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Examination Of Larval Transport, Distribution And Hybridization Patterns Of Two Blue Mussel Species Mytilus Edulis And M. Trossulus In Machias Bay, Maine

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EXAMINATION OF LARVAL TRANSPORT, DISTRIBUTION AND HYBRIDIZATION PATTERNS OF TWO BLUE MUSSEL SPECIES *MYTILUS EDULIS* AND *M. TROSSULUS* IN MACHIAS BAY, MAINE

BY

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THESIS

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ABSTRACT

Using an interdisciplinary approach, this study examined the population connectivity of the blue mussel *Mytilus trossulus* within Machias Bay, Maine, near its inshore southern boundary range. The Eastern Maine Coast Current (EMCC) flows southwestward along the northern Gulf of Maine coastline, just outside of Machias Bay and is a potential pathway for *M. trossulus* larvae. This study compared results to two historical studies that assayed *M. trossulus* and *M. edulis* species distributions to evaluate any temporal changes. Both similarities and differences were found in species composition in Machias Bay. Historical data suggested that the lower Machias Bay was supplied by larvae via the EMCC, and that conclusion was consistent with this study due to similar species composition in the lower bay. However, in upper Machias Bay, this study found a complete shift in species composition. Since earlier sampling, a *M. trossulus* population has become established in the upper bay and appears to be maintained by self-seeding via locally produced larvae. Additionally, hybrids between *M. trossulus* and *M. edulis* are now abundant in upper Machias Bay, likely due to a breakdown in conspecific sperm precedence at low densities of *M. trossulus.* The persistence of hybrids will probably be dependent upon the size of future *M. trossulus* populations.

INTRODUCTION

Evaluating population connectivity is central to understanding the local and metapopulation dynamics of sessile and sedentary invertebrates (Hastings & Harrison, 1994; Cowen *et al*., 2007). Connectivity within these metapopulations is generally established early in ontogeny by the movement of pelagic larvae. Larval dispersal is dependent upon the interaction of physical transport mechanisms such as buoyancy-, tidally-, and wind-driven currents (Cowen *et al*., 2006; Levin, 2006; Pineda *et al*., 2007) with biological mechanisms such as larval behavior (Cowen *et al*., 2007).

Population connectivity can vary in both space and time, due to variability in currents and circulation patterns. In coastal systems, buoyancy-driven currents are controlled by freshwater fluxes that change salinity conditions (Hetland and Signell, 2005). Variation in freshwater flux due to changes in river discharge can result in the advection of buoyancy-driven currents further offshore (Pineda *et al*., 2007). Such offshore movement can disrupt the alongshore currents that are a primary determinant of larval dispersal, thus affecting population connectivity. Also, tidally-driven currents are subject to increased mixing within coastal systems with increasing tidal amplitudes (Roegner 2000). Increased vertical and horizontal mixing within tidal currents can enable onshore transport of larvae during flood events (Tilburg *et al*., 2012). On the other hand, the amplitude of the tides and strength of the tidal velocities can also play a restrictive role in population connectivity. For example, increased vertical mixing associated with strong tidal velocities can result in minimal vertical stratification of alongshore currents that may restrict alongshore currents to lines of constant depth and hence prevent onshore movement (e.g. Kelley and Chapman, 1988). Coastal geography can also play a role in directing or restricting flows since alongshore currents follow the general shape of coastlines. However, the presence of bays, rivers and capes can alter the flow patterns of alongshore currents, at times resulting in disconnections of larval transport alongshore. Topography of an area can also cause spatial variation in dispersal and retention of larvae in tidal-driven currents (Sponaugle *et al*., 2002; Shanks *et al*., 2003). Wind-driven currents can alter overall connectivity patterns. The directionality of a wind event can affect connectivity patterns by shifting alongshore currents either on- or offshore via upshelf or downshelf winds (Epifanio and Garvine, 2001). On the east coast, upshelf winds (i.e. winds blowing in the upshelf direction) create upwelling that pushes currents offshore, thus reducing onshore/offshore larval exchange. However, during downshelf winds, downwelling pushes currents onshore and increases larval exchange with near shore waters (Blanton *et al*., 1989; Tilburg *et al*., 2005). In addition to the directionality of a wind event, event duration affects larval transport pathways by increasing opportunities for secondary circulation patterns such as fronts and eddies to occur (Shanks *et al*., 2003). The secondary circulation patterns can cause currents (and the larvae within these currents) to temporarily flow in the opposite direction of the long term current, thus resulting in alterations in the direction of larval transport.

Population connectivity can break down at species range boundaries due to abrupt changes in abiotic factors (i.e., temperature and circulation; Caughley *et* *al*., 1988) or variation in biotic factors (i.e., competition, predation, or facilitation; Caley *et al*., 1996). Range boundaries are often created by a combination of both biotic and abiotic factors. Abiotic range boundaries are typically characterized by a strong spatial gradient creating a dispersal barrier that may result in subpopulations that are prevented from mixing. For example, distribution boundaries may encompass areas of drastic alongshore temperature change such as those associated with the offshore movement of the Gulf Stream along the North Carolina coastline (Cerame-Vivas and Gray, 1966; Pielou, 1979; Roy *et al*., 1994; Gaylord and Gaines, 2000; Stanford *et al*., 2006; Blanchette and Gaines, 2007). In contrast, biotic interactions may occur within the confines of the suitable abiotic range boundary of a species (Sexton *et al*., 2009). For example, competition with other invertebrates in an area of finite suitable habitat can result in a biotic range boundary (Case *et al*., 2005).

Both abiotic and biotic factors can cause range boundaries to be dynamic. If abiotic and biotic conditions change, range boundaries may shift over time. For example, some abiotic factors such as the location of frontal boundaries may change within a season or with weather events (Possingham and Roughgarden, 1990; Gaylord and Gaines, 2000). Variable physical conditions, such as tidal mixing, water temperature and density (Pettigrew *et al*., 2005), can cause range boundaries formed by frontal boundaries to break down. These breakdowns of flow can result in episodic population connections where none normally exist (Gaylord and Gaines, 2000). A biotic factor that could cause a shift in a range boundary is a predator-prey interaction (Sexton *et al.,* 2009). For example, a migratory predator consuming a non-migratory prey may result in the temporary expansion of the prey range boundary.

In this study, I use the northern blue mussel, *Mytilus trossulus,* as a study system to explore larval transport processes at a range boundary via an interdisciplinary approach. My study site was Machias Bay, within the Gulf of Maine, which is a semi-enclosed continental shelf sea (Townsend *et al*., 1987) located in the northwest Atlantic Ocean. Machias Bay is the inshore southern boundary range for *M. trossulus* (Hayhurst & Rawson, 2009). Machias Bay is also occupied by *M. edulis*, a closely related congener of *M. trossulus* (Hayhurst and Rawson, 2009). Hybridization can occur among these congeners (Gosling, 1992) and some hybrids have been documented within Machias Bay (Hayhurst and Rawson, 2009). Machias Bay is influenced by tidally-driven flow and the buoyancy-driven Eastern Maine Coastal Current (EMCC; Figure 1), which is a potential pathway for *M. trossulus* larval transport in the Gulf of Maine (Rawson *et al*., 2001; Limbeck, 2003). *Mytilus* populations in Machias Bay have been previously studied (Rawson *et al.,* 2001; Hayhurst and Rawson, 2009), allowing a comparison of current and historical data to assess temporal changes in species distribution.

This study sought to (1) identify the relative composition of individual *Mytilus* populations within Machias Bay, (2) evaluate temporal changes in population composition between 1999 and 2011, and (3) examine the influence of the EMCC on the distribution of larvae within Machias Bay. The population composition was determined by collecting adult *Mytilus* mussels at several sites within Machias Bay and genotyping each mussel. Present and historical population compositions were compared to determine any temporal changes. Simultaneous temperature and salinity sampling at the collection sites determined physical conditions within Machias Bay and permitted evaluation of potential EMCC incursions. To assess the influence of the EMCC on the distribution of larvae within Machias Bay, plankton tows were conducted to quantify larval concentrations at the time of physical measurements. The results suggested that two larval sources are now supplying the Machias Bay *Mytilus* metapopulation.

MATERIALS AND METHODS

a. Study Area

Machias Bay is a relatively shallow bay with heterogeneous bathymetry, located in the northern portion of the Gulf of Maine. The depth is <3m (MLW) in the upper bay, but increases to just over 30m at the mouth of the bay. Since Machias Bay has an approximately 4m tidal range, some areas in the upper bay are completely devoid of water at low tide, resulting in a strong exchange of water throughout the bay. The EMCC is a tidally-mixed alongshore current (Townsend *et al*., 1987; Pettigrew *et al.,* 1998), whose waters originate on the Scotian Shelf, bolstered by the St. John and St. Croix Rivers (Hetland and Signell, 2005) and flows southwestward along the Gulf of Maine coast near the entrance of Machias Bay.

Machias Bay was selected due to its location at the inshore southern boundary range of *M. trossulus* and the historical abundance pattern of *M.*

trossulus within the bay. Rawson *et al.* (2001) had previously reported 0% *M. trossulus*/100% *M. edulis* in the upper bay (Machiasport - Site 1 in this study), while Hayhurst and Rawson (2009) reported 30-35% *M. trossulus/*70-65% *M. edulis* (depending on genetic marker) at the mouth of Machias Bay (Starboard Island - Site 5 in this study; Figure 2). Our study sites were chosen to coincide with the two mussel populations sampled by Rawson *et al.* (2001) and Hayhurst and Rawson (2009), with the addition of three sites between their two original sites. Four sites were located on the western shore, and one site was located on the northeastern shore (Figure 2). The eastern shore of Machias Bay contains few mussel populations due to the prevalence of soft sediment. Security issues prevented access to a military base and thus lower bay sites on the rockier portions of the eastern shore.

b. Study Species

In the Western Atlantic, *M. trossulus* ranges from Labrador to Maine (Figure 1) and *M. edulis* ranges from Newfoundland to North Carolina (Maloy, 2001). As is typical of benthic marine invertebrates in the northern temperate zones, the mussel reproductive cycle is seasonal (Maloy *et al.,* 2003). Spawning of *Mytilus* species in the northwest Atlantic is triggered by an increase in water temperature in the spring (Maloy *et al.,* 2003). After spawning, *Mytilus* larvae are then transported via currents. Larval development lasts 30 days or more prior to settlement (Rawson *et al.,* 2004). Developmental stages of *Mytilus* consist of the trochophore and the veliger stages (including prodissoconch I, prodissoconch II, eyed larvae and pediveliger). At around 300 μm, the planktonic stage ends for the pediveliger larvae and settlement occurs (Widdows, 1991). Neither juvenile nor adult *M. trossulus* and *M. edulis* are morphologically distinguishable. Genetic markers, however, can be used to identify the two species (Heath *et al.,* 1995; Rawson *et al.,* 1996).

c. **Sampling and Laboratory Analysis**

Three different types of data were collected (physical data, larval concentrations and relative abundance of adult mussels) to evaluate distribution and hybridization patterns in Machias Bay. Water temperature, salinity, wind speed and direction, precipitation and air temperature measurements were performed to determine the physical environment and evaluate the episodic presence of the EMCC within Machias Bay. Larvae were sampled and abundance calculated to determine the potential for larval settlement to result in the establishment of populations at each site. The distribution of larvae would suggest whether adult populations were sustainable within Machias Bay. Finally, adult mussels were collected, counted and genotyped to assess any change in species composition from that documented by Rawson *et al.* (2001) and Hayhurst and Rawson (2009).

i. Physical Data Collection. Temperature was recorded at 10 minute intervals using Pendant temperature loggers (Onset Instruments, Pocasset, Mass.) that were attached to concrete cinderblocks placed at 0.5m depth at all five sites (Figure 2). Additionally, a temperature logger that recorded at 10 minute intervals was placed at an offshore station in the Gulf of Maine (Figure 1) to establish the temperature signal of the EMCC in this study (Figure 3a). Air temperature (Figure 3b) for the Machias Bay region was obtained from National Oceanic and Atmospheric Administration (NOAA) Tides Online website for the Cutler Ferris Wharf location (Figure 1). Precipitation levels (Figure 3c) were obtained from NOAA National Climate Data Center (NCDC) from the Milbridge location (located approximately 50km southwest of Machias Bay; Figure 1). Sea level (Figure 3d) was obtained from NOAA Tides Online for the Cutler Naval Base (Figures 1, 2). Wind speed and direction (Figure 3e) were obtained from NOAA Tides Online for the Cutler Ferris Wharf location (Figure 1). All data were collected for yeardays 165-250 (June 15-September 8, 2011). Sea surface temperature, air temperature, sea level and wind velocities were filtered using a Lanczos filter applied with a cut off frequency of 1/36 hr^{-1} to remove any short-term variation like wind gusts or diel and tidal cycles (Tilburg *et al*., 2012). Correlations were calculated between air temperature and water temperature, wind velocity and sea level and finally wind velocity and sea temperature.

ii. **Larval Collection**. To assess larval abundance at each site, plankton tows were performed from shore at approximately 2-week intervals from June-August 2011. Tows were conducted with a 65 μm net that was 0.5 m in diameter at a depth of 0.5 m in water that was approximately 1 m deep for a tow distance of approximately 150m. The net was held in front and to the side of the human collector to reduce the amount of human disturbance. Each tow was completed in 2-3 minutes. To calculate the water volume sampled during each tow, a flow meter was attached to the net; each tow filtered approximately 120 m^3 of water.

Plankton tows at each site were replicated 3 times on each sampling date. All tows occurred within two hours of high tide within the same tidal cycle to allow for a snapshot profile of larvae in Machias Bay. At the time of collection the tidal velocities would be at the lowest or non-existent due to slack tide which allowed larval concentrations to be sampled without large tidal influences. Sieved samples were preserved in Modified Saline Ethanol (MSE) solution (Miller and Scholin, 2000) until the bivalve larvae could be counted. Counting of the bivalve larvae occurred on a dissecting microscope with species identification according to the method outlined by Fuller and Lutz (1989). Larvae were only identified to the class level Bivalvia. Bivalvia is the majority of the meroplankton composition within the Gulf of Maine (Tilburg *et al.,* 2012). Sampling occurred during known spawning times for the *Mytilus* species in the northwest Atlantic (Maloy *et al.,* 2003) to increase the likeliness of mussel larvae collection. All samples were standardized to a larval density per 100 m^3 for comparison. Variation in larval density was analyzed with a one-way ANOVA with site as factor and a Tukey's multiple comparisons of means for post-hoc comparisons using the R package statistics (version 2.15; R Core Team, 2012).

iii. **<u>Mussel Collection and Analysis</u>**. Adult mussels were collected at each site to evaluate spatial variation in the species composition of mussel populations within the bay. Samples (n=100) were collected from each site and measured using digital calipers to the nearest 0.01 mm. Mean lengths varied between 43 and 58 mm and size classes were separated into non-mature (<50mm) and mature mussels (>50mm; Rodhouse *et al*., 1986). Mantle tissue samples from each mussel were collected and stored in MSE for species identification using DNA (Henzler *et al*., 2010). QIAamp DNA Mini Kits (QIAGEN Valencia, CA) were used to isolate whole genomic DNA from the mantle tissue.

A portion of the gene encoding the mussel polyphenolic adhesive protein (*Glu-5*) was amplified according to Rawson *et al*. (1996). PCR reactions consisted of 1X PCR buffer, 200µM dNTPs, 4 mM $MgCl_2$, 0.2µM forward primer, 0.1µM of each reverse primer, 400-600ng/µL of DNA template and 1 unit of Taq Polymerase (New England Biolabs, Inc., Massachusetts) for a total reaction volume of 26µl. Samples were amplified under the following conditions: one cycle at 94°C for 10 minutes, 36 cycles of 94°C for 30 seconds, 52°C for 30 seconds and 72°C for 2 minutes, with a final extension at 72°C for 5 minutes. The resultant PCR product was size fractioned on a 2% agarose-TBE gel and stained with ethidium bromide. Successful amplifications were expected to yield a 120-bp fragment in *M. trossulus* and a 180-bp fragment in *M. edulis.* Putative hybrids yielded both 120-bp and 180-bp fragments.

An Internal Transcribed Spacer (*ITS*) PCR-based restriction fragment length polymorphism (RFLP) protocol was used to differentiate species and hybrids (Heath *et al*., 1995). PCR reactions are the same as the *Glu-5* except for the use of 0.2µM reverse primer and a total reaction volume of 20µl. Thermal cycling conditions consisted of one cycle at 94°C for 10 minutes, 36 cycles of 94°C for 30 seconds, 50°C for 30 seconds and 72°C for 2 minutes, with a final extension at 72°C for 5 minutes.

ITS PCR products (10µl) were fragmented in 1mM NEB Buffer #4, 10mM 100X BSA (New England Biolabs, Inc., Massachusetts), and 8 units of *Hha*I (New England Biolabs, Inc., Massachusetts), with overnight digestion at 37°C. The resulting products were size separated on a 2% agarose TBE gel stained with ethidium bromide. The expected digestion profiles for *M. edulis* were a 450-bp and 180-bp fragment and for *M. trossulus* a 280-bp and 180-bp fragment. Hybrids were expected to yield three fragments at 450-bp, 280-bp and 180-bp. Variation in relative frequency of *M. edulis, M. trossulus,* and hybrids among sites at the *Glu-5* and *ITS* loci was evaluated using *G*-tests and a correction was done for multiple comparisons (Sokal and Rohlf, 1981). A chi-square analysis was performed to determine if there was a change in species distribution (including hybrids) between size classes within a site.

RESULTS

a. Physical Dynamics

Figure 3 provides a comprehensive physical profile of the bay that includes sea surface temperature, air temperature, precipitation, sea level, and wind velocities. Examination of the sea surface temperature (Figure 3a) revealed a persistent along-bay spatial gradient. Overall, Site 1 (located up-bay) was the warmest and the Sites 3 and 5 were the coolest. Site 3 was colder than the two adjacent sites and comparable in temperature to Site 5, which was the site closest to EMCC temperatures.

An uncharacteristic seven-day warming period occurred throughout the whole bay during yeardays 203-210 (Figure 3a). There were 9 individual cooling events (yeardays 183, 190, 194, 210, 215, 223, 227, 235 and 244) where cold water may have entered the bay and lowered the temperature of the upper bay water (Site 1 and 2). The warmest air temperature occurred during yeardays 198-199 (Figure 3b), just prior to the warming of the bay waters. A negative correlation between air temperature and water temperature was found within each site in Machias Bay with the strongest negative correlation at Site 5 (r=-0.4168; P<0.001). The greatest precipitation occurred on yearday 219 (Figure 3c). Sea level varied with both precipitation and winds. Sea level during the warming period (Figure 3d) remained around the average sea level of 2.4 m found within the time frame of the study. The largest increases in sea level occurred on yeardays 175, 219, 221 and 240. The largest wind event was northeasterly (i.e. from the northeast) at yearday 241 (Figure 3e). During this wind event, one of the largest decreases in sea level occurred. Another large northerly wind event occurred at yeardays 200-204, just before and at the beginning of the seven-day warming period. Examination of the correlation between wind velocity and sea level (Figure 4) reveals the highest correlation (r=0.3503) occurred at 110° (0° is defined as true north), or a southeasterly wind. Wind velocity was also correlated with sea temperature. The highest negative correlations (Table 1) were found in the upper bay (Sites 1 and 2; r = -0.2463 and -0.5647, P<0.001) during easterly winds. Sea surface temperatures at the other sites were negatively correlated with westerly (Site 3 and 4; r= -0.1551 and -0.1676, P<0.001) and southwesterly winds (Site 5; r= -0.1865, P<0.001).

b. <u>Criteria for Classifying EMCC Intrusion Events</u>

The EMCC is known to periodically bring cold, nutrient rich water into the shallow Machias Bay (Hayhurst and Rawson, 2009; Figure 1). Ekman transport and/or downwind frictional transport during easterly, southeasterly or southerly wind events could result in the transport of the EMCC into Machias Bay and be classified as an EMCC intrusion event which would also cause a rise in sea level within the bay. However, the physical criteria are not able to definitively determine the presence of the EMCC in Machias Bay. Consequently, I developed a set of criteria that are consistent with the observations and probable physical mechanisms of an EMCC incursion event within Machias Bay. The first criterion is based on historical temperature patterns with the EMCC. Pettigrew *et al*. (1998) found that the across-shelf temperature range of the EMCC was approximately 3°C near Machias Bay. There is no set temperature for the EMCC; however, temperatures are normally several degrees cooler than the surrounding Gulf of Maine waters thus creating a thermal gradient (Pettigrew *et al.,* 1998; Pettigrew *et al*., 2005). Consequently, my criteria are (1) a difference in temperature from Site 1 to Site 5 that is less than or equal to 3°, (2) the presence of an easterly, southeasterly or southerly wind event, and (3) increased sea level within Machias Bay. Note that no EMCC events could be confirmed before yearday 180 because the temperature loggers in Machias Bay were deployed on yearday 180.

Wind is the primary mechanism that can cause across-shelf transport of the EMCC in the Gulf of Maine (Xue *et al*., 2000). This onshore movement causes subsequent increases in sea level. The highest correlation between sea level and wind occurred at a wind direction of 110°, or a southeasterly wind (Figure 4). Because of the overall shallow bathymetry in Machias Bay, the water is well mixed even during a flood tide. If an easterly, southeasterly, or southerly wind occurs during a flood tide, mixing would be possible throughout the whole bay. Due to the orientation of the entrance of Machias Bay along the coastline, a easterly, southeasterly or southerly wind directions are not necessarily intuitive. However, the shallow nature of this region results in transport of waters to be less than the predicted 90° due to a combination of Ekman and downwind transport allowing EMCC waters to be transported into Machias Bay. If the EMCC entered the bay due to a wind event, the sea level should increase.

From the temperature data collected in this study, nine events in which the range of water temperature from Site 1 to 5 was ≤3° in Machias Bay were initially identified as possible EMCC intrusion events (Table 2). Days 183, 223, 235 and 244 were ruled out as EMCC intrusion events because a decrease in sea level was recorded. Day 190 was eliminated as an EMCC event because the wind direction did not allow for the transport of the EMCC within Machias Bay.

The remaining four putative EMCC events (yeardays 194, 210, 215 and 227) that occurred during the course of this 85 day study might influence the distribution of larvae within Machias Bay. There are two possible ways for the EMCC to enter Machias Bay: as a wind event or as a breakdown of a frontal boundary. Since the data collected in this study are not adequate to identify the breakdown of a frontal boundary, it is possible that more EMCC intrusions actually occurred. Both of these mechanisms of the EMCC moving into Machias

Bay are consistent with the patterns of sea surface temperatures. This could suggest that more than four of the nine identified events where the range of water temperature from Site 1 to 5 was ≤3° were EMCC incursions. Sea surface temperatures (Figure 3a) in Machias Bay showed thorough mixing throughout Sites 5-3 with episodic mixing occurring in Sites 2-1.

c. Larval Distributions

Overall, larval densities were higher (Figure 5) at the mouth of the bay (Figure 2, Site 5) and tapered off to the lowest density toward the upper bay (Site 2) but were higher again at Site 1. All sites showed evidence of varying temporal patterns, with multiple peaks in larval density at different times. Larval densities during the first sample period (yeardays 165-166) were extremely low at all sites, although larval densities at Site 5 were significantly higher than at Sites 2-4 (one-way ANOVA, $P < 0.005$, Tukey post-hoc Multiple Comparison Pairwise (MCP) test). During the second larval sampling period (yeardays 177-179), the highest larval density of this study was observed at Site 5 and this density was significantly higher than at Sites 1, 3 and 4 (one-way ANOVA, $P < 0.00005$, Tukey post-hoc MCP test); note Site 2 was not sampled during this interval. During the third larval sampling period (yeardays 192-194), the highest larval density relative to other sites occurred at Site 4 and was significantly greater than density at Sites 1-3 (one-way ANOVA, $P < 0.0005$, Tukey post-hoc MCP test). During the final sample interval (yeardays 206-207), larval density at Site 5 was again significantly higher than at Sites 1-4 (one-way ANOVA, $P < 0.0001$, Tukey post-hoc MCP test).

d. Mussel Distributions

The genetic markers *Glu-5* and *ITS* were used to determine the relative abundance of settled *M. edulis*, *M. trossulus* and hybrids. Across both size classes (juvenile and adult) and genetic markers, *M. trossulus* appeared to be more abundant in the upper (Site 1 and 2) and lower (Site 5) bay and less common in the mid-bay (Site 3 and 4; Figures 6 and 7). Hybrids were more abundant in the upper bay (Figures 4 and 5) for *Glu-5*, however, with the genetic marker *ITS*, hybrids appear to be relatively abundant throughout Sites 1-4 (Figure 7). Very low hybrid relative abundance at the mouth of the bay was observed for both genetic markers. A G-test (Sokal and Rohlf, 1981) revealed that the frequency of the two pure species and hybrids (data from the two size classes combined) differed among sites (df=8, $p<0.005$). Based on the patterns established in the physical and larval sections, an additional G-test was performed comparing frequencies from the two upper bay (Sites 1 and 2, lumped) and the lower bay (Site 5) across both size classes and the genetic frequencies were significantly different (*Glu*-5 and ITS; df=2, $p<0.005$, in both cases).

In order to assess any temporal changes in population composition in Machias Bay, G-tests were used to compare genotypic frequencies from this study to genotype frequencies derived from allele frequency data collected in 2005 (Hayhurst and Rawson, 2009; personal communication from P. Rawson). There was no statistical difference between Site 5 in the present study and Starboard Island in 2005 (geographically the same location as Site 5; g-test, df=2, p>0.05) for both *Glu-5* and *ITS* (Hayhurst and Rawson, 2009). I also compared Site 5 to a site northeast of Machias Bay (South Trescott; Figure 1) in an area that is likely supplied by the EMCC (Rawson *et al.,* 2001) and no statistical difference was found (g-test, df=2, p>0.10) for either genetic marker.

The mussel distribution data were split into two separate size classes; <50mm (non-mature) and >50mm (mature) mussels. A series of chi-square analyses compared species composition between non-mature and mature mussels by site, with the null hypothesis that the distribution of pure species and hybrids did not differ between size classes. Site 2 was the only site that the species distribution between the two size classes was significantly difference (p<0.025 for both *Glu-5* and *ITS,* chi-square analysis; Figure 6 and 7).

DISCUSSION

a. Overview

Several interesting patterns emerged when comparing *M. trossulus* and *M. edulis* populations in Machias Bay to historical data. First, species composition in the lower bay was consistent with historical reports (Hayhurst and Rawson, 2009). However, species composition in the upper bay was found to have shifted from historical patterns (Rawson *et al.,* 2001). I found a *M. trossulus* population established in the upper bay, possibly due to a regional temperature shift, and evidence that mussel populations in the bay are supplied by two different sources of larvae. The formation of hybrids in the upper bay occurred as well, likely due to the breakdown of conspecific sperm precedence. Each of these results is addressed in greater detail below.

b. Similarities to Historical Data

The general hydrodynamics of Machias Bay suggest explanations for the similarities and differences between current and historical species composition (Rawson *et al.,* 2001; Hayhurst and Rawson, 2009). Similarities to historical data were found in the lower Machias Bay. Hayhurst and Rawson (2009) reported a species composition of 30-35% *M. trossulus*/70-65% *M. edulis* and a low frequency of hybrids in lower Machias Bay, specifically at Starboard Island (Site 5 for this study). Although *M. trossulus* frequencies were not as high as in 2009, the current study found *M. trossulus* frequencies at 13%/10% (*Glu-5* and *ITS*) and a low frequency of hybrids (0%/1%; *Glu-5* and *ITS*), suggesting that the larval source has not differed greatly since 2001. Hayhurst and Rawson (2009) suggested that sites directly in the path of the EMCC can be supplied by *M. trossulus* larvae due to the flow path of the EMCC. I found no significant difference in the lower Machias Bay adult population composition between 2011 and 2009 (Hayhurst and Rawson, 2009) providing support that Site 5 is directly supplied by the EMCC.

c. Differences from Historical Data

i. Establishment of *M. trossulus*. To understand the increase in the abundance of *M. trossulus* in upper Machias Bay, the establishment of this northern species needs to be explained. Establishment of *M. trossulus* in the upper bay likely occurred due to the thorough mixing between Sites 5, 4 and 3 combined with episodic mixing between Sites 2 and 1. The observed mixing among Sites 5, 4, and 3 suggests that whatever larval source is supplying Site 5 could potentially and frequently supply larvae to Sites 4 and 3. In addition, any larvae present in the bay during an episodic mixing event could also reach Sites 1 and 2. The potential for larval transport via episodic movement of the EMCC into the upper bay probably led to the initial establishment of *M. trossulus* populations in the upper bay.

Similar to the larval density patterns, water temperatures collected for this study (Figure 3a) suggest that thorough mixing routinely occurs from Site 5 at the mouth of the bay to Site 3 located in the mid-bay, but Sites 1 and 2 are consistently warmer. Sites 1 and 2 are in a portion of the bay that is shallow enough to be almost completely devoid of water during low tide (only a narrow channel remains submerged; Figure 2). There was a negative correlation between air temperature and water temperature within Machias Bay, so atmospheric heat flux is unlikely to be responsible for the variation in water temperature. This means that any warming found in the upper bay is not due to increasing air temperatures.

M. trossulus establishment in the upper Machias Bay is likely due to an EMCC intrusion event. Four episodic EMCC intrusion events occurred throughout the whole bay (including sites 1 and 2; Table 2). Assuming that this mixing pattern has been consistent over time, it is likely that a past intrusion event may have occurred when *M. trossulus* larvae were present in EMCC water.

The warming waters of the Gulf of Maine provide a possible explanation for the establishment of a *M. trossulus* population in the upper Machias Bay following a larval transport event. While changes in circulation can probably be dismissed due to the historical documentation of the EMCC (Brooks, 1985; Pettigrew *et al*., 1998; Pettigrew *et al*., 2005), larval adaptation could explain a shift in populations found in an area. Bayne and colleagues (Bayne, 1972; Bayne *et al*., 1975; Bayne *et al*., 1978) studied the relationship between average thermal exposure of parent *M. edulis* and the physiological tolerances of larval offspring. Results showed that *M. edulis* larvae whose parents were acclimated to low temperature had reduced growth rates at 16°C and above. However, parents acclimated to temperatures around 20°C produced larvae that did not exhibit reduced growth at 16°C and above. Historically, offspring larvae originating from the colder Gulf of Maine waters northeast of Machias Bay likely could not tolerate the warmer temperatures in the upper Machias Bay when transported via the EMCC, and settlement did not occur prior to 1999. Pershing *et al*. (2015) recently determined that the Gulf of Maine on average is warming at 0.03°C per year, higher than the global mean rate of 0.01°C per year. I suggest that *M. trossulus* has now been able to establish a population in the upper Machias Bay due to the increased mean Gulf of Maine temperature. Adult *M. trossulus* that supply larvae in the EMCC have now acclimated to warmer temperature, approaching those found in the upper Machias Bay. Their offspring's larvae can now successfully withstand the average upper Machias Bay temperatures due to the decrease in temperature range between the northern Gulf of Maine and the upper Machias Bay. It is likely that on average the upper Machias Bay is not warming at the same rate as the Gulf of Maine due to decreased mixing between the upper Machias Bay and the rest of Machias Bay. The upper Machias Bay is likely warming at the same rate as the atmosphere, which is slower than the Gulf of Maine (Pershing *et al.,* 2015).

ii. **<u>Maintenance of *M. trossulus*</u>.** The adult distribution data (Figures 6 and 7) suggest different larval sources for the populations within Machias Bay. Species composition, as assessed by both genetic makers, was found to be different in the upper bay (Site 1 and 2) compared to the lower bay (Site 5). Physical support for this difference comes from the water temperature data, which showed decreased mixing occurring between Sites 1-2 and Sites 3-5 (Figure 3a). Larvae were present at each of the sites (Figure 5) in this study, suggesting that each adult population within Machias Bay had a fairly continuous larval source during the 2011 sampling season. Additionally, the density of larvae at Site 1 was consistently higher than at site 2, which represents the end of a declining gradient from Site 5 (Figure 5). Likely, the decrease in larval abundance from Site 5-2 could represent the dilution of larvae arriving from the EMCC. The higher abundance at Site 1 could suggest evidence of a small larvae source in the upper Machias Bay, such as a self-seeding population.

This study suggests that both *M. trossulus* and *M. edulis* were able to achieve maturity and maintain self-sustaining, reproducing populations in upper Machias Bay. Both *M. edulis* and *M. trossulus* were present in each size class, suggesting that both species were able to reach maturity, reproduce successfully and supply larvae to seed the next generation. No ontogenetic shift in species composition was detected except at Site 2, where the pattern suggested a loss of hybrids (not *M. trossulus)* through ontogeny and was consistent with the selective pressures of reinforcement.

The maintenance of the established *M. trossulus* population in the upper Machias Bay is supported by both physical and larval evidence. The decreased mixing occurring between Sites 2 and 3 would cause any larvae produced in the upper bay to stay within the upper bay, resulting in self-seeding for *M. trossulus* populations. The higher larval densities at Site 1 compared to the decreasing trend of larval densities from Sites 5-2 (Figure 5) also suggest the possible presence of a self-seeding population in upper Machias Bay. I suggest that at some time within the past ten years the upper bay did receive larvae from the same source as Site 5 and that *M. trossulus* settlers were deposited in the upper bay, where they formed a self-sustaining population.

iii. **Formation and Possible Maintenance of Hybrids.** This study detected numerous hybrids in the upper Machias Bay, which were absent in the data set of Rawson *et al*. (2001). The formation and replenishment of hybrids within the upper Machias Bay involves two different processes. The establishment of *M. trossulus* population within the upper bay provided an opportunity for hybridization. *M. trossulus* has measurable abundances at each size class indicating that a reproducing and self-seeding population has been present for multiple generations. The bimodal northwest Atlantic hybrid zone exhibits a hybridization rate anywhere from <2% - 26% (Mallet and Carver 1995;

Comesana *et al*., 1999; Rawson *et al*., 2001), and a similar range was found within Machias Bay (Figures 6 and 7).

The process of conspecific sperm precedence reduces the chance of hybridization in mixed species communities. In conspecific sperm precedence, eggs favor conspecific sperm over heterospecific sperm (Howard 1999). Klibansky and McCartney (2014) documented both conspecific sperm precedence occurring in this hybrid zone (~50 km from this study) and a breakdown of this process as well. A breakdown of conspecific sperm precedence in upper Machais Bay probably increased resulting in hybridization.

The efficacy of conspecific sperm precedence depends on the relative abundance of the two species. In upper Machias Bay, where *M. trossulus* are relatively rare, a limited number of *M. trossulus* sperm would be available to fertilize *M. trossulus* eggs and a high level of hybridization would be expected to result. By contrast, in the populations in and upshelf of the EMCC that supply larvae to Site 5, the two species are approximately equally abundant and hybridization is rare. If this explanation is correct, the replenishment of hybrids within the upper Machias Bay is dependent upon the maintenance of the now established *M. trossulus* population. If the *M. trossulus* population continues to thrive, reproduce and increase in abundance in the upper Machias Bay, hybridization will likely decline. With more *M. trossulus* eggs and sperm in the upper Machias Bay, conspecific sperm precedence will be less likely to breakdown causing limited to no hybridization.

In this study the *ITS* genetic marker revealed more pure *M. trossulus* and hybrids than the Glu-5 marker. Toro *et al*. (2004) examined the northwest Atlantic hybrid zone with the same two genetic markers and reported a similar locus-specific pattern (Toro *et al*., 2004), which may be attributed to greater introgression of *ITS* (Comesana *et al*., 1999; Rawson *et al*., 2001). Any time backcrossing occurs, the F2 and greater hybrids are genetically biased in the direction of the backcross. The backcrossing within *M. trossulus* and *M. edulis* is likely why there is variation in abundances between the *Glu-5* and *ITS* genetic markers. Comesana *et al*., (1999) also found that the hybrids consisted of backcrosses that were *M. trossulus*-biased for small mussels and *M. edulis*-biased for large mussels. The size affinity in backcrossing could explain this differences found between the non-mature and mature mussels at Site 2 (Figures 6 and 7) and the overall differences found between the two genetic markers.

d. Implications

There are a few caveats to consider with this study. Larvae could only be identified to the class level (Bivalvia), not species level. This lack of taxonomic resolution did not allow for a determination of the species present within different portions of Machias Bay. Also, this study did not follow a cohort of *M. edulis, M. trossulus* and their hybrids to maturity. Hence apparent differences or similarities in species composition between size classes could have been confounded by different initial recruitment densities. Finally, the generation of the hybrids found in this study could not be identified. If the degree of introgression could be determined, then a better understanding of hybridization patterns would be possible.

There are a few next steps that can be considered for further exploring the population connectivity within Machias Bay. Maintaining sampling of adult mussels, larval concentrations and physical dynamics within Machias Bay would be critical to determine if the species composition is shifting at other sites. To improve this study, it would be helpful to further explore the caveats. First, genotyping larvae from sample sites throughout Machias Bay. Second, following a cohort to determine which species are supplying each site and which species are surviving to reproduce at each site. Thirdly, other genetic markers could be used to determine hybrid generations and therefore the introgression patterns across the bay. Finally, further research should be considered in bays surrounding Machias Bay if similar physical dynamics occur to determine if the results of this study can be duplicated.

In conclusion, the physical data suggest that the EMCC is episodically present throughout the entirety of Machias Bay. Combining all physical factors (i.e. temperature, wind, sea level) enables a better understanding of the study area and the effects that physical processes have on biological systems. The physical, larval and genetic measurements are consistent with all populations originating from larvae that entered via the EMCC, but now some populations appear to be self-seeding, or exchange larvae only within portions of the bay. The self-seeding populations in the upper bay have developed due to the lack of physical mixing between the upper bay and the rest of Machias Bay and the spatial variation in larval distribution and species composition. The similar species composition of different size classes suggests recruitment is occurring and the populations are reaching reproductive maturity and are self-sustaining. Range boundaries have likely caused the formation of hybrids in the upper Machias Bay due to the breakdown of conspecific sperm precedence. However, the establishment of the *M. trossulus* populations within the upper Machias Bay will determine if the hybrids get replenished or if conspecific sperm precedence is reestablished in the upper Machias Bay. Overall, the conclusions made in this study would not have been able to been made without both larval and adult mussel data. An interdisciplinary approach with physical dynamics, biological factors and genetic components were essential to allow for a complete analysis of the population connectivity for the area of Machias Bay.

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FIGURES

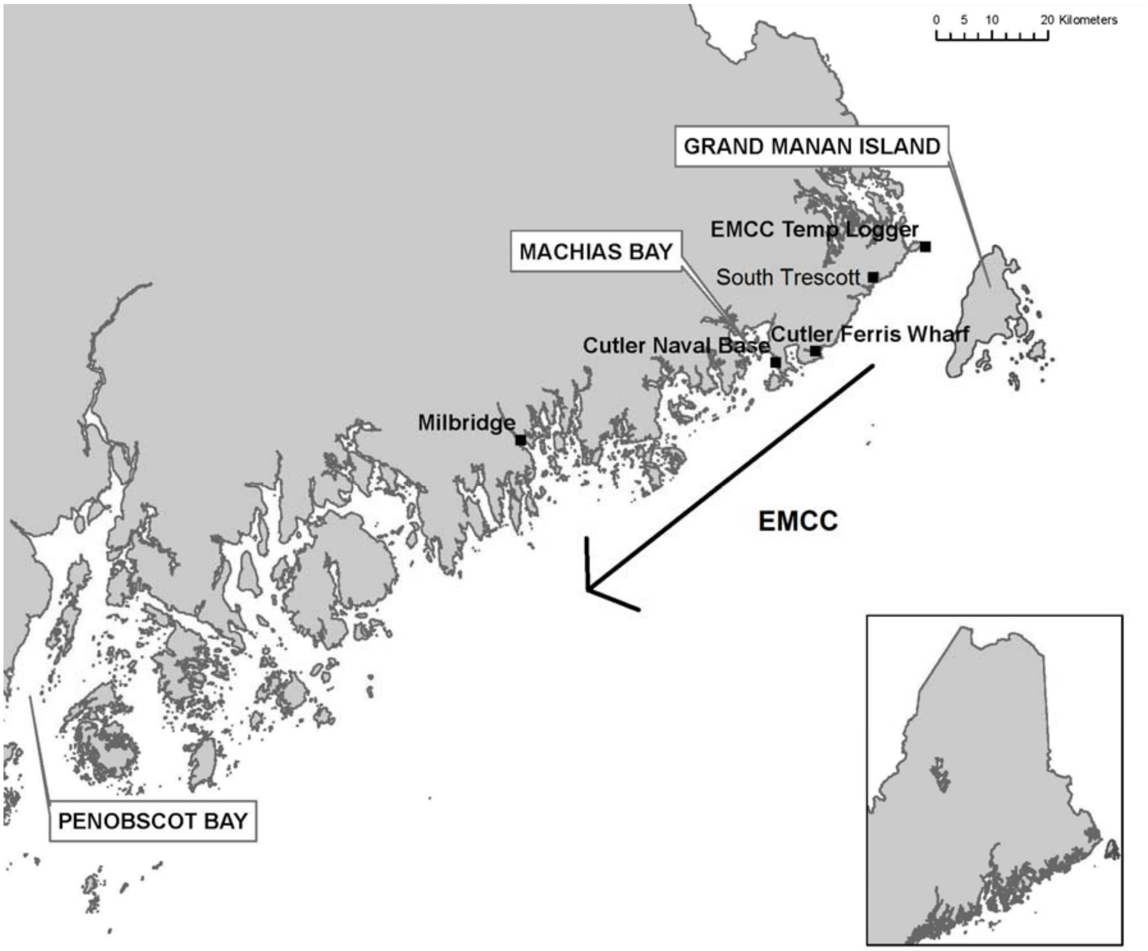

Figure 1: Location of the Gulf of Maine in reference to the eastern coast of the United States of America and the location of the Eastern Maine Coastal Current (EMCC) in reference to Machias Bay.

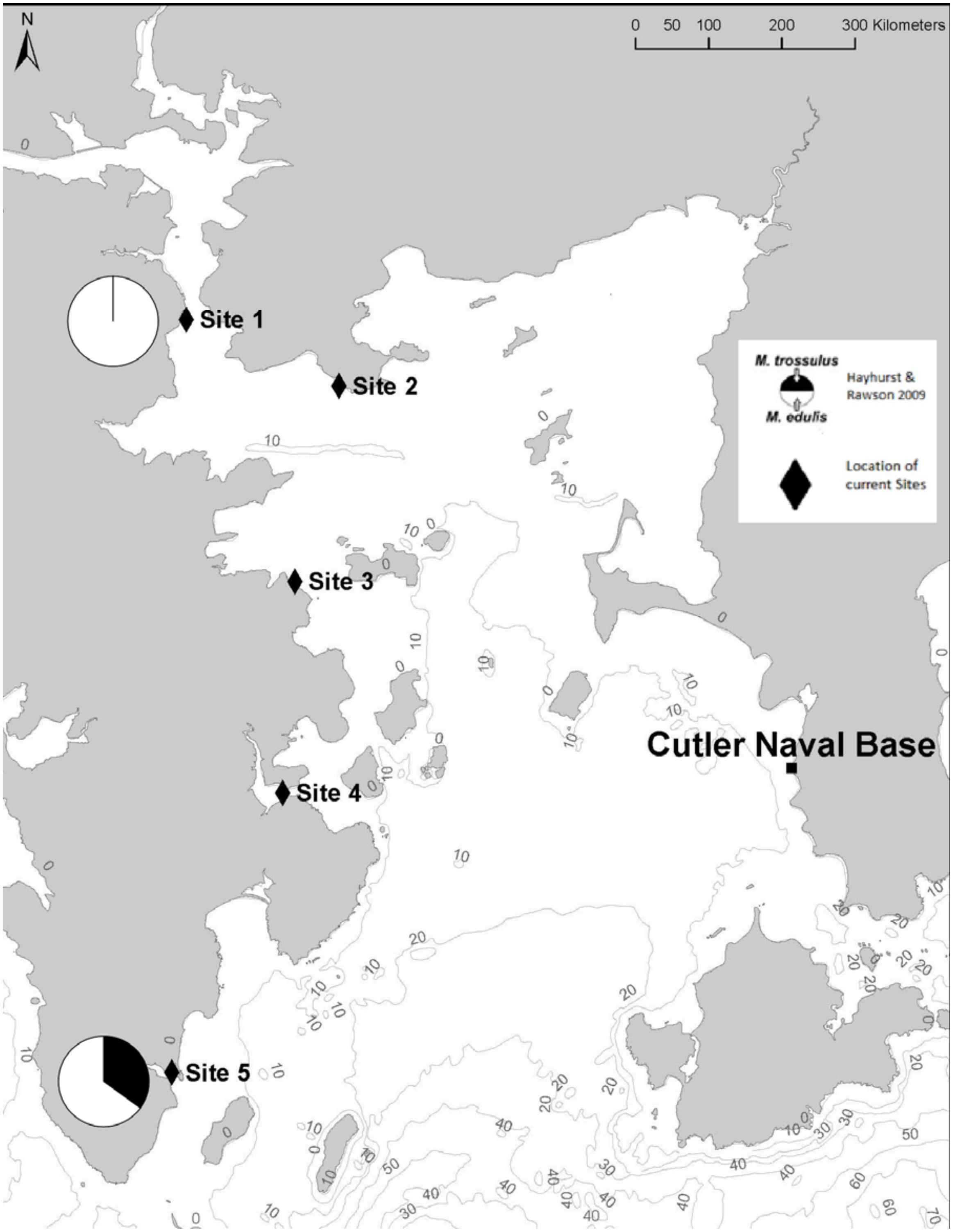

Figure 2: Site Map. Black diamonds indicate current study's locations. Black and white pie diagrams represent the relative species composition in historical samples from Hayhurst and Rawson (2009). Note Site 1 is Machiasport and Site 5 is Starboard Island mentioned in text. Contour lines are displayed in meters.

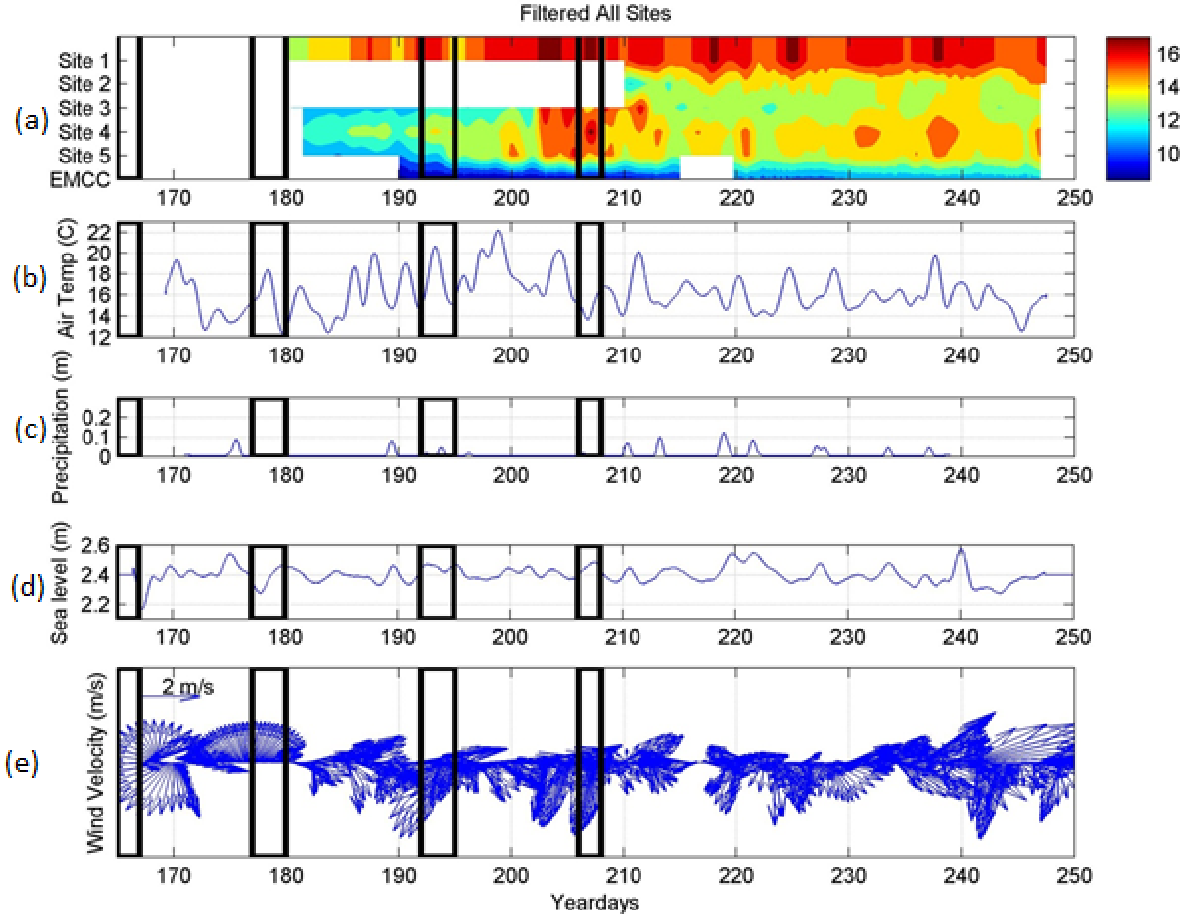

Figure 3: (a) Filtered sea surface temperature for Machias Bay, Maine. Site 1 is located in the upper bay and sites increase moving toward the mouth of the bay at Site 5. The EMCC is located at the bottom of the Figure for reference. Four black boxes are present indicating days that larval sampling occurred. (b) Air temperature filtered (°C), (c) precipitation (m), (d) sea level (m), (e) wind speed and direction for Machias Bay (m s^{-1}).

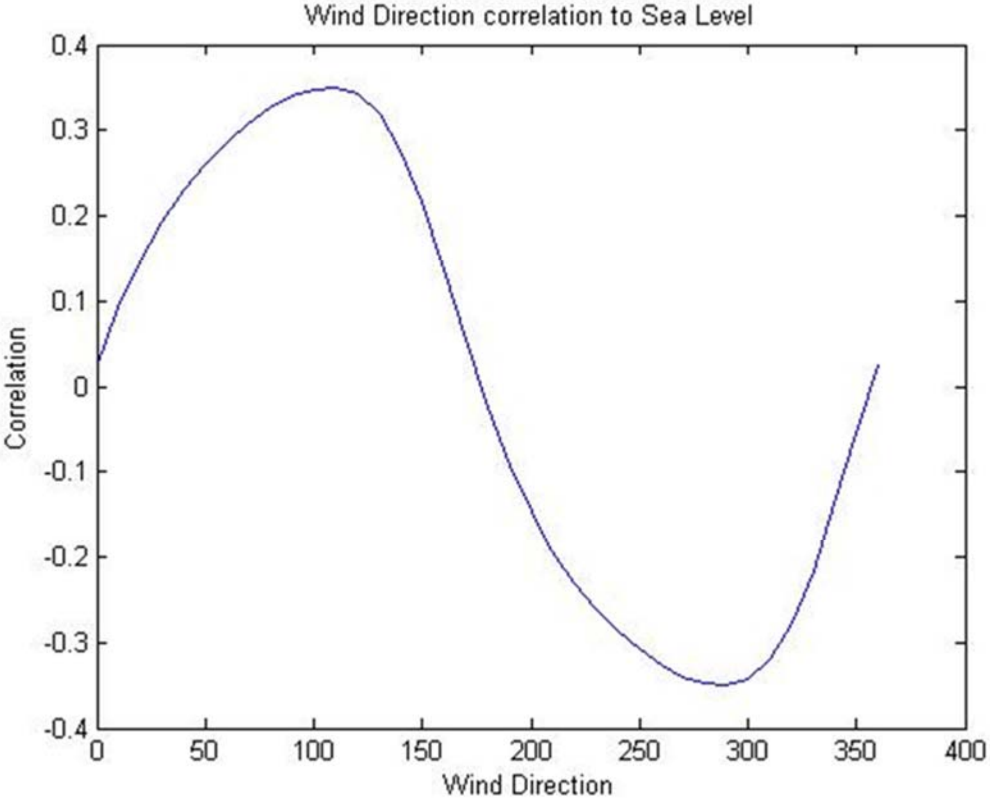

Figure 4: Correlation between Wind Direction and Sea Level

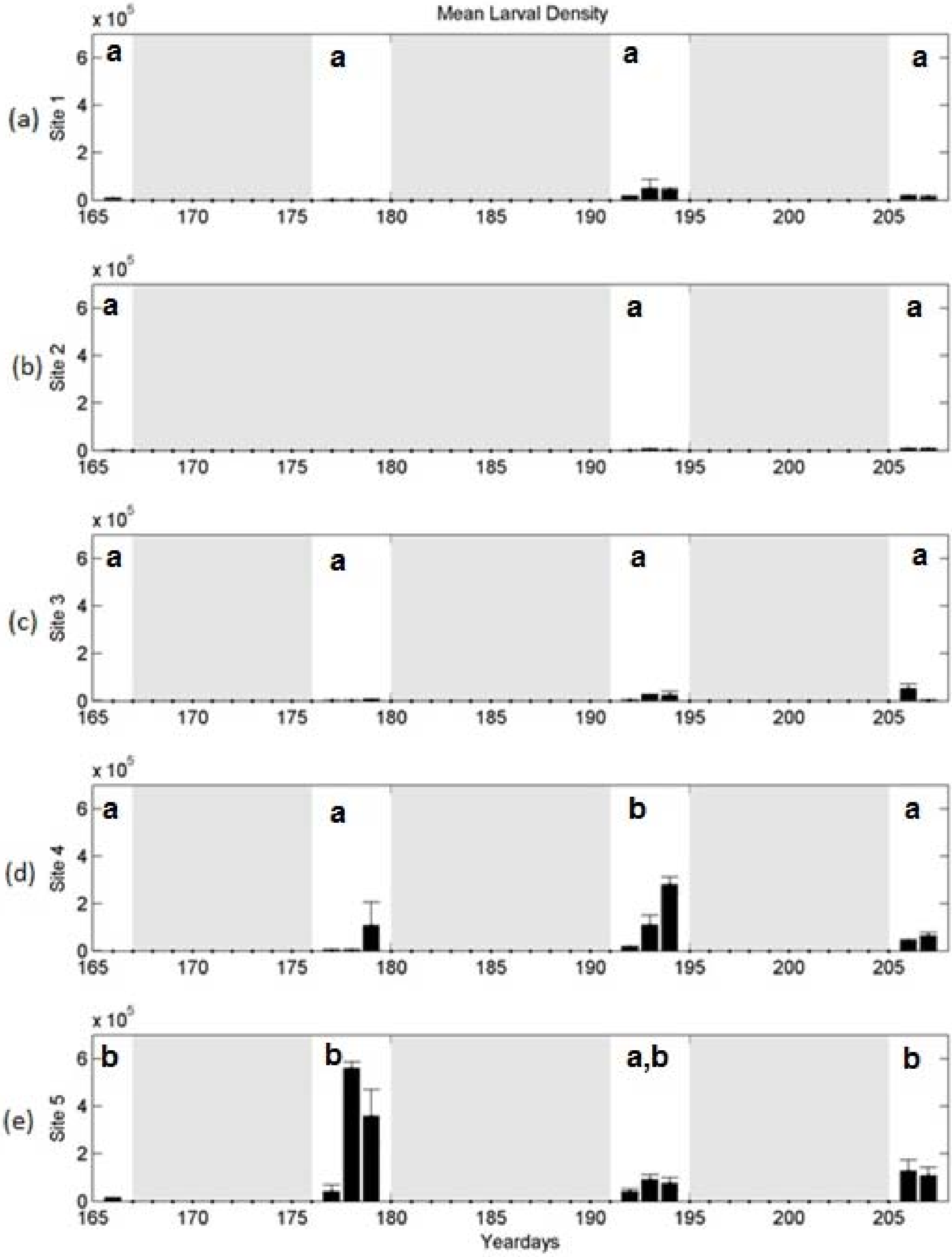

Figure 5: Mean Larval Densities for (a) Site 1, (b) Site 2, (c) Site 3, (d) Site 4, and (e) Site 5. Densities with different letters are significantly different from one another.

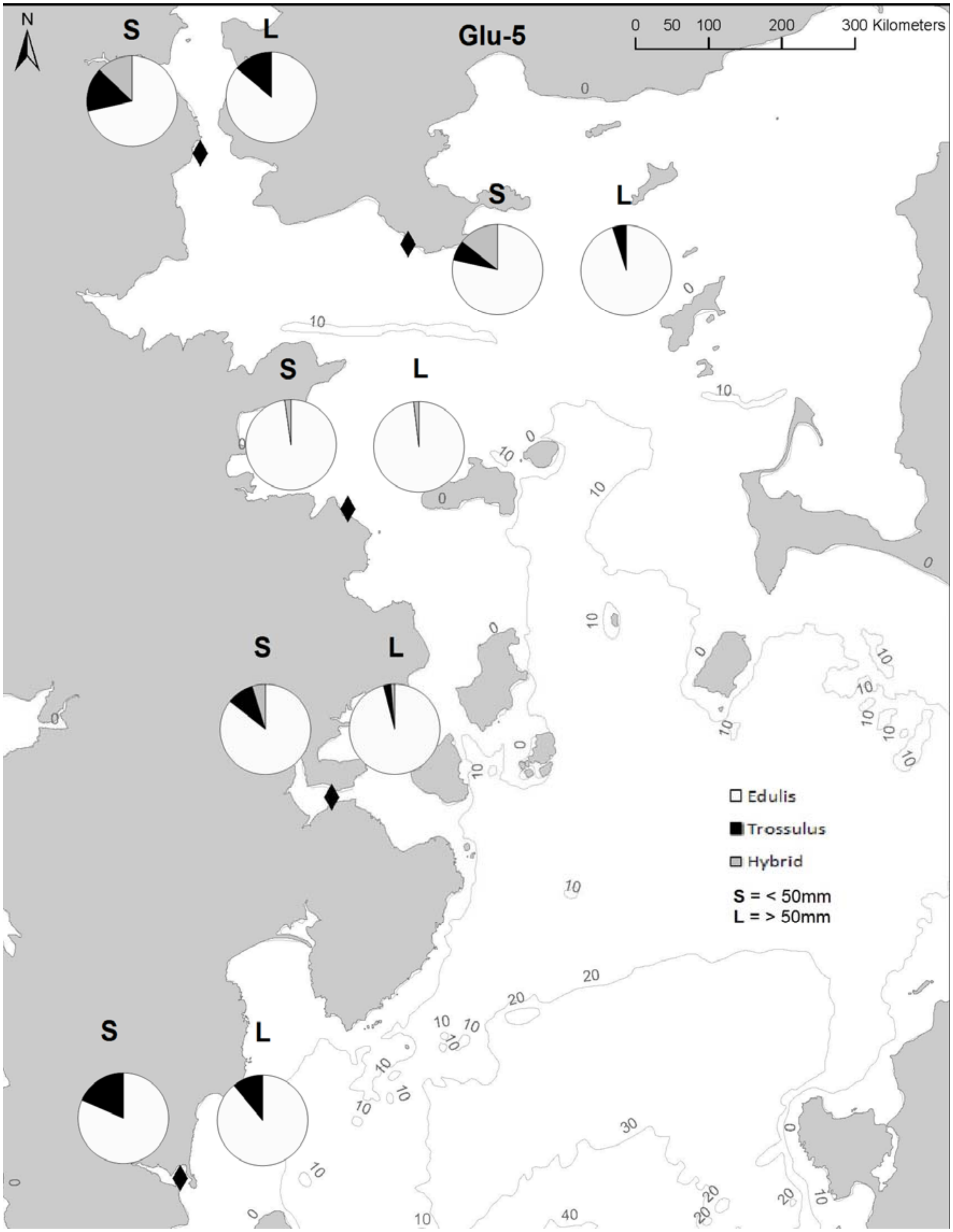

Figure 6: *Glu-5* (genetic marker) *Mytilus* distribution throughout Machias Bay. Black diamonds indicate site location. The black diamond at the top of the figure is Site 1 (upper bay) and sites increase moving to the bottom of the figure, the black diamond at the bottom of the figure is Site 5 (mouth of the bay). Pie graphs are colored by species: White = *M. edulis,* Black = *M. trossulus,* Grey = Hybrid. S and L indicate size, S = < 50mm and L = > 50mm.

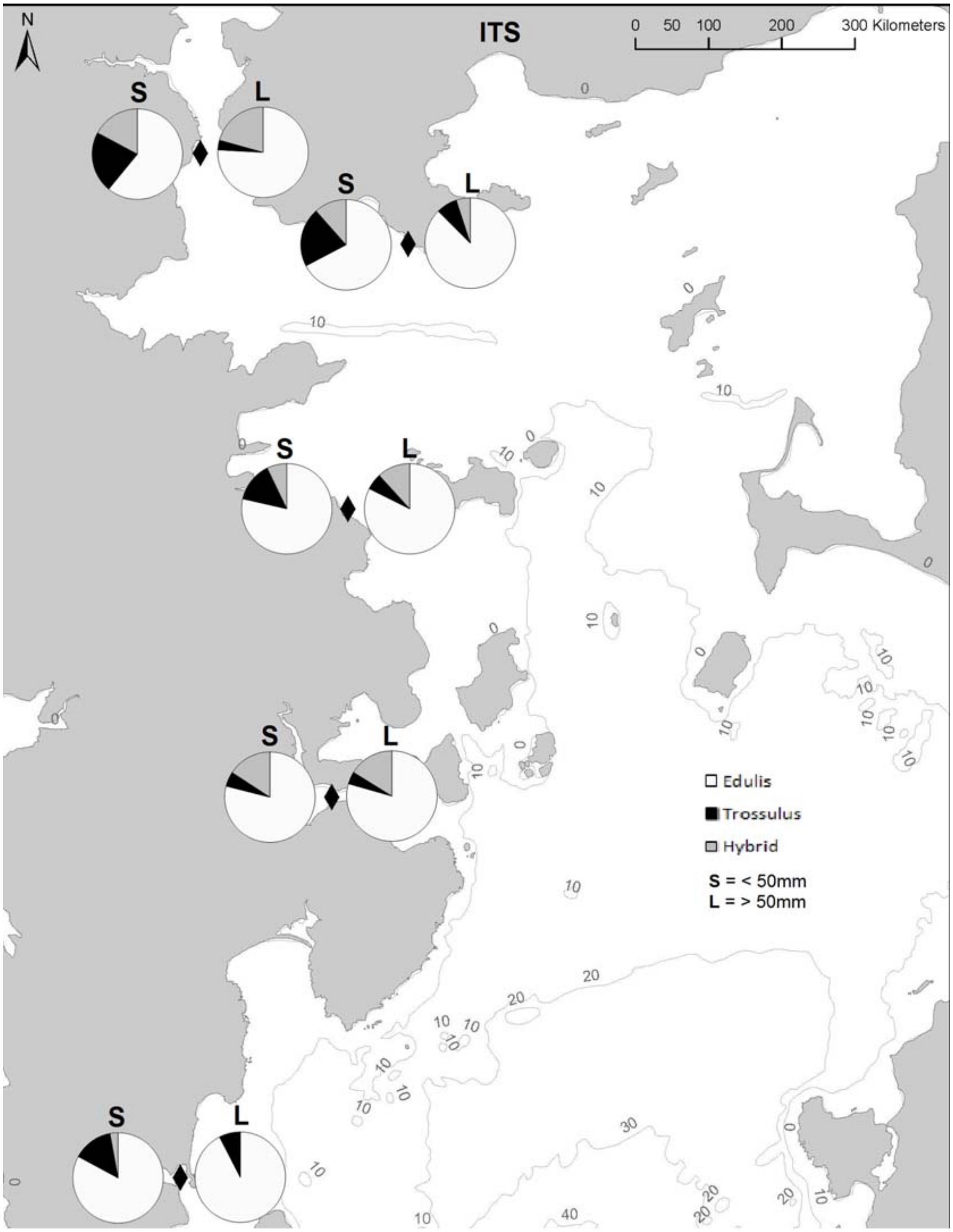

Figure 7: *ITS* (genetic marker) *Mytilus* distribution throughout Machias Bay. Black diamonds indicate site location. The black diamond at the top of the figure is Site 1 (upper bay) and sites increase moving to the bottom of the figure, the black diamond at the bottom of the figure is Site 5 (mouth of the bay). Pie graphs are colored by species: white = *M. edulis,* Black = *M. trossulus,* Grey = Hybrid. S and L indicate size, S = < 50mm and L = > 50mm.

TABLES

Site	**Negative Correlation**	**Lag Time (hr)**	**Degree**	**Wind Direction**	**P Value**
Site 1	-0.24625	16	100°	E	< 0.001
Site 2	-0.56471	25	80°	E	< 0.001
Site 3	-0.1551	0	270°	W	< 0.001
Site 4	-0.16758	0	260°	W	< 0.001
Site 5	-0.18647	21	240°	SW	< 0.001

Table 1: List of correlations of wind velocity and water temperature

Day (Yearday)	∆ Temp (°C)	Air Temp	Precipitation	Sea Level	Wind Velocity	EMCC Event (Y/N)
Control: EMCC	**3**					
183	3	↓	-	↓	↑SW	N
190	3	↓	+	↑	↑ N	N
194	1	↓	-	↑	↑NE	Y
210	2	↓	+	↑	↑SW	Y
215	2	↑	-	↑	↑ E	Y
223	2	↓	-	↓	↑ N	N
227	2	↓	+	↑	↑NE	Y
235	1	↓	-	↓	↑NW	N
244	1	↓	-	↓	↑ N	N

Table 2: 9 cooling events with the possibility of being EMCC events. Pettigrew *et al*. (2005) found a 3°C range in which the EMCC could be defined; this was used as a control to determine EMCC events in this study. ∆ Temperature (°C) is the range of temperature found within Machias Bay for the respective yearday. Air Temperature (°C), Sea Level (m) and Wind Velocity (m/s; directions indicate where wind is coming from) are reported as increased or decreased as compared to non-cooling events. Precipitation is reported as presence/absence event. Blue shading indicates decreasing trends and red shading indicates increasing trends.