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The Role of Larval Thermal Tolerance in the Distribution of Blue Mussel Species within the Gulf of Maine

Susan J. Limbeck

THE ROLE OF LARVAL THERMAL TOLERANCE IN THE DISTRIBUTION OF BLUE MUSSEL SPECIES WITHIN THE GULF OF MAINE

By

Susan J. Limbeck

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Advisory Committee:

Paul D. Rawson, Assistant Professor of Marine Sciences, Advisor

Bruce Barber, Professor of Marine Sciences

J. Malcolm Shick, Professor of Oceanography and Zoology

THE ROLE OF LARVAL THERMAL TOLERANCE IN THE DISTRIBUTION OF BLUE MUSSEL SPECIES WITHIN THE GULF OF MAINE

By Susan J. Limbeck

Thesis Advisor: Dr. Paul D. Rawson

An Abstract of the Thesis Presented
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Two species of blue mussel, *Mytilus edulis* and *Mytilus trossulus,* are sympatric throughout much of the Canadian Maritime Provinces and into the Gulf of Maine. While the distribution of *M. edulis* extends south to the Mid-Atlantic, that of *M. trossulus* ends abruptly in the Gulf of Maine. I have hypothesized that these differences in adult distribution are the result of species-specific variation in larval thermal tolerances. Previously, it has been shown that when reared at 20 °C, from 36 hour post-fertilization through settlement, *M. trossulus* had significantly higher mortality rates than *M. edulis*. This study examined whether species-specific differences in thermal tolerance vary during larval development. Larvae of both species were exposed to three experimental temperatures at three time points during development and growth and mortality were monitored. Larval thermal tolerance for both species changed significantly as a function of age. Instantaneous mortality was highest during the first ten days of development and

decreased to the lowest rate during the second ten days of development. Unexpectedly, there were no significant differences in mortality between *M. edulis* and *M. trossulus* larvae in any of the age-temperature treatments used in this experiment. These results stand in contrast to those from previous experiments and raise doubt as to whether the steep thermal gradient created by the Eastern Maine Coastal Current limits the distribution of *M. trossulus*.

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INTRODUCTION

Marine Biogeography

There is a consensus among biogeographers that there are fewer biogeographical provinces in the sea than in terrestrial ecosystems (Pielou, 1979). Pielou (1979) suggests that marine biogeographic provinces are less common because the ocean is composed of a fluid medium, sources of primary production are less structured than on land, and many marine habitats are three-dimensional. Even so, well-recognized marine biogeographical breakpoints such as those at Point Conception, CA on the Pacific coast and Cape Hatteras, NC on the Atlantic coast of North America (Valentine, 1966; Pielou, 1979) have been documented and typically occur at locations where prevailing current patterns bring distinct water masses together. Because surface temperatures associated with these water masses differ substantially it has been hypothesized that steep thermal gradients generate marine biogeographic breakpoints.

Given the association between species range boundaries and thermal gradients, several authors have suggested that temperature is an important factor controlling many species' range limits. For example, Cerame-Vivas and Gray (1966) state that the distribution of a marine species is largely controlled by the minimum and maximum temperatures limiting survival and reproduction. The range limits of numerous marine benthic invertebrates coincide off the continental shelf of North Carolina, thereby creating a faunal breakpoint the position of which correlates with water temperature and climate (Cerame-Vivas and Gray, 1966). While Engle and Summers (2000) debate the exact location of this faunal breakpoint along the North Carolina coast, they agree that

distinct biogeographical provinces along the Atlantic Coast and into the Gulf of Mexico are based on temperatures that limit the survival and reproduction of benthic macroinvertebrates.

Alternatively, many marine invertebrates have a biphasic life history, which includes a substantial period of larval development in the plankton (Strathmann, 1985). Because larvae are typically weak swimmers, they are likely to be transported by prevailing currents, with an expected dispersal distance related to the duration of the planktonic phase. Thus, oceanic currents may affect species range limits simply as a consequence of their effect on larval dispersal. If so, species with longer periods of planktonic development are expected to have broader ranges than those with shorter periods. Consistent with this view, Scheltema (1986; 1992) has suggested that large discrepancies in geographical ranges for tropical benthic invertebrates are a result of marked differences between species' capacity for dispersal. In addition, a recent model developed by Gaylord and Gaines (2000) suggests that hydrodynamic features themselves can limit range expansion even when larvae spend up to 21 days in the plankton.

Phylogeographic studies, however, do not provide strong support for the role of hydrodynamics in limiting larval dispersal. As alluded to above, a well-recognized biogeographical boundary occurs along the Point Conception, CA coastline where El Niño Southern Oscillation events and a glacial recession historically have favored northward migration and generally created a southern range limit for many northern species (Gaylord and Gaines, 2000). In contrast, for species whose ranges span Point Conception, there appear to be significant levels of southward migration. Wares et al.

(2001) used DNA sequencing to examine patterns of gene flow for three invertebrates, *Balanus glandula*, *Chthamalus fissus*, and *Strongylocentrotus purpuratus*. They found that southward gene flow for these species. Other phylogeographical studies have suggested that historical processes, such as changing sea levels, are perhaps more important in structuring marine boundaries than present-day current patterns. For example, Nelson et al. (2000) examined genetic variation among populations of the false clownfish, *Amphiprion ocellaris*, in the Indo-Malayan region. They found that interpopulation differentiation in this species was correlated with Pleistocene sea level changes but not with present-day surface currents. These findings oppose the more accepted beliefs that Pacific reef fish distributions are influenced by oceanic currents (Scheltema, 1986; Scheltema, 1992).

Other authors have concluded that it is too simplistic to focus on individual environmental characteristics when considering species range limits. Roy et al. (1994; 1998) have examined the range limits of molluscan species in the eastern Pacific and proposed that factors such as geographical area, climatic trends, the timing of barrier reef formation, and solar radiation affect habitat quality. These qualitative factors include temperature, productivity, and seasonality, which in turn have played a role in controlling the present-day latitudinal diversity of tropical communities. Thus, a complex mixture of factors structures the geographical distributions of tropical marine invertebrates and likely determines regional variation in species diversity.

Even so, when considering the forces affecting the distribution of any single species, several specific factors appear to be of greater importance. This is the case for a species of blue mussel, *Mytilus trossulus*, for which Rawson at al. (2001) hypothesized

that temperature and current patterns are the two factors most likely limiting the range of this species within the Gulf of Maine.

Blue Mussel Populations in the Gulf of Maine

Blue mussels in the genus *Mytilus* are common intertidal inhabitants in temperate regions of both the Northern and Southern Hemispheres (Gosling, 1992). Two species of blue mussel, *Mytilus edulis* and *M. trossulus*, are sympatric throughout much of the Canadian Maritime Provinces and into the Gulf of Maine (Koehn et al., 1984; McDonald et al., 1991). *M. edulis* occurs along most of the Atlantic coast of North America, ranging from Labrador to North Carolina. In contrast, *M. trossulus* is considered primarily a cold-water species because its distribution is restricted to sub-polar regions (Koehn, 1991). Throughout the region of sympatry, blue mussel populations typically contain a mixture of *M. edulis* and *M. trossulus* individuals (Bates and Innes, 1995; Comesaña et al., 1999; Rawson et al., 2001).

Recent work by Rawson et al. (2001) has documented an abrupt shift in species composition from mixed populations in eastern Maine to pure *M. edulis* populations in central and western Maine. This shift appears to coincide with prominent oceanographic features in the Gulf of Maine. Coastal hydrodynamics within the Gulf are dominated by a current system that consists of two major branches, the Eastern and Western Maine Coastal Currents (Pettigrew et al., 1998). The Western Maine Coastal Current is typically linked with deep-water movement from Penobscot Bay, Maine to Cape Cod, Massachusetts. In contrast, the Eastern Maine Coastal Current (EMCC) is a prominent cold-water feature that originates from the southwestern Scotian shelf, moves across the

mouth of the Bay of Fundy, and along the eastern coast of Maine to Penobscot Bay (Pettigrew et al., 1998). Satellite imagery indicates that the EMCC often diverges offshore near Penobscot Bay (Figure 1). Although the exact position of this divergence may vary, this movement coincides with peak spawning activity of blue mussel populations in eastern Maine during July and August (Maloy et al., 2003). The movement of herring larvae and distributional patterns of phytoplankton and zooplankton are strongly correlated with the EMCC (Brooks and Townsend, 1989; Townsend, 1992). Based on these observations, Rawson et al. (2001) hypothesized that the offshore plume of the EMCC limits westward dispersal of *M. trossulus* and maintains the change in species composition observed among mussel populations in central Maine.

To investigate this hypothesis, an expanded genetic survey of nearshore and offshore mussel populations was conducted by Rawson et al. (in prep.). Results from this survey indicate that the frequency of *M. trossulus* mussels increases with distance from shore along two sample transects running south from Mount Desert Island and Stonington, Maine (unpublished data; Figure 2). Thus, the highest frequencies of *M. trossulus* are found in populations that are most likely impacted by EMCC (e.g. Mt. Desert Island). In addition, the frequency of *M. trossulus* decreases to the west of Penobscot Bay, even among populations well offshore that are directly in the path of the EMCC. This decrease coincides with the offshore divergence of the EMCC in the vicinity of Penobscot Bay. Both observations are consistent with the hypothesis that larval dispersal and thus range expansion for *M. trossulus* is limited by the EMCC.

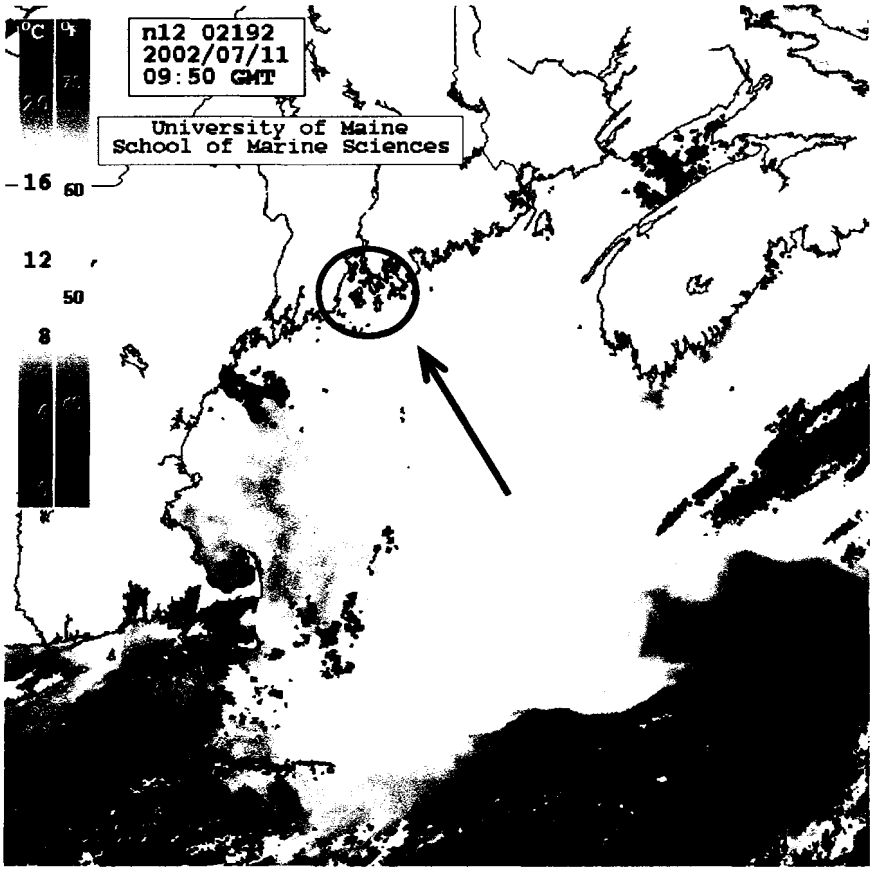

Figure 1. Satellite image of summer sea surface temperatures in the Gulf of Maine. This image is from August 20, 2000 and is provided by University of Maine Satellite Oceanography Data Laboratory. The blue/green coloring along the coast designates the Eastern Maine Coastal Current and the arrow indicates the section of the tail end of the current that will periodically move offshore east of Penobscot Bay (open circle).

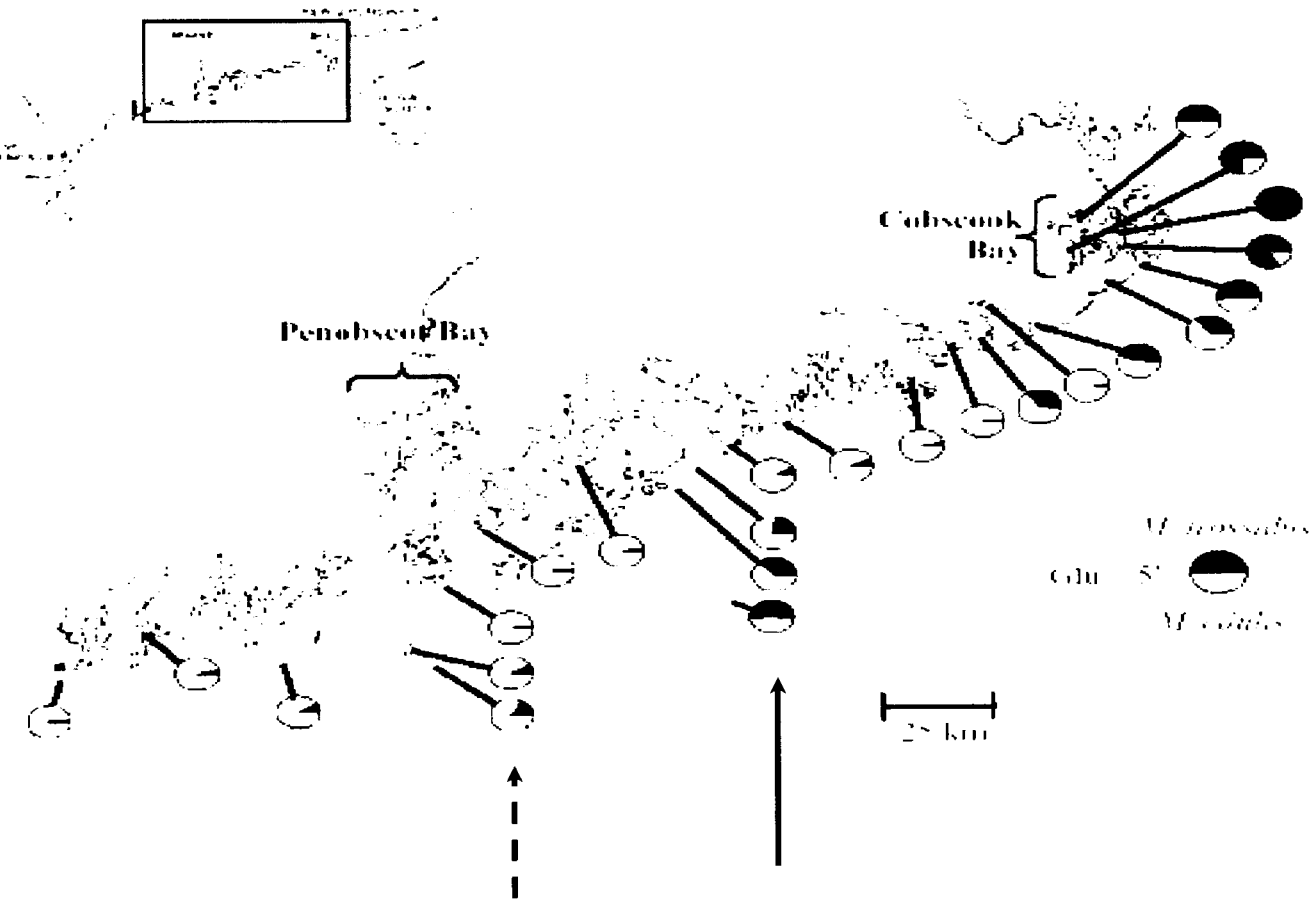

Figure 2. Distribution of adult mussels, *M. edulis* and *M. trossulus* in the Gulf of Maine. Species-specific distributions are inferred from the allelic frequency variation at a series of diagnostic molecular markers. The frequency of *M. edulis* and *M. trossulus*-specific Glu-5' alleles observed at 24 locations in central and eastern Maine are shown here (P. Rawson, unpublished data). The arrows indicate transect lines off Mt. Desert Island (solid) and Stonington (dashed) discussed in the text.

The EMCC, however, is cold-water feature that can directly influence biological processes in the Gulf of Maine (Pettigrew et al., 1998). Cooler waters exist around offshore islands and portions of eastern Maine, which are directly influenced by the current while warmer waters are found to the west of Penobscot Bay and in nearshore habitats in central Maine. Based on these observations, Rawson et al. (2001) suggested an alternative hypothesis that temperature variation associated with the EMCC might be structuring Gulf of Maine blue mussel populations. Unfortunately, the results of the expanded genetic survey, discussed above, cannot discern between the temperature variation and dispersal hypotheses. The only study to date (Mallet and Carver, 1995) that has addressed the relative survival of *M. edulis* and *M. trossulus* found little evidence of species-specific variation in thermal tolerance among adults. In contrast, Bayne (1976a) has suggested that the larval thermal tolerance is a major determinant of blue mussel ranges. While Sprung (1984a) has shown that *M. edulis* larval development proceeds normally over a range of 15-20 °C, much less is known regarding relative thermal tolerances of *M. trossulus* larvae.

In previous work investigating species-specific larval thermal tolerances, Hayhurst (2001) measured relative survival and growth for *M. edulis* and *M. trossulus* larvae reared at 5, 10, 15, and 20 °C. She noted that *M. trossulus* experienced significantly higher mortality at 20 °C, consistent with the hypothesis that species-specific larval thermal tolerance limits the distribution of *M. trossulus* in the Gulf of Maine. However, the decreased survival of *M. trossulus* larvae may have been inadvertently biased by the unknown parentage during spawning and fertilization of the experimental larvae (Rawson, pers. comm.). In addition, the larvae were primarily fed

algal paste, rather than live algae, which may have hindered successful growth and development. The presence of preservatives and the oxidation of lipids, which is occasionally found with algal paste, may have decreased the quality of the food (Davis, pers. comm.). More importantly, the design of Hayhurst's experiment exposed larvae to experimental temperatures from 2 days post-fertilization through development. Therefore, it was not able to address whether larval thermal tolerance changes with ontogeny.

Larval Metabolic Activity

Sprung (1984 a-c) and Widdows (1991) have shown there is a complex relationship between mass-specific metabolic rate and mass for mussel larvae. A high metabolic rate for newly fertilized eggs is due to the rapid utilization of stored nutrients by actively dividing cells. With the development of a velum, larval swimming activity results in an increasing metabolic rate relative to larval mass up to the veliger stage generating a positive correlation between mass and metabolic rate among the earliest larval stages (Widdows, 1991). After the veliger stage, specific metabolic rate and mass become inversely proportional because of a decrease in activity coupled with a substantial increase in mass (Figure 3).

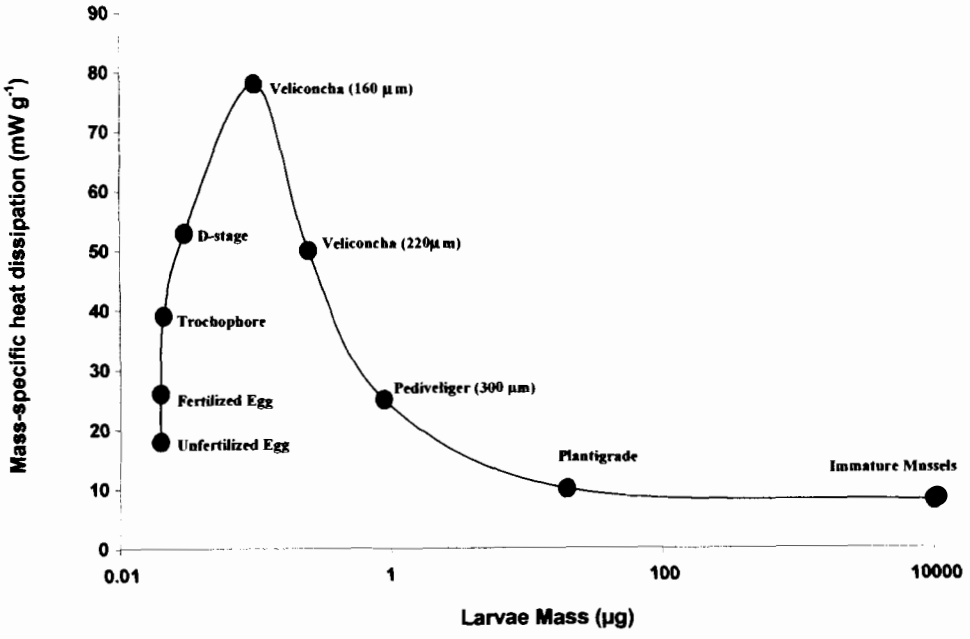

Figure 3. Relationship between mass-specific rate of heat dissipation and biomass during development of *Mytilus edulis*. Note the log scale used for plotting larval mass. (Redrawn from Widdows, 1991).

The dramatic changes in mass-specific metabolic activity suggest that the ability of larvae to tolerate thermal stress may vary through ontogeny as well. Given that larval activity and metabolic rate differ substantially through development, I have hypothesized that the ability to tolerate thermal stress will vary as a function of larval age in a species-specific manner. Using a lab-based approach, my research has investigated three specific hypotheses: 1) Mortality of *M. trossulus* larvae is higher than the mortality of *M. edulis* larvae at elevated temperatures; 2) Growth of *M. trossulus* larvae is lower than the growth of *M. edulis* larvae at elevated temperatures; and 3) Comparative patterns of mortality and growth rates in blue mussel larvae are age-dependent.

MATERIALS AND METHODS

Collection and Identification of Broodstock

The adult mussels used in this experiment were collected from two sites in eastern Maine (Figure 4) where *M. edulis* and *M. trossulus* are sympatric. Approximately five hundred adults were sampled from low intertidal sites in East Bay, Cobscook Bay, Washington County (latitude 44° 56' 30" N; longitude 67° 07' 50" W), on April 25th, 2002, and Lubec, Johnson Bay, Washington County (latitude 44° 56' 30" N; longitude 67° 00' 82" W), on May 14th, 2002. Previous work (Rawson et al., 2001) has demonstrated that mussels at these locations are a mixture of *M. edulis*, *M. trossulus*, and hybrid genotypes.

Immediately after collection, mussels were placed on ice for transport to the Darling Marine Research Center, Walpole, Maine, where they were held in tanks receiving ambient (~8 °C) seawater. Mussels were fed algal paste (Innovative Aquaculture Products Ltd.) to supplement the natural concentrations of phytoplankton in the water. As each mussel began to gape and filter water, a small sample of mantle tissue was biopsied and one valve was marked with an individual-specific fluorescent paint tag. DNA was immediately isolated from the biopsied tissue using QIAamp DNA minikit (Qiagen) or Mammalian Genomic DNA miniprep kit (Sigma). The genotype of each individual was identified by using the extracted DNA in a series of four PCR-based genetic assays (mt16S-F, Glu'-5, ITS, and MAL-1) following the protocols of Rawson and Hilbish (1995), Rawson et al. (1996a), Heath et al. (1995), and Rawson et al. (2001). A total of 200 mussels were successfully genotyped at all four markers.

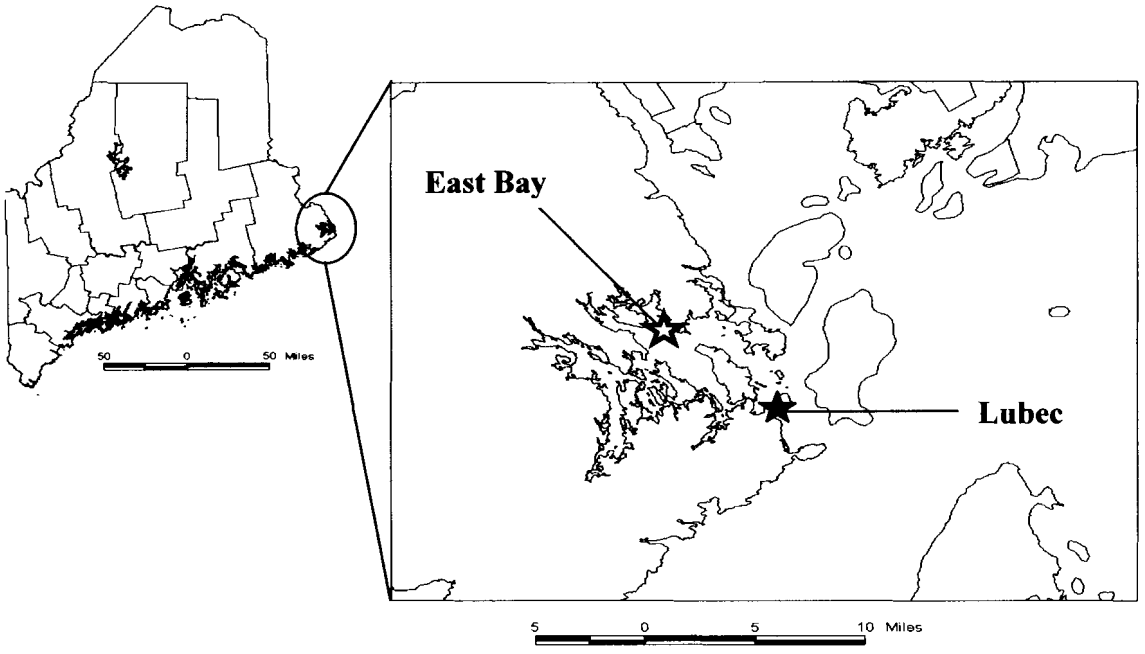

Figure 4. Sampling sites for *M.* edulis and *M.* trossulus broodstock. (Adapted from University of Maine, Fogler Library, GeoScan Services.)

Only those mussels displaying *M. edulis* or *M. trossulus* homozygous genotypes with three nuclear PCR markers (Glu-5', ITS, and Mal 1) and the corresponding species-specific mitochondrial haplotype were used in this experiment. All mussels with either mixed genotypes or those that had PCR failures with one or more of the markers were discarded. The broodstock, which consisted of 92 *M. edulis* and 93 *M. trossulus* individuals, was held in ambient seawater, which increased in temperature to ~ 14 °C, until June 20th, 2002, when they were induced to spawn.

Spawning and Fertilization

Spawning was induced by placing all *M. edulis* and *M. trossulus* adults into separate (species-specific) spawning trays containing ambient temperature seawater. Several *M. trossulus* individuals immediately released gametes upon transfer to a spawning tray. Females were quickly rinsed with 1 µm filtered seawater and placed into individual 1 l beakers containing 1 µm filtered seawater at ambient temperature to collect their eggs. Males were rinsed, wrapped in wet paper towels, and put on ice to stop the release of sperm. Spawning was induced for the remaining mussels of each species by gradually raising the water temperature to ~ 20 °C over a period of approximately 20 minutes and increasing the concentration of algae in the water. After 45 minutes at the elevated temperature, the water in each spawning tray was replaced with ambient seawater and the temperature cycle repeated. Diluted egg and sperm suspensions, from each species, were also added to the water of the corresponding trays to stimulate the release of gametes in the remaining adults.

Spawning activity was monitored for approximately 12 h. Gametes were obtained from 47 *M. trossulus* individuals and 40 *M. edulis* individuals. To maximize the number of individuals contributing to the larval pool, the suspension of sperm from each *M. edulis* male (n=24) was added to an aliquot of eggs from each *M. edulis* female (n=16) at a ratio of approximately 100:1. At 9 h post-fertilization, embryos from all fertilizations were combined and placed in a 20 l bucket containing 5 μm filtered seawater held at 14 °C. An identical protocol was followed for the 25 male and 22 female *M. trossulus* mussels. The resulting embryos from both species were initially maintained at a density of ~ 1000 ind·ml^{-1}.

Larval Treatments and Rearing

Larvae were exposed to experimental temperatures at 36 h post-fertilization (36 hpf), 11 d post-fertilization (11 dpf), and 21 dpf to test whether larval temperature tolerance varies with age. At 36 hpf, a subset of larvae from the stock of each species was transferred to 1 l experimental chambers maintained at three experimental temperatures: 14, 18, or 22 °C; the remaining larvae of each species were maintained in separate stock containers held at 14 °C. The experimental chambers held 1 l of UV-treated, 5-μm filtered seawater and were aerated continuously with a gentle stream of bubbles. Each container was stocked at larval densities of approximately 150 ind·ml^{-1} and fed live *Isochrysis galbana* at concentrations of 50 cells·μl^{-1}; this density and food regime followed the methods of Sprung (1984a-c), Widdows (1991), and Conte et al (1994). The larvae that remained in the stock containers were fed the same food but at 100 cells/μl, due to the higher larval densities. The water in the stock containers and

experimental chambers was changed every other day. For the 18 and 22 °C containers, each chamber gradually warmed to its respective temperature over approximately 4-6 h. The same transfer procedures were repeated with larvae introduced to experimental treatments at 11 and 21 dpf.

Overall, there were 18 treatments with five replicates of each treatment. Thirty experimental chambers were held at each temperature and were randomly assigned a larval treatment (Figure 5). The position of containers within each tank was randomly assigned. Each treatment consisted of three variables: species, time period at which the temperature challenge was applied (age-block), and water temperature.

Estimating Larval Mortality and Growth

After the larvae were introduced to the experimental temperatures, they were left undisturbed for a 24-hour adjustment period before sampling commenced. As the water in each container was changed, the larvae were concentrated into 200 ml of seawater. A 1 ml sample was taken from this concentrate and the number of larvae were counted under a dissecting microscope (100x) using a Sedgewick-Rafter counting cell. Approximately five larvae from each sample were recorded on SVHS videotape using a video camera mounted on the microscope. Maximum shell lengths and widths were determined by analysis of the pictures using the Scion Image software package as well as the SPOT RT slider camera software package. Larvae were sampled in this manner until the conclusion of the experiment on July 21, 2002.

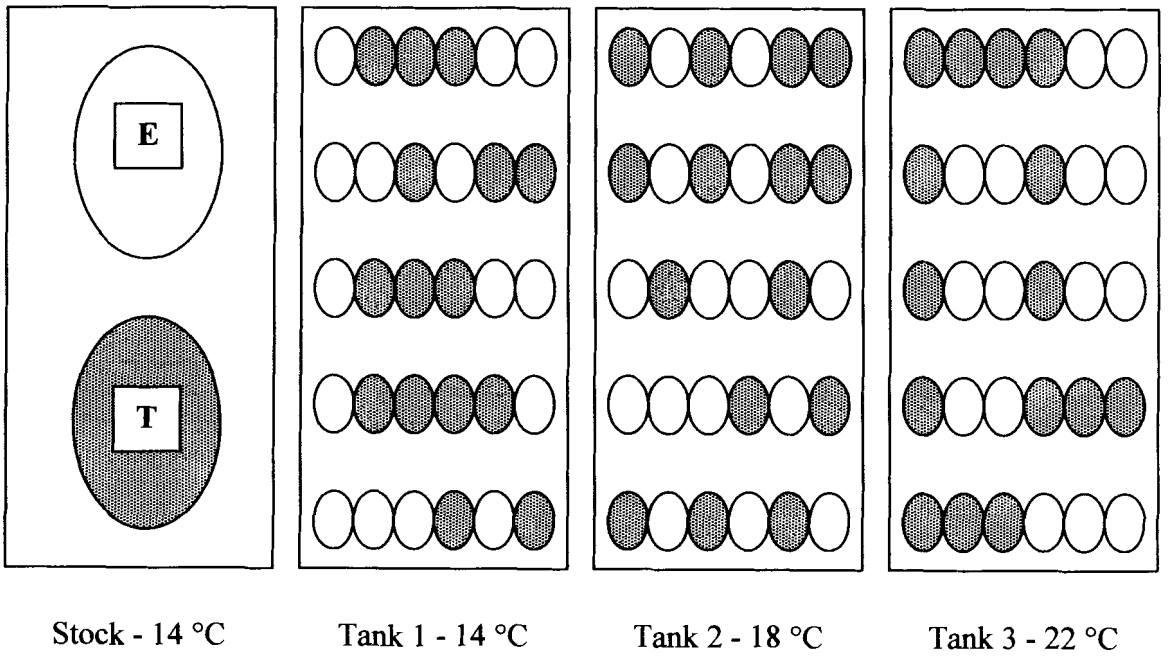

Figure 5. Experimental design for testing relative thermal tolerance of *M. edulis* and *M. trossulus*. Unshaded ovals represent containers holding *M. edulis* while shaded ovals represent *M. trossulus* containers.

Statistical Methods

Variation in the density of larvae within the experimental containers was analyzed in two ways to evaluate the effect of temperature on species-specific mortality. Cumulative mortality was estimated as the proportion of initial density remaining within each replicate for each of the treatments. Means and standard deviations were calculated for each replicate using an arcsine transformation and then plotted using the back-transformed data. LT_{50} values, which estimate the time required for cumulative mortality to reach 50%, were also calculated for each treatment using average larval survival.

Instantaneous mortality within each larval chamber was estimated from the regression of ln(density) against time. For each replicate container, separate regressions were fitted for the data from three age-blocks (days 1-10, days 11-20, and days 21-30). The slope from each regression provides an estimate of instantaneous mortality within each container during a given age-block. Regression slopes were used in a 3-factor analysis of variance (3-way ANOVA) to test whether the main effects of species, age-block, and temperature as well as for all possible 2-way and 3-way interactions significantly affected variation in instantaneous mortality.

Statistical comparisons were restricted to treatments that introduced larvae to experimental temperatures at the same time point (i.e. 36 hpf, 11 dpf, and 21 dpf). The validity of each ANOVA was evaluated by examining the normality of the error terms using the Shapiro-Wilk test as well as visually inspecting the plots of residuals. The homogeneity of the variances was also examined by incorporating the Levine test, which calculates the variance based on transformed error terms. In all cases, these tests

indicated the normality and equality of variance terms and the appropriateness of each ANOVA model.

Individual *F*-tests were performed on the regression slopes to determine the significance of species-specific mortality regression slopes at each temperature/age-block combination. The *p*-values from these *F*-tests were then evaluated against an α-level appropriate to each experiment using the standard Bonferroni correction (Rice, 1989) (Appendix A). The standard Bonferroni technique divides the experiment-wide α-level by the number of tests carried out within each experiment. For example, for larvae that were introduced at 36 hpf, there were nine statistical tests performed (one species comparison at each of three age-blocks and three temperatures). Therefore, the α-level used for those particular tests was calculated by dividing the pre-determined experiment-wide α-level of 0.05 by nine. For those larvae introduced at 11 and 21 dpf, the experiment-wide α-level of 0.05 was divided by 6 and 3, respectively.

Maximum shell lengths and shell widths were highly correlated (r^2=0.94) so only shell length was incorporated into a 3-way ANOVA, comparable to those used for the instantaneous mortality regression slopes, to test for the effects of species, age-block, temperature, and interactions of those factors. To facilitate comparisons between this experiment and published values, growth rates were estimated from average size in each treatment according to the formula: (size at day N – size at day 0) / (day N – day 0).

RESULTS

Mortality

Larval mortality varied as a function of age for both *M. edulis* and *M. trossulus* in all treatments in this experiment. This was particularly evident for larvae introduced to the experimental temperatures at 36 hpf, where LT_{50} values ranged from 1.9 to 2.2 days post exposure. The one exception was for the larvae of *M. trossulus* exposed to 14 °C where the LT_{50} value was 3.7 days post exposure (Table 1). Although there was little variation among the LT_{50} values for *M. edulis* larvae, LT_{50} values for the larvae of *M. trossulus* decreased with increasing temperature. Even so, the larvae of both species experienced a dramatic increase in cumulative mortality, which exceeded 70% at all three temperatures by 5 days post-exposure (Figure 6). This pattern of high mortality early in larval development is further emphasized when expressed in terms of age-specific instantaneous mortality. The sharp decline in larval density during age-block 1 resulted in large estimates of instantaneous mortality for all treatments. Mortality rates, however, declined uniformly during the 2nd age-block, in one case (*M. trossulus* at 22 °C) decreasing up to 40 fold when compared to the instantaneous mortality in age-block 1 from the same replicate (Table 2).

Age at Introduction	Species	Temperature	LT_{50}
36 hpf	*M. edulis*	14 °C	1.9
		18 °C	2.1
		22 °C	1.9
	M. trossulus	14 °C	3.7
		18 °C	2.2
		22 °C	2.0
11 dpf	*M. edulis*	14 °C	12.1
		18 °C	12.9
		22 °C	11.0
	M. trossulus	14 °C	10.3
		18 °C	9.6
		22 °C	8.7
21 dpf	*M. edulis*	14 °C	4.3
		18 °C	3.3
		22 °C	6.5
	M. trossulus	14 °C	4.5
		18 °C	5.0
		22 °C	4.7

Table 1. LT_{50} values estimates for *M. edulis* and *M. trossulus* larvae when introduced to three water temperatures at 36 hpf, 11, and 21 dpf. Values were estimated from cumulative mortality averaged over all replicate containers within each experimental treatment. LT_{50} values are expressed as days post-exposure.

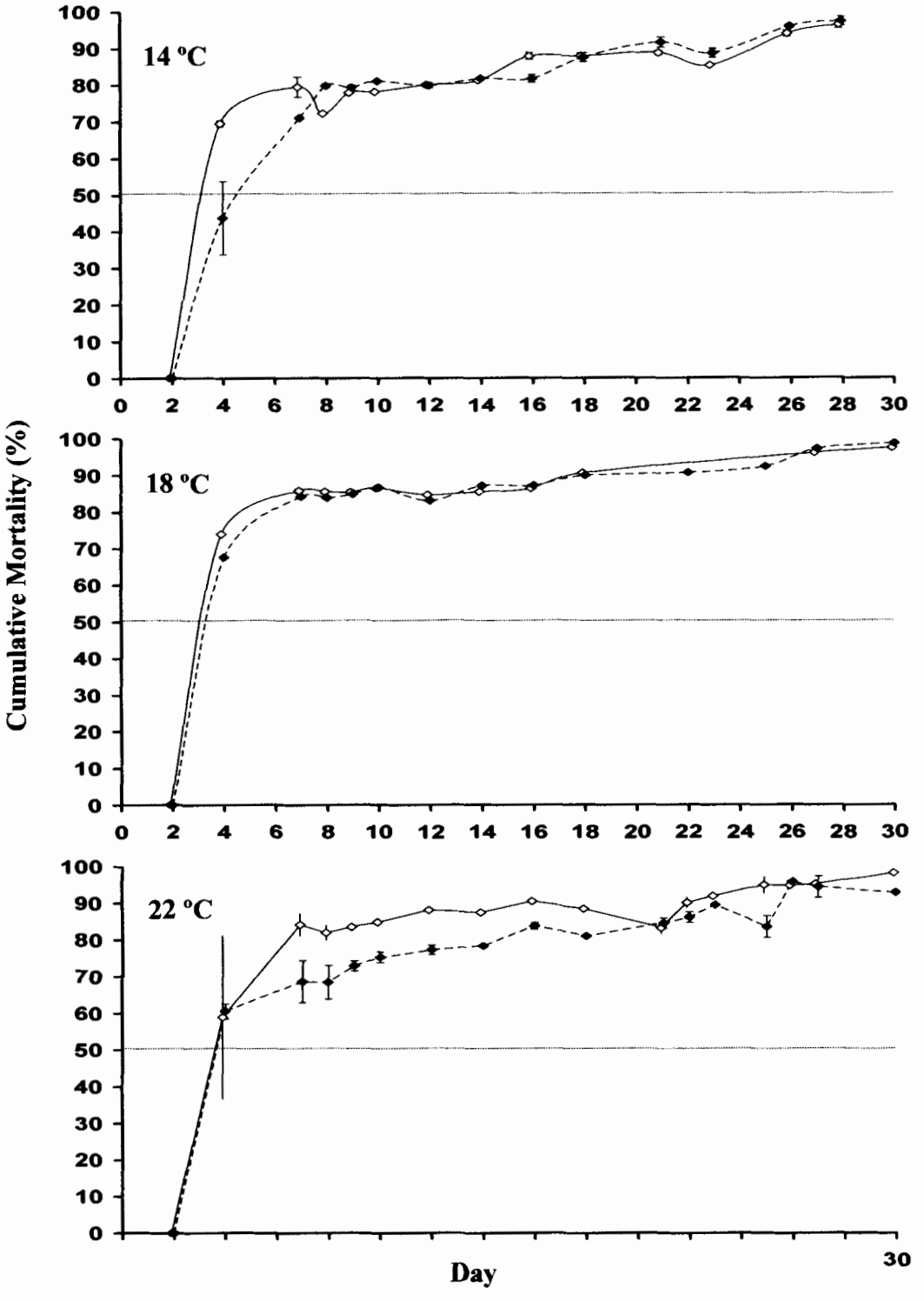

Figure 6. Cumulative mortality for *M. edulis* (open diamonds) and *M. trossulus* (closed diamonds) larvae when introduced to three water temperatures at 36 hpf. Values are expressed as the mean and SD for 3-5 replicates for each species at each temperature. A line is drawn at 50% mortality to correspond to LT_{50} values listed in Table 1.

Species	Age-block	Temp	Instantaneous Mortality					Mean	Std. Error
			1	2	3	4	5		
M. edulis	1	14 °C	-0.051	-0.063	-0.076	-0.065	N.A.	-0.063	0.005
	2	14 °C	-0.021	-0.067	-0.013	-0.078	N.A.	-0.045	0.016
	3	14 °C	-0.060	-0.070	-0.116	N.A.	N.A.	-0.082	0.017
	1	18 °C	-0.070	-0.085	-0.082	-0.060	-0.081	-0.075	0.005
	2	18 °C	-0.050	-0.035	-0.030	-0.054	N.A.	-0.042	0.006
	3	18 °C	-0.087	-0.016	-0.045	-0.067	N.A.	-0.054	0.017
	1	22 °C	-0.067	-0.058	-0.130	-0.078	-0.075	-0.082	0.013
	2	22 °C	-0.035	-0.054	-0.008	N.A.	N.A.	-0.032	0.013
	3	22 °C	-0.063	-0.066	-0.044	-0.074	N.A.	-0.062	0.006
M. trossulus	1	14 °C	-0.125	-0.100	-0.083	-0.085	N.A.	-0.098	0.010
	2	14 °C	-0.010	-0.033	-0.072	-0.058	N.A.	-0.043	0.014
	3	14 °C	-0.140	-0.170	-0.065	N.A.	N.A.	-0.123	0.030
	1	18 °C	-0.096	-0.107	-0.132	-0.098	-0.087	-0.104	0.007
	2	18 °C	-0.010	-0.054	-0.035	-0.014	N.A.	-0.028	0.010
	3	18 °C	-0.065	-0.040	-0.093	-0.056	-0.179	-0.086	0.025
	1	22 °C	-0.081	-0.079	-0.098	-0.052	-0.035	-0.069	0.011
	2	22 °C	-0.002	-0.039	-0.034	-0.056	N.A.	-0.033	0.011
	3	22 °C	-0.079	-0.055	-0.239	-0.049	-0.023	-0.089	0.038

Table 2. Instantaneous mortality estimates for *M. edulis* and *M. trossulus* larvae introduced to three water temperatures at 36 hpf. Instantaneous mortality was estimated as the slope of the regression of ln density versus time. Slopes were estimated for 5 replicates for each of three age blocks at each water temperature. Age blocks 1, 2, and 3 correspond to mortality estimates during 0-10, 11-20, and 21-30 days post exposure, respectively. N.A. – data not available.

During the final age-block, instantaneous mortality rates increased to rates similar to those observed in the first block for nearly all the experimental treatments. An ANOVA indicated that the decrease in instantaneous mortality during age-block 2 relative to mortality in age-blocks 1 and 3 was statistically significant (Table 2). Likewise, the ANOVA demonstrated that overall, average instantaneous mortality varied significantly as a function of temperature, with the highest mortality at 14 °C and lowest at 22 °C for the 36 hpf treatments. A major goal of this research was to examine whether there was evidence for differences in mortality when larvae of *M. edulis* and *M. trossulus* were exposed to elevated temperatures (Figure 7). There were no significant species-specific differences in instantaneous mortality at any temperature by age-block combination.

Effect		Instantaneous Mortality	Std. Deviation
Age-block	1	-0.082 (a)	0.023
	2	-0.037 (b)	0.022
	3	-0.070 (a)	0.035
Temperature	14 °C	-0.073 (a)	0.039
	18 °C	-0.063 (ab)	0.031
	22 °C	-0.057 (b)	0.028

Table 3. Average instantaneous mortality rates for *M. edulis* and *M. trossulus* larvae combined when introduced to three water temperatures at 36 hpf. Instantaneous mortality was estimated as the slope of the regression of ln density versus time. Age-blocks refer to days post exposure as in Table 2. The letters in parentheses indicate values significantly different from each other (Duncan's multiple comparisons, α=0.05, df=55, MSE=0.000583).

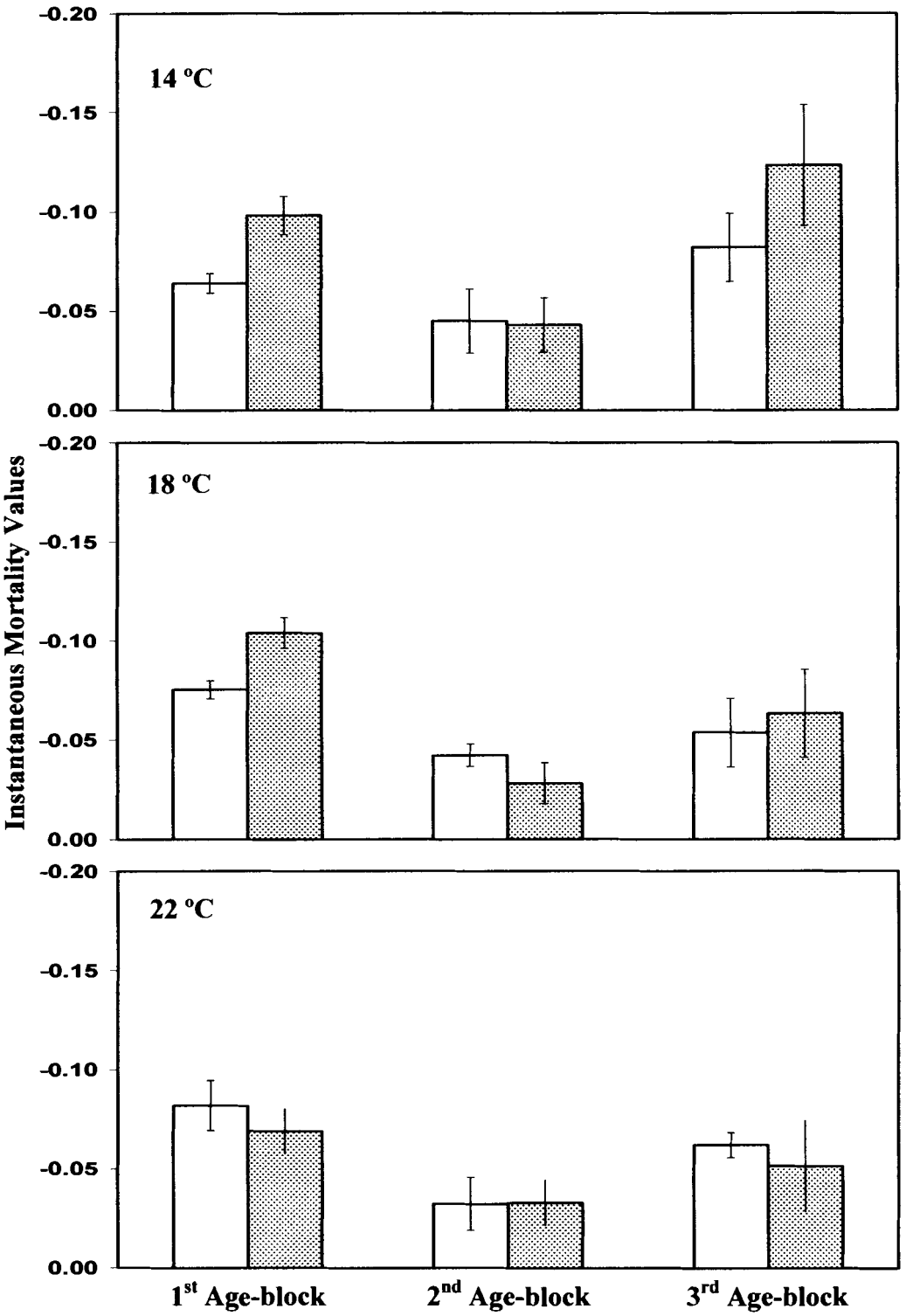

Figure 7. Average instantaneous mortality for *M. edulis* (open bars) and *M. trossulus* (closed bars) larvae introduced to three water temperatures at 36 hpf. Each bar corresponds to the mean instantaneous mortality for five replicate containers in each age-block at each temperature. Age-blocks refer to days post exposure as in Table 2. Error bars represent means ± 1 SD.

Cumulative mortality for larvae exposed to experimental temperatures 11 dpf did not increase as dramatically as it did for larvae exposed at 36 hpf (Figure 8). LT_{50} values ranged from 8.7 to 12.9 days post-exposure with the lowest value for *M. trossulus* at 22 °C and the highest for *M. edulis* at 18°C (Table 1). There was no apparent correlation between water temperature and LT_{50} for *M. edulis*. However, *M. trossulus* larvae experienced lower LT_{50} values with increasing temperatures; similar to the patterns observed for *M. trossulus* larvae introduced at 36 hpf. The patterns of age-specific instantaneous mortality, however, were qualitatively similar to those for larvae in the 36 hpf treatments. The instantaneous mortality for *M. edulis* and *M. trossulus* was significantly lower in age-block 2 relative to block 3 (Table 3). An ANOVA indicated that overall average instantaneous mortality varied significantly as a function of temperature. As seen with larvae introduced at 36 hpf, the highest mortality was seen at 14 °C (Table 4). However, the lowest mortality was seen at 18 °C, with 22 °C being intermediate. There were no species-specific differences in instantaneous mortality rates at each temperature by age-block combination for larvae introduced at 11 dpf (Figure 9).

The overall average instantaneous mortality for larvae exposed at 21 dpf was as high as seen in age-block 3 for larvae exposed at 36 hpf and 11 dpf (Table 5). However, there were no species-specific differences in instantaneous mortality at any temperature by age-block combination (Figure 10). For larvae exposed at 21 dpf, there was a striking increase in cumulative mortality in both species within the first few days after exposure (Figure 11), although not as dramatic as with larvae introduced at 36 hpf. LT_{50} values ranged from 3.3 to 6.5 days post-exposure, which is greater than the LT_{50} values for larvae introduced at 36 hpf but smaller than for those introduced at 21 dpf (Table 1).

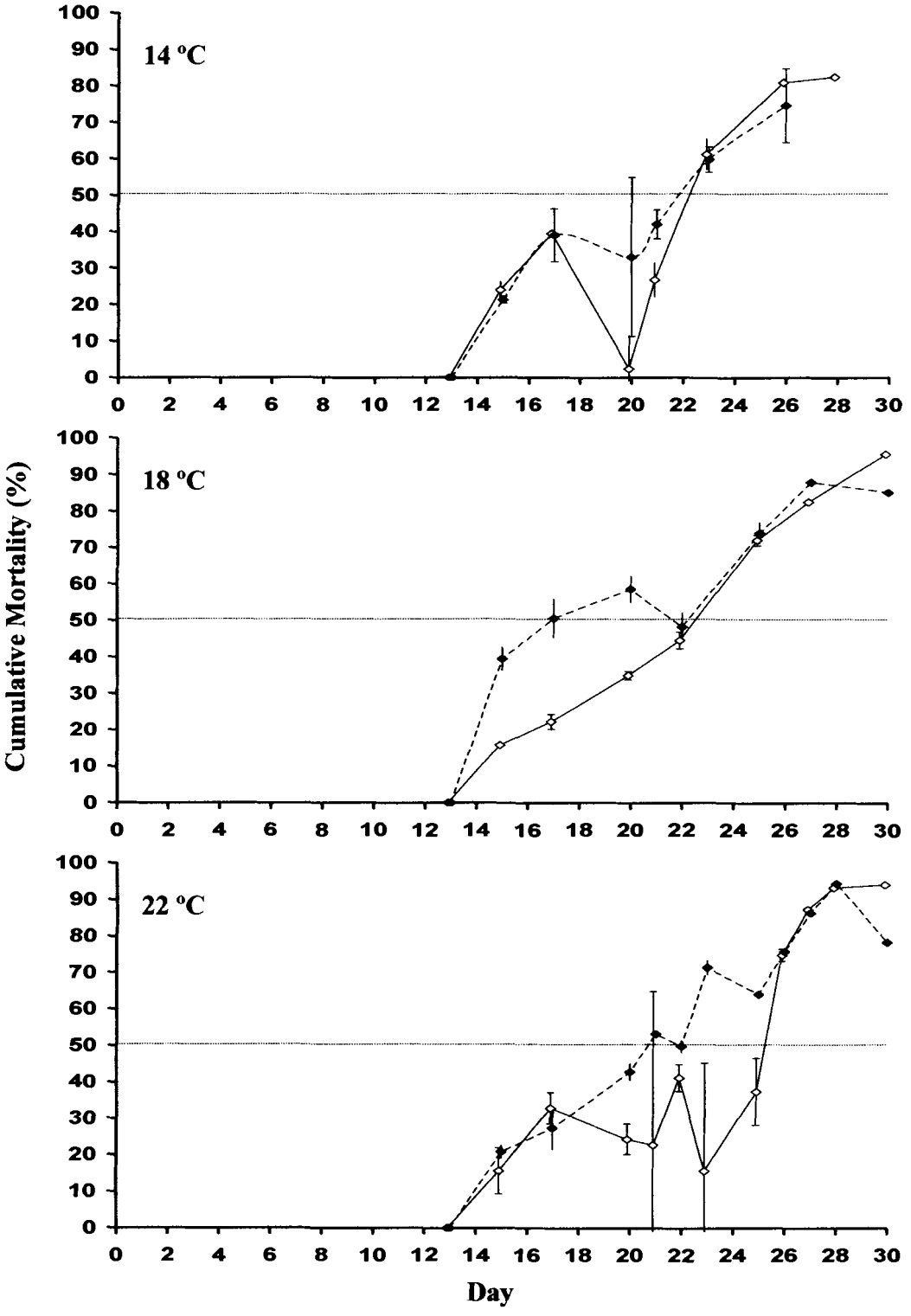

Figure 8. Cumulative mortality for *M. edulis* (open diamonds) and *M. trossulus* (closed diamonds) larvae when introduced to three water temperatures at 11 dpf. Values are expressed as the mean and SD for 3-5 replicates for each species at each temperature. A line is drawn at 50% mortality to correspond to LT_{50} values listed in Table 1.

Species	Age-block	Temp	Instantaneous Mortality 1	2	3	4	5	Mean	Std Error
M. edulis	2	14 °C	-0.066	-0.030	-0.016	-0.045	-0.007	-0.033	0.010
	3	14 °C	-0.114	-0.113	-0.106	-0.183	-0.152	-0.134	0.015
	2	18 °C	-0.021	-0.021	-0.018	-0.017	-0.020	-0.019	0.001
	3	18 °C	-0.023	-0.083	-0.100	-0.015	-0.075	-0.059	0.017
	2	22 °C	-0.039	-0.014	-0.021	-0.014	-0.046	-0.027	0.007
	3	22 °C	-0.185	-0.090	-0.091	-0.143	N.A.	-0.127	0.023
M. trossulus	2	14 °C	-0.061	-0.027	-0.018	-0.052	-0.014	-0.034	0.009
	3	14 °C	-0.190	-0.029	-0.162	N.A.	N.A.	-0.127	0.050
	2	18 °C	-0.046	-0.029	-0.121	-0.063	-0.020	-0.056	0.018
	3	18 °C	-0.053	-0.050	-0.050	-0.036	-0.045	-0.047	0.003
	2	22 °C	-0.032	-0.030	-0.032	-0.029	-0.055	-0.035	0.005
	3	22 °C	-0.031	-0.062	-0.024	-0.123	-0.022	-0.052	0.019

Table 4. Instantaneous mortality estimates for *M. edulis* and *M. trossulus* larvae introduced to three water temperatures at 11 dpf. Instantaneous mortality was estimated as the slope of the regression of ln density versus time. Slopes were estimated for 5 replicates for each of three age blocks at each water temperature. Age blocks 2, and 3 correspond to mortality estimates during 11-20, and 21-30 days post exposure, respectively. N.A. – data not available.

Effect		Instantaneous Mortality	Std. Deviation
Age-block	2	-0.034 (a)	0.023
	3	-0.087 (b)	0.055
Temperature	14 °C	-0.077 (a)	0.062
	18 °C	-0.045 (b)	0.030
	22 °C	-0.057 (ab)	0.048

Table 5. Average instantaneous mortality rates for *M. edulis* and *M. trossulus* larvae combined when introduced to three water temperatures at 11dpf. Instantaneous mortality was estimated as the slope of the regression of ln density versus time. Age-blocks refer to days post exposure as in Table 4. The letters in parentheses indicate values significantly different from each other (Duncan's multiple comparisons, α=0.05, df=55, MSE=0.001117)

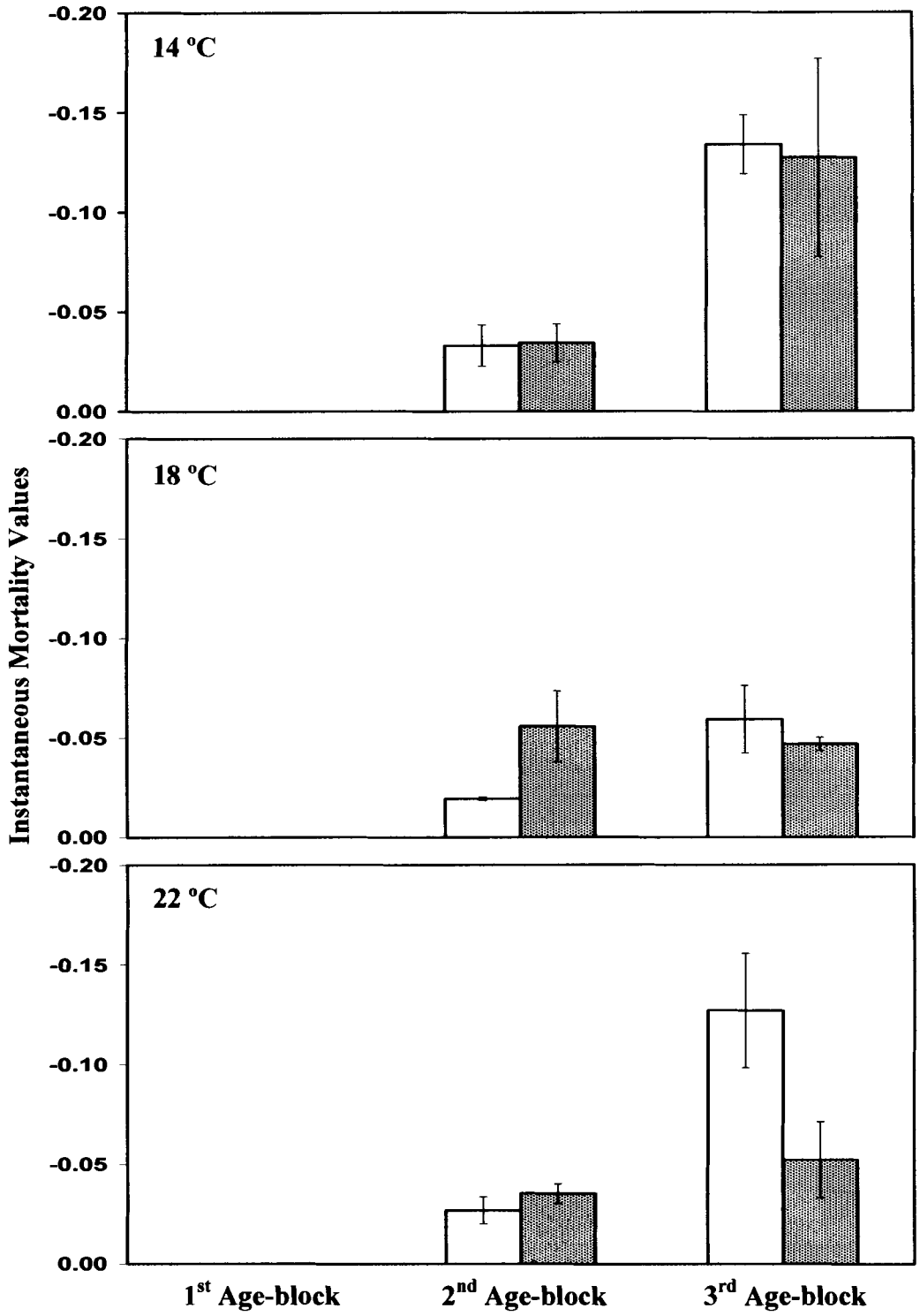

Figure 9. Average instantaneous mortality for *M. edulis* (open bars) and *M. trossulus* (closed bars) larvae introduced to three water temperatures at 11 dpf. Each bar corresponds to the mean instantaneous mortality for five replicate containers in each age-block at each temperature. Age-blocks refer to days post exposure as in Table 4. Error bars represent means ± 1 SD.

Species	Temp	Instantaneous Mortality					Mean	Std Error
		1	2	3	4	5		
M. edulis	14 °C	-0.130	-0.177	-0.109	-0.040	N.A.	-0.114	0.028
	18 °C	-0.100	-0.074	-0.107	-0.042	N.A.	-0.081	0.015
	22 °C	-0.106	-0.108	-0.112	-0.122	-0.080	-0.105	0.07
M. trossulus	14 °C	-0.126	-0.030	-0.145	-0.124	-0.107	-0.106	0.020
	18 °C	-0.103	-0.061	-0.076	-0.160	N.A.	-0.100	0.022
	22 °C	-0.100	-0.098	-0.103	-0.103	-0.103	-0.101	0.001

Table 6. Instantaneous mortality for *M. edulis* and *M. trossulus* larvae when introduced to three water temperatures at 21 dpf. Instantaneous mortality was estimated as the slope of the regression of ln density versus time. Slopes were estimated for 5 replicates for each of three age blocks at each water temperature. N.A. – data not available.

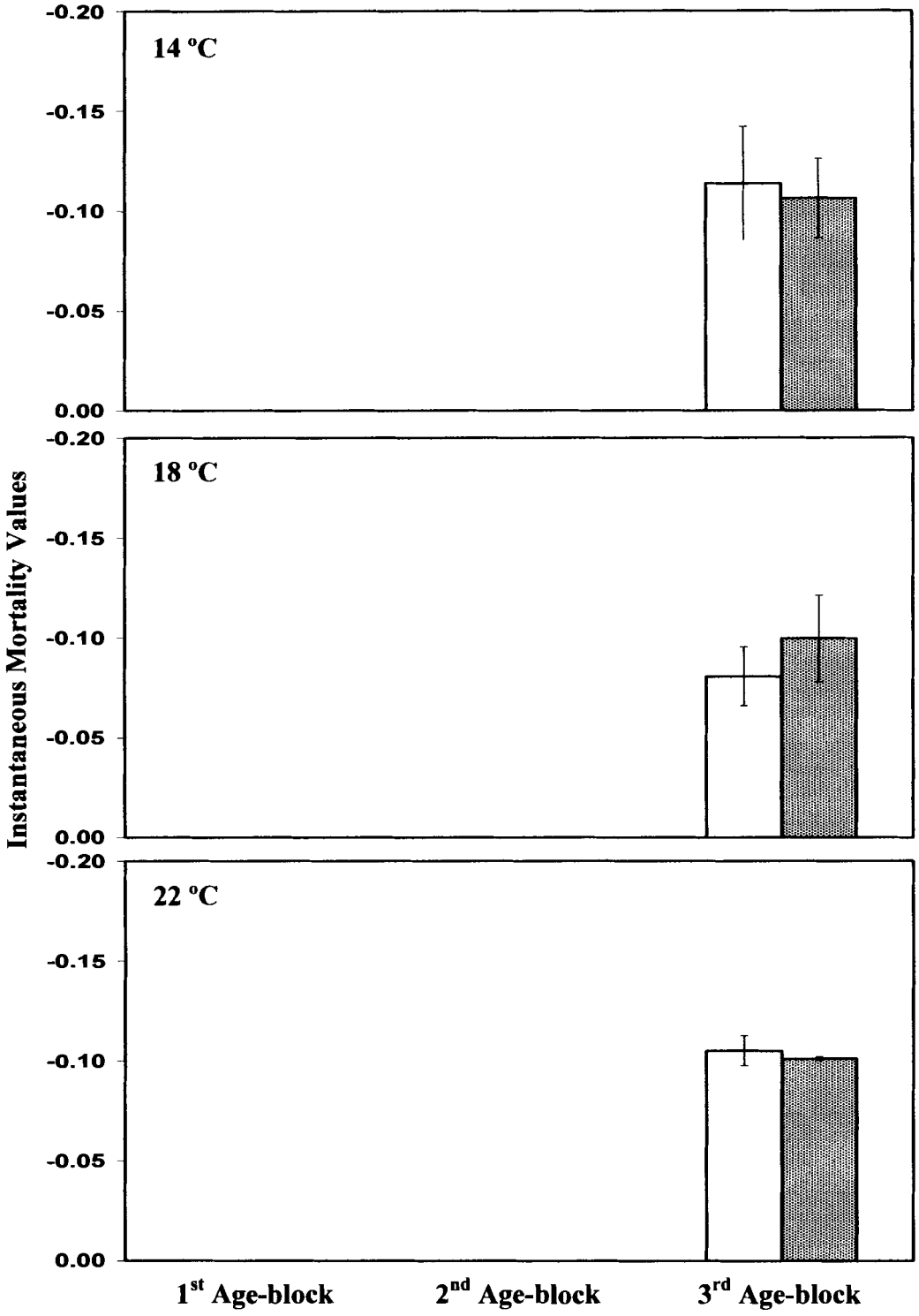

Figure 10. Average instantaneous mortality for *M. edulis* (open bars) and *M. trossulus* (closed bars) introduced to three water temperatures at 21 dpf. Each bar corresponds to the mean instantaneous mortality for five replicate containers in each age-block at each temperature. Error bars represent means ± 1 SD.

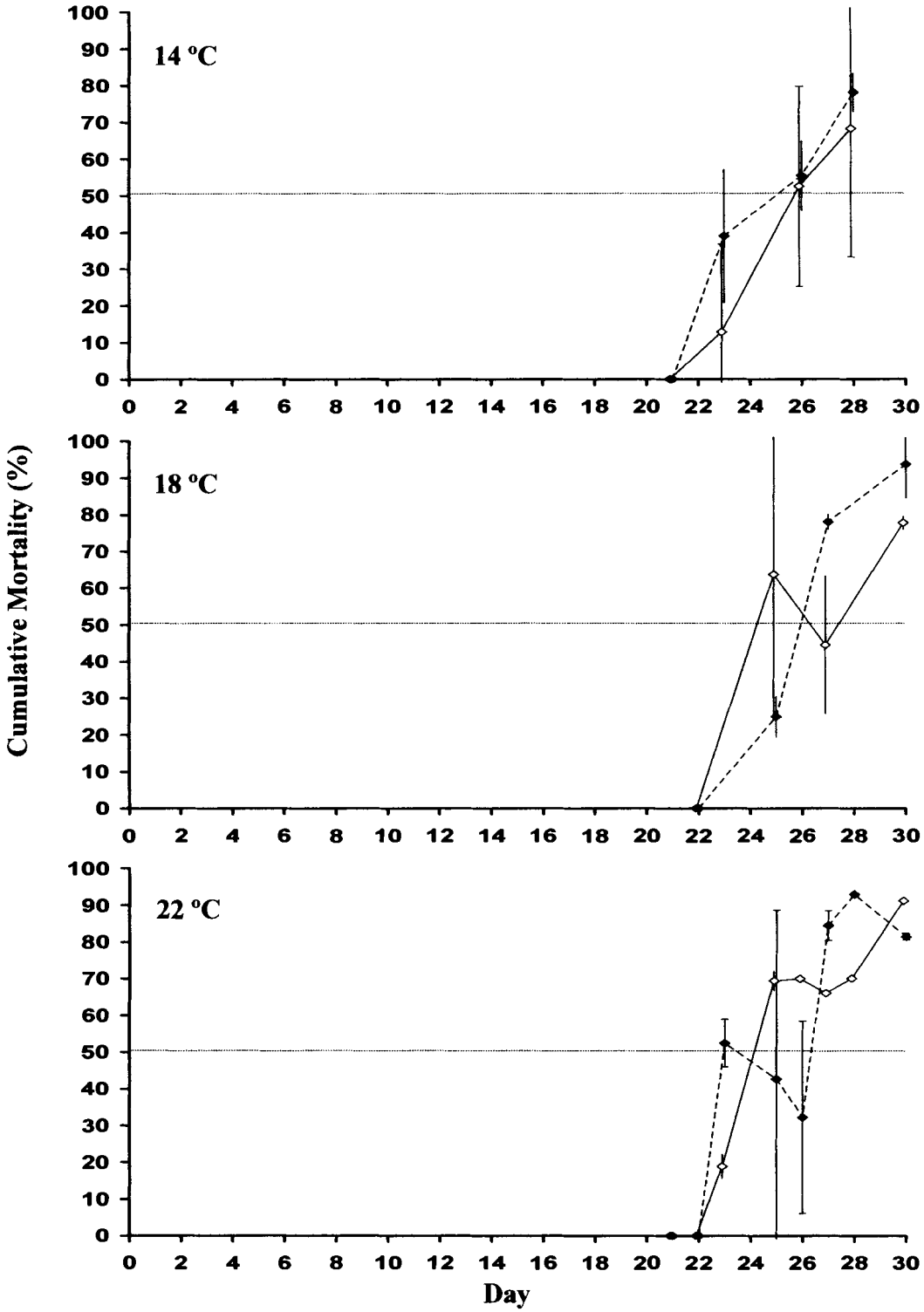

Figure 11. Cumulative mortality for *M. edulis* (open diamonds) and *M. trossulus* (closed diamonds) larvae when introduced to three water temperatures at 21 dpf. Values are expressed as the mean and SD for 3-5 replicates for each species at each temperature. A line is drawn at 50% mortality to correspond to LT_{50} values listed in Table 1.

Growth

Analysis of variance indicated that there were no statistically significant differences in the initial size of larvae whether introduced to experimental temperatures at 36 hpf, 11 dpf, or 21 dpf. When larvae were introduced at 36 hpf, shell length increased significantly with age. In addition, temperature showed a significant effect on the larval size ranging from 127.21 μm at 14 °C to 141.04 μm at 18 °C, when averaged over age-block (Table 7). There were no significant species-specific differences at any of the temperature by age-block combinations (Figure 12). Similarly, for larvae introduced at 11 dpf shell length varied significantly as a function of age-block. There was a significant species-specific difference in larval size during age-block 2 at 22 °C, with *M. trossulus* larvae reaching larger sizes than *M. edulis* larvae (Figure 13).

A significant species effect was detected for larval size when larvae were introduced at 21 dpf (Table 7). However, there were no significant differences due to temperature or any differences in species-specific size at the temperature/age-block combination (Figure 14). Growth rates based on changes in larval shell length were also calculated and presented in Table 8 to allow for a direct comparison between this experiment and those presented in the literature.

Introduction			Size	Std. Deviation
36 hpf	**Species**	*M. edulis*	131.70	33.75
		M. trossulus	138.87	34.14
	Age-block	1	125.81	23.42
		2	157.26	42.50
		3	188.34	35.87
	Temperature	14 °C	127.21	32.11
		18 °C	141.04	32.67
		22 °C	138.77	36.06
11 dpf	**Species**	*M. edulis*	136.32	26.99
		M. trossulus	148.54	31.75
	Age-block	2	138.47	26.75
		3	167.57	33.90
	Temperature	14 °C	126.33	22.81
		18 °C	154.18	30.14
		22 °C	151.74	31.11
21 dpf	**Species**	*M. edulis*	167.40	28.76
		M. trossulus	178.90	24.20
	Temperature	14 °C	170.44	25.27
		18 °C	180.47	24.64
		22 °C	180.33	30.12

Table 7. Average shell length for *M. edulis* and *M. trossulus* larvae in each of three age-blocks introduced to three water temperatures at 36 hpf, 11 and 21 dpf. Mean shell length and the standard deviation were estimated from measurements of five larvae in five replicate containers for each age-block at each temperature. Age-blocks refer to days post exposure as in Table 2.

Introduction	Temperature	Age-block	Growth Rate (µm d^{-1})	
			M. edulis	*M. trossulus*
36 hpf	14 °C	1	3.51	3.70
		2	1.34	2.36
		3	5.93	4.95
	18 °C	1	6.32	6.13
		2	3.25	2.26
		3	3.02	5.76
	22 °C	1	5.44	5.40
		2	2.24	8.02
		3	3.41	-3.37
11 dpf	14 °C	2	0.01	1.19
		3	4.71	5.02
	18 °C	2	2.25	2.98
		3	2.46	3.28
	22 °C	2	2.81	2.75
		3	4.47	1.52
21 dpf	14 °C	3	1.68	1.84
	18 °C	3	3.09	3.13
	22 °C	3	1.38	3.47

Table 8. Average growth rate of shell length for *M. edulis* and *M. trossulus* larvae when introduced to three water temperatures at 36 hpf, 11 and 21 dpf at three temperatures. Age-blocks refer to days post exposure as in Table 2.

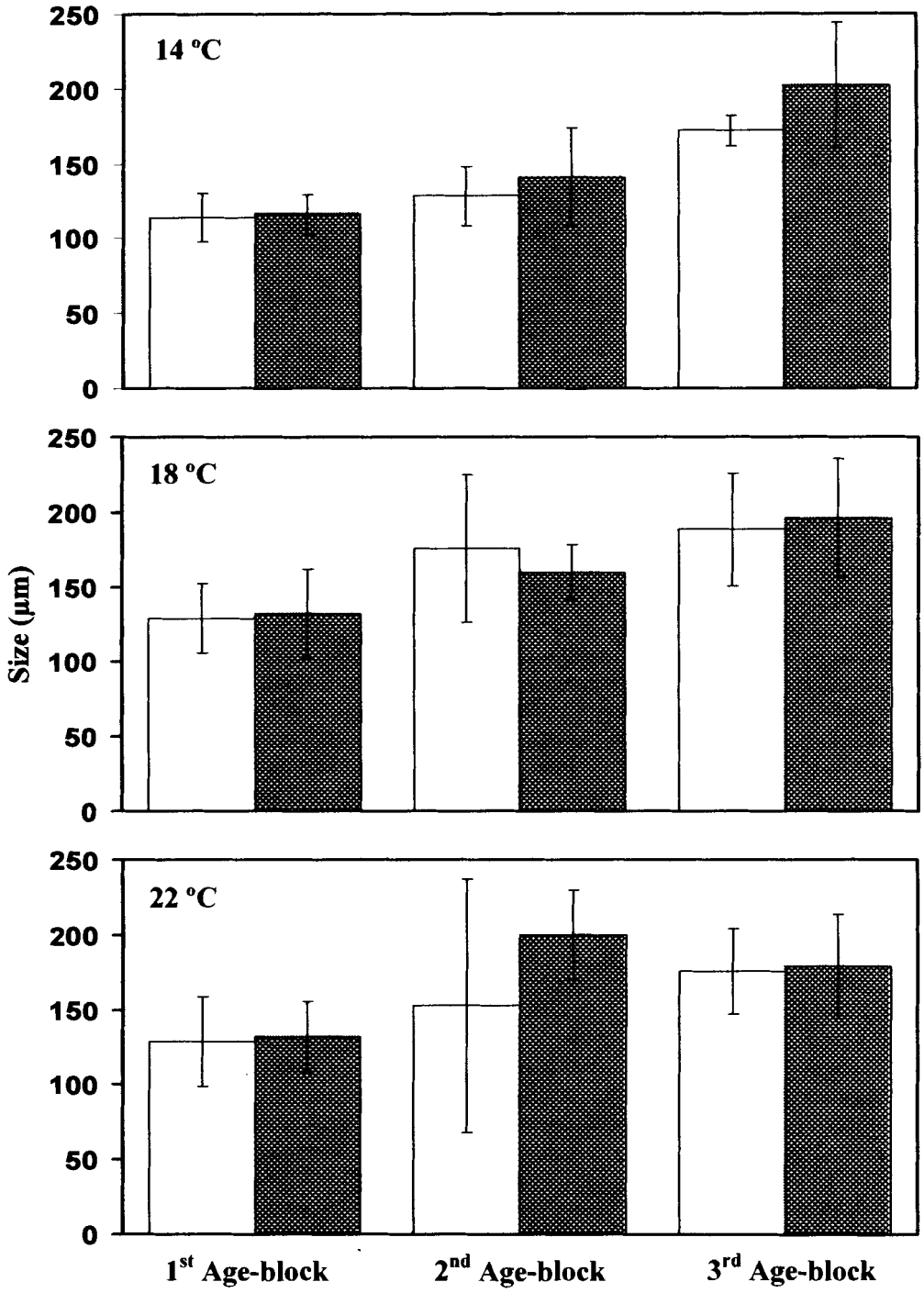

Figure 12. Average shell length for *M. edulis* (open bars) and *M. trossulus* (closed bars) larvae when introduced to three water temperatures at 36 hpf. Age blocks 1, 2, and 3 correspond to length estimates during 0-10, 11-20, and 21-30 days post exposure. Error bars represent means ± 1 SD.

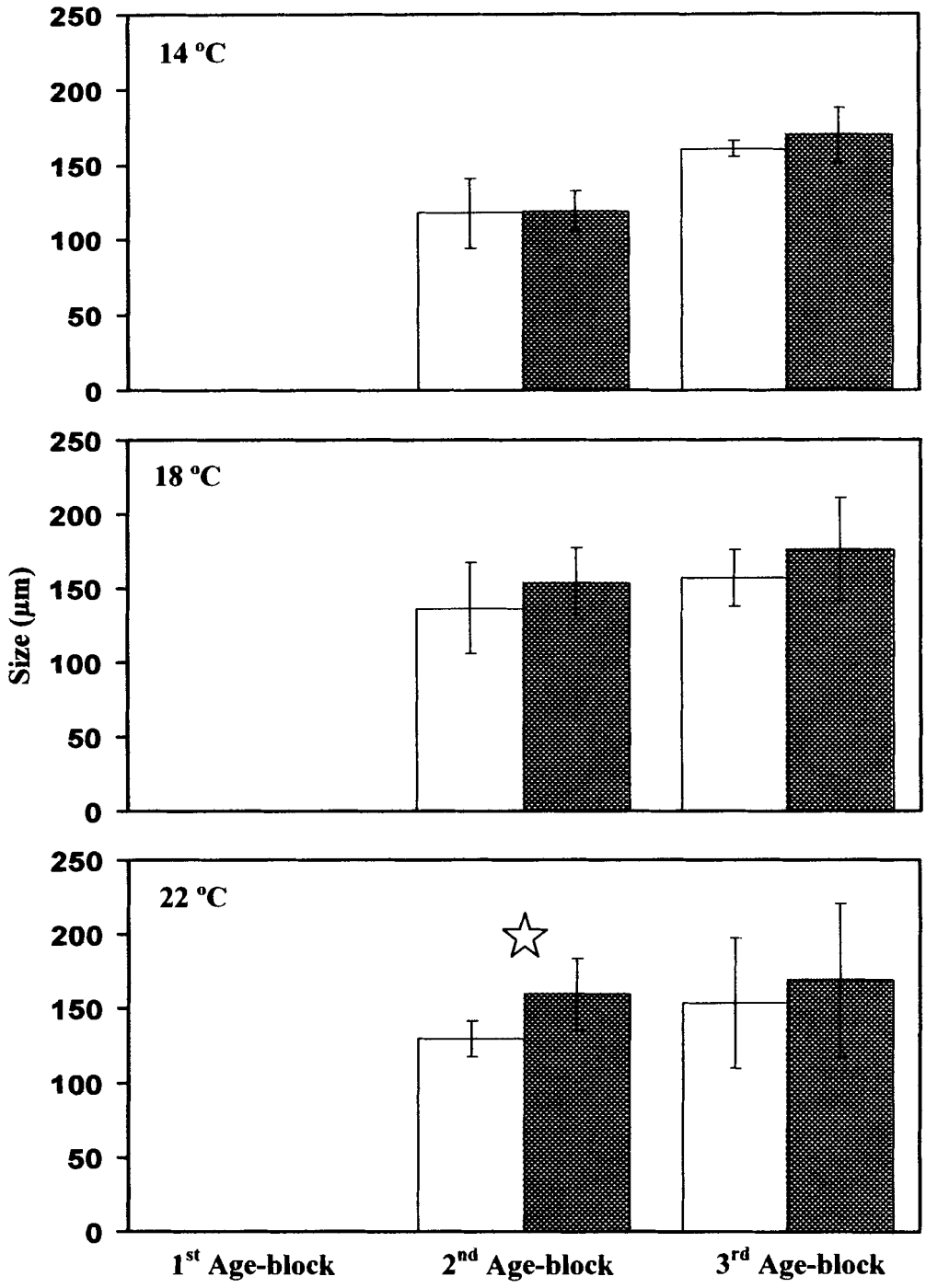

Figure 13. Average shell length for *M. edulis* (open bars) and *M. trossulus* (closed bars) introduced to three water temperatures at 11 dpf. Error bars represent means ± 1 SD. The star represents significant species differences (Duncan's multiple comparisons, df = 28, MSE = 440.1237).

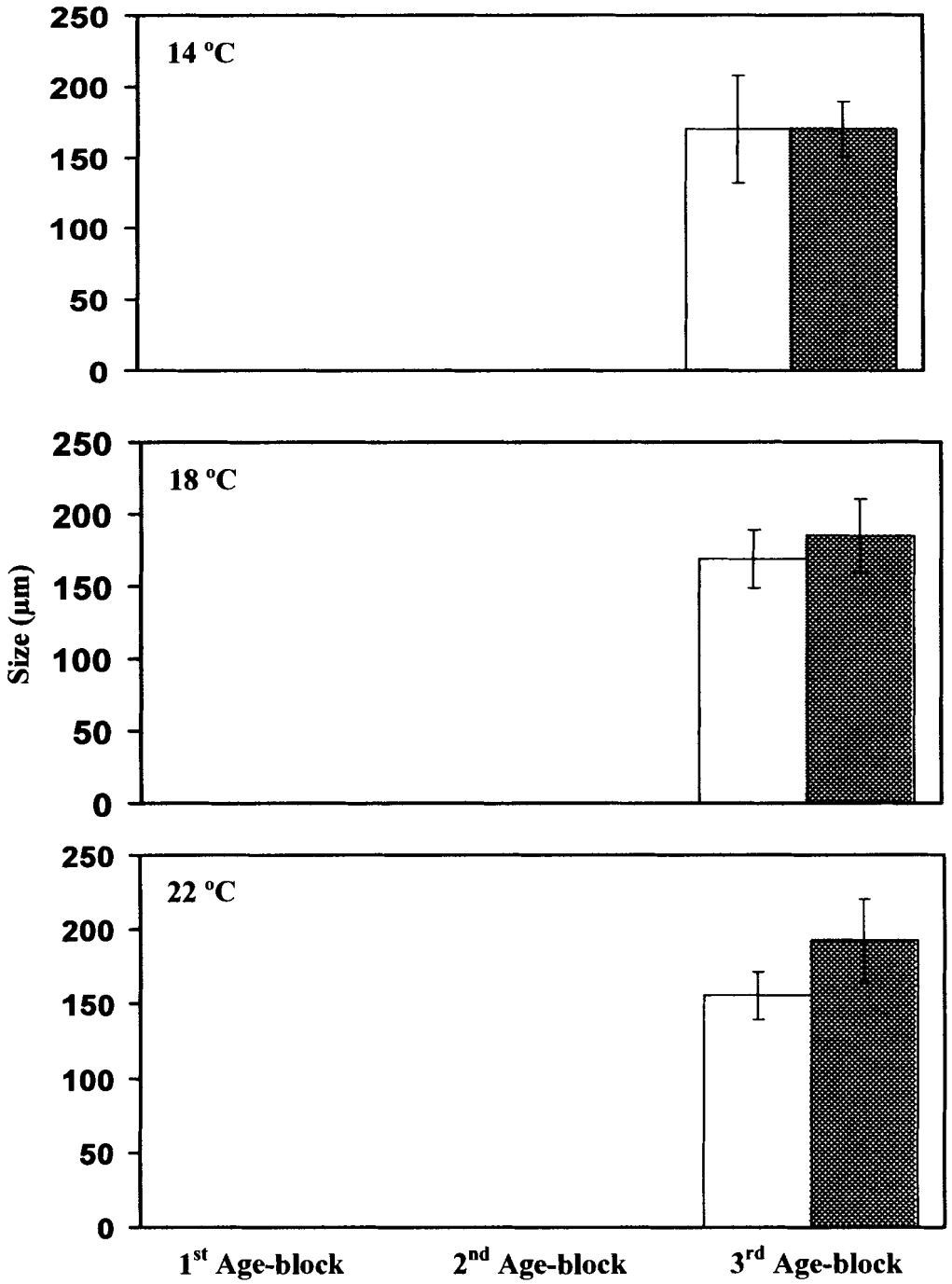

Figure 14. Average shell length for *M. edulis* (open bars) and *M. trossulus* (closed bars) larvae when introduced to three water temperatures at 21 dpf. Error bars represent means ± 1 SD.

DISCUSSION

The work of Sprung and Widdows (1986) and Widdows (1991) has demonstrated that mass-specific metabolic rate for *M. edulis* changes dramatically during larval development. Based on their observations, I hypothesized that the effects of temperature on species-specific mortality would vary during larval development. Because logistically it would have been difficult to isolate larvae into nine individual stages as done by Widdows (1991), this experiment introduced larvae to temperature challenges at times that bracketed prominent physiological and morphological changes identified by Widdows (1991). Thus, larvae were challenged at 36 hpf, 11 dpf, and 21 dpf, representing transitions to D-stage larvae, early stage veligers, and late stage veligers, respectively.

The results of this research indicate that mortality varies substantially as a function of larval age for *M. edulis* and *M. trossulus*. Initial mortality rates for both species were quite high over the first 6-8 days for larvae challenged at 36 hpf and then declined with larval age, reaching the lowest values between 11 and 20 dpf. Similarly, for larvae introduced at 11 dpf, mortality rates were significantly lower during this second age-block. There were also significant effects of temperature on larval mortality for both species when introduced at 36 hpf and 11 dpf. However, the patterns of temperature specific mortality rates I observed were unanticipated. For larvae exposed to experimental temperatures at 36 hpf and 11 hpf, the highest mortality occurred at 14 °C, a temperature which was intended to emulate water conditions experienced by larvae in eastern Maine where *M. edulis* and *M. trossulus* are sympatric and where the adult mussels for this study were collected. Surface temperatures in these areas typically peak

at 14 – 15 °C (Rawson, unpublished data) so, in this experiment, the 14 °C treatment was considered to be a permissive or control temperature. In fact, several studies have shown that survival and developmental rates are optimal for *M. edulis* at temperatures of 12 -15 °C (Bayne, 1976b; Sprung, 1984 a-c; Widdows, 1991). Although the distribution of adult *M. trossulus* populations suggest it is a cold water species with a more restricted sub-polar distribution (Koehn, 1991), much less attention has been paid to the larval ecology of this species. In one of the few studies comparing the relative thermal tolerance of *M. edulis* and *M. trossulus*, Hayhurst (2001) exposed larvae of both *M. edulis* and *M. trossulus* to four temperatures (5, 10, 15, and 20 °C) from 2 to 30 dpf. She found that both species had the highest survivorship at 15 °C but that survival decreased at 20 °C, especially for *M. trossulus*. Thus, it was surprising to see the highest average instantaneous mortality in this experiment occurred at 14 °C for larvae challenged at 36 hpf and 11 dpf.

When designing this experiment, I anticipated that increased temperature would have the greatest effect on mussel larvae in the early stages of development, particularly through the veliger stage when mass-specific metabolic rate and larval mass are positively correlated. While I observed the highest rates of larval mortality during the first age-block, I also observed mortality rates in the third age-block that were nearly equal to those seen in the first age-block. One possible explanation for this increase is that mortality was confounded by settlement. Blue mussel larvae typically have a well-developed foot and are competent to settle and metamorphose as early as 25-28 days post-fertilization. While the larval containers did not provide favorable settlement substrate for the larvae, Pechenik et al. (1990) have noted that larvae will settle

fortuitously on sides of containers, including glass beakers. In the absence of suitable substrates, studies suggest that larvae can and will delay metamorphosis for an extended period of time (Bayne, 1965; Bayne, 1976; Pechenik et al., 1990). In this experiment, larvae were monitored continuously for eyespot development as well as any other morphological changes that would indicate the onset of metamorphosis and settlement. The inside surface of the replicate containers were examined for settled mussels at the end of the experiment. I found little evidence of eyespot development and very few settled larvae. Thus, given little evidence of actual settlement, it was surprising to see such large changes in larval density when cultures had reached pediveliger stage but had not yet developed eyespots.

Although there were significant effects of temperature and larval age on mortality, the main objective of this study was to examine the role of species-specific larval mortality in the population composition of blue mussels in the Gulf of Maine. In this experiment, there were no significant differences in mortality between *M. edulis* and *M. trossulus* larvae for any of the temperature by age-block comparisons. However, there were some notable trends. The larvae of *M. trossulus* tended to have higher rates of mortality relative to *M. edulis* larvae over the first and second age-blocks when introduced to experimental temperatures at 36 hpf. In addition, when introduced at 11 dpf the mortality rates of *M. trossulus* larvae were higher at 18 and 22 °C during the second age-block. A power analysis suggests that these differences would have been significant with the addition of one or two more replicates to the experimental design (data not shown). Although not statistically significant, these observations suggest that

any species-specific differences in larval mortality are likely to occur soon after introduction to experimental temperatures.

The crucial question, however, is whether the differences I have observed are likely to have a major impact on the species composition of blue mussel populations. The differences in larval mortality between *M. edulis* and *M. trossulus* seen at 14 °C are hard to reconcile with the distribution of adult mussels in eastern Maine. In this experiment, 14 °C was considered the control treatment since both species occur at high frequencies in locations where temperatures typically peak at 14 or 15 °C. In contrast, species-specific differences in larval mortality at 18 °C, as well as those differences seen by Hayhurst (2001) at 20 °C, would seem to support the hypothesis that larval thermal tolerance plays an important role in structuring mussel populations. However, these differences occurred primarily during the first age-block of this experiment. Although the strength of the EMCC is seasonally variable, typical current speeds are in the range of 12 -20 km/day (Townsend, 1992). Any *M. trossulus* larvae spawned in eastern Maine would need several days to disperse westward into warmer waters found in central Maine. Thus, *M. trossulus* larvae would encounter elevated temperatures at an age where my experiment indicates species-specific differences in mortality are less pronounced and larval mortality for both species is at its lowest. Based on the results of my experiment, I believe it is unlikely that larval thermal tolerance by itself is structuring the species composition of blue mussel populations in the Gulf of Maine.

It should be noted, however, that neither my experiment nor that of Hayhurst (2001) examined mortality through metamorphosis and settlement. The period of metamorphosis is highly stressful for larvae and differences in thermal tolerance may be

more pronounced during this stage. Other factors that may have limited my ability to detect species-specific differences in larval mortality include the thermal history of the adults used in this experiment. Blue mussel adults possess physiological mechanisms, such as heat shock proteins, which enable cellular processes to proceed virtually unaffected in the presence of cyclic temperatures (Widdows and Bayne, 1971; Widdows, 1976). Exposure to adverse environmental conditions, such as temperature extremes, may cause cellular protein damage. The expression of heat shock proteins aids in minimizing this damage. Adults in this experiment were collected from the same location and thus, have a common thermal history. Bayne (1976a) suggests that the thermal history of parental stocks can affect the thermal tolerances of the larvae they produce, perhaps by passing on the same heat shock protein profile to offspring. In this experiment, the comparable levels of mortality for *M. edulis* and *M. trossulus* larvae may be a result of a common thermal history for the broodstock. Future experiments can test this hypothesis by comparing larval thermal tolerance for *M. edulis* and *M. trossulus* sampled from eastern Maine with *M. edulis* sampled from central Maine.

The growth data in this experiment may provide some additional insight regarding the lack of significant differences in species-specific larval mortality. Originally, I intended to use shell growth as an indicator of sub-lethal stress. However, mussel larvae can survive a wider range of temperature and salinity than is satisfactory for growth, and morphological and physiological differentiation can proceed in the absence of shell growth (Brenko and Calabrese, 1969; Pechenik et al., 1990). In fact, shell growth and tissue growth are not tightly coupled (Widdows, 1991) and shell growth can continue during periods of starvation. Indeed, Loosanoff (1963) saw substantial variation in the

size of larvae at the time of eyespot development, metamorphosis initiation, foot development, as well as other internal morphological organization, even among full siblings.

Even so, larval shell length and growth rates in this experiment were not comparable to those reported in other studies on *M. edulis* larvae. In general, the average sizes expected of larvae in the D-stage is approximately 93 μm (Loosanoff, 1963; Bayne, 1976b; Sprung 1984a-c; Pechenik et al, 1990; and Lutz and Kennish, 1992). In this experiment, the average size of larvae at day 4 (D-stage larvae) was approximately 89.4 μm, which is comparable to those reported in the literature. By day 10 (veliger stage), the average size across all temperatures was 119.7 μm; larvae continued to grow throughout the experiment and by day 20 (pediveliger stage) the larvae had reached a mean size of 147.2 μm. At the conclusion of the experiment, the larvae were approximately 175.1 μm in size, which is much lower than the reported values of approximately 320μm. The larval stocking densities, feeding rates, and concentration of live macroalgae were comparable to the methods of Sprung (1984 a-c), Widdows (1991), and Conte et al. (1994). Thus, my rearing methods should not have limited growth of the larvae. Even so, the reduced growth seen in all treatments may be an indication that the larvae were under another source of physiological stress not related to the temperature treatments in this experiment. This stress could have manifested itself not only as sub-optimal growth but also in overall lower survivorship of both species of larvae.

Alternatively, the apparent correlation between the EMCC and the distribution of *M. trossulus* in the Gulf of Maine may be an historical artifact. After the last glacial recession (approximately 12 – 18,000 years ago), the re-opening of the Bering seaway

appears to have facilitated the recolonization of the Northwest Atlantic (Vermeij, 1991). Biotas of the North Atlantic and Arctic underwent a dramatic transformation during this time, with a flood of invading species from the North Pacific entering the Arctic and Atlantic Oceans through the Bering Strait. In fact, Vermeij (2001) indicates that this effect was particularly great on the American side of the North Atlantic, with some 83% of rocky, intertidal molluscs and 50% of sandy and muddy shore molluscs showing ancestral lines to Pacific invaders. Indeed, genetic evidence suggests that *M. trossulus* has recently re-invaded the Northwest Atlantic (Rawson, pers. comm.). Thus, there is a possibility that neither dispersal nor thermal tolerance is creating a range boundary for this species but rather the range of *M. trossulus* is still expanding and the current southern range limit merely coincides with the hydrographic features in the Gulf of Maine.

In conclusion, this experiment examined the effects of elevated temperatures on the survival, development, and growth of *M. edulis* and *M. trossulus* larvae throughout development. Larval survival varied significantly with age and temperature had a significant impact on survival for both species. However, there were no significant differences in larval mortality between *M. edulis* and *M. trossulus*. In general, the results of this experiment do not support the hypothesis that the range limit of *M. trossulus* is structured by the temperature differential created by the EMCC in the Gulf of Maine.

LITERATURE CITED

Bates, J. A., and D.J. Innes. 1995. Genetic variation among populations of *Mytilus* spp. in eastern Newfoundland. Marine Biology. **12**:417-424.

Bayne, B.L. 1976a. Growth and delay of metamorphosis of the larvae of *Mytilus edulis* (L.). Ophelia. **2**:1-47.

Bayne, B.L. (ed). 1976b. Marine mussels: Their ecology and physiology. Cambridge University Press, Cambridge.

Begon, M., J.L. Harper, and C.R. Townsend. 1986. Ecology: Individuals, populations, and communities. Sinauer Assoc., Inc., Sunderland, MA.

Brenko, M. H., and A. Calabrese. 1969. The combined effects of salinity and temperature on the larvae of the mussel *Mytilus edulis*. Marine Biology. **4**:224-226.

Brooks, D.A., and D.W. Townsend. 1989. Variability of the coastal current and nutrient pathways in the eastern Gulf of Maine. Journal of Marine Research. **47**:303-321.

Cerame-Vivas, M. J., and Gray, I. E. 1966. The distributional pattern of benthic invertebrates of the continental shelf off North Carolina. Ecology. **47**:260–270.

Comesaña, A.S., J.E. Toro, D.J. Innes, and R.J. Thompson. 1999. A molecular approach to the ecology of a mussel (*Mytilus edulis – M. trossulus*) hybrid zone on the east coast of Newfoundland, Canada. Marine Biology. **133**:213-221.

Engle, V. D., and J.K. Summers. 2000. Biogeography of benthic macroinvertebrates in estuaries along the Gulf of Mexico and western Atlantic coasts. Hydrobiologia. **436**:17–33.

Gaylord, B., and S.D. Gaines. 2000. Temperature or Transport? Range limits in marine species mediated solely by flow. The American Naturalist. **155**:769-789.

Gosling, E. (ed). 1992. The mussel *Mytilus*: Ecology, physiology, genetics, and culture. Elsevier, Amsterdam.

Hayhurst, S. 2001. The effects of temperature on the survival, growth, and development of larvae of two blue mussel species (*Mytilus edulis* and *Mytilus trossulus*). MS Thesis, University of Maine.

Heath, D.D., P.D. Rawson, and T.J. Hilbish. 1995. PCR-based nuclear markers identify alien blue mussel (*Mytilus* spp.) genotypes on the west coast of Canada. Canadian Journal of Fish and Aquatic Sciences. **52**:2621-2627.

Koehn, R.K. 1991. The genetics and taxonomy of species in the genus *Mytilus*. Aquaculture. **94**:125-145.

Koehn, R.K., J.G. Hall, D.J. Innes, and A.J. Zera. 1984. Genetic differentiation of *Mytilus edulis* in eastern North America. Marine Biology. **79**:117-126.

Loosanoff, V.L., and H.C. Davis. 1963. Rearing of bivalve mollusks. In: Russell, F.S., 1963. Advances in marine biology, Volume 1. Academic Press, London and New York: 2-136.

Lutz, R.A., and M.J. Kennish. 1992. Ecology and morphology of larval and early postlarval mussels. In: Gosling, E. (ed) The mussel *Mytilus*: Ecology, physiology, genetics, and culture. Elsevier, Amsterdam: 53-80.

Mallet, A.L., and C.E. Carver. 1995. Comparative growth and survival patterns of *M. trossulus* and *M. edulis* in Atlantic Canada. Canadian Journal of Fish and Aquatic Sciences. **52**:1873-1880.

Maloy, A., B.J. Barber. and P.D. Rawson. 2003. Gametogenesis in a sympatric population of blue mussels, *Mytilus edulis* and *Mytilus trossulus* from Cobscook Bay (USA). Journal of Shellfish Research. **22**:119-123.

McDonald, J.H., R. Seed and R.K.Koehn. 1991. Allozymes and morphometric characters of three species of *Mytilus* in the Northern and Southern Hemispheres. Marine Biology. **111**:323-333.

Nelson, J.S., R. J. Hoddell, L. M. Chou, W. K. Chan, and P. E. Phang. 2000. Phylogeographic structure of false clownfish, *Amphiprion ocellaris*, explained by sea level changes on the Sunda shelf. Marine Biology. **137**:727-736.

Pechenik, J.A., L. S. Eyster, J. Widdows, and B.L. Bayne. 1990. The influence of food concentration and morphological differentiation of blue mussel *Mytilus edulis* L. larvae. Journal of Experimental Marine Biology and Ecology. **136**:47-64.

Pettigrew, N. R., D.W. Townsend, H. Xue, J.P. Wallinga, P.J. Brickley, and R.D. Hetland. 1998. Observations of the Eastern Maine Coastal Current and its offshore extension in 1994. Journal of Geophysical Research. **103**:30623-30639.

Pielou, E.C. 1979. Biogeography. Wiley, New York.

Rawson, P.D., S. Hayhurst, and B. Vanscoyoc. 2001. Species composition of blue mussel populations in the Northeastern Gulf of Maine. Journal of Shellfish Research. **20**:31-38.

Rawson, P.D., and T.J. Hilbish. 1995. Evolutionary relationships among the male and female mitochondrial DNA lineages in the *Mytilus edulis* species complex. Molecular Biology and Evolution. **12**:893-901.

Rawson, P.D., K. Joyner, and T.J.Hilbish. 1996. Evidence for intragenic recombination within a novel genetic marker that distinguishes mussels in the *Mytilus edulis* species complex. Heredity. **77**:599-607.

Rice, W.R. 1989. Analyzing tables of statistical tests. Evolution. **43**:223-225.

Roy, K., D. Jablonski, and J.W. Valentine. 1994. Eastern Pacific molluscan provinces and latitudinal diversity gradient: No evidence for "Rapoport's rule". Proceedings of the National Academy of Science USA. **91**:8871-8874.

Roy, K., D. Jablonksi, J.W. Valentine, and G. Rosenberg. 1998. Marine latitudinal diversity gradients: Tests of causal hypotheses. Proceedings of the National Academy of Science USA. **95**:3699-3702.

Saunders, N. C., L. G. Kessler, and J. C. Avise. 1986. Genetic variation and geographic differentiation in mitochondrial DNA of the horseshoe crab, *Limulus polyphemus*. Genetics. **112**:613-627.

Scheltema, R.S. 1992. Passive dispersal of planktonic larvae and the biogeography of tropical sublittoral invertebrate species. In: Colombo et al., 1992. Marine eutrophication and population dynamics. Olsen and Olsen, Denmark: 195-202.

Sprung, M. (1984a). Physiological energetics of mussel larvae (*Mytilus edulis*). I. Shell growth and biomass. Marine Ecology Progress Series. **17**:283-293.

Sprung, M. (1984b). Physiological energetics of mussel larvae (*Mytilus edulis*). II. Food Uptake. Marine Ecology Progress Series. **17**:295-305.

Sprung, M. (1984c). Physiological energetics of mussel larvae (*Mytilus edulis*). III. Respiration. Marine Ecology Progress Series. **18**:171-178.

Sprung, M., and J. Widdows. 1986. Rate of heat dissipation by gametes and larval stages of *Mytilus edulis*. Marine Biology. **91**:41-45.

Strathmann, R.R. 1985. Feeding and nonfeeding larva development and life-history evolution in marine invertebrates. Annual Review of Ecology and Systematics. **16**:339-361.

Townsend, D.W. 1992. Ecology of larval herring in relation to the oceanography of the Gulf of Maine. Journal of Plankton Research. **14**:467-493.

Vermeij, G. L. 1991. Anatomy of an invasion: The trans-Atlantic interchange. Paleobiology. **17**:281-307.

Vermeij, G. J. 2001. Community assembly in the sea: Geologic history of the living shore biota. In: Bertness, M.D., S.D. Gaines and M.E. Hay. 2001. Marine community ecology. Sinauer Assoc., Inc., Sunderland, MA: 39–60

Wares, J. P., S. D. Gaines, and C. W. Cunningham. 2001. A comparative study of asymmetric migration events across a marine biogeographic boundary. Evolution. **55**:295-306.

Widdows, J. 1976. Physiological adaptation of *Mytilus edulis* to cyclic temperatures. Journal of Comparative Physiology. **105**:115-128.

Widdows, J. 1991. Physiological ecology of mussel larvae. Aquaculture. **94**:147-163.

Widdows, J., and B. L. Bayne. 1971. Temperature acclimation of *Mytilus edulis* with reference to its energy budget. Journal of Comparative Physiology B. **51**:827-843.

APPENDIX

Independent F-tests of Species-Specific Mortality

Introduction	Age-block	Temperature	*P*-value
36 hpf	1	14 °C	0.0189
$\alpha = 0.0056$	2	14 °C	0.5273
	3	14 °C	0.3007
	1	18 °C	0.0126
	2	18 °C	0.2742
	3	18 °C	0.6224
	1	22 °C	0.4693
	2	22 °C	0.9784
	3	22 °C	0.4566
11 dpf	2	14 °C	0.9238
$\alpha = 0.0083$	3	14 °C	0.8796
	2	18 °C	0.0757
	3	18 °C	0.4914
	2	22 °C	0.3372
	3	22 °C	0.0392
21 dpf	3	14 °C	0.8302
$\alpha = 0.0167$	3	18 °C	0.4972
	3	22 °C	0.6141

Independent *F*-test results determining significant differences between species-specific mortality regression slopes. For each age at introduction, the experiment-wide α was held at 0.05. The significance level for each comparison was adjusted using the Bonferroni correction. Thus, α-levels of 0.0056, 0.0083, and 0.0167 were used for comparisons at 36 hpf, 11 dpf, and 21 dpf, respectively.

BIOGRAPHY OF THE AUTHOR

Susan J. Limbeck was born in Rochester, New York on September 10, 1977. She was raised in Rochester and graduated from Gates Chili High School in 1995. She attended the University of South Carolina in Columbia, South Carolina. She graduated with a Bachelor's degree in Marine Science in May 1999. After working for two years for a private environmental laboratory, she entered the Master's program in marine biology in the School of Marine Sciences at The University of Maine.

After receiving her degree, Susan is looking forward to her upcoming wedding as well as a promising career in marine science. Susan is a candidate for the Master of Science degree in Marine Biology from The University of Maine in December, 2003.