during periods of low oxygen, regulates respiration by increasing efficiency of oxygen extraction from water rather than by increasing water flow across the gills (Griffiths and Griffiths 1987). Many bivalves, as well, are facultative anaerobes, and are able to induce some tissues to function anaerobically even in aerobic conditions, a balance which may be regulated by sensing partial pressures (Griffiths and Griffiths 1987).

Temperature plays a significant effect on intertidal organisms. In addition to the decline in nutrient absorption in elevated temperatures, temperature also has an impact on respiration, both in instances of acute extremes and over a seasonal cycle. Oxygen consumption increases consistently with temperature, but only to a certain point. At temperatures above 20°C, oxygen consumption for bivalves becomes largely constant, an adaptation which certainly helps bivalves limit energy expenditure during periods of high temperature and exposure, such as during low tide, particularly in the summer months (Griffiths and Griffiths 1987). The 20°C mark also designates a threshold above which growth drops dramatically and mortality increases greatly, particularly under conditions of low food abundance, as frequently occurs in portions of the GOM during summer months (Incze et al. 1980). Long-term, or seasonal-scale, changes are more variable; some species do not acclimate, while others, including *M. edulis*, generally respond by shifting their metabolism gradually to maintain as close to a constant metabolism rate as possible. The ability of *M. edulis* to regulate both its respiration and its filtration rates means that, given the opportunity to acclimate following a temperature change, *M. edulis* will return to and continue to maintain these as constants. Absorption, however, does not fall under the regulatory capacity of *M. edulis*, and declines as temperature increases; over 20°C, individuals, even following acclimation, are unable to maintain a positive scope for growth (Widdows and Bayne 1971). There is, as well, recent evidence that suggests that *M. edulis* is not as capable of maintaining a constant filtration rate over a wide range of temperatures as was previously believed, but that *M. edulis*, like many other bivalves, declines in filtration rate as temperature decreases (Kittner and Riisgård 2005). Even

after allowing time for acclimation, Kittner and Riisgård (2005) found that groups of bivalves maintained within certain temperature environments did not approach a common filtration rate for all groups, but maintained a constant filtration rate proportional to the environmental temperature. Regardless of the effects of temperature on filtration, temperature does impact metabolic costs for intertidal organisms, linking higher temperatures with increased costs and a reduction in surplus energy that may be used for growth (Widdows and Bayne 1971, Widdows and Johnson 1988).

Salinity, as well, has an affect on growth; the low salinity of the Baltic Sea is responsible for the small size and slow growth of *M. edulis* found there (Griffiths and Griffiths 1987). Temperature and food intake are considered the two major factors in determining growth rate, but an organism's growth rate is also a function of exposure and submergence times, as well, which influence filtration and respiration. Organisms located higher in the intertidal have less time in which to feed. The warm temperatures and the ready availability of surface nutrients that occurs in the spring have a strong affect on growth rates: Shell growth is at a maximum during spring and summer, and next to no growth occurs during winter months.

Aside from both climatological and environmental influences on *M. edulis* growth, genetics plays a role as well, and may cause local variations for populations. After sexual maturity, which appears to be the result of individual size rather than age, organisms devote increasingly more energy to reproduction, and somatic growth declines sharply, approaching zero (Griffiths and Griffiths 1987). On top of both the genetic factors and

natural environmental conditions affecting growth potential in intertidal organisms, human activity plays a significant impact; there is a clear relationship between increasing environmental concentrations of copper, diesel oil, and contaminant hydrocarbons and decreasing scope for growth (Widdows and Johnson 1988). Consideration of human impact and pollution on the intertidal community of the GOM takes on heightened importance when viewed in conjunction with the large coastal human populations around the eastern Gulf as well as the issue that chronic exposure to pollution, as opposed to an acute event such as an oil spill, largely influences embayed coastlines (Thompson et al 2002).

The critical position and abundance of *Mytilus edulis* in the simplified trophic structure of the GOM and its nature as an organism easily subject to both bottom-up affects as well as top-down controls makes it an ideal study subject for ecological studies. The circulation dynamics of the GOM composed of two gyres largely divided by the Penobscot River outflow, provides two rocky intertidal habitats which, although largely similar in physical structure, environment, and community populations, show marked differences as well. Focus on the physiological performance of individual organisms can be indicative of the characteristics of populations as a whole, which can then be compared spatially and temporally in order to investigate physiological, ecological, and oceanographic trends along a coastline. This work described here investigates differences in *M. edulis* populations in the GOM intertidal both east and west of the Penobscot River, and examines the apparent affects of benthic-pelagic coupling both spatially and temporally, and attempts to describe any observed differences within the context of the nutrient-

productivity model. As a unique ecosystem, the GOM may exhibit marked differences to those community ecology models, such as the nutrient-productivity model, which have been built based on data from the Pacific Coast, New Zealand, and Europe.

As populations within a primarily contained and open ecosystem, we expect to find that:

H₀: There will be no differences in the molecular genetics of *M. edulis* across populations in the GOM, both spatially and temporally.

H₀: There will be no phenotypic variation in *M. edulis* in respect to metabolic state or trophic status as determined by RNA/DNA ratios and stable isotopes, or in respect to growth based on condition indices.

H₀: Phenotypic variation in populations of *M. edulis*, if observed, is the result of phenotypic plasticity and environmental impacts.

CHAPTER II

MATERIALS AND METHODS

Specimen Collection

Specimens of *M. edulis* were collected haphazardly at two spatially distant sites in the Gulf of Maine on two dates, once during the late winter and once during the late spring. Samples of *M. edulis* for 2006 were collected haphazardly at four distinct sites (Figure 2) on the Gulf of Maine coast in mid-May 2006. Approximately 35 – 50 samples of varying size were taken from the lower rocky intertidal at each location, as summarized in Table 1. Samples were identified based on morphological characteristics and were frozen at -80°C for future analysis.

Collection site	Latitude/Longitude	Collection date	N _{CI}	N _P
Black Duck Cove, Great Wass Island, ME	44° 28' 45" N 67° 36' 01" W	02 March 2004	50	5
Fort McClary, Kittery, ME	43° 04' 59" N 70° 42' 34" W	04 March 2004	54	6
Black Duck Cove, Great Wass Island, ME	44° 28' 45" N 67° 36' 01" W	05 May 2004	46	6
Fort McClary, Kittery, ME	43° 04' 59" N 70° 42' 34" W	05 May 2004	49	11
Black Duck Cove, Great Wass Island, ME	44° 28' 45" N 67° 36' 01" W	12 May 2006	36	26
Clark Point, Prospect Harbor, ME	44° 23' 49" N 68° 01' 11" W	12 May 2006	46	13
Pemaquid Point, Bristol, ME	43° 49' 53" N 69° 30' 56" W	14 May 2006	52	15
Fort McClary, Kittery, ME	43° 04' 59" N 70° 42' 34" W	16 May 2006	52	16

Table 1. List of mussel collections by location and date. N_{CI} indicates the number of individual specimens used in condition indices calculation and analysis. N_P indicates the number of individuals sequenced and used in population genetic analysis.

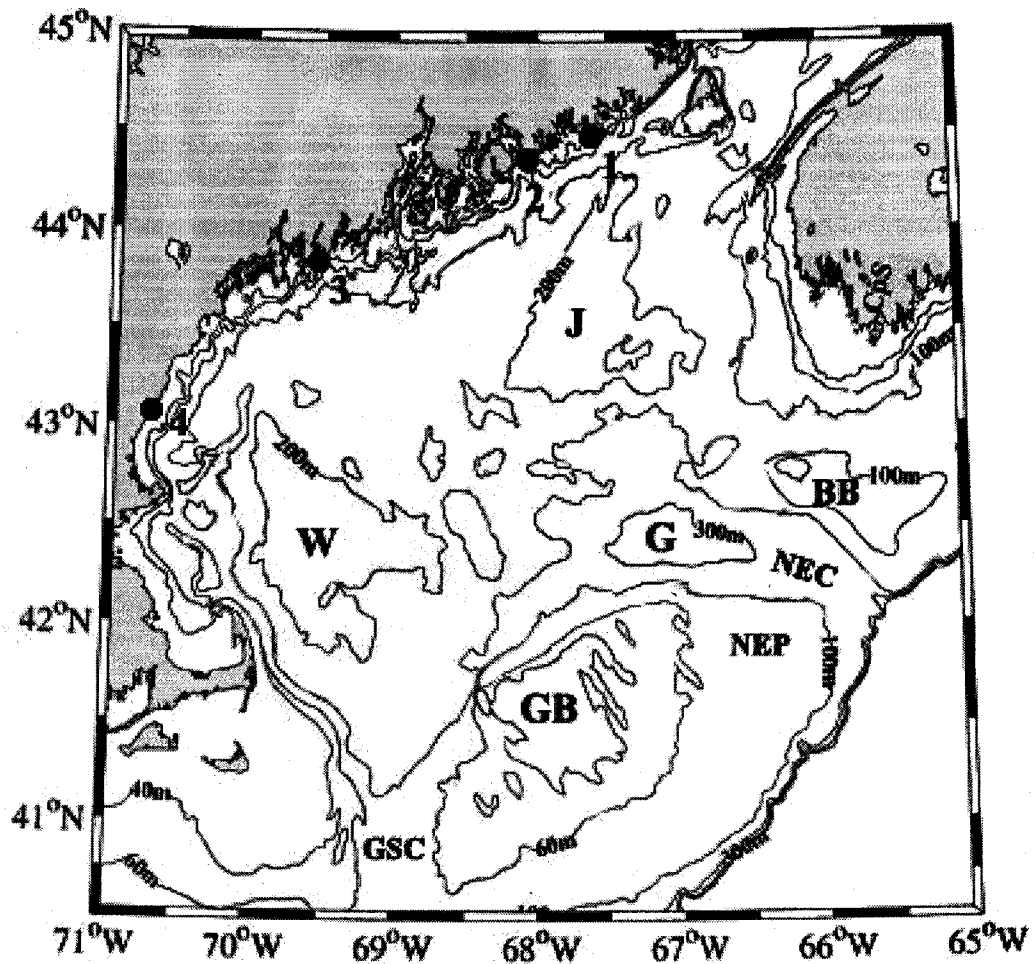


Figure 2. Collection sites on the Gulf of Maine coast. Sampling locations are designated as 1: Black Duck Cove, 2: Clark Point, 3: Pemaquid Point, and 4: Fort McClary as described by latitude and longitude in Table 1. Other abbreviations are as Figure 1. Figure redrawn with permission from Johnson et al.

Frozen samples were measured for length, width and height, followed by measuring the wet weight of collected somatic and gonadal tissue and the weight of the shell separately to the nearest 0.1 g. Specimens were also sexed based on presence and development of gonadal tissue. Adductor muscle tissue was excised and stored at -80°C for future genetic, stable isotope, and DNA/RNA ratio analysis.

Condition Indices

Condition indices (CI) were calculated based on wet weight of combined somatic and gonadal tissue (W) expressed as a percentage of the shell weight (S) as by the formula $CI = W \cdot 100 / S$ (Crosby and Gale 1990). Locations with greater CI indicate that more energy is being channeled into somatic/gonadal growth versus shell growth.

Stable Isotopes

The stable carbon (^{13}C) and nitrogen (^{15}N) isotope compositions of the total organic carbon and nitrogen in sample tissue for three randomly-selected individuals from each of the 2006 collection sites were determined using a Europa ANCA-SL elemental analyzer - gas chromatograph preparation system attached to a continuous-flow Europa 20-20 gas source isotope ratio mass spectrometer. Dried and pulverized *M. edulis* adductor muscle tissue samples were packed into tin capsules and weighed to ± 0.01 mg. Aliquots contained 10 – 200 μmoles C and 5–10 μmoles N.

Samples and standards were loaded into the autosampler on the ANCA-SL elemental analyzer combustion system. During the run, the samples were sequentially dropped into a quartz combustion tube held to a temperature of 1000°C . The samples were then flash combusted at about 1800°C in the presence of the tin, oxygen gas, Cr_2O_3 and CuO . The combustion products, principally CO_2 , N_2 and NO_x , and H_2O , were transported by a helium carrier gas through a reduction tube, filled with Cu metal and held at 600°C , where any NO_x was converted back to N_2 . The gases were then passed through an MgCl_4 trap to remove H_2O .

Following separation by gas chromatography, the N₂ and CO₂, in this order, were passed into the mass spectrometer to the collectors where the masses of interest (28 and 29 for N₂, 44, 45, and 46 for CO₂) were continuously monitored. The mass peaks were plotted, the area under each mass peak then determined, and the isotope ratios calculated. These ratios were referenced to ratios determined on in-house (Marine Biological Laboratory, Woods Hole, MA) reference materials analyzed in the same analytical run.

The data were corrected for blank contributions, drift over the course of the run, linearity effects if present, and then normalized to the international standards. Stable isotope ratios were reported in the Delta (δ) notation. Delta values are the difference in per mil (‰) between the sample isotope ratio and that of an international standard of known isotope composition. Positive δ values are enriched in the rarer, heavy isotope relative to the international standard, whereas negative δ values are depleted in the heavy isotope relative to the international standard. The carbon isotope results are reported relative to the international PDB standard. The nitrogen isotope results are reported relative to the international AIR standard. Analytical precisions on well-homogenized samples are usually better than +/- 0.2 ‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$.

RNA/DNA Ratios

Sub-samples of adductor muscle tissue from six randomly-selected individuals were thawed on ice, weighed, and homogenized in 1.0% Sarcosil Tris-EDTA buffer at room temperature and nucleic acids were extracted using Tris-EDTA buffer. Samples were

serially diluted and 75.0 μl of supernatant were used for analysis in a Millipore Cytofluor 2300 fluorometer using 96 well microplates. Concentrations of nucleic acids were determined by ethidium bromide (EB) fluorescence with an EB concentration of 2.0 $\mu\text{g ml}^{-1}$ (Dahlhoff et al. 2001). Fluorescence was recorded at 510 nm excitation and 595 nm emission, which was repeated following the addition of 7.5 μl RNase. The concentration of DNA and RNA in mussel tissues were calculated based on a standard curve of known quantities of DNA and RNA.

Statistical Analysis

An analysis of variance (ANOVA) with location as a fixed factor was applied to all measured parameters as described above at a significance level of 5%. No unequal variances were detected using the F_{max} test, and individual treatment differences were assessed using the Student-Newman-Keuls (SNK) multiple comparison test. Ratios and percentages were arcsine or log transformed for analysis and back transformed for presentation.

Remote Sensing

L1A SeaWiFS data from the 5.1 reprocessing collection was obtained from the NASA Ocean Biology Processing Group (Feldman and McClain 2006) and processed to L2 data with SEADAS (version 5.0.2) using standard atmospheric correction and chlorophyll *a* algorithms (OC4v4, O'Reilly et al. 2000). The chlorophyll *a* data were then remapped onto a common Lambertian conic projection centered at 40°N and 70°W with a mean pixel size of 1.25 km^2 with SEADAS. Eight-day composites were generated as the

arithmetic mean of daily data with pixels screened based on a select set of L2 flags including cloud and land masks. Visual checking of images was also used to remove images that contained spurious data. Chlorophyll concentrations less than 0.032 mg m^{-3} and greater than 50.0 mg m^{-3} were masked. Eight-day climatological averages were calculated as the arithmetic mean from the 9 years of 8-day composites.

Changes in climatological and yearly data at each of the four locations were investigated with data from an 11 by 11 box surrounding the central pixel (Table 1). Arithmetic means of pixels, which were $1.5 \text{ km} \times 1.5 \text{ km}$, with data within these windows were calculated for both sea-surface temperature and chlorophyll *a* concentration.

DNA Extraction, Amplification and Sequencing

Adductor muscle tissue DNA was extracted using CTAB/chloroform technique (France et al. 1996) and a 700+-bp region of the mitochondrial DNA gene cytochrome oxidase I was amplified using the universal invertebrate primers LCO1490 and HCO2198 (Folmer et al. 1994).

LCO1490: 5'-ggtaacaaatcataaagatattgg-3'

HCO2198: 5'-taaacttcagggtgacccaaaaaatca-3'

As per Folmer's designations, the L refers to the light DNA strand and H refers to the heavy DNA strand; CO designates the cytochrome oxidase gene, and the numbers describe the 5' position on the *Drosophila yakuba* genome. Primers (Invitrogen, Inc.)

were produced by Invitrogen and used at 10.0 μM concentration and reaction volumes were 30.0 μl . Polymerase chain reactions (PCR) used AmpliTaq polymerase from Applied Biosystems with the manufacturer's 10X PCR buffer containing 15.0 mM MgCl_2 , and 10.0 μM dNTPs (Promega, Inc). All samples were sequenced in both directions using the PCR primers and were performed by DNA Sequencing Core, University of Michigan (Ann Arbor, MI). Sequences were edited using FinchTV 1.4.0 (Geospiza Inc., www.geospiza.com/finchtv), 4Peaks 1.7.2, and LaserGene, and were trimmed to a 659-bp contig to remove ambiguous end regions and establish a consistent length for all sequences. Successful sequence results were compared to published sequence data using the BLAST function of the National Center for Biotechnology Information (NCBI, <http://www.ncbi.nlm.nih.gov/blast/>). Those individuals that were determined to be *M. trossulus* were removed from all analyses.

Population Genetic Analysis

Sequences were aligned using the European Bioinformatics Institute ClustalW (<http://www.ebi.ac.uk/clustalw/>). Sequences for unique haplotypes were imported into Phylogenetic Analysis Using Parsimony 4.0b10 (PAUP) for phylogenetic analysis and analyzed by two methods, maximum parsimony and maximum likelihood as described by Wares and Cunningham (2001, Castelloe and Templeton 1994). Maximum parsimony analysis was performed via a heuristic search with stepwise random addition and tree-bisection reconnection branch swapping with 1000 replicates, and bootstrapped (50 replicates) as per Curole and Kocher (2005). The Tamura-Nei model was used to estimate maximum likelihood with gamma correction ($\alpha = 0.3$, proportion of invariable

sites = 0), without the assumption of a molecular clock, and bootstrapped at 10 replicates (Curole and Kocher 2005). Trees were rooted using a representative of *M. trossulus* as an outgroup, which was collected from Black Duck Cove, Maine, in 2004, and extracted, amplified, and sequenced as above.

Due to the volume of data, the multiple sampling-year tree was created by maximum parsimony via close neighbor interchange branch swapping and random addition of trees (10 replicates) at a search level of 2 using Molecular Evolutionary Genetics Analysis v. 3.1 (MEGA) and bootstrapped at 100 replicates. Analysis in this instance was restricted to mutations in the third codon position, which assumes that these mutations are neutral and avoids the necessity of estimating among site variation via gamma correction (Wares and Cunningham 2001).

CHAPTER III

RESULTS

2004 Sampling Data

Analysis of the winter sampling for Black Duck Cove (BDC) and Fort McClary (FMC) showed that *Mytilus edulis* at both locations had similar size ranges at both locations, with shell lengths between 15.0 mm and 65.0 mm (Fig. 3a). Only one individual at BDC fell outside this range, into the 70.0-75.0 mm size class, and the distribution for both sites is bimodal. At BDC, there are two clusters of mussels, one falling in the 20.0-40.0 mm range, and the other between 40.0 mm and 65.0 mm; at FMC, the distribution is similar, although within a smaller size range, with the first group at 15.0-35.0 mm and the second at 35.0-65.0 mm. In contrast, the spring sampling collection (Fig. 3b) revealed a very broad distribution of sizes at BDC, with samples in every size class from 10.0 mm to 80.0 mm that produced a narrow unimodal distribution at FMC, with all samples falling between 15.0 mm and 50.0 mm. At FMC during the spring, over 85% of collected individuals had shell lengths between 20.0 and 40.0 mm. The size class in which the most individuals were collected at each site was also much higher at BDC (35.0-40.0 mm) than at FMC (20.0-25.0) during the spring collection.

For the winter sampling date, *M. edulis* collected at BDC had a mean shell length of 43.2

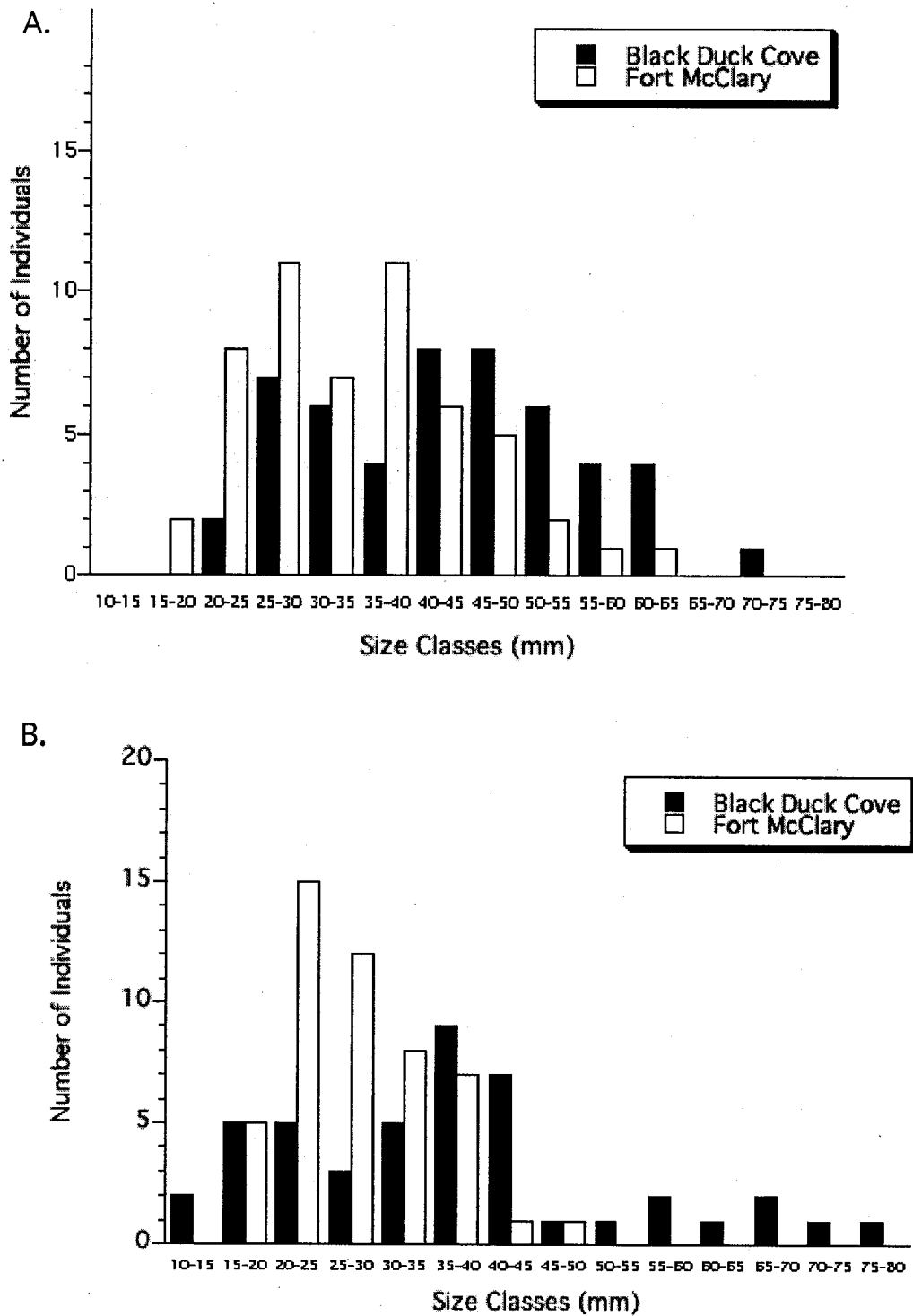


Figure 3. *Mytilus edulis* samples by size class for 2004. A. Samples collected during the winter collection dates. B. Samples collected on the spring collection date. Size classes are based on shell length in mm.

mm, significantly greater than the mean shell length of 34.8 mm found in the FMC samples, and the mean CI for BDC, at 33.4, was also higher than the mean CI for FMC, at 28.0 (Fig. 4). There was a significant effect of location on shell lengths in both winter (ANOVA: $P=0.0003$) as well as spring (ANOVA: $P=0.0007$). However, in the spring, although the BDC samples had a larger mean shell length of 37.0 mm than the FMC spring mean shell length at 28.2 mm, the FMC samples had a much higher CI, 50.6, than the samples from BDC, 35.8, which together suggest that more energy is going into tissue versus shell growth in FMC mussels. These population differences in CI were also significant for both seasons (winter sampling ANOVA: $P=0.0017$, spring sampling ANOVA: $P= <0.0001$).

Analysis of stable isotope data (Fig. 5) showed a significant difference (ANOVA: $P= <0.0001$) in $\delta^{13}\text{C}$ between the two sites BDC and FMC over the collection dates. The mean $\delta^{13}\text{C}$ for BDC in winter (-17.00‰) differed from the mean spring level (-16.73‰) by less than 0.3‰ ; mean $\delta^{13}\text{C}$ for FMC exhibited a seasonal difference of a comparable minor amount (winter mean= -18.40‰ , spring mean= -18.73‰). Seasonal differences in $\delta^{13}\text{C}$ were not significant (ANOVA: $P=0.871$), while differences between populations for each collection date were significantly different (ANOVA: $P=<0.0001$).

A significant (ANOVA: $P=0.0488$) difference in $\delta^{15}\text{N}$ between sites was also observed. Looking at each site based on season, the mean $\delta^{15}\text{N}$ for BDC in spring (mean $\delta^{15}\text{N} = 7.40\text{‰}$) was 1.0‰ higher than in winter (mean $\delta^{15}\text{N} = 8.40\text{‰}$), and the mean $\delta^{15}\text{N}$ for

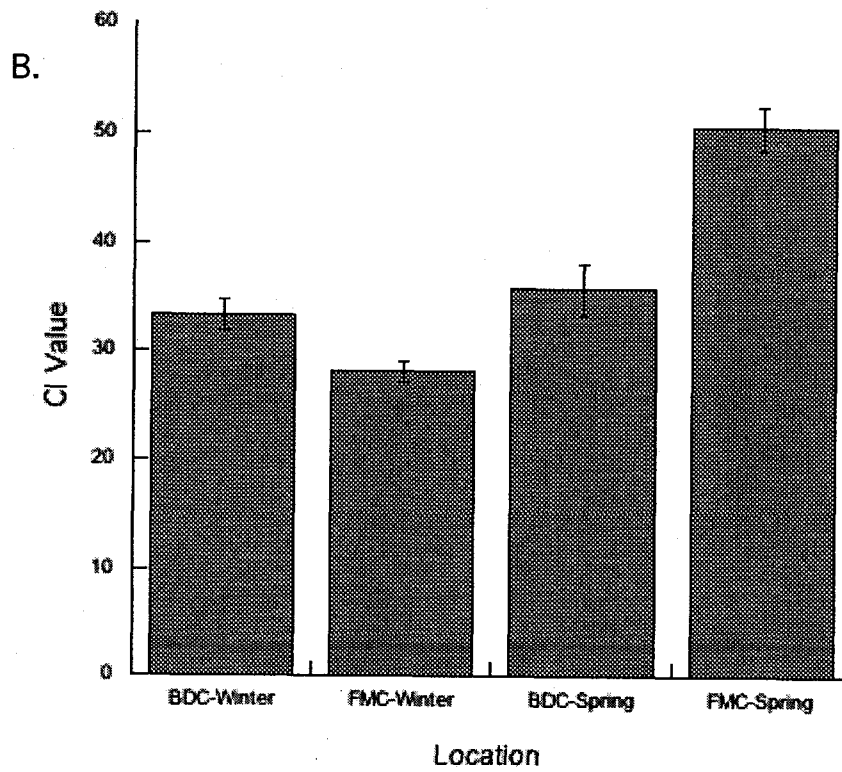
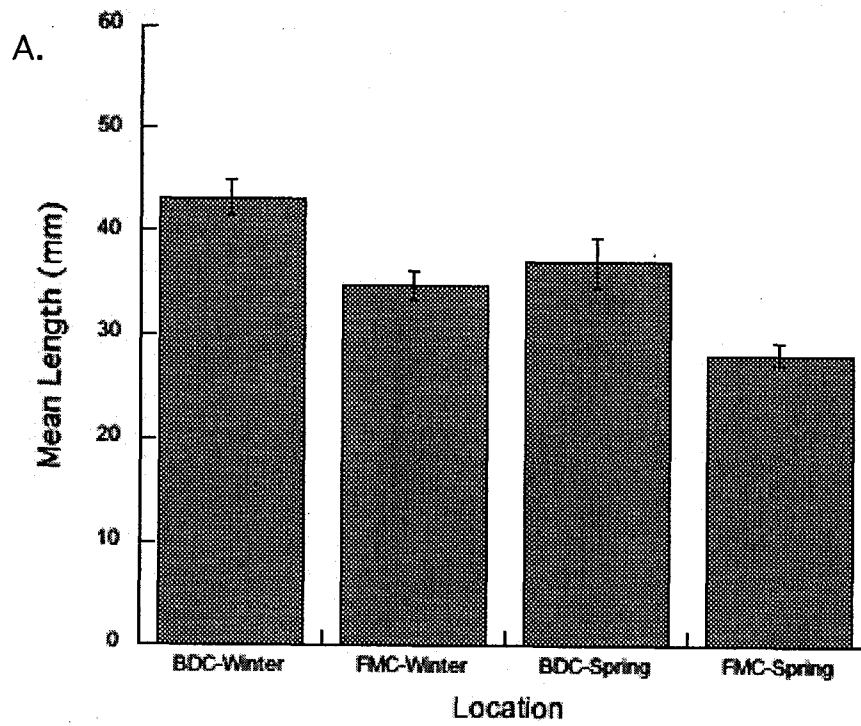


Figure 4. Mean (\pm SE) shell lengths A, and condition indices B, for winter and spring, 2004, sampling at BDC and FMC.

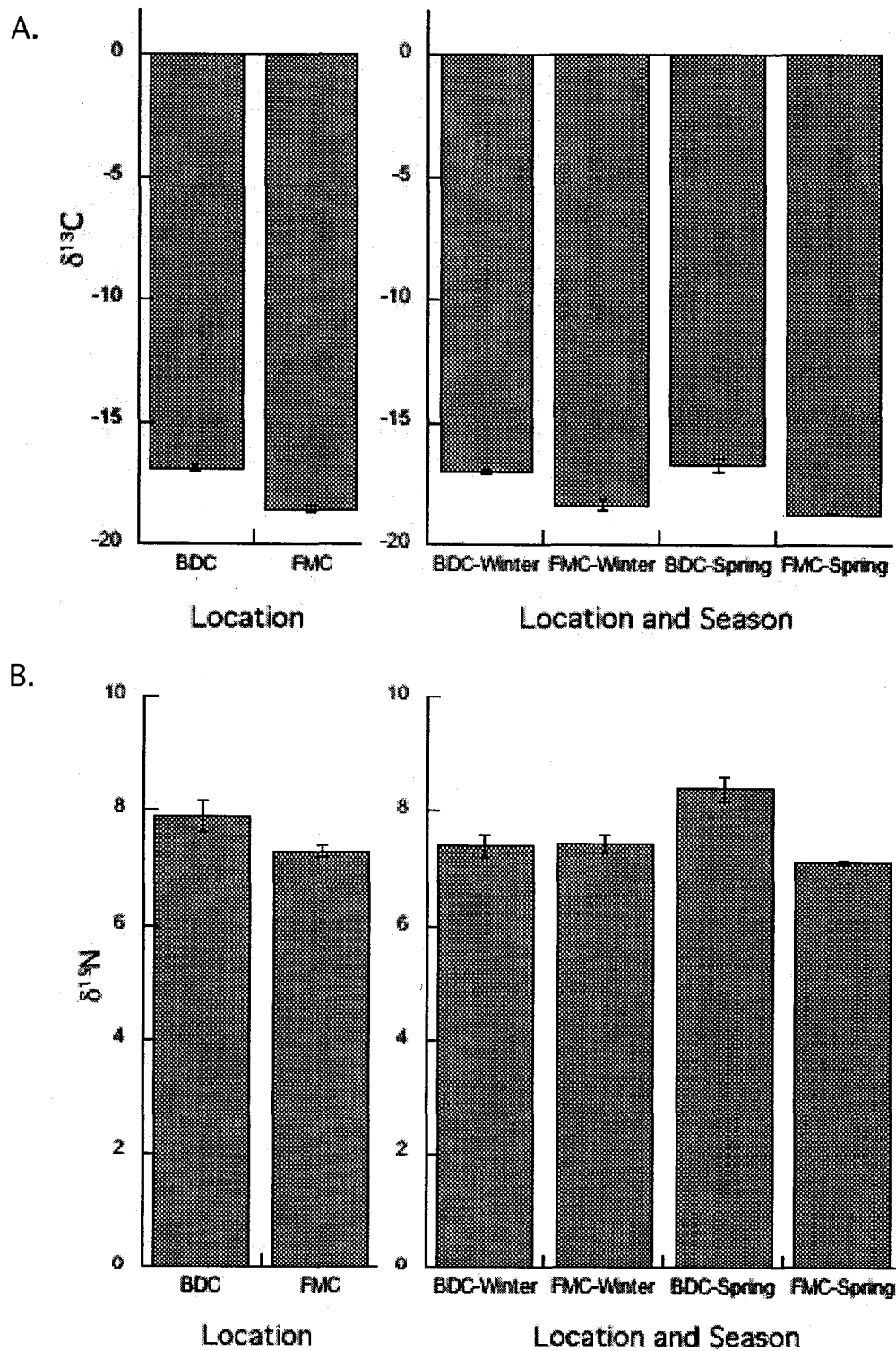


Figure 5. Mean (\pm SE) isotope values for 2004 based on all samples by location regardless of season, and on location and season. A. $\delta^{13}\text{C}$ values. B. $\delta^{15}\text{N}$ values.

FMC in winter (mean $\delta^{15}\text{N}=7.43\text{‰}$) was 0.3‰ higher than in spring (mean $\delta^{15}\text{N}=7.13\text{‰}$). Differences between mussel populations based on location in winter and in spring were found to be significant (ANOVA: $P=0.005$), however, as with carbon, differences between season were not significant (ANOVA: $P>0.05$).

Samples from BDC had a mean RNA/DNA ratio of 2.66, and FMC samples had a mean RNA/DNA ratio of 1.83 in the winter. These population differences were found to be significant (ANOVA: $P=0.038$). Analysis of the spring samples showed RNA/DNA ratios for BDC of 2.32 and for FMC of 2.24, which were not significantly different from each other (ANOVA: $P=0.748$).

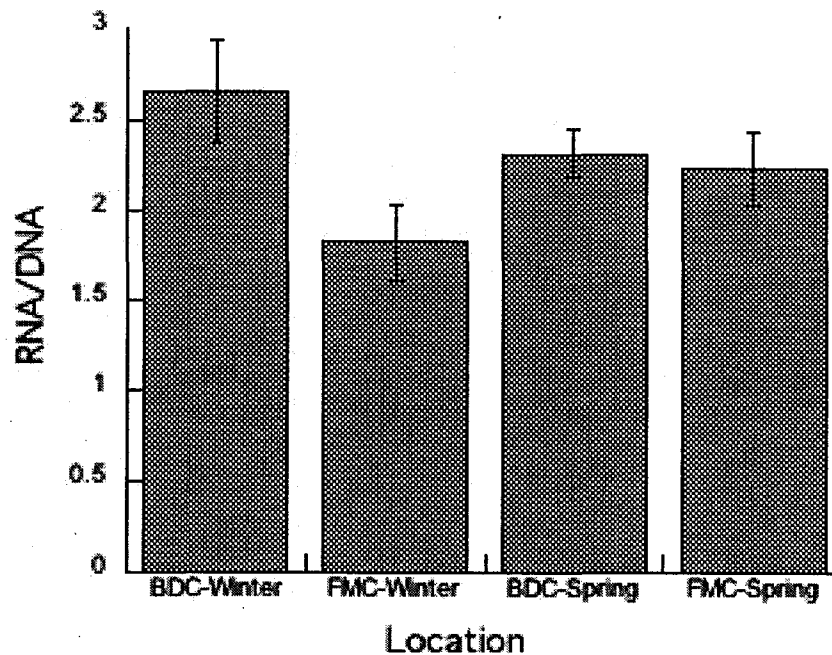


Figure 6. Mean (\pm SE) RNA/DNA ratio values for 2004 based on location and season.

SeaWiFS satellite remote sensing of chlorophyll *a* (Fig. 7) shows a large increase in chlorophyll *a* between the winter and spring sampling periods at FMC, reaching levels between sampling times as high as 16.0 mg m⁻³ in early May, fourfold higher than levels observed during the winter. At the times of the two sampling dates, levels of chlorophyll *a* in the water column were nearly the same at FMC, approximately 4.0 mg m⁻³ in spite of the large rise in chlorophyll *a* that occurred in the interim. In contrast, there is no similar increase in chlorophyll *a* observed for BDC; chlorophyll *a* levels remain relatively constant at roughly 3.0 mg m⁻³ both at the time of both the winter and spring sampling and during the interim period.

Sea surface temperature measured by SeaWiFS was higher at FMC than at BMC for both winter and spring sampling dates (Fig. 8). During winter sampling, difference in sea surface temperature between the two sites was minor, with BDC at approximately 1.5°C and FMC approximately a degree warmer, at 2.5°C. During spring sampling, in contrast, sea surface temperature at BDC had warmed to about 5.0°C while FMC was over 3 degrees warmer, at nearly 9.0°C. The temperature profile presented for 2004 is typical of recent annual patterns of sea surface temperature, as will be shown at the end of this section.

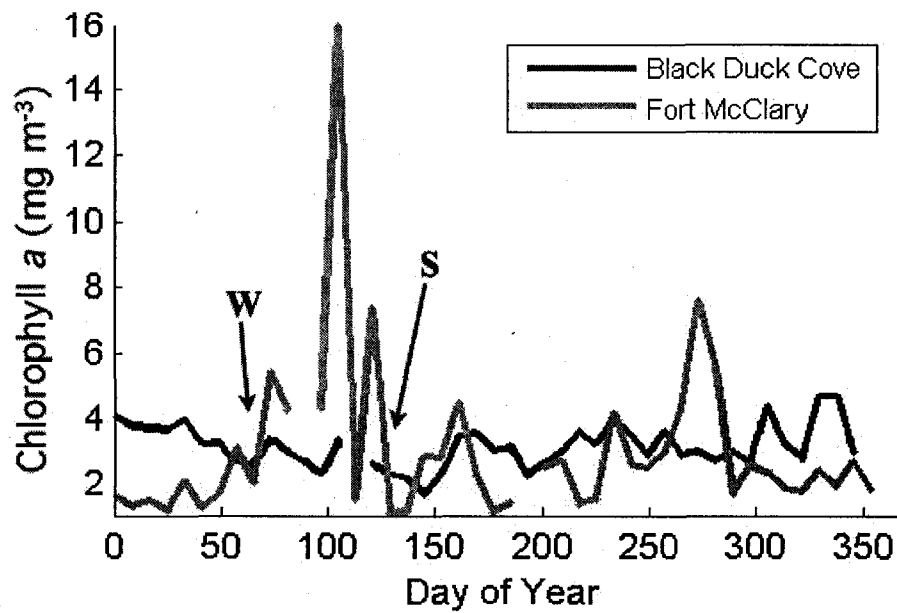


Figure 7. SeaWiFS data for chlorophyll *a* for 2004. W indicates the dates of winter sampling (02 and 04 March) and S indicates the date of spring sampling (05 May).

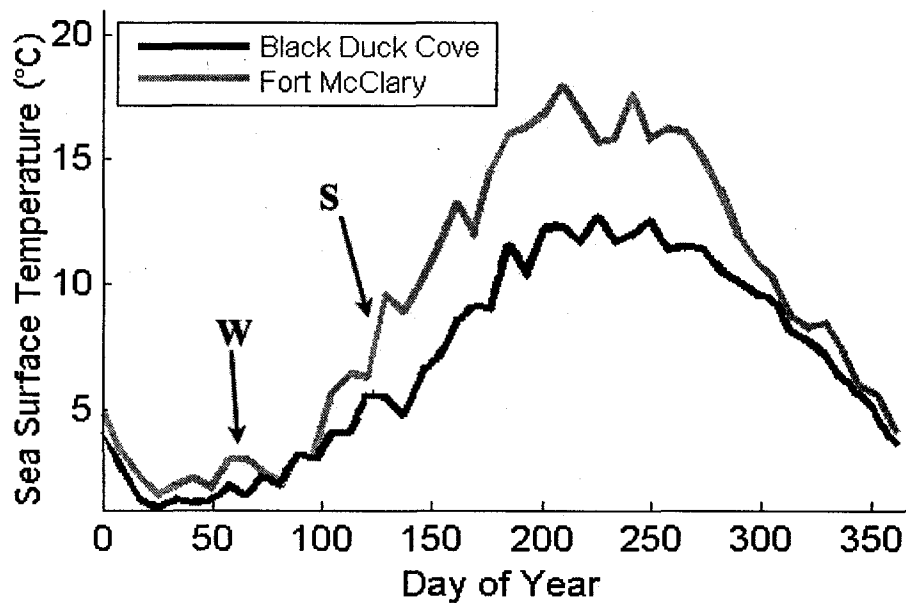


Figure 8. SeaWiFS data for sea surface temperature for 2004. W and S designations are as for Fig. 7.

Population genetic analysis of COI via maximum parsimony methods for 2004 samples produced a tree with two significant haplotypes, one represented by only a single individual, and the other represented by a number of less-resolved haplotypes, many of which differ from each other by less than 5 base pair changes out of the 659 base pairs used in the analysis (Fig. 9). Although there are minor clusters of regionally unique haplotypes within this larger branch (e.g. Group E as exclusive to FMC; Group B and immediate neighbors as exclusive to BDC), these differences are minute enough to be insignificant; the entire major haplotype differs by less than 2%. The second haplotype, represented by a single individual from BDC, is very different from the other *M. edulis*, showing a deeper division between the two *M. edulis* clades than is observed even between the major clade and the *M. trossulus* included as an outgroup.

Maximum likelihood analysis methods produced a tree in which major divisions between haplotypes were conserved (Fig. 10). Most minor groups were also maintained; some reshuffling of branches was exhibited among individuals in which sequence difference was less than 2%. Such minor differences between the phylogenetic trees as well as the lack of distinct genetic groupings based on location indicate that the populations of *M. edulis* in the GOM are genetically homogenous.

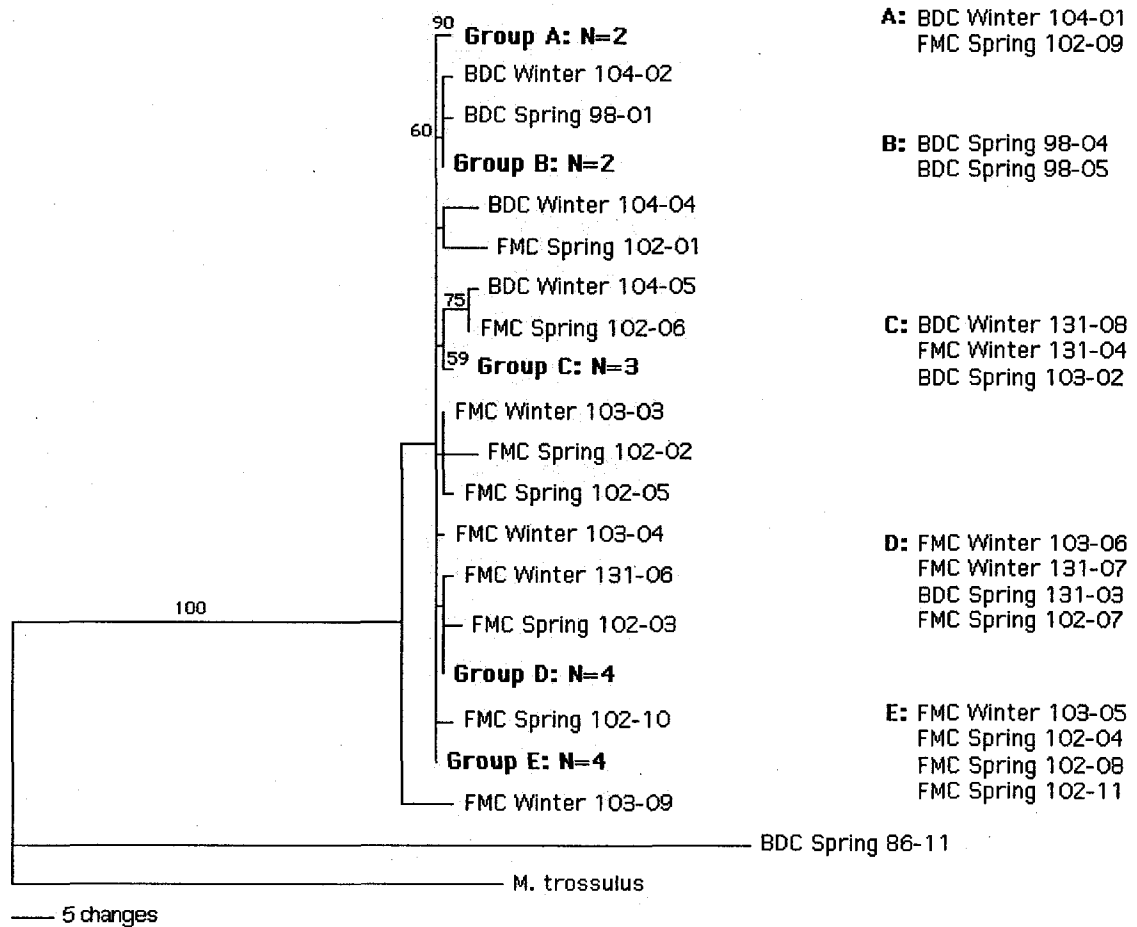


Figure 9. Maximum parsimony tree for *Mytilus edulis* collected in winter and spring of 2004. Numbers on branches indicate bootstrap values. Individuals within a group have identical haplotypes; individuals within each group are listed at right.

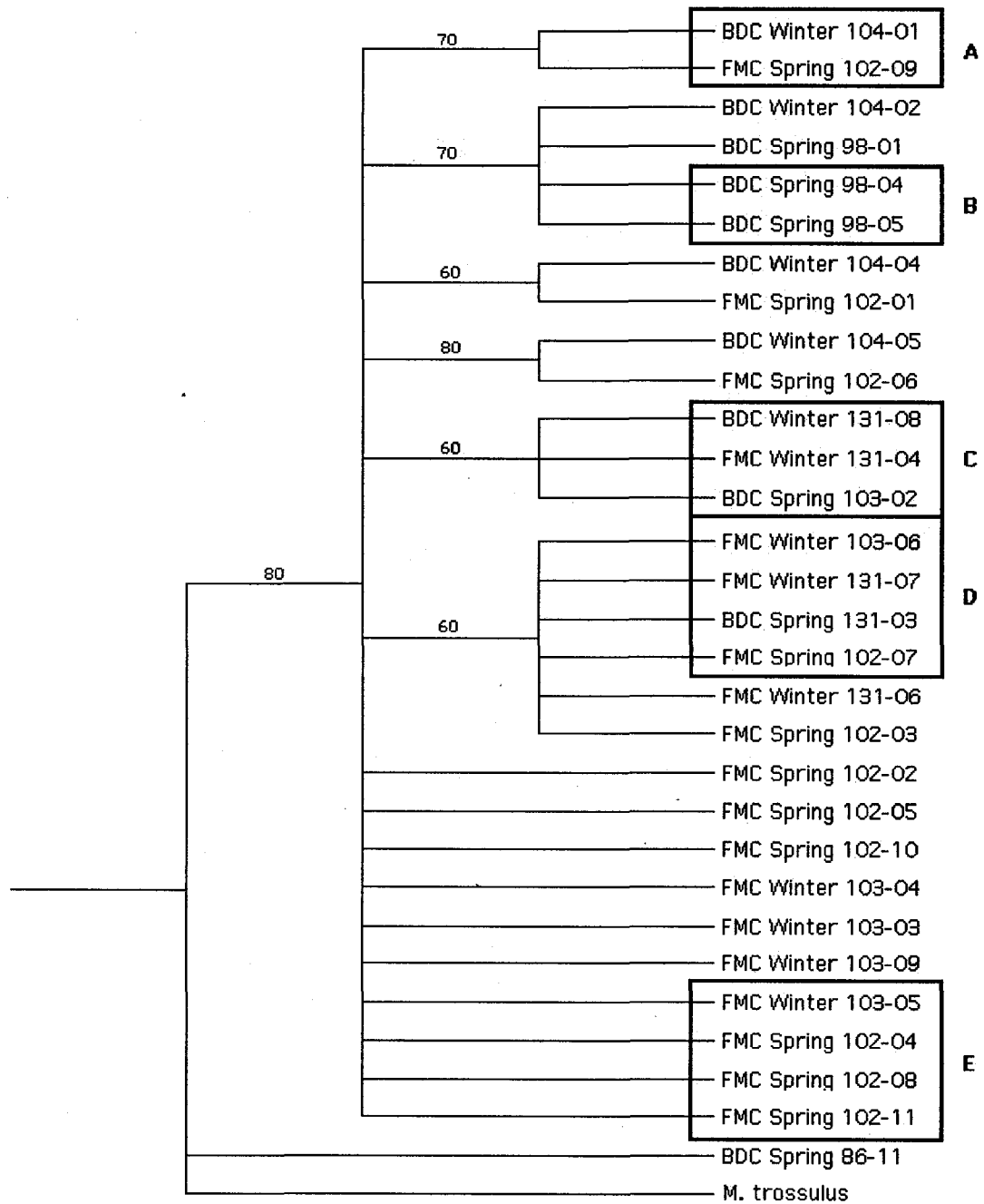


Figure 10. Maximum likelihood tree for *Mytilus edulis* collected in winter and spring of 2004. Numbers on branches are bootstrap values; black outlines indicate identical haplotype groups as labeled and listed in Fig. 9.

2006 Sampling Data

Unlike 2004 where mussels were sampled during the winter and spring at the extremes of their distribution in the GOM, in 2006 mussels were collected only during the spring, and two additional populations, Pemaquid Point, Bristol (PPB) and Clark Point, Prospect Harbor (CPH) were added to examine finer scale latitudinal differences. The size frequency distribution data for *Mytilus edulis* for the four populations surveyed in spring 2006 are presented in Fig. 11. Mussels collected from BDC grouped together in a narrow, unimodal size distribution with all collected individuals having shell lengths between 30.0 and 55.0 mm. Mussels collected from CPH were also unimodal in size distribution and were mostly 40.0-70.0 mm in length, with one outlying sample in the 15.0-20.0 mm range. PPB samples showed a size distribution ranging from 15.0 mm to 50.0 mm, however, nearly 85% of the samples collected from this location are within three size categories between 30.0 mm and 45.0 mm. Samples from FMC were all within the range of 35.0 mm and 65.0 mm and were distributed, as the other sample sites, in a unimodal fashion.

There was a significant effect (ANOVA: $P < 0.0001$) of location on shell size with mean lengths of the mussels from the sampling collection, measured as 40.2 mm for BDC, 51.8 mm for CPH, 37.6 mm for PPB, and 46.0 mm for FMC (Fig. 12). Post-hoc multiple comparison testing revealed that all populations were significantly different (SNK: $P < 0.05$) from each other.

The CI for these samples were 38.2 for BDC, 45.8 for CPH, 44.7 for PPB, and 32.5 for

FMC. There was a significant effect of location (ANOVA: $P < 0.0001$) with BDC and FMC significantly different (SNK: $P < 0.05$) from each other and both significantly different (SNK: $P < 0.05$) than CPH and PPB. CPH and PPB were not different from each other (SNK: $P > 0.05$).

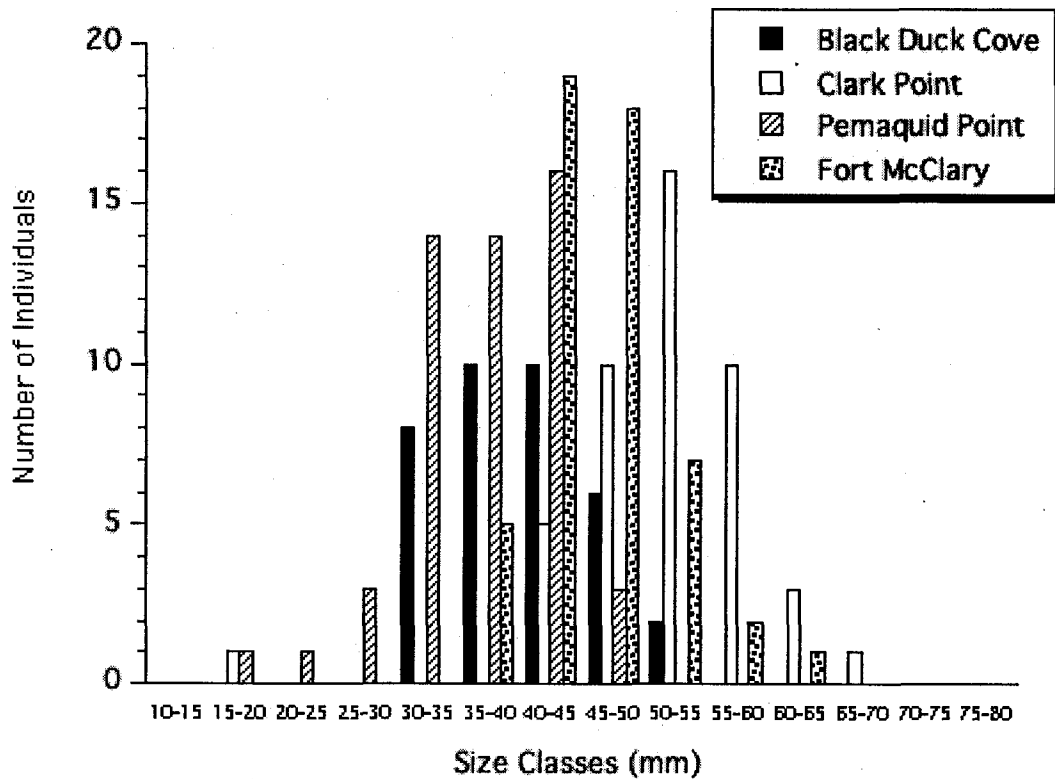


Figure 11. *Mytilus edulis* samples by size class for 2006. Size classes are based on shell length in mm.

Mean stable isotope results for 2006 are summarized in Figure 13; the mean $\delta^{13}\text{C}$ for BDC was -16.63‰ , for CPH = -17.10‰ , for PPB = -17.43‰ and for FMC = -16.53‰ .

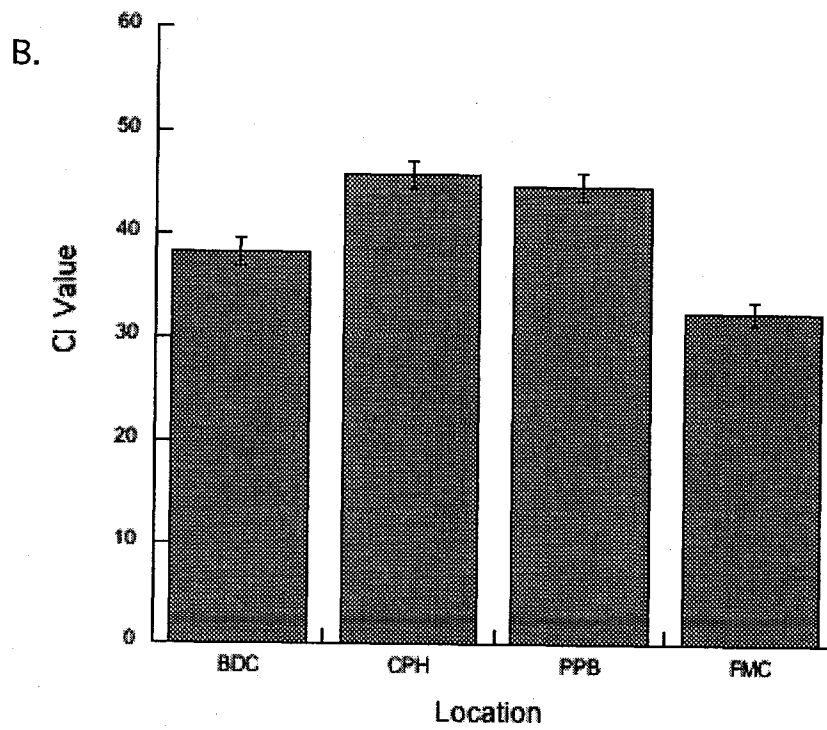
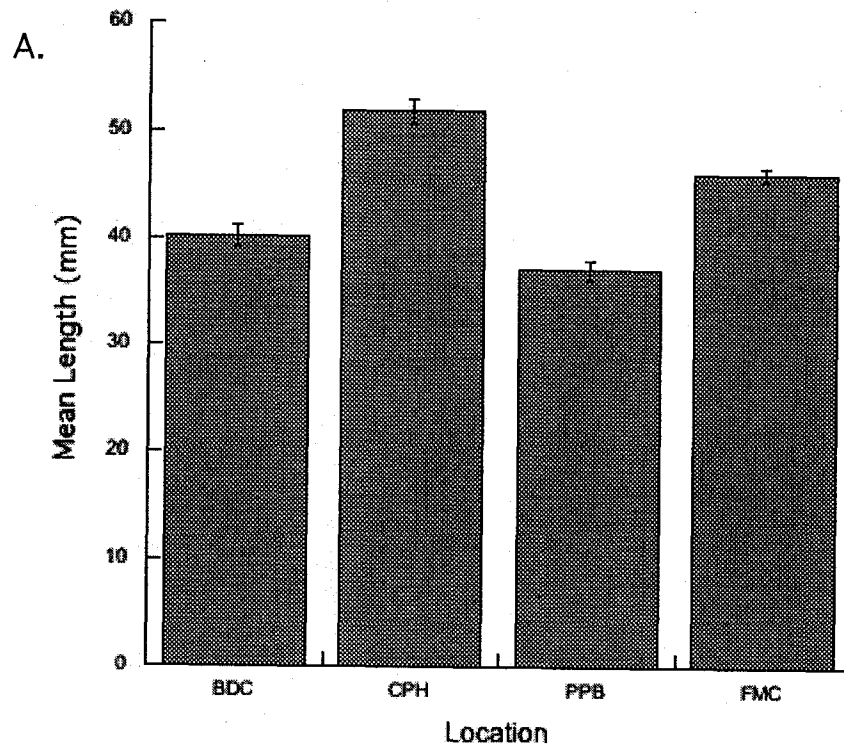


Figure 12. Mean (\pm SE) shell lengths, A, and mean condition indices, B, for 2006 collection sites.

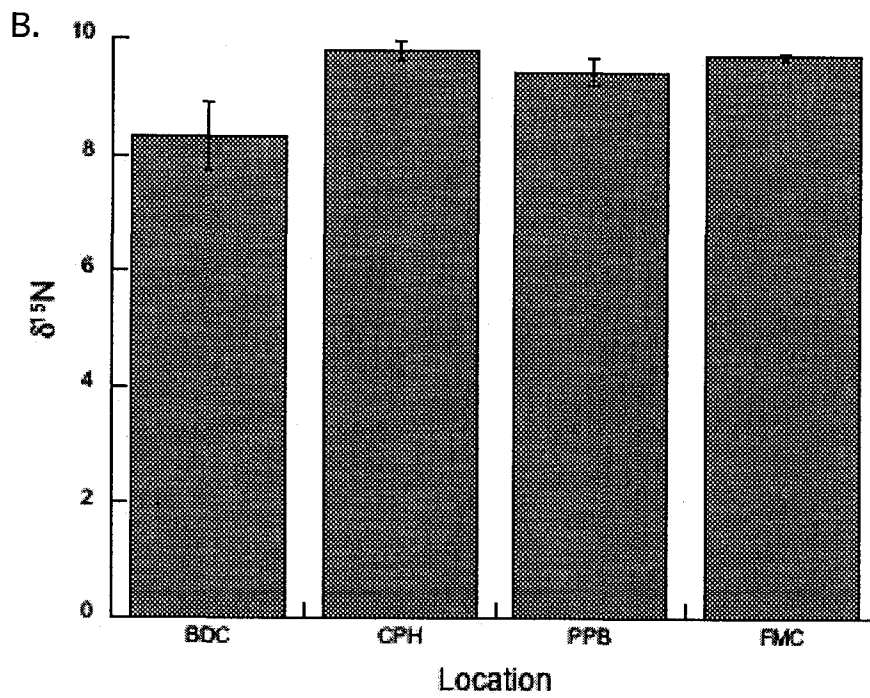
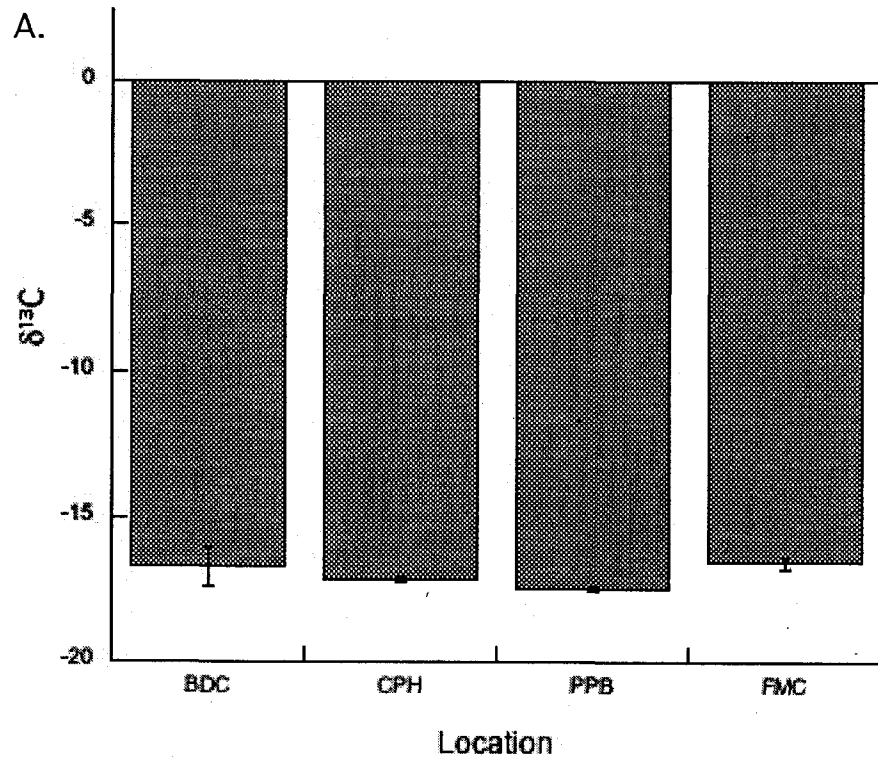


Figure 13. Mean (\pm SE) $\delta^{13}\text{C}$, A, and mean $\delta^{15}\text{N}$, B, for 2006 by collection site.

Differences in $\delta^{13}\text{C}$ were not significant with respect to location (ANOVA: $P=0.325$), however, location did produce significant differences in $\delta^{15}\text{N}$ analysis (ANOVA: $P=0.042$). The mean $\delta^{15}\text{N}$ for BDC was for BDC = 8.33‰, for CPH = 9.80 ‰, for PPB = 9.43‰, and for FMC = 9.70‰. Multiple comparison testing revealed significant differences between BDC and all other populations (SNK: $P<0.05$) that are not significantly (SNK: $P>0.05$) different from each other.

Mean RNA/DNA ratios for 2006 sampling were as follows: BDC = 2.137, CPH = 2.318, PPB = 2.18, and FMC = 2.33 (Fig. 14); differences were found to be significant with respect to location (ANOVA: $P=0.0034$). BDC differed from CPH and FMC significantly (SNK: $P<0.05$), and PPB differed from both CPH and FMC significantly as

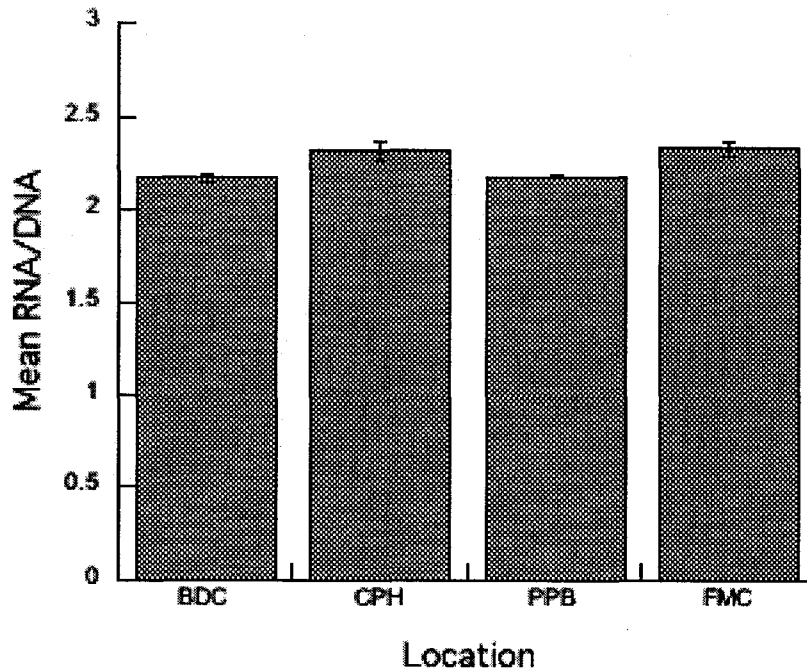


Figure 14. Mean (\pm SE) RNA/DNA ratio values for 2006 based on location.

well (SNK: $P < 0.05$), however there was no significant difference between BDC and PPB or between CPH and FMC (SNK: $P > 0.05$).

The satellite remote sensing data for 2006 (Figure 15) show a period of elevated chlorophyll *a* for about 20-30 days prior to sampling at both PPB and FMC. There was no corresponding elevation of chlorophyll *a* at CPH or BDC, the two collection sites in the eastern GOM during the same time period.

Sea surface temperature during the 2006 sampling period showed a smaller variation in the temperatures between the sites in comparison to 2004 (Fig. 16). Both BDC and CPH had temperatures at about 7.5°C during the time of sampling, and PPB and FMC had temperatures of about 9.0°C, less than a 2°C difference between the sites.

Maximum parsimony population genetic analysis on 2006 samples (Fig. 17) demonstrated the presence of two distinct haplotypes, as was observed in the 2004 sample and the larger sampling and analysis size allowed for the haplotypes to be more well-represented than in the 2004 samples. The majority of the individuals within the primary haplotype branch differ from each other by less than 5 bp, less than 1%, and the difference between the individuals in the secondary haplotype branch exhibit a similar minute level of difference. As with the 2004 samples, there is a deep difference between the two *M. edulis* major haplotype branches. The difference between the primary and secondary *M. edulis* haplotype branches is nearly 5.5%; this difference is higher than that found between either haplotype and the outgroup species *M. trossulus* (around 4% for the

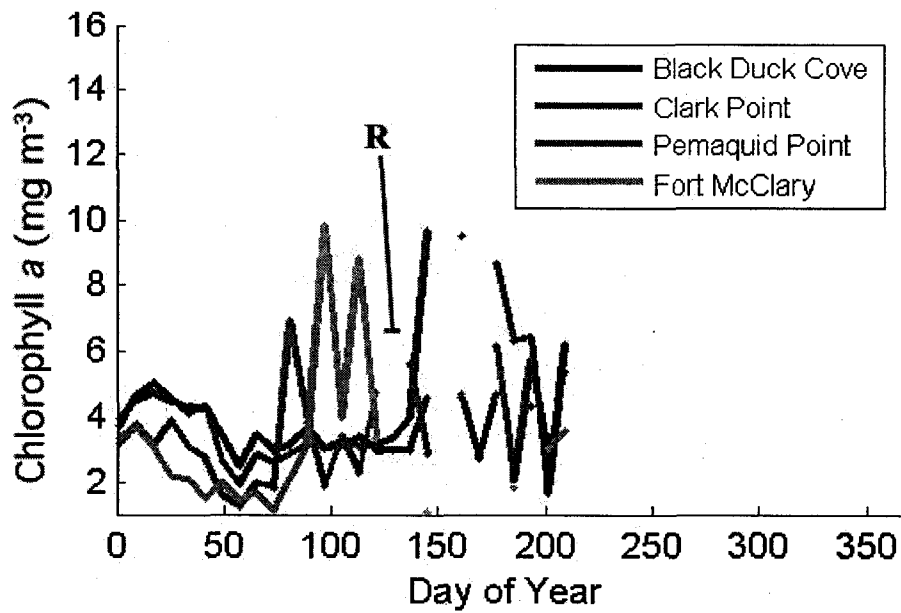


Figure 15. SeaWiFS data for chlorophyll *a* for 2006. R indicates the 4-day sampling range in 2006 (12, 14, and 16 May).

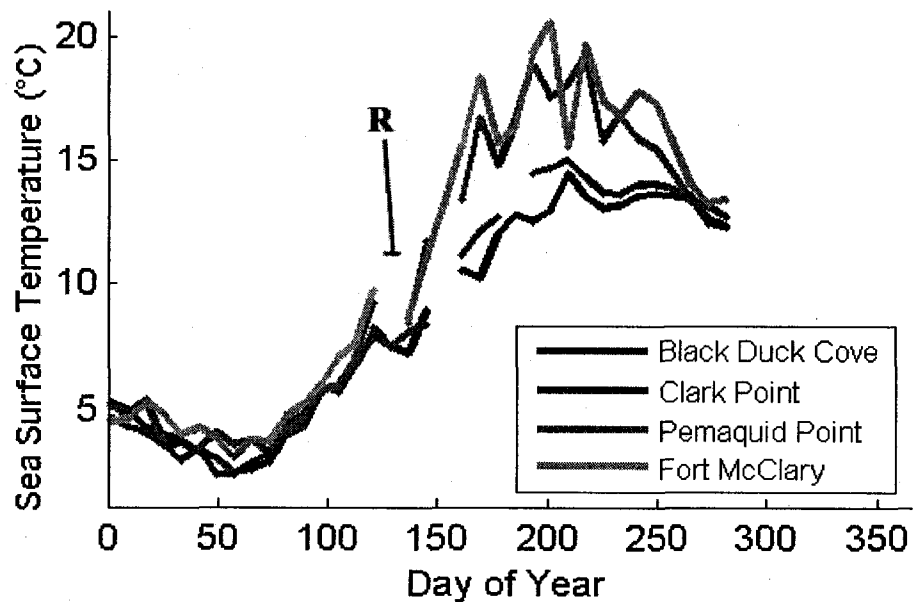


Figure 16. SeaWiFS data for sea surface temperature during 2006. R designation is as for Fig. 15.



Figure 17. Maximum parsimony tree for *Mytilus edulis* collected in 2006. Numbers appearing on branches indicate bootstrap values. Individuals within a group have identical haplotypes; individuals within each group are listed right of tree. Grouped haplotype labels are independent of labels used in analysis of 2004 data. Known males and females are marked as M and F, respectively. Asterisks designate individuals that show evidence of possible doubly uniparental inheritance.

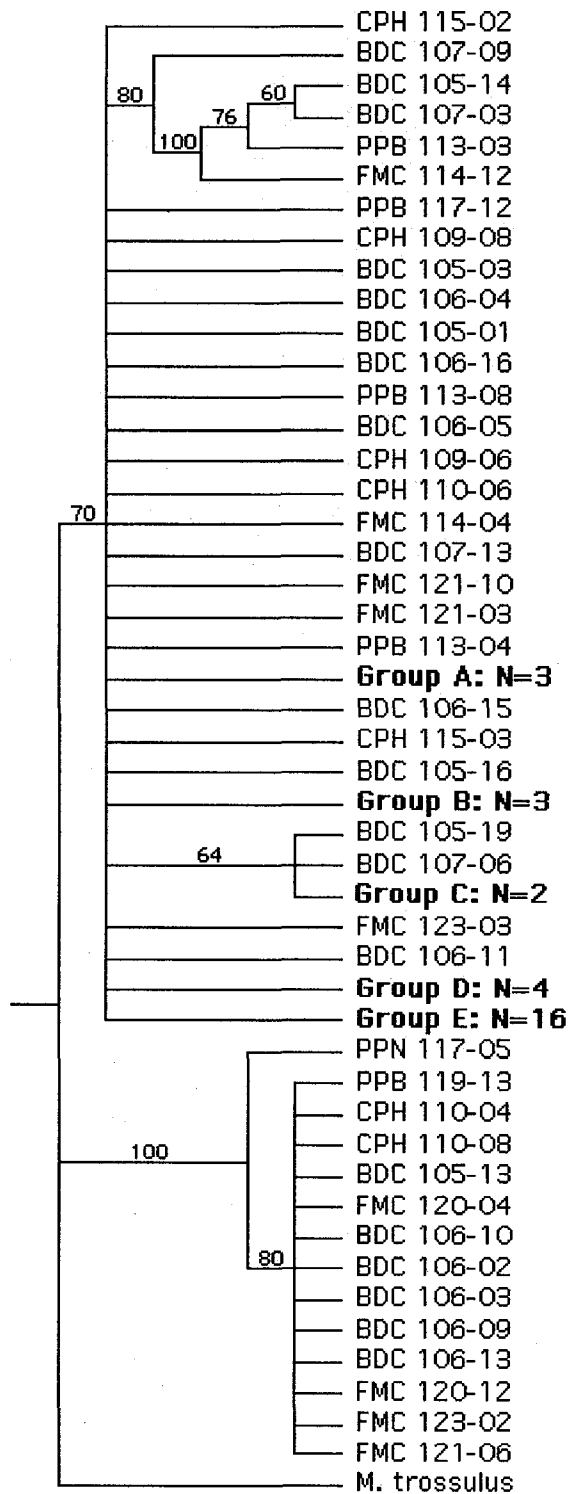


Figure 18. Maximum likelihood tree for *Mytilus edulis* collected in 2006. Numbers on branches are bootstrap values; groups are as listed for Fig. 17.

primary haplotype and 5% for the secondary haplotype), indicating that these two haplotypes are as divergent as sequences observed in fully recognized species. As with the 2004 samples, major groupings were conserved in the maximum likelihood analysis as indicated by bootstrap values (Fig 18). Although fine-scale resolution of relationships within each branch is still obscure, the deep division between the two groups as well as between each group and the outgroup, is maintained.

Four individuals, indicated by asterisks in Fig. 17, exhibited sequences that seemed to be intermediates between examples of the two major haplotype groupings. The two samples from BDC were not successfully sexed; PPB 117-12 was sexed as male and PPB 117-05 was sexed as female. Chromatograms for these four individuals frequently showed two co-occurring peaks at base-variable sites, with one base agreeing with the primary haplotype group sequence and the other possibility in consensus with the secondary haplotype group. This is possible evidence for doubly uniparental inheritance, a phenomenon of mitochondrial genome inheritance that occurs in male *Mytilus*; PPB 117-05 may have been miss-sexed.

Interannual Variation in the GOM

Patterns in chlorophyll *a* levels and sea surface temperature from SeaWiFS data (Fig. 19) show that there is a pattern of slightly lower levels of chlorophyll *a* at BDC in comparison to the other three sites, with winter 2006 being an aberrant season in the context of the long-term climatology. 2004 shows elevated chlorophyll *a* levels for FMC during the spring in comparison to previous years. It is a consistent pattern, however,

that BDC and CPH average around 5.0°C cooler during summer months than PPB and FMC, and that both sites have comparable temperatures during the winter.

There were 98 individuals sequenced for sampling done in 2004 and 2006 resulting in 478 conserved sites and 181 variable sites for sequences 659 bp in length. Maximum parsimony tree-building (Fig. 20) produced a population tree with two major haplotype groups, as was seen in both 2004 and 2006 analyses. Individuals representative of the primary haplotype from each year of study were grouped together, and the one representative of the secondary haplotype group from 2004 was placed within the secondary haplotype group of 2006, confirming that this secondary haplotype group appears to be less abundant in the studied populations throughout the period of the study. There is as much as 16% difference between the two haplotype groups based on third codon position; the difference between the primary haplotype group and the outgroup *M. trossulus* is around 13% and the difference between the secondary haplotype and the outgroup is approximately 18%. The analysis gave no evidence for spatial or temporal lineage sorting.

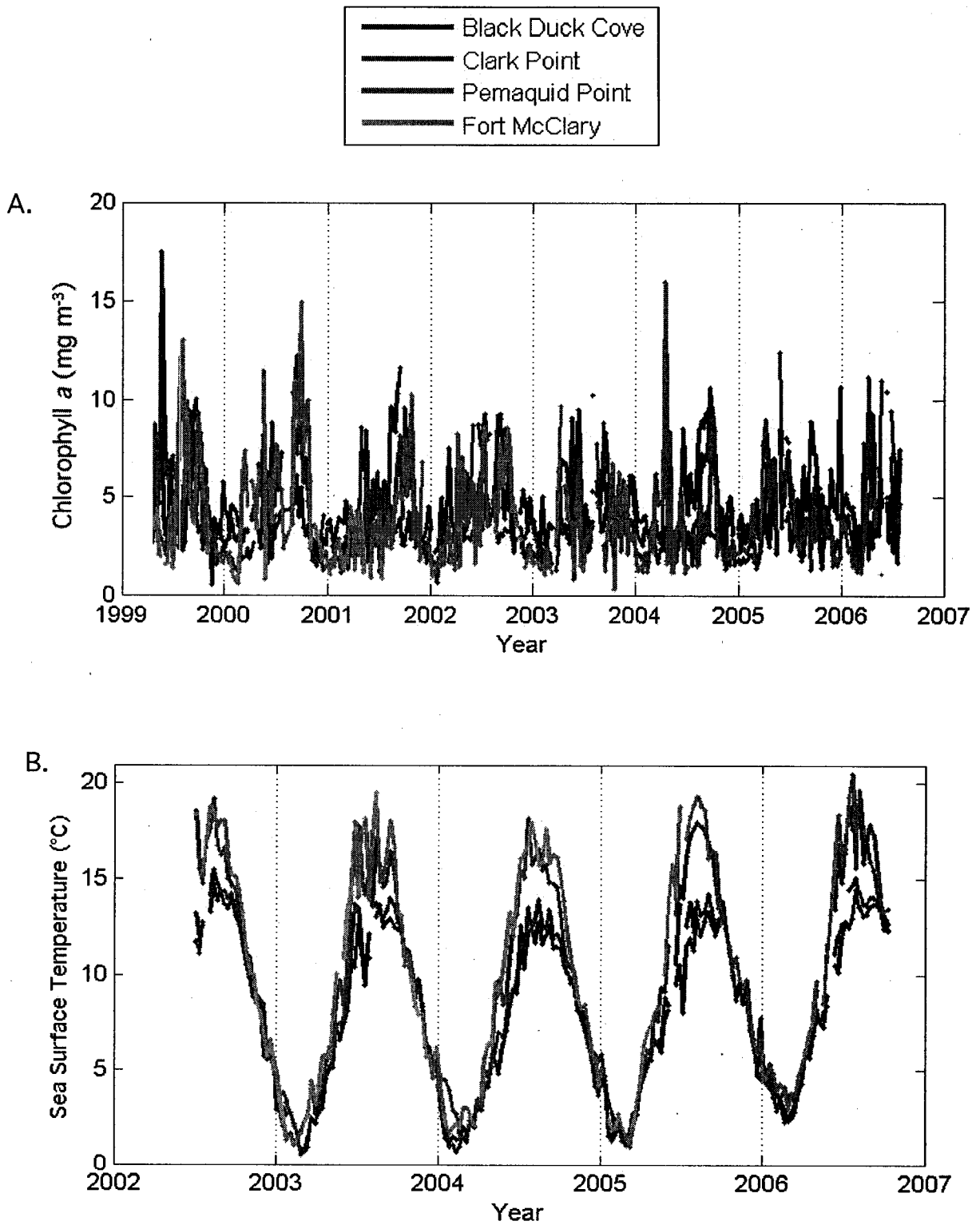


Figure 19. SeaWiFS long-term climatology data for A. chlorophyll *a* and B. sea surface temperature.

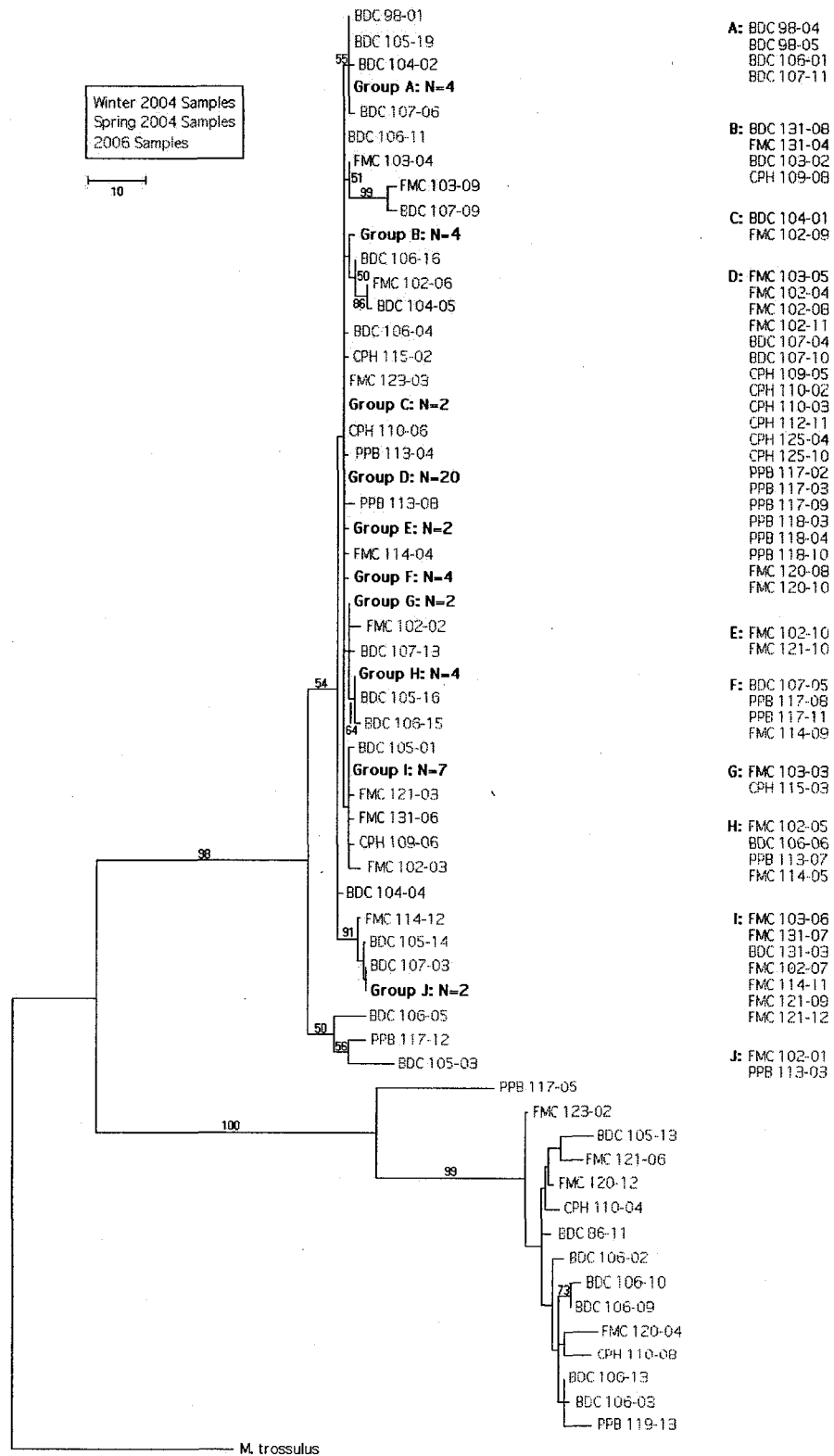


Figure 20: Maximum parsimony analysis of *M. edulis* sequence data for 2004 and 2006. Bootstrap values higher than 50% are reported on associated branches.

CHAPTER IV

DISCUSSION

Multiple analytical techniques were employed to study the environmental conditions that affect the physiological performance and therefore the growth of blue mussel populations in the GOM. Those measurements associated with the variability in the physical environment of mussels will be discussed for each year of sampling during the study, and then discussed in its entirety in the context of multiple year and long-term consideration of GOM near-shore ecology and benthic-pelagic coupling.

2004 Sampling Data

In the GOM, the surface circulation patterns responsible for the distribution of invertebrate and fish larvae also influence chlorophyll *a* concentrations and sea surface temperatures that in part drive the physiological differences observed between *Mytilus edulis* populations. During the winter, when the water column of the GOM is well-mixed, temperatures, both in magnitude and variability, at both collection sites in 2004 and for the two months prior to sampling are similar, between 0.0°C and 2.5°C. Chlorophyll *a* concentration, while at the same concentration at both sites (approximately 3.0 mg m⁻³) at the time of winter sampling (2 and 4 March), is significantly higher throughout the winter at BDC (4.0 mg m⁻³) and declines throughout the spring to a low of

2.0 mg m⁻³ at the end of May, while at FMC, chlorophyll *a* levels are beginning to rise with the advent of the spring phytoplankton bloom which stretches from approximately from 15 March to 04 May and achieves maximum chlorophyll *a* concentration of 16 mg m⁻³ in mid April. By the time of the spring sampling date (5 May), the sea surface temperature in the western GOM is significantly higher than that at BDC; the phytoplankton bloom and elevation of chlorophyll *a* in the western GOM is the product of the increasing stratification of the water column as flow progresses to the southwest (Townsend et al. 1987). This may or may not be dependent on water temperature in the GOM (Townsend and Cammen 1988) as production of a bloom in the western GOM depends largely on the stability of the water column, solar radiation, and availability of nutrients (Sverdrup 1953, Townsend and Spinrad 1986). The amount of food for *M. edulis* at specific locations, then, is not a direct function of temperature; temperature does, however, play a role in filtration rate of *M. edulis* both during sudden temperature changes and in terms of long-term acclimation (Kittner and Riisgård 2005), and in metabolic rate, which tends to increase with temperature (Dahlhoff et al. 2001, Whiteley and Faulkner 2005). If the change in temperature is too extreme, producing a stress response, more energy may be devoted to expression of heat shock proteins and responding to other temperature-induced stresses (Dahlhoff et al. 2002, Halpin et al. 2002) than to growth or reproduction. Energy budgets for mussels in the western GOM, which is consistently warmer at the surface than the eastern, may require dedicating more resources to standard rates of metabolism and heat-induced stress responses, and therefore less energy would be available for growth (e.g., scope for growth, Widdows and Johnson 1988).

In addition to the physical environment, the genetics of mussel populations are also known to play a role in their physiological performance and survival under stressful conditions (KoeHN and Hilbish 1987, McDonald and Siebenaller 1988). The genetic structure of all mussel populations examined in this study was determined in order to assess the possibility of a genetic component in physiological performance. The cytochrome *c* oxidase subunit I (COI) gene, located on the mitochondrial genome, is frequently used in molecular genetic analyses of invertebrate populations, and has been used previously as a diagnostic molecular marker to distinguish other co-occurring bivalve populations (Caterino and Sperling 1998, Gaunt and Miles 2002, Baldwin et al. 1996). Using mtDNA for population genetics studies offers several advantages, such as a comparatively rapid mutation rate, and a single, maternally-inherited genome, which provides only one locus for any gene and greatly reduces the opportunity or likelihood of recombination in most species. Several mtDNA genes, including COI, have been reliably calibrated to molecular clock models for determining evolutionary branching times for a number of invertebrate species (Stillman and Reeb 2001, Wares and Cunningham 2001, Gaunt and Miles 2002).

Work by Wares and Cunningham (2001) using COI has demonstrated the presence of several unique lineages of *M. edulis* on New England and southeastern Canadian coasts, with only one shared allele, the root haplotype, and with lineages that are distributed between Iceland, Norway, Ireland, and France. Both the North American and the European populations show evidence of having long histories in both locations,

indicating that the presence of *M. edulis* in the eastern Atlantic is unlikely to be the result of a recent, post-glacial founding population (Wares and Cunningham 2001). The populations on both coasts show wide genetic diversity and low instances of alleles derived from a founding population, both contrary to indications of recent founding events (Wares and Cunningham 2001), suggesting that *M. edulis* may not have been affected by the genetic bottleneck that characterizes some other North Atlantic invertebrate species (Bucklin and Wiebe 1998).

Complicating the issue of *M. edulis* genetics is the ability of *M. edulis* to inherit mtDNA paternally as well as maternally in a process known as doubly uniparental inheritance (DUI), providing a second locus for any given mitochondrial gene, such as COI (Zouros et al. 1992, Zouros et al. 1994, Curole and Kocher 2002). While both male and female *Mytilus* offspring inherit mtDNA maternally, referred to as the “F” type, male *Mytilus* individuals inherit a second mitochondrial genome, the “M” type, paternally, with as much as 20% divergence between the two genomes (Stewart et al 1996, Kijewski et al. 2006). The hybridization found by Kijewski et al. (2006) indicated that in the Baltic Sea, a female *M. edulis* mitochondrial genome had usurped the paternal genome in male *M. trossulus*, introducing an additional level of complexity in the hybridization and DUI patterns of the *Mytilus* species complex. Both of the F- and M-specific lineages were present in the common ancestor to *M. edulis*, *M. trossulus*, and *M. galloprovincialis* prior to species divergence, and estimation for the divergence time between the F- and M-type lineages is between 5.3 and 5.7 MYA (Rawson and Hilbish 1995). *M. edulis* is also known to have an elevated mutation rate (Hoeh et al. 1996, Wares and Cunningham

2001); this rate is not constant even between the F- and M-type lineages but has been estimated as 0.54% Myr⁻¹ for the female lineage and as 0.96% Myr⁻¹ for the male lineage (Rawson and Hilbish 1995). The difference in evolution rate may be the result of a relaxation of selective constraint on the M lineage in comparison to the F (Rawson and Hilbish 1995, Stewart et al. 1996).

Based on the analysis of haplotypes from the GOM mussel populations in this study for 2004, there is no lineage sorting within the GOM; populations of *M. edulis* are homogenous with respect to location throughout the area encompassed by the study. With 30 individual sequences distributed across 20 different *M. edulis* haplotypes, even the small sampling sizes studied in 2004 show a wide range of haplotype diversity at each study site. The small number of samples studied during 2004 may account for the single representative of the second major haplotype group. Of the 8 total individuals sequenced from BDC in winter, 25% were found to be *M. trossulus*, and 29% of 7 individuals sequenced at BDC in spring; *M. trossulus* were not found at FMC. *M. edulis* has a long-dispersing planktonic larval form which may account not only for the absence of lineage sorting between the populations in the GOM, but may have played a role in generating the wide genetic diversity among broadly distributed populations (Wares and Cunningham 2001). The haplotype diversity exhibited at each site suggest that the surface currents of the GOM promotes larval dispersal and there are no barriers to larval dispersal in the GOM, maintaining the high haplotype diversity across several homogenous populations. Local retention of *M. edulis* larvae, as in other intertidal organisms with planktonic larval stages, requires recruitment and settlement mechanisms

to prevent the dispersion of larvae downstream and the population becoming locally extinct; this mechanism, when it occurs, may involve exploitation of fluctuating minor currents, even to the extent as to allow upstream dispersal (Byers and Pringle 2006). Larval retention is lower in the eastern GOM, and the strong coastal current along the coast of eastern Maine may be responsible for distributing larvae further downstream into the western GOM (Incze and Naimie 2000) while other circulation patterns, perhaps from Nova Scotia, brought larvae to the eastern Gulf, which would establish allele flow throughout the GOM and create the genetic widespread diversity observed. A product of this homogeneity with respect to allele distribution is the consideration that differences in physiological performance observed between populations are the result of their phenotypically plastic response to the environment and ecological interactions rather than genetic differences between mussel populations.

Analysis of size frequency distributions, shell length, and condition indices provides a picture of the physiological performance of mussels that can then be related to differences in quality and quantity of food and shifts in energy allocation in mussels (Crosby and Gale 1990). The shift to observing smaller individuals from winter to spring indicates the occurrence of some recruitment event between these seasons, and the lack of larger individuals in the spring, especially at FMC, suggests the occurrence of removal of larger organisms. This may be via a biotic factor such as predation, disease, or disturbance. Like some other bivalves and species of *Mytilus*, *M. edulis* is susceptible to haemic neoplasia, or leukemia, the onset of which has been linked to abnormal temperatures, seasonality (Elston et al. 1992), viral transmission (McGladdery et al.

2001), and genetics (Muttray et al. 2005), any of which may factor into mortality in wild populations of mussels. The consistent presence of larger *M. edulis* samples collected from BDC during both seasons, as assessed using size frequency distributions and mean shell length, than those collected from FMC suggests that mussels at BDC are devoting more energy to shell growth. This is not supported by the CI data, which show a higher CI in the winter, indicating greater energy diverted to tissue growth in the winter. This pattern is reversed in the spring where FMC mussels have a higher CI than BDC mussels. The bimodal distribution of size classes seen at BDC and FMC during the winter collection may be an indication that individuals within each mode are of similar ages by year, however, in light of the absence of distinct bimodality in the spring collection, either of these patterns may be the result of other processes such as post-settlement survival, predation, or physical disturbance (e.g. dislodgment from wave action, Menge and Sutherland 1987). At least three significant severe weather events occurred on the coast of the GOM in February of 2004, prior to the winter sampling date, whereas only one occurred in the beginning of April, a month prior to the spring collection (National Weather Service), which would suggest that intertidal organisms were subjected to more storms, and thus more wave action, in winter than in spring. Interspecific competition may also be a factor in the size frequency distributions seen at each site, as a negative correlation exists between body size and density in many intertidal organisms, including mussels (Gaines and Roughgarden 1985, Petraitis 1995). Although no estimate of percent cover was made during this study, this principle of self-thinning may play a role in the distribution of size groups of *M. edulis*: many small mussels can dominate an intertidal landscape as effectively as fewer, larger mussels, and vice versa. One effect of

this is the percentage of free space available in the habitat, which then, when coupled with recruitment, determines larval settlement rate and thus species abundance (Connell 1985, Gaines and Roughgarden 1985). This may account for the shift to smaller-sized individuals at both locations between winter and spring, particularly in consideration of prior studies done by Mallet and Carver (1993), which indicate that an increase in water temperature had an immediate positive effect on shell growth in populations of Nova Scotian mussels. With larger mussels removed between winter and spring, smaller mussels would then be able to take advantage of the free space at the same time when warming ambient temperatures are increasing their shell growth rate.

Whereas gut content analyses provide discrete snapshots of short-term feeding patterns, stable isotope analyses can be used to trace macronutrient movements through individuals and populations, and then to estimate long-term food quality, reconstruct diets, or examine trophic interactions (Peterson and Fry 1987, Gannes et al. 1997, Dunton 2001, West et al. 2006). Isotopic signatures in organisms above the primary trophic level are intrinsically linked to isotopic signatures found in their diet. Both carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) were measured in this study; the first provides information on the sources of organic carbon obtained by herbivores and grazers, and the second provides information on sources of nitrogen and trophic interactions of consumers (West et al. 2006). Although isotopic signatures are an indicator of food quality and not food quantity, some studies do suggest a correlation between nitrogen availability and both size and mass, and that $\delta^{15}\text{N}$ signatures increase with nutritional stress as existing nitrogen is recycled within the tissues (Adams and Sterner 2000). With a feeding mode

that relies on ambient seston in the water column, *M. edulis* occupies a trophic position that potentially exposes it to a wide assortment of particulate carbon and nitrogen sources.

Typically, phytoplankton and particulate organic material has a carbon isotopic signature of -20 to -24‰ and a nitrogen isotopic signature of $+4$ to $+6\text{‰}$ (Kieckbusch et al. 2004, Dunton 2001). Periphyton and macrophytes have a typical carbon isotopic signature between -12 and -17‰ and a nitrogen signature between $+3$ and $+6\text{‰}$ (Kieckbusch et al. 2004, Hill et al. 2006). Detritus generally has a carbon isotopic signature range between -11‰ and -15‰ and a nitrogen isotope signature of $+3\text{‰}$ to $+5\text{‰}$ (Peterson and Fry 1987, Kieckbusch et al. 2004). Mussels collected from FMC had more depleted $\delta^{13}\text{C}$ signatures than mussels from BDC. In 2004, both populations had depleted $\delta^{13}\text{C}$ relative to pure phytoplankton diets, but FMC populations appear to be consuming a diet with a higher percentage of phytoplankton than BDC samples, whose diet consists of a larger fraction of macrophytes or detritus.

For mussels collected from FMC, $\delta^{15}\text{N}$ was consistently less enriched than for mussels from BDC. Both populations of mussels had enriched $\delta^{15}\text{N}$ tissue values relative to pure phytoplankton diets that reflect the trophic enrichment of nitrogen ($\sim 3\text{‰}$ - 4‰ per trophic level, Kieckbusch et al. 2004) from a mixture of phytoplankton and detrital material, but BDC populations appear to be consuming a diet with a higher percentage of phytoplankton than FMC samples, whose diet is more reflective of a larger fraction of detritus. This is contrary to the indications given by the $\delta^{13}\text{C}$ signatures, and mussels at

each site are most likely consuming a mixed diet, with possible enrichment of detritus by bacteria. The marine bacteria *Vibrio harveyi* has shown $\delta^{15}\text{N}$ enrichment as much as +22‰ and depletion of up to -12‰ relative to substrate when cultured under certain conditions (Adams and Sterner 2000, Macko and Estep 1984). The absence of significant differences for the $\delta^{15}\text{N}$ isotopic signatures between seasons indicates that regardless of the source of organic material within the water column at each site, and in spite of minor seasonal variations such as the $\delta^{15}\text{N}$ signature for BDC in the spring, the quality of diet over time for mussels at both sites was similar.

Biochemical indicators, such as RNA/DNA ratios, can be used to assess cellular activities and protein synthesis, providing indicators as to metabolic condition or level of physiological stress (Dahlhoff 2004). An RNA/DNA ratio is a biochemical condition index, calculated from total DNA amount, which is a function of cell number and rate of division, and the varying degree of transcription of RNA for expressed genes per individual, which is largely a function of protein synthesis (Dahlhoff 2004). Acute environmental changes (e.g. temperature) can result in a compensatory adjustment to protein and enzyme production in affected organisms, and organisms acclimated to different locations along an environmental gradient may display a range of RNA/DNA ratios that are correlated with their physical, food, and climatological conditions. Biochemical studies on a range of marine vertebrates and invertebrates have revealed that RNA/DNA ratios decline with decreasing food availability, indicating a drop in protein synthesis capacity in food-limited individuals; for sessile intertidal invertebrates, this food availability can also be correlated to primary productivity as measured by satellite

remote sensing imagery (Dahlhoff 2004). However, when using RNA/DNA ratios, it is necessary to consider all environmental variables, such as stress caused by temperature, salinity, and desiccation that can influence the metabolic status of an organism.

The significant differences observed for RNA/DNA ratios between BDC and FMC mussel populations during the winter indicates a large difference in protein synthesis that disappeared by spring. This may be a function of both sea surface temperature and chlorophyll *a*; during the winter months, sea surface temperature was comparable between sites, however BDC mussels were exposed to twice as much chlorophyll *a*, which may account for the ability of *M. edulis* at BDC to synthesize more protein than those individuals at FMC during the same time period. By the spring, sea surface temperature at FMC was elevated as much as 3° C above the sea surface temperature observed at BDC, and a large phytoplankton bloom had created a chlorophyll *a* spike as high as 16.0 mg m⁻³ at FMC, which did not occur at BDC (3.0 – 4.0 mg m⁻³). This provided individuals at FMC with greater food, which was then reflected in enhanced protein synthesis post-bloom, while at BDC, the lack of similar concentrations of chlorophyll *a* and the persistence of the colder sea surface temperatures resulted in a decrease of protein synthesis. The increase in RNA/DNA at FMC in the presence of a bloom and the decrease at BDC in the absence of a bloom between winter and spring suggests that mussel metabolism responds seasonally to changes in coastal environmental conditions, which agrees with other reports on the plasticity of mussel physiology (Dahlhoff and Menge 1996). This RNA/DNA increase at FMC may be reflected in the higher CI found at that site in the spring, and while there is an increase in CI for mussels

at BDC in the spring, the lack of a bloom may account for that increase being slight.

2006 Sampling Data

The environmental patterns of chlorophyll *a* and sea surface temperature observed in 2006 exhibit similar patterns to those observed in 2004. Temperature profiles for all four sites are similar for the first 100+ days of the year, and it is only just before mussel sampling, which occurred between 12 and 16 May, that the surface temperatures in the western GOM (approximately 10.0°C) begin to rise above those observed in the eastern GOM (approximately 8.0°C). While the eastern GOM is enriched in chlorophyll *a* in comparison to the western GOM throughout the first 2 months of the year, chlorophyll *a* concentration in the western GOM increases sharply at mid-March. Since the 2006 bloom begins, and by 5 May is nearly finished, before there is a significant difference in sea surface temperature between the eastern and western GOM, this demonstrates that temperature itself was not a direct factor for initiating a bloom as in 2004, and that other processes are affecting the timing of the spring phytoplankton bloom which provides *M. edulis* in the western GOM with a significant portion of its diet (Sverdrup 1953, Townsend and Spinrad 1986).

The lack of evidence for lineage sorting in the collected samples for 2006 again indicates the homogeneity of distribution of multiple haplotypes throughout the GOM; there were 70 individuals distributed across 47 different haplotypes, which grouped into two major branches. While relationships within these two major groupings remain unclear, the major divisions are distinct and well-supported by bootstrap analysis with over 98% of

constructed trees producing these same two major groups. With the larger sampling size, the secondary haplotype group contained many more individuals than seen in the 2004 study, and these individuals comprised 20% of the total *M. edulis* sequenced in 2006. Based on the individuals that were successfully sexed, there appears to be no correlation between the two major lineage groups and gender, however this is an incomplete data set and some individuals may have been incorrectly sexed. Sequence chromatogram data from four individuals showed evidence for possible DUI; their positions in the phylogenetic trees may be at least partially the result of base pair confusion. There were 29 total individuals sequenced from BDC in 2006, and 10% of these were classified as *M. trossulus*. Again, as with the 2004 sampling, no *M. trossulus* were found at other sampling locations.

The 2006 samples, like the spring 2004 samples, lacked the bimodality observed in the winter 2004 samples. The generally smaller size of individuals collected from BDC may be a function of the observed lack of a phytoplankton bloom in the eastern GOM in the months preceding the collection period, or may indicate the presence of a young cohort. Although winter mixing maintained a largely constant coastal sea surface temperature at all four collection sites for the approximately 100 days prior to the 2006 sampling, the lack of a spring phytoplankton bloom in nearshore waters in the eastern GOM indicates that the mixing responsible for bringing nutrients from the benthos to the surface is preventing the stratification necessary to initiate a bloom and is acting as a limiting factor on *M. edulis* growth in this region. The National Weather Service recorded six storm events that potentially impacted the coast of the GOM during the five months prior to the

collection dates of this study, however no storm events were recorded after mid-February, indicating a general lack of high wave disturbance at the time or, or immediately prior to, the period of 2006 collection.

The difference in mean length observed between individuals collected at BDC and at CPH may show the influence of the Penobscot River outflow, the freshwater runoff of which plays an important role in the development of coastal circulation and stratification of the water column, and hence phytoplankton blooms, each spring (Townsend 1991). In addition, chlorophyll *a* data for CPH indicated the presence of a summertime bloom, beginning at day 25 May, ten days after the time of sampling. This bloom may be the product of water stratification caused by the freshwater of the Penobscot River and, if present each year, may account for the increased size of mussels at CPH in comparison to BDC (Sverdrup 1953, Townsend and Spinrad 1986, Townsend 1991). This midsummer bloom may also be connected to the higher CI observed in the CPH mussels, which are devoting more energy to tissue growth and exhibiting greater protein synthesis than those at BDC. Mussels from CPH also show a carbon isotopic signature that indicates a slightly better, although comparable, diet than those at BDC, which, in contrast to the 2004 samples, have a signature which indicates a diet with a higher percentage of detritus. Both the increased depletion of $\delta^{13}\text{C}$ signature at CPH and the enrichment of $\delta^{15}\text{N}$ in comparison to BDC supports the idea of a diet richer in phytoplankton for the CPH mussels, which correlates with the larger size, higher condition index, and higher level of protein synthesis as measured by RNA/DNA ratios. It is possible as well that the difference in size and condition index observed between the BDC and CPH *M. edulis*

may be partly a function of the amount of food consumed, which currently cannot be estimated on the basis of isotopic signatures.

The larger elevation in chlorophyll *a* observed at FMC, in comparison to the other western GOM sampling location, PPB, coupled with the higher temperatures observed at this location, correlates with the larger individuals observed at FMC; the abundance of nutrients at FMC may account for some of the difference in mussel size between the populations. In addition, although no assessment of exposure at each location was estimated, PPB is an exposed site subject to heavy wave action, whereas FMC is more protected and is adjacent to an industrialized freshwater river; this increased wave action at PPB may play a role in the reduced size of the mussels found at that location. In contrast, however, while the PPB mussels were smaller than those at FMC, those found at PPB devoted significantly more energy to soft tissue growth than shell growth; this, coupled with the more depleted $\delta^{13}\text{C}$ at PPB in comparison to FMC indicates that although more exposed, individuals at PPB may have a better diet, richer in phytoplankton, than those at FMC. Although $\delta^{15}\text{N}$ for PPB was slightly lower than the nitrogen signature for FMC, both are sufficiently high to suggest a phytoplankton-rich diet, particularly in comparison to the nitrogen signature seen at the easternmost site, BDC. FMC mussels were less depleted in $\delta^{13}\text{C}$ and, although slightly more enriched in $\delta^{15}\text{N}$ than those at PPB, still suggests that the diet of *M. edulis* at FMC contains a higher percentage of detritus. Mussels from FMC may have the benefit of a more protected location, but are subject to a suite of influences that are the result of its proximity to an

estuarine system, which may result in lower food quality. Variations in $\delta^{15}\text{N}$ signatures could be due to bacterial content as well (Adams and Sterner 2000).

CPH and FMC both exhibited higher RNA/DNA ratios, indicating a greater amount of protein synthesis, which may then have lead to their larger shell sizes, an increase in metabolic rate in response to rising temperatures and increased food supply, or energy budget adjustments necessary to compensate for the increase in ambient temperature (e.g. expression of heat shock proteins). Expression of HSP-70 proteins is positively correlated with seasonal temperature change and thermal tolerance in *M. edulis*, and has been observed in natural populations as early as March and increases throughout the spring into the summer (Chapple et al. 1998). The lack of a bloom at BDC prior to sampling, resulting in low food abundance for *M. edulis* at that location, accounts for the lower RNA/DNA ratio observed there. Mussels at PPB had a lower RNA/DNA ratio as well, in spite of the presence and consumption of chlorophyll *a*, as observed both by satellite imagery and $\delta^{13}\text{C}$ signature, and the warmer temperatures of the western GOM; when placed within the context of the high CI and low length observed in the PPB mussels, the most likely answer for this apparent contradiction in condition and metabolic health may lie in efficiency. Post-bloom, at the time of sampling, mussels at PPB showed evidence of having a diet enriched in phytoplankton compared to the other study sites, and this increase in quality as well as supply may allow mussels at PPB more efficient feeding, metabolism, and growth. As slow-growers, in terms of size, mussels at PPB may require more energy allocation to maintenance, but reducing the costs of protein turnover (lower RNA/DNA ratios) helps to reduce maintenance cost, increasing potential

for growth as reflected by the high CI (Bayne 2004). Faster growth is achieved by decreasing energy requirements overall and increasing ingestion and absorption efficiency, and tends to be observed in more heterozygous individuals (Hawkins et al. 1986), which, while not directly measured, may be reflected in the high genetic diversity observed. However, mtDNA, being maternally inherited, is not a reflection of hetero- or homozygosity. Additionally, when considering the DUI phenomenon, the genetic disposition towards faster growth in heterozygotes may provide an advantage in males only.

Recent Trends and Projections for the Future

While there is seasonal variation in both the timing and the intensity of the spring phytoplankton bloom, there are trends which demonstrate consistency over the five years prior to this study. BDC regularly shows little evidence for a spring bloom, or in years where one is seen to occur (2002) it is less intense than at other locations in the GOM. In the western GOM, FMC shows the occurrence of a fairly large bloom consistently, often earlier than at more eastern locations. The temperature profiles observed for 2004 and 2006 show no deviance from patterns established by other recent years; sea surface temperatures and chlorophyll *a* observations for the years included in the study may be considered normal in the context of recent environmental trends.

The widespread genetic diversity found in GOM *M. edulis* populations throughout this study supports, precluding any genetic bottleneck as the result of a large-scale disaster, the long-term variation and presence of mussels on North Atlantic rocky coastlines.

Health and condition of *M. edulis* populations, as products of phytoplankton blooms, is intrinsically linked to circulation patterns, temperature cycles, and stratification of the water column, and it is these factors driving the physical evidence of phenotypic plasticity in mussels. The west-running Maine Coastal Current plays a critical role in delivering nutrients, phytoplankton, and larvae to the western GOM where the spring bloom occurs in greatest magnitude.

Bottom-up effects are having a significant impact on the size and condition of mussel populations in the GOM, similar to recent Pacific Coast findings by Menge et al. (1997). Continual monitoring of the coastal GOM is necessary to determine if the patterns in size and health of *M. edulis* continue as observed or change. Not all aspects of mussel physiology were taken into account in this study; the role of reproductive costs in the energy budget for *M. edulis* was not considered, and differences in fecundity may factor heavily into physiology assessments made during the spring, when mussels are reproductively active. *Mytilus* loses a significant amount of tissue weight following spawning, which occurs in May and June, immediately after the time of spring sampling during both years of this study (Mallet and Carver 1993). In addition, this study made no assessment of the many biotic factors influencing intertidal habitats, such as assessing percent cover, intraspecific and interspecific competition, and predation. Although storm events during the seasons of sampling were noted, direct assessments of wave exposure and disturbance, two abiotic factors that also play a role in structuring communities and populations, were not encompassed in this study, nor were the effects of sampling bias or human activity; the proximity of FMC to an industrialized and populated estuary may

have an influence on mussel physiology for those populations. Although no estimation of these top-down controls and secondary environmental effects was made in this study, between-site and temporal differences show significant influence of bottom-up effects on *M. edulis* populations. General health and metabolic indicators correlate to both chlorophyll *a* concentration and sea surface temperature, both of which are partially functions of the broad-scale circulation events and seasonal patterns of mixing and stratification, comparable to hypotheses of west coast upwelling patterns being a primary influence on phytoplankton and physiological status of mussels (Menge et al. 1997). Future investigations of predation and competition may reveal that, as observed by Menge et al. (1997), top-down processes vary positively with bottom-up processes; a study undertaking this aspect of *M. edulis* populations would be a logical next step in illustrating a full picture of mussel ecology in the GOM.

Although there is distinct interannual variability, physiological performance of populations of *M. edulis* in the GOM does correlate to environmental conditions, circulation patterns, and seasonal climatology, as found by Menge (1997) on the west coast. The broad genetic diversity found in mussel populations throughout the GOM does not support a genetic basis for differences in mussel performance, indicating that phenotypic plasticity linked with environmental conditions, controls the size and health of *M. edulis* individuals and, as a result, populations. Environment does not, however, explain all characteristics of *M. edulis* populations; mussels at CPH exhibit larger size and higher physiological performance than expected from environmental conditions as reported by satellite imagery, underscoring the need to make in situ estimates of local

chlorophyll *a* conditions at the time of sampling at each location to provide better resolution. Focusing on local topography and eddies may provide clues to understanding differences seen between sampling sites. Future studies should incorporate physiology, ecology, and oceanography together, and should be continued for multiple years in order to truly ascertain trends and to investigate their causes.

REFERENCES

- Adams TS, Sterner RW (2000) The effect of dietary nitrogen content on trophic level ^{15}N enrichment. *Limnol Oceanogr* 45(3): 601-607
- Baldwin BS, Black M, Sanjur O, Gustafson R, Lutz RA, Vrijenhoek RC (1996) A diagnostic molecular marker for zebra mussels (*Dreissena polymorpha*) and potentially co-occurring bivalves: mitochondrial *COI*. *Mol Mar Biol Biotech* 5(1): 9-14
- Bayne BL (2004) Phenotypic flexibility and physiological tradeoffs in the feeding and growth of marine bivalve mollusks. *Integr Comp Biol* 44: 425-432
- Bayne BL, Widdows J, Newell RIE (1977) Physiological measurements on estuarine bivalve molluscs in the field. In: Keegan BF, Ceidigh PO, Boaden PJS (eds.) (1977) *Biology of benthic organisms/11th European symposium on marine biology*. Pergamon: pp 57-68
- Brown WS, Beardsley RC (1978) Winter circulation in the western Gulf of Maine: Part 1. Cooling and water mass formation. *J Phys Oceanogr* 8: 265-277
- Bucklin A, Wiebe PH (1998) Low mitochondrial diversity and small effective population sizes of the copepods *Calanus finmarchicus* and *Nannocalanus minor*: Possible impact of climatic variation during recent glaciation. *J Hered* 89(5): 383-392
- Butman B, Beardsley RC (1987) Long-term observations on the Southern Flank of Georges Bank. Part I: A description of the seasonal cycle of currents, temperature, stratification, and wind stress. *J Phys Ocean* 17: 367-384
- Butman B, Beardsley RC, Magnell B, Frye D, Vermersch JA, Schlitz R, Limeburner R, Wright WR, Noble MA (1982) Recent observations of the mean circulation on Georges Bank. *J Phys Oceanogr* 12: 569-591

- Byers JE, Pringle JM (2006) Going against the flow: Retention, range limits, and invasions in advective environments. *Mar Ecol Prog Ser* 313: 27-41
- Castelloe J, Templeton AR (1994) Root probabilities for interspecific gene trees under neutral coalescent theory. *Mol Phyl Evol* 3(2): 102-113
- Caterino MS, Sperling FAH (1998) *Papilio* phylogeny based on mitochondrial cytochrome oxidase I and II genes. *Mol Phyl Evol* 11(1): 122-137
- Chapple JP, Smerdon GR, Berry RJ, Hawkins AJS (1998) Seasonal changes in stress-70 protein levels reflect thermal tolerance in the marine bivalve *Mytilus edulis* L. *J Exp Mar Biol Ecol* 229: 53-68
- Connell JH (1972) Community interactions on marine rocky intertidal shores. *Ann Rev Ecol Syst* 3: 169-192
- Connell JH (1985) Consequences of variation in initial settlement vs post-settlement mortality in rocky intertidal communities. *Evol Mar Biol Ecol* 93: 11-45
- Crosby MP, Gale LD (1990) A review and evaluation of bivalve condition index methodologies with a suggested standard method. *J Shellfish Res* 9(1): 233-237
- Curole JP, Kocher TD (2005) Evolution of a unique mito-type specific extension of the cytochrome *c* oxidase II gene in freshwater mussels (Bivalvia: Unionoida). *J Mol Evol* 60: 1-10
- Dahlhoff EP (2004) Biochemical indicators of stress and metabolism: Applications for marine ecological studies. *Annu Rev Physiol* 66: 183-207
- Dahlhoff EP, Buckley BA, Menge BA (2001) Physiology of the rocky intertidal predator *Nucella ostrina* along an environmental stress gradient. *Ecol* 82(10): 2819-2829
- Dahlhoff EP, Menge BA (1996) Influence of phytoplankton concentration and wave exposure on the ecophysiology of *Mytilus californianus*. *Mar Ecol Prog* 144: 97-107

- Dahlhoff EP, Stillman JH, Menge BA (2002) Physiological community ecology: Variation in metabolic activity of ecologically important rocky intertidal invertebrates along environmental gradients. *Integ Comp Biol* 42: 862-871
- Dayton PK (1971) Competition, disturbance, and community organization: The provision and subsequent utilization of space in a rocky intertidal community. *Ecol Mono* 41(4): 351-389
- Dunton KH (2001) $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ measurements of Antarctic Peninsula fauna: Trophic relationships and assimilation of benthic seaweeds. *Amer Zool* 41: 99-112
- Elston RA, Moore JD, Brooks K (1992) Disseminated neoplasia in bivalve mollusks. *Rev Aqua Sci* 6: 405-466
- Feldman GC, McClain CR (2006) Ocean Color Web, SeaWiFS Reprocessing 5.1. In: Kuring N, Bailey SW (eds.) NASA Goddard Space Flight Center. <http://oceancolor.gsfc.nasa.gov/>
- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Mol Mar Biol Biotech* 3(5): 294-299
- Foster-Smith RL (1975) The effect of concentration of suspension on the filtration rates and pseudofecal production for *Mytilus edulis* L., *Cerastoderma edule* (L.) and *Venerupis pullastra* (Montagu). *J Exp Mar Biol Ecol* 17: 1-22
- France SC, Rosel PE, Agenbroad JE, Mullineaux LS, Kocher TD (1996) DNA sequence variation of mitochondrial large-subunit rRNA provides support for a two subclass organization of the Anthozoa (Cnidaria). *Mol Mar Biol Biotech* 5(1): 15-28
- Gaines S, Roughgarden J (1985) Larval settlement rate: A leading determinant of structure in an ecological community of the marine intertidal zone. *Proc Natl Acad Sci USA* 82: 3707-3711
- Gannes LZ, O'Brien DM, del Rio CM (1997) Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory experiments. *Ecol* 78: 1271-1276

- Gaunt MW, Miles MA (2002) An insect molecular clock dates the origin of the insects and accords with palaeontological and biogeographic landmarks. *Mol Biol Evol* 19(5): 748-761
- Geyer WR, Signell RP, Fong DA, Wang J, Anderson DM, Keafer BA (2004) The freshwater transport and dynamics of the western Maine coastal current. *Cont Shelf Res* 24: 1339-1357
- Griffiths CL, Griffiths RJ (1987) Bivalvia. *Animal Energetics* 2: 1-88
- Halpin PM, Sorte CJ, Hofmann GE, Menge BA (2002) Patterns of variation in levels of hsp70 in natural rocky shore populations from microscales to mesoscales. *Integ Comp Biol* 42: 825-824
- Hawkins AJS, Bayne BL, Day AJ (1986) Protein turnover, physiological energetics and heterozygosity in the blue mussel, *Mytilus edulis*: the basis of variable age-specific growth. *Proc R Soc Lond* 229: 161-176
- Helmuth BST, Hofmann GE (2001) Microhabitats, thermal heterogeneity, and patterns of physiological stress in the rocky intertidal zone. *Biol Bull* 201: 374-384
- Hennigar P, Sowles J, Crawford R, Harding G (1996) Evaluation of Gulfwatch 1993. Augusta, ME: The Gulf of Maine Council on the Marine Environment, State Planning Office.
- Hill JM, McQuaid CD, Kaehler S (2006) Biogeographic and nearshore-offshore trends in isotope ratios of intertidal mussels and their food sources around the coast of southern Africa. *Mar Ecol Prog Ser* 318: 63-73
- Hoeh WR, Stewart DT, Sutherland BW, Zouros E (1996) Multiple origins of gender-associated mitochondrial DNA lineages in bivalves (Mollusca: Bivalvia). *Evol* 50(6): 2276-2286
- Hopkins TS, Garfield N (1979) Gulf of Maine intermediate water. *J Mar Res* 37(1): 103-139

- Incze LS, Lutz RA, Watling L (1980) Relationships between effects of environmental temperature and seston on growth and mortality of *Mytilus edulis* in a temperate northern estuary. *Mar Biol* 57: 147-156
- Incze LS, Naimie CE (2000) Modeling the transport of lobster (*Homarus americanus*) larvae and postlarvae in the Gulf of Maine. *Fish Ocean* 9: 99-113
- Innes DJ, Bates JA (1999) Morphological variation of *Mytilus edulis* and *Mytilus trossulus* in eastern Newfoundland. *Mar Biol* 133: 691-699
- Johnson C, Pringle J, Chen C (Unpublished data) Transport and retention of dormant copepods in the Gulf of Maine.
- Kieckbusch DK, Koch MS, Serafy JE, Anderson WT (2004) Trophic linkages among primary producers and consumers in fringing mangroves of subtropical lagoons. *Bull Mar Sci* 74(2): 271-285
- Kijewski TK, Zbawicka M, Väinölä R, Wenne R (2006) Introgression and mitochondrial DNA heteroplasmy in the Baltic populations of mussels *Mytilus trossulus* and *M. edulis*. *Mar Biol* 149: 1371-1385
- Kjørboe T, Møhlenberg F, Nøhr O (1980) Feeding, particle selection and carbon absorption in *Mytilus edulis* in different mixtures of algae and resuspended bottom material. *Ophelia* 10: 193-205
- Kittner C, Riisgård HU (2005) Effect of temperature on filtration rate in the mussel *Mytilus edulis*: no evidence for temperature compensation. *Mar Ecol Prog* 305: 147-152
- Koehn RK, Hilbish (1987) The adaptive importance of genetic variation. *Amer Sci* 75: 134-141
- Macko SA, Estep MLF (1984) Microbial alteration of stable nitrogen and carbon isotopic compositions of organic matter. *Org Geochem* 6: 787-790

- Mallet AL, Carver CE (1993) Temporal production patterns in various size groups of the blue mussel. *J Exp Mar Biol Ecol* 170: 75-89
- McDonald JH, Siebenaller JF (1988) Similar geographic variation at the *LAP* locus in the mussels *Mytilus trossulus* and *M. edulis*. *Evol* 43(1): 228-231
- McGladdery SE, Reinisch CL, MacCallum GS, Stephens RE, Walker CL, Davidson JT (2001) Haemic neoplasia in soft-shell clams (*Mya arenaria*): Recent outbreaks in Atlantic Canada and discovery of a *p53* gene homologue associated with the condition. *Bull Aquacult Assoc Canada* 101: 19-26
- Menge BA (1976) Organization of the New England rocky intertidal community: role of predation, competition, and environmental heterogeneity. *Ecol. Mono* 46(4): 355-393
- Menge BA (1992) Community regulation: Under what conditions are bottom-up factors important on rocky shores? *Ecol* 73(3): 755-765
- Menge BA, Daley BA, Wheeler PA, Dahlhoff E, Sandford E, Strub PT (1997) Benthic-pelagic links and rocky intertidal communities: Bottom-up effects on top-down control? *Proc. Natl Acad Sci* 94: 14530-14535
- Menge BA, Sutherland JP (1976) Species diversity gradients: Synthesis of the roles of predation, competition, and temporal heterogeneity. *Amer Nat* 110(973): 351-369
- Menge BA, Sutherland JP (1987) Community regulation: variation in disturbance, competition, and predation in relation to environmental stress and recruitment. *Amer Nat* 130(5): 730-757
- Muttray AF, Cox RL, St-Jean S, van Poppelen P, Reinisch CL, Baldwin SA (2005) Identification and phylogenetic comparison of *p53* in two distinct mussel species (*Mytilus*). *Comp Biochem Biophys Part C* 140: 237-250
- National Weather Service Forecast Office (2006) Storm event database. <http://www4.ncdc.noaa.gov/cgi-win/wwcgi.dll?wwEvent~Storms> NOAA Satellite and Information Service and National Climatic Data Center

- O'Reilly JE, Evans-Zetlin C, Busch DA (1987) Primary production. In: Backus RH (ed.) (1987) *Georges Bank*. MIT Press: pp. 220-233
- O'Reilly JE and others (2000) Ocean color chlorophyll *a* algorithms for SeaWiFS, OC2, and OC4: Version 4. SeaWiFS postlaunch calibration and validation analyses, Part 3. In: Hooker SB, Firestone ER (eds) Nasa Tech Memo 2000-206892, Vol 11, SeaWiFS postlaunch technical report series. NASA Goddard Space Flight Center. pp. 9-27
- Page HM, Ricard YO (1990) Food availability as a limiting factor to mussel *Mytilus edulis* growth in California coastal waters. *Fish Bull US* 88: 677-686
- Paine RT, Levin SA (1982) Intertidal landscapes: Disturbance and the dynamics of pattern. *Ecol Mono* 51(2): 145-178
- Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. *Annu Rev Ecol and System* 18: 293-320
- Petraitis PS (1995) The role of growth in maintaining spatial dominance by mussels (*Mytilus edulis*). *Ecol* 76(4): 1337-1346
- Ramp SR, Schlitz RJ, Wright WR (1985) The deep flow through the Northeast Channel, Gulf of Maine. *J Phys Oceanogr* 15: 1790-1808
- Rawson PD, Hayhurst S, Vanscoyoc B (2001) Species composition of blue mussel populations in the northeastern Gulf of Maine. *J Shellfish Res* 20(1): 31-38
- Rawson PD, Hilbish TJ (1995) Evolutionary relationships among the male and female mitochondrial DNA lineages in the *Mytilus edulis* species complex. *Mol Biol Evol* 12(5): 893-901
- Sanders HL (1968) Marine benthic diversity: A comparative study. *Am Nat* 102(925): 343-282

- Schiel DR (2004) The structure and replenishment of rocky shore intertidal communities and biogeographic comparisons. *J Exp Mar Biol Ecol* 300: 309-342
- Smith JR, Fong P, Ambrose RF (2006) Long-term change in mussel (*Mytilus californianus* Conrad) populations along the wave-exposed coast of southern California. *Mar Biol* 149: 537-545
- Somero GN (2002) Thermal physiology and vertical zonation of intertidal animals: Optima, limits, and costs of living. *Integ Comp Biol* 42: 780-789
- Stewart DT, Kenchington ER, Singh RK, Zouros E (1996) Degree of selective constraint as an explanation of the different rates of evolution of gender-specific mitochondrial DNA lineages in the mussel *Mytilus*. *Genetics* 143: 1349-1357
- Stillman JH, Reeb CA (2001) Molecular phylogeny of eastern pacific porcelain crabs, genera *Petrolisthes* and *Pachycheles*, based on the mtDNA 16S rDNA sequence: phylogeographic and systematic implications. *Mol Phyl Evol* 19(2): 236-245
- Sverdrup HU (1953) On conditions for the vernal blooming of phytoplankton. *J Cons Int Explor Mer* 18: 286-295
- Thompson RC, Crowe TP, Hawkins SJ (2002) Rocky intertidal communities: past environmental changes, present status, and predictions for the next 25 years. *Env Conserv* 29(2): 168-191
- Townsend DW (1991) Influences of oceanographic processes on the biological productivity of the Gulf of Maine. *Rev Aqua Sci* 5(3-4): 211-230
- Townsend DW (1998) Sources and cycling of nitrogen in the Gulf of Maine. *J Mar Sys* 16: 283-295
- Townsend DW, Cammen LM (1988) Potential importance of the timing of spring plankton blooms to benthic-pelagic coupling and recruitment of juvenile demersal fishes. *Biol Ocean* 5: 215-229

- Townsend DW, Christensen JP, Stevenson DK, Graham JJ, Chenoweth SB (1987) The importance of a plume of tidally-mixed water to the biological oceanography of the Gulf of Maine. *J Mar Res* 45: 699-728
- Townsend DW, Spinrad RW (1986) Early spring phytoplankton blooms in the Gulf of Maine. *Cont Shelf Res* 6: 515-529
- Townsend DW, Thomas AC, Mayer LM, Thomas MA, Quinlan JA (2004) Oceanography of the Northwest Atlantic continental shelf (1, W). In: Robinson AR, Brink KH (ed.) (2004) *The Sea: The Global Coastal Ocean: Interdisciplinary Regional Studies and Syntheses*. Harvard University Press: Chapter 5
- Väinölä R, Hvilsum MM (1991) Genetic divergence and a hybrid zone between Baltic and North Sea *Mytilus* populations (Mytilidaw: Mollusca). *Biol J Linn Soc* 43: 127-148
- Ward JE, Shumway SE (2004) Separating the grain from the chaff: particle selection in suspension- and deposit-feeding bivalves. *J Exp Mar Biol Ecol* 300: 83-130
- Wares JP, Cunningham CW (2001) Phylogeography and historical ecology of the North Atlantic intertidal. *Evol* 55(12): 2455-2469
- West JB, Bowen GJ, Cerling TE, Ehleringer JR (2006) Stable isotopes as one of nature's ecological recorders. *Trends in Ecol Evol* 21(7): 408-414
- Whiteley N, Faulkner LS (2005) Temperature influences whole-animal rates of metabolism but not protein synthesis in a temperate intertidal isopod. *Phys Biochem Zool* 78(2): 227-238
- Widdows J, Bayne BL (1971) Temperature acclimation of *Mytilus edulis* with reference to its energy budget. *J Mar Biol Assoc UK* 58: 109-124
- Widdows J, Bayne BL, Livingstone DR, Newell RIE, Donkin P (1979) Physiological and biochemical responses of bivalve mollusks to exposure to air. *Comp Biochem Physiol A* 62A: 301-308

- Widdows J, Johnson D (1988) Physiological energetics of *Mytilus edulis*: Scope for growth. *Mar Ecol Prog* 46: 113-121
- Winter JE (1976) Feeding experiments with *Mytilus edulis* L. at small laboratory scale. II. The influence of suspended silt in addition to algal suspensions on growth. *Eur Mar Biol Symp Proc* 10th 1: 583-600
- Xue H, Chai F, Pettigrew NR (2000) A model study of the seasonal circulation in the Gulf of Maine. *J Phys Oceanogr* 30: 1111-1113
- Zouros E, Ball AO, Saavedra C, Freeman KR (1994) An unusual type of mitochondrial DNA inheritance in the blue mussel *Mytilus*. *Proc Natl Acad Sci USA* 91: 7463-7467
- Zouros E, Freeman KR, Ball AO, Pogson GH (1992) Direct evidence for extensive paternal mitochondrial DNA inheritance in the marine mussel *Mytilus*. *Nature* 359: 412-414