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The Effects of Temperature on the Survival, Growth and Development of Larvae of Two Blue Mussel Species (Mytilus edulis and Mytilus trossulus)

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THE EFFECTS OF TEMPERATURE ON THE SURVIVAL, GROWTH AND DEVELOPMENT OF LARVAE OF TWO BLUE MUSSEL SPECIES

(*Mytilus edulis* and *Mytilus trossulus*)

By

Susan Hayhurst

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Factors affecting the distribution of *Mytilus edulis* and *Mytilus trossulus* in the Gulf of Maine are of great interest because of the blue mussel's economic and ecological importance. Genetic surveys of blue mussel populations indicate that eastern Maine represents the southern distributional limit for *M. trossulus* but that *M. edulis* is common throughout the Gulf of Maine. Because hydrographic features in the Gulf of Maine confound temperature variation and larval dispersal patterns, the relative importance of these potential range-limiting mechanisms cannot be ascertained from the distribution of adult mussels. Given that larvae are more vulnerable than adults to temperature extremes, this study focused on comparing the effects of temperature on *M. trossulus* and *M. edulis* larval growth and survival.

Molecular techniques were employed to determine species identity of adult mussels collected from a mixed population in eastern Maine. Mussels were induced to

spawn in the laboratory and larvae of both species were reared at 5, 10, 15 and 20°C for 30 days. Observations were made on larval mortality, growth and development. The results indicate that the effects of temperature are not identical for larvae of the two species, with higher mortality rates observed for *M. trossulus* at 5 and 20°C and higher mortality for *M. edulis* at 15°C. In addition, at 20°C *M. trossulus* larvae demonstrated decreased growth relative to *M. edulis*. Taken together, the growth and survival observations suggest that 5°C is sub-optimal for both species and that *M. trossulus* larvae may experience thermal stress at 20°C. These differences have important implications for mechanisms limiting the range of *M. trossulus* in the Gulf of Maine.

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BACKGROUND

Determinants of Marine Geographic Ranges

The question of what factors act to structure the range limits of marine benthic invertebrate populations is a fundamental one in marine science. Among marine invertebrates there is a tremendous diversity of complex life histories with very different adult and larval forms. The study of range limitation in such species can be as complex as their life histories. The physical, biotic, and historical factors that influence their ranges may operate in varied ways on the pelagic and benthic forms.

Many authors have proposed that developmental mode plays a major role in structuring species ranges (*e.g.* Jablonski and Lutz 1983; Strathmann 1985). Although larval developmental strategies cover a continuum of space and time that ranges from completely benthic to pelagic and from hours to months of development time, the reproductive strategies of marine invertebrates have been grouped into three major categories: direct development, lecithotrophy, and planktotrophy (Thorson 1950; Vance 1973). Benthic species with direct development do not release larvae into the water column but instead release fully developed juveniles into the habitat. Both lecithotrophic and planktotrophic modes of development include a free-living larval stage that spends time in the plankton. In the case of lecithotrophy ("yolk feeding"), offspring are supplied with enough endogenous energy reserves to develop to metamorphosis. In contrast, planktotrophic ("plankton feeding") species produce larvae that require exogenous food in order to develop to metamorphosis and therefore must feed during their time in the plankton.

Each of these reproductive strategies is associated with its own suite of life history and developmental traits. Much emphasis has been placed on the dichotomy between planktotrophic and lecithotrophic development (Thorson 1950). Planktotrophic larvae generally have a longer development time (weeks – months) than lecithotrophs (hours – days) and therefore are considered to have greater dispersal potential. It is often argued that planktotrophs should, as a result, have larger geographic ranges than lecithotrophs. Most evidence, however, only supports a difference between the ranges of species with planktonic versus non-planktonic development. Along the east coast of the U.S., for example, prosobranch gastropods with non-planktonic (direct) development have more restricted ranges than those with planktonic development, but range sizes do not differ among gastropod species with planktonic development of different durations (Scheltema 1989). Because larval form, developmental strategy, and development time are highly variable, Hadfield and Strathmann (1998) argue that the importance of the distinction between lecithotrophy and planktotrophy should be re-evaluated. Alternative dispersal mechanisms, including adult migration, rafting (*e.g.* on algae or logs), and anthropogenic introduction (*e.g.* in ballast water), may limit the correlation between larval development type and range (Bhaud 1993; Hadfield and Strathmann 1998). Despite developmental variation and alternative modes of dispersal, larval form can impact geographic range, especially in the face of a major dispersal barrier such as an ocean basin (Scheltema 1989).

Alternatively, Bhaud (1983) argues that physical parameters have a greater effect than development type on species range. The mortality hypothesis suggests that physical or biotic factors may make the environment outside of the existing range inhospitable

(Gaines et al. 1997). Biotic factors may act to limit a species range through limited food availability or interactions such as predation or competition. Physical extremes with respect to salinity, light, temperature, and wave exposure may restrict a species range by having a negative impact on survival or reproduction. In contrast, the dispersal barrier hypothesis holds that barriers act to prevent dispersal beyond the current range (Gaines et al. 1997). Possible barriers include hydrographic features (*e.g.* offshore currents or coastal gyres) and the size of ocean basins (Sheltema 1986).

The debate about the relative importance of mortality versus dispersal is a complex one. An extreme in temperature, resulting in mortality of individuals dispersing or migrating outside of the range limits, is often cited as the most important factor affecting a species' geographic range (Bayne 1976; Gaines et al. 1997). In many cases, this hypothesis is generally supported by the observed distributions of species. For example, the north-south distributions of many invertebrate species correlate well with temperature variation (see Kinne 1970). However, many of these observations are correlative and do not demonstrate clear evidence that temperature is the mechanism limiting distribution (Kinne 1970; Gaines et al. 1997). Moreover, there are many examples of marine invertebrate ranges that do not correlate with temperature variation. For example, range limits of littorine snails along the West Coast of the U.S. do not correspond to any change in sea temperature and appear to be dependent on predation, habitat suitability and currents more than temperature variation (Yamada 1976).

Many of the same physical and biological processes that can affect adult survival also impact larval survival. Many researchers suggest that an absence of larval settlement outside a species range limit is evidence of a dispersal barrier at this location (*e.g.* Efford

1970; Gaines et al. 1997). However, this view disregards the possibility that larvae may be transported across a potential barrier but unable to reach settlement due to mortality or abnormal development. These processes are encompassed by the larval mortality hypothesis, or "sub-hypothesis" (Gaines et al. 1997). The larval mortality hypothesis has not received much attention, perhaps because it is difficult to test. Although transplant experiments can examine mortality in adults outside their geographical range, it is impractical to transplant microscopic, planktonic larvae, which are often difficult to identify to species. Thus, the larval mortality hypothesis has traditionally been underemphasized in favor of the (adult) mortality and dispersal barrier hypotheses.

There is growing attention to the idea that the ranges of some invertebrate species may not be in equilibrium with current hydrographical features and that in these cases historical events may be important (Benzie and Williams 1995; Hilbish 1996). Examples of historical processes that can affect geographic range include climate change, sea level change, the opening or closing of sea passageways due to plate tectonics, and sea floor spreading (Scheltema 1986). For example, the opening of an arctic seaway between the Pacific and Atlantic Oceans 3.5 million years ago may have allowed interchange between these oceans and affected the distribution and genetic structure of a wide variety of northern temperate species (Vermeij 1991).

The conundrum inherent in the study of range limits is summed up by Gaines et al. (1997): "Unfortunately, the underlying climactic mechanisms potentially responsible for these two causes of range limits – steep physical gradients versus hydrographic barriers to dispersal – are typically confounded." The study of species ranges traditionally has fallen into the fields of ecology and biogeography (Gaines et al. 1997).

While ecologists tend to use an experimental approach to study the factors determining the local fine scale distribution and abundance of organisms, biogeographers document large scale patterns. Experimental studies aimed at understanding factors that operate at the edge of species ranges represent the "missing link" between the two approaches and have the potential to separate out the effects of physical gradients and dispersal barriers (Gaines et al. 1997). The intent of this research was to experimentally separate the confounded temperature and dispersal effects in the Gulf of Maine and examine the hypothesis that larval thermal tolerance is a potential factor controlling the southern range limit of *M. trossulus*.

Blue Mussel Biogeography in the Gulf of Maine

Blue mussels are common rocky intertidal and shallow subtidal bivalve mollusks found in the temperate regions of both the Northern and Southern hemispheres (Gosling 1992). In many parts of the world, including the Northeastern United States and Eastern Canada, there is an active blue mussel fishery and aquaculture industry. In addition, the blue mussel (*Mytilus edulis*) figures prominently as a sentinel species in biomonitoring efforts to assess coastal contaminants in both the Mussel Watch and Gulfwatch programs in Maine and elsewhere in the U.S. Given the economic and ecological importance of blue mussels, there is great interest in understanding factors affecting their distribution.

Globally, there are three species of blue mussel within the genus *Mytilus*, *M. edulis*, *M. trossulus* and *M. galloprovincialis*. Of these three sibling species, *Mytilus edulis* has the widest distribution and ranges from estuarine to oceanic sites in Eastern North America and Western Europe (Seed and Suchanek 1992). *Mytilus trossulus*, the

other blue mussel which occurs on the Atlantic coast of North America, is restricted to a sub-polar distribution and is generally considered a cold water species (Koehn 1991). Its proposed range is the Baltic Sea, Northeastern and Northwestern North America, and the Pacific coast of Siberia (McDonald et al. 1991, Seed 1992, Bates and Innes 1995). Because *M. trossulus* is the most recently defined taxon in the *M. edulis* complex (McDonald el al. 1991), it has not been studied as thoroughly as the other two species. Based on the genetic surveys of Koehn et al. (1976, 1984), it was assumed that mussels found throughout the Gulf of Maine were exclusively *M. edulis*. Sowles et al. (1996), however, used allozyme analysis to show that, in fact, the range of *M. trossulus* extends into the Bay of Fundy and Passamaquoddy Bay, Canada. More recently, Rawson et al. (2001) used a series of DNA-based genetic markers to document that appreciable frequencies (>10%) of *M. trossulus* can be found well into the Gulf of Maine, as far west as Little Machias Bay, Maine.

This survey by Rawson et al. (2001) has since been expanded upon to include an additional ten sampling sites. Among these new sites are two offshore transects; one of which extends from Mount Desert Island to Mount Desert Rock and the second from Penobscot Bay to Matinicus Rock (see Figure 1). As in the initial survey, several near-shore sites along the central Maine coast (Petit Manaan Point, Little Moose Island, Burnt Cove, data not shown) contain predominantly *M. edulis* alleles and haplotypes. In contrast, the offshore sites contained significant frequencies of *M. trossulus* alleles. In particular, *M. trossulus* alleles were as common in the samples from Mt. Desert Rock and Frenchmans Bay South as in eastern Maine and the frequency of *M. trossulus* alleles appears to increase with distance offshore.

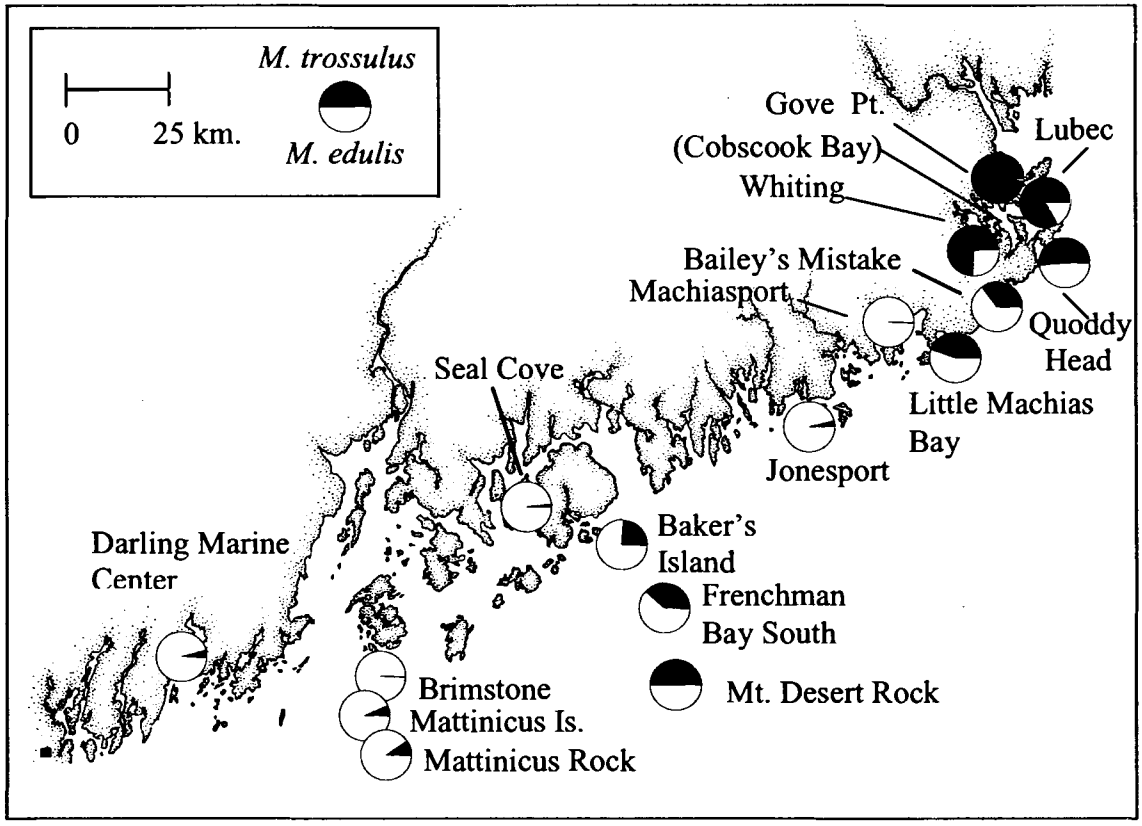

Fig. 1: The Distribution of Blue Mussels in Central Maine. Each pie chart represents the frequency of *M. trossulus* (in black) and *M. edulis* (in white) alleles at each site sampled. (Data from Rawson et al. 2001 and Hayhurst et al., unpublished data.)

The presence of *M. trossulus* in eastern Maine is of practical importance to both the mussel industry and government biomonitoring programs. *Mytilus trossulus* is considered less commercially desirable than *M. edulis* because of its slower growth, lower condition index (less meat), and higher shell fragility that causes it to fracture during processing (Freeman et al. 1994). In fact, Mallet and Carver (1995) estimate that *M. edulis* has an economic value 1.7 times that of *M. trossulus*. The presence of *M.*

trossulus in coastal Maine is of concern to the Gulfwatch program because *M. edulis* and *M. trossulus* differ in seasonal accumulation of lead, chromium, zinc and mercury (Mucklow 1996). For these reasons, the culture industry and biomonitoring programs are interested in factors that may regulate the distribution of *M. trossulus*. While the recent offshore survey (Hayhurst et al., unpublished data) gives strong evidence that *M. trossulus* occurs well into the central Gulf of Maine, it provides no explanation about what ecological or physical processes may be responsible for the observed distributions.

It is impossible to determine whether the current presence of *M. trossulus* in eastern Maine represents a historical presence or a recent migration. During the last glacial maxima (12-18,000 years ago), ice sheets covered the intertidal zone in the Gulf of Maine. Blue mussels recolonized the Gulf of Maine after these ice sheets receded, and genetic evidence suggests that *M. trossulus* has become established by a recent trans-Arctic migration (Cunningham, pers. comm.). Genetic surveys of blue mussels in the Gulf of Maine prior to that of Rawson et al. (2001) analyzed blue mussel samples from Bar Harbor, Maine and farther North in the Canadian Maritimes. Given a lack of historical information on frequency of *M. trossulus* in the eastern Gulf, it is impossible to determine whether the present range endpoint near Machias Bay, ME represents an equilibrium distribution. Although the current range of *M. trossulus* in the Gulf coincides with modern day hydrographic features, this correlative evidence does not rule out the possibility of ongoing range expansion.

Gardner (1996) proposes that differential adaptation to environmental conditions is the primary factor determining the geographical distribution of blue mussel species and maintaining genetic identity of the species despite high potential for larval dispersal. He

reasons that the location of blue mussel hybrid zones at ecotones is evidence that environmental factors limit the distribution of blue mussel species. Several authors have proposed that differential tolerance to salinity (Sarver and Foltz 1993; Gardner 1996), wave exposure (Bates and Innes 1995; Hunt and Sheibling 1996, 1998), or temperature (Sarver and Foltz 1993) may provide an explanation of distributional differences in *M. trossulus* and *M. edulis* in North America. The highest frequency (greater than 90 percent) of *M. trossulus* in mixed populations of *M. trossulus* and *M. edulis* was found at two wave-exposed sites in eastern Canada (Bates and Innes 1995).

While wave exposure and salinity variation may affect the microscale distribution of blue mussels, they are not factors that satisfactorily explain the observed transition along coastal Maine from *M. trossulus* to *M. edulis*-dominated populations. Although salinity and wave exposure may vary on a fine spatial scale, there is no evidence of any significant shift in salinity along the coast of Maine (Canadian DFO 2001). According to Bayne (1976), factors such as salinity, substratum, and wave exposure serve mainly to limit the local abundance and distribution of a species, and it is primarily sea temperature that controls its overall distribution.

Based on the hydrographic features of the Gulf of Maine, the effects of dispersal and temperature variation are the two most likely candidates for mechanisms controlling the distribution of *M. edulis* and *M. trossulus*. Blue mussels release large numbers of free-living, planktotrophic larvae into the water column. Given a larval life span of 3 weeks to as long as 3 months (Widdows 1991), there is a significant opportunity for mussel larvae to disperse away from the population in which they originate. In the eastern Gulf of Maine, the Eastern Maine Coastal Current (EMCC) plays a major role in

the movement of water and water-borne particulates, including larvae. This current (see Figure 2) is a cold water feature that originates in eastern Maine near Grand Manaan Island and moves offshore in the vicinity of Jonesport, ME (Townsend 1992) during times of the year when larvae are present in the water column.

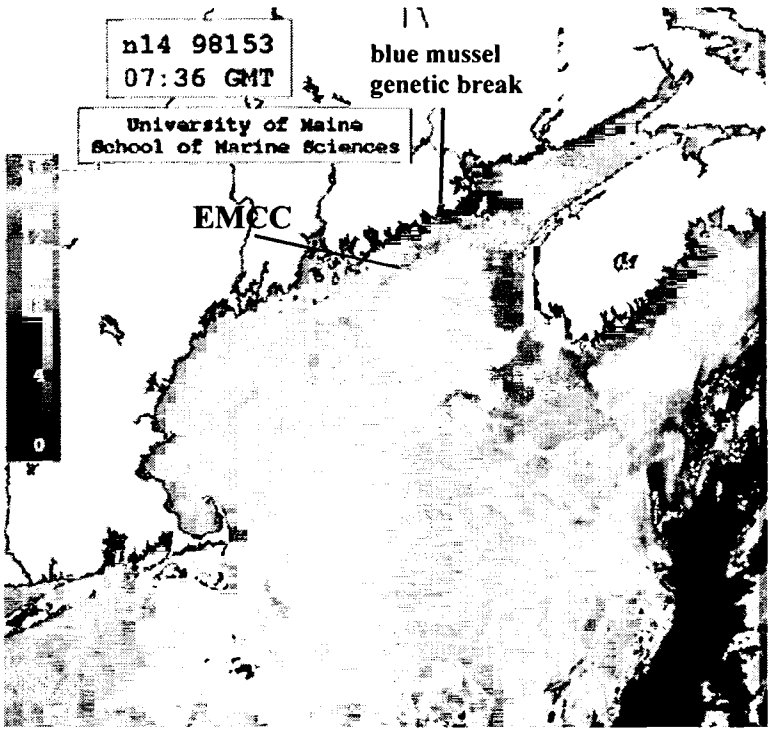

Figure 2: The Eastern Maine Coastal Current. Sea surface temperature satellite image of the Gulf of Maine in June 1998. The temperature scale (°C) is shown on the left. The Eastern Maine Coastal Current (labeled) is visible as cold water moving south along the coast and then heading offshore over the central portion of the Gulf. The genetic break in *M. trossulus* alleles observed near Machiasport is also highlighted. (Adapted from color satellite image courtesy Andrew Thomas.)

There is some evidence that the EMCC represents a dispersal barrier to planktonic organisms. For example, the EMCC may have important consequences for herring larvae, transporting some of the larvae away from the coastline when the plume of cold

water turns offshore (Townsend 1992). The current system may also affect the distribution of holoplanktonic organisms including species of the genus *Alexandrium*, a dinoflagellate responsible for paralytic shellfish poisoning (PSP) in humans. In central Maine, where PSP is reported to be lowest (Shumway et al. 1988), the distribution of *Alexandrium* is off shore and coincides with the EMCC (Dave Townsend, pers. comm.). Similarly, the EMCC may entrain *M. trossulus* larvae spawned in the east, carry them downstream and ultimately sweep them offshore. The hypothesis that the EMCC may be a barrier to the dispersal of *M. trossulus* larvae is difficult to address. Because the point of origin of the offshore plume is variable and the fate of larvae entrained in the current unpredictable, the EMCC may not represent a complete barrier to coastal dispersal (Townsend 1992). In addition, Scheltema has suggested that coastal hydrographic features may affect the rate of range expansion more than absolute range limits in species with planktonic development.

The EMCC creates a temperature break along coastal Maine that may be a physical mechanism restricting *M. trossulus* from the central Maine coast by negatively affecting survival or reproduction. The average temperatures along coastal Maine shift in conjunction with major hydrographic features such that, in the summer and fall when the EMCC turns and heads offshore, the water temperatures in eastern Maine are typically several degrees colder than in central and southern Maine (appendix A).

Temperature is considered to be the main factor limiting *M. edulis* distribution on the east coast of North America because *M. edulis* is not found south of the maximum surface isotherm of 27°C at Cape Hatteras, North Carolina. The temperature limit observed in the field corresponds well to laboratory estimates of the upper lethal

temperature (27-29°C) for adult *M. edulis* (Wells and Grey 1960, Read and Cummings 1967). The northern limit of *M. edulis* is less well defined. Populations have been observed in Labrador, where they are frozen in ice for 6-8 months each year (Kanwisher 1966; Williams 1970). In general, the distributions of *M. edulis* and *M. trossulus* in the Northwest Atlantic are correlated with temperature variation. In the Canadian Maritimes, *M. trossulus* is found in Nova Scotia and Newfoundland but is entirely absent from the warmer waters of the Northumberland Straits between the coasts of New Brunswick and Prince Edward Island (McDonald et al. 1991). Moreover, the distribution of *M. trossulus* both onshore and offshore in the Gulf of Maine corresponds to the temperature variation in the Gulf established by the EMCC.

Despite a general correlation of the *M. trossulus* distribution with temperature variation, experimental evidence concerning the effects of temperature on *M. trossulus* is limited and ambiguous. Mallet and Carver (1995) examined the comparative growth and survival of *M. trossulus* and *M. edulis* in adult size classes at two locations in Nova Scotia. They found that *M. edulis* experienced high mortality at the warmer location and concluded that, contrary to what would be expected from their distributions, *M. trossulus* can tolerate higher temperatures better than *M. edulis* (Mallet and Carver 1995). These enigmatic results are not consistent with the traditional characterization of *M. trossulus* as a cold-water species (Koehn 1991).

Because the EMCC affects both hydrographic and temperature variation in the Gulf, the distribution of *M. trossulus* adults alone cannot distinguish between the mortality and dispersal hypotheses. In an effort to determine the relative merits of the mortality versus dispersal hypotheses, this research examines the comparative survival,

growth and development of *M. trossulus* and *M. edulis* larvae exposed to a range of temperatures commonly observed along the coast of Maine.

INTRODUCTION

Sea temperature is one of the main factors that limit the macro scale distribution of marine organisms. Among other things, temperature can affect survival and growth of larvae and adults, reproductive physiology and behavior, and metamorphosis (Kinne 1970; Bayne 1976). The mechanisms by which temperature affects these processes operate at several levels, including organismal (alteration of metabolic rate and energetic requirements), cellular (alteration of cell membrane) and molecular/biochemical (alteration of enzyme quaternary structure and enzyme/substrate binding) (Kinne 1970; Hochachka and Somero 1984). At a critical size, juvenile marine invertebrates become much less vulnerable to physical factors such as temperature (Gosselin and Qian 1997). Despite evidence across phyla that the range of thermal tolerance is often narrowest in early stages of development and highest in adults (Kinne 1970; Bayne 1976; Bourget 1983), much of the scientific literature has focused on the effects of temperature on adults. Brown et al. (1992) suggest that an important factor regulating the distribution of a species may be tolerance limits of early life stages to environmental conditions. For example, the effects of temperature, salinity, and food abundance on larval survival and behavior may influence the distribution and gene flow of littorine snails (Berry and Hunt 1980) and lobsters (Hedgecock 1986). Further, Bayne (1976) suggests that the environmental requirements for normal embryonic development are a major factor limiting the distribution of blue mussel populations.

The vast majority of research on the effects of temperature on blue mussels has focused on adults (Lutz and Kennish 1992). While some work has investigated the physiological ecology of mussel larvae, most has focused on *M. edulis* or on populations whose taxonomic affinity is uncertain in light of the reclassification of blue mussels suggested by McDonald et al. (1991). Fertilization of *M. edulis* eggs occurs normally over the range of 5 to 22°C (Bayne 1965). In the laboratory, *M. edulis* larvae tolerate temperatures between 5 and 20°C but develop normally only within the temperature range of 15 to 20°C (Brenko and Calabrese 1969). Several studies of *M. edulis* larvae indicate that early developmental stages are more vulnerable than later ones to temperature conditions. Shelled larvae have a greater resistance to environmental change than embryonic stages and young larvae develop successfully over a more narrow range of temperatures than older larvae (Bayne 1976). This trend persists after settlement; juvenile *M. edulis* have a lower median lethal temperature (MLT) of -12.5°C, while the MLT for adults was as low as -16°C (Bourget 1983). Despite these and other studies of involving *M. edulis*, little is known about the physiological ecology of *M. trossulus* larvae or juveniles.

The goal of this study was to address the possible factors limiting blue mussel distribution in the Gulf of Maine. In particular, this research assessed the effects of temperature on daily instantaneous mortality rate, growth and development characteristics, and percent deformity for *M. edulis* and *M. trossulus* larvae. The results of this experiment provide the first observations concerning the effects of temperature on the developmental biology of *M. trossulus* and contribute to the limited understanding of the comparative biology of these two sibling species. Moreover, they provide

preliminary insight into how temperature may function in structuring the species composition of mussel populations in the Gulf of Maine.

MATERIALS AND METHODS

Collection and Preparation of Spawning Stocks

The thermal history of parental stocks can affect the thermal tolerances of the larvae that they produce (Bayne 1976). For this reason, approximately 200 adult blue mussels (*Mytilus* spp.) were collected from a mixed population in the lower intertidal zone of a single site in Sipp Bay, on June 6, 2000. Sipp Bay is located within the East Bay portion of Cobscook Bay in eastern Maine, where *M. edulis* and *M. trossulus* are sympatric (Figure 1). The mussels were transported on ice to the University of Maine, Orono where each mussel was individually tagged by engraving a number on its shell. The mussels were then placed in a tray of seawater; as each mussel opened and began to filter water a small piece of mantle edge tissue was clipped and immediately preserved in 70% ethanol for DNA-based species identification. After the tissue biopsy, individual mussels were placed back on ice and kept damp under rockweed until they were transported to the Darling Marine Center, Walpole, ME. Thus, within 48 hours of the time of their collection, adult mussels were placed in seawater at ambient temperature (approximately 10°C) in the Flowing Seawater Laboratory (FSL) at the Darling Marine Center.

DNA-based Species Identification

Blue mussel species are difficult to distinguish solely on the basis of external morphology, and allozyme electrophoresis typically requires that mussels be sacrificed prior to analysis. In this study, each mussel was non-destructively genotyped using DNA-based methods. DNA was prepared by boiling a portion of the clipped mantle edge from each mussel in 5% Chelex (Biorad). After centrifugation, 0.5 μl of supernatant was used as template in 3 polymerase chain reaction-based markers (ITS, MAL-I, and Glu5') all of which are diagnostic for *M. edulis* and *M. trossulus* (Heath et al. 1996; Rawson et al. 1996, 2001). Based on the results of these assays, mussels were classified as having *M. edulis, M. trossulus*, or mixed genotypes. Those with *M. edulis* or *M. trossulus* genotypes were subsequently maintained in separate trays while the mixed genotype mussels were removed from the experiment.

Spawning and Fertilization of Species-Specific Stocks

Adult mussels were maintained at 9-10°C with frequent water changes and fed high concentrations of algae from June 7 to June 10, 2000. After completion of a water change on the morning of June 10, multiple *M. trossulus* spawned spontaneously. It was visually estimated that at least 15-20 individuals contributed gametes to this unanticipated spawning event. The water was gently passed through 125 and 80 μm sieves and then through a 15 μm sieve to retain fertilized eggs. The sieved eggs were gently rinsed into 8-10 L of filtered aged seawater and set aside in a 10° C water bath.

Two hours post fertilization, the eggs were examined under a compound microscope and >90% fertilization was observed.

Immediately following the spawning of *M. trossulus*, *M. edulis* mussels were induced to spawn using a combination of the methods described by Utting and Spencer (1991), Breese and Malouf (1975), and Loosanoff and Davis (1963), as follows. Mussels were placed in seawater chilled to 10°C, which was then allowed to equilibrate with room temperature (18°C), then repeatedly cycled down to 10° and allowed to equilibrate to 18°C. The duration of these thermal cycling treatments is short enough that they should not affect larval thermal tolerances (Bruce Barber, pers. comm.). In addition, the posterior adductor muscle of each mussel was pricked with fine forceps, a dense algal solution was added to the water in the spawning trays to encourage mussel activity, the mussels were periodically gently agitated, and, in some cases, a small solution of conspecific mussel sperm, the presence of which may chemically induce spawning (Barber and Blake 1991), was added to the water adjacent to the inhalent siphon.

In all, 3 female and 4-5 male *M. edulis* adults were successfully induced to spawn. As each female mussel began to spawn, it was immediately transferred to a 1 L beaker containing filtered, aged seawater and placed in a 10°C water bath. Male spawners were removed from the water and a highly concentrated sperm solution was placed into 1.5 ml. tubes, which were labeled and temporarily stored at 4°C. Under these storage conditions, sperm and eggs are viable for upwards of 10-12 hours following spawning. Fertilization of the *M. edulis* eggs was initiated at 12:00 a.m. on June 11, 2000 by first passing them through an 80 μm sieve and retaining them on a 15 μm sieve, then gently rinsing them into 8-10 L of filtered, aged sea water. To this, 2 ml of dense sperm solution was added

and the eggs and sperm gently agitated for one minute using a plunger. The mixture was maintained in a 10° C water bath; fertilization was verified microscopically 2.5h later. *M. edulis* fertilization was also >90% successful. The embryos of both species were left undisturbed in the 10 L containers to allow for normal early development.

Larval Rearing

Larval density was estimated for each species by averaging the number of trochophores (see Figure 4) in three separate 1 ml samples drawn from the 10 L fertilization containers after the contents of each container was thoroughly mixed. All larval counts during this experiment were done under a dissecting miscrosope (63X to 100X magnification) using a Sedgewick-Rafter counting cell. Based on these estimates, an appropriate volume of larvae was distributed to each of the larval rearing containers (1 L aged, plastic beakers) and 5 μm filtered seawater was added to obtain 800 ml cultures containing 30 trochophores·ml^{-1}. An initial density of 30 developing larvae·ml^{-1} was chosen so that the density of later stage veligers would be no greater than 10-15 D-stage larvae·ml^{-1}, as recommended by Utting and Spencer (1991) and Breese and Malouf (1975). Loosanoff and Davis (1963) cite similar logic behind setting initial densities of bivalve larval cultures at 30,000 larvae·L^{-1}, or 30 larvae·ml^{-1}.

The initial dilution of all larval cultures was conducted at 10°C after which they were gradually (over several minutes) brought to the four experimental temperature treatments, 5, 10, 15 and 20°C. Temperatures were maintained in the 5 and 10°C water tables using recirculating water cycled through chillers sensitive to 1°C changes in temperature; the 15 and 20°C tables were maintained with heated seawater. A total of 10

replicate containers were maintained for each species in water baths adjusted to these four temperatures so that the entire experiment was comprised of 80 individual rearing containers. The location of the 20 containers (10 *M. edulis* and 10 *M. trossulus*) within each of the water baths was assigned randomly using the random number table in Sokal and Rohlf (1995). Each of the experimental containers had a wax paper lid to prevent material from falling into the container and the contents were continuously gently mixed by bubbling air from the bottom of each container.

Beginning 36 hours post-fertilization, larvae were fed algal paste (Innovative Aquaculture Ltd.) at a concentration of 100 cells·ml^{-1}. This concentration was based upon those recommended by Breese and Malouf (1975; 45 cells·µl^{-1}) and Utting and Spencer (1991; approximately 150 cells·µl^{-1}) for a culture with 15 larvae·ml^{-1}. The estimated density of feeding larvae was based on the prediction that, due to high mortality, an initial density of 30 trochophores·ml^{-1} would yield 15 D-veligers·ml^{-1} (Loosanoff and Davis 1963). Cultures containing later veligers (determined by visual inspection) were fed 200 cells·µl^{-1} while those containing pediveligers (determined by visual inspection) were fed 250 cells cells·µl^{-1}. Although it is likely that larvae of different species and at different temperature treatments have different feeding rates, the high concentration of algae ensured that all larvae were fed to excess.

Water in all of the larval cultures was changed every four days by carefully passing the culture through a sieve that retained the larvae but allowed water with metabolites and dead algal cells to pass through. Conservative sieve sizes (15µm for trochophores and early D-veligers, 48µm for larger veligers and pediveligers) were used. After sieving, the larvae were gently rinsed, the larval rearing container rinsed twice

(over the sieve) and partially refilled with 5μm filtered seawater of the appropriate temperature, the sieve inverted over the container and contents rinsed back into the container. Finally, the volume in each container was slowly brought up to a total of 800 ml by carefully adding additional filtered seawater. During the water change, larvae were never subjected to seawater of a different temperature and were out of the water for only a few seconds. In this manner, the experiment was maintained for a total of 30 days.

Data Collection

The larval rearing containers were periodically sampled throughout the experiment in order to estimate larval survival, growth, development, and percent deformity. The containers were first sampled immediately after adjusting the initial densities (18 hours post-fertilization) to confirm that each container received a similar density of larvae and establish initial density, size and stage. Each container was sampled again at 54 hours post-fertilization; thereafter the cultures were sampled every 48 hours. Because the *M. trossulus* fertilization took place roughly 16 hours prior to the *M. edulis* fertilization, the sampling for each species was offset by one day. Sampling was conducted by gently but thoroughly mixing each beaker, sampling 1 ml, and preserving the larvae contained in that 1 ml in a 1.5 ml tube containing 200 μls of 95% ethanol. When possible larvae were examined live and observations regarding the behavior of the larvae (presence or absence of swimming) and general appearance of the larvae (color, presence or absence of food in gut) were made. The experiment was concluded on July 9, 2000 after a total of 15 samples were taken for all replicates of both species. At this time the rearing containers were emptied and rinsed and the presence or absence of settled

juveniles was recorded and, if present, their size range was estimated. Settled juveniles were defined as shelled bivalves that remained attached to the rearing beaker after rinsing, with the assumption that these individuals were attached by byssal threads.

The preserved larvae were later removed with a micropipette and placed on glass slides. The number of larvae in each sample was counted under magnification (between 63X and100X) and 4 to 5 larvae from each sample were recorded on SVHS videotape using a video camera mounted on the dissecting scope. Size estimates were obtained by analysis of the videotaped larvae using a frame grabber and the Scion Image software package. It appeared that the larval shell preserved well in ethanol. Length data were based on the whole larva in the case of a trochophore and on one valve in profile in the case of a shelled larva (veliger or pediveliger). For each larva, shell length was estimated by considering the shell to be an ellipse and estimating the length of its major axis. An extended velum or foot, etc., was not included in size data. Developmental stage and presence of deformity was also recorded for each of the measured larvae. Deformity was defined as any irregularity or asymmetry of the larval shell (Figure 3).

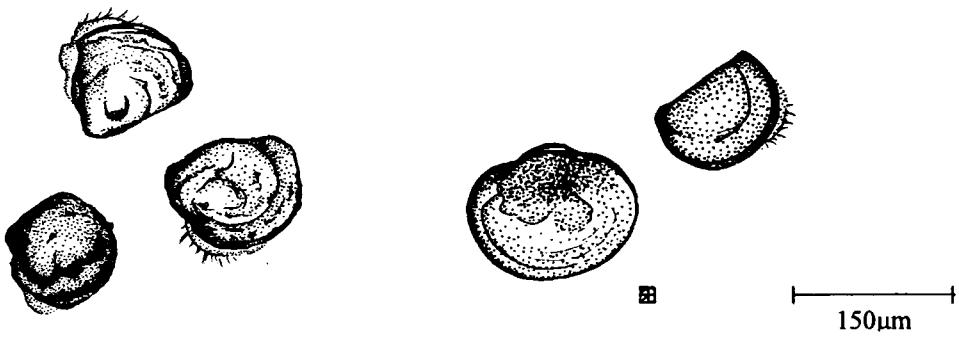

Fig. 3: Larval Deformity. Illustrations representing examples of three deformed/abnormal larvae (left) versus two normal healthy larvae (right).

Developmental stage was quantified using a developmental index where each stage corresponded to numbers 1-6 (Figure 4). As pointed out by Barber and Blake (1991) and Pechenik (1987), bivalve larval stages are in reality a continuum and the determination of stages is somewhat subjective. The staging table used in this study was based on several sources (Bayne 1976, Utting and Spencer 1991, Breese and Malouf 1975) as well as personal observation. Stage 1 was defined as a ciliated trochophore, stage 3 as a D-veliger with a characteristic straight hinge, stage 5 as a later veliger with rounded edges and umbo, and stage 6 as a pediveliger with ratio of major and minor axes close to 1 and often a visible foot or eyespot. Stages 2 and 4 were transitional stages, with stage 2 sharing characteristics of a trochophore and a veliger (roughly spherical with a partially formed shell) and stage 4 sharing characteristics of a D-stage and a later veliger (with a shell bearing a straight hinge, rounded edges and early umbo.) Veliger larvae are characterized by a wing-like, ciliated velum that was not used in staging and therefore is not shown in the illustrations below.

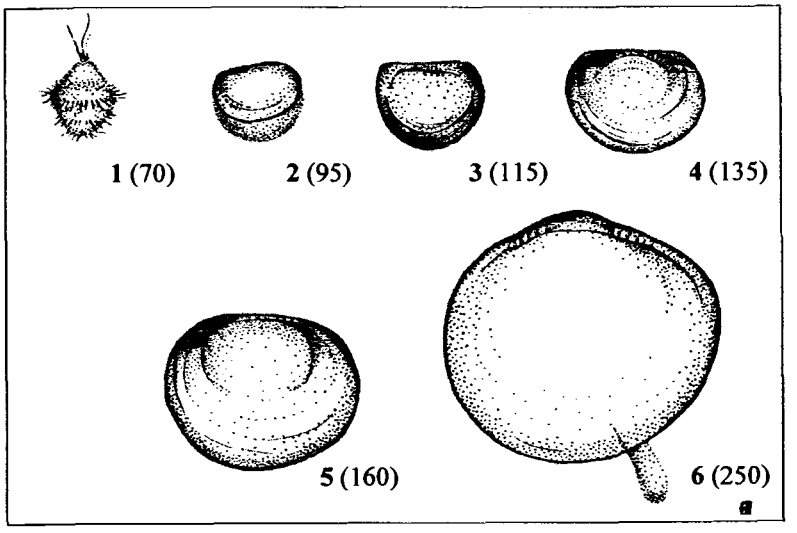

Fig. 4. Larval Staging Table. Below each illustration is the number assigned to its stage and, in parentheses, a number representing the main axis length (μm) for each illustrated larva. Actual size varied somewhat among individuals of the same stage. (Redrawn from Lutz and Kennish (1992) and personal observations.)

Statistical Analysis

All statistical analyses (with the exception of regressions) were performed using the statistical analysis package SYSTAT 9. Because larval density may affect mortality, growth and development of larvae, a two-factor analysis of variance (2-way ANOVA) was performed to test the assumption that there were no differences in initial densities of the larvae between species and among temperatures.

Daily instantaneous mortality rates, or z values, were evaluated as changes in larval density over time. This measure of mortality is used widely in fisheries biology (Houde 1989, 1997), as most mortality functions are characterized by exponential decay. Mortality was estimated separately for each replicate beaker by regressing log transformed larval density (ln[density +1]; Sokal and Rohlf 1995) against time, with t=0 representing the time at fertilization and t=28.25 days, the end of the experiment.

Estimates of density from the sample at t=2.25 days post fertilization were not used for any treatment because the samples contained a mixture of trochophores and D-stage larvae, and the former did not preserve well making it difficult to ascertain accurate density estimates. Regressions were performed with the graphing program SigmaPlot 2000. All analyses were performed with 10 replicates for each treatment (8 total treatments) with the following exception. Only 8 replicates were analyzed for *M. trossulus* larvae at 15°C; two replicates were removed from the analysis due to accidental outside contamination that may have contributed to mortality. (The data from these two replicates sampled prior to contamination were considered in growth and development analyses.) The appropriateness of the linear regression model was examined by testing whether regression residuals were distributed normally; three statistical tests, the Durbin-Watsin statistic, the normality test, and the constant variance test, were performed.

The instantaneous mortality rates (z values) were analyzed to determine the effects of temperature and species using a two-way ANOVA and pairwise comparisons. The regression slope of one variable on another is a fundamental statistic that, like the mean and standard deviation of a sample, can be important for comparing among treatments (Sokal and Rohlf 1995). The ANOVA model's assumptions of normality were tested by visually inspecting plots of the residual and estimated values and by performing a Lillifors test for normality. It is possible to perform multiple comparisons (a.k.a. unplanned comparisons) of regression coefficients to test for differences among samples much in the same way that multiple comparisons (e.g. Tukey's) are made among pairs of means (Sokal and Rohlf 1995). Thus, Tukey's pairwise comparisons were performed in order to detect significant differences between treatment pairs. An analysis

of covariance (which tests for significant differences among y-intercepts in the relationship of two variables) was not performed because the assumption of parallelism of all slopes within each group (Sokal and Rohlf 1995) was not met.

A 2-way ANOVA was performed with raw length data taken at the first larval sample (0.75 days post fertilization) to determine whether or not initial lengths were significantly different among treatments. In addition, a 2-way ANOVA was performed with the raw length data from the sample taken 2.25 days post fertilization (36 hours post treatment) in order to determine if any length differences were present at the first sample subsequent to treatment. Larval length data collected over time was converted to values for initial growth rate and final maximum length by performing non-linear regressions. The raw data were regressed to fit a three parameter equation (shown below), which is characterized by an exponential rise to a maximum Y value.

(1) $y = y0 + a(1-e^{(-bx)})$

where y equals length of main axis of the larval shell at time x; x equals time (days post fertilization). This equation is based on the von Bertalanffy function (shown below), a typical growth equation that has been used to model growth in *M. edulis* (Thompson 1984).

(2) $lt=L\infty\ [1-e^{(-K(t-to))}]$

where lt=length at time t; L∞=mean asymptotic length; K=body growth coefficient; to=time when lt=0. The third parameter (y0) used in this study adapts the von Bertalanffy equation to the study of larvae, which have a finite size at fertilization (x=0). From the regression analyses, values for the initial slope (=a*b) and the Y aymptote (=y0 +a) of

each curve were obtained. These values represented the initial growth rate and the theoretical maximum length, respectively.

The effects of temperature and species on the initial growth rates and maximum lengths from the non-linear regressions were analyzed using a 2-way ANOVA. The vast majority of regression parameters were highly significant. For a limited number of replicates, certain regression parameters were not significant at the α=0.05 level and were not included in further analyses. These parameters were also biologically unrealistic. Each of these cases was characterized by high mortality and, as a result, the length-time regressions were comprised of too few data points to establish significant relationships. Sample numbers (N) for each treatment in the analyses of variance are shown in appendix H. Tukey HSD multiple comparisons were performed to detect significant differences between treatment pairs. The appropriateness of the regression and ANOVA models were tested with the same statistical tests of normality described for the mortality analyses.

Percent deformity and developmental stage were analyzed using nonparametric statistics because of the nominal/ordinal nature of the raw data. A median test was used to determine whether there were differences in the frequency of deformed larvae among the replicates for a given species at each of the four temperatures. For this analysis, the grand median was calculated from all observed values and the number of observations less than or equal to versus greater than the grand median was compiled for each species. Observed versus expected values were compared on a 2x2 contingency table (Conover 1999).

Spearman's rank correlation test was used to test the correlation between length and developmental stage. Spearman's rank correlation coefficient, rho (ρ), is a measure of rank correlation and detects a significant correlation in a monotonic relationship (the relationship need not be linear) (Conover 1999; Haltemann, pers. comm.). Spearman's rho ranges between –1 and +1, with negative and positive values representing a negative and positive correlation, respectively, and 0 representing no correlation. For each treatment, values for ρ were calculated for the correlation between length and stage. Due to the similarity of growth and development trends, no further statistical analyses of development data were performed.

Finally, the ratio of initial growth rate to mortality rate (G/Z) was compared among treatments. This measure is used in fisheries biology to assess whether growth and mortality vary in the same way with temperature (Houde 1987). A two-way ANOVA and Tukey's HSD multiple comparisons were performed to assess the effects of species and temperature on the ratio of growth to mortality.

RESULTS

Temperature data recorded during the course of the experiment indicated that temperatures remained constant within +/- 1°C of the mean for all treatments (Table 1). The 5 and 10°C treatments were maintained slightly lower than the target temperatures. The 15 and 20°C treatments had greater accuracy but also greater variation than the low temperature treatments. Although no systematic attempt was made to monitor water temperature in the randomly distributed rearing containers, these temperatures were

periodically recorded and it was observed that they were consistent with water bath temperatures.

Analysis of initial larval densities (Table 1) by 2-way ANOVA indicated that there were no significant differences among temperatures (F-ratio=1.443; df=3; p=0.237) and no species x temperature interaction (F-ratio=49.38; df=3; p=0.448), but that there was a significant difference between species (F-ratio=16.655; df=1; p=0.000). Analysis of multiple pairwise comparisons revealed that this species effect was significant only at 5°C (Appendix B). At this temperature, *M. trossulus* had significantly higher initial larval densities (p = 0.027) than *M. edulis*.

Table 1: Mean temperatures and initial larval densities for all treatments.

treatment	mean temperature ± s.d.	*M. trossulus* initial density ± s.d.	*M. edulis* initial density ± s.d.
5	4.0785 ± 0.3035	36.800 ± 11.535	25.700 ± 5.618
10	9.6677 ± 0.2142	30.400 ± 5.602	26.800 ± 8.203
15	15.0114 ± 0.8900	31.000 ± 4.570	24.500 ± 7.397
20	20.1720 ± 0.7942	34.800 ± 8.954	28.900 ± 4.677

Mortality

Mortality of both *M. edulis* and *M. trossulus* larvae was relatively high for all experimental treatments (Figure 5). Because all conditions (save species and temperature) were standardized among all treatments, comparisons among treatment mortalities were made despite generally high mortality levels.

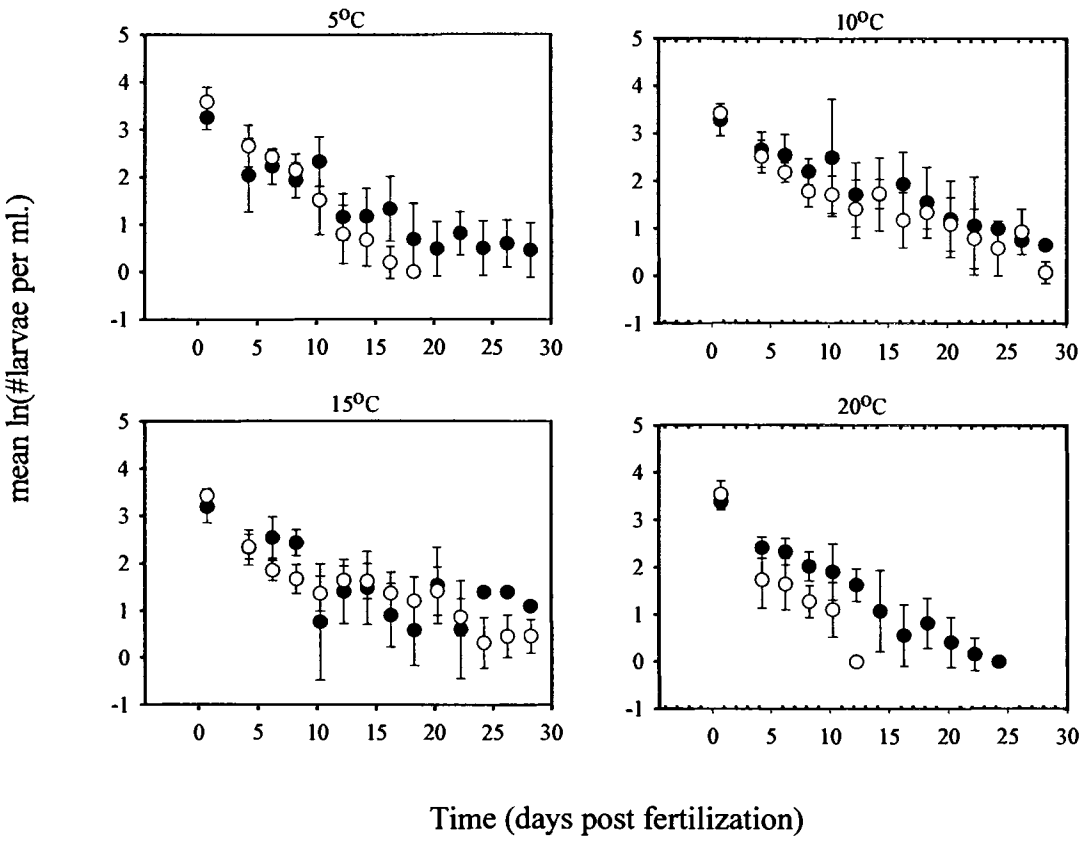

Fig. 5: Larval Mortality. Mean larval abundance (± 1 s.d.) of *M edulis* (●)and *M. trossulus* (○) at 5, 10, 15 and 20°C.

The linear regression model explained the variation in the ln(x+1) transformed larval abundance data relatively well (see appendix C). The r^2 values for replicates of *M. trossulus* ranged from 0.52 to 0.97; r^2 values for *M. edulis* were similar, ranging from 0.41 to 0.94. In addition, 75 of 78 mortality regressions satisfied either two or three of three normality tests, suggesting that the linear regression of log-transformed data was an appropriate model for mortality data. An analysis of the residuals and estimated values from the 2-way ANOVA of z values indicated no departure from normality. The p-value for the Lillifors test for normality was greater than 0.1, further suggesting that the linear

model was appropriate for the data and that the assumptions of normality of the 2-way ANOVA were satisfied.

Daily instantaneous mortality rates (z) of larvae demonstrated definite trends among temperatures and between species (Figure 6; Table 2). The significance of the interaction term stems from species-specific patterns in mortality across temperatures. *Mytilus trossulus* larval mortality was highest at temperature extremes (5 and 20°C) and lowest at intermediate temperatures (10 and 15°C), while *M. edulis* larval mortality was lowest at 5 and 10°C and higher at the two highest temperatures (15 and 20°C). These trends resulted in significant species differences at 5 and 20°C, with *higher M. trossulus* mortality, and at 15°C, with higher *M. edulis* mortality (Appendix D). The larval density of *M. trossulus* at 20°C dropped so rapidly that it was impossible to detect the presence of any larvae after only 12 days post fertilization.

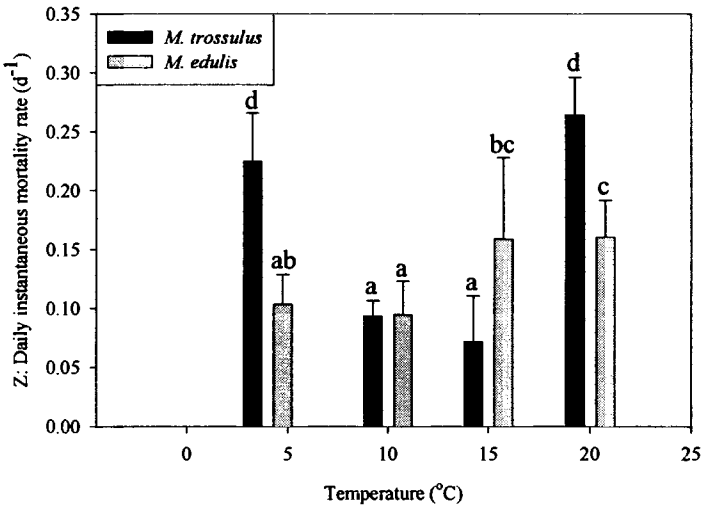

Fig. 6: Daily Instantaneous Mortality Rate. Mean daily instantaneous mortality rates (± 1 s.d.) of *M. edulis* and *M. trossulus* larvae at all four temperatures. Treatments that share the same letter are not significantly different (Tukey HSD multiple comparison test, experiment wise error of α=0.05).

Table 2: Results of the 2-way ANOVA of daily instantaneous mortality rates.

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
TEMPERATURE	0.163	3	0.054	53.073	0.000
SPECIES	0.034	1	0.034	33.730	0.000
TEMPxSPECIES	0.099	3	0.033	32.238	0.000
Error	0.071	69	0.001		

Growth

Larval sizes increased over time but the growth rates tended to decrease over time in all treatments (Figure 7). The asymptotic nature of the relationship between shell length and time was most pronounced at low temperatures.

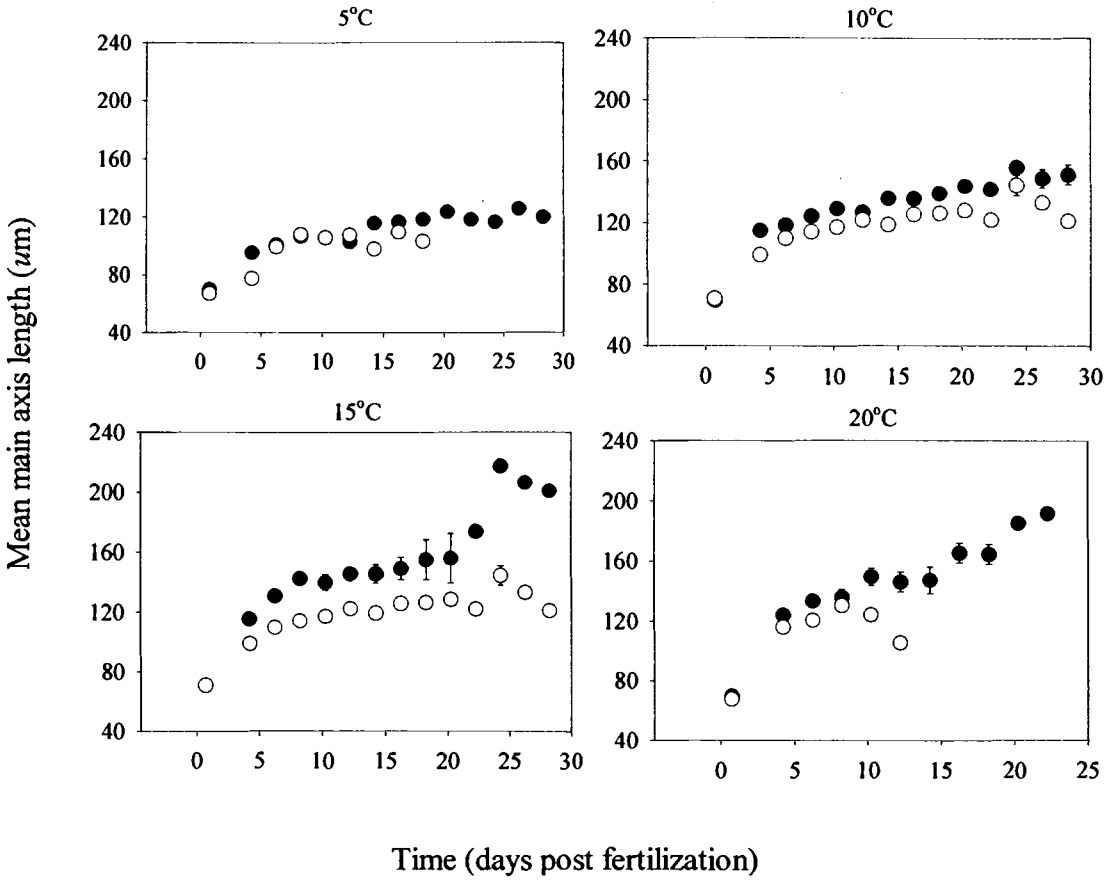

Fig. 7: Larval Growth. Mean shell length (± 1 S.E.) for *M. edulis* (●) and *M. trossulus* (○) larvae at 5, 10, 15, and 20°C.

Statistical analysis of initial lengths of larvae (just prior to exposure to experimental temperatures at 0.75 days post fertilization) revealed no significant differences due to species (F-ratio=0.992; df=1; p=0.323) or temperature (F-ratio=1.619; df=3; p=0.193). 36 hours post-exposure, substantial species differences in size were apparent (Figure 8; Table 3).

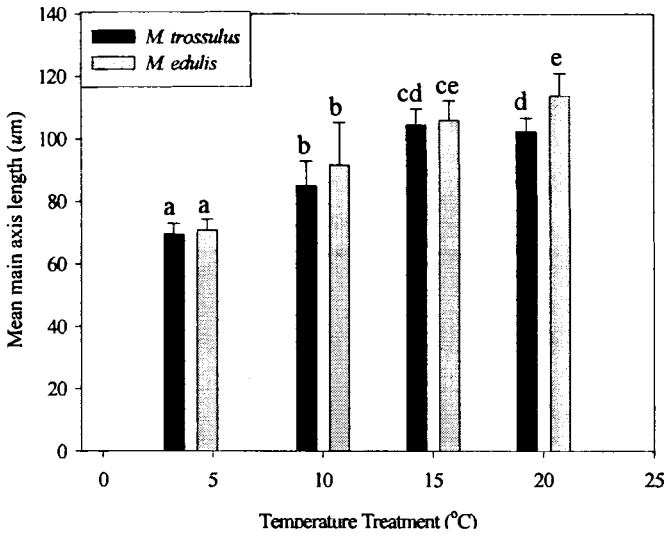

Fig. 8: Lengths 36 Hours Post Exposure. Mean shell length (±1 S.E.) of all treatment groups 36 hours post-treatment. Treatments that share the same letter are not significantly different (2-way ANOVA and Tukey HSD multiple comparison tests, experiment wise error of α=0.05).

Table 3: Results of the 2-way ANOVA of larval lengths 36 hours post exposure.

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
SPECIES	457.582	1	457.582	10.925	0.002
TEMPERATURE	17704.404	3	5901.468	140.898	0.000
SPECIESxTEMP.	310.426	3	103.475	2.470	0.070
Error	2680.623	64	41.885		

Pairwise comparisons (Appendix E) indicated that the significant species difference occurred at 20°C, where larval lengths were significantly larger for *M. edulis* than for *M. trossulus*. For both species, lengths at 36 hours post-exposure increased significantly (for all pairs) with increasing temperature until 15°C, with was no significant difference between lengths at the two highest temperatures.

Non-linear regressions provided two separate growth estimates, the initial growth rate (slope of the regression curve at time x=0) and the asymptotic maximal size (Appendix H). The r^2 values from the non-linear regressions of time and growth data indicated a fairly good fit of the regression model to data from each replicate. The r^2 values for *M. trossulus* ranged from 0.55 to 0.93, with a mean of 0.74. The r^2 values for *M. edulis* were similar, ranging from 0.53 to 0.95 with a mean r^2 of 0.79. In addition, 73 out of 80 regressions satisfied two or more of three tests for normality. Visual inspection of a scatter plot of the residuals versus the estimated values from the 2-way ANOVA of initial growth rates did not reveal any apparent trends; however, the Lillifors test for normality of the residuals resulted in a p-value slightly less than 0.05 and, for the asymptotic maximum shell length ANOVA, a p-value of 0.07.

For both species, there was a trend of increasing initial growth rates (regression initial slopes) with increasing temperature (Figure 9).

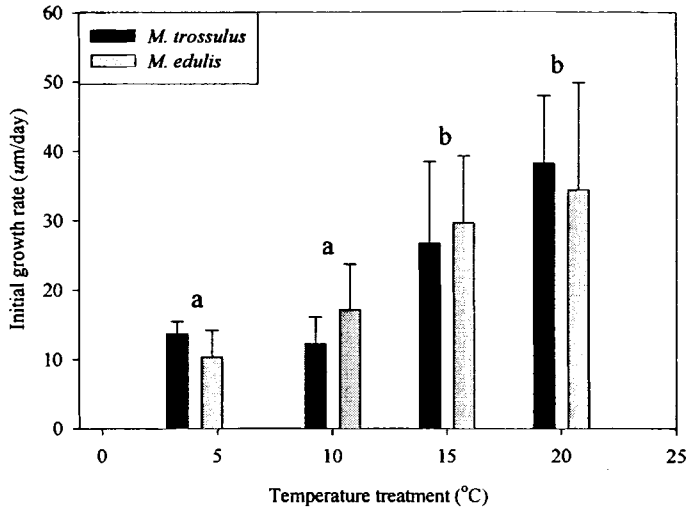

Fig. 9: Initial Growth Rate. Mean (±1 s.d.) of initial growth rates of all treatment groups. Initial growth rates correspond to the means of the slope of the non-linear length-time regression relationships when x=0. Treatments that share the same letter are not significantly different (2-way ANOVA and Tukey HSD multiple comparison tests, experiment wise error of α=0.05).

A two-factor ANOVA of initial growth rates (initial regression slopes) revealed significant differences among different temperatures (Table 4). Species had absolutely no effect on the initial growth rates of larvae and there was no significant interaction of temperature and species. Pairwise comparisons among temperature treatments (Appendix F) indicated that initial growth rates were lower at both 5 and 10°C than at either 15 or 20°C.

Table 4: Results of the 2-way ANOVA for initial growth rates (log transformed) of larvae of different treatment groups.

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
TEMPERATURE	9.949	3	3.316	22.550	0.000
SPECIES	0.001	1	0.001	0.005	0.946
SPECIESxTEMP.	1.020	3	0.340	2.311	0.086
Error	7.941	54	0.147		

Theoretical maximum larval shell lengths were estimated from the y-asymptote values of the equations from the non-linear regressions of shell length and time data. In general, maximum shell lengths were small for larvae reared at 5°C and tended to increase with increasing temperature (Figure 10).

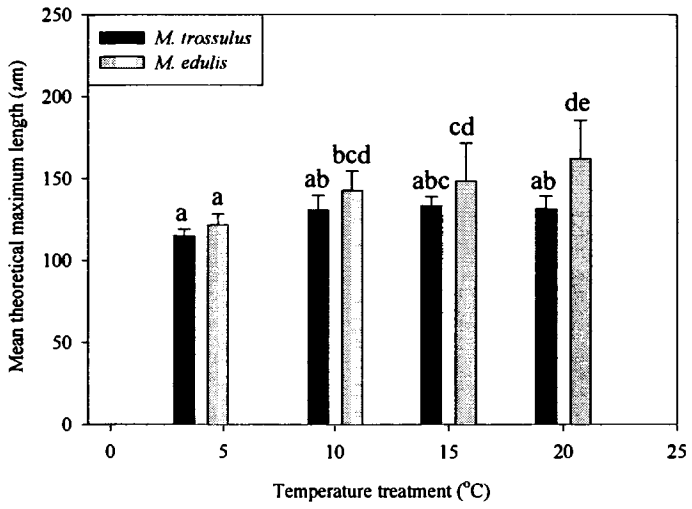

Fig. 10: Maximum Shell Length. Mean maximum shell lengths (±1 s.d.) of all treatment groups. The maximum lengths correspond to the y asymptotes of the non-linear length-time regressions. Treatments that share the same letter are not significantly different (Tukey HSD multiple comparison tests, experiment wise error of α=0.05).

Both species and temperature had a significant affect on asymptotic maximum shell lengths but there was no indication of a significant interaction between the two (Table 5).

Table 5: Results of the 2-way ANOVA for maximum shell lengths (y asymptotes of shell length-time regression) of larvae of different treatment groups.

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
TEMPERATURE	7041.195	3	2347.065	13.357	0.000
SPECIES	4561.517	1	4561.517	25.959	0.000
SPECIESxTEMP.	1108.801	3	369.391	2.102	0.109
Error	10718.801	61	175.718		

Pairwise comparisons (Appendix G) indicated that the significant species differences occurred at 20°C, with smaller *M. trossulus* than *M. edulis* larvae. *Mytilus edulis* larvae at 5°C were significantly smaller than larvae reared at the three higher temperatures but above 5°C there was no effect of temperature on maximum length. There was no significant effect of temperature on the maximum lengths of *M. trossulus* larvae.

Development

Analysis of percent deformity (Table 6, Appendix I) revealed significant species effects on deformity levels at 10°C and 15°C. At these intermediate temperatures, *M. trossulus* had a significantly higher percentage of deformed larvae than did *M. edulis*.

There was no difference in initial deformity levels of the different treatments at the time the larvae were distributed to the experimental system.

Table 6: The median percent deformity and median test results. T = test statistic for the median test. A significant result ($p<0.05$) indicates a significant species difference at that experimental temperature.

Temperature	*M. edulis* median	*M. trossulus* median	T	p-value
5	39.31	34.67	0.8	$p>0.25$
10	10.79	37.30	20	$p<0.001$
15	7.28	24.70	12.8	$p<0.001$
20	19.23	25.82	0.8	$p>0.25$

In all treatments, developmental stage tended to increase over time while developmental rate decreased with time (Figure 11). There were no differences among treatments in the initial developmental stage of larvae. While development rate slowed through time in all treatments, development still continued at 10, 15 and 20°C. At 5°C, however, development was completely arrested approximately 8 days post fertilization. At this temperature, no *M. trossulus* larvae and only 14 individual *M. edulis* larvae were observed to progress beyond the D veliger (straight-hinge / stage 3) development stage. These developmental trends parallel the general trends for larval growth.

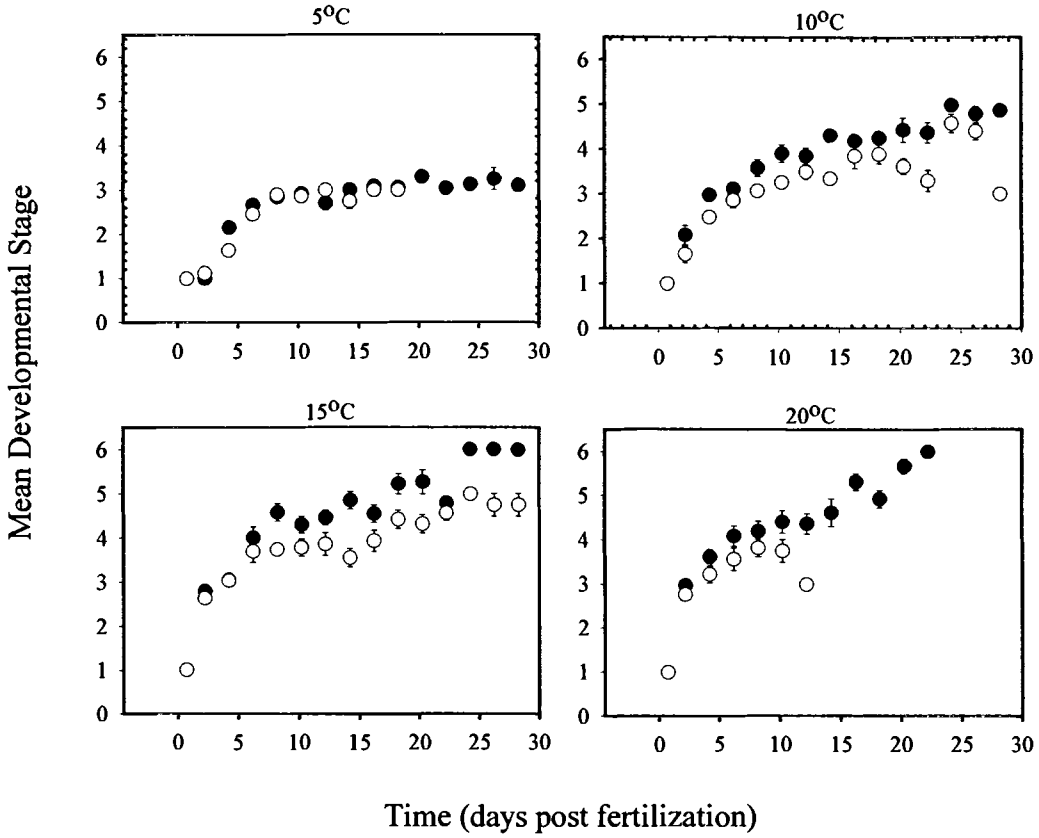

Fig. 11: Larval Development. Mean developmental stage (±1 S.E.) of *M. edulis* (●) and *M. trossulus* (○) at 5, 10, 15 and 20°C. In all four graphs, the first data point for the two species (stage 1) overlaps.

In all cases, there was a very high degree of positive correlation between developmental stage and larval shell length (Table 7). For this reason, separate statistical analyses are not presented for larval developmental characteristics.

Table 7: Spearman's coefficient for correlation between length and stage for *M. edulis* and *M. trossulus* larvae at 5, 10, 15, and 20° C.

Temperature	*M. edulis*	*M. trossulus*
5	0.865	0.887
10	0.938	0.922
15	0.946	0.914
20	0.948	0.929

Growth/Mortality Ratio

The response of a species to temperature may be viewed in terms of the ratio of growth and mortality rates at each temperature. Analysis by 2-way ANOVA revealed that there was a significant effect of temperature (F-ratio=10.037; df=3; p= 0.000) but not species (F-ratio=2.065; df=1;p=0.157) on the ratios of initial growth rates to instantaneous mortality rates. Pairwise comparisons (Appendix I) revealed that the significant differences in G/Z ratios occurred in more temperature pairs for *M. trossulus,* which significantly increased from 5 and 10 to 15°C and decreased from 15 to 20°C, than *M. edulis*, which significantly increased only from 5 to 20° (Figure 12). The ratios of growth to mortality for *M. edulis* did not differ significantly with temperature, indicating that growth and mortality had similar relationships with temperature.

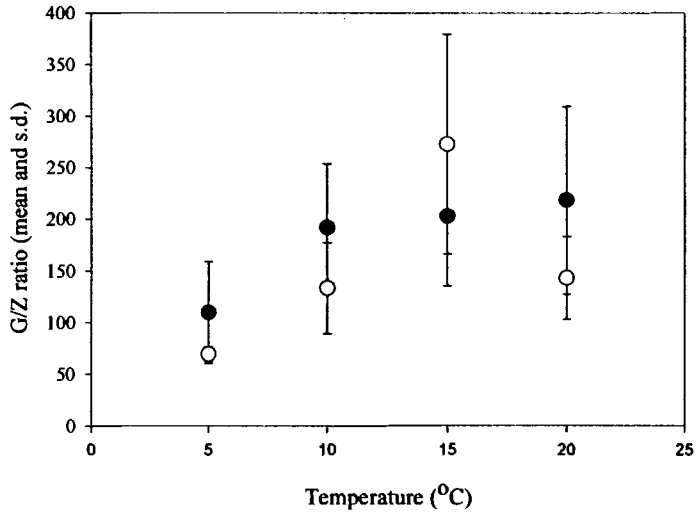

Fig. 12: Growth/Mortality Ratio. Mean ratio of initial growth rate/instantaneous mortality rate (±1 s.d.) of *M. edulis* (●) and *M. trossulus* (○) at 5, 10, 15 and 20°C.

Settlement

Very few settled juveniles were recorded at the conclusion of this experiment. No juveniles of either species were observed in any replicate culture beakers at 5°C. A single *M. edulis* juvenile was recorded in one beaker at 10°C; no *M. trossulus* juveniles were recorded at this temperature. At 15°C, four beakers contained settled *M. edulis* juveniles; two of these contained appreciable numbers (>5). Five 15°C beakers contained several (1-5) *M. trossulus* juveniles. At 20°C, several (1-5) *M. edulis* juveniles were observed in 5 beakers. No settled *M. trossulus* individuals were recorded in any replicate beakers at 20°C. Settled juveniles were in the range of 250-300μm with the following exception; one replicate beaker at 20°C contained two *M. edulis* juveniles in the range of 300-400μm.

DISCUSSION

This experiment examined the general hypothesis that the survival, growth and development of blue mussel larvae vary with respect to species and temperature. Growth in this experiment was somewhat slower than in other blue mussel larval studies. For example, Bayne (1965) found that after 30 days the average length of larvae at 11°C was roughly 200 μm and 250μm at 17°C compared to means in this experiment of approximately 150μm at 10°C and 200μm at 15°C after 30 days. The levels of mortality in this experiment tended to be high, although it is difficult to make direct comparisons to rates in the literature; the majority of studies of *M. edulis* larvae address growth and development (see Pechenik 1987), and the method of assessing and analyzing data varies among studies that do investigate mortality. The slow growth and high mortality rates in

this experiment could be attributed to nutritional or genetic (*e.g.* inbreeding) factors. Larval growth in this experiment was comparable to that observed in *M. edulis* larvae under conditions of low food ration (Pechenik et al. 1990), raising the possibility that algal paste may have lower nutritional value than live algal cultures used for feedings in other studies. However, all conditions except temperature and species were similar for all treatments, allowing for comparisons among them.

Settlement may have also had an impact on mortality and growth rates estimated in each replicate beaker. However, only *M. edulis* larvae at 15°C demonstrated appreciable amounts of settlement in any replicate beaker. For this treatment, settlement may have resulted in an overestimate of the overall larval mortality.

The survival results of this study were somewhat unexpected and enigmatic. Based on the global distribution of these two species, many researchers consider *M. trossulus* to be a cold-water species (Bates and Innes 1995); given this characterization, *M. trossulus* larvae might be expected to have greater or at the very least comparable tolerance of cold conditions than *M. edulis* larvae. On the contrary, *M. trossulus* larvae had higher mortality at the lowest experimental temperature. These results suggest that *M. edulis* is able to withstand the sub-optimal conditions at 5°C longer than *M. trossulus*, an observation consistent with the results of Bayne (1965), who found that *M. edulis* larvae are able to survive at 5°C. Temperature tolerances of larvae may not be correlated with species range, perhaps because variation in the timing of reproduction can compensate for effects of latitude on the development temperature of larvae. In fact, spawning of blue mussels in many locations in Newfoundland occurs in the temperature range of 10-15°C (Innes, pers. comm.).

One explanation of these survival results is that *Mytilus trossulus* larvae may be less tolerant of temperature extremes than *M. edulis*. Observed temperature-mortality relationships for the two species are shown in a simplified diagram below (Fig. 13). *Mytilus trossulus* has a U-shaped relationship, with higher mortality at the two extremes. In contrast, *M. edulis* has a more monotonic relationship, with generally increasing mortality with increasing temperature.

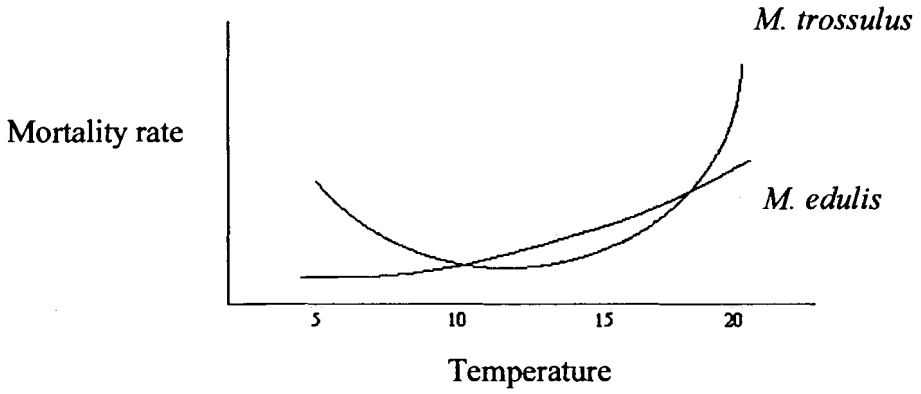

Fig. 13: Conceptual Model of Temperature-Mortality Relationships.

The temperature range used in this experiment extends beyond the typical range of temperatures encountered by summer-spawned blue mussel larvae along the Atlantic coast of the U.S. so that the relationships demonstrated in this study are relevant to the Gulf of Maine.

Another factor that might account for the high mortality of *M. trossulus* at temperature extremes is the potentially deleterious effect of temperature change. In this experiment, all fertilization and early development took place between 10 and 15°C for logistical reasons. Larvae reared at 5 and 20°C underwent greater temperature change (at the trochophore stage) when distributed to the experimental system than did those raised

at 10 and 15°C. The potential importance of changing conditions is emphasized by the fact that environmental conditions in nature often vary with diurnal or seasonal cycles or random events such as storms (Richmond and Woodin 1996). Effects of temperature change on blue mussel larvae may have important implications for the distribution of blue mussel species in the Gulf of Maine, where temperature varies on both temporal and spatial scales. Beaumont and Budd (1982) showed that *M. edulis* larvae could be raised for 2 months at 5°C and then grown to metamorphosis at 17°C with similar mortality rates and metamorphosis levels (although decreased growth rates) compared to larvae raised entirely at 17°C. The mortality results of this experiment raise the question of whether or not *M. trossulus* has a similar tolerance of temperature change

One final factor that should be taken into account is the significant difference in initial larval densities between species at the 5°C treatment. According to Loosanoff and Davis (1963), there is an inverse relationship between larval concentrations and growth in clam and oyster larvae even when food is not a limiting factor. Despite this caveat, it is unlikely that the high mortality of *M. trossulus* at this temperature can be solely attributed to initial larval concentrations. The initial stocking densities of *M. trossulus* at 5°C were still within the recommended range and the negative effects of crowding on survival of bivalve larvae are mainly observed at extremely high stocking densities (Loosanoff and Davis 1963). The increased excretory products and frequency of collisions associated with high density cultures (Loosanoff and Davis 1963) are most likely of reduced importance at 5°C, where observed larvae displayed limited swimming and feeding activity.

There was a relatively large amount of variation in larval sizes and stages among individuals of the same treatments. A high degree of individual variability in larvae is common (Loosanoff and Davis 1963; Pechenik 1987) and is often attributed to endogenous factors (Widdows 1991). Variation among individuals may account for the observation in some replicate beakers of small numbers of settled individuals when the majority of larvae did not settle.

The effects of temperature on growth appear to be a matter of rate. In general, it appears that growth and development rates increase with increasing temperature but that the effect tapers off at high temperatures. Initial growth rates and lengths 36 hours post-treatment increased from 10 to 15 and 20°C but not from 15 to 20°C. Bayne (1965) found that the growth rate of *M. edulis* larvae significantly increases from 10 to 18°C but not from 13 to 18°C and estimated that the maximum rate of larval growth occurs in the range of 16-22°C. The observed increase in initial growth rates (but not final sizes) with temperature leads to the intuitive conclusion that the energetic needs of larvae are higher at high temperatures. At 20°C, species significantly affected length at 36 hours post-treatment and maximum length. This suggests that the species length differences at this temperature were established early and lasted. It is interesting to view the size differences 36 hours post-treatment in light of early ontogenetic changes. Trochophore development is characterized by morphological differentiation with no growth; therefore, size differences at the 2nd sample provide an indication of temperature and species effects on the rate that larvae progressed from early morphological differentiation to an actual growth phase.

Growth and developmental differences between the species in this experiment manifested themselves largely after larvae were moving and feeding actively. Species had no effect on initial growth rate; at 10,15, and 20°C, the initial development rates appear to be similar between species but the maximum stage appears to be slightly lower in *M. trossulus*. These results are interesting when viewed in light of the biology of different stages. In the early cleavage stages and trochophore stage, developing mytilids still utilize the limited energy reserves from the egg. Differences in larval viability, and therefore species differences at some temperatures, might become more pronounced as larvae face the demands of actively swimming and feeding. McEdward (1985) showed that temperature variation has a greater effect on the feeding pluteus stages than the early non-feeding stages of echinoid larvae. Deformities or physiological stress may have a greater effect as the larva becomes independent of endogenous energy reserves.

By examining the ratio of growth and mortality, it is possible to summarize the impact of temperature on these two parameters. Marine fish larvae from high and low latitudes have similar G/Z ratios because their growth and mortality rates have similar relationships to temperature (Houde 1989). Although highly variable, the ratios of growth to mortality rates suggest that the relationship of growth and mortality may vary with temperature for *M. trossulus* larvae more than for *M. edulis*. Above 10°C, both growth and mortality increase with temperature at similar rates for *M. edulis* so that the growth to mortality ratio remains relatively constant.

An overall picture of the viability of *M. edulis* and *M. trossulus* larvae at the experimental temperatures can be garnered from a synthesis of the survival, growth and development results. Larvae of both species appeared to be viable at the two

intermediate temperatures, with slightly higher initial growth and development rates at 15°C. Taken together, the mortality, growth and development results suggest that 5°C was below the optimum temperature range for larvae of both species. At 5°C, larvae did not progress beyond the D stage or grow larger than the range of 110-120 μm. Observations made on live samples during the course of the experiment suggested that larvae had limited feeding and swimming activity at 5 °C. These results are similar to the findings of Bayne (1965), who showed that larval growth at 5°C ceased after three days, and Brenko and Calabrese (1969), who found that mussel larvae can survive a wider range of temperatures (5-25°C) than is satisfactory for growth (15-20°C). With significantly higher mortality and smaller (36 hours post-treatment and maximum) size, *M. trossulus* larvae appear to be less viable than *M. edulis* larvae at 20°C. *Mytilus trossulus* larvae also reached a lower maximum developmental stage at this temperature. Given that no settled individuals were observed, the drop-off characteristic of the *M. trossulus* growth and development curves at 20°C is perhaps due to selective mortality of the fastest growing/developing individuals. The sharp decline from 15 to 20°C of the *M. trossulus* G /Z ratio is strong evidence that *M. trossulus* larvae may experience thermal stress at 20°C.

Because this experiment was not designed to quantify settlement, it is unknown whether the observed pre-settlement differences among treatments in this experiment result in differential recruitment. Although growth and development in this experiment were highly correlated, temperature significantly affects the average size of competent *M. edulis* larvae (Pechenik et al. 1990). A comparative study of the two species using histological techniques found that *M. trossulus* adults produce ova of a smaller size than

M. edulis (Maloy, unpublished data). This raises the possibility that a smaller larval size in *M. trossulus* may be a simple species difference in size, perhaps accompanied by smaller size at settlement as well. In this study, however, there were no size differences between species for trochophore-stage larvae, and the observed species differences in size only occurred at 20°C. These observations suggest that the size differences in this study are due to differential temperature effects rather than a fundamental size difference between the species.

The results of this study provide the first indication that the physiological ecology of blue mussel larvae is not identical for *M. edulis* and *M. trossulus*. The focus of this study was to assess whether temperature affects larvae of *M. trossulus* and *M. edulis* differently, and to discuss the results in terms of the potential effects of pre-settlement factors on species ranges. Temperature varies in the Gulf of Maine on both temporal and spatial scales, with several degrees Celsius difference between the eastern and central coasts of Maine during the time of year when larvae are found in the water column. Although the differences in larval mortality, growth and development rates of the two species are not large, changes of as little as 5% day^{-1} may ultimately result in order-of-magnitude differences in recruitment (Houde 1989). If the findings of this experiment can be applied to a natural setting, they suggest that differential tolerance to elevated temperature may be one important factor controlling the distributions of *M. edulis* and *M. trossulus* in the Gulf of Maine. In addition, the results are consistent with previous suggestions (Brown et al. 1992, Bayne 1976) that pre-settlement factors may have important ecological effects for species range.

It is important to consider the complexity of the interactions of environmental factors as well as genotype-environment interactions during any application of this experiment's findings to a discussion of larval biology in a natural setting. In contrast to many studies that investigated the combined effects of salinity and temperature (Berry and Hunt 1980; Brenko and Calabrese 1969; Brown et al. 1992; Chen and Chen 1993; Lough 1974), this experiment assessed only the effects of temperature on mussel larvae. In the wild, there are most likely complex interactions between temperature, other physical factors (e.g. salinity, density, light), and biotic factors (e.g. predation, food abundance); these interactions most likely affect larval survival and growth (Bayne 1983; Pechenik 1987). Genetic variation is also a factor that may have affected the results of this study. It is unknown whether the genetic diversity of larvae that were batch spawned and fertilized from a limited number of parents represents the genetic makeup of a cohort of wild mussel larvae within or beyond the Gulf of Maine. The small number of parents employed in the study raises the possibility that inbreeding may have contributed to the high mortality and low growth rates seen in this experiment. Further, the influence of temperature is not fixed for any given species, but varies among different populations (Widdows et al. 1984; Newell 1989). For example, Bayne (1965) showed that the growth rates of *M. edulis* larvae with parents from a moderate temperature environment did not decline at temperatures up to 20°C but that larvae with parents from a low temperature environment had reduced growth rates above 16°C. Despite these caveats, laboratory experiments such as this one are valuable in that they can begin to elucidate general trends by simplifying the complexity inherent in a natural setting.

The 20°C results of this experiment may shed some light on the results of an experiment by Freeman et al. (1994) that assessed growth and survival of pure *M. edulis* and *M. trossulus* as well as hybrid larvae using a culture temperature of 20°C. They observed that while some percentage of *M. edulis* and hybrid larvae survived to metamorphosis, the entire set of pure *M. trossulus* larvae failed to reach metamorphosis and experienced total mortality within 22 days post fertilization. Freeman et al. (1994) made no mention of the potential effects of temperature in their study but instead attributed the high mortality of *M. trossulus* larvae to bacterial and ciliate infections. However, mortality of mussel larvae at high temperatures may be a result of reduced viability of the larvae due to sub-lethal thermal stress combined with an increased amount of bacteria and bacterial toxins (Brenko and Calabrese 1969). In light of the findings of this experiment, temperature effects should be taken into account in any evaluation of the results of Freeman et al. (1994).

There may be practical applications if *M. trossulus* larvae are truly not viable at 20°C, as the results of this study together with Freeman et al. (1994) suggest. Differential larval survival at this temperature may provide an exploitable biological difference between the two species. Such a difference is sought after by the mussel aquaculture industry in order to select for the more favored species, *M. edulis* (Freeman et al. 1994).

More importantly, the results of this experiment suggest several potential, non-exclusive mechanisms that may limit the range of *M. trossulus* in the Gulf of Maine. Both steady state and fluctuating temperature conditions may have differential effects on blue mussel larvae of *M. edulis* and *M. trossulus* that may affect their geographic ranges. For example, the high mortality of *M. trossulus* larvae at 20°C suggests that *M. trossulus*

larvae may have a slightly lower upper thermal limit than *M. edulis,* indicating that temperature-induced larval mortality may be a straightforward mechanism limiting southern range expansion for *M. trossulus*.

This explanation does not clearly account for the exact location of the current range limit of *M. trossulus* in the Gulf of Maine. In July, August and September, when larvae are potentially in the water column following a mid-summer spawning event (Maloy, unpublished data), the temperatures on either side of the *M. trossulus* distribution limit are in the range of 10-15°C (Appendix A). According to the results of this experiment, *M. trossulus* larvae are able to survive within this temperature range. However, the critical temperature for an organism with respect to mortality may be lower in the wild than in the laboratory due to sub-lethal thermal stress and the interaction of different environmental variables (Incze et al. 1980).

Temperatures of 20°C along coastal Maine are only found in shallow water for limited time periods. For example, shallow water temperatures of 22°C were recorded in mid-July 2000 at the Darling Marine Center. This raises the question of whether pulses of warm water affect the larval mortality of *M. trossulus* similarly to steady-state conditions. Any larvae that disperse from eastern to central or southern Maine would experience a temperature change of several degrees (Appendix A). Therefore, larval intolerance to temperature change could limit *M. trossulus* larvae from colonizing central and southern Maine. The effects of both macro- and micro-scale fluctuations in temperature on *M. trossulus* larvae would be an interesting topic for future investigation.

While mortality is a straightforward factor that affects a larva's ability to reach settlement, growth and development are more subtle factors that affect population

ecology. With regard to temperature, these characteristics are intricately related to larval survival as well as ability to settle and recruit in the wild. In this experiment, mussel larvae at 5°C survived but did not continue to grow or develop after a few days. Dependent on temperature and other physical factors, the larval duration of blue mussels can last between three weeks and three months (Widdows 1991). Under extreme circumstances, the pelagic phase of *M. edulis* larvae can extend past six months (Lane et al. 1985). In addition, Bayne (1965) found that pediveliger larvae are able to delay metamorphosis for up to 40 days at 10°C. In contrast, delay of metamorphosis at 20°C lasts 2 days. The long development time and delay of metamorphosis at low temperatures may have ecological implications for blue mussels by allowing for greater larval dispersal in the colder, more northern part of their range. Widdows (1991) points out that although the larval duration can be extended in theory, mortality due to starvation, predation, and other factors can be high (0.1-0.2/day) and that any factor, such as temperature, that lengthens the development time therefore has a major effect on survival. In light of these arguments, the ability of *M. edulis* larvae to delay metamorphosis for long periods (Bayne 1965; Beaumont and Budd 1982) may not be relevant outside the laboratory. Development and growth at different temperatures may therefore be as important as outright survival in terms of species range.

These arguments have interesting implications for the distribution of *M. trossulus* given the recent observations by Maloy (unpublished data) that spawning of blue mussels in eastern Maine does not appear to occur in a single peak. While spawning primarily occurs in the summer, apparent secondary peaks in the gametogenic cycle of these species may indicate that the reproductive phase extends into the fall. In the fall, the

EMCC travels entirely along the coast of Maine and therefore would not pose a barrier to dispersal of fall-spawned larvae. However, temperatures along the coast of Maine are near 10°C in the fall and quickly drop to 5°C or less (appendix A). In light of the arguments made by Widdows (1991) and the 5°C findings of this experiment (slow developmental rates of larvae of both species), it is unlikely that fall-spawned larvae (if they exist) ever reach settlement.

Sub-lethal thermal stress in both larvae and adults can have important ecological effects (Newell 1989; Incze et al. 1980; Karouna-Renier 1999). The high levels of deformity seen in *M. trossulus* larvae in this experiment may be an indication of sub-lethal stress. Abnormality of larvae is often not an important topic in research of bivalve larvae because it is a common practice to discard small and misshapen larvae. A subtle factor that could account for the high percentage of deformed *M. trossulus* larvae in this experiment as well as potentially limit the distribution of *M. trossulus* in the Gulf of Maine is the effect of temperature on the reproductive condition of adults and resulting condition of offspring. An extreme reduction in gamete viability is sometimes seen in individuals comprising "pseudopopulations," populations established by larvae settling continually outside of the optimal depth or geographic range limits of the species. Pseudopopulations have been observed for a number of marine invertebrate species, including *M. edulis* (Wells and Gray 1960; Miliekovsky 1971). It is possible that environmental conditions in eastern Maine present sub-lethal stresses that negatively affect the reproductive condition of *M. trossulus* adults, including those used as broodstock in this experiment. The offspring of stressed *M. edulis* adults (held at high temperatures with low food rations) have a higher percentage of abnormal early

development (Bayne 1972) and lower growth rates (Bayne et al. 1975) than the offspring of non-stressed adults. The high levels of deformed *M. trossulus* larvae observed in this experiment and the smaller ova size for *M. trossulus* than *M. edulis* (Maloy, unpublished data) are consistent with this hypothesis, but a comparison of the reproductive condition and larval viability of *M. trossulus* from populations at its southern distributional limit versus farther north is necessary to directly test this hypothesis.

A comparative study of nutrition, metabolic demand, and molecular/biochemical stress indicators of individual larvae could reveal more subtle effects of sub-lethal thermal stress than this study was able to detect. Using a variety of techniques, researchers have assessed sub-lethal stress in invertebrate larvae at the organismal (*e.g.* respiration rate, Sprung 1984; Marsh et al. 1999), cellular (*e.g.* electron transport system activity, McEdward 1985; mitochondrial density, Marsh et al. 1999) and molecular (*e.g.* heat shock protein expression, Carroll 1996) levels. In this experiment, as in many other studies of bivalve larvae, the length of the larval shell was used as a measure of growth. However, larval mass and shell length are not tightly coupled (Pechenik 1987; Widdows 1991). Nile Red is a fluorescent lipid specific stain used for analysis of larvae in a non-harmful way (Jackson 1993); this technique would facilitate the assessment of larval nutrition and growth over time.

The relative importance of larval dispersal versus temperature effects is a topic best addressed by a combined laboratory and field study approach. Future study of the differential settlement patterns of these two species along coastal Maine would shed light on the results of this study and help to clarify whether larval (as proposed here) or post-settlement processes control the distribution of *M. trossulus* in this region. With the aid

of new molecular techniques used to identify the two species (Rawson et al. 1996), a settlement study of this type would be relatively straightforward.

In conclusion, temperature affects larvae of *M. edulis* and *M. trossulus* in different but complex and often subtle ways. The results of this study suggest that temperature effects on larvae may be a factor limiting the range of blue mussel species in the Gulf of Maine. The mechanisms, namely temperature (steady state versus changing) or maternal stress, by which this may happen are questions left to future research.

The results of this study raise the point that the larval mortality hypothesis for range limitation is potentially important for benthic marine invertebrates. Larvae are typically less tolerant than adults of physical extremes and changing conditions. With a few notable exceptions (*e.g.* rafting and human intervention), range expansion by benthic marine invertebrates depends upon the successful development and subsequent settlement of larvae outside the current species range. Taken together, these points suggest that the effect of larval mortality on species range should be raised from the "sub-hypothesis" level (Gaines et al. 1997) and given equal consideration to the dispersal barrier and adult mortality hypotheses.

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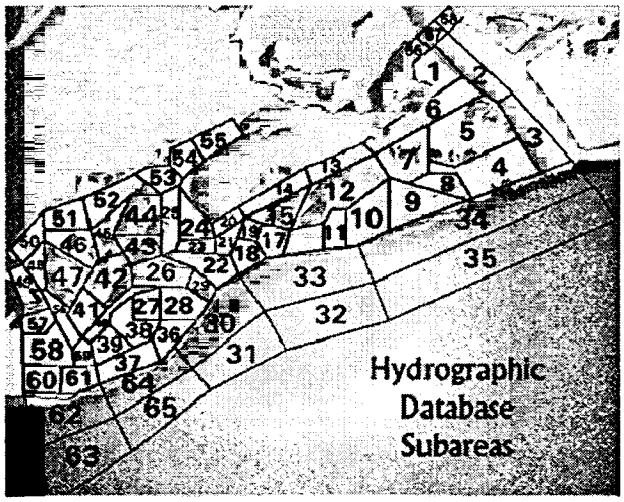
APPENDIX A

DFO TEMPERATURE DATA FROM COASTAL GULF OF MAINE

	S. Maine		Platts Basin		central Maine		S.W. Fundy		central Fundy		N.E. Fundy	
	DFO area 50		DFO area 51		DFO area 52		DFO area 53		DFO area 54		DFO area 55	
	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
Jan.	4.29	1.02	4.37	1.17	4.78	1	4.73	1.35	4.97	1.15	3.38	1.06
Feb.	2.97	0.94	3.3	0.84	3.55	1.19	4.08	0.85	3.41	1.19	1.83	0.91
Mar.	2.76	1	2.47	0.71	3.05	0.98	3.08	1	3.07	0.69	2.12	0.87
Apr.	4.91	1.14	4.38	0.98	4.06	0.86	4.15	0.95	3.6	0.9	2.71	0.64
May	8.17	1.24	7.43	1.36	6.09	0.97	5.6	0.96	5.36	1.05	5.08	1.23
June	12.2	1.39	11.4	1.6	9.07	1.63	8.57	1.74	8.29	1.61	7.73	0.79
July	15.16	1.86	14.97	1.38	12.44	1.42	10.42	1.23	10.8	1.02	10.55	0.89
Aug.	16.21	1.25	15.24	1.81	13.03	1.7	11.57	1.44	12.4	1.08	12.68	0.9
Sept.	14.83	1.65	13.68	1.12	12.49	1.21	11.62	1.27	11.89	1.14	12.15	0.83
Oct.	11.55	1.02	11.33	0.95	10.62	0.84	10.73	0.88	10.7	0.85	11.3	0.84
Nov.	9.55	1.01	9.43	1.05	9.6	0.94	9.65	0.92	9.4	1.02	9.85	0.81
Dec.	6.9	1.38	7.4	1.07	7.73	1.17	7.51	1.11	7.15	1.22	7.8	0.47

This data, representing surface temperatures, was borrowed from a Canada's Department of Fisheries and Oceans website of ocean science data for the Scotian Shelf/Gulf of Maine,
http://dfomr.mar.dfo-mpo.gc.ca/science/ocean/scotia/ssmap.html.
The above regions correspond to the numbered regions on the following map:



APPENDIX B

PAIRWISE COMPARISON PROBABILITIES OF INITIAL LARVAL DENSITIES

	5T	5E	10T	10E	15T	15E	20T	20E
5T	1.000							
5E	**0.027**	1.000						
10T	0.537	0.847	1.000					
10E	0.066	1.000	0.958	1.000				
15T	0.657	0.751	1.000	0.909	1.000			
15E	0.009	1.000	0.637	0.997	0.517	1.000		
20T	0.999	0.127	0.886	0.253	0.944	0.053	1.000	
20E	0.267	0.978	1.000	0.998	0.998	0.886	0.637	1.000

Matrix of pairwise comparison probabilities: Tukey HSD multiple comparisons for initial larval densities. In bold is the only probability that indicates a significant species difference at the same temperature or temperature difference for the same species.

APPENDIX C

REGRESSION RESULTS (TIME AND ln LARVAL CONCENTRATION)

	5T			5E		
replicate	a (-z)	b	R^2	a (-z)	b	R^2
1	-0.2022	3.6469	0.9509	-0.11927	2.938	0.71978
2	-0.2006	3.3204	0.86404	-0.1326	3.075	0.8689
3	-0.1762	3.38431	0.79922	-0.11859	2.56656	0.70969
4	-0.1885	3.38375	0.850445	-0.0645	2.0367	0.4072
5	-0.20176	3.82219	0.979	-0.0942	3.1389	0.8191
6	-0.22877	3.6814	0.73184	-0.0809	2.69098	0.67239
7	-0.23868	3.90929	0.85484	-0.1073	3.01358	0.64521
8	-0.2882	4.44768	0.92503	-0.124	2.96334	0.83776
9	-0.225	3.84799	0.88192	-0.1263	3.3421	0.8966
10	-0.29922	4.2773	0.8423	-0.0685	2.964	0.6732
	10T			10E		
replicate	a	b	R^2	a	b	R^2
1	-0.0899	2.8516	0.54987	-0.0779	3.0243	0.687017
2	-0.0804	2.84684	0.653446	-0.13619	3.439196	0.802756
3	-0.09867	3.10416	0.7823	-0.10566	3.1688	0.76327
4	-0.10312	2.949418	0.76872	-0.0615	3.060576	0.62732
5	-0.06318	2.4189	0.6075	-0.1298	3.5943	0.9019
6	-0.1018	2.9944	0.901271	-0.072	3.2162	0.713
7	-0.10463	3.038	0.71013	-0.065	2.9599	0.4157
8	-0.09189	3.00224	0.79538	-0.0735	3.06889	0.6886
9	-0.0957	2.8911	0.6718	-0.0995	2.767	0.584899
10	-0.10532	2.9156	0.73314	-0.12545	3.52254	0.850959
	15T			15E		
replicate	a	b	R^2	a	b	R^2
1	-0.0899	2.9672	0.75831	-0.0608	2.948	0.7478
2	-0.07259	2.58209	0.52388	-0.16687	3.597	0.69866
3	-0.10717	2.87011	0.71271	-0.1857	3.44966	0.7543
4	-0.08867	2.69718	0.761	-0.1087	2.66473	0.6017
5	-0.094	2.922473	0.72605	-0.2943	4.003	0.6576
6	-0.08769	2.6894	0.6772	-0.14903	3.15388	0.53215
7	-0.0937	2.83656	0.81185	-0.104	2.67297	0.35302
8	-0.0836	2.6349	0.6374	-0.17305	3.4171	0.65604
9				-0.2356	3.53153	0.8037
10				-0.1118	3.18369	0.47015

APPENDIX C

REGRESSION RESULTS (TIME AND ln LARVAL CONCENTRATION)

	20T			20E		
replicate	a (-z)	b	R^2	a (-z)	b	R^2
1	-0.244	3.378	0.6086	-0.1675	3.326	0.896
2	-0.23669	3.24516	0.7637	-0.1209	3.0699	0.7309
3	-0.25283	3.28984	0.6182	-0.20489	3.6555	0.94307
4	-0.30679	3.4492	0.94626	-0.146	3.3033	0.932
5	-0.2699	3.52429	0.91375	-0.1394	3.41109	0.8868
6	-0.2732	3.5543	0.96775	-0.199	3.661	0.72883
7	-0.20371	2.56098	0.7198	-0.11845	3.2798	0.6898
8	-0.30555	3.6188	0.86579	-0.1466	3.53095	0.8286
9	-0.26146	3.5836	0.88141	-0.19285	3.3211	0.7591
10	-0.28932	3.8419	0.8988	-0.168	3.202	0.8561

APPENDIX D

PAIRWISE COMPARISON PROBABILITIES OF MORTALITY RATES

	5T	5E	10T	10E	15T	15E	20T	20E
5T	1.000							
5E	**0.000**	1.000						
10T	0.000	0.996	1.000					
10E	0.000	0.998	1.000	1.000				
15T	0.000	0.983	1.000	1.000	1.000			
15E	0.000	0.127	0.021	0.027	**0.018**	1.000		
20T	0.123	0.000	0.000	0.000	0.000	0.000	1.000	
20E	0.001	0.004	0.000	0.000	0.000	0.951	**0.000**	1.000

Matrix of pairwise comparison probabilities: Tukey HSD multiple comparisons for instantaneous mortality (z). In bold are probabilities that indicate that the two species are significantly different at the same temperature.

APPENDIX E

PAIRWISE COMPARISON PROBABILITIES OF LENGTHS 36 HOURS POST-EXPOSURE

	5T	5E	10T	10E	15T	15E	20T	20E
5T	1.000							
5E	1.000	1.000						
10T	0.000	0.000	1.000					
10E	0.000	0.000	0.565	1.000				
15T	0.000	0.000	0.000	0.012	1.000			
15E	0.000	0.000	0.000	0.003	1.000	1.000		
20T	0.000	0.000	0.000	0.085	0.997	0.942	1.000	
20E	0.000	0.000	0.000	0.000	0.057	0.170	**0.014**	1.000

P-values for pairwise comparison matrix of Tukey's HSD test for larval length at count 2 (2.25 days post fertilization and 36 hours post-exposure). In bold are probabilities that indicate that the two species are significantly different at the same temperature.

APPENDIX F

PAIRWISE COMPARISON PROBABILITIES (AMONG TEMPERATURES) OF INITIAL GROWTH RATES

	5	10	15	20
5	1.000			
10	0.623	1.000		
15	**0.000**	**0.000**	1.000	
20	**0.000**	**0.000**	0.286	1.000

P-values for pairwise comparison matrix of Tukey's HSD test for (log-transformed) initial growth rate of larvae. In bold are probabilities indicating significant differences.

APPENDIX G

PAIRWISE COMPARISON PROBABILITIES OF MAXIMUM LENGTHS

	5T	5E	10T	10E	15T	15E	20T	20E
5T	1.000							
5E	0.932	1.000						
10T	0.258	0.880	1.000					
10E	0.002	0.025	0.455	1.000				
15T	0.114	0.610	0.999	0.862	1.000			
15E	0.000	0.001	0.046	0.950	0.243	1.000		
20T	0.308	0.891	1.000	0.643	1.000	0.118	1.000	
20E	0.000	0.000	0.000	0.084	0.003	0.547	**0.001**	1.000

P-values for pairwise comparison matrix of Tukey's HSD test. In bold are probabilities that indicate that the two species are significantly different at the same temperature.

APPENDIX H

REGRESSION RESULTS (TIME AND SHELL LENGTH)

	5T					
replicate	yo	a	b	initial rate	max length	R^2
1	48.2827	63.863	0.2068	13.20687	112.1457	0.776
2	39.9737	73.5721	0.2134	15.70029	113.5458	0.773
3	57.5406	62.7759			120.3165	0.709
4	55.1673	58.9726	0.2097	12.36655	114.1399	0.742
5	61.8609	48.5699			110.4308	0.567
6	53.4292	67.9023			121.3315	0.693
7	58.3116	55.8958			114.2074	0.805
8						
9						
10						
ANOVA N				3	7	
	5E					
replicate	yo	a	b	initial rate	max length	R^2
1	68.9634	56.6228	0.0998	5.650955	125.5862	0.692
2	62.9102	56.5953			119.5055	0.573
3	54.7639	66.315	0.2264	15.01372	121.0789	0.834
4	58.0905	57.5682	0.2257	12.99314	115.6587	0.821
5	61.8835	70.7995	0.104	7.363148	132.683	0.701
6	62.6189	56.693	0.1239	7.024263	119.3119	0.711
7	49.6746	60.248	0.2635	15.87535	109.9226	0.623
8	57.4418	66.9433	0.1589	10.63729	124.3851	0.867
9	60.9896	72.7985	0.1167	8.495585	133.7881	0.835
10	61.8293	65.4913	0.1549	10.1446	127.3206	0.79
ANOVA N				9	10	

Only significant (p</=0.05) parameters are shown here. Non-significant parameters were not used in graphs or further statistical analyses.

APPENDIX H

REGRESSION RESULTS (TIME AND SHELL LENGTH)

	10T					
replicate	yo	a	b	initial rate	max length	R^2
1	67.1137	57.6724	0.1999	11.52871	124.7861	0.66
2	57.5725	67.4239	0.2199	14.82652	124.9964	0.716
3	74.7033	75.3131	0.0822	6.190737	150.0164	0.756
4	64.5647	59.2183	0.181	10.71851	123.783	0.789
5	58.2416	68.5726	0.1735	11.89735	126.8142	0.726
6	64.0417	76.9067	0.1472	11.32067	140.9484	0.77
7	62.8617	62.6198	0.2057	12.88089	125.4815	0.73
8	60.182	69.6438	0.1544	10.753	129.8258	0.783
9	63.6939	73.8133	0.1479	10.91699	137.5072	0.772
10	51.8576	72.5978	0.2929	21.2639	124.4554	0.796
ANOVA N				10	10	
	10E					
replicate	yo	a	b	initial rate	max length	R^2
1	67.2544	83.4515	0.1589	13.26044	150.7059	0.651
2	47.012	81.8792	0.3928	32.16215	128.8912	0.91
3	48.4174	88.3134	0.2325	20.53287	136.7308	0.83
4	58.7537	94.6085	0.1725	16.31997	153.3622	0.748
5	60.4179	84.3721	0.1401	11.82053	144.79	0.786
6	69.5282	70.9167	0.2043	14.48828	140.4449	0.815
7	61.5426	70.44	0.2759	19.4344	131.9826	0.743
8	70.9032	94.2102	0.1054	9.929755	165.1134	0.67
9	61.9063	82.9383	0.1949	16.16467	144.8446	0.781
10	53.6516	79.9064	0.26	20.77566	133.558	0.831
ANOVA N				10	10	

Only significant ($p</=0.05$) parameters are shown here. Non-significant parameters were not used in graphs or further statistical analyses.

APPENDIX H

REGRESSION RESULTS (TIME AND SHELL LENGTH)

	15T					
replicate	yo	a	b	initial rate	max length	R^2
1	72.448	65.4967	0.1527	10.00135	137.9447	0.586
2	66.318	70.432	0.2028	14.28361	136.75	0.807
3	93.2382	46.9029			140.1411	0.492
4	50.9711	76.811	0.3751	28.81181	127.7821	0.723
5	61.4356	80.923	0.2959	23.94512	142.3586	0.673
6	54.2044	76.6149	0.4183	32.04801	130.8193	0.825
7	50.0732	77.5447	0.4419	34.267	127.6179	0.676
8	43.9813	87.4666	0.5002	43.75079	131.4479	0.546
9						0.817
10						
ANOVA N				7	8	
	15E					
replicate	yo	a	b	initial rate	max length	R^2
1	67.8087	138.0447	0.1059	14.61893	205.8534	0.829
2	47.1096	86.9976	0.3759	32.7024	134.1072	0.838
3	43.4176	92.8371	0.4612	42.81647	136.2547	0.858
4	67.1774	75.5196	0.2264	17.09764	142.697	0.779
5	46.6008	90.1721	0.4531	40.85698	136.7729	0.933
6	55.7314	86.529	0.3101	26.83264	142.2604	0.525
7	45.4774	116.3223	0.3065	35.65278	161.7997	0.855
8	48.5828	84.3532	0.3236	27.2967	132.936	0.87
9	51.0035	109.5833	0.2645	28.98478	160.5868	0.95
10	54.0179	89.4755	0.3321	29.71481	143.4934	0.875
ANOVA N				10	10	

Only significant (p</=0.05) parameters are shown here. Non-significant parameters were not used in graphs or further statistical analyses.

APPENDIX H

REGRESSION RESULTS (TIME AND SHELL LENGTH)

	20T					
replicate	yo	a	b	initial rate	max length	R^2
1	54.4417	74.9561			129.3978	0.76
2	37.0433	89.7742	0.4868	43.70208	126.8175	0.68
3	52.4873	70.8369	0.4947	35.04301	123.3242	0.812
4	37.564	86.3461	0.6161	53.19783	123.9101	0.757
5	46.361	98.4521	0.2982	29.35842	144.8131	0.93
6	48.5116	89.5572	0.3033	27.1627	138.0688	0.895
7	44.0576	90.0303	0.4556	41.0178	134.0879	0.827
8						0.859
9						
10						
ANOVA N				6	7	
	20E					
replicate	yo	a	b	initial rate	max length	R^2
1	43.096	95.8876	0.4624	44.33843	138.9836	0.844
2	76.2689	119.3104	0.1012	12.07421	195.5793	0.778
3	40.6571	92.0649	0.5952	54.79703	132.722	0.761
4	67.9882	104.0121	0.1445	15.02975	172.0003	0.78
5	46.6162	112.2311	0.3351	37.60864	158.8473	0.769
6	40.5515	144.4352	0.257	37.11985	184.9867	0.923
7	45.6754	106.4459	0.3748	39.89592	152.1213	0.788
8						
9						
10						
ANOVA N				7	7	

Only significant (p</=0.05) parameters are shown here. Non-significant parameters were not used in graphs or further statistical analyses.

APPENDIX I

PERCENT DEFORMED LARVAE (ALL REPLICATES)

	5T	5E	10T	10E	15T	15E	20T	20E
1	39.13043	47.61905	52.5	11.36364	18.91892	3.636364	28.57143	23.07692
2	40	50	52.38095	5.714286	24.39024	7.142857	44.44444	20.51282
3	27.27273	11.76471	48.78049	10.25641	15.78947	20.83333	14.28571	16.66667
4	36	46.15385	40.625	11.32075	22.22222	11.11111	23.07692	24.13793
5	55.55556	36.95652	39.47368	14.89362	26.31579	0	40	17.94872
6	33.33333	61.29032	23.52941	9.302326	35.71429	6.25	22.72727	33.33333
7	39.13043	36.66667	31.42857	23.25581	11.42857	3.030303	6.25	3.030303
8	18.18182	41.66667	35.13514	10	26.92308	7.407407	20	14.28571
9	25	32.43243	31.03448	3.448276	25	10	31.81818	43.47826
10	25	33.33333	32.35294	13.51351	26.66667	7.5	36.36364	17.3913

APPENDIX J

PAIRWISE COMPARISON PROBABILITIES OF G/Z RATIO

	5T	10T	15T	20T	5E	10E	15E	20E
5T	1.000							
10T	0.814	1.000						
15T	0.001	0.003	1.000					
20T	0.753	1.000	0.023	1.000				
5E	0.982	0.993	0.000	0.977	1.000			
10E	0.106	0.490	0.260	0.832	0.135	1.000		
15E	0.057	0.269	0.445	0.638	0.057	1.000	1.000	
20E	0.035	0.168	0.798	0.452	0.035	0.992	1.000	1.000

P-values for pairwise comparison matrix of Tukey's HSD test for G/Z (initial growth rate: instantaneous mortality rate) ratio

BIOGRAPHY OF THE AUTHOR

Susan Hayhurst was born in Mayfield Heights, Ohio on November 20, 1973. She spent her early years in Indiana, attending Canterbury School for part of the year and (during the rest) exploring outdoors and looking for turtles at Lake Tippecanoe. Despite her landlocked roots, she had an early interest in marine invertebrates. Every year her family visited Florida, where each morning she and her grandmother spent hours exploring beaches. She attended Stanford University where she studied the effects of harbor seal haul-out on the rocky midtidal community at Hopkins Marine Station. She graduated with a B.S. in Biological Sciences in June, 1997. Following graduation, she worked at the Monterey Bay Aquarium education department, the California Department of Fish and Game, and Echo Hill Outdoor School. While studying blue mussel larvae at the University of Maine, she has continued to join her interests in science and education by teaching part time in local schools as part of the NSF Graduate Teaching Fellows in K-12 Education program. After receiving her degree, she looks forward to continuing to share with others her enthusiasm for the marine world and its diverse inhabitants. She is a candidate for a Masters degree in Marine Biology from the University of Maine in December, 2001.