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

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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 post-recruitment 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 diramphus, 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 quantiative 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 & Sweareng 2009a; Messier, McGill &
Nevertheless, an apparent trend in weak phenotypic relationships among species is that environmental conditions experienced (Kaplan 1992; Arnvist & 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. diramphus 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 planktrophic 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). Then we 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 prerroughened 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 x 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 effects 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 ± 0.23) survival after 10 days in the field, compared to 63.5% (SE ± 0.37) 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 (low or high) on postmetamorphic survival and 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.

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<th>Source</th>
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<th>F</th>
<th>P</th>
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<tr>
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<td>0.421</td>
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<tr>
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<tr>
<td>(b) Tube length</td>
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<td></td>
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<td>0.613</td>
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**Fig. 1.** The effect of phytoplankton concentration on mean survival of *Hydroides diramphus* recruits after 10 days in the field. 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
and the individual jar that larvae wear reared in on post-

postmetamorphic density, phytoplankton did not influence

centration, despite worms fed the lower phytoplankton
di

(Fig. 3). But recruits from larger eggs did not significantly

tubes postmetamorphosis that those from small eggs

ever, when fed the low concentration of phytoplankton,

phytoplankton were 10⁰⁰⁰ or 40⁰⁰⁰ cells mL⁻¹.

Fig. 3). When we compared the effects of 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 (F₁,₅₆ = 8.344, P = 0.005; 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 (F₁,₅₂ = 5.989, P = 0.018; 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 (F₁,₅₅ = 15.001, P < 0.001; 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 (F₁,₅₇ = 2.23, P = 0.141; Fig. 3).

There were strong effects of both the experimental run and the individual jar that larvae wear reared in on post-

metamorphic 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).

**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
Context-dependent phenotypic links

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; Mono, 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 manuscript).

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 *Hydroides dianthus* (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**

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**References**


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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

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<td>1.000</td>
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<td>Run x Density x Egg size</td>
<td>15.397</td>
<td>&lt;0.001</td>
<td>1.000</td>
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<tr>
<td>Run x Egg size</td>
<td>15.397</td>
<td>&lt;0.001</td>
<td>1.000</td>
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<td>Run x Food</td>
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<td>Run</td>
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<tr>
<td>Jar(Run)</td>
<td>17.240</td>
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<td>Error</td>
<td>17.963</td>
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### Post-metamorphic size

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<tbody>
<tr>
<td>Run x Density x Egg size x Food</td>
<td>377.488</td>
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<tr>
<td>Run x Density x Food</td>
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<tr>
<td>Run x Egg size x Food</td>
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<td>1.000</td>
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