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FITNESS CONSEQUENCES OF LARVAL TRAITS PERSIST ACROSS THE METAMORPHIC BOUNDARY

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Metamorphosis is thought to provide an adaptive decoupling between traits specialized for each life-history stage in species with complex life cycles. However, an increasing number of studies are finding that larval traits can carry-over to influence postmetamorphic performance, suggesting that these life-history stages may not be free to evolve independently of each other. We used a phenotypic selection framework to compare the relative and interactive effects of larval size, time to hatching, and time to settlement on postmetamorphic survival and growth in a marine invertebrate, *Styela plicata*. Time to hatching was the only larval trait found to be under directional selection, individuals that took more time to hatch into larvae survived better after metamorphosis but grew more slowly. Nonlinear selection was found to act on multivariate trait combinations, once again acting in opposite directions for selection acting via survival and growth. Individuals with above average values of larval traits were most likely to survive, but surviving individuals with intermediate larval traits grew to the largest size. These results demonstrate that larval traits can have multiple, complex fitness consequences that persist across the metamorphic boundary; and thus postmetamorphic selection pressures may constrain the evolution of larval traits.

KEY WORDS: **Adaptive decoupling, complex life cycles, fitness surface, metamorphosis, nonlinear selection, phenotypic selection.**

Understanding how selection shapes phenotypic variation is particularly complicated for species with complex life cycles, as dramatic changes in the selective environment between life-history stages are likely to cause a shift in the fitness value of traits across ontogeny (Moran 1994). Metamorphosis is traditionally considered to provide an adaptive decoupling between traits at each life-history stage, with developmental compartmentalization allowing developmental independence of larval and adult stages (Wilbur 1980; Moran 1994; Parichy 1998). Hence, by breaking the link between life-history stages, metamorphosis may allow selection to shape the traits of pre- and postmetamorphic stages independently—thereby maximizing overall lifetime fitness. However, variation in traits at one life-history stage can have significant fitness consequences for later stages (Schluter et al. 1991; Chippindale et al. 1996; De Block and Stoks 2005; Ficetola and De Bernardi 2006; Pechenik 2006; Podolsky and Moran 2006), suggesting that pre- and postmetamorphic stages are not free to evolve independently of each other. One method of testing whether metamorphosis provides an adaptive decoupling between pre- and postmetamorphic traits is to measure whether larval traits have fitness consequences that persist across the metamorphic boundary.

Phenotypic selection occurs when individuals with particular phenotypes survive or reproduce at higher rates than individuals with other phenotypes, and can lead to evolution if the traits under selection are heritable (Pfennig and Kingsolver 2009). Phenotypic

selection can be quantified by regressing phenotypic trait values of individuals with a component of fitness (Lande and Arnold 1983). The strength and mode of selection acting on each trait can then be determined from the slope (linear selection) and shape (nonlinear selection) of the regression line. Directional (linear) selection shifts the mean trait value up or down; stabilizing (nonlinear) selection reduces trait variation by selecting for individuals with intermediate trait values; and disruptive (nonlinear) selection increases trait variation by favoring individuals with extreme trait values (Brodie et al. 1995; Kingsolver and Pfennig 2007). Thus, we can predict how selection is likely to shift the distribution of traits in a population over time simply by measuring the relationship between phenotype and fitness under natural conditions (Lande and Arnold 1983; Kingsolver and Pfennig 2007).

Selection often acts on multiple traits simultaneously (Lande and Arnold 1983; Phillips and Arnold 1989). Measuring selection on multiple traits of the same individual simultaneously therefore allows us to distinguish direct selection on traits from the indirect effects of correlated traits (Lande and Arnold 1983; Kingsolver and Pfennig 2007). Furthermore, it allows us to quantify correlational selection gradients, which act on the covariance between traits, and represent nonlinear selection along axes that are not parallel to the individual trait axes (Brodie et al. 1995; Blows and Brooks 2003). Failure to account for selection acting on multiple traits simultaneously may have led to an under-appreciation of nonlinear selection in nature (Blows and Brooks 2003). Moreover, patterns of selection acting on trait combinations can be complex and impossible to predict based on examinations of each trait in isolation (Blows and Brooks 2003; Blows et al. 2003). These complex patterns of selection can be explored and interpreted by visualizing multivariate fitness surfaces (Phillips and Arnold 1989; Blows et al. 2003).

Phenotypic selection analyses provide an ideal framework to investigate whether metamorphosis allows an adaptive decoupling between life-history stages in species with complex life cycles, but this approach has not been applied to this issue. Central to the adaptive decoupling debate is the question of whether selection acting on larval traits can influence postmetamorphic fitness. Phenotypic selection analyses allow us to quantify and visualize the postmetamorphic fitness consequences of larval traits. Thus, assuming these traits are heritable, we can predict how postmetamorphic selection pressures will influence evolutionary responses of larval traits and whether such traits are free to evolve independently.

Marine invertebrates exemplify the complexity of alternative selection pressures acting on species with complex life cycles, as each life-history stage can have different (potentially conflicting) optimal phenotypes depending on their environment (Levitan 2006; Marshall and Keough 2008). There is increasing evidence that variation in egg and larval traits can carry over to affect performance in postmetamorphic stages of marine invertebrates (Podolsky and Moran 2006). Larval traits that may influence postmetamorphic fitness include morphological, physiological, and behavioral traits such as larval size, development rate, and time until settlement. The effects of egg size can propagate through the larval stage to influence postsettlement growth, survival, and even subsequent reproduction (Emlet and HoeghGuldberg 1997; Marshall et al. 2003a; Marshall 2005; Dias and Marshall 2010). Although we expect selection to minimize development time due to the extremely high rates of mortality of developing embryos and larvae while in the plankton (Morgan 1995), selection studies in insects (Chippindale et al. 1996; Chippindale et al. 1997; Prasad et al. 2000) and amphibians (Ficetola and De Bernardi 2006) with complex life cycles suggest that increases in larval development rate may incur fitness costs in later life-history stages. It is unknown whether marine invertebrates suffer similar fitness costs of rapid development, but it is possible they may also exhibit trade-offs between development rate and postmetamorphic performance. Settlement behavior can influence postmetamorphic fitness through the choice of settlement habitat (Thorson 1950; Pechenik 1990), and by changing the amount of energy reserves remaining for postsettlement growth and survival (Wendt 1998; Marshall et al. 2003b; Pechenik 2006).

Although the majority of studies that examine postmetamorphic fitness consequences of larval traits consider the effect of each trait in isolation; the effects of these larval traits on fitness could be highly dependent on each other. Egg size (and therefore larval size) is typically positively correlated with development time (McLaren 1966; Kohn and Perron 1994; Marshall and Bolton 2007), and time until settlement (Marshall and Keough 2003b). Larval size can also affect settlement behavior (Burgess et al. 2009), and delaying metamorphosis can change the relationship between larval size and postmetamorphic performance (Dias and Marshall 2010). Thus, examining postmetamorphic fitness consequences of larval traits in isolation is likely to obscure important interactive effects, which may constrain or push the evolution of traits in unexpected directions. Furthermore, examining multiple traits simultaneously is essential to estimate the relative influence of each trait on postmetamorphic fitness.

Here, we use the novel application of phenotypic selection analyses to understand the relative and interactive effects of larval size, time from fertilization to hatching, and time from hatching to settlement on postmetamorphic performance. Larval traits of the marine invertebrate, *Styela plicata*, were measured in the laboratory, and the postmetamorphic performance of individuals was tracked in the field. Two different components of fitness were measured—survival until reproduction and the size of survivors. Clearly, if an individual does not survive until reproduction, they cannot pass on their genes to the next generation, and therefore survival to reproductive age is a major fitness

component. However, the size of survivors (measured as final weight) was measured as a secondary fitness measure. In ascidians, size at reproduction strongly affects fecundity and second-generation offspring size (Millar 1952; Marshall et al. 2000). Hence, growth is likely to be an important additional fitness component acting beyond survival. Additionally, performance was measured in both low- and high-density environments, as the influence of larval traits on postmetamorphic performance can differ depending on levels of competition (Marshall and Keough 2003a; Marshall et al. 2006). To summarize, in this study we quantify the effects of larval size, time to hatching, and time to settlement on postmetamorphic survival and growth in low- and high-density environments.

Methods

STUDY SPECIES AND FIELD SITE

Styela plicata is a hermaphroditic, solitary ascidian that reproduces by broadcast spawning (where both eggs and sperm are shed into the water and fertilization occurs externally). Gametes are released in the late afternoon, and fertilized eggs hatch into lecithotrophic (nonfeeding) larvae the following morning, which are competent to settle within a few hours. *Styela plicata* is considered to be invasive to eastern Australia (Kott 1972), and is abundant on man-made structures such as piers and docks. Collections and experiments were done at the East Coast Marina (Manly, Brisbane, Australia; 27°467′E 153°183′S)—a private access marina that is protected from wave action by a large breakwater. At this site, reproduction occurs throughout most of the year (no reproduction observed from July until September—the Austral winter), individuals develop mature gonads within 4 months of settlement, and they typically live for less than one year (A. J. Crean, pers. obs.).

PRODUCTION OF FOCAL INDIVIDUALS BY IN VITRO FERTILIZATION

Quantifying larval phenotypes of marine invertebrates in situ is virtually impossible due to their small size and high mortality rates whilst in the plankton (Levin 1990; Thorrold et al. 2002). Therefore, to obtain accurate measures of premetamorphic traits, we generated embryos and larvae with in vitro fertilization techniques in the laboratory. Gametes were harvested from adult *S. plicata* on the day of collection by strip-spawning (Crean and Marshall 2008). Gonads were dissected from the visceral mass into a Petri dish with a few drops of filtered seawater (FSW), and the gonad extract was diced to release gametes. This extract was washed with FSW through a 500 μm and then 100 μm filter into a beaker; so excess material was retained in the 500 μm filter, eggs were retained in the 100 μm filter, and sperm was passed through to the beaker. We collected sperm and eggs from different individuals for use in each fertilization assay, and therefore individuals are referred to as male and female, respectively. Eggs were soaked in 0.5 M potassium chloride (KCl) for 10 s and thoroughly rinsed in FSW, to kill self-sperm and prevent self-fertilization, while leaving the eggs undamaged.

Due to logistical constraints, we were unable to track sufficient numbers of individuals for selection analyses in a single run, and therefore data were collected over five replicate runs between May and October 2009. To maintain genetic variation across runs, we produced focal individuals by crossing the gametes of five randomly selected females and five random males. For each run, we harvested 2 mL of eggs and 2 mL of sperm (by washing the gonad extract through the appropriate filter with 2 mL of FSW) from each female and male, respectively, and mixed the gametes together. After 1 h (when >50% of cleaving eggs were beyond the two-cell stage), fertilized eggs were individually collected with a micro-pipette and transferred into 2 mL of FSW in individual 10-mm diameter wells in a 24-well plate (72 eggs collected per run). These plates were left undisturbed in a constant temperature cabinet at 22°C overnight.

MEASUREMENT OF PREMETAMORPHIC TRAITS

To measure time to hatching, developing embryos were observed under a microscope (30 × magnification) every 15 min (starting from 10 h postfertilization). Time to hatching was used as a proxy for development rate, as time until metamorphic competence includes the behavioral component of settlement. To measure larval size, individual larvae were transferred (in a random order) into a preroughened and bio-filmed Petri dish (60 × 15 mm) in a drop of FSW. Replicate digital images of each larva were recorded (under 45× magnification) with PixeLINK Capture SE software (PixeLINK, Ottawa, CA), and larval area later estimated from the average of at least three measurements (to minimize measurement error) with the area tool in Image-Pro Express (version 5.1, Media Cybernetics, Silver Spring, MD). We then ensured that the larva was free swimming and not stuck in the water surface layer, and left the covered Petri dish in the constant temperature cabinet (at 22°C) to allow the larvae to settle. To estimate the relative time to settlement, each Petri dish was examined approximately every 4 h (under 30× magnification) to determine whether the larva had settled. When larvae had settled, their position in the Petri dish was marked, 10 mL of FSW was added, and the dishes were left covered ready for transport to the field. Larvae that had not settled within 25 h posthatch were excluded from our analyses ($n = 3$), as we could not distinguish between larvae that could not find a suitable settlement substrate and larvae that were incapable of settlement. Although excluding individuals before a trait is expressed can bias estimates of selection (Hadfield 2008), we believe this is unlikely due to the small number of individuals involved.

POSTMETAMORPHIC SURVIVAL AND GROWTH

Half of the focal individuals in each run were randomly allocated to a high-density (high competition) treatment, and the other half to a low-density (low competition) treatment. In total, 165 focal individuals were measured and tracked, with 82 and 83 individuals in low- and high-density treatments, respectively. To create the high-density environments, a fresh batch of eggs was fertilized (using the methods described above) 24 h after the focal individuals were produced. The following morning these new larvae were added (approximately 15–30 larvae per dish) to Petri dishes with focal individuals that had been assigned to high-density treatments, and left to settle throughout the day. No extra larvae were added to low-density treatments (the low density in our study was therefore 1 individual per Petri dish). After 10 h, extra larvae that had settled in the high-density treatments were counted (mean = 18, range = 10–30) and their position in the Petri dish marked to differentiate them from the focal individual. All replicates in both treatments were transported to the field in an insulated container filled with FSW that evening. No settlers had completed metamorphosis before transport to the field, and therefore settlers had not begun to feed while still in the laboratory.

Settlers were deployed in the field in rigid plastic mesh cages (dimensions: 44 × 28 × 18 cm length × width × height; mesh size 1 cm^2) to exclude predatory fish, which were observed to be attracted to field equipment and feed on *S. plicata* settlers (A. J. Crean, pers. obs.). Petri dishes were suspended vertically within a cage (1 run per cage), as the majority of *S. plicata* were observed growing on vertical surfaces at the field site (A. J. Crean, pers. obs.). These cages were hung from floating pontoons in the marina, approximately 2 m below the water surface. Survival of focal individuals was checked weekly by observation under a dissecting microscope (30× magnification). Petri dishes were also farmed of any new animals that had settled during the week at this time, to ensure we could keep track of the focal individuals.

The strength of selection on phenotypic traits was assessed for two different fitness measures: (1) survival to reproduction, and (2) growth of survivors. Survival to reproduction was measured after 4 months in the field (at which time all individuals had developed both male and female gonads), with all individuals that had died previous to this time scored as 0 and survivors scored as 1. To measure growth, all survivors were brought back to the laboratory and weighed. Wet weight used as an estimate of growth as a pilot study showed wet weight was highly correlated with dry weight ($r = 0.97$, $P < 0.0001$, $n = 48$). We analyzed survival and growth separately because the two fitness measures appeared to trade-off against one another, but we note that combining our fitness measures and analyzing with aster models (Shaw et al. 2008) would also be appropriate.

DATA ANALYSIS

Standardized linear (β) and nonlinear (γ) selection gradients were estimated for each fitness measure in each density environment. Log-likelihood tests showed no significant interaction between run and the traits of interest on survival (trial × larval size × hatch time × settle time: $\chi^2 < 0.002$, df = 1, $P = 1.00$) or size at reproduction ($F_{4,69} = 0.47$, $P = 0.796$), and therefore traits were standardized across runs in the final analysis. Phenotypic traits were standardized within each density treatment (to a mean of zero and standard deviation of one), and relative fitness was calculated by dividing the absolute fitness by the mean fitness within each treatment (Lande and Arnold 1983; Mitchell-Olds and Shaw 1987). Differences in the strength and form of selection experienced by individuals in low- versus high-density environments were tested with likelihood ratio tests of treatment by trait interactions. Estimates of selection gradients for survival to reproduction were calculated using linear regression, but the significance of these estimates was determined using logistic regression to account for the nonnormal error structure of the fitness measure (Janzen and Stern 1998). To estimate selection acting via growth, phenotypic traits were restandardized using only the subset of individuals that survived. Quadratic regression coefficients were doubled to estimate nonlinear selection gradients (γ) (Stinchcombe et al. 2008).

It is difficult to interpret the strength and significance of nonlinear selection from examination of the γ matrix (the matrix of quadratic and cross-product terms, Lande and Arnold 1983; Blows and Brooks 2003), as nonlinear selection is frequently strongest on trait combinations (Phillips and Arnold 1989; Blows and Brooks 2003). Therefore, to assess multivariate selection, we performed a canonical rotation of the γ matrix, which assists in the interpretation of the form and strength of nonlinear selection acting on trait combinations by finding the major axes of the fitness surface (Phillips and Arnold 1989; Blows and Brooks 2003). Canonical analysis determines the normalized eigenvectors (m_i) which contain the loadings of the original traits on canonical axes, and their associated eigenvalues (λ_i) which describe the form and strength of nonlinear selection (Phillips and Arnold 1989; Blows and Brooks 2003). Positive eigenvalues indicate concave selection along eigenvectors, and negative eigenvalues indicate convex selection. Concave and convex selection gradients may be interpreted as disruptive and stabilizing selection, respectively, if a surface has an inflection point (Phillips and Arnold 1989). The significance of eigenvalues was testing using the double linear regression method of Bisgaard and Ankenman (1996), with *P*-values calculated from permutation tests (Reynolds et al. 2010). The significance of loadings of the original traits on canonical axes was tested using bootstrap confidence intervals (method 6 in Peres-Neto et al. 2003). We used thin-plate splines (with a smoothing parameter that minimized the generalized cross-validation

score) to visualize the major axes of the fitness surface identified by canonical analyses (Schluter and Nychka 1994; Blows et al. 2003). As only significant eigenvectors were visualized, the fitness surface was plotted on either a single or paired set of axes.

Results

SURVIVAL TO REPRODUCTION

A greater proportion of individuals survived to reproduction in low-density environments (52% survival) compared to high-density environments (37% survival); however we did not detect any significant treatment by trait interactions for either linear ($\chi^2 = 0.262$, df $= 3$, $P = 0.967$) or nonlinear ($\chi^2 = 2.621$, df $= 6$, $P = 0.855$) selection. Given that our sample size was relatively small (low-density environments $n = 82$, high-density environments $n = 83$), and therefore our power to detect a difference in selection between environments was low, we decided it would be best to examine patterns of selection within each environment separately.

In both low- and high-density environments, the individuals that survived were those that took more time to develop (Fig. 1 and Table 1). Standardized linear selection gradients in both environments showed significant directional selection favoring individuals that took longer to hatch, but no significant directional selection on larval size or settlement time (β, Table 1; although note the marginal nonsignificance of settlement time in high-density environments). However, there was significant correlational selection acting on the covariance between larval size and hatching time in high-density environments (γ, Table 1).

Canonical analysis showed significant concave selection acting along a single eigenvector of trait combinations in high-density environments only (m1, Table 2). All phenotypic traits load positively on this eigenvector, with both time to hatching and settlement contributing significantly to m1 (Table 2). Selection strongly favored high values of m1 (Fig. 2); indicating that in high-density environments, individuals that took a long time to hatch and settle were the most likely to survive until reproduction, and individuals with average and below average phenotypes were selected against (Fig. 2). We found no evidence of nonlinear selection acting via survival to reproduction in low-density environments (Table 2).

SIZE OF SURVIVORS AT REPRODUCTION

Log-likelihood tests suggested no difference in linear selection among environments ($\chi^2 = 1.9$, df $= 3$, $P = 0.593$) and marginal support for a difference in nonlinear selection among environments ($\chi^2 = 10.9$, df $= 6$, $P = 0.091$). We found negative, directional selection on time to hatching acting via growth in high-density environments (β, Table 3); which is in the opposite direction to viability selection acting on time to hatching. This means that the probability of surviving to reproduction is higher for individuals with a longer time to hatching, but of those individuals that do survive, those with the shortest time to hatching grow to the largest size. We also detected correlational selection

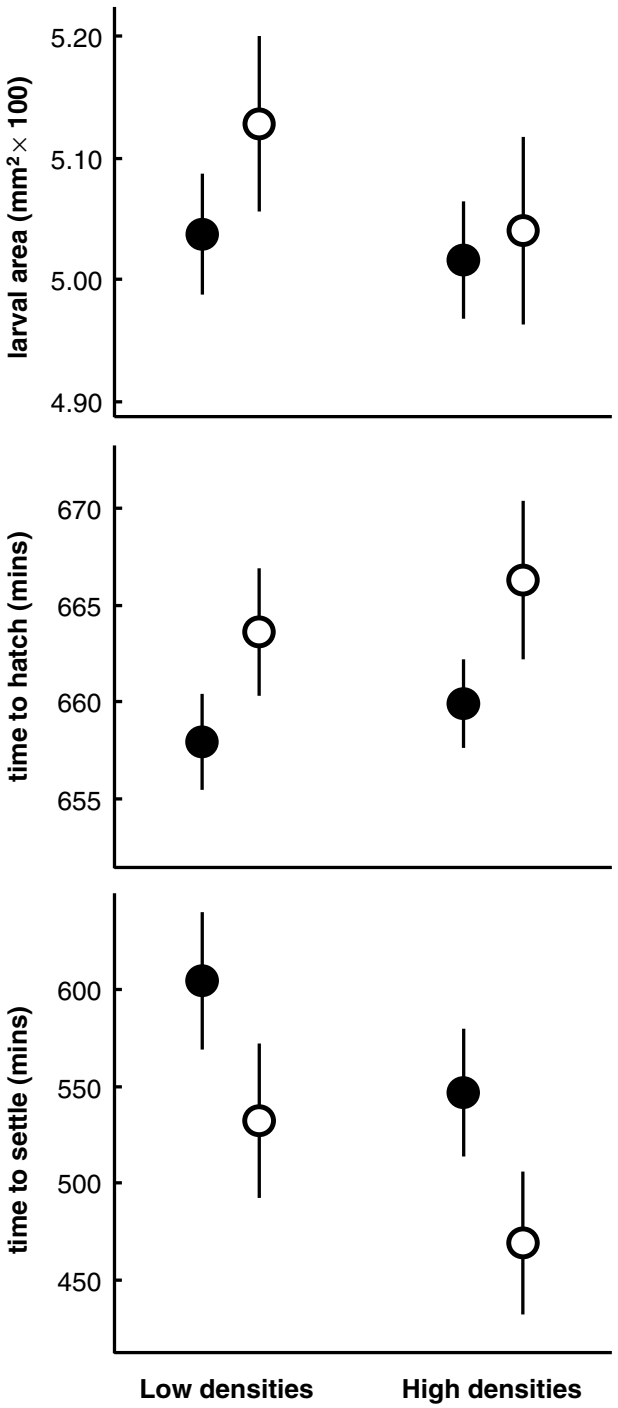

Figure 1. Mean (± SE) phenotypic traits of all individuals (before selection, black circles) and individuals that survived to reproduction (after selection, white circles) in low- and high-density environments

Table 1. Standardized gradients of directional selection (β) and nonlinear selection (γ) for survival until reproduction in low-density ($n = 82$) and high-density ($n = 83$) environments. Phenotypic traits measured are: larval area (larval size), time from fertilization to hatching (hatch time), and time from hatching to settlement (settle time). Quadratic and correlational selection gradients are the diagonal and off-diagonal elements of γ, respectively.

		γ		
	β	Larval size	Hatch time	Settle time
Low-density environment				
Larval size	0.12	0.16		
Hatch time	**0.21***	−0.05	0.18	
Settle time	−0.15	−0.10	0.04	−0.08
High-density environment				
Larval size	0.00	−0.16		
Hatch time	0.31*	**0.33***	0.17	
Settle time	−0.26**	−0.01	0.35	−0.12

(*$P < 0.05$, **$P < 0.1$)

acting between time to hatching and settlement (γ, Table 3); indicating that of the subset of individuals who survived, those that took both a long time to hatch and a long time to settle were the largest individuals at reproductive age. We did not detect any linear or nonlinear selection acting via growth on larval traits in low-density environments (Table 3).

Although initial inspection of the γ matrix suggests there is little nonlinear selection on larval traits acting via growth (Table 3); canonical analysis revealed significant nonlinear selection acting on multivariate trait combinations in both low- and high-density environments (Table 4). In low-density environments, significant convex selection acted along a single eigenvector on which both larval size and time to settlement load positively (m3, Table 4). The fitness surface suggests multivariate stabilizing selection, indicating that in low-density environments individuals with extremes (either low or high) of larval size and settlement time had the lowest growth rates; and individuals with

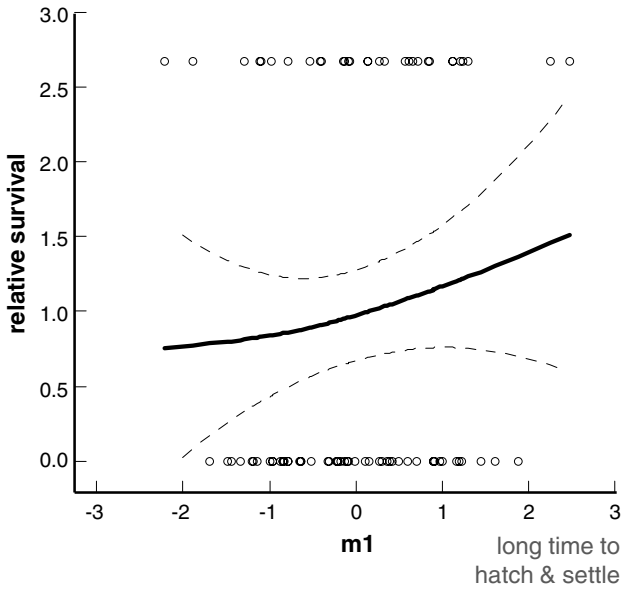

Figure 2. Predicted fitness (± 95% C.I.) for survival to reproduction in high-density environments showing the form of multivariate selection on phenotypic trait combinations. Line represents predicted fitness along the only canonical axes with significant nonlinear selection (m1), circles represent observed relative survival (absolute survival divided by mean survival). Individuals that take longer to hatch and settle are the most likely to survive until reproductive age.

Table 2. M matrix of eigenvectors (m_i) and their associated linear (θ) and quadratic (λ) coefficients, calculated from the canonical analysis of γ for survival to reproduction, in low-density and high-density environments.

			Original traits		
	θ	λ	Larval size	Hatch time	Settle time
Low-density environment					
m1	0.00	0.12	−0.69*	0.66*	0.30
m2	0.25*	0.06	0.64	0.75*	−0.17
m3	−0.13	−0.06	0.34	−0.07	0.94*
High-density environment					
m1	0.22	**0.26***	**0.39**	**0.81***	**0.44***
m2	−0.18	−0.06	−0.71*	−0.04	0.71*
m3	−0.36	−0.25	0.59*	−0.58*	0.56

*$P < 0.05$.

Table 3. Standardized gradients of directional selection (β) and nonlinear selection (γ) for the weight of survivors in low-density ($n = 43$) and high-density ($n = 31$) environments. Phenotypic traits measured are: larval area (larval size), time from fertilization to hatching (hatch time), and time from hatching to settlement (settle time). Quadratic and correlational selection gradients are the diagonal and off-diagonal elements of γ, respectively.

		γ		
	β	Larval size	Hatch time	Settle time
Low-density environment				
Larval size	0.01	−0.03		
Hatch time	−0.08	−0.14	0.08	
Settle time	0.02	−0.17	−0.06	−0.19
High-density environment				
Larval size	−0.05	−0.23		
Hatch time	**−0.18***	0.16	−0.04	
Settle time	−0.08	−0.27	**0.59***	−0.08

*$P < 0.05$.

Table 4. M matrix of eigenvectors (m_i) and their associated linear (θ) and quadratic (λ) coefficients, calculated from the canonical analysis of γ for weight of survivors, in low-density and high-density environments.

			Original traits		
	θ	λ	Larval size	Hatch time	Settle time
Low-density environment					
m1	0.00	0.09	−0.63*	0.76*	0.16
m2	−0.09	0.01	0.53	0.58	−0.62*
m3	0.02	**−0.17***	**0.56***	**0.31**	**0.77***
High-density environment					
m1	**−0.46***	**0.27***	**−0.11**	**0.70***	**0.71***
m2	0.06	−0.04	0.88*	0.40	−0.26
m3	−0.31	**−0.40***	**0.47**	**−0.60***	**0.65***

*$P<0.05$.

intermediate phenotypes had the highest weight at reproductive age (Fig. 3). In high-density environments, two eigenvectors (m1 and m3) showed significant nonlinear selection acting in opposite directions, with time to hatching and settlement loading significantly on both canonical axes (Table 4). Visualization of the concave selection along m1 combined with the convex selection along m3 (Fig. 4) shows that individuals with intermediate values of time to hatching and settlement grew to the largest size in high-density environments.

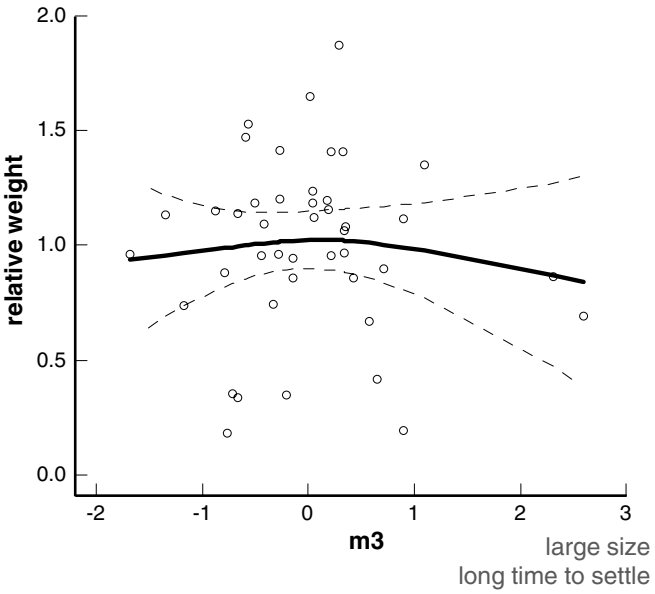

Figure 3. Predicted fitness (± 95% C.I.) for weight of survivors in low-density environments showing the form of multivariate selection on phenotypic trait combinations. Line represents predicted fitness along the only canonical axes with significant nonlinear selection (m3), circles represent observed relative fitness (absolute weight divided by mean weight). Relative weight of survivors is highest in individuals with intermediate values of larval size and time to settlement.

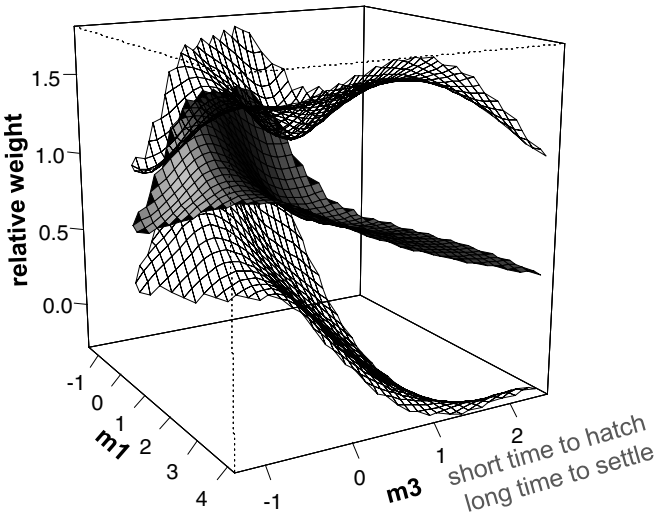

Figure 4. Predicted fitness (± 95% C.I.) for weight of survivors in high-density environments showing the form of multivariate selection on phenotypic trait combinations. Surface shows three-dimensional representation of predicted fitness along canonical axes with significant nonlinear selection (m1 and m3). Relative weight of survivors is highest in individuals with intermediate values of time to hatching and settlement.

Discussion

We found multiple and complex postmetamorphic fitness consequences of larval traits in a marine invertebrate, *S. plicata*. Individuals that took longer to develop and hatch into larvae were more likely to survive to reproduction; but of the subset of individuals that did survive, those that took less time to hatch grew to the largest size at reproduction. Nonlinear selection acted on multivariate trait combinations such that individuals with a greater than average time to hatching and settlement were the most likely to survive to reproduction. However, surviving individuals that obtained the largest size were those with intermediate values of larval traits. Hence, the direction and form of selection acting on larval traits was in opposite directions depending on whether fitness was estimated as survival to reproduction (viability selection), or the size of survivors at reproduction (which is likely to influence reproductive output). These results demonstrate that larval traits have multiple fitness consequences for postmetamorphic performance, and that with respect to selection, these life-history stages are not independent of each other.

The larval phase is undoubtedly a period of extremely high mortality for many marine invertebrates (Thorson 1950). Some studies suggest that over 99% of total mortality occurs during the larval phase, and even if mortality does not occur immediately, the

risk of advection away from suitable habitat probably increases with longer planktonic durations (Morgan 1995; Pechenik 1999). Intuitively, one might therefore expect selection to minimize the amount of time individuals spend in this highly vulnerable phase by maximizing the developmental rate of pelagic embryos and larvae (Strathmann et al. 2002). Interspecific comparisons of development rate support this expectation, as species with a pelagic larval phase develop more rapidly than related species that are brooded or aggregated into benthic egg masses (Strathmann et al. 2002; Hirst and Lopez-Urrutia 2006). As all embryos are vulnerable, the fact that development rate is not maximized in all species suggests that rapid development must come at a cost (Strathmann et al. 2002)—such as an increased risk of errors in DNA replication and repair (Karr and Mittenthal 1992) or decreased developmental stability (Arendt 1997; De Block et al. 2008). Selection studies on *Drosophila* demonstrate that accelerated development comes at the cost of reduced viability (Chippindale et al. 1997; Prasad et al. 2000). Our study also suggests that rapid development carries a fitness cost later in life: individuals that developed more slowly (took more time to hatch into larvae) were more likely to survive to reproductive age. Hence, marine organisms with pelagic larval phases may face conflicting selection pressures across life-history stages: selection acting via larval survival may favor individuals that develop quickly and leave the dangerous planktonic environment; whereas selection acting via postmetamorphic survival may favor individuals that developed more slowly as larvae. In this case, developmental rate would reflect a compromise between the premetamorphic costs of developing slowly and the postmetamorphic costs of developing quickly (Schluter et al. 1991).

Our analyses also suggest that increased development time is linked to lower growth rates, as surviving individuals with longer hatching times had the lowest weight at reproduction. Similarly, ocean pout that hatch early have higher growth rates and larger energy reserves than late hatched individuals (Methven and Brown 1991). Given that our analyses of selection acting via growth were based on only those individuals that survived, we cannot determine whether there is a trade-off between the effects of development time on postmetamorphic survival and growth. Nevertheless, it appears that phenotypes that are good for survival are not necessarily good for growth: individuals with slower developmental rates may be more likely to survive, but the slowest developing individuals are also less likely to grow well. Several studies show a trade-off between opposing fitness components such that phenotypes with the highest probability of survival have the lowest reproductive success (e.g., Schluter et al. 1991; Leroi et al. 1994; Sinervo and DeNardo 1996; Blanckenhorn 2000; Latta and McCain 2009). These studies and our findings highlight the need to carefully consider the fitness measure used in selection studies.

Interestingly, larval size had no direct effect on survival or size at reproduction. A growing number of studies have shown that larval size can influence postmetamorphic performance in marine invertebrates with complex life cycles (Marshall and Keough 2008). Although we detected no directional selection acting on larval size, we did detect correlational selection between larval size and time to hatching acting via survival in high-density environments (Table 1). Furthermore, larval size contributed to nonlinear selection on multivariate trait combinations acting via growth in low-density environments (Table 4). Traditionally, considerations of selection on offspring size have focused on this single trait alone (Fox and Czesak 2000; Marshall and Keough 2006). Such an approach probably reflects the emphasis that optimality models place on the relationship between offspring size and fitness (Smith and Fretwell 1974). Our finding that selection acted on a combination of traits rather than offspring size alone highlights that, as for any other trait, focusing solely on offspring size provides an incomplete picture of the way in which selection acts. It is increasingly apparent that the environmental context in which offspring occur changes the relationship between offspring size and fitness (Fox and Czesak 2000; Allen et al. 2008). Our findings here suggest that the offspring size-fitness relationship will also be context-dependent with regard to other offspring traits. In other words, the fitness returns of a particular offspring size depend not only on the offspring environment but also on the interaction between offspring size and other offspring traits. Correlational selection on offspring size and other larval traits could result in nonrandom associations among these traits (Lande and Arnold 1983), further contributing to offspring size variation.

As was found in linear selection analyses, the form of nonlinear selection on larval traits acting via growth was in the opposite direction to viability selection. The predicted fitness surfaces for the size of survivors (Figs. 3 and 4) suggest stabilizing selection is acting on multivariate trait combinations, such that surviving individuals with intermediate larval phenotypes achieved the greatest weight at reproductive age. We suggest that the combination of a short time to hatching and long time to settlement may be negatively associated with growth due to the interacting effects of a fast developmental rate combined with delayed settlement depleting larval energy stores (Wendt 1998; Marshall et al. 2003b). However, as phenotypic selection analyses do not provide any insight into the mechanisms of selection, this idea requires further testing. Despite the prediction that most organisms should be well adapted to their environment and therefore experience stabilizing selection (Travis 1989), there is surprisingly little evidence of stabilizing selection acting in the wild (Kingsolver and Pfennig 2007).Considering that stabilizing selection for growth was consistent across both low- and high-density environments, it would be interesting to investigate the drivers of this pattern further.

Our study clearly demonstrates that larval traits can have fitness consequences that persist across the metamorphic boundary. It appears that larval traits are not free to evolve independently of adult traits, and that metamorphosis does not provide an adaptive decoupling between life-history stages. We measured the postmetamorphic performance of individuals in the field, and therefore these analyses demonstrate how larval traits affect postmetamorphic performance in natural conditions. However, it is important to remember that due to logistical constraints, individuals were produced using in vitro fertilization and all premetamorphic stages were completed in the laboratory. Therefore, these selection analyses do not include selection on phenotypic traits acting at fertilization or during the planktonic phase—both of which may select for different trait combinations (Levitan 2006; Marshall and Keough 2008). Longer hatching times mean greater mortality due to planktonic predation and advection of eggs to unsuitable habitat. Given mortality is likely to be very high in the plankton (Morgan 1995), this could represent an important source of selection that we did not account for. Furthermore, as larvae were protected from high levels of natural premetamorphic mortality, the range of variation among individuals may have been unnaturally large, exaggerating the effects of postmetamorphic selection. Therefore, the next challenge will be to combine estimates of phenotypic selection across multiple life-history stages to understand how they interact (Arnold and Wade 1984b, a; McGlothlin 2010), and if technology advances to a point where it is logistically feasible, to estimate these selection episodes in natural conditions.

There is mounting evidence that metamorphosis is not necessarily a new beginning for marine species with complex life cycles (Pechenik et al. 1998). However, this study shows that larval traits do not just carry-over into later life-history stages, but complex patterns of multivariate larval trait combinations act together to influence postmetamorphic performance. Moreover, larval traits can have conflicting effects on different fitness components, with indications that some larval traits and trait combinations had a positive influence on postmetamorphic survival but a negative effect on growth. Clearly, marine species with complex life cycles face a multitude of opposing selection pressures on phenotypic traits across their life history. Ultimately, whether these selection pressures can act to shift trait means and variance over time depends on the patterns of genetic variance underlying these traits, and an important next step will be to determine the genetic constraints that shape the evolutionary responses to these selection pressures (e.g., Johnson et al. 2010).

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Supporting Information

The following supporting information is available for this article:

Table S1. Summary statistics for the standardized gradients of directional selection (β) and nonlinear selection (γ) for survival until reproduction in low-density ($n = 82$) and high-density ($n = 83$) environments.

Table S2. Summary statistics for the standardized gradients of directional selection (β) and nonlinear selection (γ) for the weight of survivors in low-density ($n = 43$) and high-density ($n = 31$) environments.

Supporting Information may be found in the online version of this article.