

# Microhabitats can be recruitment refuges and sinks

Paul E. Carnell<sup>1,2,\*</sup>, Michael A. Sams<sup>3</sup>, Michael J. Keough<sup>1</sup>

<sup>1</sup>School of BioSciences, The University of Melbourne, VIC 3010, Australia

<sup>2</sup>Deakin University, Geelong, Australia. School of Life and Environmental Sciences, Centre for Integrative Ecology, (Burwood Campus), 221 Burwood Highway, Burwood, VIC 3125, Australia

<sup>3</sup>Environment Protection Authority, Ernest Jones Drive, Macleod, VIC 3085, Australia

**ABSTRACT:** Recruitment is an important demographic bottleneck in the life history of many plant and animal species. Microhabitats provided by surfaces or plants or animals can reduce mortality during this critical life-history period. We investigated how microhabitats influence post-settlement processes on a range of ascidians, bryozoans and barnacles. We compared post-settlement mortality and growth on surfaces that were flat or were covered by artificial barnacle mimics or live adult barnacle microhabitats. We also tested whether the effects of surface type changed under different levels of environmental stress by manipulating the orientation of treatments. Orientation had the strongest influence on survival, growth and recruitment, with individuals on downwards-facing surfaces performing the best and those on upwards-facing surfaces with significantly higher mortality. The bryozoan *Watersipora subatra* had higher survival in microhabitats, but was not influenced by surface orientation. In contrast, barnacles showed increased mortality in microhabitats on upwards-facing panels, but were not affected by other orientations. The presence of adult barnacles caused decreased growth of barnacle recruits, but had no effect on the bryozoan. In comparison, the ascidians (*Botryllus sclosseri* and *Diplosoma listerianum*) were mostly not influenced by microhabitats. The high rates of sedimentation on upwards-facing surfaces provide the most plausible answer for the strong influences of surface orientation on survival and growth. While microhabitats can act as refuges for some species, the same microhabitat type can lead to higher mortality in other species. The response of each species to microhabitats ultimately depends on the source of mortality at each site.

**KEY WORDS:** Post-settlement mortality · Habitat characteristics · Settlement · Environmental variability · Ascidian · Bryozoan · Barnacle

— Resale or republication not permitted without written consent of the publisher —

## 1. INTRODUCTION

The recruitment of offspring back into populations is an important bottleneck that can substantially alter population and community dynamics (Lasker et al. 1998, Lopez et al. 1998). In organisms with dispersive propagules, one particular period with high mortality is the post-settlement period; a variable amount of time between propagule settlement and when juveniles enter the adult population (Keough

& Downes 1982). During early post-settlement, propagules are often tiny, undergo metamorphic changes and are extremely vulnerable to disturbance, competition and predation (Petraitis 1990, Raimondi 1990). Consequently, the early post-settlement period is often a time of extremely high mortality (Connell 1961, Gosselin & Qian 1996). Understanding the sources of spatial and temporal variation in post-settlement mortality is, thus, key for understanding variation in recruitment patterns

and subsequent adult population dynamics, both within and among species (Davis 1987).

During early post-settlement in marine organisms, recruits may avoid sources of mortality by finding refuge in microhabitats (Bergeron & Bourget 1986, Lapointe & Bourget 1999). These spatial refuges may be biologically derived via resident adults (Keough 1986), including barnacles (Osman et al. 1989), or provided by complex substrate topography, such as pits and crevices in the surface of rocks or other substrates (Keough & Downes 1982, 1986, Bourget et al. 1994). Studies involving biotic refuges often find increased settlement and reduced predation in these microhabitats (Hurlbut 1991, Walters & Wetthey 1996, Fowler-Walker & Connell 2007). However, the early advantages of particular microhabitats may not persist, because there can be reduced later growth due to competition and even increased mortality from predators using the same microhabitat (e.g. Jenkins et al. 1999, Osman et al. 1989), which could negate any potential benefits of refuges.

The benefits of particular microhabitats could also change as environmental conditions vary. One obvious way for that to occur in sessile marine communities is when the orientation of the surface changes. Orientation has a strong influence on settlement and post-settlement survival in marine communities (Young & Chia 1984, Hurlbut 1993, Babcock & Mundy 1996). Often dramatically different invertebrate communities occur on downwards-facing surfaces compared to those facing upwards (Todd & Turner 1986), or on vertical versus horizontal surfaces (Connell 1999, Glasby 2000). This difference is most often due to increased sources of stress for many sessile invertebrates on upwards-facing and vertical surfaces, including sediment deposition (Young & Chia 1984, Knott et al. 2004, Thomsen & McGlathery 2006) and light (Stoner 1990, Hart & Marshall 2013, Riedel et al. 2014). The interaction between habitat type and different environmental conditions, both on small and larger scales, has the potential to be an important source of variation in post-settlement mortality and corresponding patterns of recruitment. In particular, the habitat complexity that provides protection from predators in low-sediment environments could become a sediment trap as levels of sedimentation arise.

In this study, we investigated the interactive effects of microhabitats and environmental stress on post-settlement mortality of sessile marine invertebrates. In temperate marine sessile communities, barnacles create complex microhabitat structure from their

physical structure. When alive, they can also modify water flow and can compete for food resources (Wright & Boxshall 1999). To isolate the refuge effect, we created artificial settlement plates from surfaces colonized by barnacles and then varied the orientation of these surfaces at a study site with high sediment loads.

To understand how consistent patterns of early settlement mortality are among different species we measured mortality and post-settlement growth of 4 different invertebrate taxa. We chose taxa with different life histories and potential sensitivities. For colonial animals (e.g. ascidians and bryozoans), microhabitats may be important initially, but as the colony grows, they may grow out from the initial protection. However, for solitary animals (e.g. barnacles), their settlement location will continue to influence survival and growth over the long term. We examined survivorship of colonial invertebrates, the colonial ascidians *Diplosoma listerianum* and *Botryllus sclosseri*, the bryozoan *Watersipora subatra* (previously listed as *W. subtorquata*, but this species has been split by Vieira et al. 2014, and Victorian material appears to be *W. subatra*) and 3 barnacle species (*Amphibalanus variegatus*, *Balanus trigonus* and *Austrominius modestus*). Each species is vulnerable to different environmental stressors in different ways. Their external body coverings differ (from least protected in ascidians to most protected in barnacles) and they have different predators (a common flatworm [*Stylochus pygmaeus*] preys on barnacles and 2 different species of nudibranch [*Goniodoris meracula* and *Polycera hedgpethi*] prey on the ascidians and the bryozoan).

We first compared mortality on surfaces that mimicked the structure of a commonly found biotic microhabitat—the tests of aggregated barnacles—on surfaces oriented in 3 directions. We predicted that the complex topography of the mimic barnacle plate would provide increased survival for all species, but that solitary species would see a decrease in growth due to reduced access to food. The impact of surface orientation on the microhabitat effect would depend on the main drivers of mortality. If predation by fish was a driver, this would result in increased survival on microhabitats. If sedimentation was a driver, this would see decreased survival in microhabitats on upwards-facing panels. In a second experiment, we examined microhabitat effects in further detail by comparing both mimic and live adult barnacles as a potential refuge in 2 surface orientations. Here, we predicted that mimic barnacles would have a similar

impact as the first experiment, but that by live barnacles reducing access to food, would decrease growth and survival (particularly for solitary species) over the period of the experiment. However, we predicted this effect would change on the vertical-facing orientation due to the nature of water flow and access to food.

## 2. MATERIALS AND METHODS

### 2.1. Study Site

All field experiments were carried out at Workshops Pier, Williamstown, located within Port Phillip Bay, a temperate embayment in southeastern Australia (37°51'35"S 144°54'36"E). The pier is in an area well protected from prevailing winds and currents and has a maximum depth of 4–5 m. The mouth of the Yarra River is located close to Williamstown, resulting in high levels of sedimentation in the water surrounding the pier. The invertebrate fauna from this site is a diverse mix of native and introduced species (Todd & Keough 1994, Keough & Raimondi 1995, Sams & Keough 2013), typical of sheltered harbor environments.

### 2.2. Study taxa

Our focal species were an encrusting bryozoan, *Watersipora subatra* (hereafter referred to as '*Watersipora*'), 3 barnacles, *Amphibalanus variegatus*, *Balanus trigonus* and *Austrominius modestus*, and the colonial ascidians *Botryllus schlosseri* ('*Botryllus*', in Expt 1 only) and *Diplosoma listerianum* ('*Diplosoma*', in Expt 2 only). These are among the major space occupiers of the sessile invertebrate communities at the study site and were chosen to represent a diversity of life histories. Very newly arrived *Austrominius modestus* are difficult to distinguish from the other 2 barnacle species until the shell plates have formed, so we often grouped these together. Additionally, *A. variegatus* and *B. trigonus* are also difficult to separate in the first few days after settlement. Thus, barnacles were grouped together for settlement and mortality, but differentiated to species level for size. All 3 species are present at the study site in varying abundances and often show sharp peaks in recruitment. However, once individuals were old enough to identify, *Amphibalanus variegatus* was usually the most abundant barnacle species encountered in experiments.

### 2.3. Expt 1: Mimic barnacle experiments

We created settlement plates mimicking the surface topography of adult barnacles in a technique similar to that of Wright & Boxshall (1999). We created moulds of aggregations of adult barnacles that were obtained from settlement plates placed in the water at our study site for 8 wk using SRT30 Silicone Rubber (Dalchem). Settlement plates were cast from these moulds using Kahfil plastic putty (K&H) and coloured with black pigment paste. Flat settlement plates were made using the same method. Both flat and mimic barnacle settlement plates measured 110 × 110 × 10 mm, excluding the height of mimic barnacles. To remove most residues from the plastic putty, all plates were soaked for 2 wk in fresh water, which was changed every 3 d. Wright & Boxshall (1999) used plates made from the same material and observed no difference in settlement between Perspex (Plexiglas) settlement plates and plastic putty plates.

Three orientations were used to investigate how changing environment may influence the effect of settlement plate type. These were downwards (panel hung horizontally with plates facing downwards/seafloor), upwards (panels hung horizontally with plates facing upwards/sky) and vertical (panels hung vertically with plates attached). For each orientation, there were 2 backing panels, each with 4 replicates of each plate type (flat and mimic barnacle). Panels were suspended by ropes attached to the pier and stabilized from below with a weight at a depth of approximately 2–3 m. Each panel was separated by a distance of at least 5 m.

We mapped the individuals of each focal species by placing a 100 × 100 mm grid over each settlement plate (excluding 0.5 cm around the perimeter to exclude possible edge effects) and recorded the location of individuals.

#### 2.3.1. Expt 1a: 7 d mimic barnacle experiment

The aim of this experiment was to determine if there were substantial effects of surface topography during the very early stages of post-settlement mortality. The same experimental design was used in the 7 d experiment as for the 60 d experiment, using the same flat and mimic barnacle settlement plates.

The experimental panels and plates were deployed in the field on 5 February 2008 (Day 0). Settlement of individuals was mapped on Day 1 (for barnacles and *Watersipora*) and on Day 3 for *Botryllus* due to low

daily settlement. On Day 3, mortality of barnacles and *Watersipora* was checked. All individuals were then assessed in the field on Day 5, and the experiment was brought back into the laboratory on Day 7 and analysed for the mortality of mapped individuals.

We counted the total number of individuals of the 3 focal species on each plate at the end of the 7 d experiment. This enabled a comparison with the number of individuals mapped at the start of the 60 d experiment and an estimate of recruitment over the 7 d period.

#### 2.3.2. Expt 1b: 60 d mimic barnacle experiment

The aim of this experiment was to determine the effects of microhabitats on post-settlement mortality over a longer period. We compared the survival of our focal species on flat and barnacle mimic surfaces at each of the 3 plate orientations outlined above. Expt 1b commenced November 20, 2007 (Day 0). To allow for sufficient numbers of all the focal species, individuals were recorded on Day 6 and will be hereafter referred to as 'settlement'. Mortality of the same individuals was then assessed in the field every third day from Day 9 until Day 30. The settlement plates were then left in the water for another 30 d and mortality of mapped individuals was determined.

Size of individuals was measured on Days 30 and 60. We randomly selected up to 3 mapped individuals of each focal species per plate. The length and width of individuals was measured to calculate the area they covered. Depending on the shape of some older *Watersipora* and *Botryllus* colonies, length and width of a number of sections was measured to accurately record their size. In cases where there was partial mortality of colonies, this area was measured and subtracted from the overall size of the colony. Disturbance to the settlement plates was minimised during sampling by keeping them submerged in seawater during mapping and measurement of mortality and size.

#### 2.4. Expt 2: Live and mimic barnacle experiment

We examined the survival and growth of our focal species (outlined above) on 3 different types of settlement surface: flat settlement plates, mimic barnacle settlement plates and settlement plates covered with aggregations of living barnacles. This enabled us to determine whether microhabitats provided by live barnacles had different effects from those of the bar-

nacle moulds, which represent effects of changed surface topography only. Living barnacle settlement plates were left in the water at Workshops Pier for 8 wk, resulting in ~40% cover of adult barnacles (*Amphibalanus variegatus*, *Balanus trigonus* and *Austrominius modestus*). To facilitate the growth and presence of only barnacles on living barnacle treatments, other species were removed from plates after 1 mo and again prior to deployment. Wright & Boxshall (1999) showed no difference in settlement between the Perspex or plastic putty settlement plates, so the use of the 2 different settlement plate materials was not considered a serious issue.

Given the high mortality rates on upwards-facing panels in Expt 1 (see Section 3.2), in this experiment only 2 orientations were used: downwards and vertical. There were 4 replicate settlement plates of each treatment (flat, mimic barnacle and live barnacle) on each of 2 panels hung at each orientation. Panels were attached to the pier at the study site as described for Expt 1.

The experiment began at Workshops Pier on 25 February 2008 (Day 0). Settlement of individuals of the focal species was mapped on Day 1 using the same method as in Expt 1. Plates were assessed for mortality of mapped individuals every week for 1 mo (Days 7, 14, 21, 28). Size of all live mapped individuals per plate was measured on Day 28. This ranged from 1 to 13 individuals on each individual plate.

### 2.5. Data analysis

For all 3 experiments, the number of surviving mapped individuals was the response variable for the analyses over time. The data was positively skewed (assessed with Levene's test) and data were natural log transformed ( $\log(x+1)$ ) for all experiments. A post-hoc Tukey test was used to compare means when factors were significant ( $p < 0.05$ ). Data were analysed using a repeated-measures ANOVA. The repeated factor was time, incorporating the number of individuals at each census date. The between-subjects component of the analysis consisted of a partly nested, mixed model, with orientation and plate type as fixed factors and panel nested within orientation as a random factor. The assumption of sphericity was tested using Maculy's test and when required was overcome by using Greenhouse-Geisser adjusted p-values. In the 60 d experiment, high mortality resulted in too few data points by Day 60, reducing our confidence in detecting effects, so we also ran repeated-measures analyses from set-

Table 1. Repeated-measures ANOVA to examine effects of orientation and plate type on the number of individuals (natural log) between settlement and Day 7 in the 7 d mimic barnacle experiment and Day 30 in the 60 d mimic barnacle experiment. Threshold:  $p < 0.05$ . All significant  $p$ -values are highlighted in **bold**

Species Source	7 d experiment						60 d experiment: analysis Day 6–30			
	Barnacles		<i>Watersipora</i>		<i>Botryllus</i>		df	Barnacles p	<i>Watersipora</i> p	<i>Botryllus</i> p
<b>Between subjects</b>										
Orientation = O	2	<b>0.020</b>	1	0.261	1	<b>0.026</b>	2	0.157	0.215	<b>0.037</b>
Plate type = P	1	0.764	1	0.133	1	0.216	1	0.061	0.066	0.566
O × P	2	<b>0.011</b>	1	<b>0.022</b>	1	0.837	2	0.243	0.215	0.559
Panel (O)	3	0.158	2	0.323	2	0.707	3	<b>0.002</b>	0.388	0.152
P × panel (O)	3	0.983	2	0.915	2	0.462	3	0.065	0.426	0.372
Error	36		24		24		36			
<b>Within subjects</b>										
Time = T	3	<b>&lt;0.0005</b>	3	0.236	2	<b>0.003</b>	8	<b>&lt;0.0005</b>	<b>&lt;0.0005</b>	<b>&lt;0.0005</b>
T × O	6	0.137	3	0.758	2	<b>0.014</b>	16	<b>&lt;0.0005</b>	<b>&lt;0.0005</b>	<b>0.001</b>
T × P	3	0.337	3	0.236	2	0.988	8	<b>&lt;0.0005</b>	<b>0.015</b>	0.996
T × O × P	6	0.915	3	0.758	2	0.871	16	<b>0.001</b>	0.841	0.917
T × panel (O)	9	<b>0.049</b>	6	0.097	4	0.736	24	<b>0.007</b>	0.997	<b>0.001</b>
T × P × panel (O)	9	0.052	6	0.097	4	<b>0.009</b>	24	0.180	0.982	<b>0.006</b>
Error	108		72		48		288			

tlement to Day 30. This also enabled a comparison of subtle changes in the shorter time period of 1 mo and the broader changes over 2 mo. In the live barnacle experiment, one of the downwards panels was lost between Days 7 and 14, so repeated-measures analyses were run excluding the lost panel and excluding panel as a factor. Panels were used for logistical necessity so that plates did not have to be deployed individually, but inter-panel differences in settlement, growth and mortality were small and not the focus of our statistical analysis. Indeed, for all analyses, when the effect of panels was not significant (using a conservative  $p > 0.25$ ), panel was removed from the model and a 2-factor ANOVA conducted between orientation and plate type.

For analysis of particular time points (7 d census and size), data were analysed using ANOVA. Assumptions of normality were tested with Levene's test and size data (area) were square-root transformed to meet assumptions of normality. The 7 d census data were natural-log transformed. The model again consisted of a partly nested, mixed model, with orientation and plate type as fixed factors and panel nested within orientation as a random factor. For analysis of size data, too few individuals remained by Day 60 to run an analysis, so only the Day 30 data are presented. Due to the fact that there were different numbers of live individuals measured on each plate, the average size of each species per plate was used in analysis. Threshold levels were set at 0.05 for all statistical tests.

### 3. RESULTS

#### 3.1. 7 d mimic barnacle experiment

##### 3.1.1. Barnacles

A total of 120 barnacles settled on plates across all treatments on the first day of this experiment. By Day 7, only 43% were still alive. There was no significant interaction or main effects of orientation and microhabitat type on the survival of barnacles from settlement to Day 7 (Table 1, Fig. 1).

The total number of barnacle recruits present at the end of the experiment (those mapped on Day 1 and any that subsequently settled and survived) was influenced separately by orientation and microhabitat type (Table 2). There was a significant effect of microhabitat type, with almost twice as many barnacles on mimic barnacle plates as on flat plates. There was also an effect of orientation, with more than twice as many barnacles present on downwards- and vertical-facing panels than on upwards-facing panels (Fig. 2, Tukey's test:  $V > U$ ,  $p < 0.0005$ ;  $D > U$ ,  $p < 0.0005$ ; and  $D > V$ ,  $p < 0.0005$ ; where  $V$  = vertical-,  $U$  = upwards- and  $D$  = downwards-facing).

##### 3.1.2. *Watersipora subatra*

*Watersipora* did not settle on upwards-facing panels, but we mapped 34 *Watersipora* settlers on down-

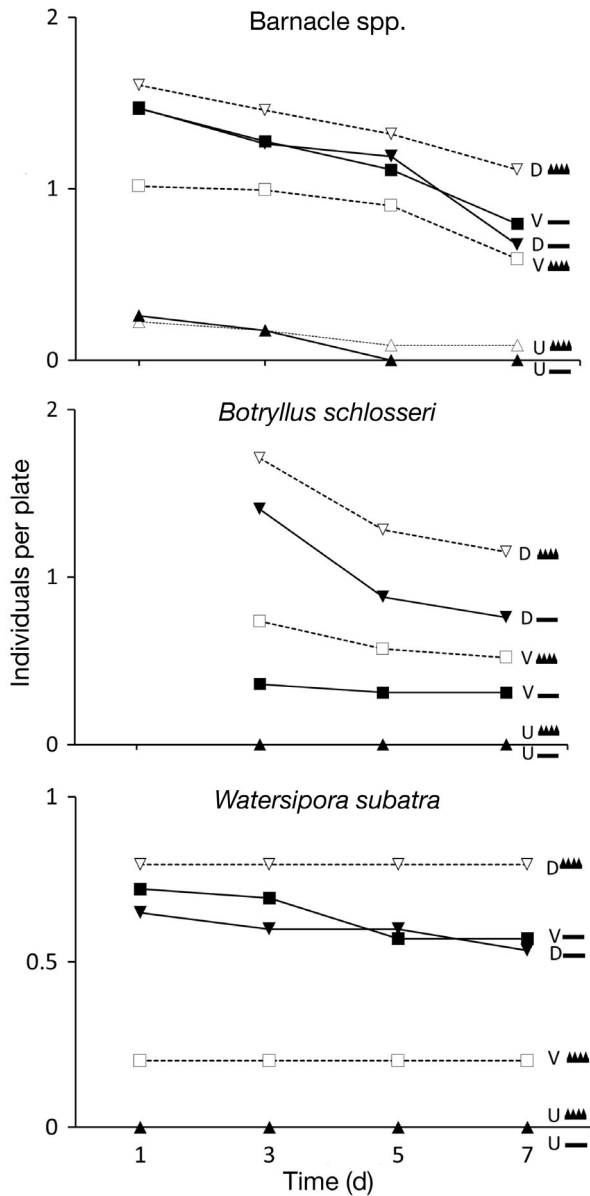


Fig. 1. Mean number of live mapped individuals (natural log) per plate in the 7 d mimic barnacle experiment. Shapes represent the 3 different orientations of experimental panels ( $\nabla$  = downwards,  $\Delta$  = upwards,  $\square$  = vertical), also represented by the letters D, U and V. The shading of shapes and line indicates settlement plate type (—■— = flat, --□-- = mimic barnacle) and is also represented by an image (—■— = flat, —▲— = mimic barnacle)

wards- and vertical-facing panels. Of these, 82% survived until Day 7. There was no interaction between orientation and plate type on mortality of *Watersipora* from settlement to Day 7 (Table 1). There was no detected effect of orientation nor of plate type (Fig. 1).

While there was no significant interaction between orientation and plate type on the total number of

Table 2. Variation in the census of the focal species populations at the end of the 7 d mimic barnacle experiment. Results of ANOVA to test the effects of orientation and plate type on the (natural log) number of individuals per plate at the end of the 7 d experiment census. Detected effects (at  $p < 0.05$ ) that are relevant to the biological hypotheses are highlighted in **bold**

Source	df	Barnacles p	<i>Watersipora</i> p	<i>Botryllus</i> p
Orientation = O	2	<b>0.003</b>	<b>0.001</b>	<b>0.037</b>
Plate type = P	1	<b>0.006</b>	0.766	0.114
O × P	2	0.101	0.612	<b>0.034</b>
Panel (O)	3	0.014	0.522	0.006
P × panel (O)	3	0.475	0.109	0.802
Error/MS residual	36	0.135	0.144	0.229

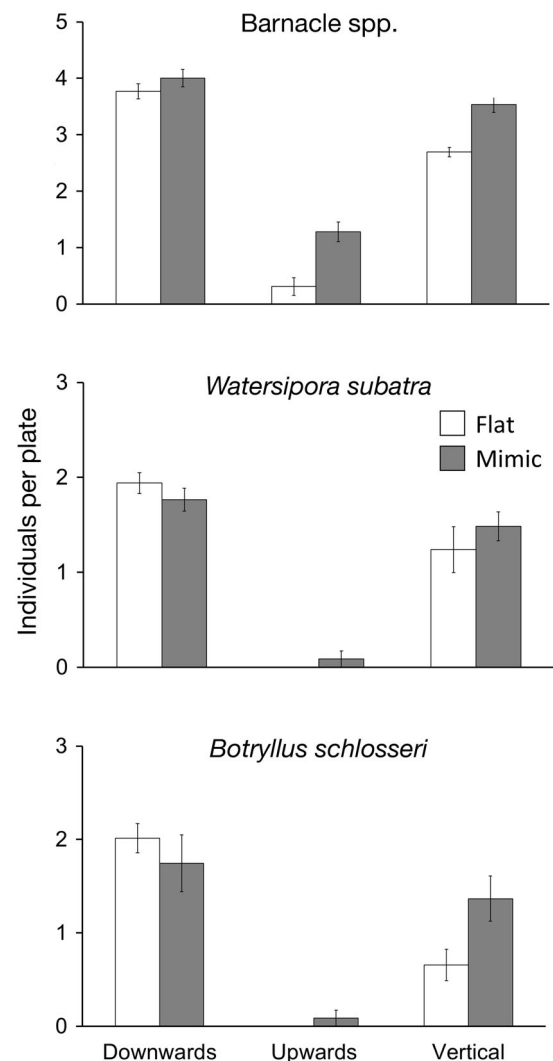


Fig. 2. Mean ( $\pm$ SE) number of individuals (natural log) per plate of the 3 focal species at the population census at the end of the 7 d mimic barnacle experiment according to the 3 different orientations the plates were hung in the water



*Watersipora* recruits present at the end of the experiment, there was a significant effect of orientation (Table 2). Two times the number of individuals were present on vertical- and 3 times the number on downwards-facing panels compared to upwards-facing panels (Tukey's test:  $V>U$ ,  $p < 0.0005$ ;  $D>U$ ,  $p < 0.0005$ ; and  $D>V$ ,  $p = 0.002$ ).

### 3.1.3. *Botryllus schlosseri*

There were too few *Botryllus schlosseri* present on Day 1 to run an analysis, so settlement was mapped again on Day 3. However, there were still no settlers on upwards-facing panels. On Day 3, 89 individuals were present and 44 % died over the next 4 d.

There was a strong effect of orientation alone on survival, with 70 % greater mortality on downwards than on vertical panels from settlement to Day 7 (Table 1). There was neither an interaction between orientation and plate type (Fig. 1) nor an effect of plate type. There was also variation among panels within orientations.

There was an interaction between orientation and plate type on the total number of *Botryllus* present at the end of the 7 d experiment (Table 2). There was no effect of plate type on downwards and upwards orientation, but on vertical panels, there were more than twice as many individuals present on the mimic barnacle plates as on flat plates. There was also an effect of panel within orientation.

## 3.2. 60 d mimic barnacle experiment

### 3.2.1. Barnacles

A total of 456 barnacles settled on plates across all treatments at the start of this experiment. Barnacles had increased mortality on more complex microhabitats and these effects varied with surface orientation. Orientation and plate type combined to affect mortality of individuals over the first 30 d (Tables 1 & A1 in the Appendix). This interaction was caused by a 20% greater mortality rate on mimic barnacle plates compared to flat plates on upwards-facing panels between settlement and Day 18 (Fig. 3a). In contrast, there were no differences in mortality between microhabitat types when facing downwards or vertical. There was also an effect of panel within orientation on barnacles (Table 1), with different overall rates of mortality between panels of the same orientation.

As there were no barnacles alive on the upwards orientation, this orientation had to be excluded from size analysis. The size of barnacles at Day 30 was affected by the combination of orientation and plate type. There was no effect of plate type on vertical panels, but barnacles on downwards-facing panels were 50 % larger when growing on flat plates than on mimic barnacle plates (Table 3, Fig. 3b).

### 3.2.2. *Botryllus schlosseri*

A total of 274 individual *Botryllus* colonies settled on plates at the start of the experiment. *Botryllus* responded strongly to surface orientation, with no colonies surviving to Day 15 on upwards-facing panels (Fig. 3a). There was no effect of plate type and an effect of panel within orientation. In the period up until Day 30, there was also an interaction between microhabitat type and panels, which indicates differential mortality between the plate types for different panels of the same orientation.

As there were no *Botryllus* colonies alive on the upwards orientation on Day 30, this orientation was excluded from size analysis. Within the remaining panels, there was no main effect of orientation or microhabitat type and no interaction on the size of *Botryllus* colonies (Table 3, Fig. 3b).

### 3.2.3. *Watersipora subatra*

A total of 129 *Watersipora* individuals settled on plates at the start of the experiment. *Watersipora* had increased survival on mimic barnacle plates compared to flat plates and also displayed strong differences between surface orientations (Tables 1 & A1 in the Appendix). All *Watersipora* died on upwards-facing panels, but 55% survived on downwards- and 75% remained alive on vertical-facing panels. There was 30% greater mortality on flat plates than on mimic barnacle plates between settlement and Day 12 (Fig. 3a).

As there were no individuals alive on the upwards orientation, this orientation had to be excluded from size analysis. Of remaining panels, there was an effect of orientation on the size of *Watersipora* colonies on Day 30 (Table 3), and colonies were twice as large on the downwards orientation than on vertical-facing panels (Fig. 3b). There was no effect of microhabitat type nor an interaction between orientation and plate type on size of *Watersipora* colonies.

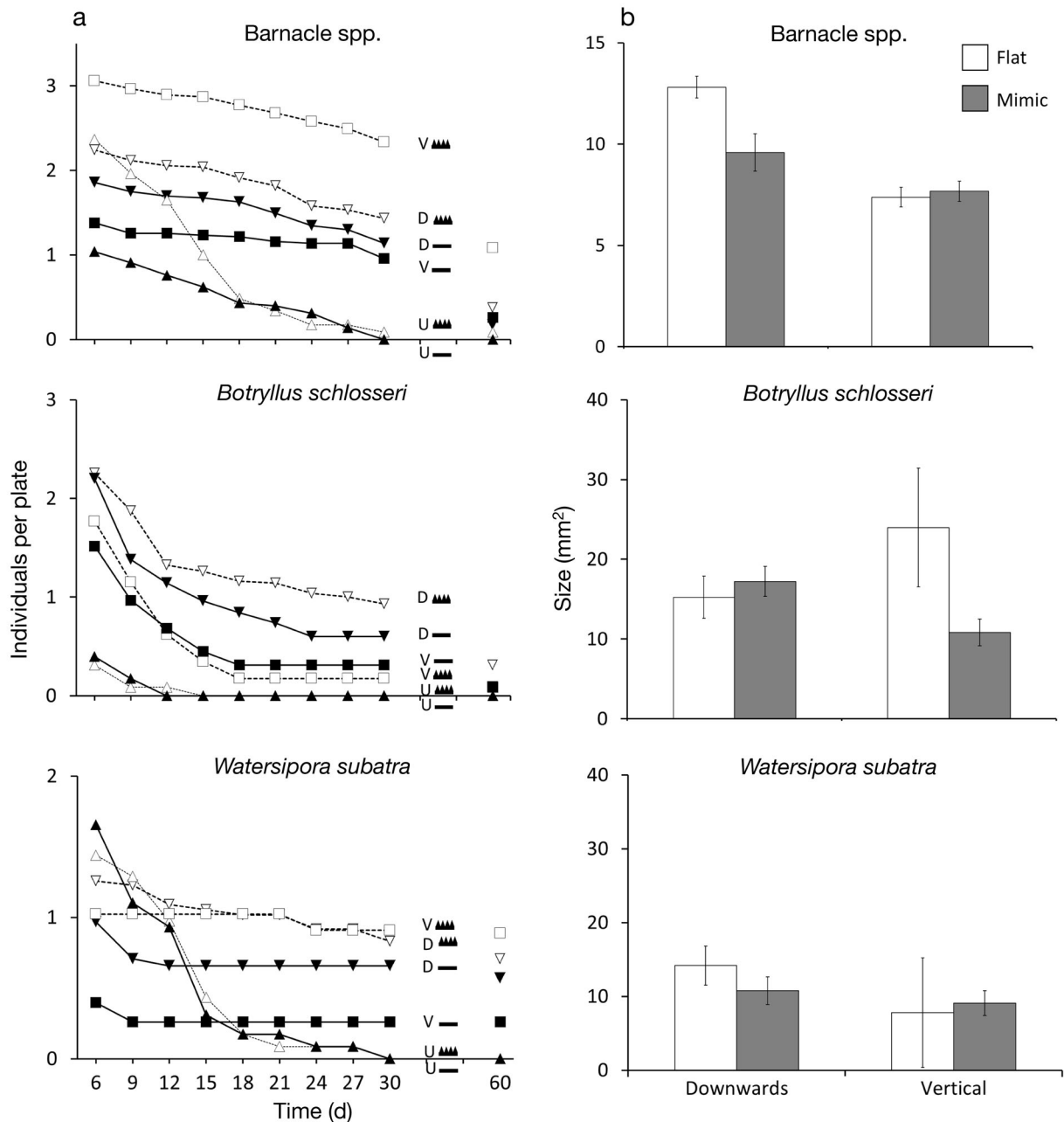


Fig. 3. (a) Mean number of live mapped individuals (natural log) per plate over the entire monitoring period in the 60 d mimic barnacle experiment. Shapes represent the 3 different orientations of experimental panels ( $\nabla$  = downwards,  $\Delta$  = upwards,  $\square$  = vertical), also represented by the letters D, U and V. The shading of shapes and line indicates settlement plate type ( $\blacksquare$  = flat,  $\square$  = mimic barnacle) and is also represented by an image ( $\blacksquare$  = flat,  $\blacksquare$  = mimic barnacle). Note the extended time interval between Days 30 and 60. (b) Mean ( $\pm$ SE) size of mapped individuals ( $\sqrt{\text{area}}$ ) per plate of the 3 focal species at Day 30 in the 60 d mimic barnacle experiment according to orientation and plate type

### 3.3. Live and mimic barnacle experiment

#### 3.3.1. Barnacles

Barnacle mortality was approximately double on vertical surfaces compared to downwards-facing surfaces (Table 4, Fig. 4a). Barnacle mortality on live bar-

nacle microhabitats also doubled compared to flat and mimic barnacle microhabitats. There was no interaction between orientation and microhabitat type (Fig. 4a).

Barnacles were identified to species level for the purpose of size analysis on Day 28. However, the only species with sufficient individuals present to run an analysis was *Amphibalanus variegatus*. Microhabitat



Table 3. ANOVA to test the effects of orientation and plate type on the ( $\sqrt{\text{area}}$ ) mean size of individuals per plate on Day 30 in the 60 d mimic barnacle experiment and at the end of the 28 d live barnacle experiment. All detected effects (at  $p < 0.05$ ) are highlighted in **bold**

Source	60 d mimic barnacle experiment				Live barnacle experiment					
	Barnacles		<i>Watersipora</i>		<i>Botryllus</i>		<i>Amphibalanus variegatus</i>		<i>Watersipora</i>	
	df	p	df	p	df	p	df	p	df	p
Orientation = O	1	<b>&lt;0.0005</b>	1	<b>0.017</b>	1	0.767	1	0.052	1	0.528
Plate type = P	1	<b>0.030</b>	1	0.498	1	0.177	2	<b>0.001</b>	2	0.259
O $\times$ P	1	<b>0.011</b>	1	0.139	1	0.077	2	0.536	2	0.993
Error	21		16		11		23		7	

type affected the size of *A. variegatus* (Table 3). Individuals on flat plates were 30% larger than those on mimics and 50% larger than on live barnacle microhabitats (Fig. 4b). *A. variegatus* showed no interaction between orientation and plate type, nor did orientation affect the size of individuals (Table 3).

### 3.3.2. *Watersipora subatra*

*Watersipora* mortality was greater on vertical- than on downwards-facing panels (Table 4, Fig. 4a). There was no effect of microhabitat type, nor did we detect an interaction between orientation and microhabitat type (Table 3). There were no effects of orientation or plate type on the size of *Watersipora* colonies on Day 28 (Fig. 4b, Table 3).

### 3.3.3. *Diplosoma listerianum*

There was no effect of orientation, microhabitat type or interaction on the survival of *Diplosoma listerianum* (Table 4, Fig. 4a). On Day 7, 84% of those individuals survived but by Day 28 only 24% survived, so size analysis was unable to be conducted.

## 4. DISCUSSION

This study provides experimental evidence that microhabitats can have both positive and negative effects on mortality and growth, and that these effects vary among species and environmental conditions. Effects were either beneficial, but only for a certain time period (e.g. *Watersipora* 60 d experiment), present only for certain orientations (interactions between plate type and orientation), or were spatially inconsistent (interactions between plate type and panel *within* orientation). The effect of microhabitats also differed between species. *Botryl-*

*lus* early post-settlement mortality was not influenced by microhabitat type. In contrast barnacles experienced increased mortality on the upwards-facing mimic barnacle plates and with live barnacles. These results highlight that there will be both winners and losers in the use of microhabitats, and this will be dependent on the drivers of mortality, which may often be species-specific.

While barnacles had higher rates of mortality in mimic barnacle plates on upwards-facing panels, other species responded quite differently. Both the size and mortality rates of *Botryllus* showed no difference on flat or mimic plates. *Watersipora* colonies exhibited no difference in size between mimic and live barnacle plates and, in fact, showed decreased mortality on mimic barnacles compared to flat plates in the first month of the 60 d experiment. This variation in species' responses to orientation and plate type is most likely a result of differences in their adult morphology (Walters & Wethey 1991, 1996). Encrusting bryozoans, sponges and colonial ascidians have

Table 4. Survival of recruits in the 28 d live barnacle experiment. Results of repeated-measures ANOVA to examine effects of orientation and plate type on the number of individuals (natural log) between settlement and Day 28 in the 28 d live barnacle experiment. All detected effects (at  $p < 0.05$ ) are highlighted in **bold**

Source	Barnacles		<i>Watersipora</i>	<i>Diplosoma</i>
	df	p	p	p
<b>Between subjects</b>				
Orientation (O)	1	<b>&lt;0.0005</b>	<b>0.005</b>	<b>0.038</b>
Plate type (P)	2	0.642	0.493	<b>&lt;0.001</b>
P $\times$ O	2	0.534	0.896	0.863
Error	30			
<b>Within subjects</b>				
Time	4	<b>&lt;0.0005</b>	<b>0.002</b>	<b>&lt;0.0005</b>
Time $\times$ O	4	<b>0.001</b>	<b>0.033</b>	0.759
Time $\times$ P	8	<b>&lt;0.0005</b>	0.117	0.099
Time $\times$ P $\times$ O	8	0.169	0.622	0.725
Error	120			

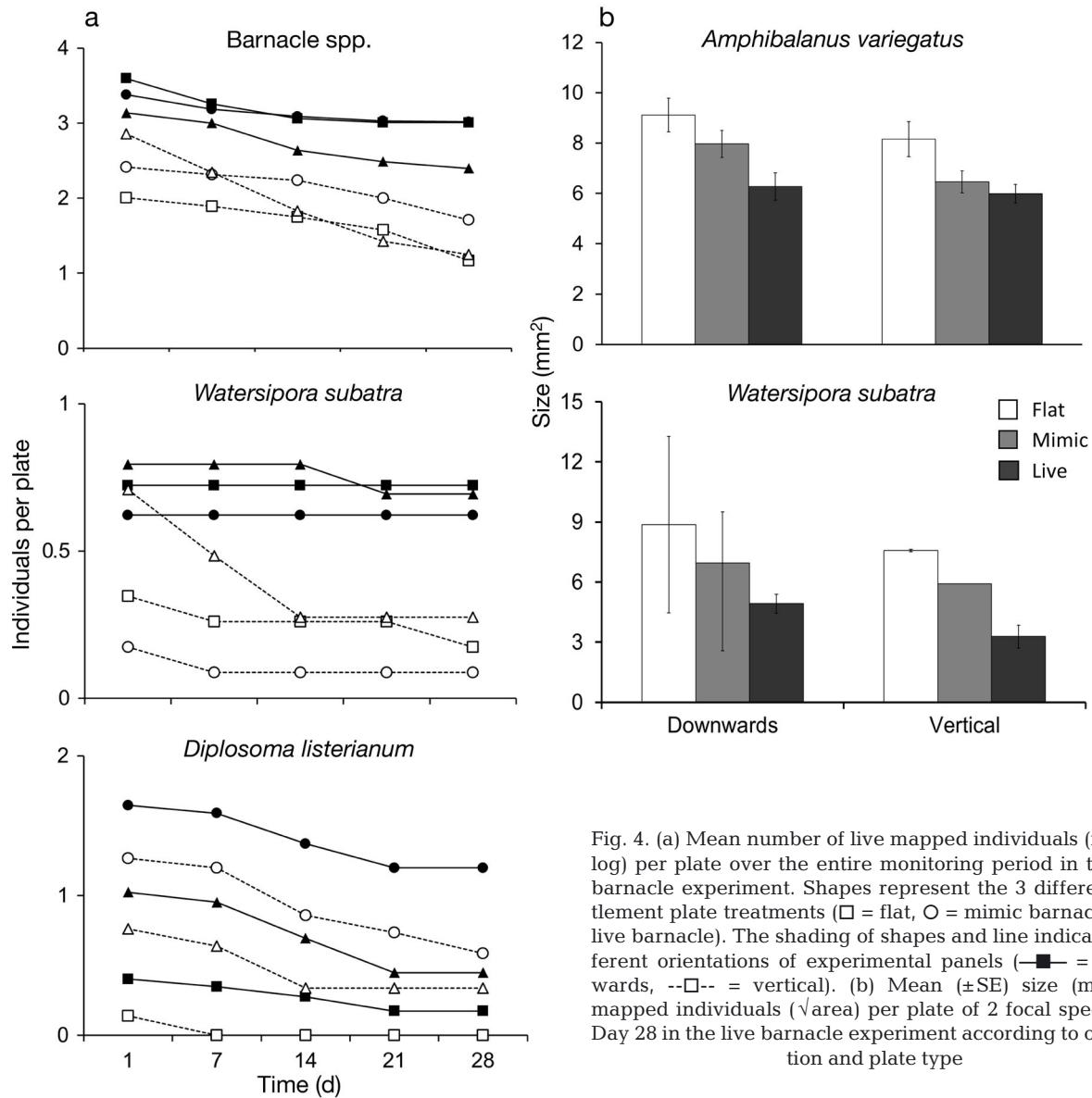


Fig. 4. (a) Mean number of live mapped individuals (natural log) per plate over the entire monitoring period in the live barnacle experiment. Shapes represent the 3 different settlement plate treatments (□ = flat, ○ = mimic barnacle, △ = live barnacle). The shading of shapes and line indicates different orientations of experimental panels (—■— = downwards, --□-- = vertical). (b) Mean ( $\pm$ SE) size ( $\sqrt{\text{area}}$ ) of mapped individuals ( $\sqrt{\text{area}}$ ) per plate of 2 focal species on Day 28 in the live barnacle experiment according to orientation and plate type

potentially 'unlimited' attachment to substrata compared to barnacles, arborescent bryozoans and other organisms characterised by 'limited' growth forms. Species with unlimited attachment can quite quickly grow away from their initial settlement site, suggesting that settlement location may only be important for a relatively short time following settlement. This was the case for *Watersipora* colonies in the 60 d experiment, but we did not observe a benefit of refuges for *Botryllus* or *Diplosoma* during early post-settlement. This is where differences in size and physical composition of species may make them differentially vulnerable to various sources of mortality. For example, soft-bodied ascidians such as *Botryllus* and *Diplosoma* may be more susceptible to predation or environmental stressors such as UV light and tem-

perature compared to *Watersipora* (calcified) or barnacles (primarily calcium carbonate shell).

This study demonstrated that microhabitats can increase post-settlement mortality by potentially enhancing an environmental stress for some species. In our experiment, there were high rates of sedimentation onto both plate types on upwards-facing surfaces, but the mimic barnacle plates appeared to trap and accumulate much greater amounts of sediment than flat surfaces (P. Carnell pers. obs.). Increased sedimentation was very likely the primary driver behind the increased mortality rates on the upwards orientation and the source of mortality that resulted in the plate type effect for barnacles on this orientation. Studies at other locations have similarly found sedimentation to be a major abiotic source of mortality on

upwards-facing surfaces (Young & Chia 1984, Knott et al. 2006, Schiel et al. 2006, Hart & Marshall 2013).

Not only can microhabitats increase mortality for some species, they can also have sublethal effects on performance by reducing size and growth. Again, this effect can vary depending on environmental conditions. In the 60 d experiment, we observed smaller barnacles on mimic barnacle plates when compared to flat plates on the downwards orientation, but size did not differ between plates on the vertical orientation. This may stem from the perpendicular nature of water flow on vertical panels, whereby individuals may experience similar water flow in both microhabitat types.

We also observed reduced growth and increased mortality of barnacles on live barnacle plates compared to flat or mimic plates on both the downwards and vertical orientations. Reduced growth can be directly linked to higher mortality because smaller individuals are likely to be more susceptible to physical disturbance and physiological stress and consequently show higher mortality rates than larger individuals (Vermeij 1972, Baker & Mann 1992, Hunt & Scheibling 1997). This higher mortality and decreased growth next to live barnacles compared to mimic barnacles indicates that the physical presence of barnacles was less influential than their negative biological effects, which could stem from decreased access to food and decreased space (Osman et al. 1989, Dalby 1995, Osman & Whitlatch 1995).

While these results indicate positive and negative effects of microhabitats depending on species, we did find similar and strong patterns between species and across experiments relating to orientation. These patterns resulted from generally lower settlement and higher post-settlement mortality of our focal species and lower numbers or even absence in 50% of species in the assemblage. This is quite often the case in studies examining recruitment and assemblages on different orientations (Glasby 2000, Fowler-Walker & Connell 2007, Hart & Marshall 2013). While orientation can be used to represent different levels of environmental stress, a number of factors co-vary in this manipulation, which makes it difficult to categorize orientations as 'low', 'medium' or 'high' stress (Hart & Marshall 2013, Riedel et al. 2014).

In studies where microhabitats were found to be beneficial for sessile invertebrate recruits, the primary source of mortality considered to be driving this pattern was predation by fishes and/or bulldozing by sea urchins (Walters & Wetthey 1996, Maldonado & Uriz 1998). In these studies, increased survival in microhabitats resulted because large pred-

ators such as fish had reduced access to individuals in crevices. At this study site, Sams & Keough (2007) found no effect of large (>2 mm) predators on the recruitment success of sessile invertebrates. Therefore, where large predators exert little mortality on individuals, microhabitats may not offer a benefit. The major predators of newly settled sessile invertebrates observed at the study site are small benthic predators, including nudibranchs and flatworms (Merory & Newman 2005, Sams & Keough 2007). Critically, these predators would be expected to forage more effectively over both flat and more complex surfaces than larger predators, due to the scale of the microhabitats studied.

The generally high but also variable post-settlement mortality in this study reflects patterns observed in other studies of benthic invertebrates (Goselin & Qian 1996) and fish (Armsworth 2002, Almany & Webster 2006, Johnson 2007). There were generally high rates of mortality for individuals in the first week of post-settlement life. The majority of mortality seemed to occur between the end of the first week and the start of the fourth. It is here that we can see variation between and within phyla in post-settlement mortality, reflected in the high level of variation between species in the assemblages. That these species displayed various effect combinations of orientation, plate type, panel or interactions between these factors supports the idea that recruitment patterns can differ between both closely and more distantly related species.

The influence of habitat on recruitment patterns has attracted much research effort, which has largely found various microhabitats to reduce post-settlement mortality (Keough 1986, Lasker et al. 1998, Maldonado & Uriz 1998). If the benefit of increased survival on complex surfaces outweighs the potential cost of reduced growth, then this surface may act as a refuge. In the case of resident adults, any potential decrease in mortality may be outweighed by associated costs to growth and survival. Results of studies that show microhabitats to decrease mortality during recruitment have been primarily driven by the consistent effect of large predators. As the results of this study demonstrate, this may not always be the case, and a number of other sources of mortality may act in complex ways to negate any net benefit of microhabitats during recruitment. This indicates that the role of microhabitats can change if they do not convey a survival benefit or if they work to reduce growth in post-settlement individuals. This inconsistent effect of microhabitats can subsequently feed into variation in post-settlement mortality. Ultimately, the loosen-

ing or tightening of this bottleneck may importantly influence recruitment of sessile marine invertebrates and community dynamics (Bourget et al. 1994).

*Acknowledgements.* We thank Allyson O'Brien and Krystina Mossop for helpful comments on the analysis and manuscript. We thank Parks Victoria for access to our study site, Workshops Pier. This project was funded by an ARC Discovery grant awarded to M.J.K. and Dustin Marshall.

#### LITERATURE CITED

- Almany GR, Webster MS (2006) The predation gauntlet: early post-settlement mortality in reef fishes. *Coral Reefs* 25:19–22
- Armsworth PR (2002) Recruitment limitation, population regulation, and larval connectivity in reef fish metapopulations. *Ecology* 83:1092–1104
- Babcock R, Mundy C (1996) Coral recruitment: consequences of settlement choice for early growth and survivorship in two scleractinians. *J Exp Mar Biol Ecol* 206–2: 179–201
- Baker SM, Mann R (1992) Effects of hypoxia and anoxia on larval settlement, juvenile growth, and juvenile survival of the oyster *Crassostrea virginica*. *Biol Bull* 182:265–269
- Bergeron P, Bourget E (1986) Shore topography and spatial partitioning of crevice refuges by sessile epibenthos in an ice disturbed environment. *Mar Ecol Prog Ser* 28: 129–145
- Bourget E, DeGuise J, Daigle G (1994) Scales of substratum heterogeneity, structural complexity, and the early establishment of a marine epibenthic community. *J Exp Mar Biol Ecol* 181:31–51
- Connell JH (1961) Effects of competition, predation by *Thais lapillus*, and other factors on natural populations of barnacle *Balanus balanoides*. *Ecol Monogr* 31:61–104
- Connell SD (1999) Effects of surface orientation on the cover of epibiota. *Biofouling* 14:219–226
- Dalby JE (1995) Consequences of aggregated living in the ascidian *Pyura stolonifera*: evidence for non-contact intraspecific competition. *Mar Freshw Res* 46:1195–1199
- Davis AR (1987) Variation in recruitment of the subtidal colonial ascidian *Podocladella cylindrica* (Quoy and Gaimard): the role of substratum choice and early survival. *J Exp Mar Biol Ecol* 106:57–71
- Fowler-Walker MJ, Connell SD (2007) Habitat heterogeneity as a consequence of sub stratum-orientation and kelp-canopy: relating interdependent responses to common patterns. *J Exp Mar Biol Ecol* 343:127–137
- Glasby TM (2000) Surface composition and orientation interact to affect subtidal epibiota. *J Exp Mar Biol Ecol* 248:177–190
- Gosselin LA, Qian PY (1996) Early post-settlement mortality of an intertidal barnacle: a critical period for survival. *Mar Ecol Prog Ser* 135:69–75
- Hart SP, Marshall DJ (2013) Environmental stress, facilitation, competition, and coexistence. *Ecology* 94: 2719–2731
- Hunt HL, Scheibling RE (1997) Role of early post-settlement mortality in recruitment of benthic marine invertebrates. *Mar Ecol Prog Ser* 155:269–301
- Hurlbut CJ (1991) Larval substratum selection and postsettlement mortality as determinants of the distribution of 2 bryozoans. *J Exp Mar Biol Ecol* 147:103–119
- Hurlbut CJ (1993) The adaptive value of larval behavior of a colonial ascidian. *Mar Biol* 115:253–262
- Jenkins SR, Norton TA, Hawkins SJ (1999) Settlement and post-settlement interactions between *Semibalanus balanoides* (L.) (Crustacea: Cirripedia) and three species of fucoid canopy algae. *J Exp Mar Biol Ecol* 236:49–67
- Johnson DW (2007) Habitat complexity modifies post-settlement mortality and recruitment dynamics of a marine fish. *Ecology* 88:1716–1725
- Keough MJ (1986) The distribution of a bryozoan on seagrass blades: settlement, growth, and mortality. *Ecology* 67:846–857
- Keough MJ, Downes BJ (1982) Recruitment of marine-invertebrates — the role of active larval choices and early mortality. *Oecologia* 54:348–352
- Keough MJ, Downes BJ (1986) Effects of settlement and post-settlement mortality on the distribution of the ascidian *Trididemnum opacum*. *Mar Ecol Prog Ser* 33:279–285
- Keough MJ, Raimondi PT (1995) Responses of settling invertebrate larvae to bioorganic films: effects of different types of films. *J Exp Mar Biol Ecol* 185:235–253
- Knott NA, Underwood AJ, Chapman MG, Glasby TM (2004) Epibiota on vertical and on horizontal surfaces on natural reefs and on artificial structures. *J Mar Biol Assoc UK* 84: 1117–1130
- Knott NA, Underwood AJ, Chapman MG, Glasby TM (2006) Growth of the encrusting sponge *Tedania anhelans* (Lieberkuhn) on vertical and on horizontal surfaces of temperate subtidal reefs. *Mar Freshw Res* 57:95–104
- Lapointe L, Bourget E (1999) Influence on substratum heterogeneity scales and complexity on a temperate epibenthic marine community. *Mar Ecol Prog Ser* 189:159–170
- Lasker HR, Kim K, Coffroth MA (1998) Production, settlement, and survival of plexaurid gorgonian recruits. *Mar Ecol Prog Ser* 162:111–123
- Lopez S, Turon X, Monterio E, Palacin C, Duarte CM, Targuero I (1998) Larval abundance, recruitment and early mortality in *Paracentrotus lividus* (Echinoidea). Inter-annual variability and plankton–benthos coupling. *Mar Ecol Prog Ser* 172:239–251
- Maldonado M, Uriz MJ (1998) Microrefuge exploitation by subtidal encrusting sponges: patterns of settlement and post-settlement survival. *Mar Ecol Prog Ser* 174:141–150
- Merory M, Newman LJ (2005) A new stylochid flatworm (Platyhelminthes, Polycladida) from Victoria, Australia and observations on its biology. *J Nat Hist* 39:2581–2589
- Osman RW, Whitlatch RB (1995) The influence of resident adults on recruitment: a comparison to settlement. *J Exp Mar Biol Ecol* 190:169–198
- Osman RW, Whitlatch RB, Zajac RN (1989) Effects of resident species on recruitment into a community: larval settlement versus post-settlement mortality in the oyster *Crassostrea virginica*. *Mar Ecol Prog Ser* 54:61–73
- Petraitis PS (1990) Direct and indirect effects of predation, herbivory and surface rugosity on mussel recruitment. *Oecologia* 83:405–413
- Raimondi PT (1990) Patterns, mechanisms, consequences of variability in settlement and recruitment of an intertidal barnacle. *Ecol Monogr* 60:283–309
- Riedel A, Monro K, Blows MW, Marshall DJ (2014) Relative influence of resident species and environmental variation on community assembly. *Mar Ecol Prog Ser* 499:103–113
- Sams MA, Keough MJ (2007) Predation during early post-settlement varies in importance for shaping marine sessile communities. *Mar Ecol Prog Ser* 348:85–101

- ✦ Sams MA, Keough MJ (2013) Early recruitment variation and an established dominant alter the composition of a temperate fouling community. *Mar Ecol Prog Ser* 486:79-91
- ✦ Schiel DR, Wood SA, Dunmore RA, Taylor DI (2006) Sediment on rocky intertidal reefs: effects on early post-settlement stages of habitat-forming seaweeds. *J Exp Mar Biol Ecol* 331:158-172
- ✦ Stoner DS (1990) Recruitment of a tropical colonial ascidian: relative importance of pre-settlement vs. post-settlement processes. *Ecology* 71:1682-1690
- ✦ Thomsen MS, McGlathery K (2006) Effects of accumulations of sediments and drift algae on recruitment of sessile organisms associated with oyster reefs. *J Exp Mar Biol Ecol* 328:22-34
- ✦ Todd CD, Keough MJ (1994) Larval settlement in hard substratum epifaunal assemblages: a manipulative field study of the effects of substratum filming and the presence of incumbents. *J Exp Mar Biol Ecol* 181:159-187
- ✦ Todd CD, Turner SJ (1986) Ecology of intertidal and sublittoral cryptic epifaunal assemblages. I. Experimental rationale and the analysis of larval settlement. *J Exp Mar Biol Ecol* 99:199-231
- ✦ Vermeij GJ (1972) Intraspecific shore-level size gradients in intertidal molluscs. *Ecology* 53:693-700
- ✦ Vieira LM, Spencer Jones M, Taylor PD (2014) The identity of the invasive fouling bryozoan *Watersipora subtorquata* (d'Orbigny) and some other congeneric species. *Zootaxa* 3857:151-182
- ✦ Walters LJ, Wethey DS (1991) Settlement, refuges, and adult body form in colonial marine invertebrates: a field experiment. *Biol Bull* 180:112-118
- ✦ Walters LJ, Wethey DS (1996) Settlement and early post-settlement survival of sessile marine invertebrates on topographically complex surfaces: the importance of refuge dimensions and adult morphology. *Mar Ecol Prog Ser* 137-3:161-171
- ✦ Wright JR, Boxshall AJ (1999) The influence of small-scale flow and chemical cues on the settlement of two congeneric barnacle species. *Mar Ecol Prog Ser* 183:179-187
- ✦ Young BL (1991) *Spartina* axil zones: preferred settlement sites of barnacles. *J Exp Mar Biol Ecol* 151:71-82
- ✦ Young CM, Chia FS (1984) Microhabitat-associated variability in survival and growth of subtidal solitary ascidians during the 1st 21 days after settlement. *Mar Biol* 81:61-68
- ✦ Yund PO, Cunningham CW, Buss LLV (1987) Recruitment and post-recruitment interactions in a colonial hydroid. *Ecology* 68:971-982
- ✦ Zamorano JH, Moreno CA, Duarte WE (1995) Post-settlement mortality in *Phragmidtopoma virgini* (Polychaeta: Sabellariidae) at the Mehuin Marme Reserve, Chile. *Mar Ecol Prog Ser* 127:149-155

#### Appendix. Additional data analysis tables

Table A1. Survival of recruits through the first 60 d of the 60 d experiment. Results of repeated-measures ANOVA to examine effects of orientation and plate type on the number of individuals (natural log) between settlement and Day 60 in the 60 d mimic barnacle experiment. All significant p-values ( $p < 0.05$ ) are highlighted in **bold**

Species Source	df	Barnacles p	<i>Watersipora</i> p	<i>Botryllus</i> p
<b>Between subjects</b>				
Orientation = O	2	0.150	0.245	<b>0.032</b>
Plate type = P	1	0.054	0.076	0.557
O × P	2	0.225	0.184	0.568
Panel (O)	3	<b>0.002</b>	0.295	0.178
P × panel (O)	3	0.066	0.466	0.436
Error	36			
<b>Within subjects</b>				
Time = T	5	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>
T × O	10	<b>0.005</b>	<b>&lt;0.001</b>	<b>0.001</b>
T × P	5	<b>0.005</b>	0.159	1.000
T × O × P	10	0.099	0.747	0.881
T × panel (O)	15	<b>&lt;0.001</b>	0.996	<b>0.011</b>
T × P × panel (O)	15	<b>0.038</b>	0.938	0.076
Error	180			