Functional Ecology

British Ecological Society

Functional Ecology 2013, **27**, 1358–1366 doi: 10.1111/1365-2435.12117

Phenotypic links among life-history stages are complex and context-dependent in a marine invertebrate: interactions among offspring size, larval nutrition and postmetamorphic density

Richard M. Allen[1]* and Dustin J. Marshall[1]†

[1]*School of Biological Sciences, University of Queensland, St Lucia, Queensland, 4072 Australia*

Summary

1. Examples of simple phenotypic relationships, where variation in one stage directly affects phenotypic variation in a subsequent stage, are documented in most taxa. However, environmental variation can mediate these relationships, and because most organisms develop through multiple life-history stages, each stage-dependent environment has the potential to create new phenotypic relationships and interfere with existing relationships.

2. Despite the likelihood of complex phenotypic interactions among life-history stages, and the potential for these interactions to resonate throughout the life history, there are few tests of the problem and few predictions of how these phenotypic interactions are resolved.

3. Here, we examined the interdependent effects of three sources of phenotypic variation on the performance of a marine tube worm. Sources of phenotypic variation included: offspring size, larval nutrition and juvenile density.

4. We found highly context-dependent relationships between these factors and postmetamorphic performance. Within the overarching result of context dependence, we found: interactions could negate and reverse relationships; early-stage phenotypes could persist to postmetamorphosis; later, life-history environments could contribute more to recruit phenotypes than early-stages; and late-stage variation can depend on early-stage phenotypes.

5. Our results demonstrate that while simple phenotypic links among the egg, larval and postrecruitment stages may be common and important contributors to growth and survival, these relationships should be considered in the context of the organism's life experience. Each phenotypic link among stages can potentially be complex and depend on prior experience, current state and the subsequent environments experienced.

Key-words: carry-over effects, complex life cycles, development, egg size, *Hydroides dirampphus*, life history, marine invertebrate, phenotypic variation

Introduction

The links between life-history stages play a critical role in ecology (Pechenik, Wendt & Jarrett 1998; Pausas *et al.* 2004; Blackburn, Cassey & Lockwood 2009; Kishida *et al.* 2010). Familiar to most ecologists are the numerical links among life-history stages: at its simplest, the abundance of individuals in one stage is a good predictor of the abundance of individuals in subsequent stages (Caley *et al.* 1996; Turnbull, Crawley & Rees 2000). More complex links are also common, where intermediate stages can strengthen, negate or reverse quantitative relationships between early- and late-stages (Fairweather 1988; Berven 1990; Webb & Peart 1999; Vonesh & De la Cruz 2002). In contrast, the ecological consequences of phenotypic links among life-history stages are less well understood (Marshall & Morgan 2011). This is surprising given phenotypic variation is ubiquitous among individuals, cohorts and populations (Taylor, Anderson & Peckarsky 1998; Johnston & Leggett 2002; Hamilton, Regetz & Warner 2008; Marshall & Keough 2008b; Shima & Swearer 2009a; Messier, McGill &

*Correspondence author. Present addresses: Department of Oceanography, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4R2; and Department of Ocean Sciences, Memorial University, St John's, Newfoundland, Canada A1C 5S7. E-mail: richard.allen@uqconnect.edu.au

† Present address. School of Biological Sciences, Monash University, Clayton, Victoria, 3800 Australia.

Lechowicz 2010), and it is now well-established that phenotypic variation can drive ecological processes at the population level (Hughes 1984; Stearns 1992; Benton *et al*. 2005; Fowler 2005, Plaistow, Lapsley & Benton 2006; Shima & Swearer 2009b; Bolnick *et al*. 2011). Similar to the complex quantitative links among stages, we might expect complex phenotypic interactions among multiple life-history stages to profoundly affect ecologically relevant phenotypes.

At every stage of the life history, phenotypic variation can be augmented or attenuated by environmental influences. Parental effects, developmental conditions and the adult environment can all generate variation in the phenotype that is ultimately expressed, which can immediately affect elements of fitness and go on to affect fitness in subsequent stages (Bernardo 1996; Pechenik, Wendt & Jarrett 1998; Lindstrom 1999; Altwegg 2003; West-Eberhard 2003; Marshall, Allen & Crean 2008, Saastamoinen *et al*. 2010; Auer 2010). Among two life-history stages, these environmentally derived phenotypic relationships are typically straightforward, where phenotypic variation in traits at one stage directly affects trait and performance variation in a later stage. For example, larger offspring in many taxa typically have higher performance as juveniles or adults (Roach & Wulff 1987; Bernardo 1996; Fox & Czesak 2000; Marshall & Keough 2008a). However, additional environmental variation can mediate relationships between early- and late-stage phenotypes, and this interaction may strengthen, negate or reverse the originally observed phenotypic relationship (Berven & Chadra 1988; Allen, Buckley & Marshall 2008; Marshall & Keough 2008b; Saastamoinen *et al*. 2010; Segers & Tabrosky 2011). For example in some plants, the benefits of larger seeds are more pronounced in highly competitive environments (Stanton 1984; Houssard & Escarre 1991); but in some frogs, the benefits of larger offspring can diminish or become negative depending on temperature and food availability (Berven & Chadra 1988; Kaplan 1992). While similar straightforward interactions between an early-stage phenotype and a later-stage environment are repeatedly demonstrated among two life-history stages, most organisms experience many environments as they mature through multiple life-history stages (Moran 1994). Hence, phenotypic relationships may increase in complexity in species with multiple life-history stages, because each stage-dependent environment has the potential to create new phenotypic relationships and interfere with existing phenotypic relationships (Auer *et al*. 2012).

Few general paradigms exist for how we might expect phenotypic interactions among multiple life-history stages to be resolved. Intuitively, increased life-history complexity may be expected to weaken phenotypic relationships across distantly connected stages, because genetic variation, environmental interactions and additional stochastic sources of variation also contribute to late-stage phenotypes, and studies to date tend to demonstrated weaker relationships in distantly connected stages (Heath, Fox & Heath 1999; Lindholm, Hunt & Brooks 2006; Wilson & Reale 2006). Nevertheless, an apparent trend in weak phenotypic relationships across multiple stages may be because few studies test for environmentally derived phenotypic interactions or persistent phenotypic relationships in species with relatively complex life histories compared to those with shorter and less complex life histories, where stronger phenotypic relationships among stages appear to be more common (Marshall & Keough 2008a,b). In addition, while studies have tracked the effects of early-stage phenotypes across multiple stages (e.g. Vonesh 2005), it is rare to independently manipulate the environments within each stage and look explicitly for phenotypic interactions among stages (but see Berven & Chadra 1988; Kaplan 1992). Hence, we must explore the nature of complex phenotypic relationships among multiple stages to understand the context of when these relationships are resilient or environmentally constrained (West-Eberhard 1989; Schmitt, McCormac & Smith 1995; Fox 1997; Lindstrom 1999; Weinig & Delph 2001; Plaistow, Lapsley & Benton 2006).

Overall, we expect the phenotypic links among life-history stages are not likely to be straightforward, because the relative strength, direction and persistence of a given phenotypic relationship is not independent of other events experienced throughout an individual's life history. Rather, phenotypic variation at any given stage depends interactively on prior and subsequent phenotypes, and the environmental conditions experienced (Kaplan 1992; Arnqvist & Johansson 1998; Marshall, Cook & Emlet 2006; Johnson 2008; Auer *et al*. 2012). These complex interactions among stages may be common and profound; yet, tests that include multiple stages are rare (but see Auer *et al*. 2012). Here, we explore phenotypic relationships among multiple life-history stages in the marine tube worm – *Hydroides diramphus*. We chose three independent sources of phenotypic variation known to occur in this species: egg size, the feeding environment experienced by developing larvae and the level of postmetamorphic competition. We hypothesized that our manipulations within each of these stages will interdependently affect two postmetamorphic performance measures, survival and tube length (tube length is an indicator of growth, fecundity and ability to occupy space in a spatially competitive environment), and that these interactions will reveal the nature of more specific phenotypic relationships, and the context in which they occur.

Materials and methods

SPECIES, COLLECTIONS AND FIELD SITE

Hydroides diramphus is a polychaete tube worm found in cosmopolitan benthic marine assemblages. Like most marine invertebrates, *H. diramphus* exhibits a complex life cycle composed of multiple morphologically and ecologically discrete phases. In this study, we focus on variation within three broadly defined stages where phenotypic variation is known to occur and can affect performance in *H. diramphus* (Allen & Marshall 2010; R.M. Allen and D.J. Marshall, unpublished manuscript). We manipulate offspring size during the egg stage, the feeding environment experienced by developing larvae (larval stage) and the density of

conspecific recruits in the postmetamorphic stage. Importantly, most species with complex life histories share relatively analogous life-history stages. Populations of maternal *H. diramphus* release broods of heterogeneously sized eggs directly into the water column where fertilization then occurs externally. Once fertilized, embryos undergo a brief period of nonfeeding development followed by a larval stage where larvae must feed to complete development. Planktonic food can be heterogeneous in space and time (Phillips 2002), and food limitation in *H. dirapmhus* can retard development and affect phenotypic variation at later stages (Allen & Marshall 2010). Once larvae acquire enough resources during planktotrophic development, larvae become competent to settle and metamorphose into the benthic adult environment. Marine benthic communities are typically characterized as being highly variable over small spatial scales in the intensity of competition for limited space and resources (Underwood & Keough 2001). In *H. diramphus,* the density of individuals similarly varies over small scales and prior evidence suggests higher densities increase competition and reduce growth in new recruits but may enhance survival (Allen & Marshall 2010). Postmetamorphic fitness can be estimated with two measures of performance: survival and the length of the adult tube. Larger tubes house larger and more fecund worms that also occupy more space in a space-limited environment (Allen & Marshall 2010, R.M. Allen personal observations).

Locally, *H. diramphus* forms part of the fouling community in commercial and recreational boat harbours. All specimen collections and field experiments were conducted from the floating pontoons of the Scarborough Marina, Redcliffe, Queensland, Australia (27°10′45″S, 153°06′18″E) between August and November 2010.

OBTAINING GAMETES AND MANIPULATING EGG SIZE

To obtain gametes, we used a standard procedure that induces spawning in polychaetes (Strathmann 1992; Allen & Marshall 2010). The calcareous tubes of adult worms were gently broken, and the adults were placed into individual petri dishes containing <5 mL of sterilized seawater (seawater microwaved at 1000 watts for 3 min past boiling and allowed to return to ambient temperature). Once out of their tubes, adults immediately released gametes into the petri dishes, ready for experimental use.

We split newly spawned eggs into two size classes: 'large' and 'small' by pooling the eggs of 15 mothers and then pouring these eggs through a nylon-mesh filter. Eggs that passed through the filter were on average 50·14 μm (±0·385 SE) diameter ('small eggs'), and those that were retained in the filter were 55·35 μm in diameter (±0·261 SE) ('large eggs'). The eggs from each size class were then separated into 16 replicate 50 mL specimen jars (8 jars of each egg size treatment) and adjusted to a concentration of *c.* 20 eggs mL^{-1}. Each jar was then fertilized separately with a few drops of dilute sperm pooled from multiple males. To ensure maximum fertilization and avoid sperm concentration selecting for particular egg sizes, the drops of sperm were added in a stepwise manner at 10–15 min intervals, so that concentration of sperm gradually increased, but allowed time for the polyspermic block to take place. After 16 h, embryos hatched into obligate-feeding larvae with a hatching success rate of >90%.

MANIPULATING FOOD AVAILABILITY DURING PLANKTONIC DEVELOPMENT

The amount of food available to developing larvae can affect the length of the planktonic feeding period in many marine invertebrate species, including *H. diramphus* (e.g. Strathmann 1992; Allen & Marshall 2010). A pilot study revealed that most *H. diramphus* larvae (adjusted to a concentration of 10 larvae mL^{-1}) reach competence at 10 days when fed a concentration of 40 000 cells mL^{-1} of *Isochrysis galbana* (Tahitian strain), and 15 days when fed 10 000 cells mL^{-1}. To ensure larvae from both treatments became competent to settle simultaneously (to avoid temporally confounding the postsettlement environment with food availability), we staggered the start date of the larval cultures as follows (Appendix S1, Supporting information). On the first day, we spawned 15 mothers, split the eggs size into 'large' and 'small', and placed them in 16 replicate jars (8 jars of each size). We then added the low concentration of phytoplankton to each jar (Low 1). After 5 days, we repeated the procedure to attain the egg size treatments, but separated the eggs of 30 mothers into 32 jars (16 of each size class); 16 jars (8 of each size class) received a high concentration of phytoplankton (High 1), and the remaining 16 jars were the second low-food treatment (Low 2). The 30 mothers were mixed together for High 1 and Low 2, hence the worms in this treatment are more genetically similar than the other treatments; however, there was no evidence that these genetic similarities (or laboratory manipulations) interacted with our treatments or survival and tube size (see Results for Run effects). After a further 5 days, another 15 mothers were spawned (split into the two size egg classes and replicate jars), and these larvae received the second allocation of the high-food treatment (High 2). Hence, Low 1 and High 1 completed development at the same time, and we refer to this pair as Run 1; Low 2 and High 2 also completed development at the same time, and we refer to this pair as Run 2. Each level of larval-food treatment was crossed with each level of egg size, such that each jar contained one combination of larval food and egg size (Appendix S2, Supporting information). The jars were maintained on a roller (for water agitation) under artificial light (14 : 10 light-dark cycle), and every two days, the water within each jar was replaced with fresh sterilized seawater and phytoplankton, until settlement. To ensure all larvae remained in the culture during water changes, we sucked the waste water through a 25 μm syringe-filter that was placed inside each culture vessel, and then discarded the waste water. The front of the filter was thoroughly rinsed to retain all larvae within the culture.

SETTLEMENT AND POSTMETAMORPHIC DENSITY

Once competent to settle, the larvae of each jar were equally split into two 60 mm petri dishes (Appendix S2, Supporting information). To encourage settlement, each dish was preroughened and placed in fresh seawater to coat the dishes with an organic biofilm (Unabia & Hadfield 1999). We allowed two days for the larvae to settle and metamorphose in the dishes. Once settled, we haphazardly culled settlers to manipulate postmetamorphic density to either a high- or low-density treatments (high: 10–20 or low: 4–9 settlers per dish). The chosen densities occur naturally and have previously been correlated with competition among settlers and affect postmetamorphic growth (Allen & Marshall 2010).

Once settled and density was manipulated, we drilled a 6 mm hole in the centre of each dish. In the field, we attached each dish to a 500 × 500 mm PVC backing panel using plastic plugs. Each run was deployed on a single backing panel; therefore, we had two runs as a blocking factor that contain random variation associated with both the temporal differences in settlement date and spatial differences in backing panel location. The panels were tied to the floating docks approximately 5 m apart and were submerged 1 m below the water surface for the duration of the field experiment. After 10 days in the field, the dishes were returned to the laboratory for worm measurements.

POSTMETAMORPHIC PERFORMANCE MEASUREMENTS

After 10 days in the field, dishes were returned to the laboratory where we estimated postmetamorphic performance by measuring

worm survival and tube length. We scored the survival of worms as the proportion of surviving worms in each dish. We then preserved the worms by placing the dishes in ethanol (50%). Next, we photographed the tubes of the surviving worms using PixeLINK Capture SE ver. 1.0. (Ottawa, ON, Canada), and measured the length of each tube with Image-Pro express ver. 5.1 (Media Cybernetics, Bethesda, MD, USA). The average tube length per dish was the unit of replication.

STATISTICAL ANALYSIS

To test the effect of egg size, phytoplankton concentration and postmetamorphic density on recruit performance, we used a split-plot analysis (alternatively called factorial and partially nested designs) in a REML estimation framework (Appendix S2, Supporting information). We had three categorical fixed-factors, each with two levels of treatment: these included egg size (large or small), phytoplankton concentration (40 000 or 10 000 cells mL^{-1}) and density (high or low). In addition, we had two random factors: two levels of run and eight replicate jars for each combination of run, egg size and food concentration. Random factors were tested using log-likelihood tests (Quinn & Keough 2002). The random factors were either an experimental convenience (Jar), or to control for the confounding effects of variation in development time (Run). Hence *a priori*, these random factors were of no biological interest, and we had no reason to expect these factors would interact with the fixed-factor treatments. This expectation was confirmed in the analysis of the full models (Appendix S3, Supporting information). We therefore removed the nonsignificant interactions containing random factors from our final models to focus on the fixed-factor hypotheses of interest. Significant fixed-factor interactions were further investigated with simple main effects tests (Quinn & Keough 2002).

Results

THE EFFECT OF EGG SIZE, LARVAL-FOOD ENVIRONMENT AND POSTMETAMORPHIC DENSITY ON SURVIVAL

Postmetamorphic survival was significantly affected by the concentration of phytoplankton available during the larval stage (Table 1a). Larvae fed high concentrations of phytoplankton had 75·9% (SE ± 0·023) survival after 10 days in the field, compared to 63·5% (SE ± 0·037) survival for those fed the lower concentrations (Fig. 1). No other main effect or any interactions among treatments had a significant effect on postmetamorphic survival (Table 1a), including the random factors of Jar and Run (Appendix S3, Supporting information).

THE EFFECT OF EGG SIZE, LARVAL-FOOD ENVIRONMENT AND POSTMETAMORPHIC DENSITY ON POSTMETAMORPHIC TUBE LENGTH

Tube length after 10 days in the field was influenced by egg size, concentration of phytoplankton during larval development and postmetamorphic density (Table 1b), as well as the random factors of Jar and Run (Appendix S3, Supporting information). However, these effects were not straightforward and often depended on interactions with the other factors.

Table 1. REML testing the effects of egg size (large or small), phytoplankton concentration (10 000 or 40 000 cells mL^{-1}) and postmetamorphic density (high or low) on postmetamorphic (a) survival and (b) tube length. Simplified model shown after nonsignificant interactions with the random factor Run omitted. Both Jar and Run were retained as main effects in the final model

Source	d.f.	*F*	*P*
(a) Survival			
Egg size	1, 58	0·658	0·421
Food	1, 58	6·492	0·014
Density	1, 56	1·273	0·264
Egg size × Food	1, 58	0·003	0·957
Egg size × Density	1, 56	1·051	0·310
Density × Food	1, 56	1·002	0·321
Egg size × Food × Density	1, 56	0·079	0·780
(b) Tube length			
Egg size	1, 58	0·617	0·435
Food	1, 58	1·01	0·319
Density	1, 53	9·452	0·003
Egg size × Food	1, 58	9·291	0·003
Egg size × Density	1, 53	2·427	0·125
Density × Food	1, 53	4·247	0·044
Egg size × Food × Density	1, 53	0·259	0·613

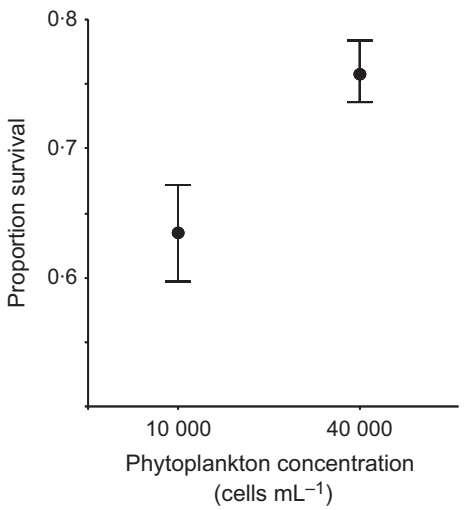


Fig. 1. The effect of phytoplankton concentration on mean survival of *Hydroides diramphus* recruits after 10 days in the field. During larval development larvae, the recruits were fed either 10 000 or 40 000 cells mL^{-1}. Descriptive means and error bars (±1 SE) presented.

Density had a strong influence on postmetamorphic tube length after 10 days in the field (Table 1b). However, the effects of density were not straightforward and depended on an interaction with the level of phytoplankton available during larval development (Table 1b, Fig. 2). Tube worms that were fed the lower concentration of phytoplankton were on average 17% larger, if they experienced the lower density of conspecific juveniles than those in the high-density treatment ($F_{1,54}$ = 4·766, P = 0·033; Fig. 2). However, tube worms that were fed the higher phytoplankton

concentration did not differ in tube-length postmetamorphosis, regardless of the postmetamorphic density experienced ($F_{1,58} = 0{\cdot}923$, $P = 0{\cdot}341$; Fig. 2). The reciprocal simple main effects test revealed that individuals that experienced high postmetamorphic density differed in tube length, where those that were fed the high concentration of phytoplankton were 10·5% larger than those fed the lower amount ($F_{1,52} = 5{\cdot}406$ $P = 0{\cdot}024$; Fig. 2). But within low postmetamorphic density, phytoplankton did not influence tube length ($F_{1,52} = 0{\cdot}074$, $P = 0{\cdot}786$; Fig. 2).

Tube length was significantly affected by an interaction between egg size and phytoplankton concentration (Table 1b; Fig. 3). When we compared the effects of egg size within each level of phytoplankton, we found that individuals fed the high phytoplankton concentration as larvae were 13·8% larger postmetamorphosis if they were from small eggs ($F_{1,56} = 8{\cdot}344$, $P = 0{\cdot}005$; Fig. 3). However, when fed the low concentration of phytoplankton, individuals from large eggs had on average 23% larger tubes postmetamorphosis that those from small eggs ($F_{1,52} = 5{\cdot}989$, $P = 0{\cdot}018$; Fig. 3). When we compared the effects of phytoplankton concentration within each egg size, we found larvae from small eggs were on average 23·5% larger postmetamorphosis if they were fed the high phytoplankton treatment ($F_{1,55} = 15{\cdot}001$, $P < 0{\cdot}001$; Fig. 3). But recruits from larger eggs did not significantly differ in postmetamorphic size among phytoplankton concentrations, despite worms fed the lower phytoplankton concentration being 13·8% larger on average ($F_{1,57} = 2{\cdot}23$, $P = 0{\cdot}141$; Fig. 3).

There were strong effects of both the experimental run and the individual jar that larvae wear reared in on postmetamorphic tube length (Appendix S3, Supporting information). Importantly, these effects were independent of all fixed-factor treatments, as no significant interactions were found (Appendix S3, Supporting information). Average tube length was highly variable among jars and the average length of worms per jar ranged from 2·38 to 11·95 mm. Worms in Run 1 had an average tube length of 3·86 mm (±SE 0·09), which was considerably smaller than the worms in Run 2 that had an average tube length of 6·22 mm (±SE 0·22).

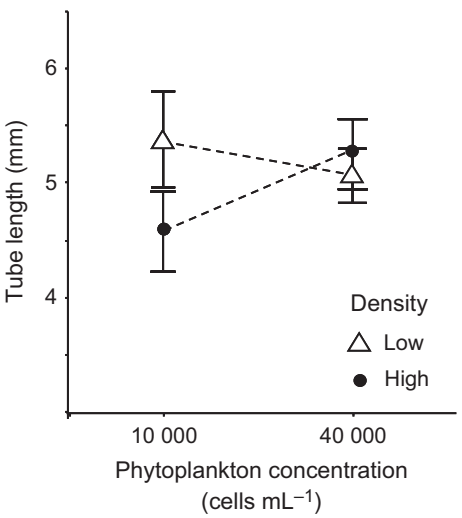


Fig. 2. The effect of phytoplankton concentration and postmetamorphic density on the mean tube length of *Hydroides diramphus* recruits after 10 days in the field. The recruits were fed either 10 000 or 40 000 cells mL^{-1} during larval development and experienced low (white triangles) or high density (solid circles) of conspecific's postmetamorphosis. Descriptive means and error bars (±1 SE) presented.

Discussion

We found context-dependent relationships between egg size, larval nutrition and postmetamorphic density, on postmetamorphic performance in *H. diramphus*. We found examples where observed phenotypic relationships could be negated or reversed depending on interactions among treatments. Within this overarching general result of context dependence, we also found evidence that early-stage phenotypes could persist and be detected postmetamorphosis; the environments in later life-history stages can contribute more to recruit phenotypes than early-stage phenotypes; and phenotypic variation in later stages can depend on phenotypic variation in earlier stages.

Environmental mediation of phenotypic relationships is common in ecology, but when these relationships span multiple life-history stages there are few general predictions for how we expect these interactions to be resolved. Explicit tests of the problem have generally been limited to experimentally tractable species with simpler life histories (Marshall & Keough 2008a,b; Auer 2010), and general observations suggest we might expect relationships between early- and late-stages to be weak in species with complex life histories (Sinervo & McEdward 1988; Heath, Fox & Heath 1999; Lindholm, Hunt & Brooks 2006; Wilson & Reale 2006). Our results, however, show that depending on the trait measured and the particular set of conditions experienced, early-stage phenotypes can persist and affect late-stage performance measures; while in other situations, early-stage phenotypes appear to dissipate and have no effect on late-stage performance. For example, the positive effects of larval nutrition on tube length persisted when individuals experienced high postmetamorphic density, but this effect was absent under low postmetamorphic density. Evidence for persistent early-stage effects was also found for survival, because the positive effects of higher larval nutrition on survival were robust and not influenced by interactions with egg size or density. Additionally, egg size effects could also persist and influence tube length, but the direction of that effect changed depending on phytoplankton concentration: egg size effects were positive at low phytoplankton concentrations, and negative at high phytoplankton concentrations. We expect that at an intermediate level of phytoplankton concentration, where the interaction lines cross, egg size may have no influence on postmetamorphic tube length, thus, further

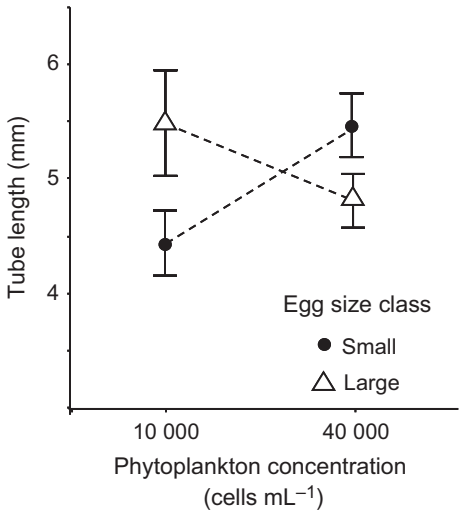


Fig. 3. The effect of egg size and phytoplankton concentration on the mean tube length of *Hydroides diramphus* recruits after 10 days in the field. The recruits were fed either 10 000 or 40 000 cells mL^{-1} during larval development and came from one of two egg size classes: large eggs(white triangle) or small eggs (solid circle). Descriptive means and error bars (±1 SE) presented.

demonstrating persistence is context-dependent. Overall, in some circumstances, it appears as though additional sources of variation associated with increased complexity in life histories can mask or overwhelm phenotypic relationships between early- and late-stages, but in other circumstances relationships persist (Kinghorn 1983; Mousseau & Dingle 1991; Lindholm, Hunt & Brooks 2006).

The high variability in our results indicates phenotypic interactions among stages are likely to be caused by a variety of mechanisms, but we suggest they appear to be the result of early-stage phenotypes dictating future responses to subsequent environments, and the overall quality or condition of individuals interacting across stages (Saastamoinen *et al*. 2010). For example in other studies, phenotypic variation induced early in development can alter developmental trajectories, and amplify or limit phenotypic variation at later stages (e.g. Schmitt, McCormac & Smith 1995; West-Eberhard 2003; Hoverman & Relyea 2007). We found worms from smaller eggs were more sensitive to low food availability, compared to individuals from larger eggs that did not differ in postmetamorphic size among the same later-stage treatment (larval nutrition). Therefore, initial egg size appears to shape larval and recruit responses to food availability. In other systems, morphological variation in early-stage phenotypes has been shown to dictate variation in later-stage phenotypes; for example, in plants, early stem elongation in response to poor light conditions compromises the structural integrity of the stem, limiting the variation in traits at later stages to only those that can be supported on a weaker stem (Weinig & Delph 2001). In our study, a similar morphological mechanism is less obvious, because our manipulations occurred prior to fertilization and either side of metamorphosis. But we suggest our reported interactions can be discussed under a general framework that considers variation in the overall state or 'quality' of individuals.

Previous theoretical and empirical studies have shown the relative benefits of better quality phenotypes increases in poorer quality environments (e.g. Parker & Begon 1986; Sibly, Calow & Smith 1988; Braby 1994; Tamate & Maekawa 2000), and our results can in part be explained under this framework. In *H. diramphus,* and marine invertebrates generally, small offspring size, low levels of planktonic food and high postrecruitment density tend to negatively affect performance (e.g. Pechenik, Wendt & Jarrett 1998; Phillips 2002; Leslie 2005; Marshall & Keough 2008a; Allen & Marshall 2010, R.M. Allen and D.J. Marshall, unpublished manuscript). Hence, we expected negative effects of egg size to be stronger in low larval-food and high postmetamorphic density environments; and similarly, negative effects of lower food levels for larvae would be stronger at a high postmetamorphic density. Our results concur, at least in part, with these expectations, because individuals from larger eggs appeared better able to cope with the harsher planktonic environment and achieved larger size postmetamorphosis than those from smaller eggs; but, when faced with more favourable conditions high food or low density, previously significant phenotypic relationships were reversed or became undetectable. Similarly, being fed higher levels of phytoplankton during larval development appeared to buffer those juveniles against variation in environmental quality postmetamorphosis; but, being fed lower levels of phytoplankton made juveniles susceptible to the harsher conspecific competition.

Interestingly, under no circumstances was survival in *H. diramphus* affected by egg size. This result may provide evidence of weakening relationships between early-stage phenotypes and later stages, but postmetamorphic density also did not influence survival. There was, however, a strong effect of larval food on survival, and the larval-food environment mediated all effects on postmetamorphic tube length. Hence, rather than confirm ubiquitous weakening relationships among multiple stages, it appears as though survival and postmetamorphic size are particularly sensitive to variation in the larval feeding environment in *H. diramphus*. That the random effect of jar strongly affected tube length, supports the argument that larval development is a particularly sensitive stage, because the 'jar' effects were imposed during larval development: something marine larval biologists have suggested for sometime (Thorson 1950). Alternatively, egg size and postmetamorphic density may affect survival in specific contexts not present among our treatments. But, we suspect postmetamorphic survival may always be independent of egg size in *H. diramphus*. In marine invertebrates, a relationship between offspring size and postmetamorphic mortality has been reported (Marshall & Keough 2008b; Monro, Sinclair-Taylor & Marshall 2010), but it is not ubiquitous, and no relationship has previously been found in *H. diramphus* (R.M. Allen and D.J. Marshall, unpublished

data) and other species: including those with nonfeeding larval stages (Allen, Buckley & Marshall 2008; Marshall & Keough 2008a; Jacobs & Sherrard 2010). Early postmetamorphic mortality in benthic marine invertebrates has been suggested by others to be dominated by other extrinsic factors, such as nondiscriminate predation by fish or extremes in the physical conditions of the postrecruitment environment (Keough 1986; Osman & Whitlatch 1996; Jacobs & Sherrard 2010). In contrast, we expected higher levels of postmetamorphic density to increase survival, because a positive relationship has previously been reported in *H. diramphus* (Allen & Marshall 2010), and a similar species show (*Hydroides dianthus*) strong preference to settle gregariously in high densities (Toonen & Pawlik 1994, 2001).

Our study was specifically designed to test the interactions of independent sources of phenotypic variation across multiple life-history stages. However, in natural conditions, the sources of phenotypic variation may not be independent, if an existing phenotype or environment creates the postrecruitment environment (Roughgarden 1986). For example, stressful environments may result in mortality in some individuals in a population, and poor quality phenotypes in the survivors; but, variable survivorship could cause additional phenotypic variation due to density-dependent interactions among the survivors (Roughgarden 1986; Fairweather 1988; Vonesh & De la Cruz 2002; Allen & Marshall 2010). Hence, nonindependent sources of phenotypic variation could potentially cause misleading correlations among direct phenotype–environment relationships, and indirect postrecruitment phenotype–environment relationships. For example, in a previous study, we conducted on *H. diramphus,* we found larvae fed lower phytoplankton concentrations had higher mortality postmetamorphosis (Allen & Marshall 2010). But, the associated reduction in postmetamorphic density caused by mortality may have confounded our ability to detect the effects of larval nutrition on postmetamorphic tube length, as survival (and therefore density) and tube length were correlated. In the present study, our manipulations of density helped to separate the independent effects of density and larval nutrition, and we have evidence both contribute to tube length. However, because mortality was again higher in individuals fed less as larvae, describing the precise contributions of each factor remains unclear. The challenge of future studies would be to partition these sources of variation in natural populations.

Examples of complex relationships are common in our results. But overall, few previous examples have demonstrated a similarly high amount of variation in relationships between an early-stage phenotype and later-stage performance within a single species (Berven & Chadra 1988; Kaplan 1992; Saastamoinen *et al*. 2010), but we reiterate that this may be because explicit tests of the problem are rare. Interestingly, we found a strong independent effect of experimental run on of tube length demonstrating that stochastic environmental effects may increase the complexity of our understanding phenotypic relationships across multiple life-history stages. This result is in keeping with numerous other field studies where temporal and spatial variation in performance appears to be common, rather than an exception (Allen & Marshall 2010; Gooley, Marshall & Monro 2010; Moran, Muniz Dias & Marshall 2010).

Conclusion

Overall, our study demonstrates the dynamic nature of the relationships between egg size, larval feeding environment and postrecruitment density on postmetamorphic survival and size. While in ecology, simple phenotypic links among life-history stages are well documented for many taxa at both the organism and population levels, we found phenotypic variation depends interactively on prior and subsequent phenotypes, and the environmental conditions experienced. Thus, we suggest complex phenotypic links across multiple life-history stages must be explored in a variety of contexts to help understand the frequency of early- and late-stage relationships, resilient phenotypic relationships, sensitive stages and shifts in phenotypic trajectories based on early phenotypic variation.

Acknowledgements

We thank Charlene Wong and Arthur Riedel for their valuable assistance in the laboratory and field. The Moreton Bay Boat Club for access to the floating docks. This project was funded by Australian Research Council (DPO556552) to DJM. RMA was supported by an Australian PostGraduate Award.

References

Allen, R.M., Buckley, Y.M. & Marshall, D.J. (2008) Offspring size plasticity in response to intraspecific competition: an adaptive maternal effect across life-history stages. *American Naturalist*, **171**, 225–237.

Allen, R.M. & Marshall, D.J. (2010) The larval legacy: cascading effects of recruit phenotype on post-recruitment interactions. *Oikos*, **119**, 1977–1983.

Altwegg, R. (2003) Multistage density dependence in an amphibian. *Oecologia*, **136**, 46–50.

Arnqvist, G. & Johansson, F. (1998) Ontogenetic reaction norms off predator-induced defensive morphology in dragonfly larvae. *Ecology*, **79**, 1847–1858.

Auer, S.K. (2010) Phenotypic plasticity in adult life-history strategies compensates for a poor start in life in Trinidadian guppies (*Poecilia reticulate*). *The American Naturalist*, **176**, 818–823.

Auer, S.K., Lopez-Sepulcre, A., Heatherly, T., Kohler, T.J., Bassar, R.D., Thomas, S.A. & Reznick, D.N. (2012) Life histories have a history: effects of past and present conditions on adult somatic growth rates in wild Trinidadian guppies. *Journal of Animal Ecology*, **81**, 818–826.

Benton, T.G., Plaistow, S.J., Beckerman, A.P., Lapsley, C.T. & Littlejohns, S. (2005) Changes in maternal investment in eggs can affect population dynamics. *Proceedings of the Royal Society B-Biological Sciences*, **272**, 1351–1356.

Bernardo, J. (1996) The particular maternal effect of propagule size, especially egg size: patterns, models, quality of evidence and interpretations. *American Zoologist*, **36**, 216–236.

Berven, K.A. (1990) Factors affecting population fluctuations in larval ad adult stages of the wood frog (*Rana sylvatica*). *Ecology*, **71**, 1599–1608.

Berven, K.A. & Chadra, B.G. (1988) The relationship among egg size, density and food level on larval development in the wood frog (*Rana sylvatica*). *Oecologia*, **75**, 67–72.

Blackburn, T.M., Cassey, P. & Lockwood, J.L. (2009) The role of species traits in the establishment success of exotic birds. *Global Change Biology*, **15**, 2852–2860.

Bolnick, D.I., Amarasekare, P., Araujo, M.S., Burger, R., Levine, J.M., Novak, M., Rudolf, V.H.W., Schreiber, S.J., Urban, M.C. & Vasseur, D.A. (2011) Why intraspecific trait variation matters in community ecology. *Trends in Ecology & Evolution*, **26**, 183–192.

Braby, M.F. (1994) The significance of egg size variation in butterflies in relation to hostplant quality. *Oikos*, **71**, 119–129.

Caley, M.J., Carr, M.H., Hixon, M.A., Hughes, T.P., Jones, G.P. & Menge, B.A. (1996) Recruitment and the local dynamics of open marine populations. *Annual Review of Ecology and Systematics*, **27**, 477–500.

Fairweather, P.G. (1988) Consequences of supply-side ecology - manipulating the recruitment of intertidal barnacles affects the intensity of predation upon them. *Biological Bulletin*, **175**, 349–354.

Fowler, M. S. (2005) Interactions between maternal effects and dispersal. *Oikos*, **110**, 81–90.

Fox, C.W. (1997) The ecology of body size in a seed beetle, *Stator limbatus*: persistence of environmental variation across generations? *Evolution*, **51**, 1005–1010.

Fox, C.W. & Czesak, M.E. (2000) Evolutionary ecology of progeny size in arthropods. *Annual Review of Entomology*, **45**, 341–369.

Gooley, T.A., Marshall, D.J. & Monro, K. (2010) Responses to conspecific density in an arborescent bryozoan. *Marine Ecology Progress Series*, **415**, 83–90.

Hamilton, S.L., Regetz, J. & Warner, R.R. (2008) Postsettlement survival linked to larval life in a marine fish. *Proceedings of the National Academy of Sciences of the USA*, **105**, 1561–1566.

Heath, D.D., Fox, C.W. & Heath, J.W. (1999) Maternal effects on offspring size: variation through early development of chinook salmon. *Evolution*, **53**, 1605–1611.

Houssard, C. & Escarre, J. (1991) The effects of seed weight on growth and competitive ability of *Rumex acetosella* from 2 successional old-fields. *Oecologia*, **86**, 236–242.

Hoverman, J.T. & Relyea, R.A. (2007) How flexible is phenotypic plasticity? Developmental windows for trait induction and reversal. *Ecology*, **88**, 693–705.

Hughes, T.P. (1984) Population dynamics based on individual size rather than age - a general model with a reef coral example. *American Naturalist*, **123**, 778–795.

Jacobs, M.W. & Sherrard, K.M. (2010) Bigger is not always better: offspring size does not predict growth or survival for seven ascidian species. *Ecology*, **91**, 3598–3608.

Johnson, D.W. (2008) Combined effects of condition and density on postsettlement survival and growth of a marine fish. *Oecologia*, **155**, 43–52.

Johnston, T.A. & Leggett, W.C. (2002) Maternal and environmental gradients in the egg size of an iteroparous fish. *Ecology*, **83**, 1777–1791.

Kaplan, R.H. (1992) Greater maternal investment can decrease offspring survival in the frog *Bombina orientalis*. *Ecology*, **73**, 280–288.

Keough, M.J. (1986) The distribution of a bryozoan on seagrass blades: settlement, growth, and mortality. *Ecology*, **67**, 846–857.

Kinghorn, B.P. (1983) A review of quantitative genetics in fish breeding. *Aquaculture*, **31**, 283–304.

Kishida, O., Trussell, G.C., Mougi, A. & Nishimura, K. (2010) Evolutionary ecology of inducible morphological plasticity in predator-prey interaction: toward the practical links with population ecology. *Population Ecology*, **52**, 37–46.

Leslie, H.M. (2005) Positive intraspecific effects trump negative effects in high-density barnacle aggregations. *Ecology*, **86**, 2716–2725.

Lindholm, A.K., Hunt, J. & Brooks, R. (2006) Where do all the maternal effects go? Variation in offspring body size through ontogeny in the livebearing fish *Poecilia parae*. *Biology Letters*, **2**, 586–589.

Lindstrom, J. (1999) Early development and fitness in birds and mammals. *Trends in Ecology and Evolution*, **14**, 343–348.

Marshall, D.J., Allen, R.M. & Crean, A.J. (2008) The ecological and evolutionary importance of maternal effects in the sea. *Oceanography and Marine Biology: an Annual Review*, **46**, 203–250.

Marshall, D.J., Cook, C.N. & Emlet, R.B. (2006) Offspring size effects mediate competitive interactions in a colonial marine invertebrate. *Ecology*, **87**, 214–225.

Marshall, D.J. & Keough, M.J. (2008a) The evolutionary ecology of offspring size in marine invertebrates. *Advances in Marine Biology*, **53**, 1–60.

Marshall, D.J. & Keough, M.J. (2008b) The relationship between offspring size and performance in the sea. *American Naturalist*, **171**, 214–224.

Marshall, D.J. & Morgan, S.G. (2011) Ecological and evolutionary consequences of linked life-history stages in the sea. *Current Biology*, **21**, R718–R725.

Messier, J., McGill, B.J. & Lechowicz, M.J. (2010) How do traits vary across ecological scales? A case for trait-based ecology. *Ecology Letters*, **13**, 838–848.

Monro, K., Sinclair-Taylor, T. & Marshall, D.J. (2010) Selection on offspring size among environments: the roles of environmental quality and variability. *Functional Ecology*, **24**, 676–684.

Moran, N.A. (1994) Adaptation and constraint in the complex life-cycles of animals. *Annual Review of Ecology and Systematics*, **25**, 573–600.

Moran, D., Muniz Dias, G. & Marshall, D.J. (2010) Associated costs and benefits of a defended phenotype across multiple environments. *Functional Ecology*, **24**, 1299–1305.

Mousseau, T.A. & Dingle, H. (1991) Maternal effects in insect life histories. *Annual Review of Entomology*, **36**, 511–534.

Osman, R.W. & Whitlatch, R.B. (1996) Processes affecting newly-settled juveniles and the consequences to subsequent community development. *Invertebrate Reproduction & Development*, **30**, 217–225.

Parker, G.A. & Begon, M. (1986) Optimal egg size and clutch size - effects of environment and maternal phenotype. *American Naturalist*, **128**, 573–592.

Pausas, J.G., Bradstock, R.A., Keith, D.A. & Keeley, J.E. (2004) Plant functional traits in relation to fire in crown-fire ecosystems. *Ecology*, **85**, 1085–1100.

Pechenik, J.A., Wendt, D.E. & Jarrett, J.N. (1998) Metamorphosis is not a new beginning. *BioScience*, **48**, 901–910.

Phillips, N.E. (2002) Effects of nutrition-mediated larval condition on juvenile performance in a marine mussel. *Ecology*, **83**, 2562–2574.

Plaistow, S.J., Lapsley, C.T. & Benton, T.G. (2006) Context-dependent intergenerational effects: the interaction between past and present environments and its effect on population dynamics. *American Naturalist*, **167**, 206–215.

Quinn, G.P. & Keough, M.J. (2002) *Experimental Design and Data Analysis for Biologists*. Cambridge University Press, Cambridge.

Roach, D.A. & Wulff, R.D. (1987) Maternal effects in plants. *Annual Review of Ecology and Systematics*, **18**, 209–235.

Roughgarden, J. (1986) A comparison of food limited and space limited animal competition communities. *Community Ecology* (eds J. Diamond & T. Case), pp. 492–516. Harper and Row, New York, New York, USA.

Saastamoinen, M., van der Sterren, D., Vastenhout, N., Zwaan, B. & Brakefield, P. (2010) Predictive adaptive responses: condition-dependent impact of adult nutrition and flight in the tropical butterfly *Bicyclus anynana*. *The American Naturalist*, **176**, 686–698.

Schmitt, J., McCormac, A.C. & Smith, H. (1995) A test of the adaptive plasticity hypothesis using transgenic and mutant plants disabled in phytochrome-mediated elongation responses to neighbors. *American Naturalist*, **146**, 937–953.

Segers, F.H.I.D. & Tabrosky, B. (2011) Egg size and food abundance interactively affect juvenile growth and behaviour. *Functional Ecology*, **25**, 166–176.

Shima, J.S. & Swearer, S.E. (2009a) Larval quality is shaped by matrix effects: implications for connectivity in a marine metapopulation. *Ecology*, **90**, 1255–1267.

Shima, J.S. & Swearer, S.E. (2009b) Spatially variable larval histories may shape recruitment rates of a temperate reef fish. *Marine Ecology Progress Series*, **394**, 223–229.

Sibly, R., Calow, P. & Smith, R.H. (1988) Optimal size of seasonal breeders. *Journal of Theoretical Biology*, **133**, 13–21.

Sinervo, B. & McEdward, L.R. (1988) Developmental consequences of an evolutionary change in egg size - an experimental test. *Evolution*, **42**, 885–899.

Stanton, M.L. (1984) Seed variation in wild radish - effect of seed size on components of seedling and adult fitness. *Ecology*, **65**, 1105–1112.

Stearns, S.C. (1992) *The Evolution of Life Histories*. Oxford University Press, Oxford.

Strathmann, M.F. (1992) *Reproduction and Development of Marine Invertebrates of the Northern Pacific Coast: Data and Methods for The Study of Eggs, Embryos, And Larvae*. University of Washington Press, Seattle; London.

Tamate, T. & Maekawa, K. (2000) Interpopulation variation in reproductive traits of female masu salmon, *Oncorhynchus masou*. *Oikos*, **90**, 209–218.

Taylor, B.W., Anderson, C.R. & Peckarsky, B.L. (1998) Effects of size at metamorphosis on stonefly fecundity, longevity, and reproductive success. *Oecologia*, **114**, 494–502.

Thorson, G. (1950) Reproductive and larval ecology of marine bottom invertebrates. *Biological Reviews of the Cambridge Philosophical Society*, **25**, 1–45.
Toonen, R.J. & Pawlik, J.R. (1994) Foundations of gregariousness. *Nature*, **37**, 511–512.
Toonen, R.J. & Pawlik, J.R. (2001) Settlement of the gregarious tube worm *Hydroides dianthus* (Polychaeta: Serpulidae). I. Gregarious and nongregarious settlement. *Marine Ecology Progress Series*, **224**, 103–114.
Turnbull, L.A., Crawley, M.J. & Rees, M. (2000) Are plant populations seed-limited? A review of seed sowing experiments. *Oikos*, **88**, 225–238.
Unabia, C.R.C. & Hadfield, M.G. (1999) Role of bacteria in larval settlement and metamorphosis of the polychaete *Hydroides elegans*. *Marine Biology*, **133**, 55–64.
Underwood, A.J. & Keough, M.J. (2001) Supply-side ecology: the nature and consequence of variations in recruitment of intertidal organisms. *Marine Community Ecology* (eds M. D. Bertness, S. D. Gaines & M. E. Hay), pp. 183–200. Sinauer, Sunderland, MA.
Vonesh, J.R. (2005) Sequential predator effects across three life stages of the African tree frog, *Hyperolius spinigularis*. *Oecologia*, **143**, 280–290.
Vonesh, J.R. & De la Cruz, O. (2002) Complex life cycles and density dependence: assessing the contribution of egg mortality to amphibian declines. *Oecologia*, **133**, 325–333.
Webb, C.O. & Peart, D.R. (1999) Seedling density dependence promotes coexistence of Bornean rain forest trees. *Ecology*, **80**, 2006–2017.
Weinig, C. & Delph, L.F. (2001) Phenotypic plasticity early in life constrains developmental responses later. *Evolution*, **55**, 930–936.
West-Eberhard, M.J. (1989) Phenotypic plasticity and the origins of diversity. *Annual Review of Ecology and Systematics*, **20**, 249–278.
West-Eberhard, M.J. (2003) *Developmental Plasticity and Evolution*. Oxford University Press, Inc., New York.
Wilson, A.J. & Reale, D. (2006) Ontogeny of additive and maternal genetic effects: lessons from domestic mammals. *American Naturalist*, **167**, E23–E38.

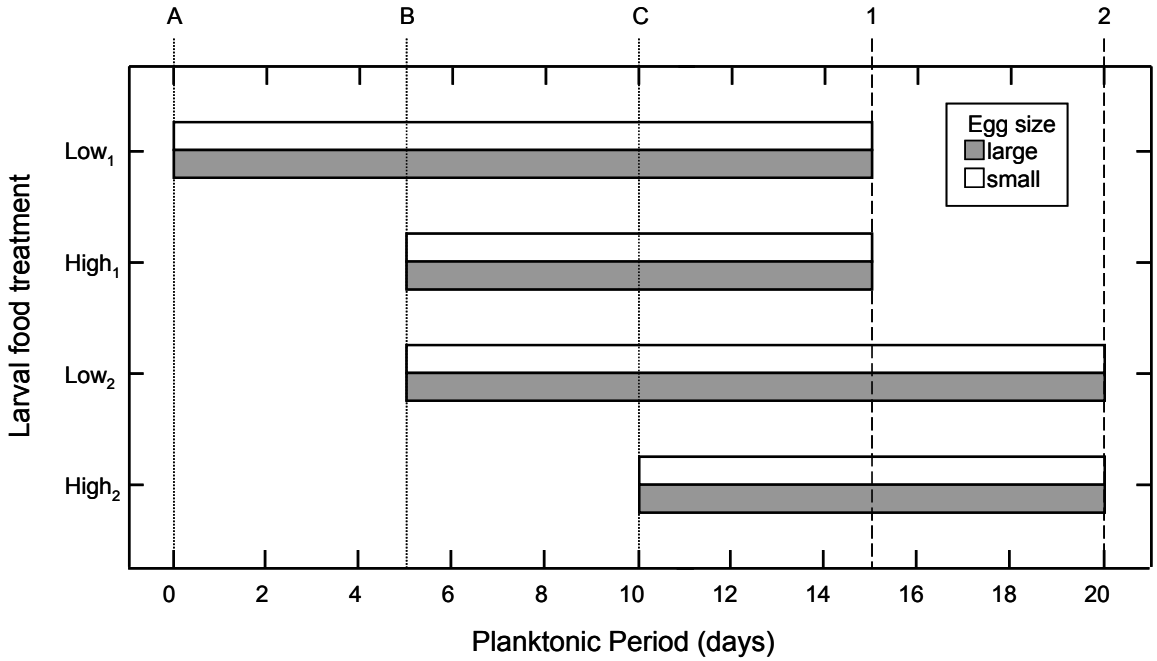
Received 6 November 2012; accepted 18 April 2013
Handling Editor: Jonathan Blount

Supporting Information

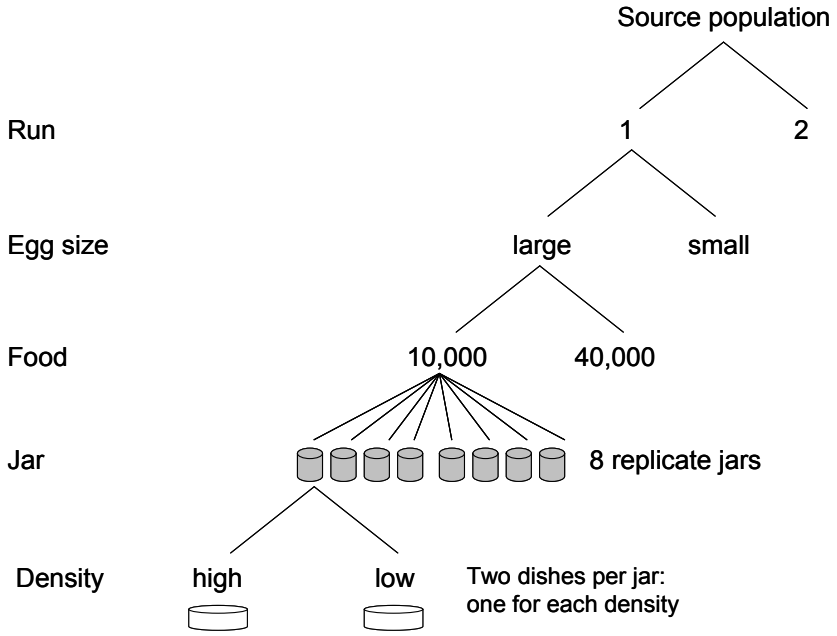
Additional Supporting Information may be found in the online version of this article:

Appendix S1. Experimental control for the effects of food availability and larval development time.
Appendix S2. Split-plot experimental design.
Appendix S3. REML analysis of Random factors.

Appendix S1. A schematic diagram of the experimental manipulations to control for the effects of larval food concentration on the development period in *Hydroides diramphus* larvae. The axes represent the total planktonic development time (x-axis), and the four groups of experimental run (y-axis). Larvae were either fed 10,000 or 40,000 cells ml^{-1} (low and high treatments respectively), and each run is indicated by the sub-script on the y-axis labels. Grey and white bars represent larvae that came from large and small eggs, respectively. Vertical lines represent the point in time when each treatment was commenced (dotted lines: A, B, and C), and the day larvae were settled and deployed into the field (dashed lines: 1 and 2).



Appendix S2. Split-plot experimental design to test the effects of egg size, phytoplankton concentration, post-metamorphic density and experimental sources of variation (run and jar) on post-metamorphic size and survival. The diagram shows one branch of all possible combinations of factors. Within each of the two runs, eggs were split into either large or small. Larvae were then fed high (40,000 cells ml^{-1}) or low (10,000 cells ml^{-1}) concentrations of phytoplankton, and each combination of egg size and food treatment was replicated in 16 culture jars. Once competent, the larvae from each jar were equally split into petri dishes for settlement then their densities were adjusted to either high or low density.



Appendix S3. REML analysis of Random factors. The non-significant random effects including interactions with Run (Egg size, Food, and Density) were omitted from the final model, however, Jar(Run) and Run as main effects were retained in the final model. Values are rounded to the nearest thousandth place.

Survival			
Source	2LL	*Chi*	*P*
Run x Density x Egg size x Food	12.696	<0.001	1.000
Run x Density x Food	12.696	2.701	0.100
Run x Egg size x Food	15.397	<0.001	1.000
Run x Density x Egg size	15.397	<0.001	1.000
Run x Egg size	15.397	<0.001	1.000
Run x Food	15.397	0.080	0.777
Run x Density	15.477	1.763	0.184
Run	17.731	0.232	0.630
Jar(Run)	17.240	0.491	0.483
Error	17.963		

Post-metamorphic size			
Source	2LL	*Chi*	*P*
Run x Density x Egg size x Food	377.488	<0.001	1.000
Run x Density x Food	377.488	<0.001	1.000
Run x Egg size x Food	377.488	<0.001	1.000
Run x Density x Egg size	377.488	1.799	0.362
Run x Egg size	378.318	<0.001	1.000
Run x Food	378.318	<0.001	1.000
Run x Density	378.318	0.254	0.614
Run	389.158	71.799	<0.001
Jar(Run)	378.572	10.586	0.001
Error	460.957		