Local Adaptation in the Atlantic silverside (*Menidia menidia*): Fine-scaled geographic variation in vertebral number and the adaptive significance of Jordan's Rule

A Dissertation Presented

by

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to

The Graduate School

in Partial Fulfillment of the

Requirements

for the Degree of

Doctor of Philosophy

in

Marine and Atmospheric Science

Stony Brook University

August 2010

Stony Brook University

The Graduate School

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Abstract of the Dissertation

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2010

The Atlantic silverside, *Menidia menidia*, is a widely distributed marine species along the east coast of North America that has been shown to exhibit local adaptation, despite evidence of high gene flow. This species displays a very strong and spatially fine scale increase in vertebral number with latitude, consistent with Jordan's Rule. Spatial and temporal variability can be seen on a microgeographic scale, potentially due to differences in site-specific developmental temperature and/or mixing among nearby locales. Most of the vertebral number variation is genetic however, and such tight clinal patterns implicate natural selection as the cause but its adaptive significance is unclear. Laboratory experiments show vertebral number responds to artificial selection on size, with populations with the largest size classes removed showing a decrease in vertebral number and vice versa. Natural selection on vertebral number is also evident in the wild, with vertebral numbers higher in juvenile populations compared to the same populations as adults. High latitude populations are thought to have evolved a greater number of vertebrae to allow for increased body flexibility in colder, move viscous water, however empirical evidence is limited. To test this theory, I hypothesized that at high temperatures, southern Atlantic silverside populations would show significantly higher critical swimming speeds than northern populations, but the reverse would be true at lower temperatures. Swimming speed experiments were conducted on southern (South Carolina) and northern (Nova Scotia) populations reared in a common environment. Each population was tested at a range of larval sizes and experimental temperatures. Vertebral number was negatively correlated with swimming speed at 28°C but such correlations at lower temperatures were non-significant. Few studies have investigated the link between vertebral number and swimming ability experimentally. The results of my research suggest extreme fine-tuning of vertebral number to natural selection in the wild and provide evidence for potential agents of selection.

Dedication

To my parents, Barry and Virginia Hice, for their unyielding support throughout all the strange decisions I have made.

To my grandfather, William Hause, who started calling me 'Doctor' when I began applying to graduate school. I am certain he is proud.

And to my husband Keith Dunton, who always makes me laugh, even when I don't want to. This would not have been possible without you.

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Introduction

The forces leading to natural selection often vary on local or regional scales. This differential selection may lead to locally adapted populations, often along a geographic gradient such as latitude or altitude (Conover et al. 2009). The central idea of local adaptation is that a proportion of the differentiation among populations within a species reflects traits adapted for the local environment. This generally occurs when genotypes in a particular habitat have higher relative fitness than genotypes from other regions (Carvalho and Hauser 1994; Policansky and Magnuson 1998; Conover et al. 2006). The level of local adaptation within a species depends on the level of gene flow among populations, environmental variability, the selection pressure for particular traits, and genetic drift (Williams 1966; Endler 1977; Kaweki and Ebert 2004; Conover et al. 2006). Local populations of the same species can express differences in phenotypic traits such as growth rate or body morphology, allowing them to maximize fitness in a given environment. The driving factor behind such variation, however, is often unknown. Investigations of the basis of intraspecific variation have greatly influenced our views on speciation, adaptation, and geographic variation (Trussell 2000).

Many studies have been done to demonstrate local adaptation in a wide range of species and traits. Geographic variation has been demonstrated in terrestrial species (Berven 1982; Arnett and Gotelli 1999), marine invertebrates (Bertness and Gaines 1993; Brown et al. 2001; Sanford et al. 2003), and freshwater fishes (Power and McKinley 1997; Arendt and Wilson 1999; Braaten and Guy 2002; Bell et al. 2004). Marine environments, however, are often thought to be open systems where geographic barriers are generally absent. In many species, certain life stages are assumed to be highly dispersive and with wide distribution ranges, which may allow for increased gene flow and homogenization of populations. Yet insights from local adaptation studies find that this is less common than originally thought. Geographic variation has been demonstrated across taxa in marine fishes (Schultz et al. 1996; DiMichele and Westerman 1997; Brown et al. 1998; Craig and Foote 2001; Lankford Jr. and Targett 2001; Lombardi-Carlson et al. 2003; Case et al. 2005; Marcil et al. 2006*b*). Prior studies on Atlantic silversides (*Menidia menidia*) by Conover and colleagues, for instance, have found that certain phenotypic traits such as growth rate and vertebral number vary with latitude and indicate local adaptation (Conover and Present 1990; Lagomarsino and Conover 1993; Billerbeck et al. 1997). Recent studies have also shown marine populations exhibit natal homing (Thorrold et al. 2001) and local self-recruitment (Jones et al. 1999; Cowen and Sponaugle 2009). If these patterns are common among marine species, then local adaptation may occur more frequently than previously believed.

Species exhibiting local adaption often exhibit genetic differences in traits along a latitudinal or altitudinal gradient. Commonly assessed traits include those related to metabolic processes (e.g. growth or development) and those related to morphological characters (e.g. body shape or body part size) (reviewed in Conover et al. 2009). Initially, the aim is often to find evidence of local adaptation occurring. However once the phenomenon has been established, the focus shifts to determining why a particular trait exhibits local adaptation and the adaptive significance of the trait, which is often a complex endeavor.

Study Species

It is difficult to determine the driving factor behind phenotypic variation in wild populations due to their migratory behavior and other life history traits. In order to do so, a good experimental system is needed. The Atlantic silverside, *Menidia menidia*, serves as an excellent model species. *M. menidia* is found in virtually all marshes, bays, estuaries, and barrier beaches along the North American east coast from northern Florida to the Magdalen Islands, Quebec, Canada (Johnson 1975). This broad geographic range exposes the species to a steep environmental gradient. *M. menidia* are also batch spawners on a semilunar cycle with high fecundity and demersal eggs. Larvae hatch after approximately one week and presumably stay in their natal estuary until they are older juveniles or adults (Conover and Present 1990). Silversides at higher latitudes overwinter on the continental shelf after migrating from estuaries in late fall (Conover and Murawski 1982). In the spring, fish return to estuaries and spawn multiple times to complete their life cycle. The timing of this pattern is different between northern and southern latitudes, where spawning occurs later at higher latitudes and the spawning season is decreased (Conover and Present 1990). Additionally, this species generally has an annual life cycle, with less than 1% of individuals surviving to breed in year two (Conover and Ross 1982; Collette and Klein-MacPhee 2002). This makes them amenable to field and laboratory studies as multiple generations can be reared and studied over a relatively short time period, and the focus of the research presented within this dissertation.

Summary of Chapters

My dissertation research uses *Menidia menidia* as a model species to gain insight on fine-scaled local adaptation, particularly in vertebral number. Vertebral number in some fish species is known to increase with increasing latitude, which is known as Jordan's Rule (Jordan 1891). When first described, Jordan's Rule demonstrated the relationship of the number of vertebrae among interspecific fish from different latitudes, however later work by Hubbs (1922) described intraspecific latitudinal variation in vertebral number. Jordan's Rule has been used exclusively to describe latitudinal variation in vertebral number among and within fish species, and there is little evidence of this phenomenon occurring in other taxa. Jordan's Rule was first discussed in the late 1800s (Jordan 1891), yet over 100 years later many details of this widespread phenomenon remain unclear. I use both field research and laboratory experiments to investigate this trait in great detail.

The focus of my research is twofold: 1- to determine fine-scaled spatial and temporal patterns of variation in *Menidia menidia* vertebral number along a broad latitudinal gradient, and 2- to investigate the adaptive significance of this trait. In chapter 1, I present data from a latitudinal study investigating local adaptation across the entire range of the species and through sampling that occurs at a very fine scale (every ~50km). I compare geographic patterns of vertebral number variation to that of growth rate as well. This data is part of a larger study which includes local adaptation in temperature-dependent sex determination (TSD) as well as molecular work on mitochondrial DNA and microsatellites. It is the most comprehensive study of local adaptation in a marine species known to date.

Although vertebral number in *Menidia menidia* is known to be genetically determined there is also a degree of plasticity that can occur due to differences in temperature during embryonic development (Taning 1952; Harrington and Crossman 1976; Billerbeck et al. 1997). In chapter 2, I expand on some of the insights gained from the broad latitudinal study and present data on temporal variation in vertebral number and spatial variation on a microgeographic scale. Looking at vertebral number in this manner allows us to gain insight on the effects of environmental temperature on vertebral number and to use a novel approach to investigate local retention and natal homing by comparing adult and juvenile populations over an extended time period.

Chapter 3 begins my investigation into the adaptive significance of variation in vertebral number. The trait is genetically determined and capable of evolving in a short time period, yet the reason this occurs has been left mostly to speculation (Billerbeck et al. 1997; Walsh et al. 2006). I present evidence for natural selection on *Menidia menidia* vertebral number in the wild, as well as data on how vertebral number evolves in response to selection on size, a continuation of research by Walsh et al. (2006).

Much of the research in vertebral number suggests a connection with swimming ability; however few studies attempt to empirically test this theory. In chapter 4, I present a study on the effect of vertebral number on critical swimming speeds (U_{crit}) in juvenile *Menidia menidia*. My goal was to determine if vertebral number provided an adaptive advantage to swimming in cold or warm water, and whether this is particularly important for a specific period in development.

Overall, this study helps further our understanding of patterns of local adaptation in *Menidia menidia* and particularly in the adaptive significance of Jordan's Rule, which has had little empirical attention. Most of the work referencing Jordan's Rule provides evidence that it occurs in or among species but leaves the benefit of this trait to speculation. Additionally, studies in fine-scaled local adaptation and the adaptive significance of phenotypic traits are not only important to enhance our understanding of evolutionary ecology, but they are also of particular importance when applied to species experiencing anthropogenic changes such as selective harvest or climate change. In a species with a broad latitudinal distribution such as *Menidia menidia*, small changes in environment or the environmental gradient can have serious impacts, especially in a species with a high degree of local adaption. *M. menidia* serves as an excellent model species for local adaptation studies, and insights gained from this species can further our understanding of evolution in marine species.

Chapter 1:

Fine-scaled Geographic Variation in *Menidia menidia*

Introduction

Historically, marine species have been thought to have open, dispersive populations with geographic variation occurring only on broad scales. More recently scientists have been investigating local adaptation in marine species. Early work by Dehnel (1955) and Arment (1979) showed evidence of latitudinal variation in growth rate in several species of gastropods. Latitudinal variation in development time and respiration rate has also been demonstrated in several species of fiddler crab (Vernberg 1959; Vernberg and Costlow 1966). A majority of the studies of local adaptation in marine species have focused on traits in fish such as growth rate, body shape, metabolic, rate, swimming performance, and vertebral number (Conover and Present 1990; Nicieza et al. 1994; Secor et al. 2000; Purchase and Brown 2001; Lombardi-Carlson et al. 2003; Finstad and Forseth 2006; Marcil et al. 2006; Yamahira et al. 2006). However, these studies are often conducted over a small geographic area or in few sample sites over a broad spatial scale. No studies to date have attempted to determine the geographic scale or fine-scaled patterns of local adaptation. In recent years there has been an increasing interest in studies of local adaptation not only to improve our understanding of evolutionary ecology but also with increased need for conservation and species management, especially in the face of climate change.

Marine environments are often believed to be open systems absent of geographic barriers. Many species are assumed to exhibit life history strategies that allow them to be highly dispersive and to have wide distribution ranges. These traits would lead to high gene flow and thus limited geographic variation between populations, yet recent insights from local adaptation studies in marine species find that this pattern is less common than originally thought. Geographic variation has been demonstrated across taxa in marine organisms (reviewed in Conover et al. 2009) and recent studies have also shown marine populations exhibit natal homing (Thorrold et al. 2001) and local self-recruitment (Jones et al. 1999; Cowen and Sponaugle 2009). If these patterns are common among marine species, then local adaptation may occur more frequently than previously believed. Despite the growing number of cases of local adaptation in marine species in the literature, many studies are quite limited in the number of populations studied or the geographic range of the populations. A large proportion of studies use only two samples sites (reviewed in Conover et al. 2009) and often the focus is either on populations over a very narrow range (Moore and Hendry 2005), or populations that are studied over large distances, often hundreds of kilometers (Jonassen et al. 2000). In reality however, the scale on which local adaptation can occur marine species may be much finer than has been shown.

Figure 1-1 shows three hypothetical patterns that may become apparent in a species showing local adaptation across a latitudinal gradient. The first is a situation where a trait shows a continuous, gradual increase with latitude. This type of pattern would result from an environmental influence that increases linearly, such as photoperiod, or from homogenizing gene flow among neighboring populations (Conover et al. 2006). Previous broad-scale studies in the Atlantic silverside (*Menidia menidia*) have shown that both growth rate and vertebral number exhibit this pattern (Conover and Schultz 1995; Billerbeck et al. 1997; Yamahira et al. 2006). The second pattern shows areas of gradual or no change separated by abrupt shifts, creating a stepwise pattern. This may be due to areas of distinct change in environmental factors such as temperature, or geographic barriers to gene flow such as those created by distinct oceanographic patterns, suggested to occur on the east coast of the United States near Cape Hatteras or Cape Cod (Adams and Rosel 2006). In the third scenario, the trait increases gradually in middle latitudes, but shows evidence of flattening at the tips. Such a pattern would likely be caused by unidirectional gene flow at the ends of the species' distribution caused by frequent extinction or bottlenecks at the extremes and recolonization from individuals from the middle of the range (Conover et al. 2006). It is also possible that different traits follow individual latitudinal patterns in the same species. Many studies focusing on the geographic patterns of local adaption only focus on a single trait, most commonly traits associate with metabolic processes such as growth rate (reviewed in Conover et al. 2009). However, by studying multiple characters, a better understanding of the true nature of local adaptation is gained. Here I will show data for two traits, growth rate and vertebral number, to demonstrate geographic patterns and the scale of local adaption.

Growth rate is a trait commonly assessed in studies of local adaptation across marine taxa (Conover and Present 1990; Lombardi-Carlson et al. 2003; Pardo and Johnson 2005). It is frequently used because it is under intense selection by various environmental factors such as temperature and predation which results in distinct, genetically-based growth rates (Jonassen et al. 2000; Lankford Jr. et al. 2001; Salvanes et al. 2004; Chiba et al. 2007). *Menidia menidia* exhibits genetic variation in growth rate that varies inversely with the length of the growing season, referred to as countergradient variation (CnGV), where the environment opposes the genetic variation between populations. Conover and Present (1990) presented evidence that northern populations have genetically faster growth rates due to shorter growing seasons. In the northern extent of their range, *Menidia menidia*'s growing season is roughly half that of populations in the south. It is believed that northern populations have evolved faster growth rates in response to shorter growing seasons to reduce winter mortality at small sizes. CnGV in growth reduces the phenotypic variation as all populations reach similar adult sizes at all latitudes (Conover 1990; Conover and Present 1990; Conover et al. 2009).

Vertebral number is a trait that has been used to study geographic variation within and among species in *Menidia menidia* and *Menidia peninsulae* (Billerbeck et al. 1997; Yamahira et al. 2006). Unlike growth rate, vertebral number exhibits cogradient variation (CoGV) where the genetic and environmental influences accentuate phenotypic differences (Conover et al. 2006; Conover et al. 2009). In many species, numbers of vertebrae increase with increasing latitude in accordance with Jordan's rule, which states that fish from higher latitudes tend to have greater numbers of vertebrae than related fish from lower latitudes (Jordan 1891). Many studies have suggested a link between vertebral number and swimming ability (Lindsey 1975, 1978; McDowall 2004, 2008), but empirical tests are limited. A common theory is that having more vertebrae and thus a more flexible anguilliform body form provides an adaptive benefit in colder, more viscous water (Spouge and Larkin 1979; McDowall 2008).

Conover and colleagues have shown latitudinal clines of local adaptation in growth rate and vertebral number on a broad scale in *Menidia menidia* through the use of "common garden" experiments, which assess the genetic component of each phenotype by rearing populations in standardized environments (Conover and Present 1990; Billerbeck et al. 1997). Previous work is based on six sites along the east coast of North

America from South Carolina to Prince Edward Island, Canada; however the large distance between sites on such a broad range may not be indicative of fine-scaled changes. To truly understand the geographic scale of local adaptation in a species one must not only look at populations on a fine scale, but also over the entire range that the species is found. Here I present a study of local adaptation on a fine scale over a broad geographic range that encompasses multiple traits in a single species.

Methods

Field Sampling

Thirty-six sites along the east coast of North America were sampled to study fine-scaled geographic variation in growth rate and vertebral number, each roughly 50 km apart or less. An additional three sites were used in the study of vertebral number only (Figure 1-2, Table 1-1). Latitude and longitude coordinates were taken onsite using a Garmin handheld GPS unit. Samples were collected during the spring spawning cycling in 2005-2007 with roughly 12 sites spanning the range of the study sampled each year. Two sites, Oregon Inlet, NC and Norfolk, VA were also repeated in 2008 to confirm outliers in the data. Up to 100 adult fish were collected at each site using a 30 meter beach seine, and with an equal number of male and female fish when possible. Ripe adults were strip-spawned onto four 10 cm x 10 cm screens at each site. Six to eight individuals of each sex were used per screen. The screens were then transferred to secure screen blocks in coolers kept at a constant, low temperature (~15°C) with ice and a low level of seawater to keep the eggs moist but not submerged. Embryos were transported to the laboratory for rearing and adult fish were frozen for vertebral analysis.

Laboratory Rearing

Common garden experiments were employed to rear eggs and larvae in the laboratory. This technique allows genetic differences between populations to become apparent after environmental variability is removed. Embryos spawned in the field were transferred to buckets submerged in 21°C water baths at the Flax Pond Marine Laboratory in Old Field, NY or at the Bluepoints Laboratory in West Sayville, NY. One week post-hatch, the larvae were split into temperature treatments of either 15°C or 28°C and were allowed to acclimate for several days. The experimental temperatures used represent typical temperature extremes experienced during development in the wild (Conover and Present 1990) and were also used for coinciding experiments on temperature dependent sex determination (TSD; results described in Duffy 2010) as these temperatures encompass the range which have the greatest effect on sex ratio (Conover and Heins 1987). Once the larvae reached a mean size of 8-10mm, 10 fish per replicate were measured for initial growth data and the remaining fish were split into three replicate treatments of equal density. After 14 days, 20 fish in each replicate were removed and measured for time-dependent growth data. In the case of high mortality prior to the 14 day measurement, the fish were live measured and returned to their respective treatments. After this point the fish remained in the treatments until they reached 25-30mm. At the end of the experiment, the remaining fish were weighed and measured for the final, size-dependent growth data. All fish were fed live *Artemia* nauplii *ad libitum* and kept on a constant 15:9 light cycle throughout the experiment.

Vertebral Analysis

To determine vertebral number, adult fish from the field were x-rayed on Kodak Industrex MX125 film using a Hewlett Packard Faxitron (50kVP/6mA/60sec) or a Kramex PX - 20N (60kVP, 20mA, 1.6sec) x-ray system. Sheets were manually developed and individual fish vertebral counts were read and scored using microfilm machine. Counts are made in the centra between the basioccipital and urostyle and differentiated between caudal and precaudal vertebrae. Any fish with obvious vertebral deformities, such as fused vertebrae, were removed from the analysis. Standard length for individual fish and sex were recorded to analyze an equal number of males and females. Sex was determined by the presence of eggs or milt in ripe adults in the field when possible.

Statistical Analyses

Site-specific mean vertebral number was corrected for body length by calculating linear regression statistics for each site. The regression equation at each site was then used to determine a predicted vertebral number for an individual of the mean size of the entire study (79mm) at each site. All fish were adult, wild-caught fish ranging from 48-123mm. A piecewise regression was performed on the growth rate data and the size-corrected vertebral number data from the field. Regressions were fit to the data with one (linear) to five segments. Akaike Information Criterion (AIC) was calculated for each regression and ΔAIC values were used to determine the best-fit model. Piecewise regression and ΔAIC analysis was also used to determine breakpoints in vertebral count ratios, which were calculated by dividing the number of precaudal vertebrae by the number of caudal vertebrae.

Results

Linear regression analysis shows that 31 of 40 sites (77.5%) show a significant relationship between individual vertebral number and size (Table 1-2). However, the 9 sizes that do not show a significant relationship also have low statistical power (<0.80) therefore enough data may not be available to show the true relationship. Additionally, mean size of each population does not increase with increasing latitude, rather the populations with the largest fish are found in the sites in the Outer Banks, North Carolina at Hatteras Inlet (35.18°N) and Oregon Inlet (35.77°N) (Figure 1-3). It should be noted that the Nova Scotia population from Annapolis Royal was removed from the analysis. These fish were maintained as a laboratory stock established from embryos collected in the wild, thus laboratory conditions may have altered adult sizes compared to what may have been found in the field. The comparison of uncorrected size-corrected vertebral number is shown in Figure 1-4.

Vertebral number data from adult fish collected in the field shows a general increase with increasing latitude, however after expanding the study to 39 sites a more complex pattern emerges when compared to previous work in this species. Overall, there is an increase of five vertebrae between the lowest mean vertebral number in the south (Edisto, SC = 39.19) and the highest mean vertebral number in the north (St. Andrews, NB = 44.31) and there is no overlap in the ranges of vertebral phenotypes found at the northern and southern extends of the range. Piecewise regression and ΔAIC analyses on size-corrected vertebral number show evidence of three breakpoints in the latitudinal distribution; one at 33.11°N (South Carolina), another at 35.00°N (North Carolina), and a third at 43.68°N (Maine) (Figure 1-5). ΔAIC analysis shows that the four segment model has the highest AIC. There is no significant difference between the four segment and five segment model (Table 1-3), however, the five segment model would add a breakpoint at 47.95°N, which is beyond the northernmost site in this study. Hence, the four segment model best fits the data.

Interestingly, there is also a latitudinal trend in the variance of the vertebral number data (Figure 1-6). Variance in vertebral number is lowest at the northern and southern tails of the distribution and increases in the middle of the range. The highest variance occurring in North Carolina (~35°N) also corresponds with the rapid increase in vertebral number seen in this region.

When comparing mean vertebral numbers of male and female fish within each population, the majority of sites show females with slightly higher vertebral counts than males (Figure 1-7). However since the population level means were determined with an equal ratio of male and female individuals, the potential for sexual dimorphism within a site should not have a significant effect on the latitudinal trend. Additionally, size correction of the data should remove any bias, since female fish tend to be larger with more vertebrae. This corresponds with previous evidence of sexual dimorphism in size and vertebral number in this species (Billerbeck et al. 1997).

There also appears to be regional differences in the types of vertebrae driving the change in mean vertebral number at a given latitude as seen by differences in the vertebral count ratio along the range (Figure 1-8). The increase in vertebral number in the region of rapid change near in North and South Carolina coincides with an increase in the vertebral count ratio, with fish exhibiting proportionally more precaudal vertebrae. In northern regions, there appears to be proportionally more caudal vertebrae, except in the populations at the northern extreme, which again exhibit a greater proportion of precaudal vertebrae. Piecewise regression analysis shows evidence of breakpoints in the vertebral count ratio at 38.54°N (Maryland) and 45.18°N (northern Bay of Fundy/Gulf of St. Lawrence) (Figure 1-9, Table 1-4).

Growth rate roughly doubles between the northern and southern extremes of the species range when larvae are reared at either 15°C or 28°C (Figure 1-10). When reared at 28°C, size-dependent growth rate in *Menidia menidia* increases linearly with latitude, despite a high degree of variation between sites. Linear regression analysis shows a significant relationship between growth rate and latitude ($p<0.001$, Figure 1-11). Piecewise regression models were unable to fit two or more segments to the data, despite attempts to constrain the model. However when *M. menidia* are reared at 15°C, piecewise regression and ΔAIC analysis show evidence of a break in the distribution of size-dependent growth rate at 39.77°N, corresponding with northern New Jersey (Figure 1-11, Table 1-5). Although the two segment model has the highest AIC, when comparing the three segment to the two segment model the ΔAIC value shows that there is no significant difference between the two segment and three segment models. The three segment model would predict an additional breakpoint at 33.59°N, corresponding with South Carolina (Table 1-5). The time-dependent growth rate was omitted from the analysis because it was found that the two week time period was too short to show true growth rate differences, particularly at 15°C and in slow-growing southern populations.

Discussion

Menidia menidia shows a clear increase in vertebral number with increasing latitude in accordance with Jordan's Rule. Previous work done by Billerbeck et al. (1997) on six field sites had shown a linear increase, however it is now apparent that when the study is expanded to encompass the entire range of the species and on a much finer scale, the pattern is much more complex and regional differences among populations become evident. For one, at the southern end of the species range, from southern Florida to northern South Carolina, there is little change in the mean vertebral number in the populations studied (Figures 1-4 & 1-5). It is possible that in the southern region there is little change in the selection factor acting on vertebral number with latitude. This region also shows low amounts of variation in vertebral number (Figure 1-6) and coincides with a region where there is thought to be little, if any, offshore winter migration of the species and where fish experience long periods of warm water temperatures (Fay et al. 1983; Conover and Present 1990). Limited migration would reduce the amount of mixing between populations offshore (and variance in phenotypes) or when they move inshore to spawn as is seen in northern populations (Conover and Murawski 1982).

From 33.31°N to 34.55°N there is a rapid increase in *Menidia menidia* vertebral number with latitude. This occurs roughly from northern South Carolina to the Southern edge of the Outer Banks in North Carolina. The estimated breakpoint at 35.00°N is only roughly 60km south of the proposed biogeographic transition zone at Cape Hatteras (35.22°N), and may correspond to rapid changes in environmental conditions imposed by the Gulf Stream (Ekman 1967; Briggs 1974). It is possible that this area marks the southern limit of offshore winter migration, and populations in this region may experience increased natural selection due to the rapid environmental change near Cape Hatteras. The northernmost breakpoint in the vertebral number data occurs at 43.68°N, roughly 75km south of Penobscot Bay in the Gulf of Maine. Interestingly, this region corresponds with the transition zone between Georges Bank and Browns Bank in the Gulf of Maine, where deeper waters from the Atlantic Ocean enter the Gulf of Maine through the Northeast Channel. This is also the southernmost limit of the tongue of cool, nutrient rich water that forms from the tides within the Bay of Fundy (Apollino and Mann 1995). These oceanographic circulation features may create a boundary to population mixing and increase the potential for locally adapted populations (Galarza et al. 2009; Watanabe et al. 2009).

The differences in vertebral number could be presumed to be due to a difference in one region of the vertebral column, such as the caudal vertebrae. However, there is no evidence that vertebral number increases occur in one section of the vertebral column- i.e. solely in precaudal or caudal vertebral (Figures 1-8 & 1-9). Most of the increase in vertebral number in the region of rapid change near in North and South Carolina appears to be due to a proportional increase in precaudal vertebrae. In northern regions, the increase appears to be in the proportion of caudal vertebrae, except in the populations at the northern extreme, which exhibit a greater increase in the proportion of precaudal vertebrae. This suggests that in certain regions of the species range, there may be an adaptive benefit to having relatively more caudal vertebrae and in others having relatively more precaudal vertebrae is beneficial. In the south for example, where *M. menidia* exhibits temperature dependent sex determination (TSD) over a long growing season

(Conover et al. 1992) and allocate more energy to growth (Billerbeck et al. 2000) it may be beneficial to have a larger precaudal region to allocate more space in the body cavity for gonads or other tissues, whereas in the north it may be beneficial to have more caudal vertebrae to have a more flexible, anguilliform swimming mode to deal with colder, more viscous water. One would expect this to continue into the populations at the extreme northern extent of the species range; however these populations show increases in precaudal rather than caudal vertebral. However, in studies on TSD in this species, populations at the northern extreme of the species range in the Gulf of St. Lawrence are more similar to mid-latitude populations. This is attributed to the Gulf of St. Lawrence being a shallow ocean basin with a longer growing season (Duffy 2010). Therefore, there could be an adaptive benefit to having more precaudal vertebrae, and perhaps consequently more gonadal space, in populations at the northern extreme of the species' range. There also is evidence that there are interspecific differences between the relationship between body depth and vertebral number. In some galaxiid fishes, there is an inverse relationship between vertebral number and body depth (McDowall 2004), however there are no known studies that investigate intraspecific differences the relationship between body depth and vertebral number. This could not be performed in this study since the specimens used to investigate vertebral were ripe adults returning to spawn, resulting in a temporal change in the proportion of the body cavity allocated to gonads and gametes during the spawning period. Additionally, the preservation method used was not amenable to morphometric analyses.

Although latitudinal differences in vertebral number have been documented in numerous fish species (reviewed in McDowall 2008), little attention has been given to whether this variation occurs due to caudal or precaudal vertebrae. Ward and Brainerd (2007) showed that across species differences in vertebral number can be attributed to either increases in precaudal or caudal vertebrae. Recent studies by Yamahira and colleagues have shown evidence of Jordan's rule in medaka, and noted that the increase in vertebral numbers in fish collected in the wild occurred primarily in the abdominal (precaudal) vertebrae (Yamahira and Nishida 2009). They also showed evidence of heritability of abdominal vertebral numbers and were able to increase the number of abdominal vertebrae in laboratory reared larvae by decreasing developmental temperature (Yamahira and Nishida 2009; Yamahira et al. 2009). Interestingly, the ratio of abdominal (precaudal) to caudal vertebrae has also been shown to affect predation survival in threespine sticklebacks (*Gasterosteus aculeatus*) (Swain 1992b, a). Within a narrow range of lengths, burst swimming speed, and potentially the ability to escape predation, was dependent on vertebral count ratio (Swain 1992a). However, studies of variation in the number of precaudal versus caudal vertebrae in relation to Jordan's rule are rare, and no known studies show a latitudinal shift in how vertebral number increase occurs as seen here in *M. menidia*. Regardless, it is possible that latitudinal variation in vertebral number in *M. menidia* is driven by selection for a particular vertebral count ratio, rather than or in conjunction with selection for the total number of vertebrae.

Although other vertebrates can exhibit differences in their number of vertebrae, the term "Jordan's Rule" has been used exclusively to describe inter- and intraspecific increases in vertebral number with latitude in fish (reviewed in McDowall 2008). In vertebrates such as reptiles and amphibians, the majority of studies use vertebral counts as a method to distinguish between species or subspecies (Jockusch 1997; Arntzen and

Wallis 1999; Van Damme and Vanhooydonck 2002; Litvinchuk and Borkin 2003). There are limited studies on intraspecific variation in vertebral number in other vertebrates, although there is evidence of such trends in reptiles (Kaliontzopoulou et al. 2008) and even mammals (Aimi 1994). However these are not related to latitude or other geographic or ecological clines as in the case of Jordan's Rule.

The growth rate data is similar to what has been previously seen in this species (Conover and Present 1990). In general there is a linear increase in growth rate with latitude; however the results differ when the larvae are reared at 28°C versus 15°C, where a breakpoint near northern New Jersey becomes apparent. It is possible that at low temperatures the slower, protracted growth rates of all populations cause differences in populations to become more evident. Additionally, measurements of growth rate often have a high degree of intrinsic variability, which may change depending on the temperature of rearing. This could also lead to breakpoints becoming more apparent at one temperature or 'hidden' at another. It is also possible, however, that some of the patterns seen in the growth rate data are due to simple measurement error. Daily fluctuations of environmental or physiological factors such as temperature, food availability, or energy acquisition could cause a high degree of variation in growth even in common garden experiments (Conover 1990; Present and Conover 1992; Billerbeck et al. 2001).

This study provides the first concrete evidence of a migratory marine species with a fine-scaled geographic pattern in local adaptation. No study has investigated local adaptation at such a fine scale over a broad geographic range. Previous work in *Menidia menidia* focused on six broadly spaced sites from South Carolina to Prince Edward Island

and did not encompass the entire range of the species (Conover and Present 1990; Billerbeck et al. 1997). These studies found a linear relationship between growth rate and vertebral number and latitude. However, when research of this species was expanded to the northern and southern extent of their range at a very fine spatial scale (every 50km or less), we find important transitional areas that had previously been overlooked (Figures 1-5 & 1-11).

This is also the first known study to simultaneously look at multiple traits at such a scale. By studying more than one trait, insights about this species that would have been overlooked if only one trait had been used become clear. For example, a major transition zone for vertebral number occurs through North and South Carolina that is not seen in the growth rate data. Geographic variation in growth rate has been widely studied (reviewed in Conover et al. 2009), yet this trait is known to have a large amount of variability among individuals and measurement error, even in controlled experiments. Growth in *M. menidia*, for example, has been shown to be effected by temperature (Conover 1990), energy acquisition rate (Billerbeck et al. 2001), and growth efficiency (Present and Conover 1992). It is possible that daily fluctuation in these and other environmental or physiological factors could cause variation in growth in common garden experiments. To combat this high degree of variation, it is often useful to study other traits in conjunction with growth rate, especially meristic characters such as vertebral number. These traits have the benefit of low measurement error as they can be discretely counted and often do not vary once they are set in an individual's larval development, regardless of changes in environment, body shape, or body size (Lindsey

1988) whereas individual and population growth rates can vary even on daily time scales.

Both traits exhibit rapid changes occurring over short distances and each trait has been show to be maintained in a common garden (Conover 1998), suggesting that each is an adaptive evolutionary response. Such a high degree of local adaptation could imply that this species has low connectivity and limited exchange between populations. However, recent studies on natal homing in this species have found that despite the fact that individuals have the highest probability of originating from the site which they were captured, there is also a large degree of mixing among populations (Clarke et al. in press). Some fish were even shown to migrate over 700km from their natal location to spawn. The patterns of migration among populations also varied in the two years of Clarke et al.'s study, providing evidence for interannual variability in migration. Interestingly, despite evidence of mixing between populations, local adaption remains high in the species, suggesting high selection pressure.

Additionally, the differences in the geographic patterns in each trait suggest that they each have independent selection factors, which are not explained by gene flow. Mitochondrial DNA samples from these populations show three genetic breaks in the population which do not correspond with the phenotypic patterns in these traits (Mach 2007). This could lead one to believe that this species has a high degree of "population persistence" due to low geographic isolation (Cowen and Sponaugle 2009); however it appears that independent selection factors establish the optimum phenotype at each location. For example the 15°C growth rate data shows a breakpoint at 39.77°N, roughly northern New Jersey, which is contrary to the breakpoints in the vertebral number data.

From a conservation perspective, this can impact management decisions and designs of MPAs. If placed in an improper location, such as along an unknown boundary of rapid change, recruitment to outside the MPA may fail. Recruits from neighboring populations may not be able to survive in adjacent environments. These boundary zones may be difficult to predict, especially since they may vary depending on the trait studied.

Many studies have inferred a link between population biology and geographic or oceanic boundaries (Adams and Rosel 2006; Galarza et al. 2009; Watanabe et al. 2009) however actual empirical evidence showing a link between environmental parameters at these particular locations and population structure is rare. Those that do show this connection are often on a broad scale and/or have relatively few samples sites (Jorgensen et al. 2008; Ritter 2009). This work is part of a larger study on the geographic scale on which we attempted to link the breakpoints in the phenotypic data to environmental breakpoints using information from coastal buoys from 2004-2008. However, after analyzing several seasonal temperature trends such as latitudinal differences in season length or mean summer or winter temperatures, we found no evidence of environmental breakpoints corresponding with the breakpoints seen in the vertebral number. However, the breakpoint in the vertebral count ratio at 38.54°N (Maryland) corresponds with a breakpoint in average summer temperature. The breakpoint at 45.18°N (northern Bay of Fundy/Gulf of St. Lawrence) overlaps with a major environmental breakpoint in factors such as season length, average temperature range, and average summer and winter temperatures. Additionally, the breakpoint in the 15°C growth rate data at 39.77°N coincides with a breakpoint in average winter temperature (Hice et al., unpublished data, Figure 1-12). It is therefore possible that these estimates of regional differences in environmental temperature could be driving the patterns of local adaptation we see in the field, perhaps by creating boundaries to mixing between populations or regional differences in selection regimes.

Despite the lack of evidence for latitudinal breaks in environmental temperature corresponding with latitudinal variation in overall vertebral number, it is possible that other factors are driving the geographic pattern. For example, although coastal buoy data does not show an abrupt change in temperature corresponding with the rapid increase in vertebral number in the wild, it is possible that the migration of the Gulf Stream at Cape Hatteras has a profound effect on regional environment. This could be a transition zone for factors such as salinity or predator/prey assemblages. And perhaps it is not each factor acting independently, but acting synergistically which causes fine-scaled local adaptation in this region. A recent study by Clarke et al. (in press) speculated that oceanographic features such as ocean currents, offshore winter water temperature, and geographic boundaries such as Cape Cod effected migration patterns of *Menidia menidia* populations from New Jersey to Maine. Although Clarke et al.'s study covered a smaller geographic area than the data presented here; it is likely that other oceanographic features are affecting connectivity and thus local adaptation within along the range of this species.

This work highlights some potential shortcomings that occur when analyses of geographic variation are based on a limited sample size. By expanding previous work from six broadly spaced sites to 36 sites fine-scaled details of this species' population biology have become apparent. This research also shows that studying a single trait can be misleading, and valuable information would have been missed if only one trait, such as growth rate, was studied. With advances in molecular biology much research has begun to focus on using mitochondrial DNA and other genetic markers such as microsatellites to gain insights to natural populations. Yet here is a clear example where the geographic pattern seen in this species is not explained by population genetics when compared to the work done by Mach (2007). Neutral markers have been used extensively to study parameters such as migration rates, however there has been an increased interest in studying genetic markers under selection (Nielsen et al. 2009). Nevertheless, by focusing purely on one genomic method without simultaneous laboratory experiments, important data may be missed. No known work has attempted to address local adaptation at such a fine scale, nor has any study addressed the adaptive significance is such detail.

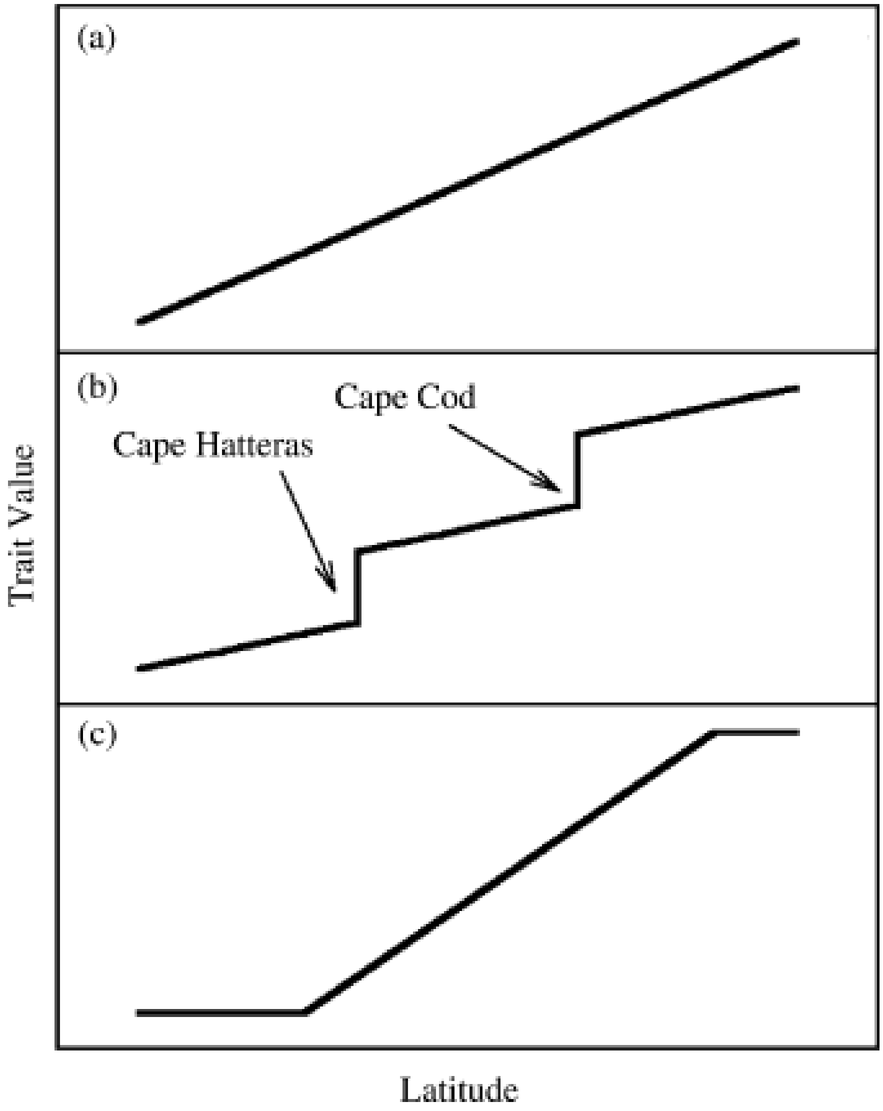


Figure 1-1: Three hypothetical patterns that may become apparent in a species showing local adaptation across a latitudinal gradient. (a) A linear increase with latitude, most likely corresponding to an environmental gradient such as photoperiod. (b) A pattern showing evidence of breaks in the distribution, such as those that are believed to occur in ecological transition zones such as Cape Hatteras and Cape Cod on the east coast of the United States. (c) A distribution that shows flattening at the tips, most likely due to unidirectional gene flow from the center of the distribution towards the ends of the range or frequent extinction and recolonization by individuals from more central populations (figure reproduced from Conover et al. 2006b).

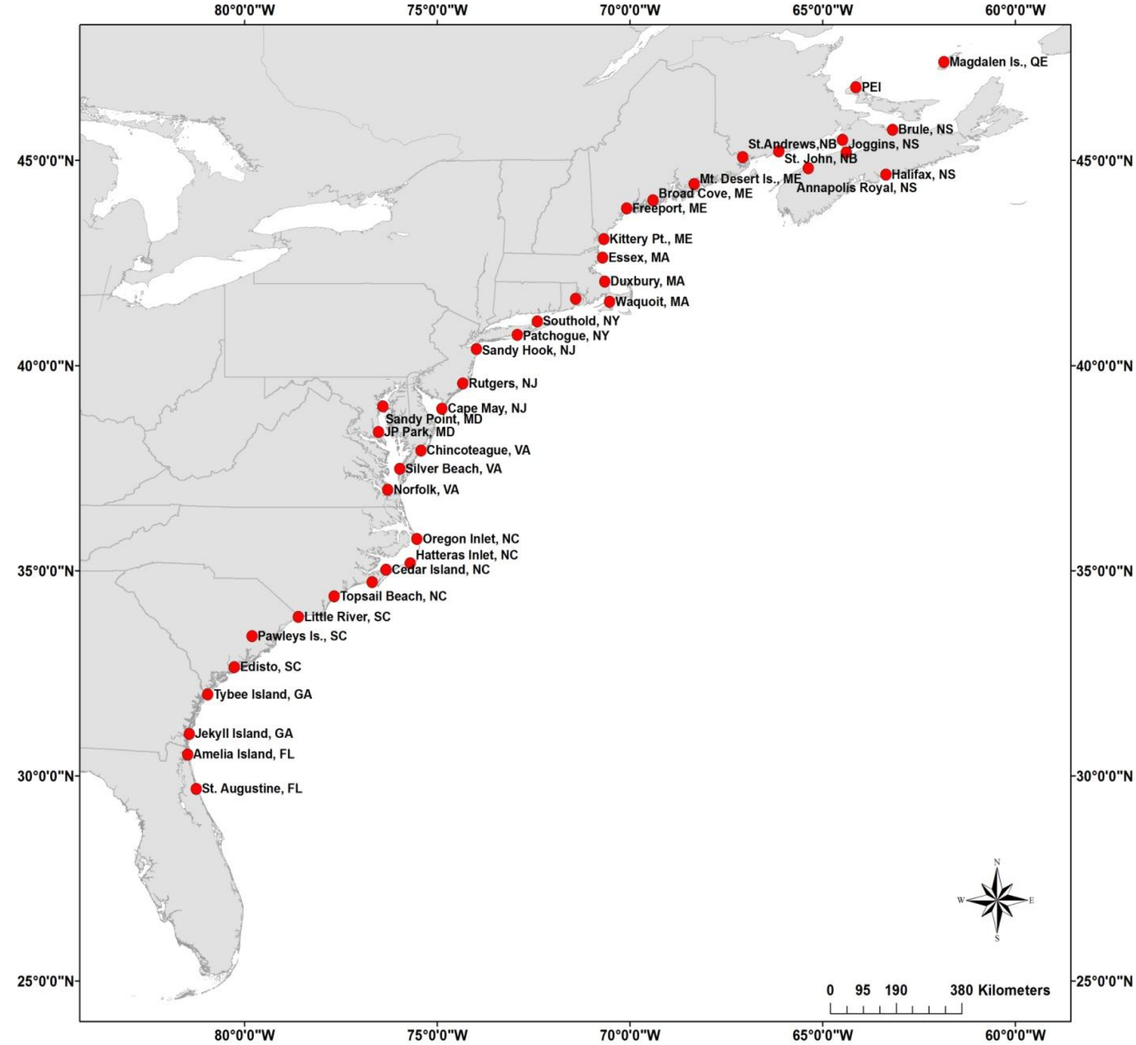


Figure 1-2: Map of the 39 sample sites used in the 2005-2008 coast-wide study.

Table 1-1: Latitude, longitude, sample size (n), and year collected for each of the sites in the study. Oregon Inlet, NC and Norfolk, VA were repeated in 2008 to confirm outliers in the data (n= 55 and 46, respectively).

Year	Site	Latitude	Longitude	n
2005	St. Augustine, FL	29.68	-81.25	100
2007	Amelia Island, FL	30.52	-81.47	86
2006	Jekyll Island, GA	31.02	-81.43	99
2005	Tybee Island, GA	31.98	-80.95	98
2007	Edisto, SC	32.65	-80.26	69
2006	Pawley's Is., SC	33.40	-79.80	98
2005	Little River, SC	33.87	-78.60	95
2007	Topsail Beach, NC	34.37	-77.67	100
2005	Morehead City, NC	34.72	-76.68	98
2007	Cedar Island, NC	35.02	-76.32	91
2006	Hatteras Inlet, NC	35.18	-75.70	77
2006	Oregon Inlet, NC[1]	35.77	-75.52	100
2007	Norfolk, VA[1]	36.97	-76.28	100
2005	Silver Beach, VA	37.48	-75.97	97
2007	Chincoteague, VA	37.93	-75.42	99
2006	JP Park, MD	38.38	-76.52	99
2007	Cape May, NJ	38.95	-74.87	25
2006	Sandy Pt., MD	39.00	-76.40	99
2005	Tuckerton, NJ	39.56	-74.33	80
2006	Sandy Hook, NJ	40.40	-73.98	98
2005	Patchogue, NY	40.75	-72.92	76
2007	Southold, NY	41.08	-72.40	73
2006	Narragansett Bay, RI	41.63	-71.40	98
2005	Waquoit, MA	41.55	-70.52	81
2007	Duxbury, MA	42.05	-70.65	78
2007	Essex, MA	42.63	-70.70	65
2006	Kittery Pt., ME	43.08	-70.67	95
2007	Freeport, ME	43.83	-70.08	95
2005	Broad Cove, ME	44.03	-69.40	95
2006	Mt. Desert Is., ME	44.42	-68.33	94
2007	Halifax, NS	44.65	-63.35	69
2007	Annapolis Royal, NS	44.81	-65.37	30
2005	St.Andrews,NB	45.08	-67.07	79
2007	Minas Basin, NS	45.20	-64.38	69
2006	St. John, NB	45.22	-66.13	19
2005	Joggins, NS	45.50	-64.48	29
2007	Brule, NS	45.75	-63.18	92
2005	Prince Edward Island	46.78	-64.13	100
2006	Magdalen Is., QE	47.40	-61.85	89

[1] repeated in 2008

<u>Table 1-2:</u> Results of linear regression of vertebral number versus length for each site. All but 9 out of 40 sites (p values in bold) showed a significant relationship between vertebral number and size.

Year	Site	n	R^2	p
2005	St. Augustine, FL	100	0.038	**0.051**
2007	Amelia Island, FL	86	0.018	**0.216**
2006	Jekyll Island, GA	99	0.092	0.002
2005	Tybee Island, GA	98	0.088	0.003
2007	Edisto, SC	69	0.023	**0.217**
2006	Pawley’s Is., SC	98	0.032	0.078
2005	Little River, SC	95	0.156	<0.001
2007	Topsail Beach, NC	100	0.216	<0.001
2005	Morehead City, NC	98	0.149	<0.001
2007	Cedar Island, NC	91	0.057	0.023
2006	Hatteras Inlet, NC	77	0.162	<0.001
2006	Oregon Inlet, NC	100	0.041	0.042
2008	Oregon Inlet, NC	55	0.095	0.025
2007	Norfolk, VA	100	0.189	<0.001
2008	Norfolk, VA	46	0.151	0.008
2005	Silver Beach, VA	97	0.376	<0.001
2007	Chincoteague, VA	99	0.079	0.005
2006	JP Park, MD	99	0.013	**0.263**
2007	Cape May, NJ	25	0.285	0.006
2006	Sandy Pt., MD	99	0.041	0.044
2005	Rutgers, NJ	80	0.088	0.008
2006	Sandy Hook, NJ	98	0.127	0.002
2005	Patchogue, NY	76	0.087	0.01
2007	Southold, NY	73	0.094	0.008
2006	Narragansett Bay, RI	98	0.138	0.001
2005	Waquoit, MA	81	0.080	0.01
2007	Duxbury, MA	78	0.107	0.003
2007	Essex, MA	65	0.188	<0.001
2006	Kittery Pt., ME	95	0.113	<0.001
2007	Freeport, ME	95	0.286	<0.001
2005	Broad Cove, ME	95	0.189	<0.001
2006	Mt. Desert Is., ME	94	0.141	0.001
2007	Halifax, NS	69	0.013	**0.347**
2005	St.Andrews,NB	79	0.007	**0.462**
2007	Minas Basin, NS	69	0.012	**0.376**
2006	St. John, NB	19	0.003	**0.828**
2005	Joggins, NS	29	0.018	**0.489**
2007	Brule, NS	92	0.083	0.005
2005	PEI	100	0.087	0.003
2006	Magdalen Is., QE	89	0.051	0.033

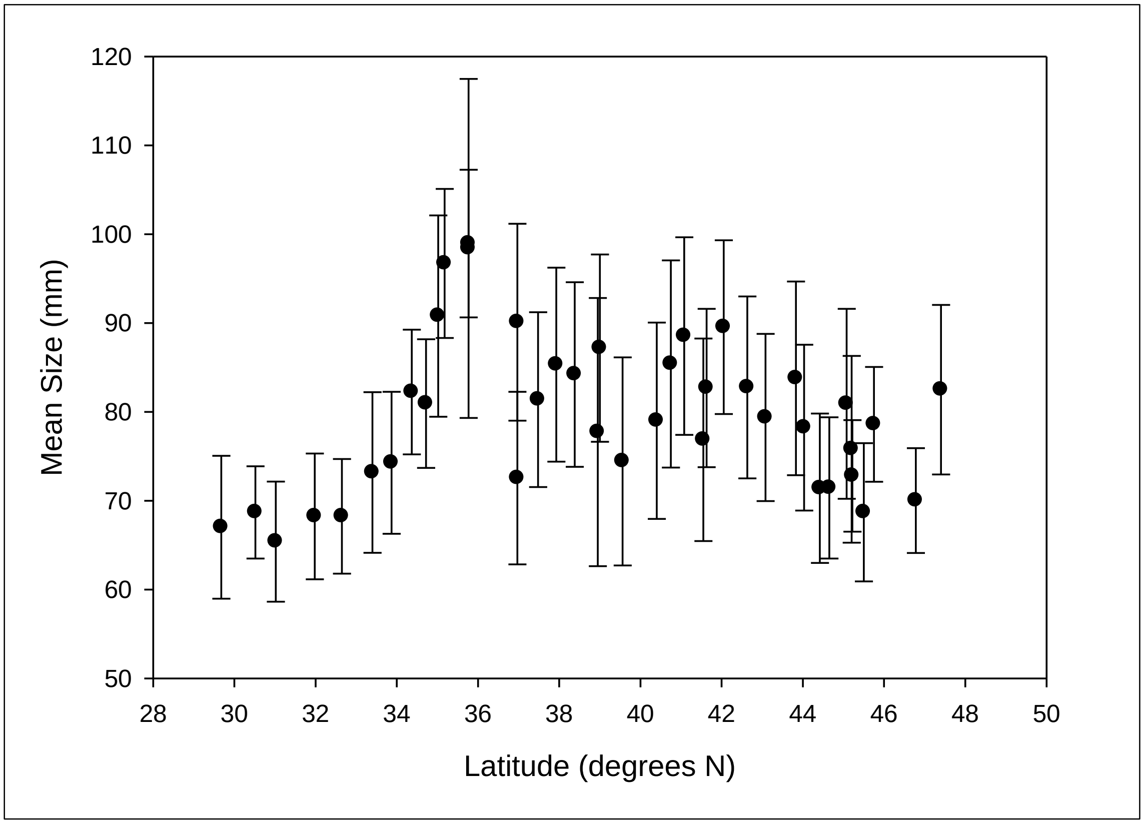


Figure 1-3: Mean size (standard length ± standard deviation) of each population along the range.

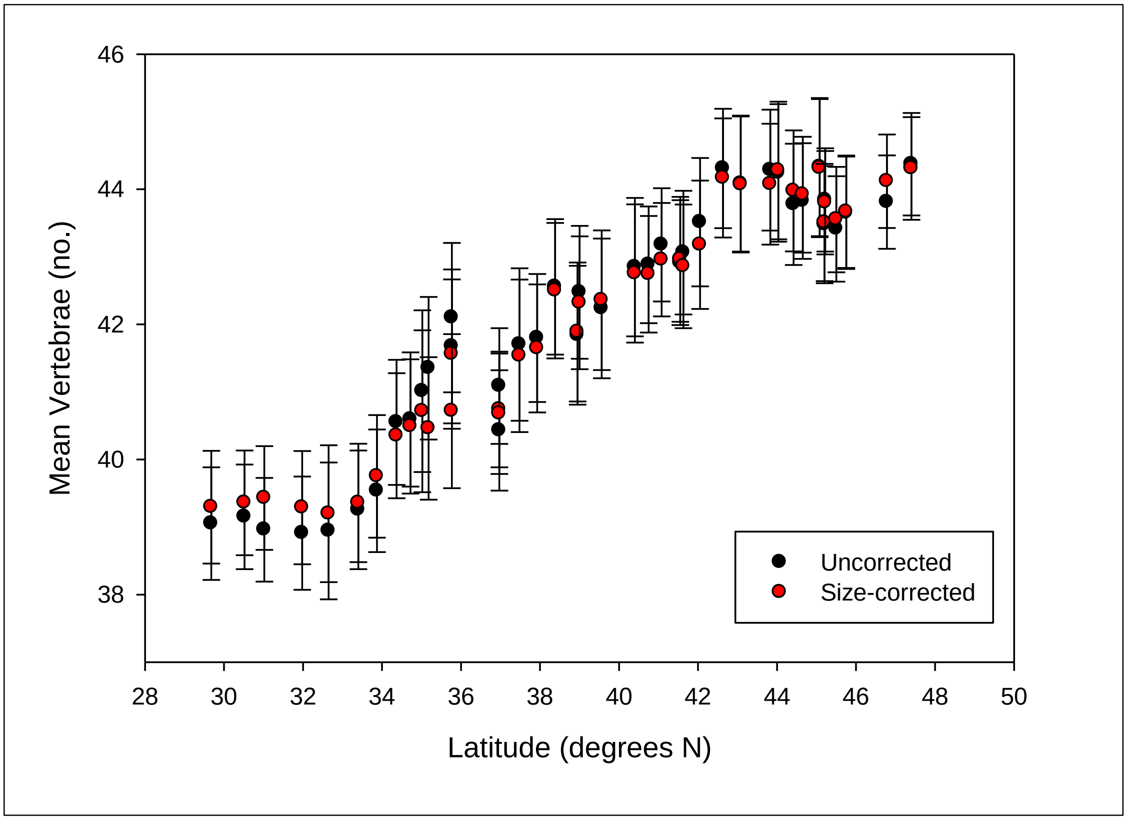
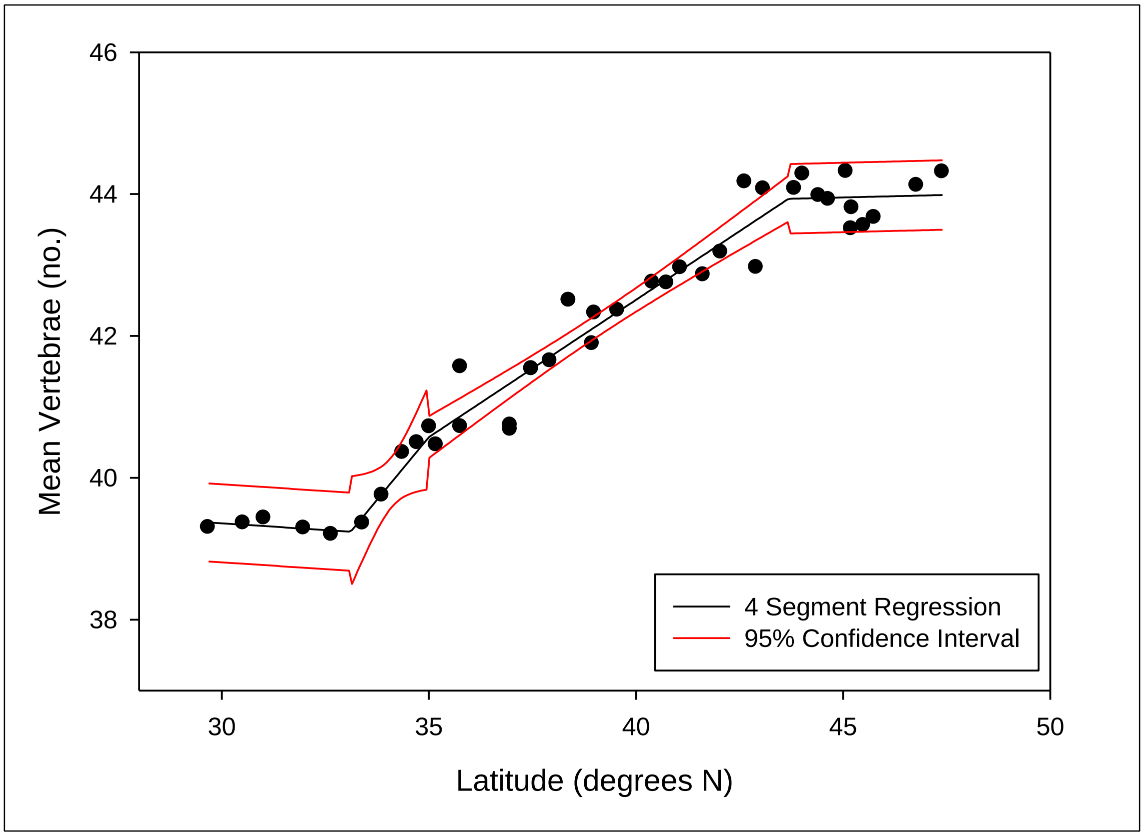


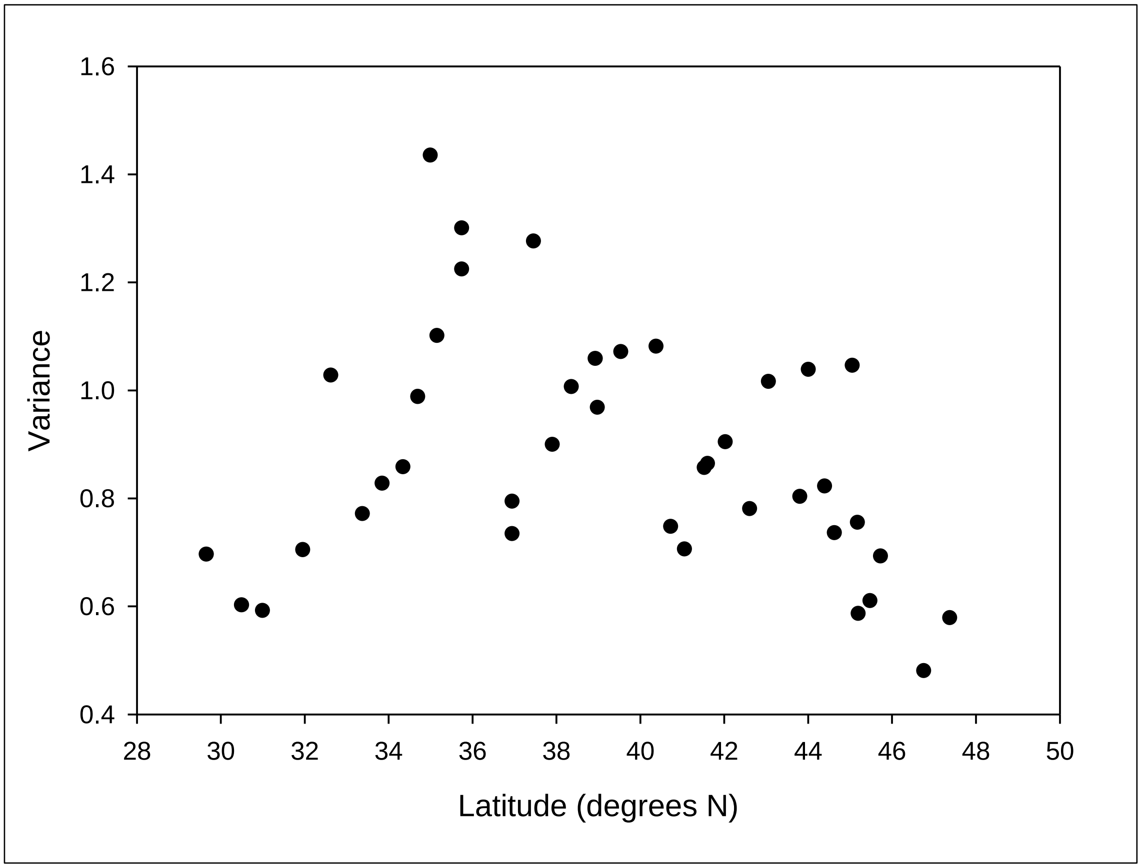
Figure 1-4: Vertebral number (± standard deviation) of uncorrected and size-corrected means at each site.



<u>Figure 1-5:</u> Four segment regression analysis of size-corrected vertebral number with latitude. Breakpoints in the data appear at 33.11°N (South Carolina), 35.00°N (North Carolina), and 43.68°N (Maine).

Table 1-3: ΔAIC analysis of piecewise regression on size-corrected vertebral number data. The ΔAIC value for the 4 segment vs. 5 segment model shows that there is no significant difference between the 4 segment and 5 segment models. However the 5 segment model adds an additional breakpoint beyond the northernmost site of the study.

	lnL	**AIC**	**ΔAIC**
5 segment	28.09457	16.044	0.584
4 segment	26.679	17.679	0
3 segment	19.689	12.689	4.990
2 segment	13.216	8.216	9.462
1 segment	13.217	10.217	7.462



<u>Figure 1-6</u>: Variance in vertebral number versus latitude. Variance is lowest at the tails of the distribution, increasing in the middle of the range. The greatest variance corresponds with an area of rapid change in North and South Carolina.

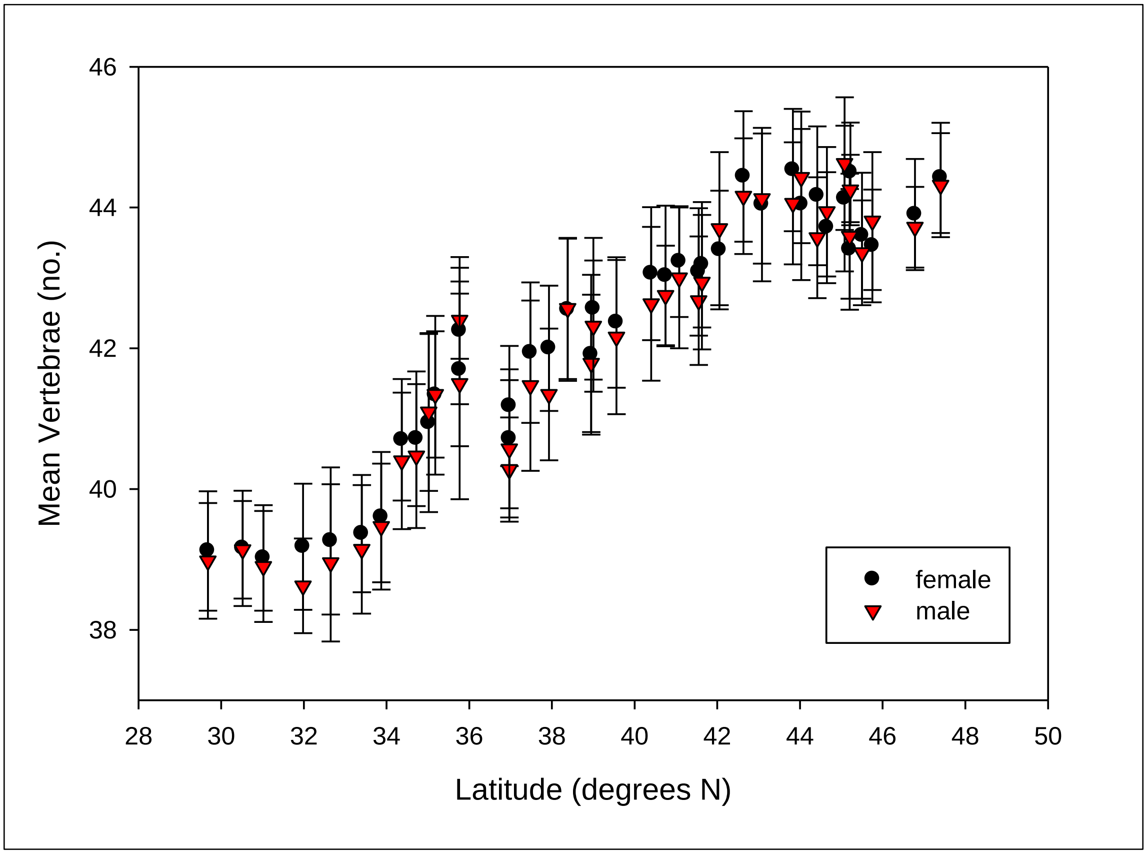
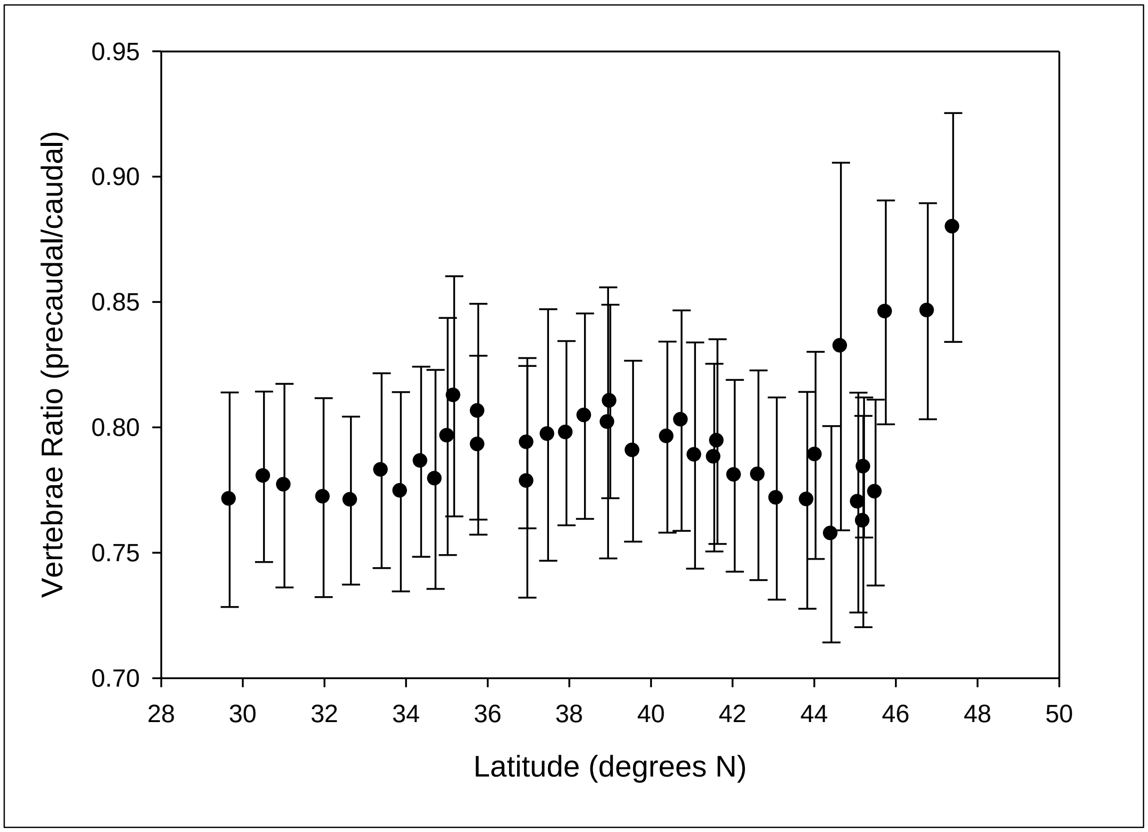


Figure 1-7: Uncorrected mean vertebral numbers (± standard deviation) of male and female fish at each sampling location.



<u>Figure 1-8</u>: Latitudinal pattern in vertebral count ratio given as the mean of each individual's precaudal vertebrae divided by caudal vertebrae (± standard deviation). Most of the increase in vertebral number in the region of rapid change near in North and South Carolina appears to be due to an increase in precaudal vertebrae. In northern regions, the increase appears to be in caudal vertebrae, except in the populations at the northern extreme, which exhibit a greater increase in precaudal vertebrae.

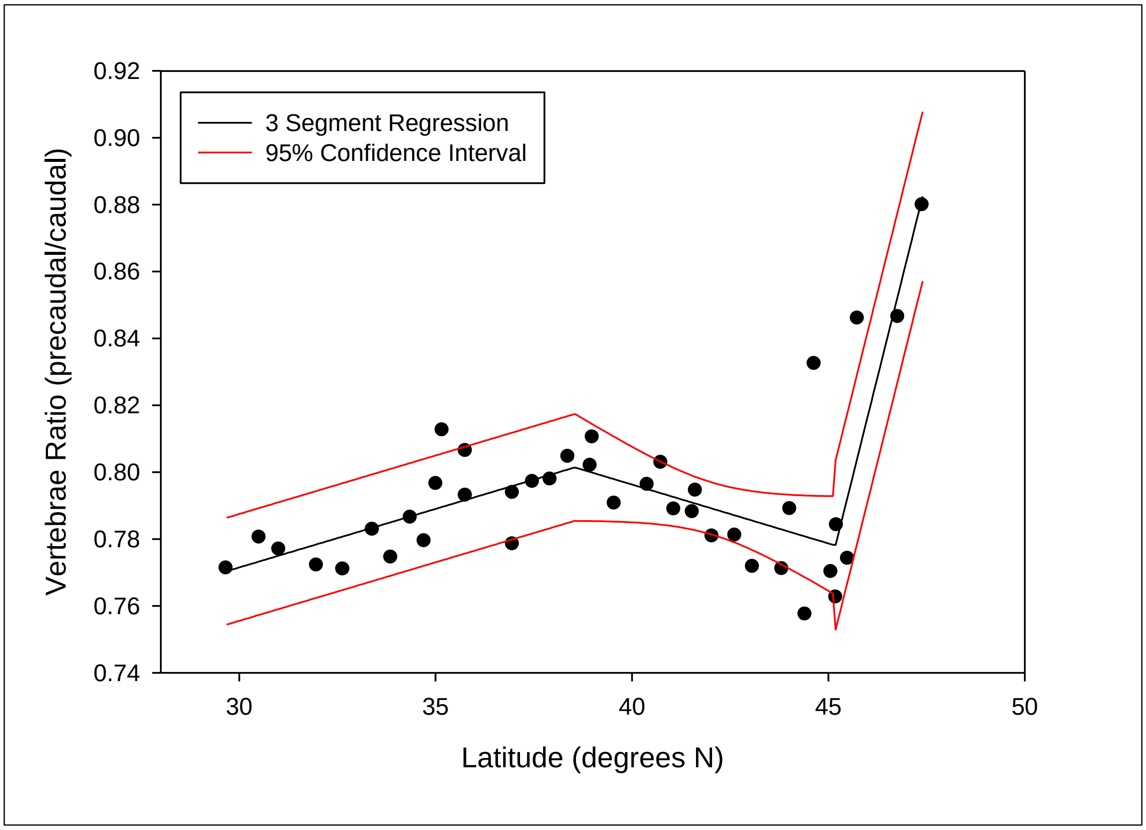


Figure 1-9: Piecewise regression analysis of vertebral count ratio suggesting breakpoints at 38.54°N (Maryland) and 45.18°N (northern Bay of Fundy/Gulf of St. Lawrence).

Table 1-4: ΔAIC analysis of piecewise regression of vertebral count ratio, showing a 3 segment model as the best fit for the data.

	lnL	**AIC**	**ΔAIC**
3 segment	150.344	143.344	0
2 segment	142.571	137.571	5.773
1 segment	131.524	128.524	14.820

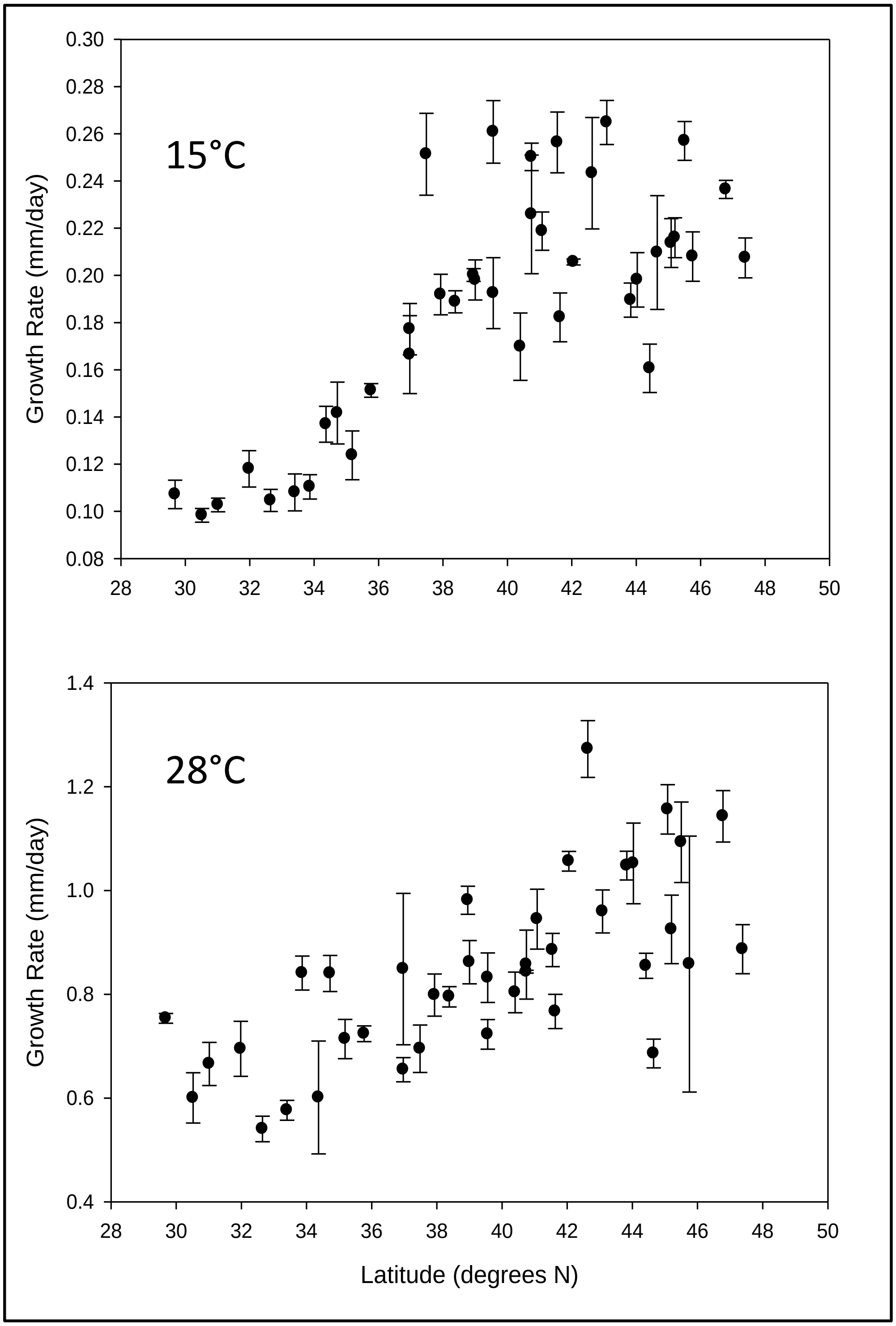


Figure 1-10: Size-dependent growth rate (±standard deviation) of *Menidia menidia* reared at two experimental temperatures.

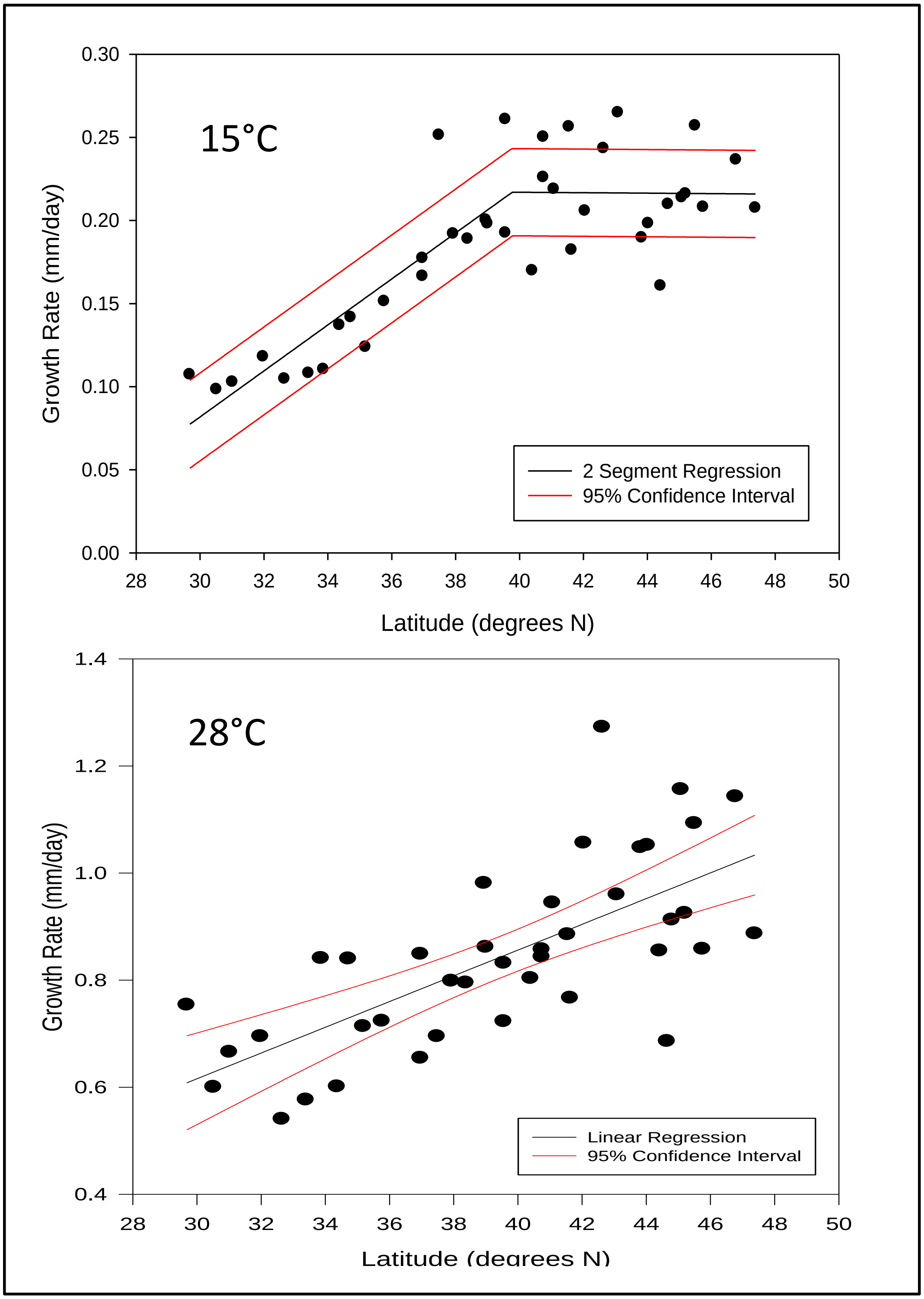


Figure 1-11: Size-dependent growth rate at 15°C and 28°C. At 28°C growth rate appears to increase linearly with latitude, while at 15°C there appears to be a breakpoint in the distribution at 39.77°N, corresponding with northern New Jersey.

Table 1-5: ΔAIC analysis of piecewise regression on size-dependent growth data at 15°C. The ΔAIC value for the 2 segment vs. 3 segment model shows that there is no significant difference between the 2 segment and 3 segment models. The 3 segment model would predict an additional breakpoint at 33.59°N, corresponding with South Carolina.

	lnL	**AIC**	**ΔAIC**
3 segment	124.669	117.669	0.068
2 segment	122.736	117.736	0
1 segment	115.352	112.352	5.385

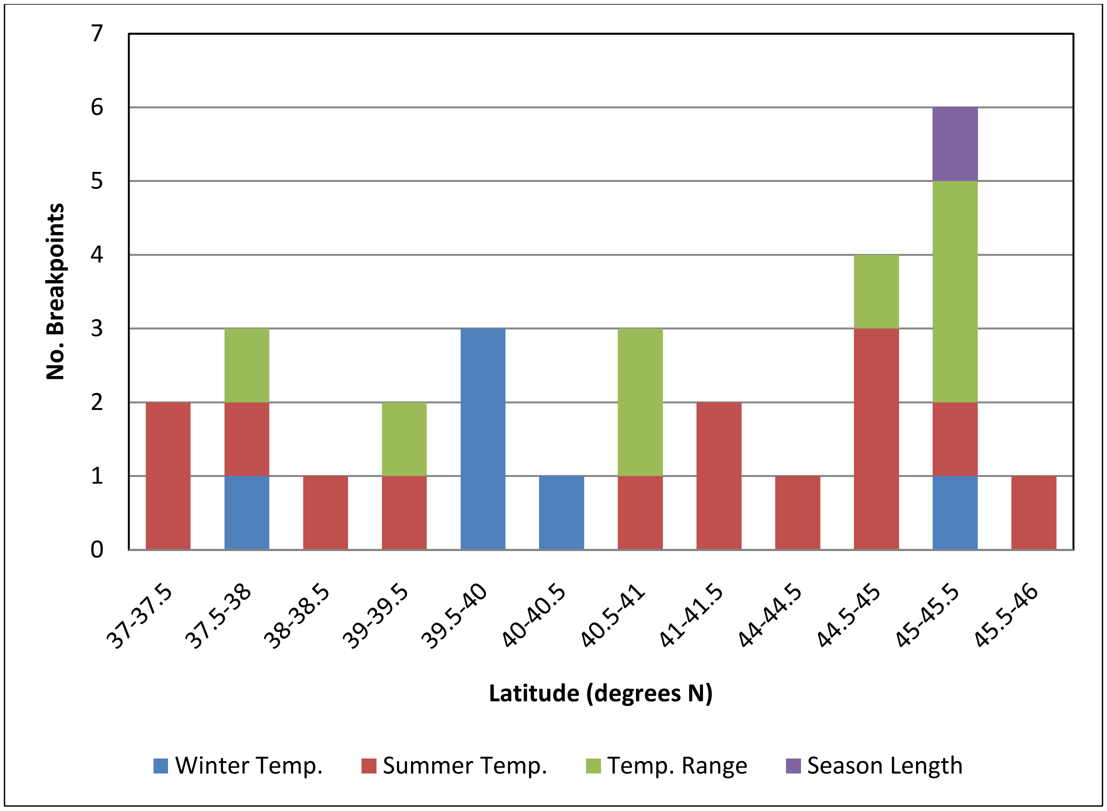


Figure 1-12: Frequency of environmental breakpoints in a given latitudinal range. Sea surface temperature (SST) information from coastal buoy data was used to calculated interannual averages at 25 sites used in the latitudinal study. Winter temperature statistics (blue bars) include average temperature February through March, average February temperature, average winter temperature (January-March), average absolute minimum temperature, and average 30 day moving average minimum temperature. Summer temperature statistics (red bars) include the average temperature July through August, average August temperature, average temperature August through September, average summer temperature (July-September), average absolute maximum temperature, and 30 day moving average maximum temperature. Temperature range (green bars) includes range August through February, range summer through winter, average range and absolute range. Season length (purple bar) is a measure of the growing season of *Menidia menidia*, defined by days where SST ≥ 12°C (Conover and Heins 1987).

Chapter 2:

Fine-scaled Spatial and Temporal Variation in *Menidia menidia* Vertebral Number

Introduction

Early studies investigating variation in vertebral number in fish followed the assertion that significant differences in average number of vertebrae indicated genetically distinct species (Taning 1952). By the early 1900s, however, scientists began to realize that vertebral number often was a locally adapted trait in the same species (Hubbs 1922; Taning 1952). Vertebral patterns have been used in an attempt to identify stocks of Atlantic cod (Nordeide and Pettersen 1998; Swain and Frank 2000; Swain et al. 2001) and Greenland halibut (Rasmussen et al. 1999) or to demonstrate correlations between related species (Yatsu 1980; McDowall 2003; Britz and Johnson 2005; Yamahira et al. 2006). Early experimental results show that vertebral number is set early in development and before hatching (Taning 1952; Lindsey 1988). This has been demonstrated in a variety of fish species including sea trout, mummichog, and plaice (Taning 1952).

A study by Billerbeck et al. (1997) found that latitudinal variation in the number of vertebrae in the Atlantic silverside (*Menidia menidia*) had a genetic basis. According to Jordan's Rule, fish from higher latitudes and colder waters tend to have greater numbers of vertebrae than related fish from lower latitudes and warmer waters (Jordan 1891). In Billerbeck's study, silverside embryos spawned from field collected adults showed vertebral numbers that were tightly correlated with latitude. The correlation persisted in laboratory stocks reared for multiple generations over a range of common environments. This strongly suggests a genetic basis for this phenotype. Although Jordan's rule is found in many fish groups, its cause is unknown.

Studies generally examine whether phenotypic variation is due to phenotypic plasticity or local genetic adaptation. Unlike genetic adaptation which evolves over multiple generations, phenotypic plasticity is a response of a genotype to its environment which occurs within a single lifespan (Pigliucci 2005). A single plastic genotype could produce multiple phenotypes, which may or may not be adapted to a particular environment. Phenotypic plasticity makes it difficult to disentangle the environmental and genetic components of phenotypic variation in the wild. However, phenotypic plasticity can be removed to a certain extent through common garden techniques which remove environmental variability and allow genetic differences to become apparent.

The Billerbeck et al. (1997) study suggests patterns that may arise when comparing field collected specimens to their progeny raised in a common environment. In Billerbeck's study laboratory populations raised at a common temperature showed a linear increase in vertebral number with latitude that was genetically based. However when a subset of the same populations were reared from fertilization at two different temperature treatments, there were significant differences in vertebral number, with colder temperatures producing fish with higher vertebral numbers. Each of three populations had significantly higher vertebral numbers at 17°C than the same population reared at 28°C. This was seen in a low latitude (South Carolina), intermediate latitude (New York), and high latitude (Nova Scotia) population (Billerbeck et al. 1997). This showed that despite being genetically determined, there was still a degree of plasticity in the trait during development. Vertebral number exhibits cogradient variation (CoGV), which is said to occur when environmental influences on phenotypic variation accentuate the genetic variation across an environmental gradient. Thus field populations of adults, which are readily exposed to environmental fluctuations during vertebral development in the egg stage, may show a different geographic pattern than laboratory populations held at constant temperature. Results from chapter 1 show that local adaptation in vertebral number from field populations shows abrupt changes in areas thought to be important regions for ecological change, such as near Cape Hatteras, NC. This pattern may be less pronounced in laboratory populations where major environmental influences are removed and genetic differences are more apparent.

The goal of this study is to investigate the annual variation in vertebral number at a given site and variation in vertebral number on a microgeographic scale within sites <30 km apart. The maximum north-south distance from Staten Island to the North Fork of Long Island is roughly 0.65° latitude, which is equivalent to approximately 72 km. However, this occurs over a broad longitudinal range of roughly 2.4° or approximately 210 km. Thus we can estimate the amount of variability within a short change in latitude, which is not possible in most areas along the eastern seaboard. The theory however, is not that vertebral number varies simply due to latitude, but rather an environmental proxy associated with a given latitudinal location, most likely temperature. Many empirical studies have shown that eggs exposed to different temperatures can develop significant differences in vertebral number (Hubbs 1922; Taning 1952; Lindsey and Ali 1965; Fowler 1970; Lindsey et al. 1984; Lindsey 1988; Pavlov and Shadrin 1997). In some fish, the temperature history of the parents before fertilization has also shown to effect vertebral number (Dentry and Lindsey 1978; Swain and Lindsey 1986).

Coastal temperature records from buoys or other methods do not give enough fine-scaled variation for this particular study. Therefore it is needed to have a method to record in situ temperature at the specific sites where sampling will occur. By placing small loggers to record temperature trends at each sampling site, we can get an idea of microgeographic changes that may influence vertebral number during embryonic development where vertebral number may be influenced by temperature (Taning 1952; Lindsey 1988). *Menidia menidia* have demersal eggs which attach to vegetation in coastal zones. Thus by recording coastal temperature during the spawning period we can get an idea of small differences between sites and possible correlations to vertebral numbers of juvenile fish found in each region and changes that may occur between years. Sampling adults and juveniles from the same sites over two year can also provide insight into site fidelity and interannual variability.

Methods

Broad-scale comparisons

A subset of 6-8 sites from the 39 sites encompassing the east coast of the United States and Canada discussed in Chapter 1 were resampled in 2006 and 2007 to investigate inter-annual variability at a broad scale (Table 2-1). All samples in the study were collected using a 10 or 30 meter beach seine. When possible, 100 specimens were collected from each sites consisting of 50 males and 50 females. Sex was determined by the presence of eggs or milt in ripe adults in the field when possible. Additionally, subsets of juvenile fish from eight populations reared in the growth rate experiments discussed in Chapter 1 were also analyzed to compare vertebral numbers of wild adult populations to their laboratory-reared offspring which were grown in a common environment (Table 2-2). Equal ratios of male and female fish were used whenever possible. Sex was determined through dissection and inspection of gonads in conjunction with a study investigating local adaptation in temperature dependent sex determination (TSD, results described in Duffy 2010).

Microgeographic Comparisons

Preliminary results from juvenile *Menidia menidia* sampled in 2005 from 7 sites spanning the length of the island on both the north and south shores of Long Island, NY showed significant differences among sites along a small latitudinal range (ANOVA, $p<0.001$). In 2006 and 2007, 5 of these sites were repeated and 5 additional sites were added for a total of 10 sites (Table 2-3, Figure 2-1). In 2007, adult *M. menidia* were collected from 8 of the sites. This created a time series of juveniles collected in late summer of 2006, those fish returning as adults in the spring of 2007, and the offspring of those adults in late summer of 2007. All samples in the study were collected using a 10 or 30 meter beach seine. When possible, 100 specimens were collected from each sites consisting of 50 males and 50 females. Sex of adult fish was determined by the presence of eggs or milt in ripe fish in the field when possible. Sex of juvenile was determined through dissection and inspection of gonads in conjunction with a study investigating the sex ratios of *Menidia menidia* in the wild (Duffy et al. 2009).

Environmental Temperature Data

Billerbeck et al. (1997) showed that the temperature that *Menidia menidia* embryos experience during development can influence vertebral phenotype, which provides evidence for phenotypic plasticity in this trait. To account for the effects of local environmental temperature on vertebral number, Hobo Pendant Temperature

Loggers (Onset Computer Corp.) were set at all 10 sample sites in 2006 and 5 sites in 2007 during the spring spawning period (Table 2-3). At each site loggers were attached to PVC pipe secured into the sediment which kept the logger suspended 0.3m below the lowest water mark. Water temperature was recorded every four hours (6 times per day). To estimate the mean temperature fish experienced during embryonic development, it was assumed that all fish exhibited a 7 day embryonic period, hatched at 5mm length, and had a growth rate of 1mm/day based on prior studies of *M. menidia* from New York (Conover and Present 1990; Walsh et al. 2006). Individual fish were grouped into 5mm size classes and the average temperature experienced for each group during embryonic development was calculated. Site-specific mean temperatures were determined by calculating a weighted average for each site based on the number of fish in each size class. Linear regression was used to investigate the relationship between temperature and vertebral number, using both uncorrected and size-corrected vertebral number. Size corrections were done following the same method described in Chapter 1, using the mean size for all juvenile populations in a single sampling year (2006 = 46mm, 2007 = 50mm).

Vertebral Counts and Statistical Analyses

Adult fish were x-rayed using the same methods discussed in Chapter 1. Fish less than 50mm in length were x-rayed using a INSPEX 20i digital x-ray system with a Kevex PXS10-16W micro-focus x-ray source and Varian PaxScan 4030R panel and software (40 kV /140uA, Resolution = 3.941p/mm (density dependent), compiled by Kodex, Inc). Images were captured with Varian Image Viewing Acquisition (VIVA), stored as TIFF files (.tif) and analyzed for length and vertebral counts using ImageJ software (Rasband 1997-2010). Vertebral numbers for all populations in this section were corrected for size as in Chapter 1, with separate correction factors used to predict vertebral number at the mean size for each adult and juvenile population group (i.e. 2007 juveniles and 2007 adults). Spatial and temporal differences in population means were tested using a one-way ANOVA with Holm-Sidak pairwise multiple comparisons. Also, mean vertebral numbers of a subset of wild adult populations were compared to their offspring reared in the laboratory using a one-way ANOVA with Holm-Sidak pairwise multiple comparisons.

Results

Broad Scale Spatial and Temporal Variation

In the eight sites sampled over two or three years, the majority showed a slight correlation between size and vertebral number (Figure 2-2). Three of these sites showed significant temporal differences in vertebral number once corrected for size (Figure 2-3). There was an overall difference of approximately four vertebrae across the range, but in Morehead City, NC (34.72°N), vertebral number was significantly higher in 2005 than in 2007 ($p<0.001$). The mean was 0.52 vertebrae higher in 2005. There were no significant differences between 2005 and 2006 or 2006 and 2007 however. In Silver Beach, VA (37.48°N) and Tuckerton, NJ (39.56°N) vertebral numbers were significantly higher in 2006 than in 2005 or 2007 ($p<0.001$). In Virginia the mean was 1.4 vertebrae higher in 2006 and in New Jersey the mean was 2.2 vertebrae higher. There were no significant differences between 2005 and 2007 in these sites or among any years in the other five sites studied. Most interestingly, the samples collected in 2006 in Silver Beach, VA and

Tuckerton, NJ were juvenile fish collected in the late summer, whereas the samples from other years were adult fish caught in the spring. The 2006 samples from Patchogue, NY (40.75°N) were also juvenile samples but these did not show significant differences from the other years sampled once corrected for size.

Additionally, when vertebral number data from adults caught in the wild was compared to their progeny raised in a common environment four of the eight sites studied show a significant difference between the mean vertebral numbers of the adult and juvenile populations (Figure 2-4). In St. Augustine, FL (29.68°N) the mean vertebral number for the wild adult fish was 0.50 vertebrae higher than the lab-reared juveniles. In Topsail Beach, NC (34.37°N) the mean vertebral number was 0.52 higher in the adults, and in Oregon Inlet, NC (35.77°N) the mean for the adults was 1.57 vertebrae higher. Conversely, in Sandy Point, MD (39.00°N) the mean number of vertebrae was 0.80 higher for the lab-reared juveniles when compared to the adults. This suggests that some of the variation seen in the field data may be due to environmental effects, presuming that laboratory rearing controlled for environmental influences on vertebral number. However, it is also possible that the subsample of adult fish selected for strip-spawning had higher or lower vertebral numbers than the overall wild adult population by chance. Only 25-50% of the individuals sampled at each site were used to create the embryos for laboratory rearing. Since the exact individuals used for spawning is unknown, it is impossible to conclusively determine if sampling error caused the discrepancy between the adult and juvenile vertebral numbers. However, this must be considered as a possibility.

Fine-scaled Spatial and Temporal Variation

There was an overall mean difference of 1.10 vertebrae among the ten populations of *Menidia menidia* studied in Long Island, NY after being corrected for size (Figure 2-4 & 2-5). Significant differences could be seen in close populations sampled in the same year (Table 2-4, $p<0.001$), but there was not a significant relationship between vertebral number and latitude in any year (2006 juveniles: $p = 0.994$, 2007 adults: $p = 0.250$, 2007 juveniles: $p = 0.685$, Figure 2-6), nor was there a significant trend with longitude (2006 juveniles: $p = 0.536$, 2007 adults: $p = 0.302$, 2007 juveniles: $p = 0.649$). There were two populations where significant differences in mean vertebral number could be seen in the same population among different years. In Mt. Sinai, NY (40.95°N) the juvenile fish studied in 2006 had a mean vertebral number that was 0.61 vertebrae higher than the juvenile fish from 2007. In Patchogue, NY (40.75°N), the juvenile fish in 2007 had a significantly higher mean vertebral number than the adult fish returning to that site in 2007 (0.86 vertebrae). Additionally, the juvenile fish from that same site had a significantly higher mean vertebral number than the adults in the same year (0.43 vertebrae). There was no correlation between 2006 juveniles and 2007 adults or 2007 adults and the 2007 juveniles at the same sites (Figures 2-7 & 2-8).

Temperature data from loggers placed in the field during the spring spawning period in 2006 and 2007 was used to test the hypothesis that small-scale difference may be due to vertebral plasticity caused by developmental temperature. There was a slight negative relationship between mean vertebral number and developmental temperature, with lower temperatures trending towards higher vertebral numbers, however the relationship was non-significant in both the size-corrected and uncorrected vertebral numbers for juvenile fish (Figure 2-9, p = 0.470 and p = 0.407, respectively). It should be noted that temperature data during the spawning period from eastern Jamaica Bay is considerably higher than other sites in the region. It is possible that this is due to improper deployment of the temperature logger in particularly shallow water or in an area where it was close to the water surface.

Discussion

The data presented shows evidence of both spatial and temporal variability in vertebral number in *Menidia menidia*. When wild adult fish were compared to laboratory populations of their offspring, four of the eight populations had significant differences between the wild adults and the lab-reared juveniles. In three out of four cases, the laboratory populations had lower vertebral numbers than the wild populations. Vertebral number is a meristic trait set early in development and thus exposed to less environmental variability than a trait that affects an individual throughout its lifecycle, such as growth rate. There is, however, a degree of plasticity that occurs during development and before hatch. It is possible then, that the differences between the adult populations and their lab reared offspring are simply an indication of environmental effects on the adult populations, which would have been removed in laboratory fish reared in a common environment. Billerbeck et al. (1997) found that raising the environmental temperature *M. menidia* embryos were exposed to by 11°C caused a decrease in 0.5-0.7 vertebrae in three different locally adapted populations. Therefore, it is possible in the current study that adult populations in the wild were exposed to lower temperatures during embryonic development than embryos collected for laboratory experiments. However, this cannot be said for certain, as the exact temperatures the adult fish or the larvae experienced as embryos are unknown. Temperature data within the coastal bays during the time when the adult fish were spawned is unavailable, and the larvae were part of a larger experiment investigating local adaptation in *M. menidia* that did not tightly control or monitor embryonic temperature during transport. In most cases, the embryos were spawned in the field and then transported in coolers kept at a low temperature (~15°C) for 1-5 days to delay development. However, it is possible that the embryos experienced temperature fluctuations throughout this period. In some cases, the embryos were spawned in the laboratory and placed directly into 21°C water baths. Even in this case, temperature was only recorded once per day, and may have fluctuated throughout embryonic development (~7 days, personal observation). Therefore, without records of temperature taken several times a day throughout transport and embryonic development, coupled with temperature data from the field when the adults were spawned, it is impossible to say for sure whether the differences in adult and juvenile populations are due to phenotypic plasticity, although the assertion is plausible.

Plasticity in vertebral number has been shown in a variety of species. Täning (1952) found that this change in environmental temperature between 4°C and 10°C caused a marked change in vertebral number in sea trout (*Salmo trutta trutta*). Fish from cold temperatures exposed to warm temperatures showed decreased vertebral numbers while fish from warm temperatures exposed to cold temperatures had an increase in vertebral number. In homozygous clones of *Rivulus marmoratus*, fish reared at low temperature had increased vertebral counts when compared to those at intermediate or high temperature (Harrington and Crossman 1976). Since these fish were genetically the same, the difference seen had to be due to their rearing environment. Many species are also known to have U- or V-shaped norms of reaction of vertebral number and temperature with intermediate temperatures causing a reduction in vertebral number (reviewed in Lindsey 1988). Temperature effects on vertebral number have also been documented in reptiles (Osgood 1978; Lourdais et al. 2004) and amphibians (Jockusch 1997).

Lindsey (1988) described an “atroposic model” for the establishment of vertebral number which accounts for differences in processes such as growth and tissue differentiation through time and at varying temperatures. This model ascertains that the final number of vertebrae is determined “by the level that one process has attained when it is suddenly terminated; termination is triggered at the moment when the other process has risen to some critical level” (Lindsey 1988). Depending on the temperature exposure of eggs, incubation time in *M. menidia* can range from 4 days at 30°C to 27 days at 15°C (Martin and Drewry 1978). At 21°C the incubation time is roughly 7 days (personal observation). The exact timing when vertebral number becomes set in *M. menidia* is unknown but it is likely to happen early in the embryonic period as vertebrae are some of the first meristic structures to appear in development (Lindsey 1988). During the embryonic phase, and certainly during the transport of embryos described above, it is likely that environmental changes in temperature could affect vertebral number, particularly if embryos experienced a range of temperatures at various stages of development.

Much of the focus on phenotypic plasticity in vertebral number has focused on developmental temperature; however it is possible that other environmental factors such as salinity or photoperiod may also affect vertebral number. Both decreased levels of dissolved oxygen and increased levels of dissolved carbon dioxide have been shown to increase vertebral number in several fish species (Taning 1952; Lindsey 1988). Decreased exposure to light has also been shown to increase vertebral number by 0.3-0.5 vertebrae in grunion (*Leuresthes tenuis*) (McHugh 1954). Salinity is known to influence vertebral number as well; however whether the effect is positive or negative varies among species (Taning 1952; Swain and Lindsey 1986; Lindsey 1988). Although none of the aforementioned environmental factors have been empirically tested in *Menidia menidia*, it is possible that any or all can influence vertebral number during development in this species. Since these environmental factors were not carefully monitored during transport or development of the experimental embryos it is impossible to rule out the possibility of their effect on vertebral phenotype. One could imagine that transport in a closed cooler, for example, could lead to an environment with higher levels of carbon dioxide and lower levels of light, both of which may lead to an increase in vertebral number.

Although plasticity may explain some of the variation in vertebral phenotypes, Billerbeck's study in *Menidia menidia* only resulted in a 0.5-0.7 vertebrae change when fish were reared in environments that differed by 11°C. This would not explain the difference of 1.57 vertebrae seen between the wild caught adult fish in Topsail Beach, NC and the larvae from the same location reared in the laboratory. This difference may be due to genetic differences in the parental subsample, or a combined effect of this and phenotypic plasticity.

When broad scale populations of *Menidia menidia* from along the east coast were studied over multiple years, three of the eight populations showed significant differences in mean vertebral number from one year to the next (Figure 2-3). Interestingly, two of these three populations were juvenile fish which with significantly higher vertebral numbers than adults in the same population. In the ten populations studied on a microgeographic scale in Long Island, NY two populations showed significant temporal differences in vertebral number. This study showed further evidence of juvenile populations showing significantly higher vertebral numbers than adults in one of the sites. One could argue that the juvenile fish have higher vertebral numbers simply because they were spawned at lower temperatures (Lindsey 1988). However, adult fish studied in 2007 would be from the same cohort as juvenile fish from 2006. The adults would presumably be the fish from 2006 returning to spawn during the spring. Therefore, a decrease in vertebral number over time in a cohort suggests that this is not a case of phenotypic plasticity, but rather a case of natural selection for lower vertebral number at a given site, a hypothesis that will be further explored in Chapter 3.

Previous studies have shown that in spring-spawning species, larger fish within a cohort which were spawned earlier in cooler temperatures have more vertebrae (Lindsey 1988). Billerbeck's (1997) study showed that southern (South Carolina) populations had a degree of sexual dimorphism, with females having higher vertebral averages than males. This pattern corresponds with known patterns of environmental sex determination in the species (Conover and Fleisher 1986). This causes fish spawned earlier in the season and hence at colder temperatures to have a highly female-biased sex ratio. It is likely that the sexual dimorphism seen in southern *M. menidia* populations is due to ESD and temperature responses of vertebral number (Billerbeck et al. 1997). Therefore, interannual variability in vertebral number can potentially be attributed to seasonal temperature differences that embryos are exposed to during the spring spawning period. However, *in situ* temperature data did not seem to be correlated with vertebral number in juvenile populations in the current study. It is possible that the high degree of variability in small scale differences on vertebral number mask the vertebral number-temperature relationship in the juveniles. Recent research investigating natal homing in this species shows evidence of local retention in Long Island populations (Clarke et al. in press); however I saw no evidence of a correlation between adult and juvenile populations at a given site showing the same vertebral phenotype. It is possible that the 3-4 vertebrae range of vertebral phenotypes found at a given site is enough to conceal the small differences of frequencies of one phenotype versus another at a nearby location.

This study has shown that vertebral number remains relatively constant across a broad geographic range when compared across years. There is a degree of plasticity that can occur, and at sites less than 30 km significant variation in vertebral number becomes difficult to determine. Interestingly, on a broad scale there seems to be a tendency for juvenile fish to have higher vertebral numbers that adult fish returning to the same site. This phenomenon will be further discussed in chapter 3.

Table 2-1: Sampling locations, samples sizes (n) and years sampled to investigate temporal variation in vertebral number.

Site	**Latitude**	**Longitude**	**n**		
			2005	**2006**	**2007**
Morehead City, NC	34.72	-76.68	98	44	100
Silver Beach, VA	37.48	-75.97	97	32[a]	84
Tuckerton, NJ	39.56	-74.33	80	26[a]	80
Patchogue, NY	40.75	-72.92	76	44[a]	99
Waquoit, MA	41.55	-70.52	81	40	98
Broad Cove, ME	44.03	-69.40	95	93	66
St.Andrews,NB	45.08	-67.07	79	44	n/s
Prince Edward Island	46.78	-64.13	100	93	n/s

a = juveniles
n/s = not sampled

Table 2-2: Location, sample size (n) and year sampled of adult and juvenile fish used for comparison of wild and lab-reared populations.

Year	Site	Latitude	Longitude	n	
				Adults	**Juveniles**
2005	St. Augustine, FL	29.68	-81.25	100	191
2007	Topsail Beach, NC	34.37	-77.67	100	100
2006	Oregon Inlet, NC	35.77	-75.52	100	95
2005	Silver Beach, VA	37.48	-75.97	97	95
2006	Sandy Pt., MD	39.00	-76.40	99	100
2005	Patchogue, NY	40.75	-72.92	76	194
2006	Kittery Pt., ME	43.08	-70.67	95	74
2005	Prince Edward Island	46.78	-64.13	100	161

Table 2-3: Site locations and sample sizes (n) for populations studied in Long Island, NY. All sites had temperature data recorded in spring 2006. Sites which had temperature data recorded in spring 2007 are noted.

Site	**Latitude**	**Longitude**	**n**		
			2006 Juveniles	**2007 Adults**	**2007 Juveniles**
Jamaica Bay West	40.60	-73.88	69	27	80
Tobay Beach[1]	40.61	-73.41	89	100	96
Jamaica Bay East	40.63	-73.78	79	n/s	73
Patchogue[1]	40.75	-72.92	85	99	88
Manhassett[1]	40.81	-73.70	92	n/s	90
Cold Spring Harbor	40.87	-73.47	93	17	96
Shinnecock	40.87	-72.49	81	25	97
Jamesport[1]	40.92	-72.576	95	32	98
Mt. Sinai	40.95	-73.03	87	63	91
Southold[1]	41.08	-72.40	90	73	50

n/s = not sampled

[1] temperature data in Spring 2007

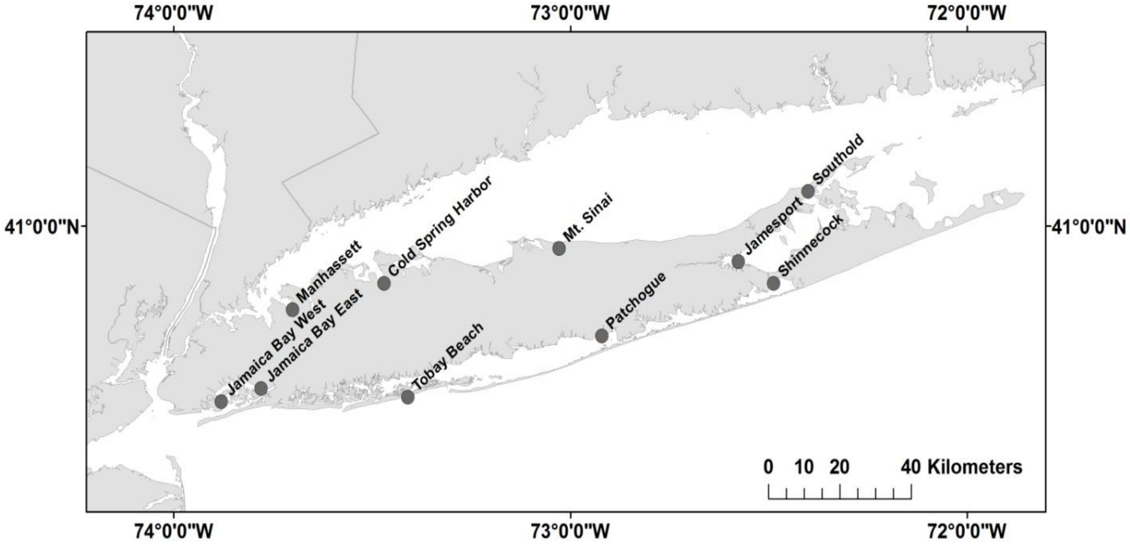


Figure 2-1: Map of ten sampling locations for 2006-2007 in Long Island, NY.

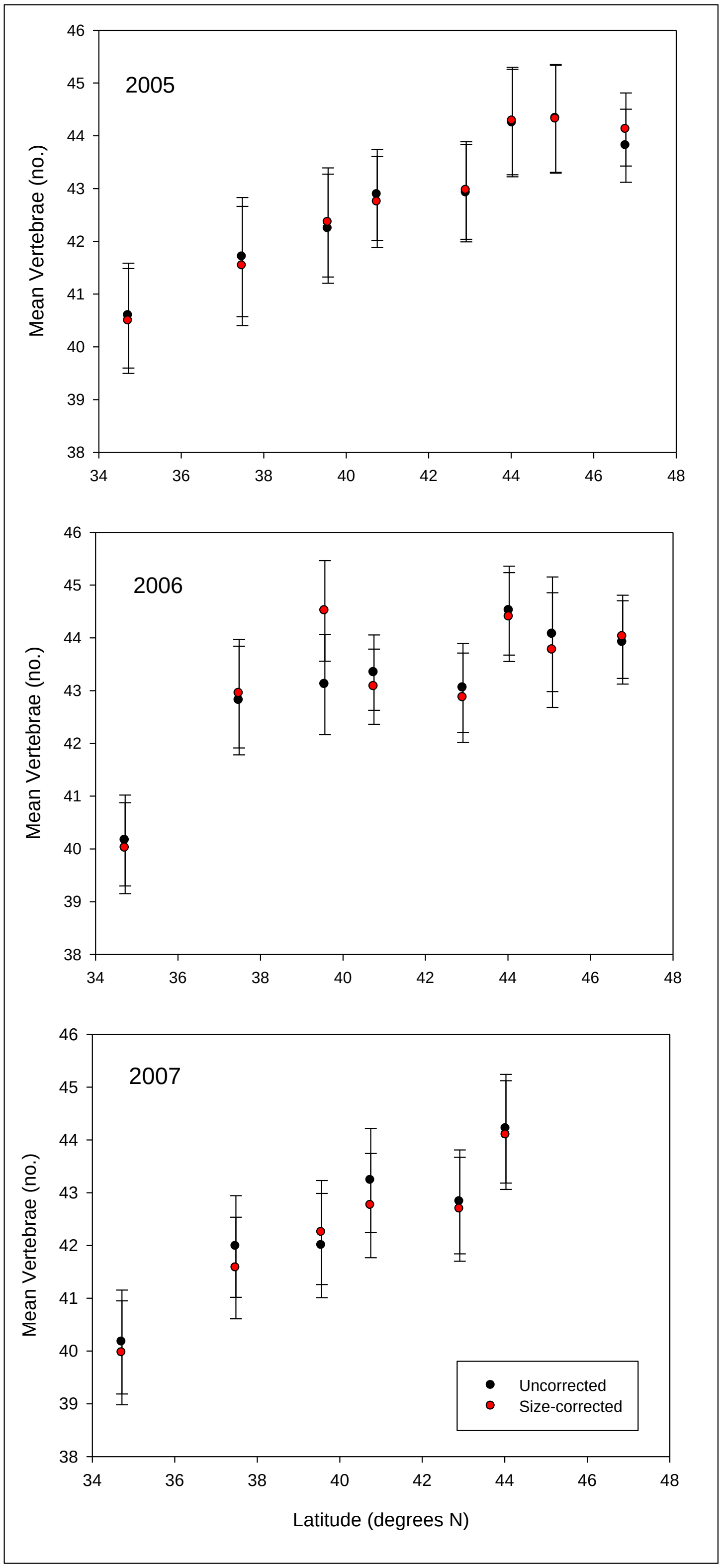


Figure 2-2: Uncorrected and size-corrected vertebral number means (±standard deviation) for each population over the three years studied.

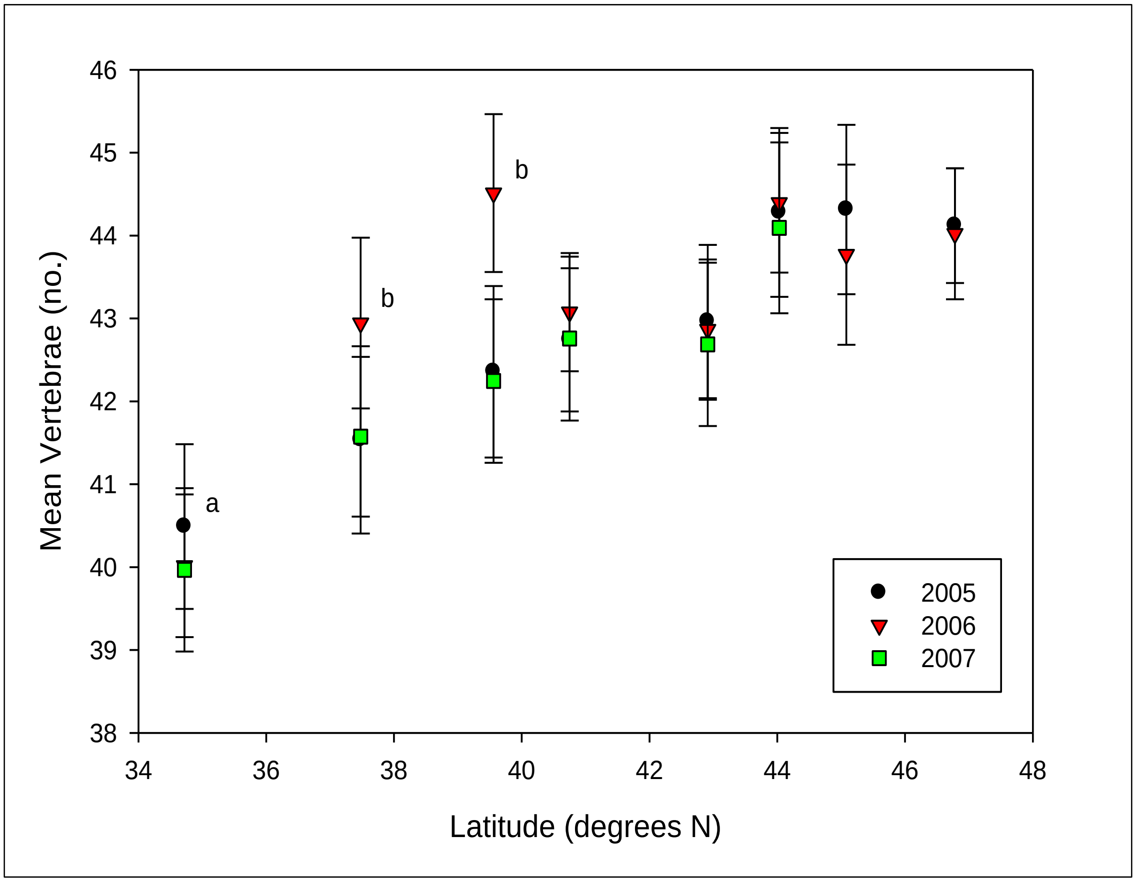
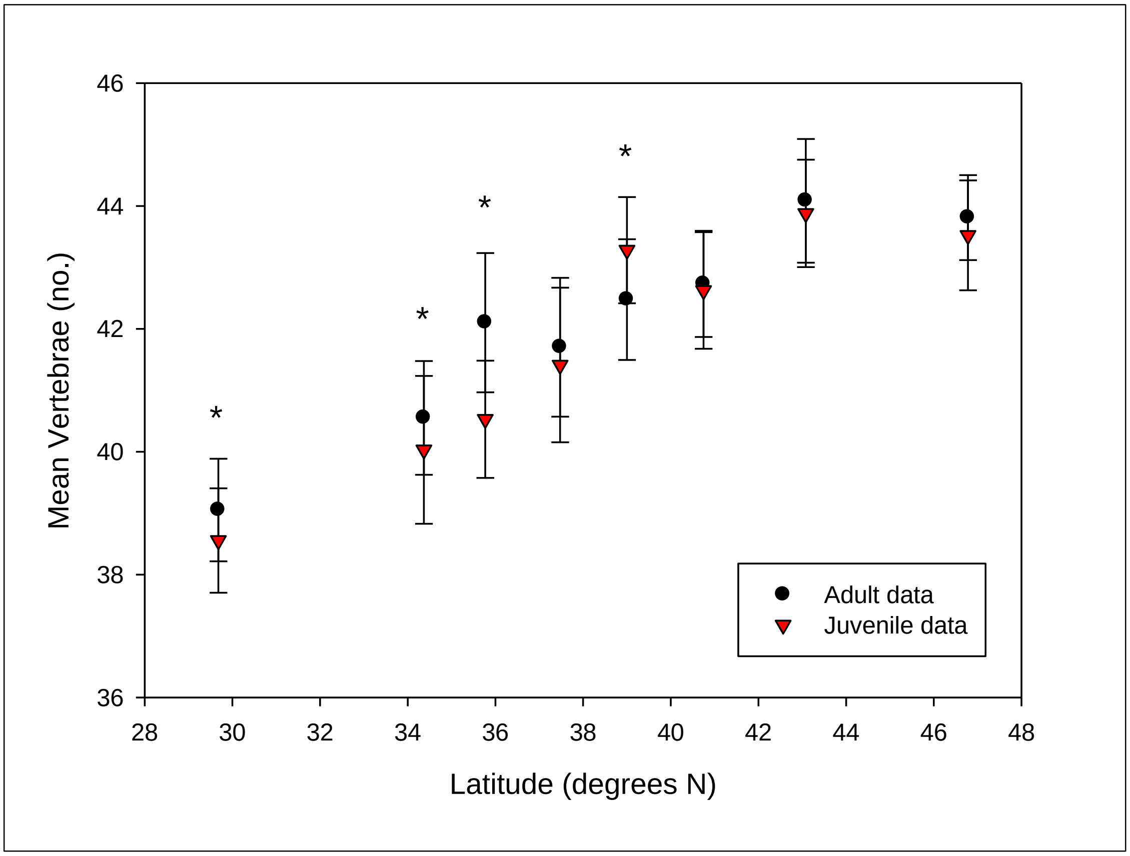


Figure 2-3: Temporal variation in size corrected vertebral number versus latitude at a broad spatial scale. [a]2005 and 2007 samples significantly different (p<0.001). [b]2006 samples significantly different from 2005 and 2007 (p<0.001).



<u>Figure 2-4</u>: Comparison of wild caught adult populations with laboratory-reared progeny from the same sites (juvenile data). (*) denotes populations where adult and juvenile data is significantly different ($p<0.001$).

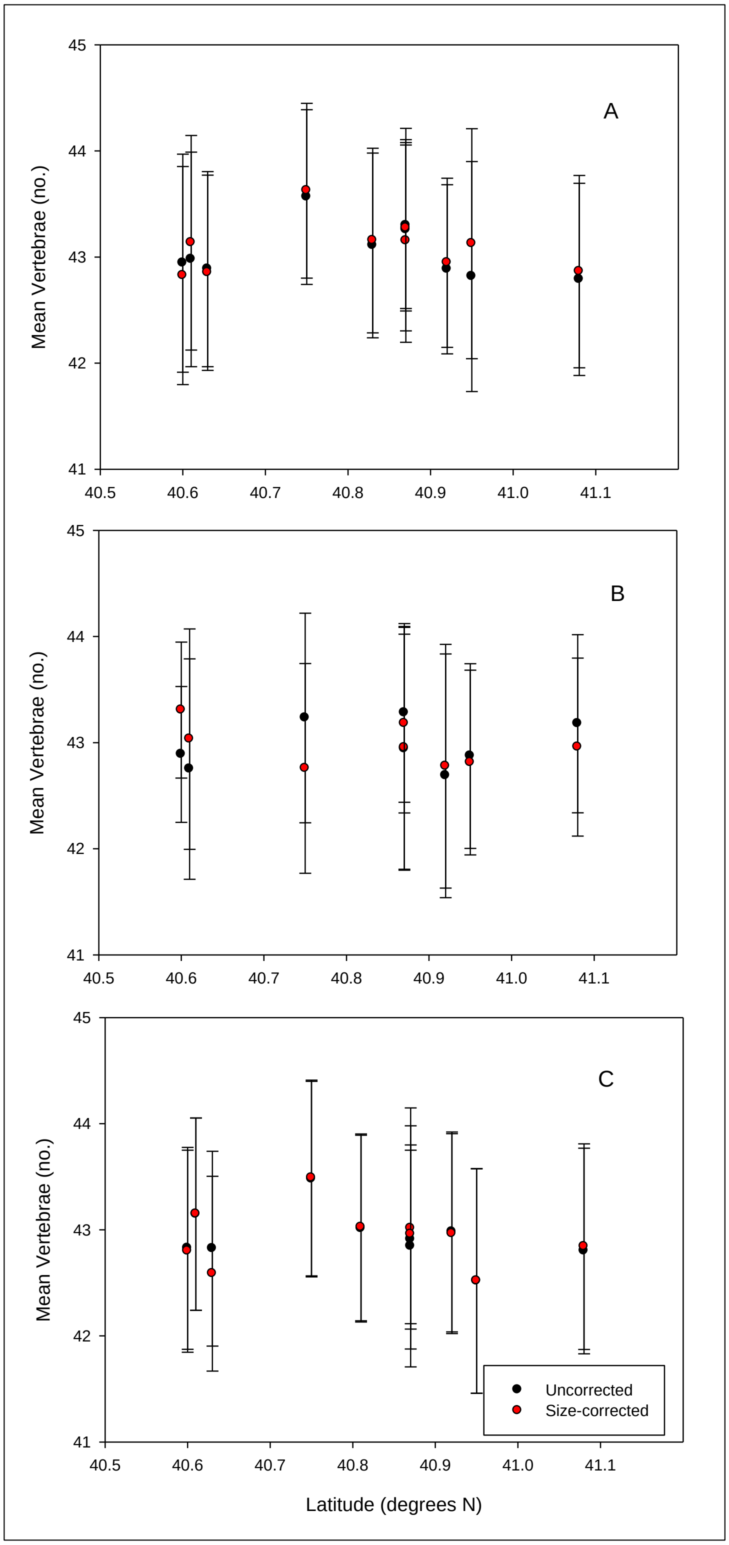


Figure 2-5: Uncorrected and size-corrected vertebral number means (± standard deviation) for (A) juvenile fish collected in late summer 2006, (B) adult fish collected in spring 2007, and (C) juvenile fish collected in late summer 2007.

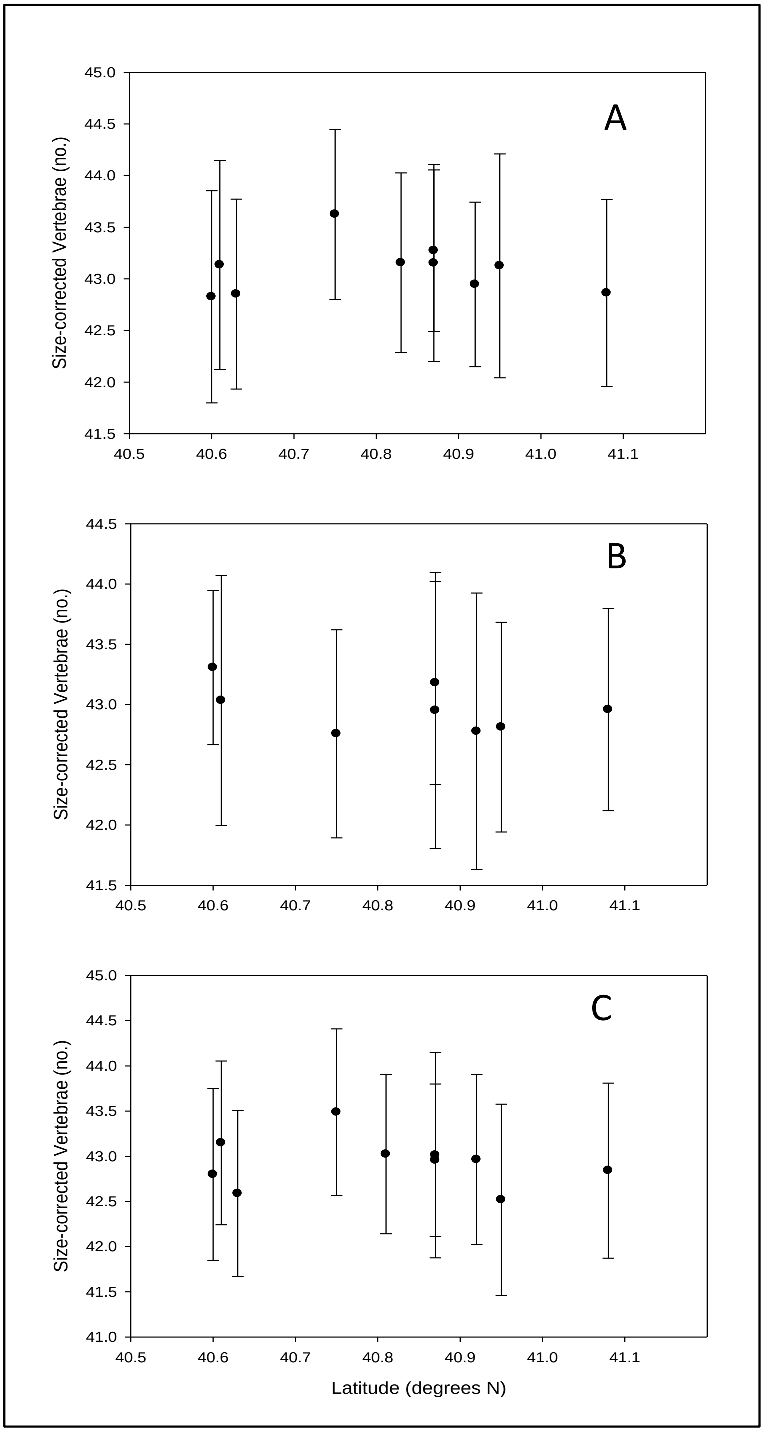


Figure 2-6: Latitudinal trends in size-corrected vertebral number for 2006-2007 time series for populations collected on a microgeographic scale around Long Island, NY. (A) Juvenile fish collected in late summer 2006. (B) Adult fish collected in spring 2007. (C) Juvenile fish collected in late summer 2007. No collections show a significant relationship between vertebral number and latitude (A: $p = 0.994$, B: $p = 0.250$, C: $p = 0.685$).

Table 2-4: Holm-Sidak pairwise comparison of 2006-2007 time series of populations collected in Long Island, NY. (■) Denotes populations that are significantly different. (🟥) Denotes populations which are significantly different when comparing two time points at the same site.

			2006 juveniles										2007 adults								2007 juveniles									
			Jamaica Bay West	Tobay Beach	Jamaica Bay East	Patchogue	Hempstead Harbor	Cold Spring Harbor	Shinnecock	Jamesport	Mt. Sinai	Southold	Jamaica Bay West	Tobay Beach	Cold Spring Harbor	Shinnecock	Jamesport	Mt Sinai	Southold, NY	Patchogue	Jamaica Bay West	Tobay Beach	Jamaica Bay East	Patchogue	Manhassett	Cold Spring Harbor	Shinnecock	Jamesport	Mt. Sinai	Southold
		Latitude (° N)	40.6	40.61	40.63	40.75	40.83	40.87	40.87	40.92	40.95	41.08	40.6	40.61	40.87	40.87	40.92	40.95	41.08	40.75	40.6	40.61	40.63	40.75	40.81	40.87	40.87	40.92	40.95	41.08
2006 juveniles	Jamaica Bay West	40.6																												
	Tobay Beach	40.61																												
	Jamaica Bay East	40.63																												
	Patchogue	40.75			■																									
	Hempstead Harbor	40.83																												
	Cold Spring Harbor	40.87																												
	Shinnecock	40.87																												
	Jamesport	40.92				■																								
	Mt. Sinai	40.95																												
	Southold	41.08				■																								
2007 adults	Jamaica Bay West	40.6																												
	Tobay Beach	40.61				■																								
	Cold Spring Harbor	40.87																												
	Shinnecock	40.87																												
	Jamesport	40.92				■																								
	Mt Sinai	40.95				■																								
	Southold, NY	41.08				■																								
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2007 juveniles	Jamaica Bay West	40.6				■																								
	Tobay Beach	40.61																												
	Jamaica Bay East	40.63				■	■	■	■													■								
	Patchogue	40.75			■					■		■						■		🟥	■		■							
	Manhassett	40.81				■																								
	Cold Spring Harbor	40.87				■																								
	Shinnecock	40.87				■																		■						
	Jamesport	40.92				■																								
	Mt. Sinai	40.95				■	■	■	■		🟥		■									■		■						
	Southold	41.08				■																		■					■	

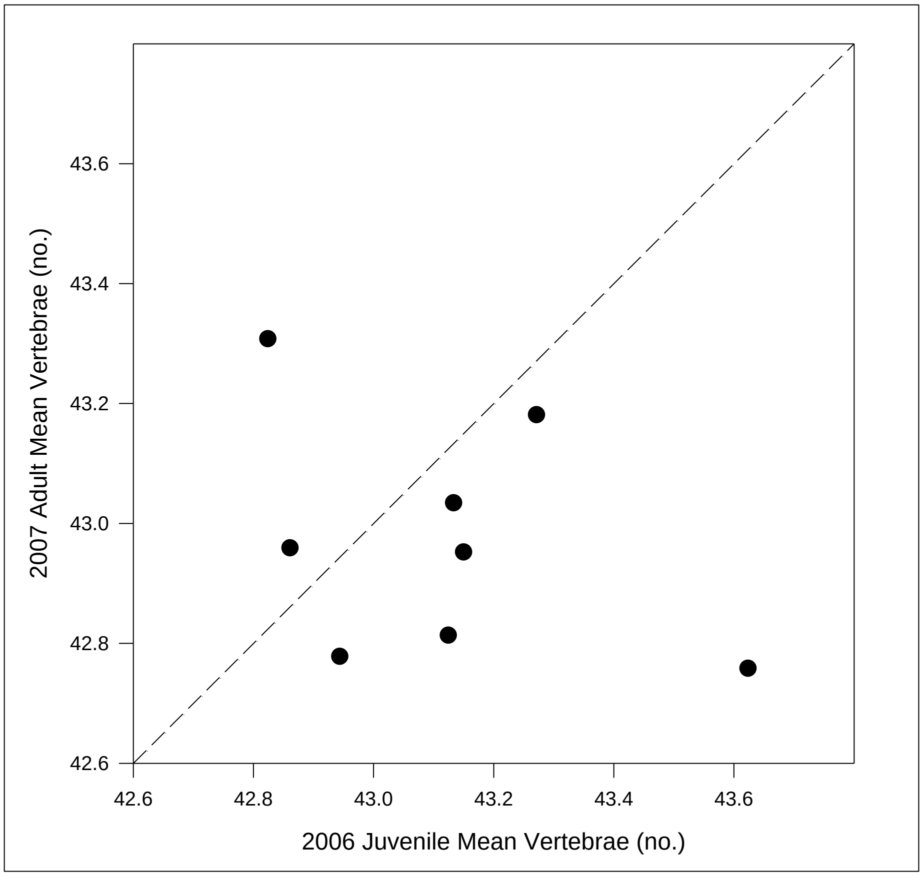

Figure 2-7: Size-corrected vertebral number of juvenile fish collected in late summer 2006 versus the adult fish returning to the same sites in 2007. Dashed line represents 1:1 correlation. There is no significant correlation between juvenile and adult vertebral numbers ($p = 0.337$), however mean vertebral counts in juveniles are higher at 6 of 8 sites studied.

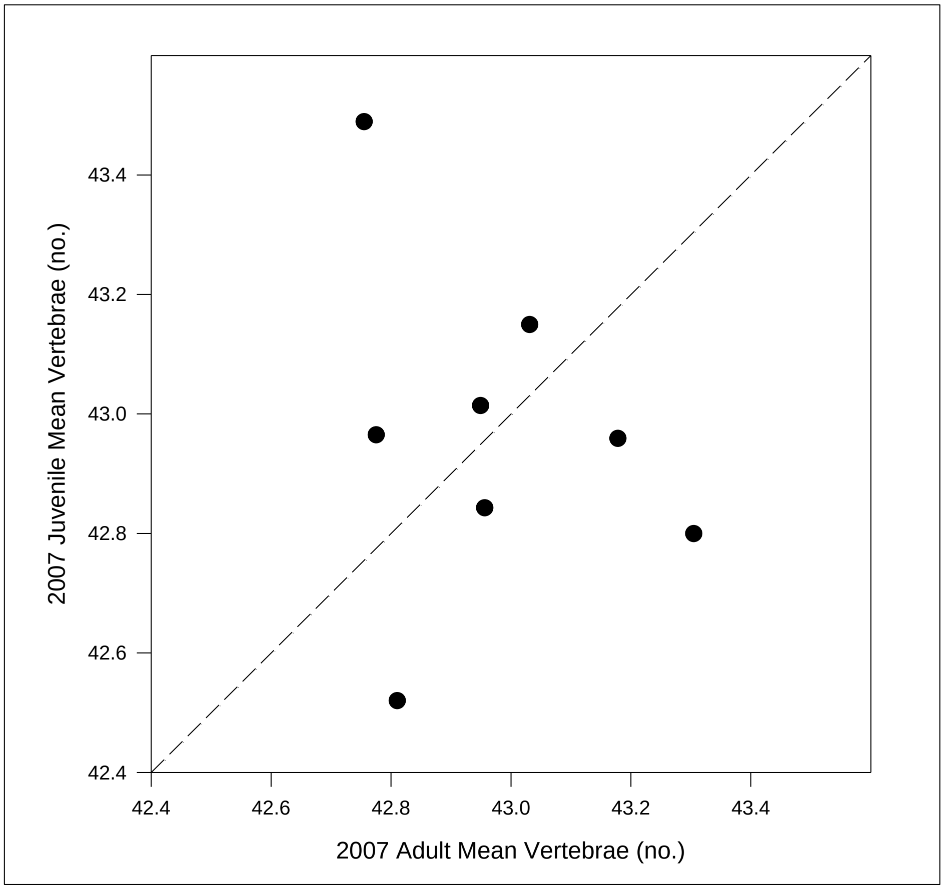

Figure 2-8: Size-corrected vertebral number of adult fish collected in spring 2007 versus juvenile fish collected at the same sites in late summer of the same year. Dashed line represents 1:1 correlation. There is no significant correlation between adult and juvenile vertebral number (p = 0.594).

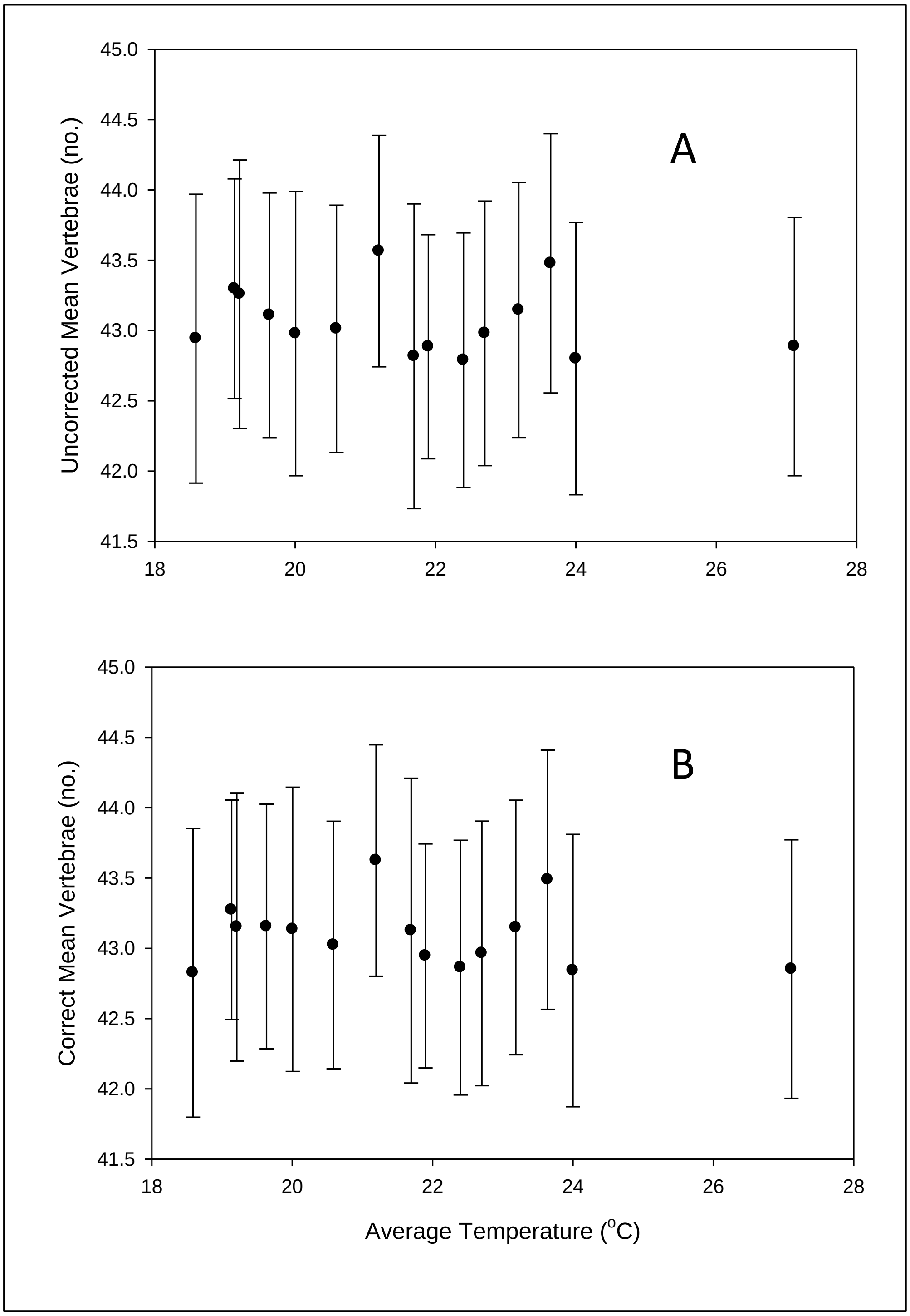

Figure 2-9: Linear regression of (A) uncorrected and (B) size-corrected vertebral number versus site-specific temperature for juvenile populations collected in Long Island, NY. Both relationships are non-significant (A: r^2=0.0535, p= 0.407; B: r^2= 0.0408, p=0.470).

Chapter 3:

The Response of Vertebral Number to Natural and Artificial Selection

Introduction

Vertebral number is a trait that can vary greatly across species of fish, ranging from as few as 16 in *Mola mola* to over 700 in some eel species (McDowall 2008). D. S. Jordan, for whom Jordan's Rule is named, is credited for recognizing that vertebral number of fish increases with latitude (Jordan 1891). His original work referred to differences among different species, but later work by Jordan and Hubbs focused on variation in vertebral number within a species (Hubbs 1922). Jordan's Rule has since been recognized in many fish species (Lindsey 1975; McDowall 2003; McDowall 2004; Yamahira et al. 2006; Yamahira and Nishida 2009). Previous studies by Billerbeck et al. (1997) and data presented in chapter 1 and 2 provide evidence that vertebral number in *Menidia menidia* is a genetically determined trait. The increase in vertebral number with increasing latitude seen in this and other species presumably serves some adaptive benefit, but what this is or the selection forces acting on vertebral number have been, in most cases, been left purely to speculation (reviewed in McDowall 2008). Several studies show that differences in developmental temperature can change vertebral phenotypes, but why this would be beneficial is unknown (Hubbs 1922; Lindsey and Ali 1965; Harrington and Crossman 1976; Dentry and Lindsey 1978; Lindsey et al. 1984; Swain and Lindsey 1986; Lindsey 1988; Yamahira and Nishida 2009).

Vertebral number itself may serve an adaptive benefit, or it may simply be carried along as a consequence of selection on a correlated character. Walsh et al. (2006) presented evidence that selecting for size in *Menidia menidia* results in corresponding selection in vertebral number. When the largest individuals were removed from the population, vertebral number was reduced. The opposite trend occurred when the smallest individuals were removed. This experiment provided evidence that vertebral number was capable of evolving, and that adaptation could occur in a short time period (5 generations). However Walsh and colleagues do not suggest any explanation for this observation, merely state that it occurs. The mechanism and adaptive benefit remain to be determined. It is possible selection on size causes a selection on vertebral number simply due to pleomerism, or the positive correlation between size and number of vertebrae, where larger fish have more vertebrae (Lindsey 1975). Although pleomerism is generally used to describe interspecific correlation, similar trends can be seen within species (Chapter 1, Billerbeck et al. 1997, Reimchen and Nelson 1987). Therefore, selecting on body size (or growth rate) in a cohort where the larger fish had faster growth rates, would consequently select on vertebral number as well. It is also possible that vertebral number has an adaptive benefit to swimming ability, where fish with more vertebrae and thus a more anguilliform swimming mode can provide a hydrodynamic advantage in colder, more viscous water (Spouge and Larkin 1979; McDowall 2004). However, no known studies empirically test this theory.

The goal of this study is first to investigate whether selection on vertebral number can be documented in the wild by following a single cohort of *Menidia menidia* through their period of juvenile development in the coastal zone. The second aim is to investigate the impacts of several generations of artificial selection on size, a correlated character of vertebral number, on evolution of vertebral phenotype. I also aim to determine whether relaxing selection pressure on size for several generations will change the vertebral phenotype.

Methods

Natural Selection of Wild Populations

To investigate selection on vertebral number in the wild, fish were collected from Patchogue, NY (40.75°N, 72.92°W). Samples were collected once every 1-3 weeks from June 26 to September 11, 2007, then again on October 22 and November 14, 2007. Fish were collected using a 10 or 30 meter beach seine as part of a larger project investigating selection on growth rate throughout the growing season (K. Perez, unpublished data).

Artificial Selection of Laboratory-reared Populations

Artificial selection was performed in a common environment in the lab. This work is part of a larger project done as a continuation of data presented in Conover and Munch (2002) and Walsh et al. (2006). The selection procedure was carried out in this study was in outlined Conover and Munch (2002). At the start of the experiment in 1998, live adult *Menidia menidia* were collected from Patchogue, NY (40.75°N, 72.92°W) and embryos were produced through captive spawning in the lab. During the first 5 generations 90% of each population was harvested on day 190 post fertilization. Three harvest regimes were used in 6 populations: 2 where the largest 90% were removed, 2 where the smallest 90% were removed, and 2 control populations were 90% were randomly harvested with respect to body size (length). The remaining 10% of each population became the spawning stock for the next generation. One hundred fish from the breeding stock of each of the three experimental groups were used for each generation. After the 5th generation selection pressure was removed and all populations experienced random harvest through the 10th generation.

Vertebral counts and statistical analyses

Menidia menidia collected in the field were x-rayed following the digital x-ray techniques described in chapter 2. Cohort data from these samples were analyzed using an ANOVA with Holm-Sidak pairwise comparisons. Laboratory populations were x-rayed using the methods described in Chapter 1. Treatment level differences in the artificial selection experiment were determined by performing a Kruskal-Wallis ANOVA (due to non-normality in the data) on each of the three separate treatments with data for the two populations within each treatment pooled for the analysis. Further statistical analysis was conducted using a two way repeated measures ANOVA to account for the six individual populations within the three treatments that were measured throughout time (generation). Multiple comparisons versus the randomly harvested control group were performed using the Holm-Sidak method. Vertebral counts for *Menidia menidia* collected from the wild and laboratory populations were not corrected for body size.

Results

Natural Selection on Vertebral Number

Results from *Menidia menidia* collected over the span of juvenile development show a significant decrease in vertebral number at the end of the growing season (Figure 3-1). Samples collected on November 14, 2007 show a difference of roughly 0.5 vertebral from the populations collected from June to September and are significantly different from all other sampling dates ($p<0.01$). Despite little change in vertebral number between June 23 and October 22 mean size of fish sampled increased by 31.6 mm between these sampling dates. Mean size decreases on September 11; however the sample size on this date was less than half of the surrounding dates, which may be misrepresentative of the true population for this time period. Mean size on November 14 continued to increase slightly from 65.23mm on the previous sampling date (October 22) to 65.48mm, despite the significant decrease in vertebral number (Figure 3-2). Size frequency data of the fish collected show a general increase through time, and a greater proportion of large individuals (>70mm) present at the site on November 22 than at earlier sampling dates suggesting the same cohort was present at the site (Figure 3-3).

Artificial Selection on Vertebral Number

When the population level data are pooled analyzed at the treatment level, as in previous reports of this large scale experiment (Conover and Munch 2002; Walsh et al. 2006), changes in vertebral number through time seem apparent. Treatment level data is presented as both mean vertebral number for each population and as the difference between the experimental treatment and the randomly harvested control (Figure 3-4). There were no differences in vertebral number in generation one, but after five generations of artificial selection there were significant differences between all treatments, consistent with the findings presented in Walsh et al. (2006). The large harvested treatment showed a decrease in 0.53 vertebrae while the small harvested showed an increase of 0.45 vertebrae when compared to the randomly harvested population (Figure 3-4). After relaxing selection for an additional five generations, large and small harvested treatments were still significantly different from each other in generation 10 ($p<0.001$). The difference between the large harvested treatment and the randomly harvested treatment was reduced to 0.45 vertebrae and the treatments remained significantly different (p=0.004). However the small harvested treatment was only marginally different from the randomly harvested treatment with a difference of 0.28 vertebrae (p=0.051) (Figure 3-4). This suggests evidence of rebounding to pre-selection levels in the small harvested treatment.

However, when assessed at the population level, no clear patterns appear between the randomly harvested control and the small and large harvested populations. There are no significant effects of treatment (large or small harvested) or population within treatments, however there is a significant effect of the treatment x population interaction (Table 3-3). This suggests that there is a large population effect within the treatments, and this was inconsistent among the replicate populations. Data is presented as both mean vertebral number for each population and as the difference between means of the experimental population and the coinciding randomly harvested control to account for variation in laboratory rearing and measurement error through time (Figure 3-5). In both the large-harvested and the small-harvested treatment, one population shows a decrease in vertebral number through time, while the other shows an increase in vertebral number. It should also be noted that the difference between the two replicate populations within each treatment is greater than the difference between the treatments (Figure 3-5).

Discussion

It is clear from the results of the artificial selection experiment that vertebral number is capable of evolving through time, and the natural selection data from the field suggests evidence of selection for lower vertebral numbers in the wild. Therefore vertebral number is either adaptive or genetically correlated with another selected trait, but the selection factor or the benefit to having a particular vertebral phenotype in *Menidia menidia* is unknown. In the wild, the decrease in vertebral number seen at the end of the growing season (Figure 3-1) may be due to migration or changes in habitat selection with age, bias in the sampling gear used, phenotypic plasticity, or natural selection.

It can be argued that in a given sampling period, fish collected later in the summer in the coastal bays may be fish born later in the season and are thus younger and presumably hatched in warmer temperature water. There is evidence that warmer developmental temperatures can decrease vertebral numbers, both in *M. menidia* and other fish species (Lindsey and Ali 1965; Harrington and Crossman 1976; Lindsey et al. 1984; Lindsey 1988; Billerbeck et al. 1997; Yamahira and Nishida 2009). However there is no evidence that the fish collected in this study are biased by gear selection or migration of older age classes (Figure 3-2 & 3-3). As part of a separate study investigating seasonal selection on growth rate, there was no evidence of larger (and potentially older) fish being collected with different sampling gear or in offshore sites during the same time period (K. Perez, unpublished data). By the end of the growing season, Perez found that fish showed evidence for selection for slower growth rate. Adult fish returning to the same site in the fall also show evidence of slower growth rates in their juvenile stages from otolith analysis (K. Perez, personal comm.). However, otolith analysis to back calculate age could be done on the fish used for the vertebral analysis to assure that they are not simply fish born later at warmer temperatures, which would likely lead to a decreased vertebral number over time.

If selection favoring lower vertebral number is indeed occurring in this species in the wild, it may be because these fish have better swimming abilities. Studies by Billerbeck et al. (2001) have shown that southern populations of *M. menidia* have better swimming abilities, both in prolonged (U_{crit} and sustained) and predator avoidance (burst) swimming. Lankford et al. (2001) and Chiba et al. (2007) have shown that northern populations of *M. menidia* are also more vulnerable to predators and take more risks when foraging for food. In Billerbeck's and Lankford's studies, manipulating growth rate in a given population also showed similar results in that slower growth lead to increased swimming performance. Although these traits are attributed to differences in growth rate, metabolism, and energy acquisition rates, these populations also had significant differences in vertebral numbers, with fish from populations with better swimming abilities having lower vertebral numbers than northern fish who were more vulnerable to predation. These studies suggest that growth rate and other metabolic effects are the main drivers of swimming ability. Billerbeck's study in particular investigated the effect of vertebral number on swimming ability within a population and found no effect. However, studies in threespine stickleback (*Gasterosteus aculeatus*) have shown that vertebral count ratio had a more pronounced effect on swimming performance than total vertebrae counts and this occurred over a small window of size during development (Swain 1992a). Similarly, vertebral count ratio may be linked to selection in *Menidia menidia* but this has not been studied in this species.

It is possible that in the wild, fish within a population with slower growth rates, and potentially lower vertebral numbers, have better swimming abilities and a greater likelihood of escaping predators. Fish with higher growth rates may be riskier when foraging for food to keep up with their metabolic demand, and may be more likely to be eaten by predators, allowing for selection to favor slower growth rates and lower vertebral numbers. The fish studied for local selection on vertebral number for this study also show evidence of selection for slower growth in juvenile and adult stages from otolith analysis (K. Perez, unpublished data).

Alternatively, it may be that vertebral number is not being selected upon directly in the wild, but is essentially being carried along due to selection on a correlated characteristic. The results from the artificial selection experiment show that selecting for size can result in selection on vertebral number as well. There is some evidence that removing the largest individuals (the large harvested treatment) can cause a decrease in vertebral number, while removing the smallest individuals (the small harvested treatment) can cause an increase (Figures 3-4 & 3-5). This suggests that that size and vertebral number must be correlated, either genetically or phenotypically. However, the significant interaction between treatment and population suggest a more complex pattern (Table 3-3). The large differences between populations with a treatment could be evidence of genetic drift occurring. There is a degree of genetic variation in vertebral number in any given population. Within each experimental group, there is potential to only have a few individuals contributing to the next generation. Individual *M. menidia* were allowed to freely spawn within their experimental tanks, so the number of individuals actually contributing to the next generation is unknown. If only a few males and/or females became part of the spawning stock, genetic drift could rapidly occur within the replicate populations. Even if genetic drift did occur however and the treatment effect is unclear, there is clear evidence of evolution of vertebral number over a short time period (10 generations).

If vertebral number does indeed respond to selection on size, this may be due to the correlation between the number of meristic parts and characteristic body size, known as pleomerism (Lindsey 1975). This relationship is most notably seen positive with the correlation between maximum body length and vertebral number in related fish species (Lindsey 1975) or within populations of the same species (Reimchen and Nelson 1987; Billerbeck et al. 1997). Pleomerism can reinforce latitudinal gradients in vertebral number due to corresponding latitudinal gradients in maximum length (Lindsey 1975). Although the cause of pleomerism is unknown, it may be linked to functional mechanics in locomotion (Lindsey 1975).

Billerbeck et al. (1997) presented evidence of pleomerism within populations from field collections and dimorphism between sexes in southern populations in *Menidia menidia*. If *M. menidia* do indeed show pleomerism within populations, then selection on size would indirectly select for vertebral number. This could serve as an explanation for geographic patterns seen in the field, because growth rate in *M. menidia* increases with increasing latitude (Conover and Present 1990, Chapter 1). It is believed that northern populations have evolved faster growth rates in response to shorter growing seasons to reduce winter mortality at small sizes. If this is true, then conceivably smaller individuals with fewer vertebrae would be expected experience higher mortality in northern populations. However, the reverse appears to be true, with selection favoring fish with fewer vertebrae.

Perhaps selection on vertebral number occurs while the fish are juveniles, rather than during the winter adult phase. Several studies have shown that selection can favor different vertebral phenotypes throughout development. Predation experiments in peamouth (*Mylocheilus caurinus*) have shown that selection for vertebral number in fry only occurs when vertebrae are well developed and that selection in the wild favored lower vertebral counts at small lengths and higher vertebral counts at larger lengths (Swain 1988). In juvenile threespine sticklebacks (*Gasterosteus aculeatus*) predation experiments favored fewer vertebrae, attributed to differences in locomotor ability (Swain and Lindsey 1984). Later studies in this same species showed that the ratio of abdominal to caudal vertebrae had a more pronounced effect on burst swimming ability, and likely escape response from predators, than total numbers of vertebrae (Swain 1992a). Predation experiments showed evidence for selection for both vertebral number and vertebral count ratio, however selection was more directly related to vertebral count ratio (Swain 1992b). Studies of *G. aculeatus* collected in the wild have also suggested differing selection for vertebral number throughout development. Reimchen and Nelson (1987) presented evidence of a decrease in vertebral number from juvenile to pre-adult sizes, followed by an increase among larger adult fish, potentially due to differences in survival from predation. These studies suggest a link between vertebral number and size and swimming ability, particularly in the ability to escape predation. They also demonstrate differing selection on vertebral phenotypes throughout development, which may be occurring in *Menidia menidia.*

The artificial selection experiments on *Menidia menidia* in the laboratory discussed here have shown that selection on body size (and consequently growth rate) has the potential to lead to selection on vertebral number. Therefore, natural selection in the wild presented within this study may be due to a selection on growth rate, and corresponding swimming ability or the willingness of fish to risk predation.

This study shows a decrease in vertebral number in a single cohort over the course of a growing season. Whether this is due to sampling error, biased migration, direct selection on vertebral number, or selection on a correlated character is unknown. There is evidence from chapter 2 that this is not a phenomenon limited to fish found in Long Island, NY as fish from a separate, independent study in NY as well as fish from VA and NJ showed evidence of lower vertebral numbers in adult fish compared to juvenile fish at the same sites (Figures 2-3 & 2-7). In all of these sites the juvenile fish sampled in the late summer had significantly higher vertebral numbers than the adults returning to the same sites in the following spring. This phenomenon needs to be studied in greater detail, perhaps by studying multiple sites along the species range throughout the growing season to look for evidence of selection on vertebral number. This could be combined with otolith analysis on individual fish to determine if differences in size or vertebral number were correlated with differences in growth rate or age. Although more work needs to be done in this area, this is the first known study to document this phenomenon in detail and it warrants further investigation.

Table 3-1: Collection dates and sample sizes (n) for 2007 cohort in Patchogue, NY.

Date	**n**
June 26	87
July 3	39
July 24	57
July 31	96
August 14	99
August 28	95
September 11	40
October 22	95
November 14	98

Table 3-2: Sample sizes for each population in the artificial selection experiment by treatment and generation.

Generation	Treatment	Population	n
1	Large harvested	1	44
		2	45
	Random harvest	1	48
		2	43
	Small harvested	1	44
		2	47
5	Large harvested	1	44
		2	48
	Random harvest	1	44
		2	44
	Small harvested	1	48
		2	46
10	Large harvested	1	44
		2	42
	Random harvest	1	42
		2	48
	Small harvested	1	45
		2	45

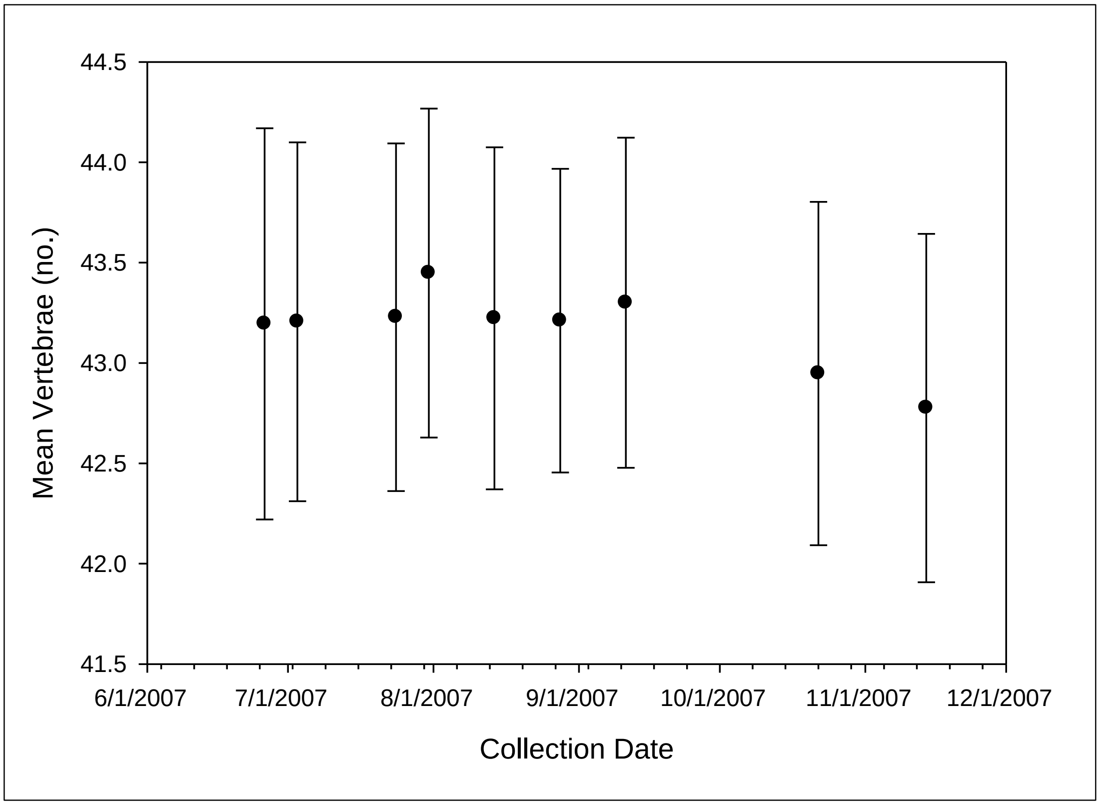

Figure 3-1: Variation in vertebral number (± standard deviation) of a single cohort followed across a growing season in Patchogue, NY (40.75°N, 72.92°W). Fish collected on the last sampling date (Nov. 14) have significantly lower vertebral numbers that all other dates ($p<0.001$).

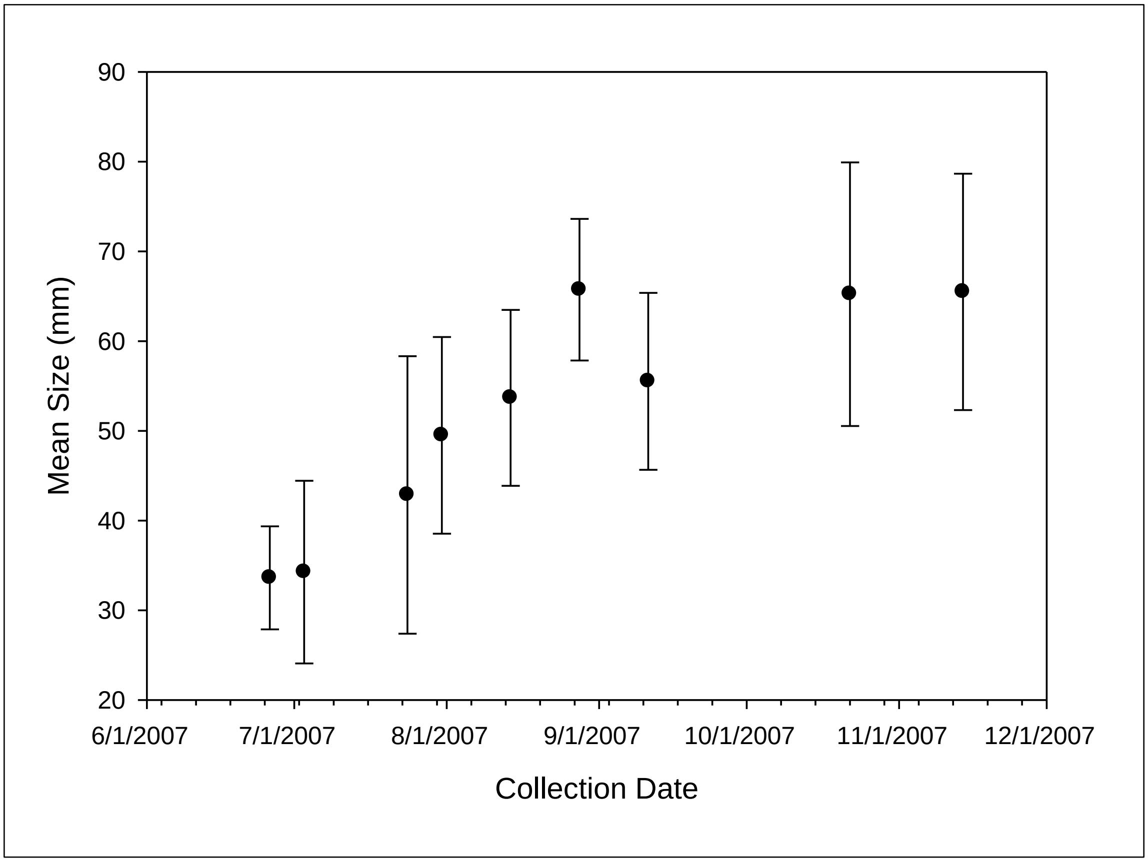

Figure 3-2: Mean size (± standard deviation) of fish collected in Patchogue, NY (40.75°N, 72.92°W) across the growing season.

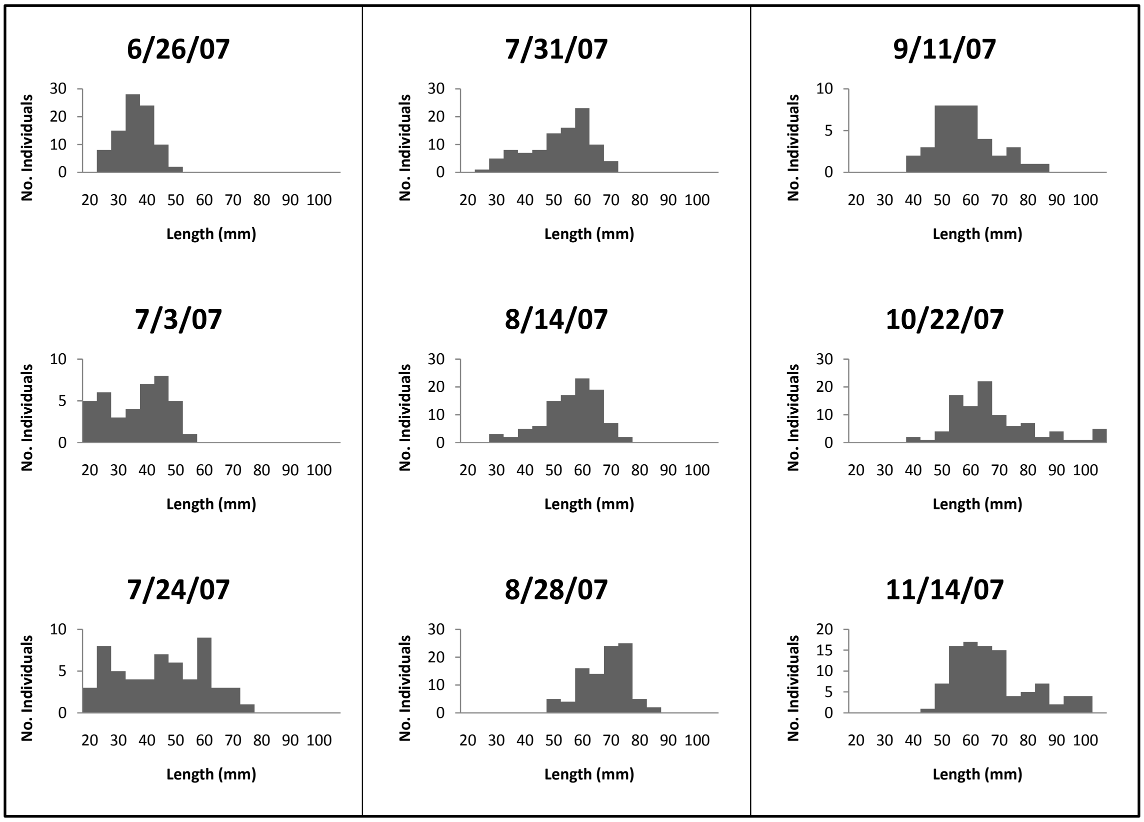

Figure 3-3: Length frequency through time of fish captured in Patchogue, NY (40.75°N, 72.92°W).

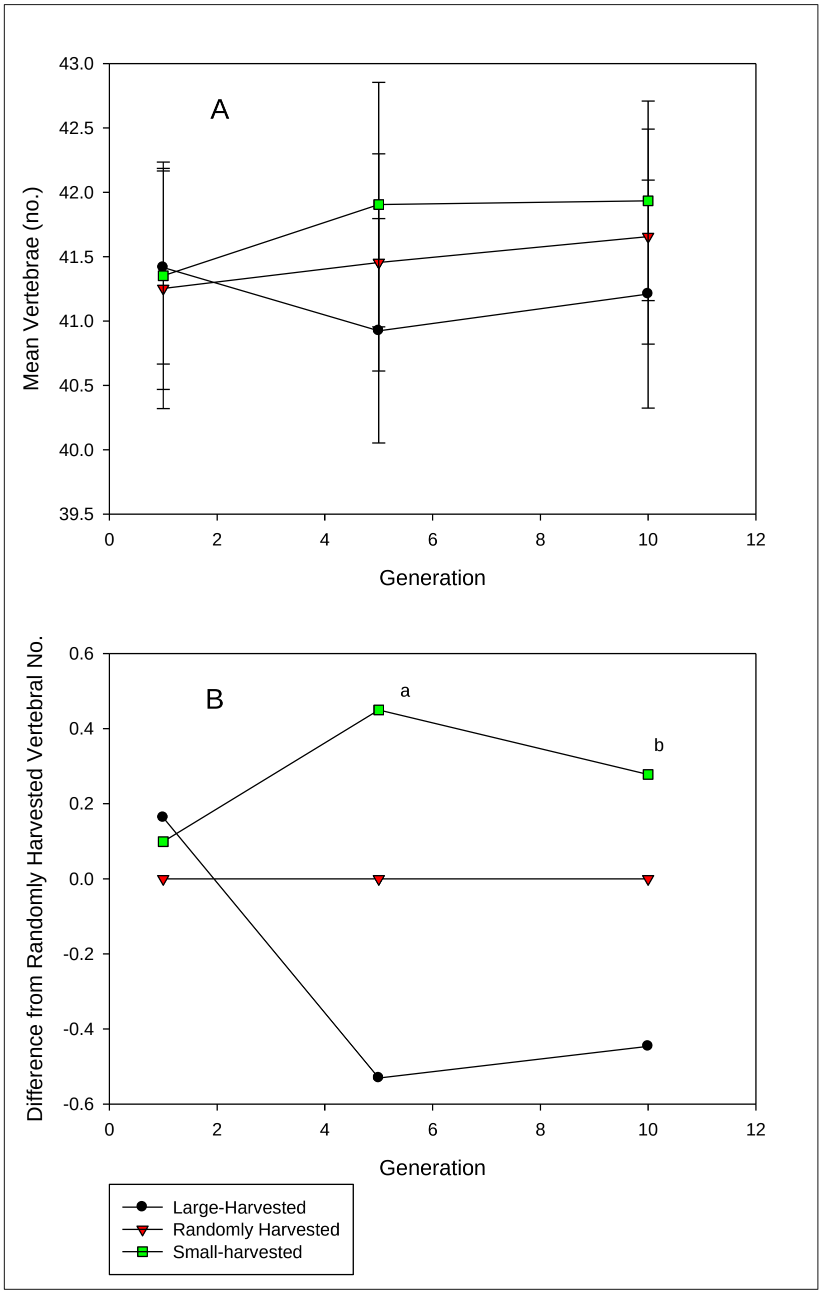

Figure 3-4: Changes in vertebral number among 3 treatments after five generations of selection on size followed by five generations of random harvest in all groups. (A) Change in mean vertebral number (± standard deviation). (B) Data is shown as the deviations from the randomly harvested control populations. (a) All populations are significantly different from each other ($p<0.001$). (b) Small harvested and large harvested populations are significantly difference from each other ($p<0.001$). Large harvested and randomly harvested populations are significant different ($p=0.004$), however the small harvested population is only marginally different from the randomly harvested populations ($p=0.051$).

Table 3-3: Results from two way repeated measures ANOVA.

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Generation	2	0.176	0.0879		
Treatment	2	0.889	0.445	2.955	0.163
Treatment x Generation	4	0.602	0.15		
Population	1	0.141	0.141	1.667	0.326
Pop x Generation	2	0.169	0.0846		
Treatment x Population	2	2.591	1.296	12.52	0.019
Residual	4	0.414	0.103		
Total	17	4.982	0.293		

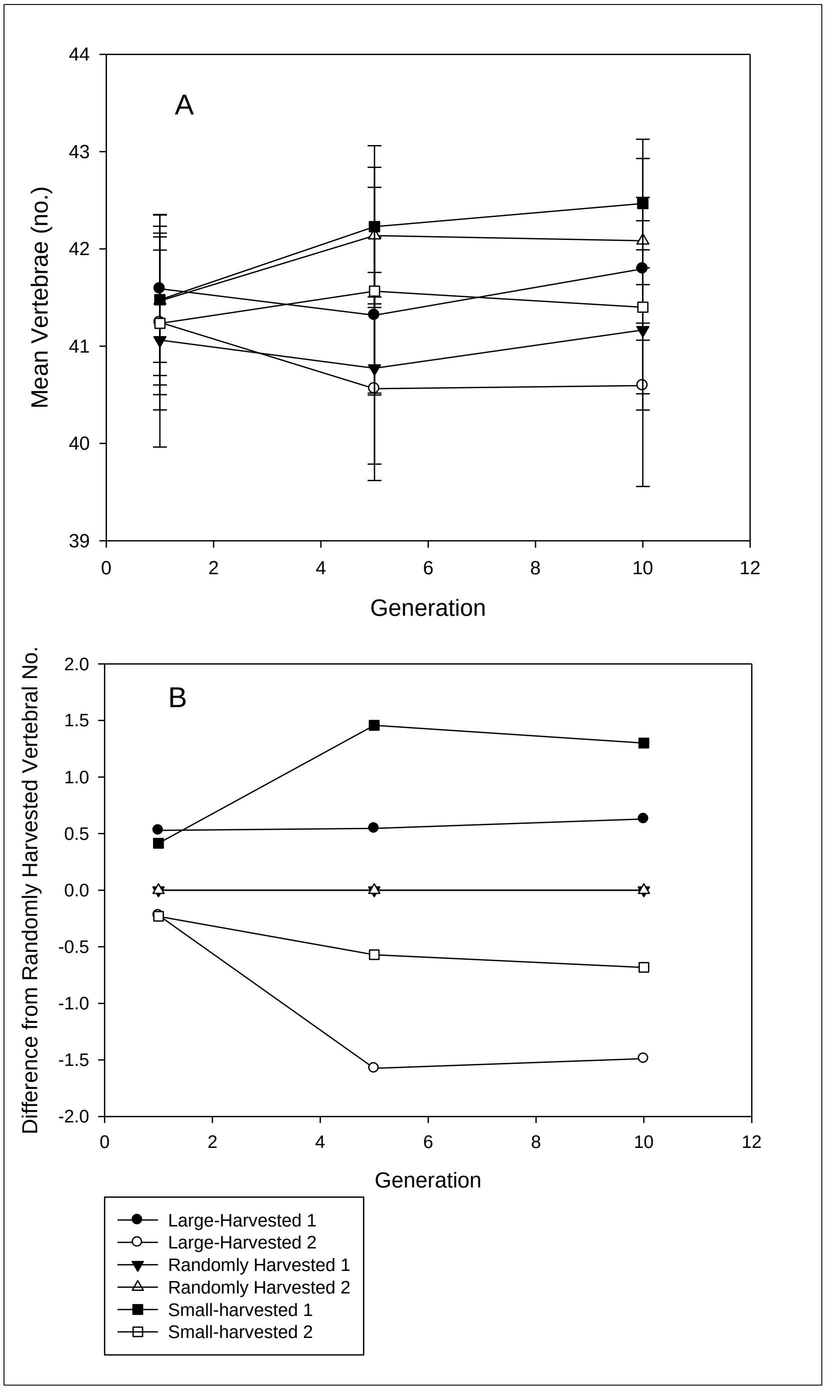

Figure 3-5: Changes in vertebral number among 6 populations after five generations of selection on size followed by five generations of random harvest in all groups. (A) Changes in vertebral number means through time (± standard deviation). (B) Difference between the mean of the experimental population and the corresponding randomly harvested control.

Chapter 4:

Investigation into the Adaptive significance of Jordan's Rule: The Relationship between Vertebral Number and Swimming Ability

Introduction

Jordan's Rule, or the tendency of fish from northern latitudes to have more vertebrae than fish from southern latitudes, has been proven to occur in many species (Jordan 1891; McDowall 2008). In some species the trait is known to have a strong genetic component, but the reason for this adaptation is often left to speculation (Billerbeck et al. 1997; Ando et al. 2008; McDowall 2008; Yamahira and Nishida 2009). Many studies have suggested a link between vertebral number and swimming ability (Lindsey 1975, 1978; McDowall 2004), but empirical tests of this theory are limited.

Studies by Swain have shown selective predation for vertebral number in larval sticklebacks (*Gasterosteus aculeatus*) and peamouth (*Mylocheilus caurinus*) and suggest a relationship between the ratios of precaudal to caudal vertebrae and burst swimming ability (Swain and Lindsey 1984; Swain 1988; Swain 1992a, b). Many studies have also shown the effect of temperature on swimming ability in larval fishes (Batty and Blaxter 1992; Swank and Rome 1999; Dickson et al. 2002; Green and Fisher 2004) and suggest a link to water viscosity at small sizes; however empirical studies on the effect of viscosity versus temperature in fish larvae are rare. To thoroughly investigate changes in swimming ability and the potential adaptive significance of vertebral number, a broad empirical study is needed that looks at both changes with larval size and effect of temperature and/or water viscosity. Few studies have attempted this in fish larvae, and among those that have the focus on the adaptive significance of vertebral number are quite uncommon.

There are two major theories with development in larval fish. Some argue that all larvae have similar anguilliform swimming behavior and therefore differences in abilities are not seen until larvae reach larger sizes, especially when attempting to equate differences due to vertebral number (McDowall 2004). In anguilliform mode, swimming is achieved by undulatory body movement through most or all of the length of the body. The amplitude of the wave created by the body is large along the whole body and increases towards the tail (Lindsey 1978). For this to occur, the body must be long and thin, generally with a reduced or absent caudal fin as is seen in eels and most fish larvae. If this is a common swimming mode among all larvae, then it may not explain fine-scaled adaptation in vertebral number. Another theory, however, is that at very small sizes and low Reynolds numbers larval fish are most affected by water viscosity (Fuiman and Batty 1997; Hunt von Herbing 2002). In this case, small difference in vertebral number could potentially increase body flexibility to combat high water viscosity, potentially in the face of environmental variation such as temperature.

Previous work investigating swimming ability in *Menidia menidia* has shown that northern (Nova Scotia) populations have significantly lower maximum prolonged and burst swimming speeds than southern (South Carolina) populations (Billerbeck et al. 2001). This, along with a study by Munch and Conover (2004), demonstrated that faster growth in *M. menidia* results in a tradeoff with critical swimming performance. Lankford et al. (2001) found that fast growing phenotypes were also more susceptible to predation, which was likely a function of their swimming ability. In all these studies however, swimming ability was assessed over a small juvenile size range (18-35mm in Billerbeck et al., 20-45mm in Lankford et al., and 19-22mm in Munch and Conover) or not considered in the analysis (as in Lankford et al.). Both Billerbeck's and Lankford's studies reported that variation in vertebral number within populations did not influence swimming performance. However, the limited size and/or temperature ranges used may overlook critical periods for the adaptive significance in vertebral number on swimming ability. Studies by Swain on selection for vertebral phenotype have shown an “optimal” phenotype that may change in development as length increase (Swain 1992b). Therefore, previous work in *M. menidia* by Billerbeck et al. (2001) and Lankford et al. (2001) may not have been broad enough to identify potential critical periods in *M. menidia* where the adaptive significance of vertebral number becomes significant. It is also possible that differences in swimming abilities between *M. menidia* populations become more pronounced at different sizes or temperatures. All previous work on swimming abilities in *M. menidia* larvae have focused on fish of a small size range and do not take into account changes in swimming morphology or energetics that may occur with development (Billerbeck et al. 2001; Lankford Jr. et al. 2001; Munch and Conover 2004; Arnott et al. 2006).

Critical swimming speed is a category of prolonged swimming designed to determine the maximum velocity a fish can maintain for a precise time period (Beamish 1978). Because *Menidia menidia* are a continuously swimming fish found in coastal zones and often have to combat tidal currents, prolonged swimming ability of larvae and juvenile fish can be used as an indicator of their ability to contend with environmental stress early in life. The goal of this study was to determine the effect of vertebral phenotype on swimming ability. If high vertebral numbers truly provide an adaptive hydrodynamic advantage in colder water, than northern populations of *M. menidia* should exhibit higher swimming speeds in cold water when compared to their southern conspecifics. This should be most pronounced at small sizes, where low Reynolds numbers allow for greater viscous effects (Fuiman and Batty 1997).

Methods

Laboratory Rearing

Tests of swimming performance were conducted on two F2 laboratory populations- a northern population from Annapolis Royal, Nova Scotia (44.81°N, 65.37°W) and a southern population from Edisto, South Carolina (32.65°N, 80.26°W). The parent (F0) populations were collected in 2007 as part of the latitudinal study of local adaptation discussed in Chapter 1. These populations have genetically distinct vertebral phenotypes and despite population variability the two populations do not have overlapping vertebral distributions (Billerbeck et al. 1997, Figure 1-4). Adult fish were placed on a 15:9 light: dark light cycle to induce spawning. Due to *Menidia menidia*'s natural tendency to spawn in coastal vegetation, laboratory stocks can be induced to spawn on clusters of yarn placed in the tanks to simulate natural vegetation. The resulting eggs and larvae were reared in 21°C water baths until they reached 8mm, at which point they were size-matched (± 1mm) and transferred to experimental buckets for swim acclimation. Each population was set up in duplicate buckets stocked at equal densities. The buckets also maintained a slow current (4-6 cm/sec). A second set of duplicate buckets was set up for each population 1-2 weeks later to allow for enough individuals to run experimental trials before fish outgrew the size class needed, for a total of 4 replicate buckets for each population. A second phase of experiments was conducted after the experiments on the first phase were completed, for a total of 8 replicate buckets to rear each population over the two phases.

Swim trials

Experiments were performed under twelve combinations of three different temperature treatments and four size classes of larvae in a 3 x 4 factorial design (Figure 4-1). Fish were reared in 21°C baths in large buckets with a slow current (4-6 cm/sec) until 2 days prior to the experimental trial at which point they were size matched (±1mm) and moved to baths at the experimental temperature of 15, 21, or 28°C. Longer durations at the experimental temperatures were avoided to reduce the known effect of rearing temperature on *Menidia menidia* growth rate (Conover and Present 1990). Fish fasted for 12-24 hours prior to swim experiments to eliminate metabolic effects on swimming ability (Billerbeck et al. 2000). Experimental trials were performed on 8 replicate groups in each block. This was done for each of the two populations studied. Because *Menidia menidia* are obligate schooling fish, swim trials were performed on groups of three. Experiments were conducted by alternating Nova Scotia and South Carolina groups whenever possible. Ninety-six trials were run for each line using 288 fish per line. In total, 576 fish were used for 192 trials across both populations.

To begin the trial, fish were placed in a 10 cm x 10 cm x 40 cm long swim chamber in a Qubit DT10 Swim Tunnel (Qubit Systems). Fine-mesh retaining screens were used to keep fish within the swim chamber and flow speed was calibrated using dye. At the beginning of the experiment, the 3 fish in each trial were placed in the chamber and allowed to acclimate without flow for 10 minutes. Flow was then increased to a slow speed (~2cm/sec) for a 10 minute acclimation in the flow. After the acclimation, speed was increased to 5 cm/sec to begin the trial. Every ten minutes the flow speed was be increased by 5 cm/sec. Fatigue time was noted as when a fish fell back against the screen and could no longer maintain swimming. Fish that did not complete the initial low-speed acclimation were not included in the analysis and no fish were able to swim faster than the maximum flow velocity of the swim flume. Critical swimming speed was calculated using the following equation:

$$U_{crit}\ (\text{cm/sec}) = V + (T/t \times v)$$

Where V is the highest speed maintained for the full time internal, v is the velocity increment (5 cm/sec), T is the time in the velocity where the fish fatigued, and t is the time increment (10 minutes) (Beamish 1978; Billerbeck et al. 2001).

X-raying and scoring

Vertebral counts were done using the INSPEX 20i digital x-ray system described in Chapter 2. Fish in the 10mm size class were too small to achieve clear resolution in x-rays therefore specimens were cleared and stained for analysis using the methods described in Potthoff (1983). This technique is commonly employed in studies investigating vertebral number in larval fish (Swain and Lindsey 1984; Swain 1988; Swain 1992b). Once the clearing and staining was complete, TIFF (.tif) images of individual fish were captured using a Nikon Eclipse 80i microscope system (4x E Plan objective) affixed with a Nikon DXM-1200CC digital camera. Images were captured with NIS Elements F package software (v2.20). Fish too large to fit in one image were captured in two images which were then stitched together using Windows Live Photo

Gallery software (Microsoft). Vertebral counts for these images and images from digital x-rays were analyzed using ImageJ (Rasband 1997-2010).

Statistical Analyses

The data from the swimming performance trails was analyzed with a multiple regression. The analysis was done using individuals with the median critical swimming speed in each replicate with individual size, growth rate, and vertebral number as covariates. Each experimental temperature (15°C, 21°C, or 28°C) was analyzed independently and all data was log-transformed to achieve normality for statistical tests.

Results

Different populations of *M. menidia* essentially appear identical, with no outward physical characteristics distinguishing separate stocks. However due to the fact that vertebral phenotypes in Nova Scotia and South Carolina fish rarely overlap in vertebral numbers, vertebral data can be used as an indicator of population. Yet the drawback is that this can only be seen in preserved specimens. There is no known way to morphometrically distinguish live fish from separate, locally adapted populations. Because of this, only once all experiments were completed did x-ray analysis show evidence of an unintentional hybridization of Nova Scotia and South Carolina laboratory stocks. Nova Scotia larvae used in experimental trials exhibited vertebral phenotypes intermediate of those found in either the Nova Scotia or South Carolina populations. Further investigation into the breeding (F1) populations showed evidence of South Carolina phenotypes within the Nova Scotia stock, most likely caused by mixing of the

South Carolina and Nova Scotia populations during laboratory rearing (Figure 4-1). Fish with intermediate phenotypes also exhibited intermediate growth rates, further indicating hybridization of the stocks, since Nova Scotia and South Carolina show local adaptation in growth rate as well (Conover and Present 1990). There was a significant correlation between growth rate and vertebral number (Pearson Product Moment Correlation: $r = 0.556$, $p < 0.0001$; Figure 4-2). To account for this unintentional and uncontrolled hybridization, population level differences were excluded from the analysis. The length, vertebral number, and growth rate for the fish with the median U_{crit} in each trial was used for the multiple regression analyses at each temperature.

Multiple regression analyses showed that there was a significant effect of length on U_{crit} at all temperatures ($p < 0.001$). There was a significant effect of vertebral number on U_{crit} only at 28°C ($p = 0.003$) and no effect of growth rate on U_{crit} at any temperature (Table 4-2, Figure 4-3).

Discussion

Although this carefully designed experiment did not go as expected due to the unplanned hybridization of the laboratory stocks, it did however create essentially a continuous range of growth rates and vertebral numbers in fish used in the study (Figure 4-2). This is beneficial since in locally adapted populations with correlated characteristics it is often difficult to determine which character is truly driving the effect seen. In this study, it was shown that at all temperatures there was a significant, positive effect of size on swimming ability (Table 4-2). Larger fish had higher critical swimming speeds, as would be expected. This has been shown in many fish species, especially in larval and juvenile reef fish in studies aiming to investigate population connectivity (Stobutzki and Bellwood 1997; Fisher et al. 2000; Leis et al. 2006).

It is interesting that growth rate did not show a significant effect on swimming ability at any temperature. Previous work in *Menidia menidia* showed that slower growing fish had higher swimming speeds. When comparing different locally adapted populations or slower growing fish in from the same populations, fast growth has been shown to lead to decreased critical and burst swimming, as well as lead to increased vulnerability to predation (Billerbeck et al. 2001; Lankford Jr. et al. 2001; Munch and Conover 2003; Munch and Conover 2004). These differences are believed to be due to tradeoffs with energy acquisition, metabolic rate, and risk taking behavior (Billerbeck et al. 2001; Lankford Jr. et al. 2001; Arnott et al. 2006; Chiba et al. 2007). The difference in results seen here may be due to the hybridization of populations, which could presumably affect growth rate and other physiological factors, as well as the interaction between these traits. It is also possible that the experiments discussed here simply did not have enough statistical power to show a significant effect on growth rate, and that more replicates would be needed to show the true effect of this trait.

Vertebral number had a significant effect on swimming ability, but only at the highest temperature (Figure 4-3, Table 4-2). At 28°C fish with fewer vertebrae have higher critical swimming speeds. If viscous effects of water were truly the driving force behind latitudinal differences in vertebral number, one would expect fish with fewer vertebrae to perform best in warm water and fish with higher vertebral numbers to perform best in cold water. However, only the fish in warm water showed a significant relationship between vertebral number and swimming ability. It is possible that at high temperatures, northern populations are already working at their maximum metabolic rate, and higher temperature may create stress and thus decrease their swimming ability further than seen at other temperatures.

Although no effect of vertebral number was seen at low temperatures, to truly determine whether viscous effects are driving swimming ability temperature and viscosity must be studied independently. Most work investigating both temperature and viscosity effects focus on zooplankton. To differentiate effects of temperature and viscosity, researchers set up a series of treatments manipulating the two factors. Inert polymers such as Ficoll (Bolton and Havenhand 1998; Loiterton et al. 2004; Bolton and Havenhand 2005), dextran (Podolsky 1994; Loiterton et al. 2004), polyvinyl pyrrolidone (Podolsky 1993), or methyl cellulose (Linley 1986; Fuiman and Batty 1997) have been used to manipulate viscosity with minimal osmotic stress on the study organism. Studies on feeding performance in serpulid polychaete larvae (*Galeolaria caespitosa*) have shown that both temperature and water viscosity effect feeding rate (Bolton and Havenhand 1998, 2005). Similar studies on feeding rates in *Daphnia galeata* and *Chydorus sphaericus* (Loiterton et al. 2004) as well as sand dollar (*Dendraster excentricus*) larvae (Podolsky 1994) found that 50-65% of the reduction in feeding rate at low temperatures was due to increase in water viscosity.

Few published studies focus on viscous effects of temperature in swimming ability. Podolsky and Emlet (1993) found that 55% of the decrease in water movement and 40% of the decline in swimming speed by sand dollar (*Dendraster excentricus*) larvae at low temperature was caused by an increase in viscosity. The only known empirical study addressing viscous effects on swimming ability in larval fish was done by

Fuiman and Batty (1997). Their study found that water viscosity had a significant effect on voluntary swimming in herring larvae (*Clupea harengus*) while water temperature at the same viscosities did not affect swimming ability.

Preliminary studies were attempted using methyl cellulose to test the effects of viscosity on swimming ability in *Menidia menidia* larvae; however it was discovered that methyl cellulose was unstable when disturbed or aerated and had a tendency to create large amounts of foam on the surface of the water. Alternative materials were sought to manipulate water viscosity, but most biological studies are done on small larvae or zooplankton in small volumes of water, and the volume needed for the swim flume used in my experiments and the cost of alternative materials deemed the experiments unfeasible. The thought was then to try manipulating water viscosity in small volumes of water that could be kept steady in burst or routine swimming experiments using methyl cellulose, similar to what was done by Fuiman and Batty (1997). However, the discovery of the hybridized lab populations caused these experiments to be abandoned. It is however, an idea that may be worthwhile to test in *M. menidia* in the future. Not only will it allow for the decoupling of temperature and viscosity, but it also would investigate an alternative swimming mode.

It is also feasible that vertebral number is more important for routine swimming, or perhaps vertebral number is more important in c-start flexibility and burst response for escape of predators. Additionally, vertebral count ratio, rather than total vertebral number, may be more important in determining swimming ability as demonstrated in threespine sticklebacks (*Gasterosteus aculeatus*) (Swain 1992b). Although this was not the focus of the current study, this warrants further investigation in *Menidia menidia*.

Additionally, it may be that even at 10mm and 15°C, larval *Menidia menidia* are already able to swim fast enough to be outside of the viscous regime. According to Leis (2006), the larger and the faster it is, the more likely a fish larvae is to be swimming in an inertial environment at a given water viscosity. Experiments in fish larvae suggests that the viscous environment extends to Reynolds numbers (Re) of 300 (Fuiman and Batty 1997; McHenry and Lauder 2006). Recent data has suggested that to reach Re > 300 at 20°C, 10mm larvae, such as those used in the critical swimming speed experiments here, only need to swim at 2 body lengths per second, or 2 cm/sec. Even if temperature decreases to 1°C, swimming speeds of only 5-6 cm/sec allow a 10mm larvae to be outside the viscous regime. Most 10mm *M. menidia* larvae in this experiment exhibited swimming speeds of 15-30 cm/sec, well beyond the speeds needed to move outside the viscous regime, even at the lowest experimental temperature of 15°C.

If viscous effects are truly driving vertebral number, it may be that vertebral number is important at very small sizes. Larval stage *M. menidia* at 5-8mm total length (TL) have been observed in randomly oriented aggregations in shallow coastal waters. This larval size has been suggested to represent a planktonic stage with limited dispersal capability however once the larval are larger than 8mm TL passive removal from coastal areas by tidal currents becomes limited. When the fish reach roughly 8-10mm total length they begin to move in oriented schools (Martin and Drewry 1978). Thus a critical shift in life history occurs between 5-10mm, for instance, and this may be when vertebral number's effect on swimming ability is most important. Although population-level differences in development in this species is unknown, in general, flexion in *M. menidia* occurs at 9.2-9.4mm (Able et al. 2006; Vasquez-Yeomans and Valdez-Moreno 2006). It is possible that vertebral number's effect on swimming ability occurs at pre-flexion sizes, most likely less than 9mm. Studies in threespine sticklebacks (*Gasterosteus aculeatus*) have shown that vertebral count ratio has a greater effect on burst swimming ability than total vertebral number and that this relationship was apparent at a small range of sizes (Swain 1992b). It is possible that the size classes used here missed this critical period in vertebral selection, assuming similar effects are seen in *Menidia menidia*. However, data from chapter 3 suggests selection on vertebral number, at least in intermediate latitude populations, occurs later in the season when fish are much larger and well beyond the viscous regime. If selection is truly on vertebral number, it may be due to a relationship with escape responses or swimming endurance later in life.

Although Jordan's rule is a relatively simple observation, the mechanism and the evolutionary significance of this trend is clearly quite complex. I did not find evidence of increased vertebral numbers providing an adaptive benefit towards higher swimming abilities in cold temperatures, however there is evidence that low vertebral numbers can have a significant effect on swimming ability at warm temperatures. More work needs to be done in this study area, particularly in decoupling temperature and viscous effects. However, this was the first in-depth study of swimming ability in *Menidia menidia* in fish that simultaneously exhibited a broad range of vertebral numbers, growth rates, and sizes, as well as at a broad range of temperatures and enhanced our understanding of swimming abilities of this species.

<u>Table 4-1:</u> 3 x 4 factorial design of swim trial experiments. Design was used for Nova Scotia and South Carolina populations separately. Numbers in boxes indicate the number of replicates performed in each experimental condition. Three fish were used for each replicate. 96 trials were run for each population using 288 fish per population in total.

		Length			
		10mm	**17mm**	**24mm**	**30mm**
Temperature	**15°C**	x8	x8	x8	x8
	21°C	x8	x8	x8	x8
	28°C	x8	x8	x8	x8

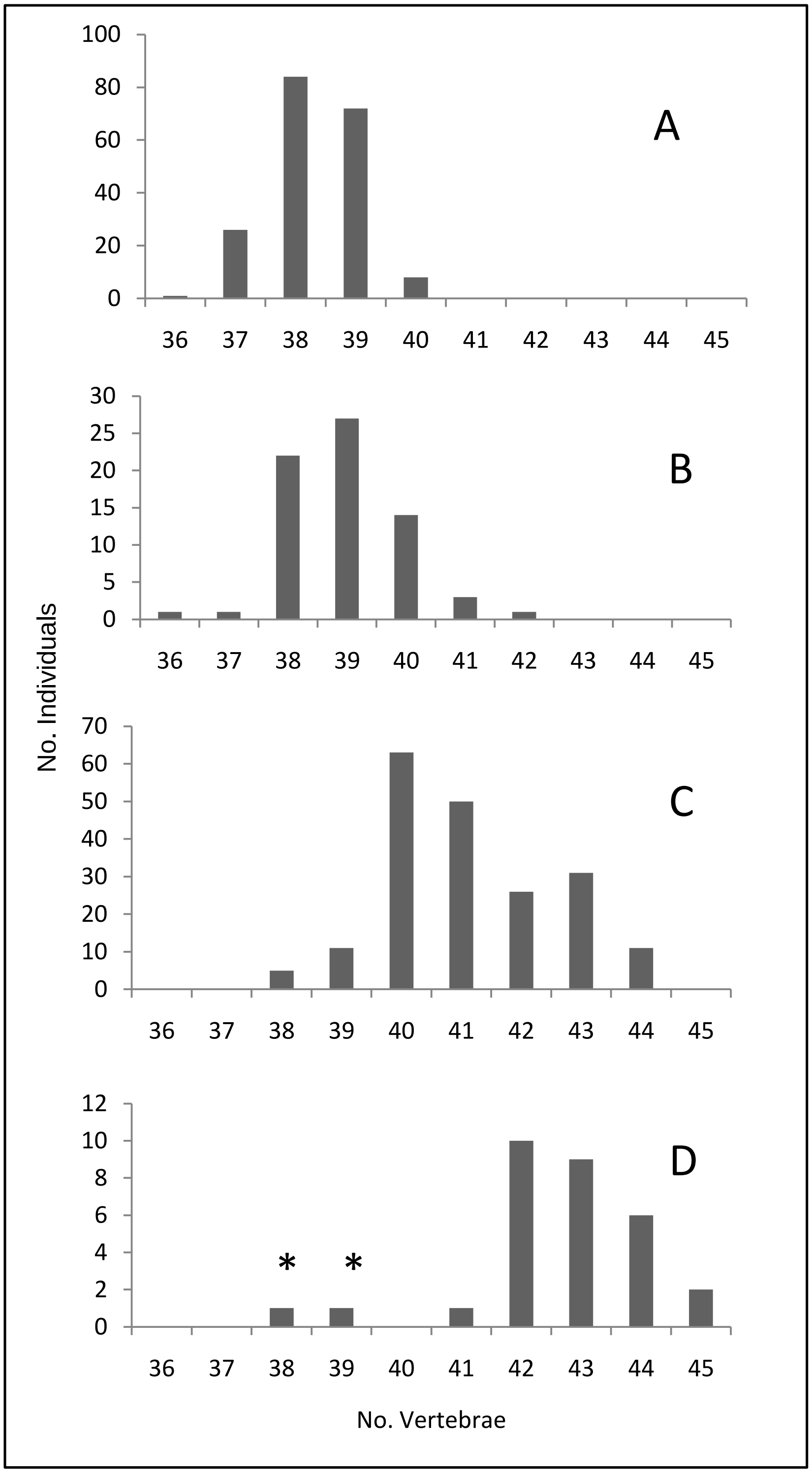

Figure 4-1: Frequency distributions for the (A) South Carolina juvenile population from swim flume experiments (mean = 38.30, n = 191), (B) South Carolina adult spawning population (mean = 38.94, n = 69), (C) Nova Scotia juvenile population from swim flume experiments (mean = 41.11, n = 197), (D) Nova Scotia adult spawning population. (*) Denotes South Carolina phenotypes found within the Nova Scotia lab stock.

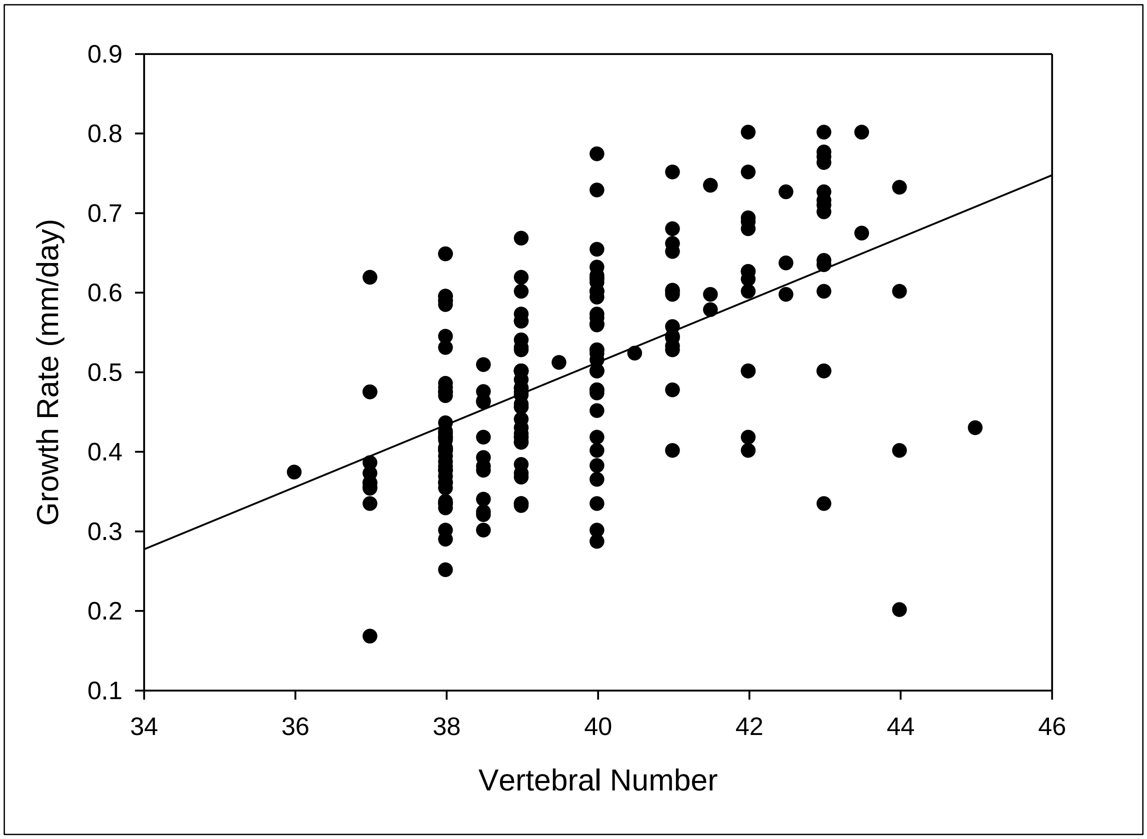

Figure 4-2: Relationship between vertebral number and growth rate of experimental fish with median U_{crit} swimming speeds. Data shows a significant correlation between vertebral number and growth rate (Pearson Product Moment Correlation: r= 0.556, p<0.0001).

Table 4-2: p-values for multiple regression analyses at each experimental temperature.

	Temperature		
Variable	**15°C**	**21°C**	**28°C**
Vertebral number	0.16	0.62	0.003
Growth rate	0.273	0.061	0.198
Length	<0.001	<0.001	<0.001

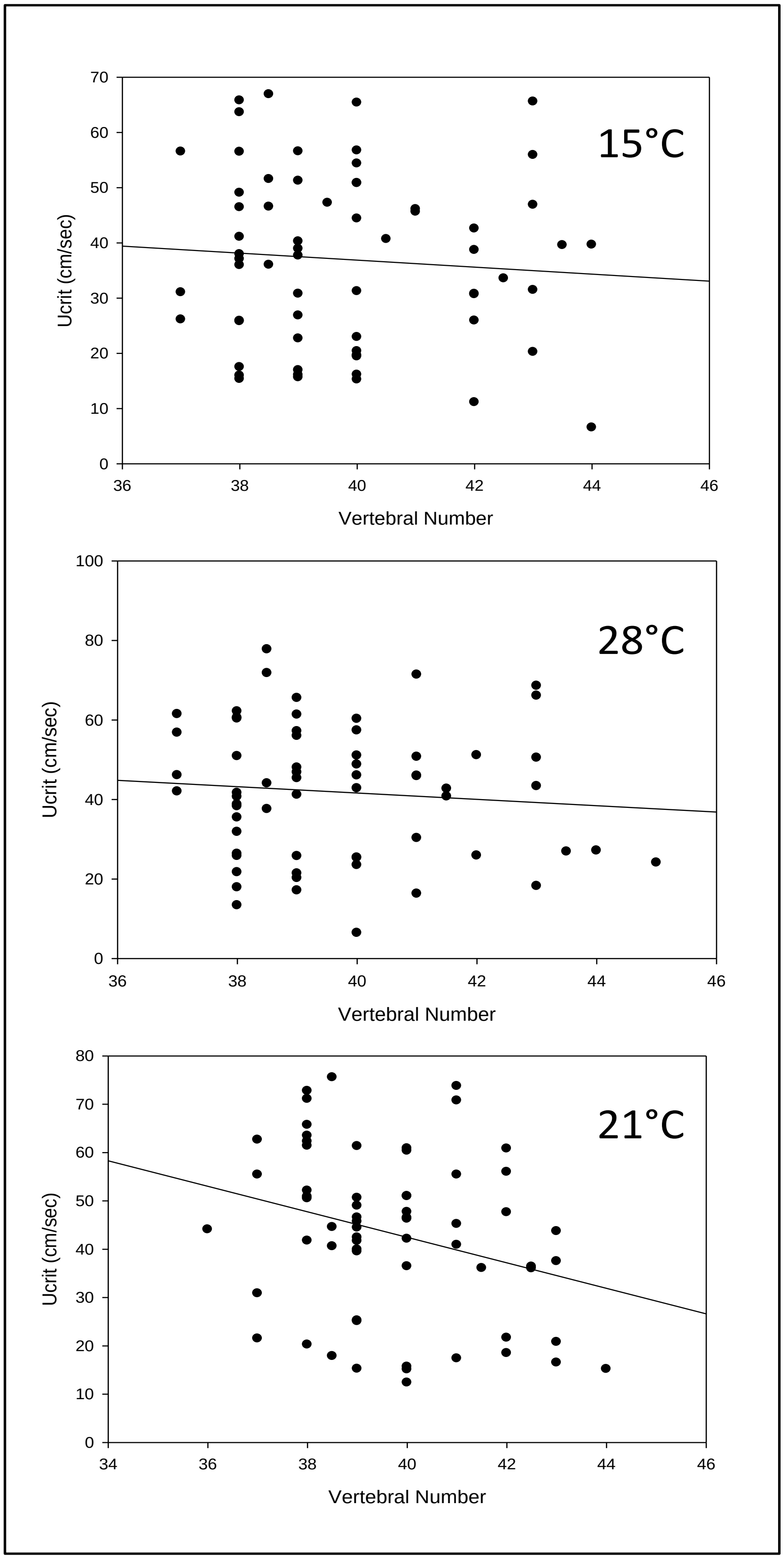

Figure 4-3: Critical swimming speed (U_{crit}) as a function of vertebral number at three experimental temperatures. Vertebral number only has a significant effect on swimming ability at 28°C ($p=0.003$).

Conclusions

This work has provided novel insight to the spatial scale of local adaptation in a migratory marine species and is likely the first of its kind. In particular, I have shown that vertebral number in *Menidia menidia* increases with latitude in accordance to Jordan's rule, but the relationship between vertebral number and latitude in this species is more complex than previously recognized. I have shown that certain locations along the coastline are important areas for rapid change in vertebral number, particularly near the Outer Banks, NC and in the Gulf of Maine. Additionally, I have shown that vertebral number exhibits a geographic pattern separate from that seen in growth rate, indicating the likelihood that there are different selection forces driving these two traits.

I have shown that despite this fine-scaled geographic pattern, there is a degree of plasticity that appears to occur in vertebral number, and that the trait can vary both spatially and temporally. However more work needs to be done to investigate this variation in further detail. It would be interesting to place temperature loggers in more broadly spaced locations throughout the coast and see if spawning temperature affects vertebral numbers along the latitudinal range. If this could be done across multiple years, we would be able to see if annual differences in temperature were correlated to annual difference in vertebral number. Although this could be accomplished using coastal buoy data, placing temperature loggers *in situ* with the embryos would give much more valid information on the temperatures the embryos were experiencing. It would also be worthwhile to expand the spatio-temporal study in Long Island, NY to a broader geographic range, perhaps at 50 km scale used in the broad latitudinal study in chapter 1, to see if vertebral numbers can provide evidence of local retention on a larger scale. It may be that the reason that it was not seen in the work in Long Island is because of local mixing. Clarke et al. (in press) found evidence of local retention in Southern Long Island, but this varied in the two years of their study. This indicates that local retention at small spatial scales is not constant, which may limit the ability to see local retention in vertebral number in a given year.

My research in selection on vertebral number shows evidence for selection for fewer vertebrae within a single site, contrary to what would be expected from current theories on the adaptive significance of vertebral number, which assert a hydrodynamic advantage to increased vertebral number in cold water (McDowall 2008). This is quite striking. Again, it would be worthwhile to expand this study to more sites to see if this pattern is consistent throughout the species range. The addition of *in situ* temperature loggers combined with otolith analysis to back-calculate birth date in several locations could provide detailed information regarding the adaptive significance of this trait. Additionally, laboratory experiments testing the vertebral development, the particular norm of reaction of vertebral number, and when the ‘window’ of vertebral number development occurs could be very useful to our understanding of this trait.

Although the experiments testing the effect of vertebral number on swimming ability did not work out as planned or give the expected results, it is still possible that vertebral number is important for swimming ability. As discussed in chapter 4, it is possible that the larvae used in the experiment already had the ability to escape the viscous regime. It would be interesting to test swimming ability on smaller fish and also look at other modes of swimming and in waters where the viscosity has been manipulated independent of temperature.

It is clear that vertebral number is adaptive and capable of evolving, but the significance of the adaptation still remains unclear and warrants further investigation. This is a phenomenon that occurs across several species, yet the reason remains left mostly to speculation (reviewed in McDowall 2008). In chapter1, I discuss latitudinal differences in precaudal and caudal vertebrae along the species range. In the south and extreme north the increase in vertebral number is due to proportionally more precaudal vertebrae while in northern populations there are proportionally more caudal vertebrae. This suggests that the adaptive benefit of increased vertebral number may be occurring for different reasons along the species range. Populations which show the majority of the increase in precaudal vertebrae are also populations which exhibit temperature-dependent sex determination (TSD). It may be that relative increases in precaudal vertebrae provide more space for gonadal development. Conversely populations in the north exhibit proportionally more caudal vertebrae, perhaps to deal with differences in water viscosity at very small sizes, essentially at hatch (3.8-5.0mm, Wang 1974). It could also be that there are differences not only in the number of precaudal or caudal vertebrae, but also in the proportion of the length of the vertebral column devoted to precaudal versus caudal vertebrae. One could argue, for example, that devoting a larger proportion of the vertebral column to precaudal vertebrae, either through more precaudal vertebral or precaudal vertebrae that are longer than caudal vertebrae, an individual may consequently increase the area of the body cavity that could be devoted to internal organs. This has not been studied in this species, but population level differences in *Menidia menidia* anatomy and morphometrics warrant further investigation.

Vertebral number and growth rate are often correlated as seen in several chapters; however evidence from chapter 1 suggests different selection forces acting on the two traits. Chapter 4 reinforces this assertion since growth rate had no significant effect on swimming ability. It is clear that the life history and evolution of this species is quite complex, but the mere recognition of this complexity furthers our understanding of evolutionary ecology.

Since the publication of D.S. Jordan's original work in 1891, Jordan's Rule has been used exclusively to describe the positive correlation between vertebral number and latitude in fish. This phenomenon, whether recognized as Jordan's Rule or not, has not be documented in other vertebrates. Interspecific differences in numbers of vertebrae in reptiles and amphibians are common and often used to distinguish between related species or subspecies (Jockusch 1997; Arntzen and Wallis 1999; Van Damme and Vanhooydonck 2002; Litvinchuk and Borkin 2003). Although not as common, intraspecific variation in vertebral number has been documented in amphibians (Jockusch 1997), reptiles (Arnold and Bennett 1988; Lindell 1996; Kelley et al. 1997; Kaliontzopoulou et al. 2008), and even mammals (Aimi 1994). However studies of geographic clines in intra- or interspecific vertebral number are rare. Interspecific geographic variation in vertebral number has been studied in slender salamanders (*Batrachoseps spp.*), yet the mechanism driving such variation is unknown (Jockusch 1997). Although Jochusch's study showed phenotypic plasticity in relation to temperature, the resultant differences among were not enough to explain the variation seen among populations in the wild, suggesting a genetic component to the trait. The factor driving this geographic variation is not known. Intraspecific differences in vertebral number have also been noted between coastal and inland populations of snakes; however the reason for these differences is unclear. It has been suggested to be an adaptation to habitat differences in push-points or due to selection on correlated body size (Kelley et al. 1997).

Studies on *Vipera aspis*, a viviparous snake, found that higher temperatures during early gestation periods led to higher numbers of ventral scales, which are tightly correlated with vertebral number in snakes and linked to the number of pairs of somites produced during development (Lourdais et al. 2004). This suggests that increased temperature increases somotisation in this species. However in *Menidia menidia* and the related *Menidia peninsulae*, increased temperature during development has the opposite effect, leading to a decrease in vertebral number (Billerbeck et al. 1997; Yamahira et al. 2006).

The evolutionary advantage to variation in vertebral number is often linked to locomotion across taxa. In lizards it has been suggested that having a larger number of vertebrae in a given body area would increase maneuverability, which could be beneficial to populations living in habitats with complex structure, such as dense vegetation. Conversely, having fewer vertebrae would benefit speed and acceleration in open, unimpeded habitats (Van Damme and Vanhooydonck 2002). However, Van Damme and Vanhooydonch (2002) were unable to link habitat variation to interspecific variation in vertebral number in habitat types occupied by lacertid lizards. Bergmann and Irschick (2010) investigated differences in body shape in related species of lizards but did not find evidence that body shape was linked to habitat substrate, as the kinematics of locomotion did not change with different substrate types. However Kelley et al. (1997) found that in gater snakes (*Thamnophis elegans*), vertebral number had a significant effect on locomotive ability among conspecifics. Snakes with more body vertebrae were slower than those with fewer, while snakes with more tail vertebrae were faster than those with fewer. Snakes with relatively more vertebrae also were suggested to have greater body flexibility through greater lateral bending. Although Jordan's Rule has not been used to describe vertebral number in other vertebrates beyond fish, it is possible that it may occur, particularly in reptiles and amphibians. Similar studies to those seen in fish in vertebral number plasticity have been demonstrated in these taxa, and analogous theories as to the adaptive significance and the effect on locomotion have been suggested.

Broad Implications

In a marine environment, the study of locally adapted traits is especially important in populations that experience heavy selective harvest. The practice of fishing can have selective effects that result in genetic changes in populations, an idea addressed as early as the 1950's (Policansky and Magnuson 1998) and more recently in work done by Conover (Conover and Munch 2002; Walsh et al. 2006) and Swain (Swain et al. 2007). From a fisheries perspective, findings such as these have implications to stock structure and harvest practices. For instance if a region experiences heavy fishing pressure and stocks decline or disappear, surrounding populations may not be able to recolonize the area due to a high degree of local adaptation to their particular environment. Studies in local adaptation can also be applied to issues such as climate change and habitat loss. Increases in water temperature can lead to shifts in a species range, or elimination of populations at the tips of ranges. In coastal species such as *Menidia menidia* or other commercially and recreationally important species, anthropogenic influences such as loss of wetlands can create patchy environments along a species range and reduce population connectivity. Empirical studies on model species such as *Menidia menidia* are essential to increase our understanding of local adaptation and potential anthropogenic impacts on marine species.

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