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ORIGINAL PAPER

Family effects on the growth and survival of congeneric blue mussel larvae (*Mytilus edulis* and *M. trossulus*)

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Abstract Intraspecific genetic variation is widely recognized to affect how bivalve larvae respond to variation in environmental conditions, but has been largely ignored in comparisons of larval performance between closely related sibling species. Replicates of five different full-sib families of larval blue mussels (*Mytilus edulis* and its more northerly congener, *M. trossulus*) were reared under different temperature (10, 13, and 17 °C) and food conditions in a full factorial design. Growth and survival were strongly affected by temperature, family, the interaction of family and temperature, and the three-way interaction of family, temperature, and food. Family means for days to 20 % survival ranged from 10.2 to 20.0 and did not cluster by species. The two *M. trossulus* families were intermediate in survival and ranked first and third for growth. The temperature by family interaction effect for survival was very strong, and the two *M. trossulus* families responded very differently to temperature manipulations. One *M. trossulus* family exhibited highest survival at 13 °C, while the other *M. trossulus* family and all three *M. edulis* families exhibited highest survival at 10 °C. By contrast, greatest growth consistently occurred at 17 °C, indicating that higher mortality in warmer water may be at least partially offset (at a population level) by more rapid growth in body size. The results illustrate the importance of assaying both growth and survival when evaluating environmental effects on larvae and the need to ensure a high level of genetic diversity when pools of larvae are used to study genetically similar species.

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Introduction

The larvae of benthic invertebrates potentially encounter a broad range of environmental conditions. Consequently, understanding how larvae respond to variation in environmental conditions (e.g., salinity, temperature, food availability) is critical for evaluating physiological stress, life history evolution, and environmental impacts on population dynamics (Hoegh-Guldberg and Pearse 1995). The dispersal consequences of environmental tolerance are beginning to be incorporated into coupled biophysical transport models used to model larval dispersal (Xue et al. 2008; Inzce et al. 2010), and information about environmental effects on growth and survival is thus also critical for evaluating scenarios in which larvae move among different water masses or in which water masses bearing larvae are subject to heating or cooling. Assessing both growth and survival is important, because environmental conditions that are optimal for growth need not be identical to those that are optimal for survival (Lough 1975).

Considerable evidence indicates that intraspecific genetic variation plays a substantial role in modulating larval response to environmental variation. Numerous studies on bivalves have explored genetic effects on larval performance using either experimentally produced full or half-sib families (Innes and Haley 1977; Hilbish et al. 1993),

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selected breeding lines (Taris et al. 2007; Curole et al. 2010), or distinct populations (Eierman and Hare 2013). In the best-studied bivalve taxon (oysters), we are beginning to understand both the physiological basis of variation in environmental tolerance (Pace et al. 2006; Pace and Manahan 2007; Tamayo et al. 2014) and the specific genes involved (Hedgecock et al. 2007).

The effect of environmental conditions on larval performance is also critical for understanding the dynamics of hybridizing sibling species. Performance of larvae from hybrid versus homospecific crosses is often assessed to evaluate potential selective pressures that might promote or deter hybridization (Bierne et al. 2002; Matson et al. 2003; Toro et al. 2004). Differences in larval and juvenile environmental tolerance have also been invoked to explain habitat preferences (Riginos and Cunningham 2005) and the location of range boundaries (Gilg and Hilbish 2003; Gilg et al. 2009; Hayhurst and Rawson 2009).

However, while genetic variation in larval performance is well documented in individual species, the role of intraspecific genetic variation has received little attention in the context of sibling species comparisons. Experiments on conspecifics are generally based on pools of larvae for the congeners that are produced from multiple parents, though both the number of parents contributing and the experimental control over mating design varies widely (Bierne et al. 2002; Hayhurst and Rawson 2009; Toro et al. 2012). An alternative approach involves creating and monitoring multiple full or half-sib families of larvae (Beaumont et al. 2005), with the results then used to explicitly evaluate within species versus between species variation in larval performance. However, this approach requires large sample sizes and is consequently very labor intensive. Yet without evaluating potential family effects, it is impossible to assess the extent to which apparent minor differences in larval performance between closely related species might be generated from random sampling involved in creating larval pools, rather than from more biologically relevant differences between species.

We explored the growth and survival of larvae from full-sib families of two sibling species of blue mussels, *Mytilus edulis* and *M. trossulus*, under a range of temperature conditions and food regimes. These closely related congeners, as well as other species pairs in the genus, have previously been the subject of larval performance studies that reported very minor apparent differences between species (Bierne et al. 2002; Matson et al. 2003; Beaumont et al. 2005; Hayhurst and Rawson 2009; Toro et al. 2012). The *M. edulis* southern range boundary lies 980 km to the southwest of the *M. trossulus* range boundary, but the two congeners overlap in eastern Maine (Rawson et al. 2007) and through most of the Canadian Maritime Provinces to northern Newfoundland (Bates and Innes 1995; Mallet and Carver 1995), while *M. trossulus* extends further north into Labrador. Most individuals can be assigned to a species on the basis of diagnostic molecular markers and first generation hybrids are rare; however, backcross hybrids are common (Slaughter et al. 2008) and many of the individuals that are nominally designated as pure species are likely to possess some alleles that have introgressed across species lines (Hayhurst and Rawson 2009). The southern range boundary of *M. trossulus* is located in eastern Maine and is associated with the divergence of the cold Eastern Maine Coastal Current (EMCC) from shore (Rawson et al. 2003, 2007; Tilburg et al. 2012; Yund et al. 2015). Consequently, the strong association between *M. trossulus* and the EMCC raises the question of whether variation in larval thermal tolerance between species contributes to the location of the range boundary (Hayhurst and Rawson 2009). Species-specific differences in larval performance have also been used to evaluate sources of selection on hybrids (Toro et al. 2004).

We created replicate cultures of each of five full-sib families (three *M. edulis* and two *M. trossulus*) and raised them at three water temperatures and under two food levels in a full factorial design, with eight replicates per family/temperature/food treatment combination. Because we were interested in how family identity interacted with abiotic and biotic conditions, the number of families tested was necessarily limited. Consequently, this experimental design assessed the potential effect of family-level genetic diversity on our evaluation of the impacts of other factors on larval performance, but does not represent a comprehensive examination of family effects on species-level performance per se. Addressing that question would require monitoring substantially more families, but would be extremely difficult in a multifactorial context (i.e., it would be feasible to monitor more families if fewer environmental variables were manipulated, or fewer levels used per variable). Cultures were monitored weekly for survival for 28 days, while growth was assessed as larval size at the termination of the experiment. Three-way analyses of variance were then used to partition variance in growth and survival into family, temperature, and food effects, plus all three pairwise interaction effects and the three-way interaction among main effects. Post hoc tests on main effects and pairwise interactions were used to further evaluate family and family interaction effects and to assess whether species identity contributed to growth or survival variation.

Materials and methods

Species identification, spawning, and creation of crosses

These two mussel species cannot be reliably distinguished by morphology, and our past experience showed the success

of spawning an individual mussel that had been genotyped in advance to be poor. Consequently, we spawned mussels from a mixed population of the two species and conducted numerous controlled crosses, then used diagnostic molecular markers to establish the species identity of the parents during the 3 d that the embryos were developing and had not yet reached a feeding stage. A subset of the initially created families was selected for the experiment.

Mussels were collected immediately prior to the natural spawn in 2013 from sites in Cobscook Bay, Maine, where *M. edulis* and *M. trossulus* are approximately equally abundant (Rawson et al. 2001, 2003, 2007; Slaughter et al. 2008). They were isolated in aged seawater and induced to spawn via heat shocks and exposure to hydrogen peroxide as described in Rawson et al. (2003, 2007) and Slaughter et al. (2008). Once numerous individuals had spawned, controlled crosses were conducted via methods described in the same two papers. We combined 0.5 ml of an egg solution (approximately 3.16×10^5 eggs ml^{-1} for *M. trossulus* and 3.46×10^5 eggs ml^{-1} for *M. edulis*) and 0.1 ml of sperm suspension (dry sperm diluted 1:100 in aged seawater; hereafter, ASW) from a unique male/female combination in 5 ml of ASW. This gamete ratio and sperm concentration results in the fertilization of circa 90–95 % of the eggs in homospecific crosses with virtually no detectable consequences of polyspermy (Slaughter et al. 2008).

A tissue sample was collected from each spawned mussel and preserved in salt-saturated dimethyl sulfoxide (Seutin et al. 1993) for immediate genotyping at three diagnostic loci (Glu-5′, Mal-1, and ITS; Heath et al. 1995; Rawson et al. 1996a, b) that distinguish the two species and hybrids. For genotyping, we used the methods described in Slaughter et al. (2008), except that DNA extractions were performed using the DNeasy kit (Qiagen, Valencia California).

Similar past experiments conducted using these methods and the same source populations have always yielded many more *M. edulis* crosses than *M. trossulus* crosses (Slaughter et al. 2008), and results from the present matings were consistent; genotyping revealed that we had conducted many more crosses involving nominally pure *M. edulis* mussels (i.e., pairs of parents that each carried six *M. edulis* alleles at the three diagnostic loci) than nominally pure *M. trossulus* animals (pairs of parents that both carried six *M. trossulus* alleles at the three diagnostic loci). Because the limited number of markers we used would have led us to misclassify backcross hybrids (especially late-generations) as pure species (Boecklen and Howard 1997), these nominally pure crosses may nevertheless have included individuals that were introgressed. With three codominant markers at loci carrying species-diagnostic alleles (as used in this study), the probability of error (P_e) for misclassifying backcross hybrids as pure species increases with the generation of backcrossing; backcross generation 1, $P_e = 0.15$; 2, $P_e = 0.45$; 3, $P_e = 0.68$; 4, $P_e = 0.85$; 5, $P_e = 0.90$ (Boecklen and Howard 1997). However, with our three markers, we can be confident that an individual we type as "*M. edulis*" is not an *M. trossulus* ($P_e = 0$), an F_1 hybrid ($P_e = 0$), or an F_2 hybrid ($P_e < 0.05$). See Boecklen and Howard (1997) for a full treatment of these assignment issues.

Three *M. edulis* families were selected from numerous ones available on the basis of two criteria: (1) They yielded >180,000 developing embryos per cross, and (2) they represented unique male and female combinations. A single nominally pure *M. trossulus* family was also included in the experiment. Due to the shortage of pure *M. trossulus* crosses, a second *M. trossulus* family was selected that was the product of mating a pure *M. trossulus* male with a female that carried five *M. trossulus* alleles and one *M. edulis* allele (i.e., a backcrossed hybrid that contained mostly *M. trossulus* genes). The progeny produced by this cross are expected to have had a genetic composition that averaged 92 % *M. trossulus*. For convenience, the three experimental *M. edulis* families are hereafter labeled E1-3 and the two *M. trossulus* families are labeled T1 and T2, with T1 representing the genetic composition that was likely more strongly biased toward *M. trossulus*.

Experimental treatments

We reared larvae of these three *M. edulis* and two *M. trossulus* families under three different temperature conditions and two different food regimes in a full factorial design and monitored growth and survival for 28 days. Eight replicates of each family, temperature, and food treatment combination were housed in separate 1-L glass jars, resulting in a total of 240 larval cultures. The planned analysis (see following section) partitioned the variance among seven effects and thus required this level of replication to estimate the variance within each treatment combination. Each replicate was initiated with 3000, 72-h-old early veliger larvae in 800 ml of filtered seawater, for a density of 3.75 larvae ml^{-1}. Three temperature treatments were established at target temperatures of 10, 13, and 17 °C via two recirculating water baths (with temperature maintained by heat pumps) and an incubator. Selected temperatures ranged from the approximate temperature at spawning time for both species (Maloy et al. 2003) in Cobscook Bay (10 °C) to the slightly elevated temperature that larvae are likely to experience just inshore of the EMCC (13 °C; Tilburg et al. 2012), to a temperature that is representative of the upper portion of local estuaries south of Cobscook Bay (17 °C, unpublished data). A temperature logger (Onset Instruments, Cohasset, MA) programmed to record at 15-min intervals was added to a blank jar in each temperature treatment to monitor actual versus nominal temperatures.

Jars in the two food treatments received either a high or low daily food ration. The food supply consisted of an equal mixture (by volume) of five different phytoplankton cultures. The density of each culture varied slightly among days, but during the experiment the component cultures averaged (±SE): *Rhodomonas salina*, 1.22×10^6 cells $ml^{-1} \pm 8.51 \times 10^4$; *Isochrysis galbana* (cultivar C-Iso), 4.67×10^6 cells $ml^{-1} \pm 8.19 \times 10^5$; *Isochrysis galbana* (cultivar T-Iso), 4.73×10^6 cells $ml^{-1} \pm 1.13 \times 10^6$; *Chaetoceros muelleri* (also known as Chagra), 5.66×10^6 cells $ml^{-1} \pm 4.41 \times 10^5$; *Chaetoceros calcitrans* (formerly known as *Chaetoceros* sp. B; Pettersen et al. 2010), 5.27×10^6 cells $ml^{-1} \pm 5.71 \times 10^5$. This combination of algae has been used to rear mussel larvae at The Downeast Institute for years, and all the component species have also been used to raise bivalve larvae in other labs (Nevejan et al. 2007; Pettersen et al. 2010; Marshall et al. 2010). The two food treatments consisted of additions of different amounts of this phytoplankton mixture to the 800 ml cultures (i.e., food quantity was manipulated, not quality), with the low food treatment composed of 50 % of the food added in the high food treatment. Our goal in the low food treatment was to subject larvae to some minor nutritional stress, but to avoid elevating mortality rates to the point that cultures in the low food treatment perished completely by the end of the experiment. As larval densities decreased, we progressively reduced the absolute amount of food added to cultures in both treatments, according to the following schedule: days 0–6, 4.6 ml jar^{-1} day^{-1} high treatment versus 2.3 ml jar^{-1} day^{-1} low treatment; days 7–14, 2.2 ml jar^{-1} day^{-1} versus 1.1 ml jar^{-1} day^{-1}; days 15–21, 1.6 ml jar^{-1} day^{-1} versus 0.8 ml jar^{-1} day^{-1}; days 22–28, 1.0 ml jar^{-1} day^{-1} versus 0.5 ml jar^{-1} day^{-1}. This reduction over time dampened temporal variation in the ratio of phytoplankton cells to mussel larvae. Due to the size of the experiment, we were unable to monitor larval densities in real time and adjust food levels individually for each jar. An inevitable consequence of this approach was to decrease the actual density of food in the culture jars over time. In the absence of field data on feeding rates in mussel larvae, it is difficult to assign environmental equivalencies to these feeding levels. However, the high food treatment reflected food levels in an aquaculture environment and likely provided more abundant food than most natural environments, while the low food treatment was somewhat more limited, but still high enough to support rapid growth.

The seawater medium was filtered to one μm and changed three times per week. Prior to each water change, replacement water was preheated or cooled to match the incubation temperature treatments. To prevent contamination among cultures, we used unique sets of sieves dedicated to the two different species. While processing replicates within a family/temperature/food treatment combination, we alternated between multiple sieves, with each sieve rinsed in freshwater before use in another jar. Each set of sieves was then soaked in an iodine sterilizing solution and rinsed before proceeding to the next family/temperature/food treatment combination. A pilot experiment indicated that in the absence of these steps, contamination among jars was likely to occur.

Survival was monitored by preserving an aliquot from each jar once per week (on days 0, 7, 14, and 21). The size of the aliquot varied among individual cultures and was based on the preceding count to yield an anticipated sample on the order of dozens of larvae. We were unable to collect larger aliquots because doing so would have driven the cultures extinct prior to the conclusion of the experiment. At the termination of the experiment (day 28), all larvae remaining in each culture were preserved to provide a final survival estimate and material for a final size determination. Raw larval counts from aliquots were multiplied by appropriate scaling factors to estimate the total count in each jar and then converted to percent survivorship by dividing by the day 0 total and multiplying by 100. However, the number of larvae removed in any earlier aliquot was first subtracted from the day 0 total so that larvae collected in earlier intervals did not register as deceased. To assess growth, larvae from day 0 aliquots and the final day 28 sample were individually picked and then measured under a compound microscope (at 100×) equipped with a digital camera (Lumenera Infinity 2) interfaced to a computer. Larval measurements included length (parallel to the valve hinge), width (perpendicular to the length), and cross-sectional area. At least three larvae were measured per aliquot unless <3 were present. A mean size was calculated for each replicate jar if >1 larva was measured. Due to constraints on partitioning the available degrees of freedom, size variation within each jar was omitted from the analysis. Although larvae had attained sufficient size to potentially settle by the end of the experiment, none had actually settled in the cue-free environment of the glass culture jars. Furthermore, the combination of repeated sampling and mortality had reduced the number of larvae in some of the cultures to the point where it was not feasible to induce settlement and calculate percent metamorphosis.

Statistical analyses

The nonlinear curve fitting platform in JMP (V9) was used to fit two-parameter negative exponential curves of the form %Survival = a × Exp(b × Day) to survival as a function of day for each replicate. Parameter estimates from the fit curves were then used to calculate the day at which survival dropped to 20 % (Fig. 1; hereafter termed S_{20}). This approach allowed us to base our survival estimate on the entire relationship between survival and time

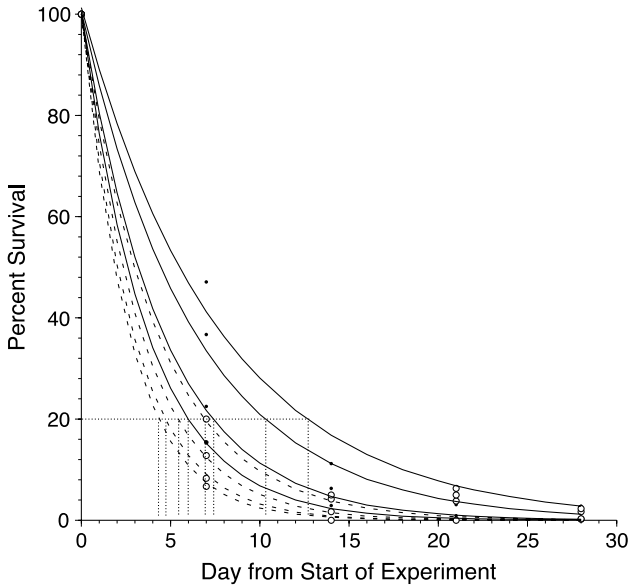

Fig. 1 Example of survival curve fitting and illustration of S_{20} calculations. *Solid circles* and *lines* represent survival data and fitted negative exponential *curves* for four replicates of family E3, while *open circles* and *coarse dashed lined* represent data and *curves* for family E1. All data are from a 17 °C temperature and low food treatment. Only *data* and *curves* from four out of the eight replicates in each family are presented in the interest of visual clarity. The *fine dashed vertical lines* provide a graphical representation of S_{20} computations, with the calculated values indicated by the intersection of the *vertical lines* and *X*-axis

within each culture, rather than a single time point, but still present survival as a single intuitive value (as opposed, e.g., to directly analyzing the parameters from the survival curves). For each S_{20} estimate we also calculated an associated coefficient of variation and then assessed the reliability of the individual S_{20} values by examining the fit of the survival curves and the distribution of the coefficients of variation among all the different replicates. Survival data that passed this quality control procedure were then analyzed via a multi-way ANOVA, with family, temperature, and food treatment as main effects. All three effects were considered fixed rather than random. Temperature and food treatments tested specific levels based on criteria a priori, not a random selection of levels. The family treatment similarly tested families with specific genetic profiles that were non-randomly drawn out of a larger pool of available families. All pairwise interactions and the single 3-way interaction term were also included, for a total of 7 factors in the model. Final size data were analyzed via a multi-way ANOVA with identical effects. For both size and survival, differences among specific levels within significant main and pairwise interaction effects were analyzed via post hoc tests. A one-way ANOVA was used to determine whether larval size on day 0 varied among the families prior to the establishment of individual culture flasks. Treatment effects were irrelevant in this analysis because the treatments had not yet commenced.

Results

Culture temperatures

Temperatures in our 10, 13, and 17 °C treatments averaged 9.9, 12.8, and 17.0 °C, respectively, during the course of the 28-day culture experiment. Standard errors were substantially <0.1 °C in all three treatments. For convenience sake, we will continue to refer to the three treatments by their nominal temperatures.

Survival

Of the 240 individual replicate cultures in the experiment, two immediately showed 100 % mortality (before the first data collection on day 7), presumably because of handling errors on setup, and so they were excluded from the analysis. The vast majority of the remaining 238 cultures exhibited an exponential decline in number that was well described by the negative exponential function. However, 11 suffered from poor curve fits and did not exhibit an exponential decline. In all 11 cases, the lack of fit was caused by one or more percent survival values at days 7–21 that were >100 %. We suspect that the source of the problem was a starting number underestimate for these cultures, which inflated subsequent survival values. As a consequence of the poor fits in these cases, the coefficients of variation for the S_{20} values were more than two standard deviations from the mean for the group. Consequently, these 11 cultures were also excluded from the analysis, producing a final sample size of 227 individual cultures.

The number of days to 20 % survival (S_{20}) was significantly affected by family and temperature, the interaction of temperature and family, and the three-way interaction of family, temperature, and food treatment (Fig. 2a, b; Table 1). Among those four significant effects, the temperature by family interaction was the strongest, followed by the main effects of family and temperature, with the three-way interaction somewhat weaker (compare sums of squares in Table 1). The dominant effect on survival was thus variation among families in how they responded to temperature. The family ranks for the least square means for survival (i.e., family means adjusted for the other effects in the model) did not correspond to species identity (Table 2); family E2 had the highest mean S_{20}, while E1 and E3 had the lowest, with T1 and T2 intermediate. Within the temperature effect, survival was significantly higher at 10 °C, but it did not differ between 13 and 17 °C

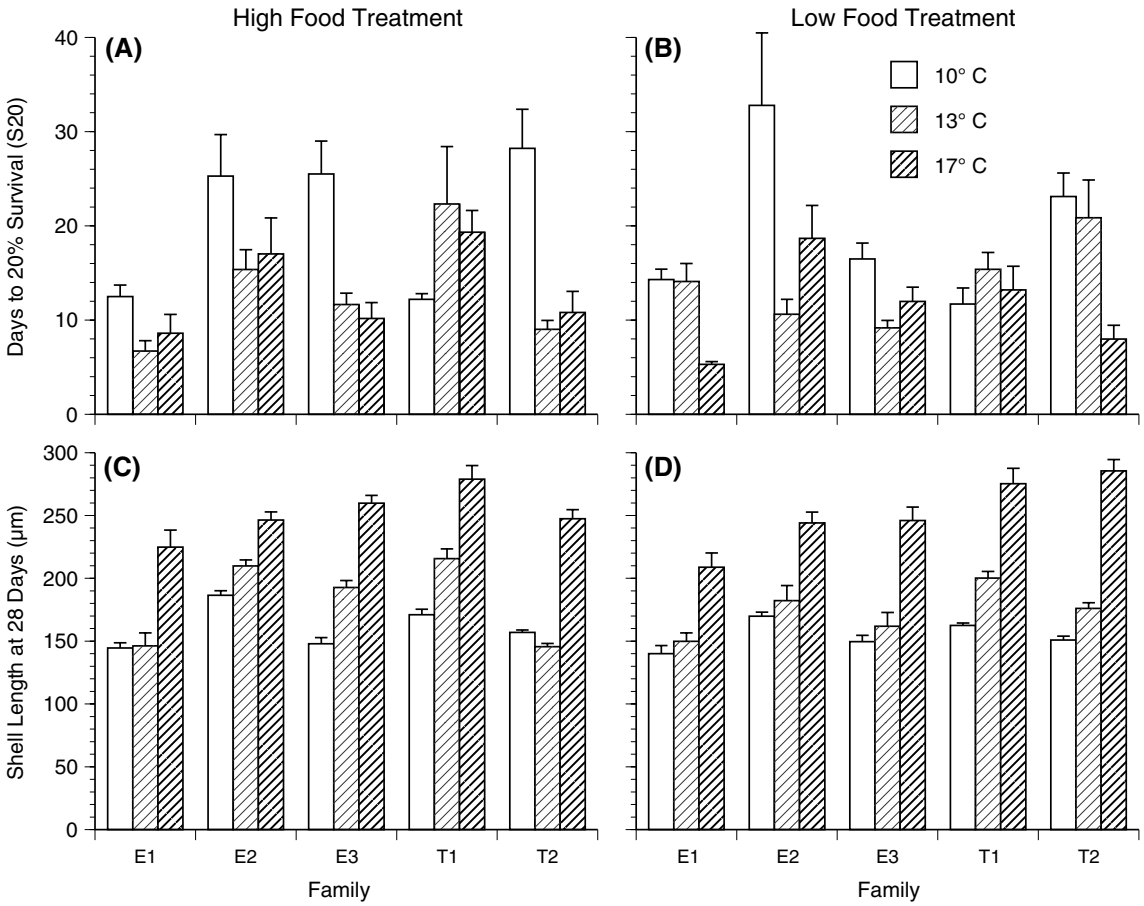

Fig. 2 Larval survival (**a**, **b**) and growth (**c**, **d**) for the five families reared under three temperature conditions and two food treatments. Families E1–E3 represent the three *Mytilus edulis* families, while families T1 and T2 represent the two *M. trossulus* families. Starting densities were 3000 larvae in 800 ml. Sample sizes for final shell lengths ranged from 1 to 3 larvae per culture and 5 to 8 cultures per treatment. *Error bars* represent one standard error

Table 1 ANOVA results for the number of days to 20 % larval survival (S_{20})

Source	*df*	Sums of squares	*F*	*P*
Family	4	2192.2	9.99	**<0.0001**
Temperature	2	2662.4	24.28	**<0.0001**
Food	1	20.1	0.37	0.5454
Temperature × family	8	3075.5	7.01	**<0.0001**
Food × family	4	416.7	1.90	0.1120
Food × temperature	2	78.5	0.72	0.4903
Food × temperature × family	8	1338.6	3.05	**0.0029**
Error	197	10,802.3		

Probability values for significant effects are highlighted in bold

(Table 2). While most individual families conformed to this general pattern with respect to temperature, family T1 exhibited highest survival at 13 °C in both food treatments (Fig. 2a, b), and this difference is reflected in the significant temperature by family interaction effect (Table 2). The other patterns that emerge from the complex interaction are the particularly high survival of families E2, E3, and T2 at 10 °C and low survival of families E1, E3, and T2 at 17 °C and E1 and E3 at 13 °C (Table 2). Contrary to simplistic expectations based on the distributional ranges of the two species, warmer temperatures did not have a greater effect on survival of *M. trossulus* families, when compared to *M. edulis* families.

Growth

Larval size did not vary among the five families at the beginning of the experiment [day 0; one-way ANOVAs; length, $F(4,38) = 1.96$, $P > 0.12$; width, $F(4,38) = 1.34$, $P > 0.27$; cross-sectional area, $F(4,38) = 1.22$, $P > 0.32$]. Consequently, growth was analyzed simply as larval size at the conclusion of the experiment (28 days). Final shell length was significantly affected by family, temperature, the interaction of family and temperature and the interaction

Table 2 Least square means and results of post hoc tests (Tukey's HSD) for the significant effects of family, temperature, and the interaction effect of temperature and family on survival (S_{20})

Effect	Level	Least square mean S_{20}	Groups
Family	E2	19.95	A
	T2	16.66	A, B
	T1	15.69	A, B
	E3	14.16	B, C
	E1	10.25	C
Temperature	10 °C	20.20	A
	13 °C	13.52	B
	17 °C	12.31	B
Temperature × family	10 °C, E2	29.02	A
	10 °C, T2	25.67	A, B
	10 °C, E3	20.99	A, B, C
	13 °C, T1	18.85	B, C, D
	17 °C, E2	17.85	B, C, D, E
	17 °C, T1	16.26	C, D, E, F
	13 °C, T2	14.93	C, D, E, F
	10 °C, E1	13.36	C, D, E, F
	13 °C, E2	12.99	C, D, E, F
	10 °C, T1	11.95	C, D, E, F
	17 °C, E3	11.07	D, E, F
	13 °C, E1	10.42	D, E, F
	13 °C, E3	10.42	D, E, F
	17 °C, T2	9.39	E, F
	17 °C, E1	6.97	F

Levels within an effect that do not share a group letter differed significantly ($P < 0.05$) from one another

Table 3 ANOVA results for larval shell length at day 28

Source	*df*	Sums of squares	*F*	P
Family	4	53,740	33.0	**<0.0001**
Temperature	2	367,293	451.7	**<0.0001**
Food	1	1151	2.8	0.0942
Temperature × family	8	21,486	6.6	**<0.0001**
Food × family	4	9585	5.9	**0.0002**
Food × temperature	2	701	0.9	0.4237
Food × temperature × family	8	7570	2.3	**0.0209**
Error	191	77,655		

Probability values for significant effects are highlighted in bold

of family and food treatment, and the three-way interaction of family, temperature, and food treatment (Fig. 2c, d; Table 3). Of the five significant effects in the model, temperature was by far the strongest (Table 3), with larvae in

Table 4 Least square means and results of post hoc tests (Tukey's HSD) for the significant main effects of family and temperature and interaction effects of temperature by family and food level by family on larval shell length at day 28

Effect	Level	Least square mean length (um)	Groups
Family	T1	217.27	A
	E2	206.53	A
	T2	193.79	B
	E3	192.97	B
	E1	169.55	C
Temperature	17 °C	251.71	A
	13 °C	178.33	B
	10 °C	158.02	C
Temperature × family	17 °C, T1	277.11	A
	17 °C, T2	266.47	A, B
	17 °C, E3	252.82	A, B
	17 °C, E2	245.27	B
	17 °C, E1	216.90	C
	13 °C, T1	207.92	C
	13 °C, E2	196.06	C, D
	10 °C, E2	178.26	D, E
	13 °C, E3	177.33	D, E, F
	10 °C, T1	166.77	E, F, G
	13 °C, T2	160.88	E, F, G
	10 °C, T2	154.01	E, F, G
	13 °C, E1	149.45	F, G
	10 °C, E3	148.74	G
	10 °C, E1	142.30	G
Food × family	H, T1	221.89	A
	H, E2	214.32	A, B
	L, T1	212.65	A, B
	L, T2	204.22	A, B, C
	H, E3	200.14	B, C, D
	L, E2	198.75	B, C, D
	L, E3	185.79	C, D, E
	H, T2	183.35	D, E
	H, E1	171.93	E
	L, E1	167.17	E

Levels within an effect that do not share a group letter differed significantly ($P < 0.05$) from one another

all families and both food treatments attaining maximum size in the warmest temperature treatment (Fig. 2c, d; Table 4). However, the magnitude of the family effect was the second highest, at approximately 1/7th the temperature effect (compare sums of squares in Table 3). As with the survival results, variation in final larval size did not reflect species identity, with families T1 and E2 attaining the longest lengths, families T2 and E3 intermediate, and family E1 the shortest (Table 4). The interaction effects for size

were quite a bit weaker than the main effects (Table 3). Nevertheless, families differed in how they responded to temperature and food and the combination of temperature and food treatment. Growth in all five families was highest at 17 °C, so most of the temperature by family interaction appears to revolve around how the five families grew at 10 versus 13 °C temperatures (Table 3). The family by food interaction was dominated by variability in the responses of families T2, E2, and E3 to the two food levels; family T1 had consistently high growth and family E1 had consistently low growth under both food levels (Table 3). We conducted identical analyses for the dependent variables of shell width and shell cross-sectional area. However, the qualitative results (significant effects of family, temperature, family by temperature interaction, and the three-way interaction) were the same as those for shell length, so the quantitative results are not presented here.

Overall, growth and survival were maximized at different temperatures, with higher temperatures leading to reduced survival (Table 2), but greater final size (Table 4). Because of this apparent ecological trade-off between growth and survival, we also calculated the percentage of larvae surviving to a size of 200 microns (near settlement size) by day 28 in each culture. Small sample sizes for calculating growth percentages and the presence of numerous zeros in the final data set precluded formal statistical analysis or evaluation of family effects, but mean survival to 200 microns increased with temperature (10°, 0.71 %; 13°, 2.62 %; 17°, 4.22 %). This pattern suggests that the positive effects of higher temperatures on growth outweighed the negative effects on survival. Family effects on growth versus survival exhibited a more complex pattern than the overall pattern. Family E2 had the highest survival and second highest growth, while E3 and E1 had the lowest survival and growth, and T1 and T2 were intermediate in both respects (Tables 2, 4).

Discussion

Different thermal response of the two *M. trossulus* families

Only two *M. trossulus* families were available for this experiment. One of these (T1) exhibited highest survival at the intermediate 13 °C temperature in both food treatments (Fig. 2a, b). By contrast, the second *M. trossulus* family and all three *M. edulis* families exhibited highest survival at 10 °C (Fig. 2a, b). With only two *M. trossulus* families exhibiting different response profiles, it is difficult for us to assess whether there are consistent differences in temperature response between the two species. *Mytilus trossulus* is the northern of the two species and is generally expected to be adapted to lower, not higher, temperatures. Thus, the temperature effect on family T1 appears less likely to be typical for this species than the temperature effect on family T2. But because family T2 had a small estimated proportion of *M. edulis* genes, its genome was not exclusively of *M. trossulus* origin. A previous study by Hayhurst and Rawson (2009) examined survival in *M. trossulus* and *M. edulis* larvae, each separately pooled from multiple homospecific crosses. Their data for *M. trossulus* indicate trends toward increased mortality early on (days prior to 50 % mortality in the cultures) at 15 and 20 °C compared to 5 and 10 °C, but overall show decreased instantaneous mortality at 10 and 15 °C compared to 5 and 20 °C (figures 3 and 4, respectively, in Hayhurst and Rawson 2009). Consequently, temperature effects for our family T1 are consistent with their instantaneous mortality pattern, but results from our T2 family are more consistent with their early survival pattern. Hence, this comparison does little to resolve the issue and the species-level temperature response remains an open question that can only be answered with a study design that adequately addresses the extensive family-level variation in both species.

Highest survival and growth occurred at different temperatures

Our results demonstrate that in all five families, highest survival versus growth occurred at very different temperatures. While survival was overall significantly higher at 10 °C than at 13 or 17 °C (Table 2), growth (assayed as final size) increased continuously with temperature and was highest at 17 °C (Table 4). As noted above, the exception to this general pattern was that one *M. trossulus* family (T1) exhibited maximum growth at the intermediate temperature of 13 °C, in both food regimes (Fig. 2a, b). But even this family exhibited highest survival at a lower temperature (13 °C) than the temperature of highest growth (17 °C; Fig. 2c, d). Overall, the ultimate effect of temperature on larval performance appeared to derive from the fact that more larvae survived to a size of 200 microns at higher temperatures. While the literature contains several examples of highest larval growth and survival occurring at different temperature or salinity conditions (Calabrese 1969; Leighton 1972; Lough 1975; de Albuquerque et al. 2012), counter-examples of highest growth and survival at the same temperature appear to be more common (e.g., Tan and Wong 1996; Talmadge and Gobler 2011; Sanchez-Lazo and Martinez-Pita 2012). When growth and survival are maximized at different temperatures, the pattern is generally in the same direction we reported; higher temperatures promote growth at the expense of survival (Lough 1975).

It is possible that temperature effects on survival may be exacerbated in laboratory cultures due to high larval

densities (relative to field conditions: Yund et al. 2015) and mediated through either the accumulation of waste products or low dissolved oxygen. However, our starting density (3.75 larvae ml^{-1}) was low relative to other laboratory studies on these or closely related species (Hayhurst and Rawson 2009, 13–60 larvae ml^{-1}; Sanchez-Lazo and Martinez-Pita 2012, 15 larvae ml^{-1}; Toro et al. 2012, 8–10 larvae ml^{-1}). If the temperature effect on survival is an artifact of laboratory culture conditions, our study should have been less susceptible to this artifact than most other studies. Overall mortality was fairly high in our study, with many treatment combinations experiencing circa 90 % mortality by 28 days. While this mortality level is high relative to some other larval mollusks (e.g., 95–100 % survival to metamorphosis in the gastropods *Crepidula fornicata* and *C. plana*, which produce much larger larvae; Lima and Pechenik 1985; Pechenik and Tyrell 2015), it is typical for both experimental mussel cultures (Hayhurst and Rawson 2009; Sanchez-Lazo and Martinez-Pita 2012; Toro et al. 2012) and mass culture in an aquaculture setting (B. Beal, personal communication) and does not appear to indicate unusual problems with our particular culture conditions. Nevertheless, mortality rates in this range suggest some room for improvement in mussel larval culture techniques.

The consistency of environmental effects on growth versus survival is impossible to assess in some taxa because some studies have assayed only growth or survival (e.g., Beaumont et al. 2005; Cataldo et al. 2005; Verween et al. 2007; Hayhurst and Rawson 2009). Yet potential discrepancies between growth and survival are critical for fully evaluating environmental effects in a dispersal context. In *M. edulis*, warmer temperatures appear to decrease larval survival, but they increase growth (Fig. 2) and hence might reduce the time to metamorphosis (not assayed in this study) if onset of metamorphosis were a simple function of size (but see also Pechenik et al. 1990). Consequently, an ecological trade-off seems to exist and at a population level, growth increases in warmer water may partially compensate for survival decreases. An important parameter would be omitted if temperature effects were assessed via growth or survival alone. This trade-off may have an additional dispersal consequence. By promoting faster growth to metamorphosis, higher temperatures should decrease larval duration and hence may decrease dispersal distances, although this would depend on other factors (e.g., the distribution of suitable habitat for settlement, local hydrography, and larval behaviors) that are likely to affect realized dispersal distance per day (see Shanks 2009).

Interaction effects involving food level

Food level alone did not have a significant effect on either survival or growth (Tables 1, 3). As noted in the methods, the lower food level was intended to impose some energetic constraints on larvae, but the level chosen represented a compromise with our goal to not increase mortality to the point that cultures would go extinct prior to the termination of the experiment. In retrospect, even our low food level treatment contained sufficient phytoplankton to support reasonable survival and growth. If phytoplankton clearance rates were saturated even in the reduced food treatment, then a greater reduction relative to the high food treatment would probably have resulted in a significant main effect of food regime. However, the effect of our minimal food manipulation cannot be completely dismissed—the three-way interaction of food, temperature, and family had a significant effect on both survival and growth (Tables 1, 3), and the two-way interaction of food and family had a significant effect on growth (Table 3). Consequently, food was sufficiently reduced in the low food treatment to create a differential growth response among families, and it generated differential survival and growth responses to temperature effects among families. So while food levels were not different enough to generate a significant main effect, they nevertheless differed enough to interact with the other factors in our multi-way manipulation.

Family effects: overall performance

Three of the families (E2, T2, and T1) exhibited no apparent association between overall growth and survival. Rankings for survival versus growth were: family E2, first and second (respectively), T2, second and third, and T1, third and first (Tables 2, 4). By contrast, the remaining two families consistently ranked fourth (family E3) and fifth (E1) in both survival and growth. Hence at a family level, there was no evidence of a trade-off with some families exhibiting high survival at the expense of growth or vice versa. Rather, the dominant pattern was one of the families that exhibited consistently higher survival and growth, or consistently lower survival and growth. It may seem surprising that we detected so much variation among families in traits like survival and growth that are strongly tied to fitness and hence subject to selection. But these results pertain only to the limited range of conditions that we controlled and manipulated in the laboratory. If other environmental parameters (e.g., salinity) had been included, a fuller picture of family-level strengths and weaknesses might have emerged. In addition, performance differences among families may reflect both genetic differences and maternal effects (Bertram and Strathmann 1998) and our design did not exclude maternal effects. Consequently, the consistently poor performance of the E1 and E3 larvae might also reflect the conditioning of the maternal mussels. Alternatively, the experimental induction of spawning might introduce some artifacts that would be manifest as family effects (e.g., variation in egg maturity).

Ramifications of family effects for species-level interpretations

The family and family by treatment interaction effects demonstrated here (Tables 1, 3) suggest that careful attention should be paid to methodologies when assembling diverse larval pools to explore environmental effects on sibling species. The family effects we demonstrated did not fall out along species lines (Tables 2, 4). However, our study also did not test enough families within each species to yield a truly robust test of interspecific differences. From our limited test, it appears that intraspecific variation in larval tolerance to temperature and food regimes in *M. edulis* and *M. trossulus* (i.e., family by food treatment and temperature interactions, and the three-way interaction effect; Tables 2, 4) has the potential to exceed and obscure any interspecific differences. We do not argue that every interspecific comparison should include an explicit exploration of intraspecific variation. Such an approach would dramatically increase the effort and expense of these studies. Rather, we simply urge that if genetically diverse larval pools are used to represent species, then those pools should be assembled to ensure adequate sampling of family-level genetic diversity.

Protocols for generating such larval pools include inducing mixed spawns, in which gametes from multiple parents are spawned simultaneously into a common pool (Hayhurst and Rawson 2009), performing a single controlled fertilization with eggs and sperm pooled from multiple individuals (Bierne et al. 2002), and mixing embryos post-fertilization following the execution of multiple paired crosses (Toro et al. 2012). If large asymmetries exist in the contribution of different males, females, or male/female pairs to the developing embryo pool, the pool of larvae produced by the former two methods will be less genetically diverse than expected on the basis of the number of parents initially spawned (a laboratory version of the "sweepstakes effect" that has been proposed for wild populations; Li and Hedgecock 1998; Hedgecock and Pudovkin 2011). Manually combining gametes from paired parents and mixing the resulting embryos (Toro et al. 2012) appear to be somewhat more reliable because lottery effects can be reduced, especially if embryo production from different crosses is quantified and adjusted to ensure equal representation of each family in the final larval mixture. However, work on oysters suggests that parentage ratios in larvae produced via mixed spawns may vary throughout ontogeny (Boudry et al. 2002), suggesting a need to reassess experimentally produced mixtures at the end of a larval rearing experiment. Genetic approaches could also be used in conjunction with less controlled spawning techniques to quantify parentage. In addition, we note that any experimental design that produces a mixture of full and half-sib larval families will not capture as much genetic diversity as a full-sib design with an equal number of families.

Regardless of the technical approach, we argue that the number of families necessary to capture existing intraspecific variation may be far higher than previously expected (e.g., 4 females and 4 males per species in Bierne et al. 2002; 2–4 females and 5–6 males in Hayhurst and Rawson 2009). A simple prospective power calculation is instructive. The mean S_{20} for the three *M. edulis* families was 14.9 days with a standard deviation of 4.0 (calculated from the family effect least square means in Table 2). If that level of variance is typical for both species, we would need a sample size of 115 full-sib families in each species to detect a 10 % difference in survival between the species (treated as a *t* test for one given set of environmental conditions, at an alpha of 0.05 and power of 0.8; Snedecor and Cochran 1980). If the true difference in survival between the species was on the order of 25 %, we could detect it with only 20 full-sib families in both species (maintaining the same alpha and power). Survival differences for larvae of these same two mussel species reported in Hayhurst and Rawson (2009) fluctuated between approximately 10 and 20 %, depending on the time period. In a scenario where differences between congeners are fairly subtle and family effects are considerable, the number of families needed to adequately represent both species is substantial. Of course, it is possible that the variance estimate from our experiment represents a worst-case scenario and family effects are lower in other bivalves. Future work might assess growth and survival in a larger number of families within a single species (albeit with fewer manipulated environmental variables, to make the experiment tractable) and use the variance from that study to better estimate the number of families needed in a pooled-embryo design to discern a biologically meaningful difference in larval performance between pairs of sibling species.

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