

Pre-Settlement Behavior in Larval Bryozoans: The Roles of Larval Age and Size

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Abstract. Larval behaviors prior to settlement are important for both dispersal and the likelihood that larvae will encounter settlement habitat. The role of endogenous factors such as larval age and size are likely to be important in determining pre-settlement behavior but are less well understood than exogenous factors. In a simple experiment we explored the role of larval age and size on pre-settlement behavior in two species of bryozoan. We then used the results of this experiment to develop a theoretical model, which explored potential fitness benefits associated with phenotype-dependent changes in larval behavior (*i.e.*, behaviors that changed depending on larval age or larval size) in a heterogeneous environment. In the experiment we delayed the metamorphosis of larvae of *Bugula neritina* and *Watersipora arcuata* and assessed the changes in the behavior of individual larvae (exploring the substratum *vs.* swimming away from it) as a function of larval age and size. In *B. neritina*, larval size had no effect on larval swimming behavior, but the youngest and oldest larvae spent more time exploring the substrate than did larvae of intermediate age. In *W. arcuata*, larval size and age had interactive effects on larval behavior. Our theoretical model predicted that phenotype-dependent behaviors carried a fitness benefit relative to phenotype-independent behaviors, but this depended strongly on the availability and quality of habitat elsewhere. We suggest that, taken together, larval age and size are important endogenous factors that act to affect pre-settlement larval behavior and that changes in behavior may act to increase fitness.

Introduction

There is a long tradition in marine biology of examining the settlement choices of marine invertebrate larvae (Thorson, 1950; Ryland, 1960; Young, 1990). Such a focus is appropriate, particularly for species with a sessile or sedentary adult phase. The quality of the site at which larvae make the permanent transition from the water column to the substrate has profound implications for individual fitness and ultimately determines distribution and abundance within populations (Keough and Downes, 1982; Raimondi and Keough, 1990; Toonen and Pawlik, 1994; Jarrett, 1997; Armsworth and Bode, 1999). As a result of this intense research effort, we now recognize that a range of exogenous and endogenous factors affect the final choices made by larvae when accepting sites for settlement and metamorphosis (Knight-Jones, 1951, 1953; Raimondi and Keough, 1990; Pawlik, 1992; Toonen and Pawlik, 1994; de Nys *et al.*, 1995; Maldonado and Young, 1996; Jarrett, 1997; Marshall and Keough, 2003; Botello and Krug, 2006). In contrast to our knowledge of the factors influencing larval settlement choices, the factors that determine larval behavior prior to the final acceptance of substrata (pre-settlement), although clearly related, are less well studied (Walters *et al.*, 1999; Miron *et al.*, 2000).

Pre-settlement larval behaviors have profound implications for larval dispersal profiles and the type of settlement habitat that larvae encounter (Raimondi and Keough, 1990; Kisdí, 2002; Morgan and Anastasia, 2008). Pre-settlement behavior also determines the probability that a larva will encounter suitable habitat at all and so is the first critical element in the settlement process. Furthermore, variability in pre-settlement larval behavior ultimately contributes to variability of recruitment into populations (Armsworth and Bode, 1999).

Variability in pre-settlement behaviors among individuals is not only important ecologically, but may also be a target

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of selection if the behavior of individuals affects their fitness. Should a larva immediately swim upward, away from a settlement site it has rejected, or should it continue to explore in the local area in the hope of finding a more suitable microhabitat? Re-entering the water column allows larvae to search over a greater area, with the possibility of encountering better habitat elsewhere, but also carries risks including increased chances of mortality in the plankton or not encountering settlement habitat at all (Thorson, 1950; Strathmann, 1985). Selection is expected to favor choices that maximize individuals' post-metamorphic performance, so the best decision will depend on the costs and benefits associated with different behaviors. In that sense, the exploration of substrata for settlement sites could be viewed as a form of optimal foraging (Pyke, 1984). When foraging, organisms use information about the rate of energy intake to decide when to leave a patch and search for food elsewhere, and this behavior can be predicted using optimal foraging theory (Krebs *et al.*, 1974; Charnov, 1976). Similarly, larvae may use information about their local environment and endogenous state to decide when to settle or when to search for settlement habitat elsewhere, in order to maximize their expected fitness (Doyle, 1975; Pyke, 1984; Ward, 1987; Stamps *et al.*, 2005; Toonen and Tyre, 2007; Clobert *et al.*, 2009). Recent reviews have highlighted the ecological and evolutionary importance of linking exogenous and endogenous factors that determine dispersal and habitat selection in terrestrial animals (Bowler and Benton, 2005; Nathan *et al.*, 2008; Clobert *et al.*, 2009). These reviews emphasized the fact that the phenotype (*i.e.*, the product of their genotype and the environment that they experience) of dispersers strongly determines both their distribution and dispersal profiles. In marine organisms, there is a lack of understanding of how experiences throughout the larval stage influence larval behavior and the extent to which larval behaviors are phenotype-dependent.

Like the factors that influence larval settlement choices, pre-settlement larval behavior is determined by exogenous and endogenous factors (Walters *et al.*, 1999; Miron *et al.*, 2000). Exogenous factors such as hydrodynamics, substratum complexity, chemical cues, and light have all been shown to have independent and interactive effects on behaviors prior to settlement (Crisp, 1955; Young and Chia, 1982; Maldonado and Young, 1996; Walters *et al.*, 1999; Wendt and Woollacott, 1999; Miron *et al.*, 2000; Hadfield and Koehl, 2004; Prendergast *et al.*, 2008). For example, Walters *et al.* (1999) showed that behavior of the bryozoan *Bugula neritina* depended on the interactive effects of water flow and substratum type. Larval behaviors can also change over time: the larvae of many invertebrate species are photopositive upon release and then become photoneutral or photonegative over time, a change that is often associated with an increase in exploring behavior (*e.g.*, Ryland, 1960; Thorson, 1964; Wendt and Woollacott, 1999).

Endogenous factors such as larval age, size, physiological

condition, developmental stage, genotype, and prior exposure to stresses can affect settlement decisions (Knight-Jones, 1951, 1953; Toonen and Pawlik, 1994; Jarrett, 1997; Marshall and Keough, 2003; Botello and Krug, 2006), so it is reasonable to expect that these factors will also affect larval behavior prior to settlement. The influence of age and size are of particular interest in species with nonfeeding larvae because these larvae cannot swim indefinitely and delaying settlement reduces post-metamorphic performance, thus carrying significant fitness costs (Pechenik, 2006). In a rare example of an examination of the influence of endogenous physiological cues on larval behavior, Miron *et al.* (2000) showed that, as nonfeeding cyprid larvae of the barnacle *Balanus amphitrite* aged and their physiological condition declined over time, the proportion of larvae that explored available settlement surfaces increased. In that study, larval physiological condition was not the sole determinant of behavior; rather there was an interplay between endogenous and exogenous factors: both young and old larvae explored preferred settlement surfaces, but only old larvae, in poorer physiological condition, explored non-preferred surfaces (Miron *et al.*, 2000). Thus, despite some indications that larval behavior is affected by factors such as larval age, we still have little understanding of how dynamic larval behavior is across the larval period. Also unknown is whether other endogenous factors such as larval size—which in species with nonfeeding larvae appears to be correlated with the amount of resources available for post-metamorphic growth (Marshall *et al.*, 2003)—modify the influence of larval age over time. Furthermore, because of the difficulty of examining the fitness consequences of different larval behaviors in the field, we have little information on whether changes in larval behavior are adaptive responses associated with gathering information about the local habitat or are simply an inevitable consequence of larvae getting older.

Our goals here are twofold. First, we empirically examine whether larval behavior (swimming or exploring) prior to settlement changes over time and whether variation among individuals relates to differences in larval size. To do this, we followed the behavior of individual larvae of two species of marine bryozoan, *Bugula neritina* and *Watersipora arcuata*. These species release nonfeeding larvae that are competent to settle immediately. Our experiments were conducted in the laboratory in still water so that we could clearly distinguish between swimming and exploring behavior. We deliberately used a low-quality settlement cue, otherwise larvae (particularly older ones) would have settled immediately upon exposure to the settlement surface, precluding the examination of larval behavior.

Second, we use a theoretical model to explore whether there are fitness benefits associated with the observed behaviors relative to behaviors that are independent of larval phenotype. Because a range of parameters such as habitat quality, probability of finding habitat, planktonic mortality,

and fitness costs associated with accepting poor settlement sites are likely to influence larval fitness, we examine changes in behavior across a range of values for these parameters. Thus our theoretical examination contained a range of factors that were not explored empirically. This approach, known as sensitivity analysis, was used to determine whether there was a broad or narrow parameter space in which changes in larval behavior carried fitness benefits.

Materials and Methods

Study species

Bugula neritina (Linnaeus, 1758) and *Watersipora arcuata* (Banta, 1969) are cosmopolitan species of arborescent and encrusting bryozoans respectively, and occur on man-made structures in protected harbors on the south and east coasts of Australia. Both species brood embryos and release nonfeeding larvae that are competent to settle upon release. Their larvae typically spend minutes to hours in the plankton before permanently attaching to suitable substrata and metamorphosing.

Study location and collection methods

All experiments were done at the University of Queensland in Brisbane, Australia. We collected reproductively mature colonies of the two species from the sides of floating docks: *B. neritina* from Manly Boat Harbor (Brisbane, Queensland, Australia; 27°27'S, 153°11'E) in August 2007, and *W. arcuata* from the Lincoln Marine Science Centre (Port Lincoln, South Australia; 34°44'S, 135°52'E) in August 2008. *B. neritina* colonies were returned straight to the laboratory, whereas *W. arcuata* colonies were transported by airfreight to Brisbane in a dark, insulated container. Transport of *W. arcuata* took 8 h; colonies arrived in good condition and subsequently survived in the laboratory for 2 weeks, showing no signs of partial colony mortality and producing larvae on two occasions. In the laboratory, colonies of both species were kept in dark, aerated aquaria for 48 h before spawning. We repeated experiments multiple times on both species. We collected new colonies of *B. neritina* for each repeat, whereas the same *W. arcuata* colonies were induced to spawn on two occasions separated by 3 days for two different runs of the same experiment.

Spawning and measurements of larval size

To collect larvae, we removed colonies from the dark and placed them in separate large beakers of seawater, which we exposed to bright light. This procedure induces colonies to release larvae within about 15 min. Larvae from 5–9 colonies of a single species were pooled and then randomly selected for the experiments. Within 15 min of release, each larva was placed on a microscope slide in a drop of seawater and then digitally photographed with the median furrow (for *B. neritina*) or eyespots (for both species) facing the camera

(PixeLINK Capture SE, ver. 1.0). Subsequently, photographs were used to measure the size of each larva (cross-sectional area) with image processing software (Image Pro Express, ver. 5.1) according to standard methods (Marshall and Keough, 2003)

Manipulating dispersal and measuring larval behavior

Larvae of both species exhibit two distinct behaviors that are visible to the naked eye, and we classified larval behavior accordingly. Swimming behavior occurs when larvae move rapidly, often erratically, through the water column away from a suitable settlement surface. Exploring behavior occurs when larvae are stationary on, or moving slowly across, a settlement surface and includes characteristic spinning and crawling behavior (Walters *et al.*, 1999). Exploring represents a fine-scale searching behavior that occurs prior to settlement.

For each larva, we manipulated dispersal time by preventing settlement. For *B. neritina* we did this by putting individual larvae into 5-ml vials filled with 0.45- μ m-filtered seawater and then constantly rotated each vial by using a mechanical roller. This procedure limits the potential for larval attachment by constantly moving potential settlement surfaces. *W. arcuata* suffered high mortality when the mechanical roller technique was used, so we prevented settlement by placing individual larvae in clear, 10-ml tissue-culture wells filled with 0.45- μ m-filtered seawater exposed to bright light from above and below. Both methods were effective at preventing settlement.

To measure the proportion of time spent swimming and exploring, we removed individual larvae from vials (*B. neritina*) or tissue-culture wells (*W. arcuata*) and placed them into a petri dish (4-cm diameter, 5-mm depth) with 30 ml of 0.45- μ m-filtered seawater. The petri dish was roughened but contained no biofilm and represents a suitable, but poor-quality, settlement surface. Changes in behavior over time in response to preferred settlement surfaces were not assessed, though many invertebrate larvae settle much sooner, and in greater numbers, on biofilmed surfaces (Marshall and Keough, 2003). Lighting was provided from above (ceiling lights) and was constant throughout the experiment. After a 1-min adjustment time, we recorded the amount of time that the larva spent swimming and exploring in the following 2-min period. For both species, our methods of preventing settlement between observation periods did not appear to affect larval behavior. Prior observations indicated that larval behavior was similar immediately after larvae were placed into the observation dishes compared to 5 min later. We then put each larva back in a vial or well to delay settlement further. We repeated this procedure at 0, 1, 3 and 5 h after larval release and so had four repeated measurements of behavior for each larva. The experiment was repeated seven times for *B. neritina* and twice for *W.*

arcuata. In total, repeated measurements were made on 31 *B. neritina* larvae and 23 *W. arcuata* larvae.

Data analysis

The effects of larval size and larval duration on the behavior of larvae were tested using repeated-measures ANCOVA. Larval duration was a fixed, repeated factor, experimental run was a random factor, and larval size was a covariate in these analyses. There were several nonsignificant terms in the model for *B. neritina*, and these were removed to generate a final model. We also tested for a quadratic trend across the levels of larval duration for *B. neritina*. All statistical analyses were done using Systat ver. 11, significance levels were set at $\alpha = 0.05$, and all ANCOVA assumptions were satisfied.

Model

To explore theoretically whether any of the observed changes in behavior could provide a fitness benefit in a heterogeneous environment, we developed a discrete-time, simulation model in which suitable habitat composed a proportion of good or poor settlement sites (Fig. 1; Table 1; Appendix). Good settlement sites are ones that result in a higher fitness than poor sites, and it is assumed that larvae have evolved to discriminate between good and poor sites by responding to cues related to expected fitness and using them as guides to settlement. The model is broadly based on that used by Stamps *et al.* (2005) and Elkin and Marshall (2007). Larvae begin at a patch where the probability of finding good or poor settlement sites is proportional to the abundance of each in that habitat. Larvae always accept good settlement sites immediately, but the probability of accepting poor habitat increases over time (*i.e.*, larval specificity decreases over time; Knight-Jones, 1951, 1953) according to a logistic function (Elkin and Marshall, 2007). Settling in poor sites confers a fitness cost relative to settlement in good sites. Those larvae that reject poor-quality habitat either continue exploring or swim away. Those that swim away then have some probability of locating a different habitat patch. There is differential mortality associated with swimming and exploring, and fitness declines over increasing larval durations as larvae consume finite energy sources.

Fitness is given by the sum of larvae that survive until reproduction in all habitat patches over time, t , according to:

$$Fitness = \sum_{t=0}^T (S_{g,t}M_{x,t}w_g) + (S_{p,t}M_{x,t}w_p)$$

where S_g and S_p are the total number of larvae that settle in good and poor settlement sites (summed over both patches) in each time step, w_g and w_p are the fitness costs in good and poor settlement sites, and $M_{x,t}$ is the probability that larvae

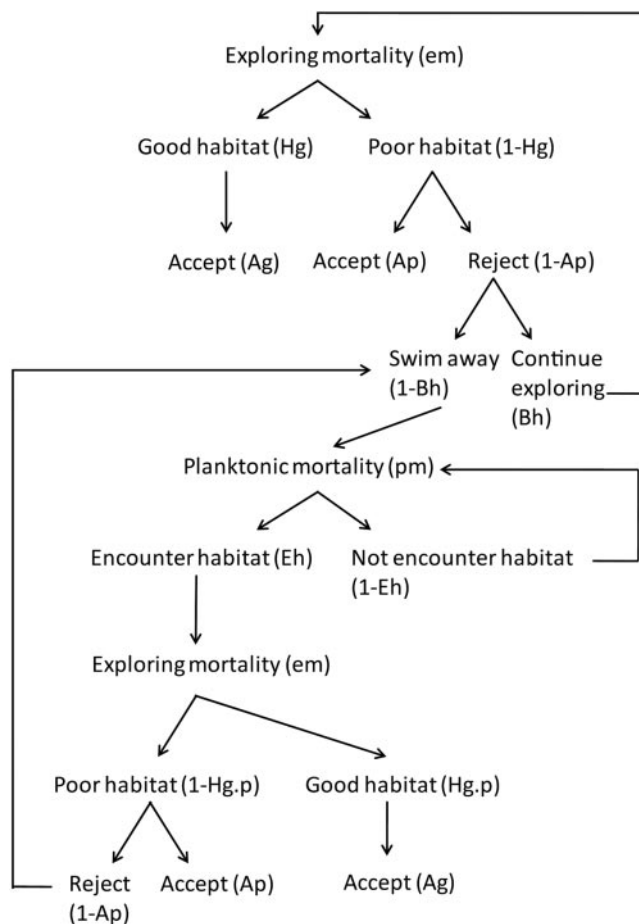


Figure 1. Conceptual design of the model explained in detail in the Appendix. Parameters are defined in Table 1. Larvae go through each level at each time step until all larvae settle or die.

survive after settlement, which is a decreasing function of larval energy reserves. For each set of parameters (Table 1), the model was run twice. In the first run, the probability that larvae explored or swam away after rejecting poor-quality settlement sites was kept constant over time at 50%. We use this first run as a null model and refer to this as phenotype-independent behavior because the behavior is independent of age or size. In the second run, we varied larval behavior with age and size on the basis of our findings in our empirical study. We modeled three behavior profiles that were observed in our empirical study: (i) that of large *W. arcuata* larvae, where the amount of time spent swimming increased over time; (ii) that of small *W. arcuata* larvae, which spent most of their time constantly exploring, regardless of larval age; and (iii) that of *B. neritina* larvae, in which young and older larvae explored more than larvae of intermediate age. We refer to this as phenotype-dependent behavior since the probability that larvae swim or explore depends on age and size. Results are presented as relative fitness, which is the fitness obtained in the second run divided by the fitness in the first run. Values greater than 1

Table 1

Parameters of the model referred to in the main text and Figure 1 as well as the range of values explored

Parameter	Explanation	Range
Eh	Probability of encountering habitat	0-1
Hg	Proportion of habitat containing good sites in habitat first encountered	0-1
Hg.p	Proportion of habitat containing good sites in habitat encountered after previously rejecting a settlement site	0-1
em	Exploring mortality rate (per time step)	0.0025-0.0225
pm	Swimming mortality rate (per time step)	0.0025-0.0225
wp	Fitness cost in poor habitats (low values equal high costs)	0.01-0.5
wg	Fitness cost in good habitats (low values equal high costs)	0.5-1
Ap	Probability of accepting good habitat	0.9-1
Apt	Probability of accepting poor habitat (logistic decline over time as function of energy reserves)	—
Bh	Probability that larvae will explore after rejecting poor habitat (fitted values from empirical data, or Bh = 0.5)	0-0.9

therefore indicate a fitness advantage of the observed behavior relative to a situation where larval behavior was independent of phenotype. Details of the model including parameters and their values are presented in Table 1 and the Appendix.

Results

Empirical results

There was no effect of experimental run ($F_{7,23} = 1.1410$, $P = 0.373$) or larval size ($F_{1,7} = 2.1623$, $P = 0.1849$) on the behavior of *Bugula neritina* larvae, and so these terms were removed from the final model (Table 2a). There was a significant quadratic relationship between larval duration and larval behavior ($F_{1,29} = 6.2994$, $P = 0.0179$; Fig. 2): immediately after being released, larvae spent substantial time exploring settlement surfaces; they then decreased exploration time at 1 and 3 h, before increasing it again at longer durations (5 h; Fig. 2).

The behavior of larvae of *Watersipora arcuata* depended on both larval size and larval duration (interaction in Table 2b, $F_{3,3} = 9.587$, $P = 0.0478$). Small larvae spent most of their time (>80%) exploring settlement surfaces irrespective of larval duration (Fig. 3). Larger larvae explored less than small larvae (<70% of the time) at all times. The time spent exploring decreased with larval duration for larger larvae (Fig. 3).

Model results

The model indicated that in an environment with varying abundance of suitable sites for settlement, the phenotype-

Table 2

Repeated-measures ANCOVA testing for the effects of larval size and larval duration on behavior: (a) reduced model (i.e., simple repeated-measures ANOVA) for *Bugula neritina* and profile analysis to detect for quadratic trend across levels of duration; and (b) full model for *Watersipora arcuata*

Source	df	MS	F	P*
(a) <i>Bugula neritina</i>				
Duration	3	0.2789	2.7811	0.0458
Error	87	0.1003		
<i>Duration effects</i>				
Quadratic function	1	0.6078	6.2994	0.0179
Error	29	0.0965		
(b) <i>Watersipora arcuata</i>				
<i>Between Subjects</i>				
Larval size	1	0.6348	1.623	0.2198
Run	1	0.4223	4.1568	0.0573
Run × Larval size	1	0.3909	3.8480	0.0664
Error	17	0.1016		
<i>Within Subjects</i>				
Duration	3	0.0550	4.296	0.1311
Duration × Larval Size	3	0.0767	9.587	0.0478
Duration × Run	3	0.0128	0.1936	0.9003
Duration × Run × Larval Size	3	0.0080	0.1205	0.9476
Error	51	0.0662		

*Values in boldface type are statistically significant at 0.05.

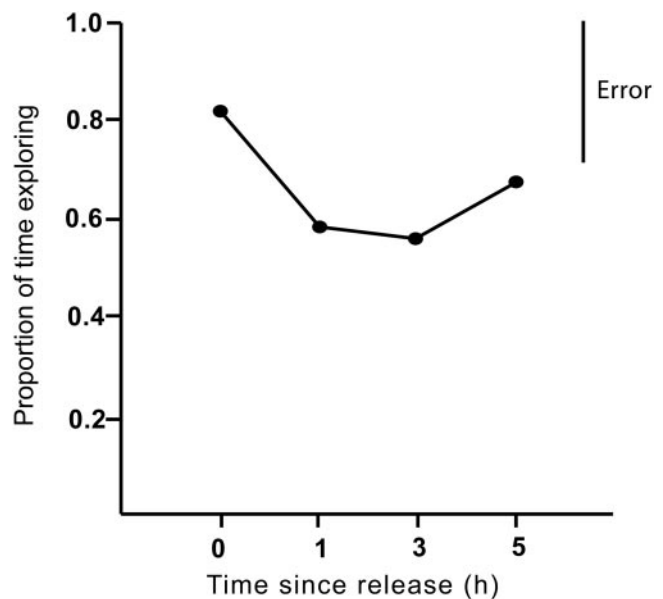


Figure 2. The influence of larval duration on larval behavior in *Bugula neritina*. Error bars are shown in the top right corner of the plot. Note that the appropriate error for this figure is the $\sqrt{MS_{error}}$ from Table 2a (for an explanation of why this error is appropriate, see p. 506, Quinn and Keough, 2002).

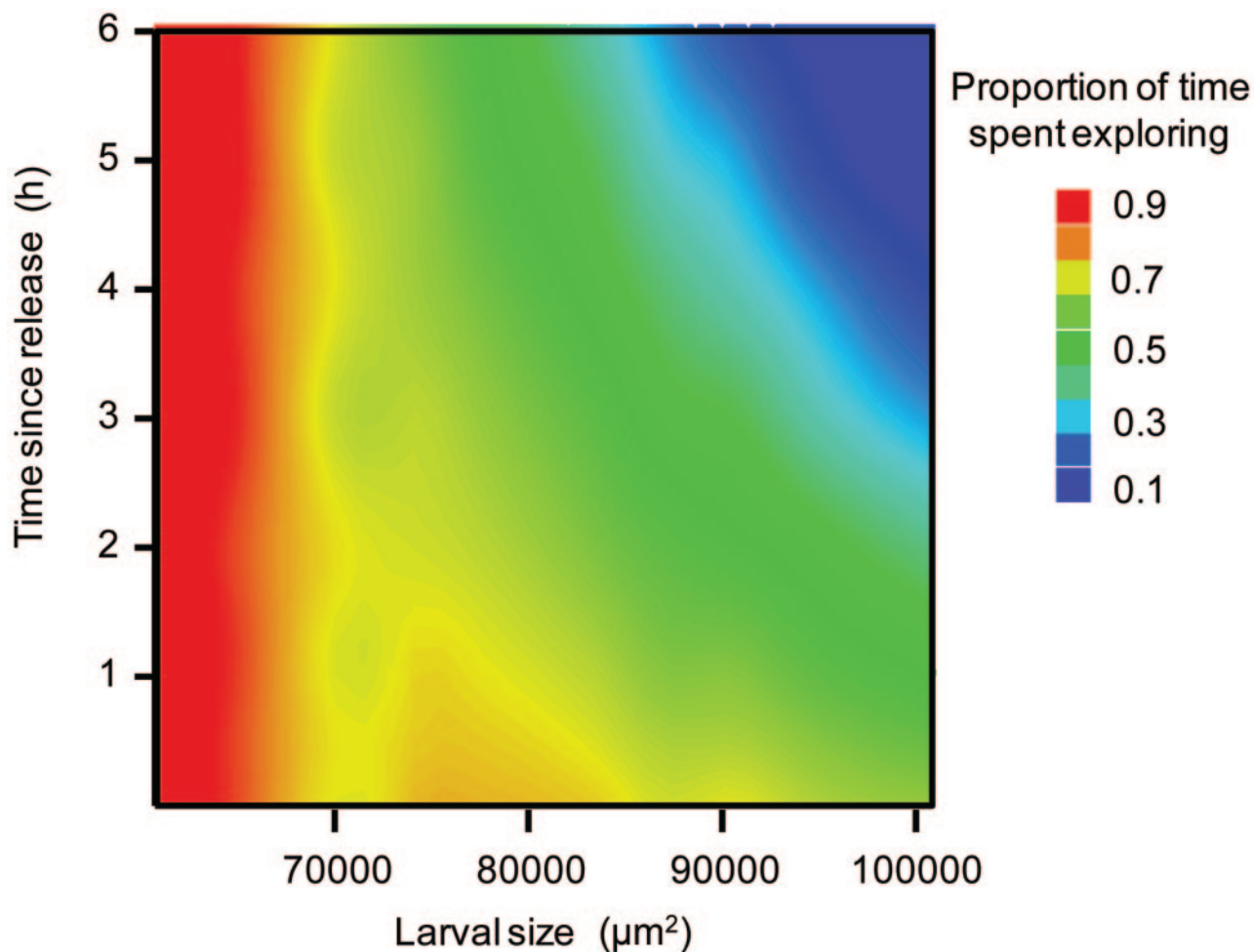


Figure 3. The influence of larval size and larval duration on larval behavior in *Watersipora arcuata*. Colors represent proportion of time spent exploring settlement surfaces estimated from the model in Table 2b.

dependent behaviors observed in the experiments resulted in a higher fitness than the phenotype-independent behaviors over much of the parameter space explored (grey areas of Fig. 4). The probability of encountering habitat after rejecting a site and swimming away (E_h) was found to be the most important factor determining when different phenotype-dependent behaviors were favored.

Continuing to explore the current habitat patch, such as was the case for *B. neritina* and small larvae of *W. arcuata*, was more beneficial than phenotype-independent behaviors when the probability of encountering other habitat was low ($E_h = 0.2$, grey areas in Fig. 4a, c). This was true even if there was a higher abundance of good habitat elsewhere (above diagonal lines). Neither of the behaviors exhibited by *B. neritina* and small *W. arcuata* were favored over phenotype-independent behaviors if there was a high probability of encountering better habitat elsewhere (white areas of Fig. 4b, d), though there were still some situations when this was not the case (grey areas above diagonal lines in Fig. 4b, d). Increases in the probability of swimming away from current habitat, as was observed in large *W. arcuata* larvae,

increased fitness only when there was a higher abundance of good habitat elsewhere and a high chance of locating that habitat (Fig. 4e, f). In other words, swimming away from poor habitat never increased fitness if there was poorer habitat elsewhere. In contrast, due to the additional costs of pelagic mortality and not encountering habitat, continuing to explore the current habitat could still be favored despite there being a slightly higher abundance of good habitat elsewhere. Sensitivity of these model results to changes in the magnitude of fitness costs in poor habitats (w_p) and the strength of planktonic mortality relative to swimming mortality were also assessed, but had little effect on the overall findings.

Discussion

We found that larval behavior was dynamic over time in *Bugula neritina* regardless of larval size, but in *Watersipora arcuata* only larger larvae changed their behavior. Our theoretical model highlighted how phenotype-dependent behaviors, such as those relating to age and size as observed in

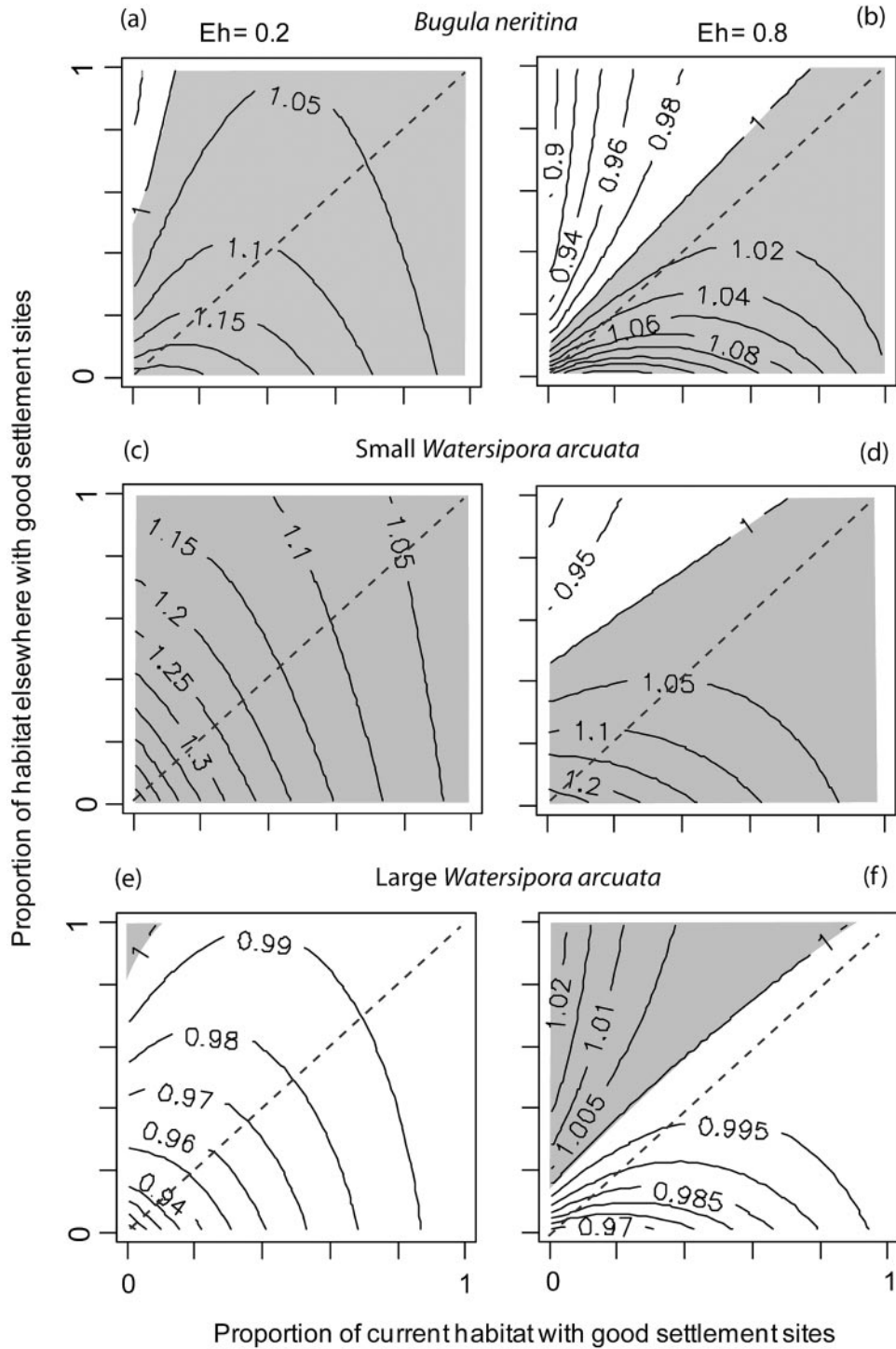


Figure 4. Contour plot of the fitness benefits of phenotype-dependent behaviors observed in *Bugula neritina* larvae (a, b), small (c, d) and large (e, f) *Watersipora arcuata* larvae, in the experiments compared to those obtained from phenotype-independent behaviors (see Methods). Fitness benefits vary depending on how good the current habitat is (x-axis) in relation to habitat elsewhere (y-axis) and the probability of encountering habitat elsewhere (Eh). Diagonal dashed line indicates that current habitat and habitat elsewhere have the same proportion of good and poor settlement sites. Results are shown when the probability of encountering habitat is low (Eh = 0.2; left panel) and high (Eh = 0.8; right panel). Grey areas show relative fitness above 1, indicating a higher fitness of the observed behaviors compared to behaviors that do not depend on larval phenotype.

the experiments, can carry a fitness advantage over phenotype-independent behaviors. Smaller *W. arcuata* larvae spent most of their time exploring the substratum regardless of age, but larger larvae, especially those that had been delayed for a longer period, spent increasing amounts of time swimming away from the substratum. Behavior in *B. neritina* depended only on larval age, but the effect of age was nonlinear: the youngest and oldest larvae spent more time exploring than did larvae of intermediate age. The theoretical model highlighted that the probability of encountering good-quality habitat was the most important factor determining when either of these behaviors might be advantageous compared to a situation in which behaviors were constant across larval age and size. Before discussing the broader implications of our model, however, we must first discuss some of the limitations of the data that we used to generate our model.

Although our empirical approach was artificial and precludes easy generalization to field situations, our experimental set-up allowed us to isolate the effects of larval age and size in the absence of other cues. These results therefore provide some of the first information on how these two endogenous factors affect larval behavior prior to settlement in marine invertebrates with nonfeeding larvae. Whereas most previous studies of larval behavior prior to settlement have demonstrated the importance of external cues, such as hydrodynamics, substratum type, and light (Ryland, 1960; Thorson, 1964; Wendt and Woollacott, 1999), we have shown that internal cues can also be important. For non-feeding larvae, these internal cues relate to larval age and size. However, many factors correlate with age and size (such as energy reserves, biomass, metabolic rate, developmental stage, or genetic changes), so it is difficult to assign proximate causes for the observed changes in behaviors. An important next step will be to separate the effects of energy reserves from other correlates of age and size so as to determine the relative contribution of these different factors in determining temporal changes in larval behavior prior to settlement.

Although exogenous and endogenous factors interact to affect larval behavior (Miron *et al.*, 2000), we minimized the number of exogenous factors to focus on the role of endogenous factors. In the field, many factors potentially influence larval behaviors prior to settlement, such as hydrodynamics, pressure, light, substratum type, resident community, or presence of predators (Cronin and Forward, 1986; Forward and Tankersley, 2001; Morgan and Anastasia, 2008; Prendergast *et al.*, 2008). Though notoriously difficult to study larval behaviors in the field, the few studies that have overcome some of the challenges have found nonrandom patterns of behavior (*e.g.*, Shanks, 1985; Young, 1986; Davis and Butler, 1989; Bingham and Young, 1991; Walters *et al.*, 1999; Leis *et al.*, 2006; Prendergast *et al.*, 2008). Laboratory studies that have attempted to include some of these additional factors found that the strength of

constant water flow mediates behavioral responses to different types of substratum (Crisp, 1955; Walters *et al.*, 1999; Miron *et al.*, 2000). For example, Walters *et al.* (1999) showed that larvae of *B. neritina* were more likely to reject and swim away from a surface in still water than in constant flow conditions ($1.3\text{--}8.3\text{ cm s}^{-1}$), which could be advantageous in the field if food availability is highest in locations with high flow. Similarly, cyprid larvae of the barnacle *Balanus amphitrite* explored less surface area in still water (Miron *et al.*, 2000). Therefore, while our study represents an important first step in examining how larval behavior changes over time, the patterns of behavior that we observed in the experiments may be modified under different flow conditions and substratum types, and future investigations of this topic should examine the interaction between larval endogenous factors and exogenous factors such as flow.

It is well known that marine invertebrate larvae respond to light, and the sign of phototaxis (positive or negative) depends on many things such as larval age, light intensity, or gravity (Ryland, 1960; Thorson, 1964; Forward, 1974; Young and Chia, 1982; Miller and Hadfield, 1986; Barile *et al.*, 1994; Wendt and Woollacott, 1999). Like many invertebrate larvae, those of *Bugula spp.* and *Watersipora spp.* are typically photopositive upon release but become photonegative over time (Ryland, 1960; Thorson, 1964; Wendt and Woollacott, 1999). The role of phototaxis in our findings therefore remains unclear. Given that larvae shift from being photopositive to photonegative with age, one would expect a similar, directional shift in exploratory behavior over time in both species, regardless of larval size. The behavioral changes that we observed were, however, more complex than those that would be expected on the basis of a simple shift in phototaxis alone. For *B. neritina*, the changes in larval behavior were nonlinear over time and in *W. arcuata*, there were complex interactions with larval size. Changes in exploratory behavior could still interact with larval age-mediated shifts in phototaxis, but evaluating this hypothesis and isolating its effects requires further experiments. But regardless of what specific mechanisms drove the shifts in larval behavior over time, it is clear that larval behavior prior to settlement is dynamic in the species studied here, and our theoretical model predicts that these changes in behavior through time can be favored by selection.

Our model predicted that, in an environment where the abundance of suitable sites for settlement varied, larvae with phenotype-dependent behavior had higher fitness than larvae with phenotype-independent behavior over much of the parameter space explored (grey areas of Fig. 4). However, the fitness benefits of phenotype-dependent behaviors did depend on the type and availability of habitat elsewhere. Continuing to explore after rejecting a settlement site, as was observed in experiments on *B. neritina* and small larvae of *W. arcuata*, often resulted in higher fitness than pheno-

type-independent behaviors, though the disparity was less if there was a high chance of encountering better habitat elsewhere. In contrast, larvae that spent increasing amounts of time swimming away from habitat, as was observed in large larvae of *W. arcuata*, benefited only if there was a high chance of encountering better quality habitat elsewhere. Given the assumptions of our model and the lack of information on the availability of good-quality and poor-quality habitat in nature, the specific predictions of our model should be interpreted with caution. Nevertheless, the purpose of the model was to show that phenotype-dependent changes in larval behavior prior to settlement can carry a fitness benefit under a variety of conditions considered in our model, suggesting that dynamic larval behavior could represent an adaptive strategy.

What is the ultimate cause of swimming away from a potential settlement site and why might larval age affect, in complex ways, the amount of time larvae spend exploring? Although we acknowledge the assumptions of the model and the limitations of extrapolating our experimental results to the field, we suggest that larvae may use their own age or time spent exploring as a cue about the local abundance of quality habitat in the area. In that sense, settlement could be viewed as a form of optimal foraging, where larvae gather information and behave in such a way as to maximize their expected fitness (given no genetic constraints; Charnov, 1976; Pyke, 1984; Clobert *et al.*, 2009). Since the 1970s, much effort has been devoted to examining the role of information acquisition in optimal foraging theory more generally (*e.g.*, Charnov, 1976; Krebs *et al.*, 1978; Pyke, 1984). Previous studies on optimal habitat selection in time-limited dispersers predict that the length of time during which larvae should reject poor-quality sites depends on the time available for searching, the abundance of poor-quality sites, the difference in fitness between the good and poor sites, and the survival rate during dispersal (Doyle, 1975; Ward, 1987). An optimal foraging strategy for marine larvae strongly depends on the grain size of quality settlement environments, the temporal reliability of cues related to expected fitness, the amount and type of information actually acquired and whether larvae can adjust behavior accordingly, and the potential for behaviors to be an evolved response to cues (Pyke, 1984). Importantly, individual larvae are unlikely to have the opportunity to sample all habitats and choose the best one. Rather, information gathered concerning their current patch and condition, as well as external factors affecting the decision to leave, is expected to play a major role in determining overall behavior. Since larvae cannot search indefinitely, the optimal allocation of time searching a particular habitat for a favorable settlement site should depend on how much foraging time remains (Krebs *et al.*, 1974; Ward, 1987), which for nonfeeding larvae could be an internal cue such as age or energy reserves. Selection is expected to act on this internal cue

when the probability of finding a better patch is unknown (Doyle, 1975; Ward, 1987; Dingle and Drake, 2007).

Viewing our results in an optimal foraging framework, repeated exposure to low-quality habitat (as was done in our experiments here) would indicate that poor-quality habitat is locally abundant and that larvae in good condition should benefit from initially swimming away from that habitat in order to increase their chances of being transported to another suite of habitats. Qualitatively similar predictions were also made by Doyle (1975). Continuing to explore a poor-quality habitat may still be optimal if larvae are in poorer condition or if other costs associated with leaving suitable habitat (*e.g.*, the probability of encountering other habitat) are strong enough to select for larvae that continue to explore. It will be interesting to determine whether exposing larvae to different indicators of habitat quality (*e.g.*, biofilmed *vs.* unbiofilmed surfaces) as they age changes their behavior over time. If larvae do indeed use their prior experience of settlement surfaces as information about habitat availability, then we would predict that larvae repeatedly exposed to high-quality habitat will increase the amount of time they spend exploring, but this remains to be tested. We also expect the fitness differences between phenotype-dependent and phenotype-independent behaviors to be greater in species with longer periods of delayed metamorphosis (*e.g.*, Ockelmann and Muus, 1978). This is because species with longer pelagic larval periods have higher accumulated costs of planktonic mortality and of being transported away from settlement habitat (Jackson and Strathmann, 1981).

Our results also have implications for maternal effects. Mothers often produce offspring of a range of sizes (*e.g.*, Marshall and Keough, 2008), and our data for *W. arcuata* show that larvae of different sizes have very different swimming behavior. By producing larvae of different sizes, mothers are in fact producing offspring with polymorphic dispersal profiles (Krug, 2001; Toonen and Pawlik, 2001). Since vertical stratification (such as boundary layers and wind-driven surface currents) of water bodies is common, larvae of different ages and sizes are likely to be transported to different places. In *W. arcuata* in particular, smaller larvae could be regarded as most likely to settle close to the maternal colony, whereas larger larvae are much more likely to disperse and colonize habitats that are farther away. Both *B. neritina* and *W. arcuata* colonies increase the size of their offspring in response to changes in the levels of competition that mothers experience (Allen *et al.*, 2008; Marshall and Keough, 2009). Our results here and earlier strongly suggest that increases in mean larval size can act as an adaptive shift in offspring provisioning in order to increase the chances that offspring will escape a poor-quality, high-competition environment in favor of colonizing a new habitat.

In summary, our empirical results suggest that larval swimming behavior is not constant over time and can vary

among individuals of different size. Our theoretical results suggest that this dynamic larval behavior may carry fitness benefits relative to larvae that pursue a constant strategy with an equal chance of either behavior. These results have important implications because if settlement behavior is viewed as a form of foraging, then factors related to larval age and size could act as an internal source of information about the relative abundance of quality habitat in the local area, and this should influence settlement decisions. Selection should favor those individuals that can accurately assess the current environment and make decisions based on their current state and past experience to increase expected fitness, but additional experiments that measure behavior and subsequent post-metamorphic success are required. Nonetheless, variation in responses to external cues coupled with complex age- and size-dependent larval behaviors have implications for estimating dispersal profiles and understanding recruitment variation, both of which are key processes in the dynamics of marine populations.

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Appendix

Model of larval behavior and fitness

The probability that larvae survive after settlement, $M_x(R, \alpha, \beta)$, was modeled as a function of energy reserves (R) using the incomplete beta function, which is defined by:

$$M_x(R, \alpha, \beta) = \frac{1}{B(\alpha, \beta)} \int_0^R t^{\alpha-1} (1-t)^{\beta-1} dt$$

Where α and $\beta > 0$, and $B(\alpha, \beta)$ is the value of the beta function:

$$B(\alpha, \beta) = \frac{\Gamma(\alpha)\Gamma(\beta)}{\Gamma(\alpha + \beta)}$$

The values of α and β were held constant at 10 and 2, respectively. Habitat-specific fitness curves are derived by multiplying $M_x(R, \alpha, \beta)$ by the habitat-specific relative fitness value (wg or wp for good or poor, respectively).

Energy reserves (R) were depleted over time (t) according to

$$R = 1 + t \cdot m^{-1/4} - c$$

where m is the mass-specific rate of energy use by larvae and c is the relative difference in initial energy reserves between largest and smallest larvae. It was assumed that different-sized larvae started with different levels of energy.

The probability of accepting good habitat (A_g) was 1, and the probability of accepting poor habitat was modeled as a logistic function of time by:

$$A_p = \frac{1}{1 + (\exp(rR - b))}$$

Where r and b are rate and shape parameters and were set to 18 and 14, respectively.