

Increased larval planktonic duration and post-recruitment competition influence survival and growth of the bryozoan *Watersipora subtorquata*

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ABSTRACT: For organisms with complex life cycles, longer time spent in the plankton by dispersing propagules can cause reduced survival, growth and fecundity, which could alter interactions between neighbours in the post-dispersal environment. We compared post-settlement performance of bryozoan *Watersipora subtorquata* colonies that developed from larvae of different natural and experimental planktonic durations over ca. 15 wk of colony growth. Settlers were situated either near established adults of the ascidian *Botrylloides leachii* or without competition. Increased larval planktonic durations reduced colony growth in the absence of competition; colonies that developed from longer or delayed larval durations were 2 to 3 times smaller than those that developed from shorter durations. Colonies that developed from longer larval periods (natural or experimental) also experienced higher mortality (75 to 100%) than those that settled quickly (20 to 42%), but these effects varied between experiments and seasons. In winter, *W. subtorquata* colonies of longer larval planktonic durations experienced greater mortality when adjacent to established *B. leachii*, whereas differences in colony growth due to planktonic duration were reduced by adjacent *B. leachii*. The influence of *B. leachii* varied between experiments in different seasons, however, and did not alter colony performance in summer. Our findings demonstrate that while increased larval planktonic duration can be costly for post-dispersal growth and survival, some differences can be mediated by species interactions and environmental variability. This suggests that while connectivity among populations that take longer to disperse may be limited, it may also be influenced in complex ways by the post-recruitment environment and not simply dispersal duration.

KEY WORDS: Larval dispersal · Planktonic duration · Competition · Marine bryozoan · Post-settlement · *Watersipora*

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INTRODUCTION

Sessile and sedentary organisms rely almost exclusively on dispersal by larvae, spores and seeds to colonise habitats and connect populations. While dispersal is crucial for population connectivity, it can be energetically and physically costly for dispersing propagules, and can negatively affect the performance of individuals well beyond the end of the dispersal phase (Pechenik et al. 1998, Phillips 2002, Mar-

shall & Keough 2003, Pechenik 2006, Bonte et al. 2012). If the costs accrued by propagules during dispersal reduce their ability to successfully survive, grow and reproduce post-dispersal, connectivity among populations can be less than would be suggested by the number of propagules capable of arriving at a given habitat (Stamps et al. 2005, Shima & Swearer 2009, Burgess & Marshall 2011). However, the quality of habitats colonised by propagules can mediate how costs associated with dispersal affect

life-stages beyond the end of the dispersal phase. In good quality habitats dispersal costs may be ameliorated, while they may be exacerbated in harsh or poor quality habitats (Stanton 1984, Marshall et al. 2010, Shima & Swearer 2010, Burgess et al. 2012). A key step towards understanding the processes that influence connectivity among populations is therefore to understand how variation in propagule dispersal history influences the colonisation success and post-settlement performance of juvenile and adult colonisers in habitats of different quality.

If propagules are planktonic and do not feed (as is the case for many marine invertebrates), greater dispersal can result in longer planktonic durations, increased swimming activity and delayed metamorphosis, leading to a decline in propagule condition as energy reserves are consumed. This can affect a propagule's response to the cues that terminate the dispersal stage (Marshall & Keough 2003) and have strong effects on post-dispersal performance (Pechenik et al. 1998, Pechenik 2006). Increased larval swimming activity can reduce the survival, growth rates and size of juveniles and adults as well as increase the time taken for metamorphosis in a range of marine invertebrates, including colonial ascidians (Marshall et al. 2003), bryozoans (Woollacott et al. 1989, Wendt 1996, 1998, Burgess & Marshall 2011, Burgess et al. 2012), sipunculid worms (Pechenik & Rice 2001), polychaetes (Qian & Pechenik 1998), gastropods (Roberts & Lapworth 2001), echinoderms (Highsmith & Emler 1986) and crustaceans (Pechenik et al. 1993, Gebauer et al. 1999).

Individual larvae may experience longer planktonic durations either because their settlement has been delayed due to the absence of suitable settlement sites, or because they have 'chosen' to spend more time dispersing in the plankton despite the availability of suitable settlement sites. The maximum swimming durations of non-feeding larvae can be determined by energetic reserves (Wendt 2000), which vary among individuals. When suitable settlement sites are readily available, differences in planktonic durations among conspecific larvae may reflect differences in energy reserves, with larvae that spend longer in the plankton possessing higher energy reserves and entering the post-settlement phase still in relatively good condition. In contrast, larvae that are unable to find suitable settlement sites may be forced to swim for durations beyond their innate preference based on their energy reserves, resulting in significantly poorer condition post-settlement (Marshall & Keough 2003). Therefore, the implications of increased planktonic duration with respect to

the condition of settling larvae could be very different depending on whether they have been delayed/prevented from settling or have preferentially spent longer in the plankton despite having opportunities to settle into suitable habitat.

Once dispersal is complete, propagules must settle and become established in habitats that can vary greatly in quality. In harsh or unfavourable habitats, larvae that arrive in poorer condition may exhibit lower survival and growth as juveniles and adults than those in good condition, while in benign environments, larval condition may be less important as all larvae can do well after settlement (Ward 1987, Marshall & Keough 2004, Marshall et al. 2010, Burgess et al. 2012). Often, larvae will settle alongside other settlers or established adults, and thus post-settlement competition with nearby individuals for space and food can present a particularly unfavourable environment (Marshall et al. 2006, Allen & Marshall 2010). If larvae of longer planktonic durations are in poorer condition, they may be more likely to experience lower survival or growth if they settle near competitors than those that are in better condition due to shorter planktonic duration (Marshall et al. 2003, 2006, Burgess & Marshall 2011). Thus, the presence of established competitors could further reduce the realised connectivity of populations among which propagules take longer to disperse. However, variation in the presence/absence of competitors could also have an important mediating influence on the performance of individuals of different larval planktonic durations if, for example, larvae arriving in good condition due to shorter planktonic durations experience lower survival and growth when they settle near established competitors than larvae that arrive in poorer condition due to longer planktonic durations but manage to avoid settling near competitors. How the causes of, and costs associated with, different planktonic durations influence post-settlement interactions between nearby individuals is still poorly understood. In particular, few studies have examined how colonisers of different larval planktonic durations perform near established adult competitors of different species.

In this study, we experimentally compared the effects of different natural planktonic swimming durations and forcibly delayed settlement on the growth and survival of colonies of a common bryozoan, *Watersipora subtorquata*, in the presence of a neighbouring established competitor, the colonial ascidian *Botrylloides leachii*, as well as in the absence of competition. In *W. subtorquata*, the effects of variation in larval condition on colony performance can emerge

weeks after settlement and can persist beyond 9 to 12 wk of colony age (e.g. Ng & Keough 2003). Therefore, we followed colony performance for >13 wk to determine how long the effects of planktonic larval durations persist and how these effects are influenced by nearby *B. leachii* colonies. We predicted that *W. subtorquata* colonies that developed from larvae of longer planktonic durations would be smaller and survive for less time than their counterparts that developed from larvae of shorter planktonic durations, and that delayed larvae in particular would have the lowest growth and survival. We also predicted that *W. subtorquata* colonies from longer planktonic durations/delay treatments would have higher mortality and lower growth rates in competition with adjacent established adult *B. leachii* colonies than those from shorter planktonic durations and, again, that delayed larvae would experience the lowest survival and growth rates. Last, we predicted that *W. subtorquata* colonies from all larval planktonic durations would perform better under conditions of no competition than those in competition with *B. leachii*.

MATERIALS AND METHODS

Study site and species

Experiments were conducted subtidally from May until late December 2011 at Workshops Jetty, Williamstown, Australia (37° 51' 39.78" S, 144° 54' 34.17" E). Workshops Jetty is located in the northern end of Port Phillip Bay, a large shallow embayment in temperate southeastern Australia (for a more detailed description of the study site see Sams & Keough 2007).

Watersipora subtorquata (Bryozoa: Cheilostomata) is a cosmopolitan encrusting bryozoan commonly found on hard natural and artificial substrates throughout southeastern Australia. Adult *W. subtorquata* colonies reproduce via internally brooded, non-feeding larvae that spend a short time (minutes to hours) dispersing in the plankton before settlement (Marshall & Keough 2003, D. J. Marshall & M. J. Keough unpubl. data). Larvae were obtained from colonies of *W. subtorquata* collected from Workshops Jetty. Colonies were collected from artificial settlement surfaces that were deployed approximately 1 yr earlier and suspended from the jetty to approximately 2 m below the low water mark. For each of the experiments outlined below we used a different set of colonies to obtain larvae.

Larval planktonic duration treatments and colony deployment

To obtain larvae, we transported reproductively mature *W. subtorquata* colonies from the field to the lab and held them in the dark within a re-circulating seawater system. Water temperature was ~15°C and lab temperature was ~22°C in all experiments. After approximately 48 h we induced spawning by placing colonies in shallow trays of seawater and exposing them to bright fluorescent lamps and natural sunlight. Larvae spawned from multiple colonies (20 colonies in Expts 1A and 1B, and 15 and 20 in Expts 2A and 2B, respectively) ranging in age from approximately 1 to 6 mo and in size from 2 to 7 cm in diameter.

When larvae were released from adult colonies, we pipetted approximately half directly into settlement trays where they could undergo natural settlement (here, we define 'settlement' as both the attachment and beginning of metamorphosis). The remaining larvae were pipetted into plastic vials that were placed on a mechanical roller mixer (Hwashin 205RM, Hwashin Technology) that rolled vials continuously at 40 rpm for 5 h, preventing larvae from settling and forcing them to remain in the plankton. Delayed larvae were spread across 10 (Expts 1A and 2A) and 12 (Expts 1B and 2B) vials, from which we obtained 1 to 4 larvae. At the end of the delay treatments, larvae were randomly assigned to settlement trays and then to experimental treatments after completing settlement.

A potential artefact of rolling larvae in vials is that they may experience greater physical stress from turbulence and impacts with the wall of vials, thus making it difficult to disentangle the effects of longer planktonic duration from physical damage to larvae. The speed of vial rotation used in this study was low, and although it was enough to prevent larvae from attaching to the vial walls, it did not cause larvae to regularly impact vial walls. Instead, we typically observed larvae swimming in the middle of the vials where water was less turbulent, although they may have impacted vial walls if they attempted to settle. Because such impacts could potentially affect the physical condition of the larvae, we also compared the delayed larvae to larvae that naturally swam for similar periods of time, allowing us to formally test for negative effects of experimental methods on larvae.

Settlement trays contained cool seawater and were lined with roughened sheets of acetate to facilitate settlement. We submerged the acetate sheets in a re-circulating seawater system for several days prior to

spawning so that they could develop microbial biofilms to encourage larval settlement. We left larvae overnight to undergo and complete metamorphosis (approximately 24 to 72 h; Ng & Keough 2003) before arranging them in experimental treatments on settlement plates.

Previous pilot studies in the lab revealed that most *W. subtorquata* larvae that were not forced to swim settled within the first 3 to 5 h of being spawned. Comparable patterns of *W. subtorquata* settlement timing have been observed in other studies (Marshall & Keough 2003). Similarly, in all of our experiments, approximately one-third of larvae of natural planktonic duration settled and began metamorphosis within 3 h of spawning and two-thirds did so after 5 h. To compare natural and longer larval planktonic durations and to disentangle the effects of delay treatment and naturally (i.e. un-manipulated) longer planktonic durations, we divided larvae into 3 treatment groups: (1) those that settled and began metamorphosis naturally within 3 h of being spawned (short natural planktonic duration, SN), (2) those that settled naturally and began metamorphosis 5 h or later after being spawned (long natural planktonic duration, LN), and (3) larvae that settled and began metamorphosis after being placed on rollers for 5 h to delay settlement for a similar amount of time as those with long natural durations (delayed, D).

Our 3 treatments provide several useful comparisons of the effects of planktonic duration on post-settlement colony performance. First, comparing naturally short and long larval durations provides a direct comparison of whether there are costs associated with longer planktonic durations under the same conditions. Second, comparing experimentally delayed and natural long planktonic durations provides both a comparison of whether being forcibly delayed from settling versus innately remaining in the plankton for a similar time period has different associated costs and also acts as a control treatment to compare whether there are artefacts associated with rolling larvae in vials that are not due to planktonic duration.

The morning after *W. subtorquata* larvae were spawned and settled, we cut small squares of acetate (ca. 1 × 1 cm) bearing successfully metamorphosing individuals and attached them to the surface of opaque acrylic settlement plates (10 × 10 cm) using a small drop of cyanoacrylate adhesive placed on the underside of acetate squares. We allowed the adhesive to air dry for approximately 5 to 10 s before placing plates into small tubs of cool seawater which were replaced frequently for approximately 1 h, at which

time plates were transferred to larger tanks supplied by a closed seawater system. The following day, settlement plates with attached *W. subtorquata* settlers were transported to Workshops Jetty in containers of cool seawater and attached to weighted PVC backing panels (50 × 50 cm) suspended from the pier by a rope to a depth of approximately 2 m below the low water mark at approximately 4 to 5 m maximum depth. Plates were attached to panels using stainless steel bolts (5 mm diameter) that were passed through a hole in the middle of the plate (colonies of both species used in the experiments readily overgrow bolts).

Expt 1: colony performance adjacent to adult *Botrylloides leachii*

Botrylloides leachii (Chordata: Ascidiaceae, Stolidobranchia) is a common colonial ascidian found on artificial and natural surfaces throughout our study area. While it can be large and competitively dominant in many systems, at our study site it tends to attain only moderate sizes (100 to 400 mm² for large colonies), and single colonies rarely dominate available space. *W. subtorquata* often attains similar or greater sizes to *B. leachii* at this site; thus they are often evenly matched space competitors as adults (M. A. Sams unpubl. data).

To examine the effects of larval planktonic duration on the performance of colonies that settle adjacent to established colonies of *B. leachii*, we placed a single, newly settled *W. subtorquata* individual from one of the 3 planktonic duration treatments on either a bare plate (control) or on a plate containing a single adult colony of *B. leachii*, in a fully crossed factorial design. Colonies of *B. leachii* were approximately 4 to 5 cm in diameter and were located in a haphazardly chosen corner of a plate. Two weeks prior to transplanting *W. subtorquata* recruits, whole *B. leachii* colonies were removed from older settlement surfaces and pier piles by sliding paint scrapers under colonies and gently prying them free. Detached colonies were then held against new plates with rubber bands and submerged beneath Workshops Jetty for approximately 1 wk, after which they attached to plate surfaces with their own naturally produced adhesives. Similar methods have been used to transplant congeners of *B. leachii* and sponges without adverse effects (Agius 2007, Johnston & Clark 2007). *B. leachii* colonies appeared healthy following attachment and continued to grow throughout the duration of the experiments. In *B. leachii* treatments, a recruit of *W. subtorquata* was

placed 2 cm away from the growing edge of *B. leachii* colonies at a haphazard location around the colony edge, at least 1 cm from the rim of the plate. In control treatments, a recruit of *W. subtorquata* was also placed at least 1 cm from the edge and 2 cm from the midline of new plates such that they were in a similar location as recruits in competition treatments.

We ran 2 separate established *B. leachii* neighbour experiments, the first from early May until late August during autumn/winter (Expt 1A), and the second from mid-September to late December during spring/summer (Expt 1B) 2011, using identical methods. There were 9 and 12 replicates of each planktonic duration \times *B. leachii* treatment in Expts 1A and 1B, respectively. In Expt 1A, there were 2 replicates of all treatments across 4 panels and a 5th panel with only 1 replicate of each treatment, while in Expt 1B there were 6 panels with 2 replicates of all treatments.

Expt 2: colony performance without competition

Initially, we designed Expt 2 to test the effects of larval planktonic durations on colony performance under intraspecific competition. We placed *W. subtorquata* settlers of different planktonic durations onto plates so that they were arranged in adjacent pairs. Each plate had a pair of adjacent colonies of either SN vs. LN or LN vs. D treatments randomly located on either the top or bottom half of the plate and separated by approximately 6 cm. We were not able to obtain enough larvae to compare all planktonic duration treatments, so rather than using all possible paired larval planktonic duration combinations, we only compared the above subset, which we consider to be the most informative comparisons.

In the field, *W. subtorquata* naturally settle at a range of distances from one another where they are likely to interact, from immediately touching to centimetres apart. The strength of effects is determined by how quickly colonies grow to encroach on each other's space relative to their distance. In this experiment, paired settlers were placed within 1 cm of one another so that they would be likely to rapidly encounter their neighbour as they grew and competed for space. However, colonies did not grow quickly or large enough to directly interact with each other during our experiments, and we found no evidence that competition occurred. Detailed methods and results comparing the size and mortality of colonies from different larval planktonic durations in adjacent competition pairs are provided in Supplement 1 at www.int-res.com/articles/suppl/m531p179_suppl1.pdf. Be-

cause competition did not occur, we pooled paired competition treatments and only present comparisons of the growth and survival of colonies from larvae of different planktonic durations. These experiments provide complementary information to Expt 1 about variation in the consequences of different larval planktonic duration since they were done at different times to Expts 1A and 1B.

We ran 2 separate experiments without competition: the first from early June until mid-September 2011 during winter/spring (Expt 2A), and the second from mid-September to December 2011 during spring (Expt 2B), using identical methods. In Expt 2A there were 22 SN, 42 LN pairs and 20 D colonies spread across 22 plates and 5 panels. In Expt 2B there were 24 SN, 41 LN and 17 D pairs spread across 21 plates and 5 panels. In both Expts 1 and 2, differences in the numbers of replicates, panels and settler pairs (Expt 2 only) were due to differences in the number of colonies that spawned and successfully settled in each experimental run.

Sampling

We recorded weekly colony survival until the end of each experiment at 14 (Expts 1A and 1B), 13 (Expt 2A) and 15 (Expt 2B) wk. Colonies were considered dead if they were no longer present on settlement plates or if they had turned completely white (all zooids appeared dead) and remained that way for 2 wk (age of death was recorded at the beginning of this 2 wk period). To measure colony growth rates, we took standardised photographs of plates and *W. subtorquata* colonies every 2 wk starting from Week 3 in *W. subtorquata* neighbour experiments and Week 1 for *B. leachii* neighbour experiments until the end of each experiment. The size of colonies was measured as colony area (mm²) using ImageJ image analysis software. *W. subtorquata* produces approximately uniform-sized zooids, and colony area is a reasonable proxy for the number of zooids forming a colony (Bone 2006). We also measured ancestrula size of all colonies in both *Watersipora* experiments (Expts 1A and 1B) and our second *Botrylloides* experiment (Expt 2B). Ancestrula size is correlated with larval size, which can have important effects on post-settlement survival and colony growth in *W. subtorquata* (Marshall & Keough 2003).

Throughout all experiments, we cleared any new recruits of all species that settled onto plates once each week so that only the individuals from our experiments were present on a plate.

Data analysis

In all experiments, we compared the survival of *W. subtorquata* colonies from different larval planktonic durations and neighbourhood treatments by generating survival curves using the Kaplan-Meier product-limit estimate of the survivorship function, and comparing them using the log rank test (further details of comparisons specific to each experiment are provided below). Our response variable was the proportion of colonies surviving to each week sampled.

The log-rank test does not allow for the inclusion of covariates, so we were not able to include ancestrula size as a predictor variable in any of our survival analyses. To determine if larvae of different planktonic duration treatments had different size distributions that could influence colony size or survival, we compared ancestrula size between larval planktonic duration treatments in Expts 1A, 1B and 2B (we did not measure ancestrula size in Expt 2A) using a 1-way ANOVA. We found no significant differences in ancestrula size among different larval planktonic duration treatments in these experiments (Expt 1A: $F_{2,118} = 1.89$, $p = 0.16$; Expt 1B: $F_{2,116} = 2.40$, $p = 0.10$; Expt 2B: $F_{2,57} = 1.04$, $p = 0.36$). The ranges of larval sizes obtained in these experiments were much smaller than those reported to cause significant differences for the survival and growth of *W. subtorquata* colonies in other studies (Marshall & Keough 2008), and may be less likely to have a strong influence on colony performance.

In all experiments, we also compared differences in the sizes of colonies (measured as colony area, mm²) that developed from larvae of different planktonic durations using linear mixed effects models (LMMs). All colony size data were log₁₀ transformed to meet assumptions of homogeneity of variance. In 3 of our experiments (Expts 1A, 1B and 2B), we found that by 7 wk our sample sizes were too small (due to colony mortality) to effectively run LMMs. Consequently, we only compared the size of colonies up to 7 wk in all experiments (colony sizes until experiment end are provided in Supplement 2 at www.int-res.com/articles/suppl/m531p179_supp2.pdf). In all linear effects models, significance of the main fixed effects was assessed using the Wald statistic. All analyses were done in R v.3.1.1 using the 'survival' and 'nlme' packages.

Survival and colony growth analysis

Expt 1

To determine whether colonies that developed from larvae of different planktonic duration had dif-

ferent survivorship in the presence/absence of adult *B. leachii*, we first used the log-rank test to compare whether there was an overall difference in survivorship between colonies of different larval planktonic durations when *B. leachii* was present and absent in separate tests. To determine whether *B. leachii* had an overall effect on colony survival that may have overwhelmed any effect of planktonic duration, we also compared differences in colony survival between *B. leachii* and no *B. leachii* treatments pooled over larval planktonic duration. The log-rank of more than 2 survival curves tests whether there is an overall difference between them, but does not indicate which curves differ. If a significant overall difference in these survival curves was detected, we then followed this analysis with a log-rank test to perform pairwise comparisons of survivorship of colonies between each planktonic duration treatment. To determine whether *B. leachii* had an overall effect on colony survival that may have overwhelmed any effect of planktonic duration, we also compared differences in colony survival between *B. leachii* and no *B. leachii* treatments pooled over larval planktonic duration treatments.

We compared the size of colonies from different planktonic duration treatments in a single factorial LMM, with terms consisting of 4 factors: larval planktonic duration (fixed with 3 levels: SN, LN, D), *Botrylloides* (fixed with 2 levels: *B. leachii* present or absent), weeks (fixed with 4 levels: 1, 3, 5 and 7 wk) and colony (random with 36 [Expt 1A] and 42 [Expt 1B] levels repeatedly sampled through time). If experimental treatments had a significant interaction with time, we determined whether there was an effect of experimental treatment that matched our hypothesis by running pairwise comparisons (Tukey's HSD) within each week sampled at the relevant treatment level. For example, for a larval planktonic duration × *Botrylloides* × week interaction, we ran pairwise comparisons of colony size amongst each larval planktonic duration treatment within each *Botrylloides* treatment and time. If there were multiple interactions, we only compared treatments within the levels of the highest order interaction.

Although we used log₁₀-transformed colony area for analyses and to interpret treatment differences detected by LMMs, we report differences in observed mean sizes of colony area (mm²) ± the observed SE of colony size within each treatment at each time period measured, as they are more intuitive to interpret. We provide figures showing the means and SE of log-transformed data that we used to interpret differences in colony size in

Supplement 2. Ancestrula size did not have an interaction with any of our main treatment effects, nor did it differ between planktonic durations in these experiments (though it had additive effects in some comparisons, but these did not change our conclusions) so we present LMMs without ancestrula size as a term for simplicity.

Expt 2

In Expt 2, we analysed the 2 runs of the *W. subtorquata* neighbour experiment separately, as patterns of colony growth and environmental conditions between the experiments were very different. We compared the survival of *W. subtorquata* colonies from each larval planktonic duration treatment using survival analyses as outlined above. We compared the size of colonies from larvae of different planktonic duration in LMMs consisting of 3 factors: larval planktonic duration (fixed with 3 levels: SN, LN and D) and weeks (fixed with 3 levels: 3, 5 and 7 wk) and colony (random and nested within larval planktonic duration, with 62 levels in Expt 2A and 64 levels in Expt 2B). If experimental treatment had a significant interaction with time, we determined whether there was an effect of experimental treatment that matched our hypothesis by running pairwise comparisons (Tukey's HSD) comparing larval planktonic duration treatments within each week sampled.

RESULTS

Expt 1: colony performance adjacent to adult *Botrylloides leachii*

Expt 1A: survival

In Expt 1A, survival of *Watersipora subtorquata* colonies that developed from larvae of different planktonic durations did not differ when *Botrylloides leachii* was absent ($\chi^2 = 0.28$, $df = 2$, $p = 0.87$; Fig. 1a). When *W. subtorquata* colonies were next to established *B. leachii* there was a strong trend for colonies from LN and D treatments to have lower survival than those of SN, but this was marginally non-significant ($\chi^2 = 6.9$, $df = 2$, $p = 0.063$; Fig. 1b). At the end of the experiments, only 11% of colonies from D and 22% from LN treatments survived, whereas 60% of colonies from SN survived next to *B. leachii* colonies (Fig. 1b). Survival of *W. subtorquata* was significantly lower overall when *B. leachii* was present ($\chi^2 = 6.79$,

$df = 1$, $p = 0.009$). At the end of the experiments at 14 wk, only 32% of *W. subtorquata* colonies adjacent to *B. leachii* survived compared to 64% survival of colonies without neighbouring *B. leachii* (Fig. 1c).

Expt 1A: growth

Larval planktonic duration and the presence of *B. leachii* had an interactive effect on *W. subtorquata* colony size that changed over time (larval planktonic duration \times time: $F_{6,94} = 2.816$, $p = 0.015$; *Botrylloides* \times time: $F_{3,94} = 3.44$, $p = 0.020$; larval planktonic duration \times *Botrylloides* \times time: $F_{6,94} = 2.547$, $p = 0.024$). In the absence of *B. leachii*, *W. subtorquata* colonies from LN and D did not differ in size at any time. However, colonies from SN were larger than those derived from LN at 5 wk and were at least twice the size of colonies from both LN and D after 7 wk (Fig. 1d). In contrast, the size of *W. subtorquata* colonies that developed from different larval durations was not significantly different at any time when they were adjacent to established *B. leachii* (Fig. 1d). Overall, *W. subtorquata* colony size increased over the first 7 wk of growth ($F_{3,94} = 470.241$, $p = 0.001$) but there were no significant overall differences due to larval planktonic duration ($F_{2,32} = 2.822$, $p = 0.071$) or *B. leachii* presence/absence ($F_{1,32} = 1.072$, $p = 0.308$) or an overall interactive effect of larval planktonic duration with *B. leachii* presence/absence ($F_{2,32} = 0.021$, $p = 0.976$) (Fig. 1d).

Expt 1B: survival

In Expt 1B, *B. leachii* did not affect survival of *W. subtorquata* colonies (overall test pooled across larval types, $\chi^2 = 1.8$, $df = 1$, $p = 0.184$; Fig. 2c) but colonies from different larval planktonic durations experienced different survival both when they were adjacent to established *B. leachii* colonies ($\chi^2 = 7.7$, $df = 1$, $p = 0.022$; Fig. 2a) and without *B. leachii* ($\chi^2 = 13.3$, $df = 1$, $p = 0.001$; Fig. 2b). When *B. leachii* colonies were present, *W. subtorquata* colonies from SN had higher survival than those that developed from larvae of LN ($\chi^2 = 5.5$, $df = 1$, $p = 0.019$) and D ($\chi^2 = 5$, $df = 1$, $p = 0.025$), but survival of colonies from both LN and D treatments did not differ ($\chi^2 = 0.1$, $df = 1$, $p = 0.808$). All colonies from D treatments had died by 10 wk, while 8% of colonies of LN and 70% of colonies from SN survived until the end of experiments (Fig. 2b). When *B. leachii* colonies were absent, patterns of survival were very similar, with similar patterns of significance (SN vs. LN: $\chi^2 = 5$, $p = 0.025$; SN vs. D: $\chi^2 = 12.2$, $p = 0.003$; LN

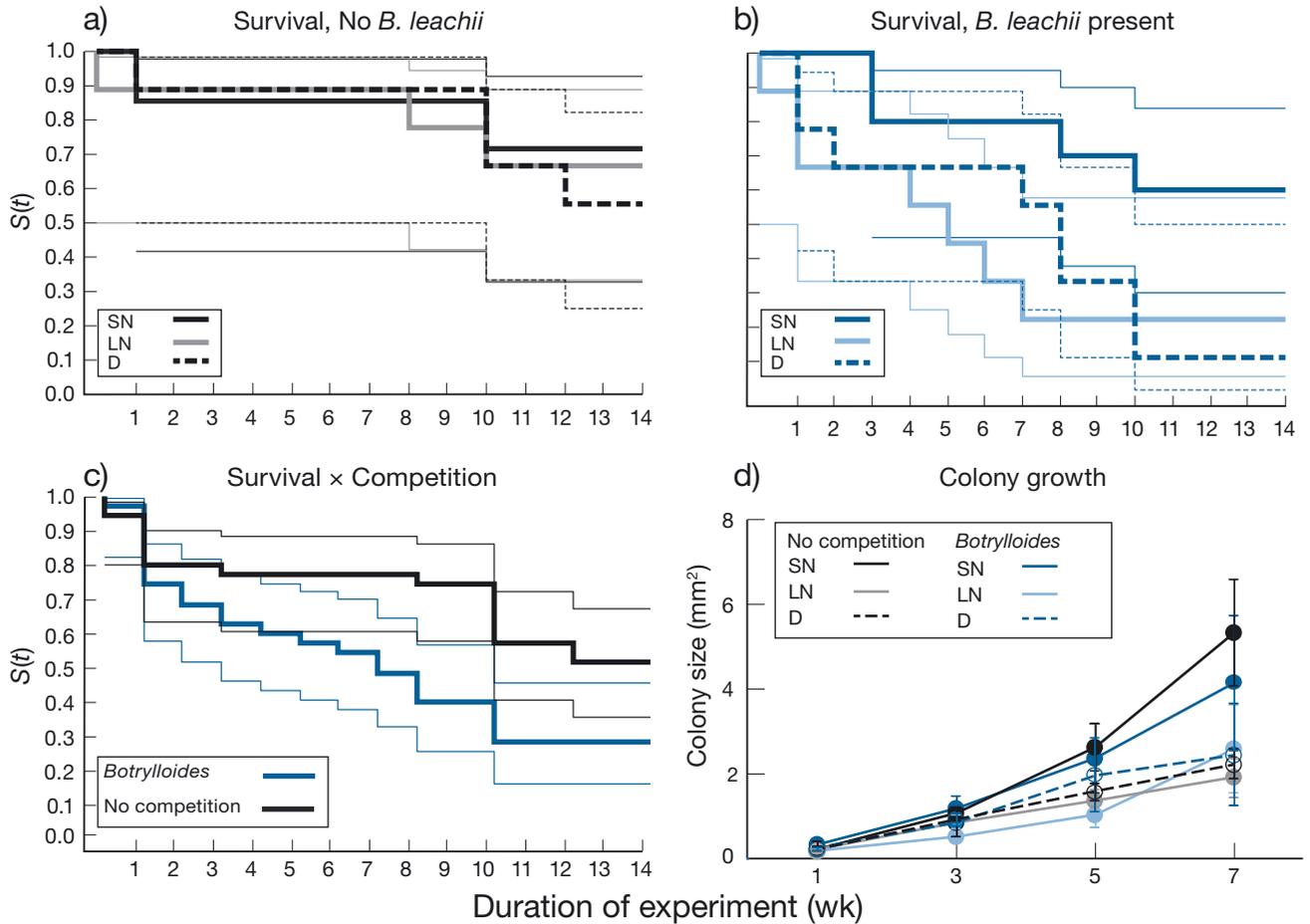


Fig. 1. Survival and growth of bryozoan *Watersipora subtorquata* colonies that developed from larvae of short natural planktonic durations (SN), long natural planktonic durations (LN) or delayed planktonic durations (D) during winter competition experiments with the colonial ascidian *Botrylloides leachii* (May to August; Expt 1A). Survival is shown with Kaplan-Meier survival curves (where $S(t)$ represents the proportion of individuals surviving at each time step) for colonies (a) without neighbours, (b) next to established colonies of *B. leachii* and (c) between *B. leachii* and no competition treatments (pooled over larval planktonic duration treatments). Thick lines: estimate of the proportion of individuals surviving; fine lines: 95% confidence limits. (d) Mean (\pm SE) colony size (area in mm^2) over the initial 7 wk of experiments from all treatments

vs. D: $\chi^2 = 0.17$, $p = 0.675$; Fig. 2a). All colonies from larvae of D treatments had died by 12 wk, while 25% of colonies from LN and 80% of colonies from SN survived beyond the end of experiments (Fig. 2a).

Expt 1B: growth

Larval planktonic duration also affected colony size ($F_{2,36} = 6.66$, $p = 0.003$) and this effect varied with time (larval planktonic duration \times time: $F_{6,106} = 7.65$, $p < 0.001$). As colonies grew, the gap between the size of colonies derived from SN and those from LN and D became steadily greater, and were at least twice the size of the other groups after 7 wk (Fig. 2d). *B. leachii* colonies did not affect colony size, with no overall effect (*Botrylloides*: $F_{1,36} = 1.392$, $p = 0.246$) or combined effects of *B. leachii* and larval planktonic

duration (larval planktonic duration \times *Botrylloides*: $F_{2,36} = 0.212$, $p = 0.809$; larval planktonic duration \times *Botrylloides* \times time: $F_{6,106} = 1.339$, $p = 0.246$).

Expt 2: colony performance without competition

Expt 2A

In Expt 2A, *W. subtorquata* colonies that developed from larvae of different natural planktonic durations survived equally well ($\chi^2 = 0.21$, $df = 1$, $p = 0.36$). Most colony mortality occurred in the first 8 wk, and 68% of colonies survived beyond the end of the experiments at 13 wk (Fig. 3a). However, larval planktonic duration affected colony size (larval planktonic duration: $F_{2,65} = 3.58$, $p = 0.034$), with colonies from SN having larger sizes than those from both LN and D

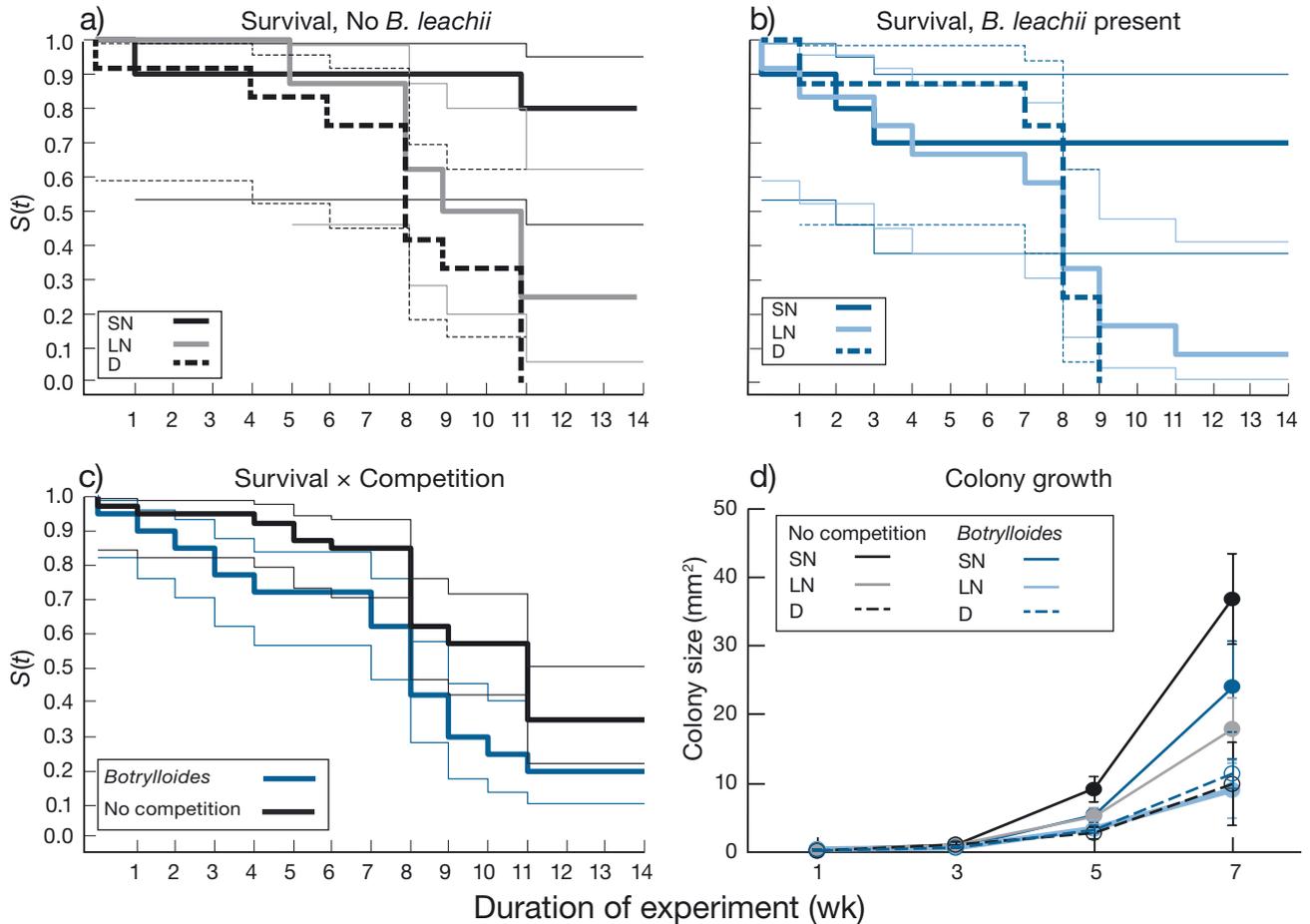


Fig. 2. (a,b,c) Survival and (d) growth of bryozoan *Watersipora subtorquata* colonies with different larval planktonic durations during spring/summer competition experiments (September to December; Expt 1B). See Fig. 1 for further details

treatments (Fig. 3c). Colonies increased in size overall (time: $F_{4,130} = 118.9$, $p < 0.001$) but the effect of larval planktonic duration did not vary significantly over time (larval planktonic duration \times time: $F_{2,130} = 0.41$, $p = 0.792$).

Expt 2B

In Expt 2B, larval planktonic duration affected the survival of *W. subtorquata* colonies ($\chi^2 = 36.3$, $df = 2$, $p < 0.001$), with colonies from SN having higher survival than those from LN ($\chi^2 = 24.16$, $df = 1$, $p < 0.001$) and D treatments ($\chi^2 = 23.12$, $df = 1$, $p < 0.001$), while survival of colonies from LN and D did not differ ($\chi^2 = 0.68$, $df = 1$, $p = 0.40$). All D colonies had died by the end of the experiments, while 5% of LN and 58% of SN survived until the end of the experiments (Fig. 3b).

Similarly, larval planktonic duration affected colony size (larval planktonic duration: $F_{1,61} = 6.84$, $p = 0.002$) and the effects of larval planktonic duration and over-

all colony growth changed over time (larval planktonic duration \times time: $F_{4,121} = 5.88$, $p < 0.001$; time: $F_{2,121} = 565.84$, $p < 0.001$). Colonies from different larval planktonic durations did not differ in size at 3 wk, but colonies from SN were larger than colonies from LN but not D treatments at 5 wk, and were larger than both LN and D at 7 wk (Fig. 3d). Colonies from LN and D treatments did not differ at any time (Fig. 3d).

DISCUSSION

Increased planktonic larval duration generally had a strong negative effect on the survival and growth of *Watersipora subtorquata* colonies; however, these effects varied depending on whether *W. subtorquata* colonies were adjacent to established adults of the colonial ascidian *Botrylloides leachii*, and also varied between seasons.

In our experiments, longer planktonic larval durations often caused lower survival and growth rates

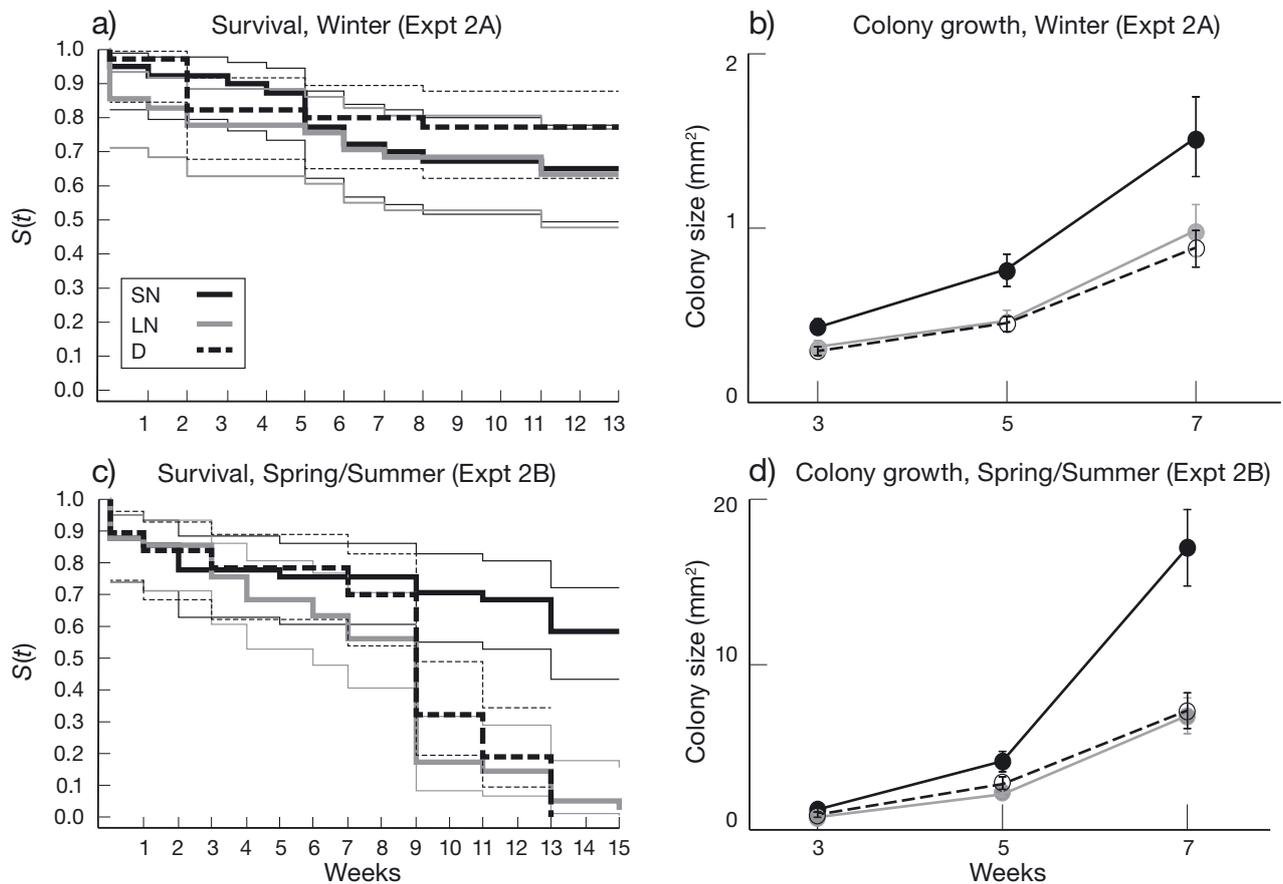


Fig. 3. (a,c) Survival and (b,d) growth of bryozoan *Watersipora subtorquata* colonies that developed from larvae of short natural planktonic durations (SN), long natural planktonic durations (LN) or delayed planktonic durations (D) and experienced no competition in both winter (a,b; May to August; Expt 2A) and summer (c,d; September to December; Expt 2B). See Fig. 1 for further details

that persisted as *W. subtorquata* colonies matured. Most other studies on the effects of planktonic duration in other marine invertebrates only measured colony performance during early life history stages (i.e. within the first days to <2 mo of post-settlement; e.g. Woollacott et al. 1989, Wendt 1998, Marshall et al. 2003, Burgess et al. 2012). In our study, differences in survival due to larval planktonic durations became most apparent 7 to 8 wk after settlement, while differences in growth only became apparent at 5 to 7 wk. Though we could only formally compare colony size up to 7 wk due to decreasing sample sizes, the effects of larval planktonic duration on growth also persisted well beyond this time (see Fig. S2-2 in Supplement 2 at www.int-res.com/articles/suppl/m531p179_supp2.pdf). This suggests that the costs associated with increased dispersal can emerge and persist far beyond the end of the dispersal phase in *W. subtorquata*, and may not necessarily be expressed during early post-settlement. Costs accrued due to long planktonic durations may also

emerge and persist at later life-history stages in other organisms with complex life cycles (particularly if they produce non-feeding propagules), and potentially be missed in studies that only follow early post-settlement.

In winter, *B. leachii* had several important mitigating effects on differences in colony performance due to planktonic larval duration (Expt 1A). First, colonies from long planktonic larval durations experienced similar survival as colonies from short planktonic larval durations without competition, but exhibited higher mortality when they were adjacent to *B. leachii*. Thus, variation in planktonic larval duration may not always result in differences in colony survival but can be exacerbated when larvae settle near an established competitor like *B. leachii*. Second, colonies from long planktonic larval durations that did not experience competition exhibited similar survival as colonies from short planktonic larval durations that were adjacent to *B. leachii* (Fig. 1a,b). This demonstrates that variation in the presence/absence

of *B. leachii* can also mediate some effects of planktonic larval duration on survival. Lastly, differences in growth between surviving colonies of different planktonic larval durations were also reduced by the presence of nearby *B. leachii*, thus demonstrating that the presence of an established competitor can reduce differences in post-settlement growth rates associated with different dispersal durations.

W. subtorquata colonies were generally much smaller in the winter experiments (Expts 1A and 2A) compared to those run in spring/summer (Expts 1B and 2B), likely due to seasonal differences in temperature, light availability and productivity. These differences in overall colony growth rates between experiments/seasons likely contributed to the differences in the effects of larval planktonic duration on colony performance we observed between experiments. The relatively small size of *W. subtorquata* colonies in Expt 1A likely made them more vulnerable to indirect competition or overgrowth by *B. leachii*, thereby mitigating the effects of larval planktonic duration on colony performance. In contrast, *W. subtorquata* colonies grew more quickly and to larger sizes in spring/summer (Expt 1B), and *B. leachii* did not have a strong overall influence on colony survival and growth nor did it change the differences in colony performance related to larval planktonic duration. Though we were only able to formally compare colony growth to 7 wk, these effects persisted until the end of the experiments (Fig. S2-2).

The different effects of *B. leachii* on the performance of *W. subtorquata* colonies between experiments may not only be associated with differences in *W. subtorquata* growth rate but might alternatively be explained if *B. leachii* colonies performed better in winter than summer. However, we did not observe differences in *B. leachii* colony performance in our experiments, and studies conducted in other regions have shown that *B. leachii* colonies are generally larger and have higher growth rates when water temperatures are warmer, similar to those experienced in spring/summer at our site (Epelbaum et al. 2009, Grey 2011); therefore this explanation is unlikely.

Effects of natural vs. delayed longer larval planktonic durations

We did not find strong differences in the growth and survival of *W. subtorquata* colonies that developed from larvae that were forced to remain in the plankton for longer time periods versus those that re-

mained there naturally for the same amount of time before settlement. This result suggests that delaying larvae by rolling them in vials at the speeds and time periods used in this study does not physically damage larvae any more than those that were not rolled. It could also suggest that larvae in the LN and D treatments may not have initially possessed or expended different energy reserves over longer planktonic durations. However, *W. subtorquata* larvae could potentially spend longer in the plankton compared to our experiments. Planktonic durations of up to 15 h followed by successful settlement and metamorphosis have been reported in laboratory experiments (Marshall et al. 2003, D. J. Marshall pers. obs.) although natural planktonic durations of this length appear to be quite rare. In our study, almost all larvae had settled by nightfall in the lab and all larvae completed settlement before nightfall in the field (D. J. Marshall & M. J. Keough unpubl. data). However, if the energy reserves of larvae that are forced to remain in the plankton are more depleted than those that naturally remain there over longer durations than what was observed in these experiments, greater differences in post-recruitment performance between these 2 treatment types could occur. Similarly, artificially rolling larvae for much longer periods than was used here could potentially lead to physical damage, and should be controlled for in studies adopting similar methodology.

Implications for population connectivity

The effects of different planktonic larval durations and the influence of established *B. leachii* on *W. subtorquata* colony performance have important implications for understanding how increased planktonic duration can influence population connectivity in *W. subtorquata* and other organisms with complex life cycles. The results of our experiments suggest that *W. subtorquata* populations among which larvae take longer to disperse are likely to have reduced ecological connectivity compared to those with shorter dispersal times, because colonies arising from long larval planktonic durations will be less likely to survive, grow, persist and contribute to longer-term population and community dynamics. Reduced colony growth associated with longer larval planktonic periods could also potentially limit the number of larvae that colonies subsequently produce (e.g. Hall & Hughes 1996), thereby reducing future connectivity, although we were not able to directly measure fecundity in this study. However, our exper-

iments demonstrate that the effects of planktonic larval duration on colony survival and growth can be both exacerbated and mitigated by a neighbouring adult competitor, but that even these effects can vary among seasons. More broadly, these findings highlight the fact that population connectivity cannot always be simply predicted by the time taken for propagules to disperse among populations, since differences in growth and survival associated with larval planktonic durations are likely to be mediated by the presence of incumbent competitors as well as background environmental conditions. Increased planktonic larval duration can cause reduced post-settlement performance in other marine invertebrates (e.g. Pechenik et al. 1993, Pechenik & Rice 2001, Roberts & Lapworth 2001, Marshall & Keough 2003), and our findings are likely to be relevant to a range of organisms, particularly colonial invertebrates with non-feeding larvae. How dispersal costs are altered by competition among species of different functional types and varying densities is still relatively poorly understood and further detailed studies are required to better understand the relationship between planktonic larval duration, post-dispersal performance and species interactions.

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