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Blue Mussel Hybrid Complex Shifts in the Gulf of Maine towards a Dominance of *Mytilus edulis*

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ABSTRACT

The Gulf of Maine (GOM) in the United States is the fastest warming body of water in the world. Due to a complex history of glaciation, the GOM has restricted circulation that causes it to function more akin to an enclosed sea. As climate change has disrupted the stable flow of the Gulf Stream, more warm-water currents are entering the GOM at higher frequency. This warming has had a profound impact on the distribution of the blue mussel, *Mytilus spp.*, which inhabits the rocky intertidal habitat within the GOM as the main prey species to a keystone predator. Two sister species, *M. edulis* and *M. trossulus*, both inhabit the GOM and hybridize in areas of sympatry. However, in recent years, the population of both hybrids and *M. trossulus* have been retreating northward in the GOM towards the Canadian coast. This study builds upon previous assessments of *Mytilus* species distribution along the GOM using two genetic markers, SOD and TAUT, that differentiate between members of each species. Results indicate a dominance of *M. edulis*, with few remaining refuges for either *M. trossulus* or hybrid individuals. These refuges are mostly islands, suggesting a potential correlation between exposure and/or temperature caused by increased distance from the mainland. The trends in the GOM are troubling for the intertidal ecosystem, as decreased biodiversity in a key prey species could lead to increased vulnerability to disease and other population threats, which could lead to drastic negative effects on the rest of the local food web.

INTRODUCTION

The earth's atmosphere and seas have warmed due to human influence, with each of the last four decades breaking new records for the warmest decades since 1850 (IPCC, 2021). Studying the effects of this warming is important to understand how anthropogenic emissions and pollution impact the biodiversity of the oceans. In 2015, the Gulf of Maine (GOM) was documented as the fastest warming body of water in the world, with warming rates exceeding those of 99% of the world ocean (Pershing et al., 2015). Glacier formation influences the shape and depth of the GOM, which contributes to the warming, as does the interaction between the two main currents that influence the GOM, the Gulf Stream and Labrador Current. Intertidal species within the GOM have also been affected by the glacial history of the region. At least two rocky intertidal invertebrate species that inhabit the GOM are experiencing hybridization, or the interbreeding of two separate species, in areas of secondary contact (Rawson *et al.*, 2001; Harper and Hart, 2007). Secondary contact refers to the interaction between two species which have relatively recently speciated from a common ancestor (Kangas et al., 2015). In the GOM, the speciation occurred as a result of the same glacial activity that formed the GOM. Due to its hybrid populations and rapid warming, the GOM is a unique region of environmental importance. In this study, I examine the effects of temperature increases on the distribution of the blue mussel *Mytilus edulis*, and its sister species, *Mytilus trossulus*, and how these temperature changes may impact biodiversity of the Atlantic rocky intertidal. Based on the faster warming in the GOM in comparison to other coastlines, the changes in distribution observed in the blue mussel may be indicative of future trends for other coastlines worldwide.

Geological History of the Gulf of Maine

Located in the northwest Atlantic, the GOM is a feature of the continental shelf bordered by the US states of Massachusetts, New Hampshire, and Maine, together with Canada's New Brunswick and Nova Scotia (Figure 1). The GOM is separated from the continental shelf mainly by Georges Bank, a submerged rise that reaches shallow waters, forming a lip that is cut by two narrow channels, named the Northeast and Great South channels (Figure 1;Uchupi and Bolmer, 2008). The Northeast Channel is the only source of deep-water exchange between the GOM and the north Atlantic. The GOM is also separated from the Atlantic by numerous shoals between Cape Cod, MA and Georges Bank (Figure 1), as well as the Scotian Shelf on its eastern side (Uchupi and Bolmer, 2008). The isolation of the GOM from the Atlantic and the presence of one deep-water channel, the Northeast Channel, has important implications for the temperature patterns of the GOM, as the presence of only one deep connection to the Atlantic limits the movement of water into the GOM (Lower-Spies et al., 2020). Warm water flows into the GOM at depth from the Atlantic through the Northeast Channel, and cool water enters the GOM from northern subsections of the Labrador Current over the Scotian Shelf and Georges Bank (Seidov *et al.*, 2021).

The GOM began to form as early as the formation of the Atlantic, but its development was heavily influenced by glaciation in the Pleistocene epoch (Fulton and Prest, 1987; Uchupi, 1966; Uchupi and Bolmer, 2008). The Pleistocene is a broad category of geological time just before our current epoch (the Holocene), starting 2.58 million years ago and ending around 12,000 years ago (Cohen et al., 2016). The Pleistocene was defined by significant global glaciation, and is divided into three main stages: the Illinoian, the Sangamonian, and the Wisconsinan (Fulton and Prest, 1987). The period of greatest influence on the GOM is the Wisconsinan stage (30-12,000 years ago), in which a massive ice sheet known as the Laurentide Ice Sheet formed and extended south through the modern-day Great Lakes, covering parts of the United States and most of Canada, including what is now the GOM (Fulton and Prest, 1987). Five glaciation events impacted the GOM throughout the Pleistocene epoch (Uchupi and Bolmer, 2008). These events alternatively cut and built upon Georges Bank during glacial advance and regression, respectively, ultimately shaping it to its modern dimensions and limiting open water exchange with the Atlantic (Uchupi and Bolmer, 2008). The same processes altered the Northeast Channel (Figure 1), with deposition eventually narrowing it to its current width (Uchupi and Bolmer, 2008).

The Mytilus Complex in the Gulf of Maine

The glaciation of the GOM also affected the species inhabiting it. Two closely related species of blue mussel inhabit the GOM: *M. edulis* and *M. trossulus*. The two species originated with the opening of the Bering Strait around 2.6 million years ago, allowing *M. trossulus* to migrate from the Pacific and colonize the north Atlantic Ocean (Vermeij, 1991). During the Pleistocene era, glaciers created geographic separation between Pacific and Atlantic *M. trossulus* populations, and allopatric speciation occurred in the Atlantic resulting in the modern *M. edulis*, and eventually *M. galloprovincialis* in the Mediterranean (Riginos and Cunningham, 2005). Allopatric speciation refers to the process in which one species diverges into two because of geographic isolation. This forced isolation into two separate habitats causes speciation as natural selection acts upon each population differently, causing different traits of the original species to be selected for. Eventually, these differences become great enough to prevent the two populations from interbreeding to produce fertile offspring, and they become separate species. Allopatric speciation can be caused by either natural disasters, or by population dispersal into habitats that are isolated from one another. As the Laurentide Ice Sheet covered the GOM, the newly speciated *M. edulis* was extirpated from the region (Wares and Cunningham, 2001), but populations persisted along the ice-free east coast of the US and in Europe (Wares and Cunningham, 2001). After the Pleistocene ended and the Laurentide Ice Sheet retreated, both *M. trossulus* from the Pacific and *M. edulis* from the Atlantic reentered the Gulf of Maine and established secondary contact, creating a hybrid zone (Innes and Bates, 1999). Hybrid zones are geographic areas in which two species interbreed with one another, resulting in shared genes and hybrid offspring. (Gardner, 1996; Kangas et al., 2015). There are at least two other instances of these *Mytilus* hybrid zones, including *M. trossulus* and *M. galloprovincialis* off the coast of California and *M. edulis* and *M. trossulus* in the Baltic Sea (Innes and Bates, 1999; Riginos and Cunningham, 2005). The characteristics and extent of the hybridization within these zones have been studied for at least 50 years (Barsotti and Meluzzi, 1968; Innes and Bates, 1999; Rawson and Hilbish, 1995, 1998).

It is most likely that a combination of environmental and genetic factors has led to the *Mytilus* species distribution gradient along the GOM. Within the GOM hybrid zone, *M. edulis* and *M. trossulus* appear morphologically very similar. Small differences do exist, but these are difficult to discern due to the hybridization and similar environmental conditions (Innes and Bates, 1999). There are important genetic distinctions between the two species, and the two species occupy slightly different micro-habitats, depending upon salinity, wave exposure, and average temperature. In the GOM, a gradient of species distribution occurs moving northward, with *M. edulis* populations off the southern coast of Maine gradually giving way to *M. trossulus* populations off the northern coast of Maine (Rawson *et al.*, 2001). The explanation for these differences in distribution has been greatly debated. Early studies suggested that salinity tolerance caused the gradation, noting that the northern waters of the hybrid zone where *M. trossulus* dominate are characterized by lower salinity (34 ppt) than the south (36 ppt) (Gardner, 1996; Stammer et al., 2021). Wave exposure preferences were also identified, with *M. trossulus* able to thrive in more wave-exposed environments in the GOM than *M. edulis* (Bates and Innes, 1995; Tam and Scrosati, 2014). Both the salinity and wave exposure hypotheses were not supported by other studies that found either inconclusive or contradicting results to the assertions that *M. trossulus* survived better in low salinity and/or in areas with high wave exposure (Comesaña *et al.*, 1999; Rawson *et al.*, 2001; Rawson *et al.*, 2003). Identifying one factor that causes *M. trossulus* to dominate in the north of the GOM and *M. edulis* to occupy the south has been difficult because of the confluence of multiple environmental factors that are present along the northeast coast of North America.

Climate Change in the Gulf of Maine

As oceans experience net warming, high latitude waters are affected the most (Shearman and Lentz, 2010). The GOM is experiencing the most rapid warming in the world's oceans in recent times, thus serving as a prominent and useful location for studies looking to assess the impacts of climate change (IPCC, 2021). The GOM is influenced by the cold Labrador Current moving southwest along the continental slope from the poles and the warmer Gulf Stream located further southeast, moving northeast (Figure 1). The two currents are adjacent to one another, and move in opposite directions. The interactions and variation between these two currents as they occasionally intersect create the water temperature patterns found within the GOM. Average surface temperatures have risen north of Cape Hatteras, North Carolina from 0.5 to 1.3 degrees Celsius since 1905 (Shearman and Lentz, 2010). This warming is attributed to the weakening of the Atlantic Meridional Overturning Circulation (AMOC), the global current system that transports water throughout the world ocean (Caesar et al., 2018). The AMOC system involves the net movement of warm waters north in the Atlantic Ocean, which then cool and sink in the Northern Ocean, moving south past the equator into the southern Atlantic (Frajka-Williams et al., 2019). The AMOC is crucial for nutrient exchange between latitudes and depths, and the Gulf Stream is responsible for the AMOC's movement of warm water in the north Atlantic (Frajka-Williams et al., 2019). A northern deflection in the Gulf Stream into waters previously associated with the Labrador Current east of 50°W (Newfoundland) within the past few decades is evidence for the weakening of the AMOC (Seidov *et al.*, 2021). Recent data tracking the strength of the AMOC since the late 19th century suggests that the weakening of the AMOC is anthropogenic in nature, stemming from rising CO_2 levels causing the seawater at the poles to have lower salinity and thus slowing the AMOC, which is driven by differences in temperature and salinity (Caesar et al., 2018). Increases in CO_2 levels cause more heat retention in the atmosphere, which in turn causes the ice at the poles to melt (Haskins et al., 2020). Less ice at the poles decreases the albedo of the earth's surface, or reflection of the Sun's energy, which leads to increased heating of the ocean. Melting glaciers in Greenland also introduce fresh water to the north Atlantic, reducing the water's density and disrupting the density gradient necessary for the sinking of water in the north Atlantic (Haskins et al., 2020). In addition, the past decade has seen anomalous behavior in the Gulf Stream that coincides with the warming of the GOM (Seidov et al., 2021). As the flow of cold polar water from the Labrador Current decreases due to the AMOC weakening, the adjacent Gulf Stream shifts northward to take its place (Seidov *et al.*, 2021).

Warming in the GOM cannot be directly attributed to the Gulf Stream's shift, however, since the GOM is located west of the observed Gulf Stream deflection. Instead, the warming has been correlated with the incursion of warm eddies created by the interaction of the Gulf Stream with the Labrador Current. Since they are adjacent to one another and flow in opposite directions, the Gulf Stream and Labrador current are in constant contact with one another, mixing to create eddies (Hansen, 1970). These eddies consist of a core of either warm or cold water encircled by the warm Gulf Stream, and are warm and cold core eddies, respectively (Hansen, 1970). Usually, eddies remain within the main flow of the Gulf Stream current, but they can also detach and travel north or south. Warm core eddies travel west, moving inshore via deep channels, such as the Northeast Channel of the GOM (Figure 1)(Brickman *et al.*, 2018; Gangopadhyay *et al.*, 2019). Since 2000, the frequency of these warm core eddies has increased, and thus the GOM has experienced more consistent warming events (Brickman *et al.*, 2018; Gangopadhyay *et al.*, 2019; Seidov *et al.*, 2021). This increase in warm core eddies has been tied to the northward shift of the Gulf Stream, caused by a weakening of the Labrador Current and the subsequent decrease in the flow of polar water along the Scotian Shelf into the GOM (Seidov *et al.*, 2021). Both the northerly shift of the Gulf Stream and the increase in warm core eddy frequency play a role in the warming of the GOM, separately and in combination, and it is debated which factor plays a larger role.

Brickman *et al.* (2018) suggest that the variability in GOM temperatures due to the effects of warm core eddies is natural, and thus warming of the GOM is not a cause for preventative action or concern in line with climate change examples. Previous studies of the AMOC variability, which were limited to the past few decades, would most likely lead to this same conclusion because they too show oscillating strength of the AMOC (Caesar et al., 2018). At present, the future trends of the Gulf Stream, AMOC, and GOM temperatures are not certain. The Intergovernmental Panel on Climate Change (IPCC) predicts a high likelihood of further AMOC weakening throughout the next 80 years, with the magnitude of such change needing more research to predict with greater confidence (IPCC, 2021). The further weakening of the AMOC is concerning because the warming within the past two decades has already caused a change in the distribution of the *Mytilus* complex in the GOM. The distribution of *M. edulis, M. trossulus*, and their hybrids is affected in some capacity by latitude, sea surface temperature, and salinity. As the GOM continues to warm, large populations of the colder-water *M. trossulus* may disappear (Rawson *et al.*, 2001). The implications of this loss for the biodiversity of the region depend largely upon the ecological role of mussels within the intertidal community of the GOM.

The genetics of the hybridization between *M. edulis* and *M. trossulus* in the GOM also play a role in maintaining an observable distribution gradient from *M. edulis* in the south to *M. trossulus* in the north. The hybrid zones in both Europe and the GOM are closely aligned with ecotones, or transitions between environmental conditions. The GOM is a location of confluence between the warmer Gulf Stream and the polar Labrador Current, creating an ecotone, while the hybrid zone in northern Europe occurs where the low-salinity waters of the Baltic Sea combine with the polar North Sea (Gardner, 1996; Saba et al., 2016). The GOM hybrid zone differs from the *Mytilus* hybrid zone in northern Europe in that the hybridization is not nearly as extensive (Rawson *et al.*, 2003). Contrary to European examples of high hybrid abundance and shared genes, the GOM populations of *M. edulis* and *M. trossulus* remain distinct, with lower rates of hybridization than expected (Gardner, 1996; Rawson *et al.*, 2003). The low hybridization of *Mytilus* within the GOM has been attributed to the relatively young age of the hybrid zone, beginning secondary contact after the Pleistocene era (Rawson, *et al.*, 2003). Another explanation for the low hybrid numbers found in the GOM is evolutionary fitness. Gardner (1996) observed that hybrids in the GOM experience higher mortality than their pure counterparts due to their intermediate traits providing less of an advantage than either pure species, inhibiting hybrid genes from being passed across the hybrid zone (Gardner, 1996). Thus, the hybrid zones themselves serve as a barrier to gene flow between *Mytilus* species (Gardner, 1996). Rawson *et al.* (2003) expanded upon this research, testing gamete compatibility between species and fertilization rates. Fertilization rates were very low between species, and gamete incompatibility was identified as the main contributor to gene flow inhibition in the GOM hybrid zone. It has now been established that both prezygotic (occurring before fertilization) and postfertilization barriers exist in the hybridization of the *Mytilus* complex (Miranda *et al.*, 2010)

Due to its heterogenous nature, the *Mytilus* complex in the GOM can be used as an indicator of the ecotone present between Atlantic and polar waters in the GOM. Relatively distinct environments contain mostly pure *M. edulis* and *M. trossulus*, while ecotones contain higher concentrations of hybrids. Although the contribution of changing environmental conditions, including salinity and wave exposure, are not yet known, sea surface temperature is likely to have a role (Tam and Scrosati, 2014). Thus, as climate warms and the GOM is disproportionately affected, the distribution of *Mytilus* can be expected to change.

Intertidal Ecology of the Gulf of Maine

The rocky intertidal ecosystem in the GOM is zoned from high to low according to physical stressors like desiccation and wave shock, as well as biological factors such as predation and competition. The spray zone is at the very top, above the highest high tide line, meaning that it is never fully submerged by the tides. The upper intertidal zone below is submerged during high tide, but experiences high amounts of air and sun exposure. In the GOM, the upper intertidal zone is dominated by barnacles of the genus *Semibalanus* (Menge, 1976). Descending from the high intertidal, the dominance of species shifts to mussels of the genus *Mytilus* (Menge, 1976). Mussels are able to outcompete barnacles, but they cannot withstand desiccation pressure at higher elevations, thus limiting their vertical distribution (Menge, 1976). The middle intertidal is periodically submerged, being located between high and low tide lines. The lower intertidal is almost always submerged, only being exposed to air during the lowest of low tides. Especially in areas with low wave exposure, the near constant submersion allows fucoid (*Fucus*, *Ascophyllum*) algae to grow and provide habitat and refuge for mobile predators such as the sea star *Asterias* and the dogwhelk *Nucella* (Lubchenco and Menge, 1978; Menge, 1976, 1978). These two predators play the dominant role in controlling the populations of *Mytilus* mussels, which allow algae such as *Chondrus crispus* to colonize the region (Lubchenco and Menge, 1978). Notably, this effect is only observed in wave-protected shorelines; in more wave-exposed regions, predation is low, and *Mytilus* beds dominate the substrate (Petraitis et al., 2004). Mussels like *Mytilus* are molluscan invertebrates within the class Bivalvia. Mussels inhabit rocky intertidal habitats at temperate latitudes (Rawson and Hilbish, 1995). Mussels are filter feeders, obtaining nutrients by filtering out edible material from ambient seawater. Mussel beds provide several benefits to the surrounding biological community as ecosystem engineers (Jones *et al.*, 1994). The dense mat of byssal threads that mussels use to anchor themselves to rocky substrate, as well as the mussel shells themselves, provide surface area and refuge for many species of invertebrates, including amphipods, polychaetes, and nemerteans (Tsuchiya and Nishihira, 1985). Mussels increase biodiversity in areas where they are present compared to areas of the shore without them (Borthagaray and Carranza, 2007).

Overall, the distribution of *M. edulis, M. trossulus,* and their hybrids may be good indicators for the effects of climate change upon the GOM, and thus for other temperature-limited species elsewhere which share similar ecological roles. If the populations of *Mytilus* continue to shift, the entire rocky intertidal ecosystem they inhabit in the GOM may be affected. As *Mytilus* is prey to a keystone predator, *Asterias*, the population of this sea star could be negatively affected by the changing range of *Mytilus* species. If warming is extreme enough to extirpate *Mytilus* from the GOM, the loss of an ecosystem engineer such as *Mytilus* would undoubtedly cause a decrease in the biodiversity of the region, regardless of the benefit to species which have historically been outcompeted, such as *Semibalanus* barnacles (Borthagaray and Carranza, 2007).

This research seeks to determine the distribution of *M. edulis*, *M. trossulus*, and their hybrids quantitatively using two genetic markers that distinguish each species and their hybrids. The results of this research will provide an idea of the rate of change in the *Mytilus* complex distribution over time due to climate change in comparison with the results of Rawson *et al.* (2001). The markers, SOD (super oxide dismutase) and TAUT (taurine transporter), are novel markers which are effective in differentiating between *M. edulis*, *M. trossulus*, and their hybrids (Rawson unpub.).

METHODS

Sample Collection and DNA Extraction

Whole mussels were collected from 16 sites along the Gulf of Maine in June 2019 and stored on ice before transfer to the laboratory (Table 1). Samples were collected from rocky substrate and from boat docks. Mantle tissue was excised from each mussel and stored in separate tubes containing 95% ethanol solution, then shell length and width were measured. DNA was extracted from mussel mantle tissue using the Quiagen Mini-kit, according to Rawson protocol (2004).

PCR Analysis: TAUT Marker

Polymerase chain reactions (PCR) were used to amplify the novel TAUT marker that differentiates *Mytilus* species (Rawson, unpub.). Each assay contained 25µL total volume consisting of 1 µL sample DNA, 1X PCR buffer from Life Tech, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 1 U Invitrogen DNA Taq polymerase, 0.3 µM each of marker-specific forward and reverse primers. Primer sequences were TAUT FOR5: GGCGATCTTCACTCCCCTTTT, TAUT REV6: GTTTCCTGCCTGGGTAAGAATG.

Reactions were placed in the thermocycler and denatured at 94°C for 3 minutes, then incubated for 30 cycles of denaturation at 94°C for 20s; annealing at 55°C for 30s; and extension at 72°C for 2 min.

PCR Analysis: SOD Marker

Polymerase chain reactions (PCR) were used to amplify the novel SOD marker that differentiates *Mytilus* species (Rawson, unpub.). Each assay contained 25µL total volume consisting of 1 µL sample DNA, 1X PCR buffer from Life Tech, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 1 U Invitrogen DNA Taq polymerase, 0.3 µM each of marker-specific oligonucleotide primers. Primer sequences were SOD 1L:

AATGGTGATTCAGCAGTGAC, SOD TR1: CATACAAACCCCCTAAAGTGA, SOD ED1: GCTAATACATTTCCCAGATCACC.

Reactions were placed in the thermocycler and denatured at 94°C for 3 minutes, then incubated for 30 cycles of denaturation at 94°C for 20s; annealing at 50°C for 30s; and extension at 72°C for 2 min.

A positive control for *M. edulis* DNA and *M. trossulus* DNA was used for both markers, originally sourced outside of the GOM. After depleting previous *M. trossulus* positive control DNA, a known sample of *M. trossulus* from RE was used as a substitute (RE11).

Analysis

In order to visualize PCR products, they were combined with 6X Thermo DNA Loading Dye and pipetted into 1.5% agarose gels containing 0.5X TAE stained with ethidium bromide. Invitrogen TrackIt 1Kb DNA Ladder and 1Kb Plus DNA Ladder were used to measure product size. Gels were run in 0.5X TAE at 75 V for 75-80 minutes. UV light was used to image products. For the SOD marker, *M. edulis* produced a single PCR product at 320 bp while *M. trossulus* could yield two alleles at 200 bp and 250 bp. For the TAUT marker, *M. edulis* produced a single PCR product at 500 bp while *M. trossulus* could yield two alleles at 350 bp and 520 bp. Pure individuals either had one *M. edulis* allele or up to two *M. trossulus* alleles in both SOD and TAUT assays. Hybrid mussels were identified in two ways. First, the presence of two PCR products representing an allele from each species indicated a hybrid using only one marker. Hybrids were also identified by examining results across markers: if an individual scored as *M. edulis* in one marker and *M. trossulus* in another, then this individual was also considered a hybrid. After scoring, results were compiled and analyzed using Microsoft Excel. Each location's samples were grouped by size according to four size bins (length, mm): 15-35, 36-50, 51-65, and 66-90.

RESULTS

A total of 535 *Mytilus* individuals were sampled and assigned as either *M. edulis*, *M. trossulus*, or a hybrid over 16 collection sites along the coast of Maine using two genetic species-specific markers (Table 1). Sample size for each population averaged 33 individuals, with a minimum of 24 and a maximum of 40 mussels collected. Mussel shell length varied from 20 mm to 85 mm. A majority of locations (69%) entirely consisted of pure *M. edulis* individuals. Both *M. trossulus* and hybrid individuals making up less than 5% of the total sampled population (Figure 2).

M. trossulus were found only at five locations: WI (2.8%), HI (4.3%), PC (29.6%), RE (16.7%), and RW (4.2%). Three of the locations with pure *M. trossulus* individuals, HI, RE, and RW, were located on offshore islands, while WI and PC are located in the northeast region of the GOM. Hybrids were present at HI (7.7%), PC (18.5%), RE (3.3%), and RW (4.2%). WI was the only location with *M. trossulus* and no hybrids present. Six hybrids (67% of hybrids) were detected by the presence of both *M. edulis* and *M. trossulus* alleles on a single gel row from HI, PC, and RE. Three hybrids (33% of hybrids) were identified across both SOD and TAUT markers from PC and RW.

In addition to the previously identified allesles for SOD, I found individuals from *Mytilus* populations at RE and HI (both islands) that had novel alleles not present in other sampling locations (Figure 3). There are one to two previously unidentified alleles of around 1000-1500 bp. These alleles were most visible in sample RE 23, but present in at least five individuals across RE and HI, including RE 1, 11, 15, and 23; HI 4.

Size, as quantified in shell length, also varied in mussels collected from around 20 to 85 mm. *M. edulis* was represented in all size categories (Figure 4). All *M. trossulus* and hybrids identified at WT, HI, RE, and RW had small shell lengths of less than 50 mm. At both RE and RW, located far offshore, shell length for all individuals was also less than 50 mm. At GS and PP, the two southernmost sampling sites, shell lengths were all less than 65 mm.

In contrast, at BI, BL, and PC, all located on the northern half of the coast, all samples were large, measuring above 35 mm in length. No small mussels were collected at these three sites, despite careful searching.

DISCUSSION

The species distribution of *M. edulis, M. trossulus,* and their hybrids in the GOM is heavily weighted towards pure *M. edulis* individuals. The majority of samples, 95% of the 535 individuals, displayed homozygous *M. edulis* alleles for both SOD and TAUT markers. This result indicates dominance of *M. edulis* alleles within the *Mytilus* population in the GOM. When grouped by location, each population, including those with other *Mytilus* representation, had a *M. edulis* population of >50%. Furthermore, at 69% of 16 locations, populations consisted of 100% *M. edulis*. These pure populations were spread all along the GOM, ranging from Prince's Point (PP) at the southern end, through Staple Point (SP) around the middle, up to Sipp Bay (SB) at the northern edge of the coast of Maine. In contrast to previous observations that species distribution favored *M. trossulus* in wave-exposed headlands, I observed multiple locations, including PP, SP, Pemaquid Point (PQ), and Beal's Island (BI), consisting of only *M. edulis* in protruding coastlines (Tam and Scrosati, 2014). This finding suggests that factors other than wave exposure alone have influence over the patterns of *Mytilus* distribution, a conclusion that aligns with the previous studies (Rawson et al., 2001, 2003).

Mytilus trossulus and hybrid individuals are present in a pattern that is not easily discerned along the GOM. The westernmost population of *M. trossulus* and hybrids was identified at Hurricane Island (HI), an island around the middle of the Maine coastline. Although these individuals constitute 11.5% of the total *Mytilus* population sampled, their presence is interesting because surrounding locations like SP, PQ, and Stockton Springs (STK) located on the mainland are fully populated by *M. edulis*. This correlation between population distribution and geographic placement is continued in two other populations containing *M. trossulus* and hybrids, Mount Desert Rock East (RE) and Mount Desert Rock West (RW). Both sampling locations are located on either side of one small island located far offshore. The closest mainland sampling site is SP, a population of only *M. edulis.* In addition, the frequency of *M. trossulus* and hybrids on the east side of the island, RE, is 2.5 times higher than that of the west at RW. Based on the presence of *M. trossulus* and hybrids on the three island sites surrounded by mainland areas of 100% *M. edulis* populations, some inferences can be made about factors influencing *Mytilus* species distribution.

The first potential factor affecting *Mytilus* species distribution is water temperature. Due to their distance from the mainland, HI, RE, and RW are likely to be subject to more of the colder surface water flowing over the Scotian Shelf, as previously discussed (Hayhurst and Rawson, 2009; Seidov *et al.*, 2021). Previous work has suggested that *M. trossulus* is better adapted to survive colder temperatures than *M. edulis*, and that *M. edulis* may be able to outcompete *M. trossulus* in warmer waters (Tam and Scrosati, 2014). The species distribution on HI, RE, and RW support this hypothesis. Another factor that may influence *Mytilus* species distribution is wave exposure, favoring *M. edulis* in sheltered regions (Comesaña et al., 1999; Tam and Scrosati, 2014). The higher relative frequency of *M. trossulus* and hybrids at RE compared with RW may provide evidence that wave exposure does influence distribution to some degree. Given the southwestward flow of the Labrador Current over the Scotian Shelf, higher proportions of *M. edulis* on the leeward side of the island align with the hypothesis that *M. edulis* have a preference for sheltered coastlines (Hayhurst and Rawson, 2009; Tam and Scrosati, 2014). However, further testing of these ideas is necessary to provide more concrete evidence for the effect of wave exposure on *Mytilus* species distribution.

The northernmost four sampling sites, Whiting (WT), Cobscook Bay Landing (BL), Pirate's Cove (PC), and Sipp Bay (SB), present convoluted results which do not follow any discernible patterns. WT has a very small presence of *M. trossulus* (2.8%), which may be lower than expected for a northern site with colder waters conducive to *M. trossulus*, but does not reveal much on its own (Rawson et al., 2001). The remaining three sites, BL, PC, and SB, are very close to each other within the greater Cobscook Bay area. However, PC demonstrates drastically different *Mytilus* assemblages than BL and SB. While PC has the highest proportion of *M. trossulus* and hybrid species, at 48%, its two neighboring sites are 100% *M. edulis*. The massive difference between the three sites cannot be explained by any of the previous factors, warranting future research on *Mytilus* species distribution specifically within the Cobscook Bay.

Another set of results which may provide insight into the factors affecting distribution of *Mytilus* species is the size ranges observed at each location. In particular, the mussels collected from RE and RW were all small, measuring less than 50 mm in length. A previous study explored how the mechanical stress of wave exposure could limit the optimum size of intertidal species, and this hypothesis could apply to the isolated RE and RW sites (Denny et al., 1985). The physical force of constant wave action affects larger organisms more than it does smaller ones due to a larger surface area, threatening to dislodge and kill individuals that grow to be too large (Denny et al., 1985). In mussels, size is also directly correlated with age, as an observable growth ring is added to the shell of the animal annually (Hunt and Scheibling, 1995). Taking size into account, it is possible that RE and RW exhibit smaller sizes than other locations due to increased wave action dislodging larger and older individuals, leaving only small, younger specimens behind of all *Mytilus* species.

Using the size to age correlation, the population history of other locations can also be inferred. At PP and GS, two southern locations, there is a lack of larger *M. edulis* individuals above 65 mm in length. This suggests a younger population spread than in other sites with representation from all four size categories, like Darling Marine Center (DMC) and Little Machias Bay (LMB). Conversely, BI, BL, and PC only yielded larger (older) individuals above 35 mm in length. These locations are in the northern half of the GOM, creating a pattern of younger to older populations moving north through the GOM. As a broadcast spawning species, a pattern of decreasing age moving downstream in the Labrador Current can be expected (Miranda et al., 2010). However, these patterns of age distribution require further examination and statistical analysis beyond the scope of the present study.

The species distribution of *Mytilus* also appears to be dependent on size and therefore age. At HI, WI, RE, and RW, all *M. trossulus* and hybrid individuals are small, measuring below 50 mm in length. These results suggest that the *M. trossulus* and hybrids are relatively new recruits to the locations. However, at PC, *M. edulis, M. trossulus,* and their hybrids are large, measuring longer than 35 mm. These results contrast with the trend of younger, smaller *M. trossulus* and hybrids at other sites, instead representing an old and diverse population. As with species distribution itself, PC represents an outlier from all other sampling sites.

In comparison with both 2001 and 2009 species distribution studies, the pattern of *M. trossulus* disappearance from the GOM is apparent (Rawson et al., 2001; Hayhurst and Rawson, 2009). In both 2001 and 2009, all study sites had some population of *M. trossulus,* including sites with a majority consisting of *M. trossulus* (Rawson et al., 2001; Hayhurst and Rawson, 2009). Now, less than a third of sites have any *M. trossulus* or hybrids at all, and none are dominated by them. In 2001, DMC had an *M. trossulus* frequency of 5%, decreased to 0% in this study (Rawson et al., 2001). LMB also decreased drastically from 44% *M. trossulus* in 2001 to 0% in 2019 (Rawson et al., 2001). WI did not lose all of its *M. trossulus*, but still dropped from 76% in 2001 to 2.7% in 2019 (Rawson et al., 2001). Finally, RE and RW combined dropped from 50% in 2001 to 14.7% in 2019 (Hayhurst and Rawson, 2009).

This shift in population composition may be traced to the rapid warming in the GOM providing an evolutionary advantage to the warmer-water *M. edulis* to outcompete both *M. trossulus* and hybrid individuals (Caesar et al., 2018; Rawson et al., 2001; Seidov et al., 2021). The warming may be caused by the increased frequency of warm-core eddies branching off entering the GOM through the deep Northeast Channel, or by the disruption and weakening of the AMOC through anthropogenic polar warming (Brickman et al., 2018; Caesar et al., 2018; Seidov et al., 2021). In either case, there appears to be a correlation between the warming of the GOM and the distribution of the *Mytilus* complex. However, a multitude of microenvironmental factors that may be influenced by climate change also may play a role in the shifting species distribution. The data gathered in this experiment appear to support the hypothesis that *M. trossulus* are more prevalent on wave-exposed islands (RW, RE), but the colder temperatures associated with islands are also likely to play a role in this distribution (Tam and Scrosati, 2014). In addition, the lack of *M. trossulus* on sampled headlands and other exposed landforms prevents any conclusions concerning wave exposure's independent influence on distribution from being made.

Another factor thought to be involved in species distribution is salinity, though there has not been a consensus reached as to the extent of its influence in *Mytilus* spp. (Comesaña et al., 1999; Gardner, 1996; Riginos and Cunningham, 2005; Tam and Scrosati, 2014). Some studies (Johannesson et al., 1990; Väinölä and Hvilsom, 1991) observed greater survivability of *M. trossulus* in low-salinity environments, while others came to the opposite conclusion (Gardner and Thompson, 2001; Qiu et al., 2002). No inferences about salinity are able to be made within this experiment to support or reject previous conclusions about a correlation with species distribution (Gardner, 1996).

Data collection and analysis in this study is ongoing. Another location, Greenhead, exists in the genotype database, yet lacked DNA samples to perform PCR. Although the original tissue may have been available, time restraints prevented the analysis of this location. In addition, a small number of samples from RW, HI, and GS also had missing DNA. In the GS samples, only one individual was missing, and all other GS individuals were marked as *M. edulis*. Thus, the missing sample was most likely another *M. edulis*. However, both RW and HI were missing multiple samples, and both locations consisted of all three *Mytilus* species. The missing information could therefore change the species proportions at these sites.

The shift in 18 years from 2001 to 2019 from a gradient of *M. edulis* in the south to *M. trossulus* in the north to complete dominance of *M. edulis* in the GOM represents a loss of biodiversity that may cause changes in the larger ecosystem due to the mussels' position as prey to a keystone predator, the common sea star (Borthagaray and Carranza, 2007). If the current pattern of *M. trossulus* and hybrid loss continues, even current refuges like HI, PC, RW, and RE may become unsuitable within the next few decades, leading to virtually complete extirpation of *M. trossulus* genes from the GOM (Hayhurst and Rawson, 2009; Rawson et al., 2001). If this occurs, the biodiversity within the entire GOM rocky intertidal is likely to decrease as a result of bottom-up influence (Borthagaray and Carranza, 2007; Menge, 1976). Future study of this region should provide more insight on this loss of biodiversity and serve as predictions for the upcoming effects on other marine ecosystems experiencing warming at a slower rate than the GOM.

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FIGURES

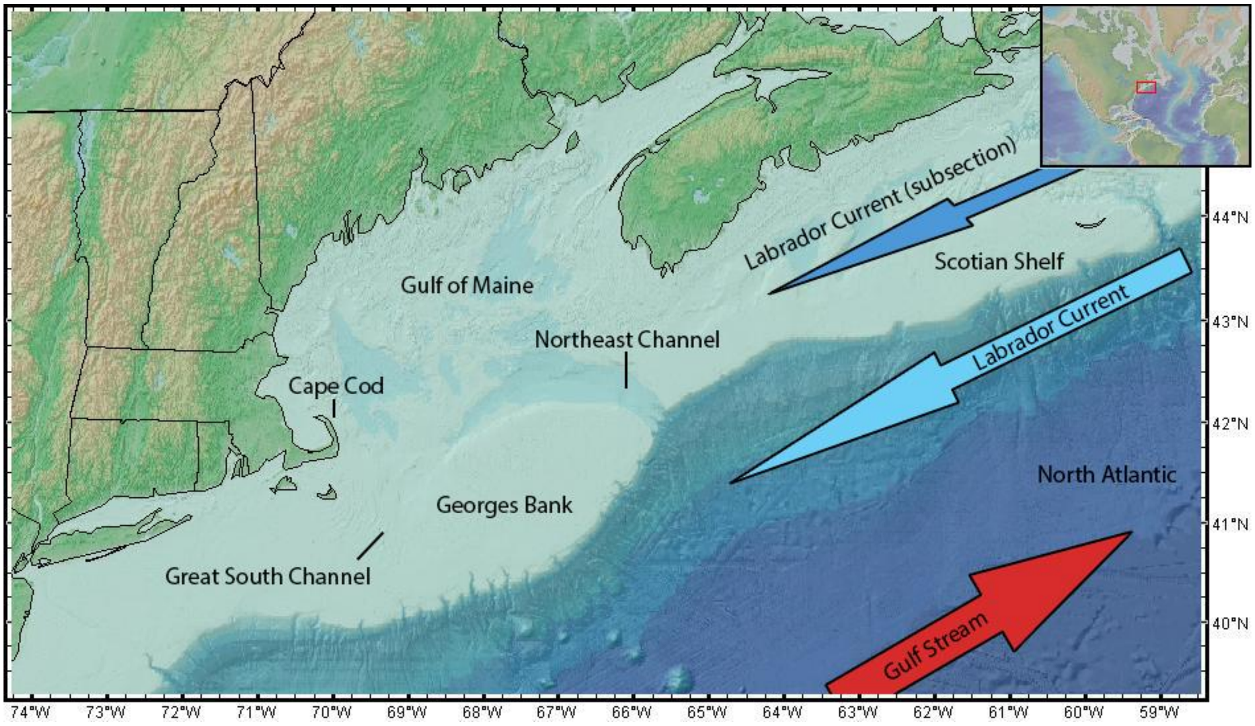
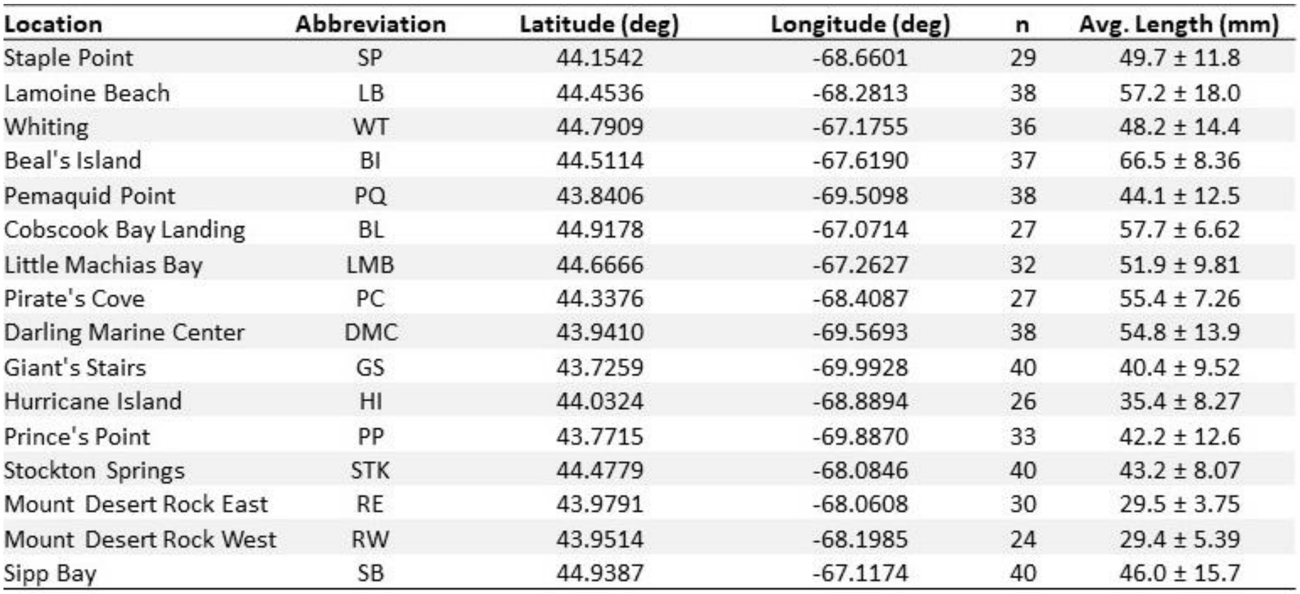


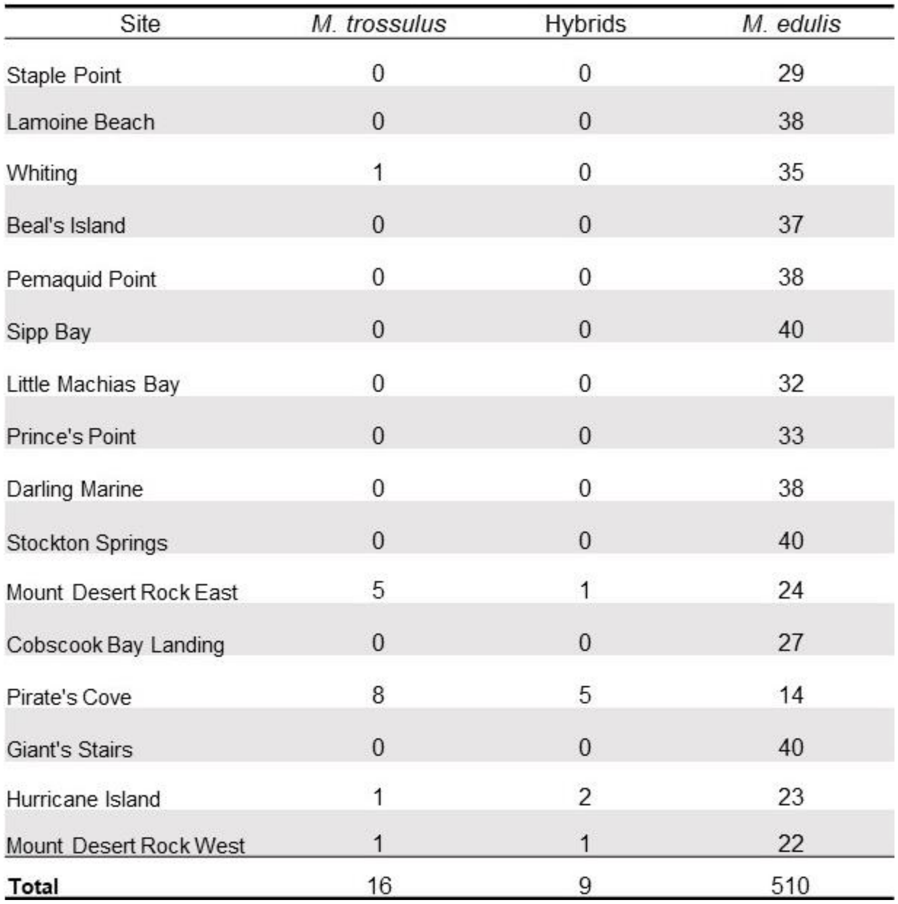
Figure 1. Map of Gulf of Maine region, with the warm Gulf Stream, two portions of the cold Labrador current, and key topographic features labeled. Figure made using GeoMapApp (www.geomapapp.org)

Table 1. Coordinates, sample size values, and size averages for 16 sampling sites of *Mytilus* spp. along the Gulf of Maine. Samples were collected manually in June 2019. Average length (mm) is standard deviation values are reported.



Location	Abbreviation	Latitude (deg)	Longitude (deg)	n	Avg. Length (mm)
Staple Point	SP	44.1542	-68.6601	29	49.7 ± 11.8
Lamoine Beach	LB	44.4536	-68.2813	38	57.2 ± 18.0
Whiting	WT	44.7909	-67.1755	36	48.2 ± 14.4
Beal's Island	BI	44.5114	-67.6190	37	66.5 ± 8.36
Pemaquid Point	PQ	43.8406	-69.5098	38	44.1 ± 12.5
Cobscook Bay Landing	BL	44.9178	-67.0714	27	57.7 ± 6.62
Little Machias Bay	LMB	44.6666	-67.2627	32	51.9 ± 9.81
Pirate's Cove	PC	44.3376	-68.4087	27	55.4 ± 7.26
Darling Marine Center	DMC	43.9410	-69.5693	38	54.8 ± 13.9
Giant's Stairs	GS	43.7259	-69.9928	40	40.4 ± 9.52
Hurricane Island	HI	44.0324	-68.8894	26	35.4 ± 8.27
Prince's Point	PP	43.7715	-69.8870	33	42.2 ± 12.6
Stockton Springs	STK	44.4779	-68.0846	40	43.2 ± 8.07
Mount Desert Rock East	RE	43.9791	-68.0608	30	29.5 ± 3.75
Mount Desert Rock West	RW	43.9514	-68.1985	24	29.4 ± 5.39
Sipp Bay	SB	44.9387	-67.1174	40	46.0 ± 15.7

Table 2. Species distribution of *Mytilus* from 16 locations along the coast of the Gulf of Maine from 2019. Specimens were manually collected by boat, processed, and shipped to Rollins College for genetic analysis and species identification using two genetic markers, SOD and TAUT, and PCR.



Site	*M. trossulus*	Hybrids	*M. edulis*
Staple Point	0	0	29
Lamoine Beach	0	0	38
Whiting	1	0	35
Beal's Island	0	0	37
Pemaquid Point	0	0	38
Sipp Bay	0	0	40
Little Machias Bay	0	0	32
Prince's Point	0	0	33
Darling Marine	0	0	38
Stockton Springs	0	0	40
Mount Desert Rock East	5	1	24
Cobscook Bay Landing	0	0	27
Pirate's Cove	8	5	14
Giant's Stairs	0	0	40
Hurricane Island	1	2	23
Mount Desert Rock West	1	1	22
Total	16	9	510

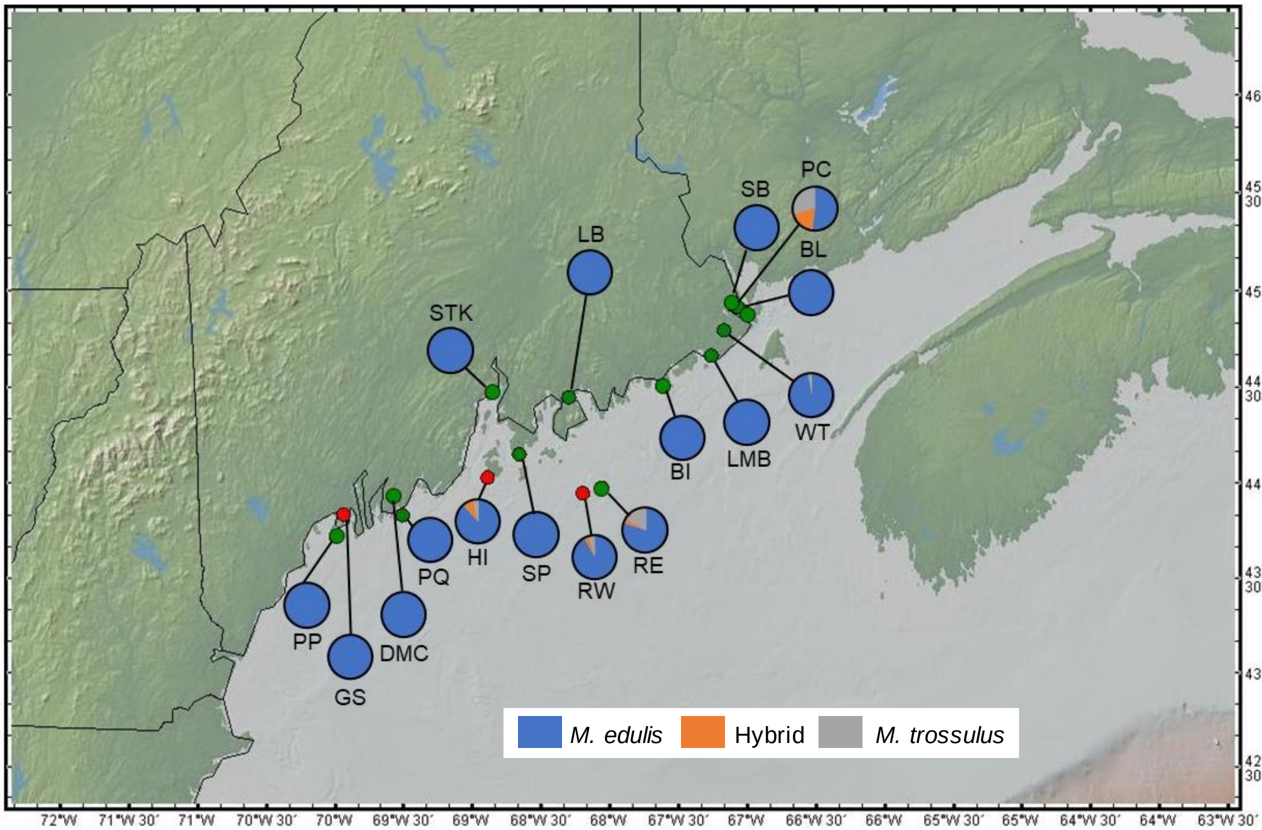


Figure 2. Geographic distribution of *Mytilus* species showing the dominance of *M. edulis* at sixteen locations along the Gulf of Maine in pie charts. Species was determined using two genetic markers and PCR on isolated DNA from mantle tissue. Green dots represent completed datasets, while red dots represent incomplete sets. Map created with GeoMapApp (geomapapp.com).

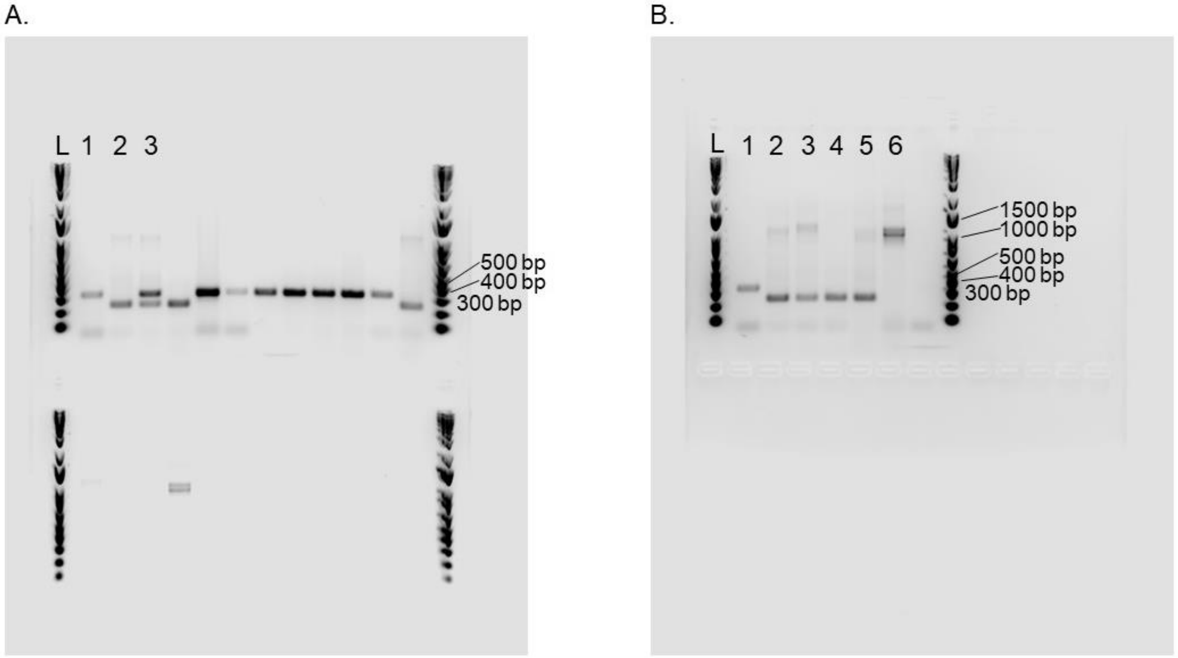


Figure 3. 1.5% agarose gel electrophoresis products showing (A) the appearance of *Mytilus edulis, Mytilus trossulus,* and a hybrid, respectively, in the first three wells, and (B) three samples displaying large bands indicating an unidentified allele in the SOD marker (wells 2, 3, 6). Gels were stained with ethidium bromide and imaged under UV light. Ladder used was Invitrogen 1kb Plus DNA Ladder, but did not function optimally resulting in "smiling".

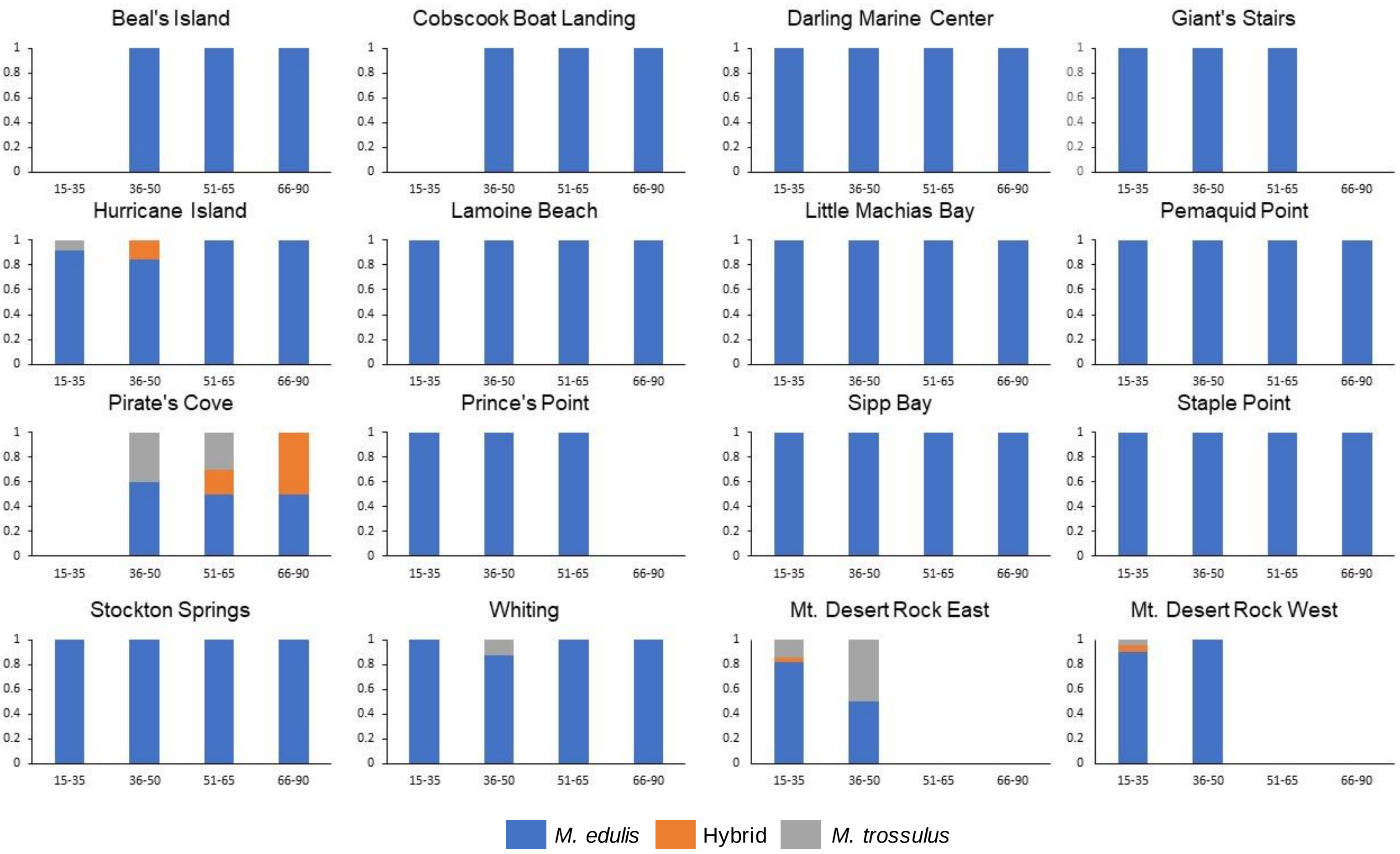


Figure 4. Frequency chart for *Mytilus spp.* sampled from 16 locations along the Gulf of Maine. Size, indicated on the x-axis, was defined as shell length (mm) and grouped into four bins. Frequency is noted on the y-axis.

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