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Living on the edge: Early life history phases as determinants of distribution in *Pyura praeputialis* (Heller, 1878), a rocky shore ecosystem engineer

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ABSTRACT

The distribution of intertidal organisms can depend on processes operating early in their life history. The ascidian *Pyura praeputialis*, a mid- to low-intertidal habitat-forming ecosystem engineer, was strongly associated with specific types of habitat (biogenic vs. bare rock). We examined field patterns and performed laboratory and field experiments to assess the nature of this association. Recruits were frequently found on the tunics of conspecifics and clumps of turfing coralline algae. Larvae preferred these same habitats in a series of laboratory settlement assays. Laboratory-reared juveniles (20- & 50-days-old) survived poorly on bare rock in the laboratory, while those on rugose surfaces - the tunic of adults and turfing corallines - showed high survivorship. Field-collected juveniles (< 2 cm) affixed to these rugose habitats also exhibited high survivorship in the field. We conclude that both pre and post-settlement processes determine spatial pattern in this important habitat-forming taxon. The acute sensitivity of juveniles to desiccating conditions was unexpected in an intertidal organism.

1. Introduction

The intertidal zone is a demanding environment. It is characterised by strong patchiness as well as marked physical and biological gradients. The role of thermal stress and desiccation as structuring forces on rocky shores is well established in the marine ecological literature (see reviews by Connell, 1972; Raffaelli and Hawkins, 1996). Despite the strong abiotic gradients, small-scale variation in the spatial and temporal distribution of stressors is also apparent (e.g., Helmuth and Hofmann, 2001; Lathlean et al., 2016) and may have important impacts on the survival and abundance of individuals (Brawley and Johnson, 1991).

Space is often an important limiting resource in these systems, but as a habitat, bare rock offers a distinct set of challenges, as it is frequently much hotter than surrounding areas (Lathlean, 2014). Biogenic structure can play an important role in ameliorating the temperature regime, even at scales of relevance to larvae (Lathlean et al., 2013). The role of biogenic structure in facilitating neighbouring organisms is certainly not new; Menge (1978) explored the role of canopy forming algae in providing shade. Examples of positive effects, particularly in stressful environments, are now commonplace in the literature (Bertness and Leonard, 1997) with the realisation of the group benefits associated with the ameliorating effects of neighbours (Bertness et al., 1999). For sessile taxa that do not have an opportunity to move once

settled, the selection of appropriate microhabitats is critical to their fitness (Lathlean et al., 2013).

Ascidians are group of chordates that are largely restricted to the subtidal-zone. Solitary ascidians in the genus *Pyura* represent an exception, with several taxa monopolising space and forming extensive bands on the lower intertidal-zone of the three main southern hemisphere continents; Australia, Africa and south America (Fig. 1A, Kott, 1985, Davis, 1995, Castilla et al., 2004, Branch et al., 2016). Members of this genus can form the highest biomass per square meter of any intertidal organisms (Rius et al., 2017). In addition, they are important ecosystem engineers, providing biogenic habitat that supports a diversity of organisms. The capacity of *Pyura* species to monopolize space is notorious, even beyond their natural distribution range, as some species have proven to be successful invaders (Rius et al., 2017). Recent molecular work has confirmed that at least three taxa have established large self-sustaining populations many thousands of km from their source (Teske et al., 2011). This includes the focus of our research, *Pyura praeputialis* (Heller, 1878), which has taken up residence in northern Chile (Castilla et al., 2004; Teske et al., 2011).

For such an important group of organisms there has been little attention paid to the role of early life history phases in determining patterns of adult distribution and abundance. There are a series of largely anecdotal observations of larval retention (Marshall, 2002) and of recruits associated with adults (Alvarado et al., 2001; Monteiro et al.,

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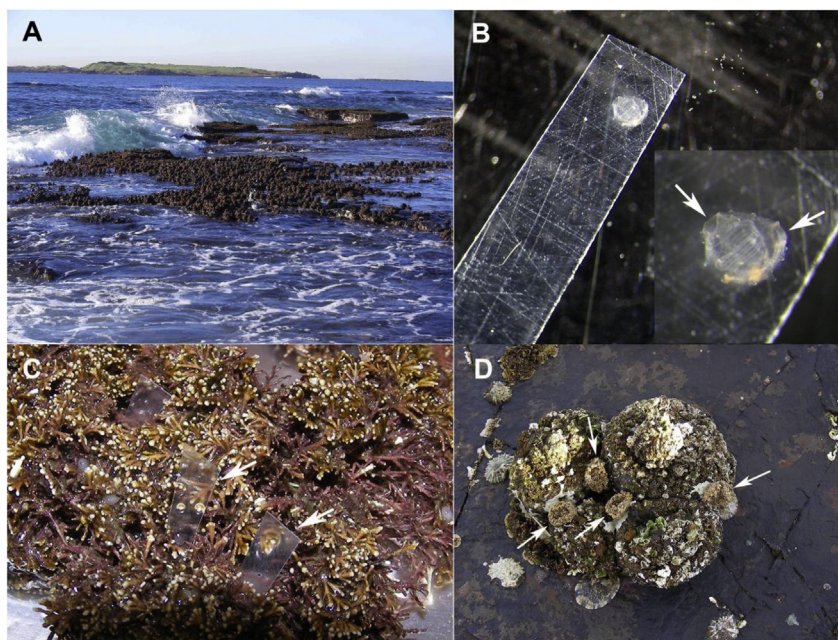


Fig. 1. A. A thick band of adult *Pyura praeputialis* at MM Point, near Wollongong. B. One of the 20-day-old juveniles used in assessments of survivorship in the laboratory. It was settled at the tip of an acetate strip – note the open siphons (arrows in inset). C. Assessment of lab survivorship on turfing coralline algae – note the acetate strips (arrows) with juveniles at the tips placed inside the turf. D. Assessment of juvenile (< 2 cm) survivorship in the field. Four recruits (arrows) can be seen epoxied to adult conspecifics. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2002). The most compelling evidence for adult conspecifics affecting patterns of conspecific recruitment comes from an examination of the invasive *Pyura praeputialis* in Chile; Manríquez et al. (2016) report strong positive relationships between adult cover and recruitment at four of their five study sites.

In this contribution, our focus was on a single species that dominates the lower shore of eastern and southeastern Australia, *Pyura praeputialis* (Heller, 1878). Although there is some information on broad-scale spatial patterns for this species (Underwood et al., 1991; Glasby et al., 2017) the processes that underpin these patterns are poorly resolved. A clearer understanding of these processes may also contribute valuable knowledge applicable to invasive populations of this species and potential control options, particularly in a future ocean.

We examined patterns of small-scale distribution for adults and recruits, with a focus on habitat use by this ascidian. We then looked for determinants of the patterns found using a series of laboratory and field experiments. We first tested the role of pre-settlement processes by examining larval selection of different types of habitat in the laboratory. Finally, we explored post-settlement processes by testing survival of early (20–50 days) juveniles in the laboratory and survival of recruits (< 2 cm in diameter) in the field as a function of microhabitat.

2. Materials and methods

2.1. Study organism and study location

Pyura praeputialis (Heller, 1878) is a member of the *Pyura stolonifera* species complex, a group of at least 5 closely related and morphologically very similar solitary ascidian species (Rius et al., 2017). Formerly known as *P. stolonifera*, molecular approaches have confirmed that this species is distributed from southern Queensland, down the Australian east coast to Victoria (Teske et al., 2011; Rius and Teske, 2013). Another species of the complex, *Pyura doppelgangers*, has recently been transported across Bass Strait from Tasmania and occupies some sites in the Victorian portion of the range of *P. praeputialis* (Rius et al., 2017). *P. praeputialis* has also invaded the coast of Chile, occupying 70 km of an embayment near Antofagasta (Castilla et al., 2004).

We sampled and made collections from reefs in the vicinity of the city of Wollongong in southern New South Wales, Australia. This equates to the southern portion of the range of this species. We worked at four sites; Bulli Point (34.3302° S, 150.9289° E), Towradgi Point

(34.3858° S, 150.9158° E), North Beach Rock platform (34.4181° S, 150.9030° E) and MM Point (34.4771° S, 150.9149° E). All of these sites are dominated by wave-cut sandstone platforms. *P. praeputialis* (*Pyura* henceforth) forms dense bands on the lower shore at each of these sites (Fig. 1A).

2.2. Assessing spatial patterns in the field

We assessed patterns of adult distribution at MM Point and Towradgi Point with 5 m long transects running parallel to the shore at each site. Five transects were placed at different tidal heights, dividing the distance from low to mid shore level into 5 evenly spaced levels. On each transect we counted the number of adult (> 3 cm diameter) *Pyura* in each of 5 randomly placed 0.25 m² quadrats. We then calculated mean:variance ratios pooling across the two sites.

To measure the distribution of recruits in relation to the available substrata, we tossed 5 quadrats haphazardly in the band of distribution of *Pyura*, ranging from the low to mid shore at MM Point and Bulli Point. Each quadrat was subdivided into a 10 × 10 grid, and we scored the type of substrate below 20 randomly selected intersections of the grid. Substratum was categorized as bare rock, *Pyura* and turfing algae; the main substrata at this shore level. In each of these quadrats, the number of recruits (< 2 cm in diameter) found on each of the substrata was counted.

2.3. Laboratory settlement trials

We reared larvae of *Pyura* in the laboratory to provide a source of larvae for settlement experiments and juveniles for survival assessment (see below). Mature adults were collected at Towradgi Point in August and gametes were obtained by dissection from 10 individuals and combined in filtered seawater (0.45 microns). Fertilised ova were washed five times with filtered seawater (FSW) in the first hour and then left to develop for the next 24 h at 18 °C in small glass fingerbowls. Only actively swimming larvae were used in the experiments.

In settlement trials, larvae were offered surfaces or ‘conditioned’ water (see below) from surfaces drawn from the common habitat types present at the low shore level. Trials were done in 24-well polystyrene culture plates (Nunc). A single larva was placed in each well along with 1 ml of FSW. Larvae were exposed to one of three treatments within the wells of culture plates; (i) small pieces of conspecific tunic (2 × 2mm),

(ii) pieces of the same size of the dominant frondose coralline in turfs, *Corallina officinalis* and (iii) chips of rock of the same size. Twenty larvae were used for each treatment, which were interspersed in the plates, and the experiment was run twice. The number of larvae that had settled 24 h later was recorded, thereby ensuring that effects due to the degeneration of tissue were minimised.

In a second set of trials, we wanted to ascertain whether water-borne cues from conspecifics could elicit the response observed in the first trial. To this end, we exposed larvae to water conditioned with *Pyura*. Specimens of the ascidian were steeped in FSW for 24 h prior to placing 1 ml of this water into each well and recording the number of settled larvae 24 h later. Controls consisted of non-conditioned water, and 20 larvae were used per treatment as before. Only one run was conducted for this experiment.

2.4. Juvenile survival

For survivorship trials with juveniles we allowed larvae to affix naturally onto pre-roughened acetate sheets laid on the bottom of petri dishes. Dishes were then maintained in aquaria with unfiltered seawater into which air was bubbled. Regular water changes provided food for the juveniles and they were maintained in the laboratory for up to 50 days prior to being used in trials. We did two sets of survivorship trials; the first at 20 days and the second at 50 days. Just prior to survivorship trials, the acetate sheets were cut in strips with a juvenile affixed near the tip (Fig. 1B). The strips were placed onto three habitat types in air in the laboratory: adult conspecifics, coralline algal turf and rock, with the tip of the acetate sheet holding the juvenile inserted within the rugose surfaces of the first two treatments (Fig. 1C). For the rock treatment the acetate strips were laid flat with the juvenile on the upward-facing side.

Time of exposure to air was a factor in the experiment, with sets of juveniles left for 15 min, 30 min, 1 h, 2 h, 4 h. For each treatment and time, 10 different juveniles were employed (totalling 150 juveniles). After the respective exposure times, the juveniles were brought back to the aquaria and monitored regularly for the following 7 h to determine if they were alive (relaxed with open siphons and reacting to touch with a probe) or dead (strongly contracted with closed siphons, no reaction to the probe). A further examination at 48 h was made to confirm these assessments. Importantly, the laboratory in which all of these experiments took place was not air conditioned, and the laboratory temperature during the experiments was ca. 18 °C. After recording the outcome of this trial (see Results), we decided to perform a second experiment consisting of repeating the rock treatment for 1 h of exposure using another set of 10 juveniles. These juveniles were sprayed with a mist of seawater every 15 min. Post-experiment assessment was performed as above.

Finally, the assessment of survivorship was repeated for 50-day-old juveniles. After the previous results they were placed onto a single habitat type – rock – again in the laboratory and exposed to the air for four periods of time (15 min, 30 min, one hour, two hours). Ten juveniles were used for each exposure period (for a total of 40 juveniles). Survivorship was assessed as above, which allowed comparison with the 20-day-old juvenile trial described earlier. Our hypothesis was that older juveniles would be more resistant to desiccation.

2.5. Recruit survival

We also assessed survivorship of individuals in the field. We collected recruits (< 2 cm diameter) from Towradgi Point in November. These recruits were collected amid the *Pyura* bed during low tide at the mid-tidal height. We then used epoxy (Araldite™) to fix them to the three habitats of interest; the tunic of adult conspecifics, frondose coralline algae turf and rock at MM Point (Fig. 1D). The same tidal height (mid-tide) was selected to deploy the experiments. Five sets of five individuals were affixed, a total of 25 recruits for each habitat type.

We assessed survivorship at daily intervals for the following four days. At the conclusion of the experiment individuals were returned to the laboratory and placed in seawater aquaria for 48 h to ensure that our assessments of survivorship in the field were correct.

2.6. Microhabitat associated amelioration of temperature

A handheld thermal IR gun (3M) was used to measure temperature in the three habitats of interest – the external surface of *Pyura* tunics, coralline algae and bare rock, both wet and dry, with 10 measures each. This was done at two locations (North Beach rock platform and MM Point) on two occasions – late in the Austral spring (November) and mid-summer (January). As Lathlean and Seuront (2014) have highlighted, wet surfaces on rocky shores affect emissivity and hence temperature estimates based on IR readings. Our temperate measures on wet rock should be regarded with caution.

2.7. Statistical analyses

We used two-way ANOVA (factors: site and transect) to analyse abundance data in the field. The variance to mean ratio in the distribution of adult ascidians was used as a dispersion measure and departures from a Poisson (random) distribution were examined by comparing the variance/mean*(n-1) statistic to a χ^2 distribution with n-1 degrees of freedom (Elliot, 1977).

Goodness of fit tests were used to compare the number of recruits on the different substrata with the expected frequency based on the proportion of each substratum available.

Contingency table analysis was used for the settlement experiments using log-linear models and the likelihood ratio G-test. When the fit of the model without interaction was significantly poorer than the saturated model (indicating lack of independence between treatments and settlement success), a pairwise G test was made across levels of treatment adding a multiple comparison correction with the Benjamini-Yekutieli method (Narum, 2006). No statistical analysis was necessary for the juvenile survival experiment as results were mostly either zero or 100% survival.

T-test and one-way ANOVA were used to test the effect of substratum on the survival and dislodgment rates of recruits. A two-factor (site and substratum) ANOVA design was used to test effects on our field estimates of microhabitat temperature. In all ANOVAs, normality of the data was assessed with the Kolmogorov-Smirnov test and homoscedasticity with Levene's test. No transformation of data was needed. *Post-hoc* tests were performed when necessary using the Student-Newman-Keuls method. Statistical analyses were done with Sigmacast v 3.5 (Systat software Inc.) for ANOVA and with the R Stats package, v 3.6.0 (R Core Team, 2016) and RVAideMemoire (<https://CRAN.R-project.org/package=RVAideMemoire>) for contingency table analyses.

3. Results

3.1. Spatial patterns in the field

As anticipated, adult *Pyura* increased in abundance with decreasing shore height. There were three times as many individuals in quadrats at the low shore level, relative to mid shore and this was consistent between sites (Fig. 2A). The ANOVA (Table 1) confirmed a significant effect of transect level on *Pyura* density, while neither the locality or interaction were significant. *Post-hoc* comparisons across transect levels showed that the low shore level had significantly higher abundances of *Pyura* than the two uppermost levels ($P < 0.05$, all other comparisons were not significant). Given the non-significant site effect, we pooled both localities for the analysis of the spatial arrangement of individuals: the variance to mean ratio decreased drastically (from ca. 95 to ca. 7) as we moved towards lower shore levels (Fig. 2B). However, in all cases

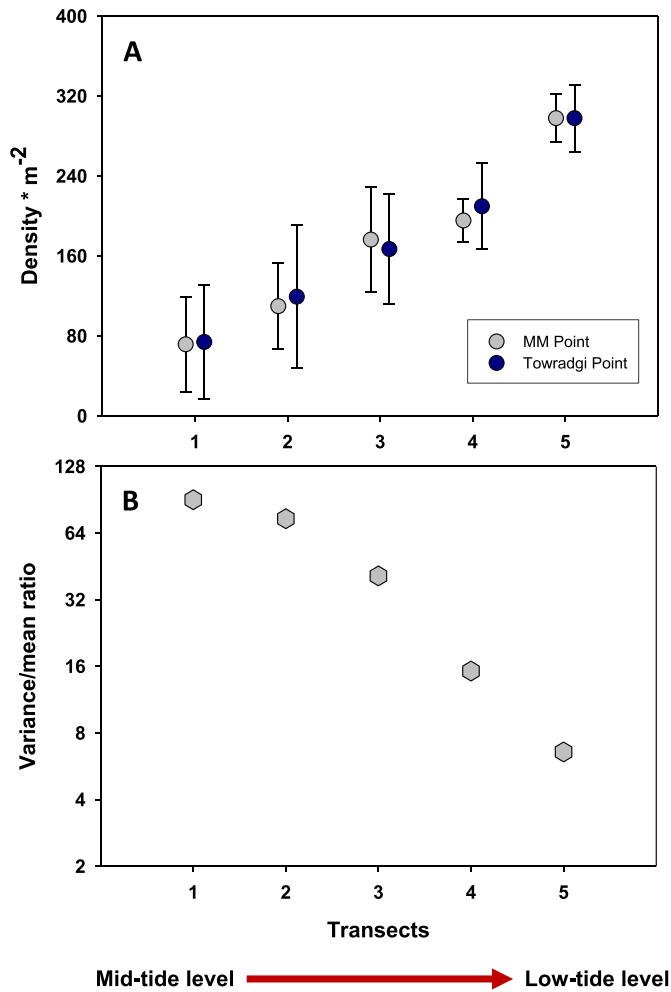


Fig. 2. Patterns of spatial abundance for adult (> 3 cm diameter) *Pyura praeputialis* at two sites in southern NSW. A. Mean (\pm sem) density. B. Variance to mean ratios (pooled for the two sites). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1

Summary of two-way ANOVA for estimates of the mean number of adult *Pyura praeputialis* found across five tidal heights (Transect factor) in the two localities sampled in southeastern Australia.

	SS	DF	MS	F	p
Locality	0.32	1	0.32	0.007	0.934
Transect	1186.68	4	296.67	6.482	< 0.001
Loc*Tran	3.08	4	0.77	0.017	0.999
Error	1830.80	40	45.77		

this ratio corresponded to a clumped or aggregated distribution as tested with a χ^2 approximation. We noted that clumps were almost invariably centred on patches of coralline algal turf consisting mostly of *Corallina officinalis* and *Amphiroa anceps*.

Recruits of *Pyura* were strongly associated with the tunics of conspecific adults and clumps of turfing coralline algae. Rarely were recruits present on bare rock, despite the prevalence of this microhabitat. Patterns were consistent for the two sites we assessed (Fig. 3). The observed distribution of recruits was significantly different from that expected considering the availability of substrata (goodness-of-fit test, $\chi^2 = 45.975$ in Port Kembla, $\chi^2 = 73.464$ in Bulli, $df = 2$ and $p < 0.001$ at both localities).

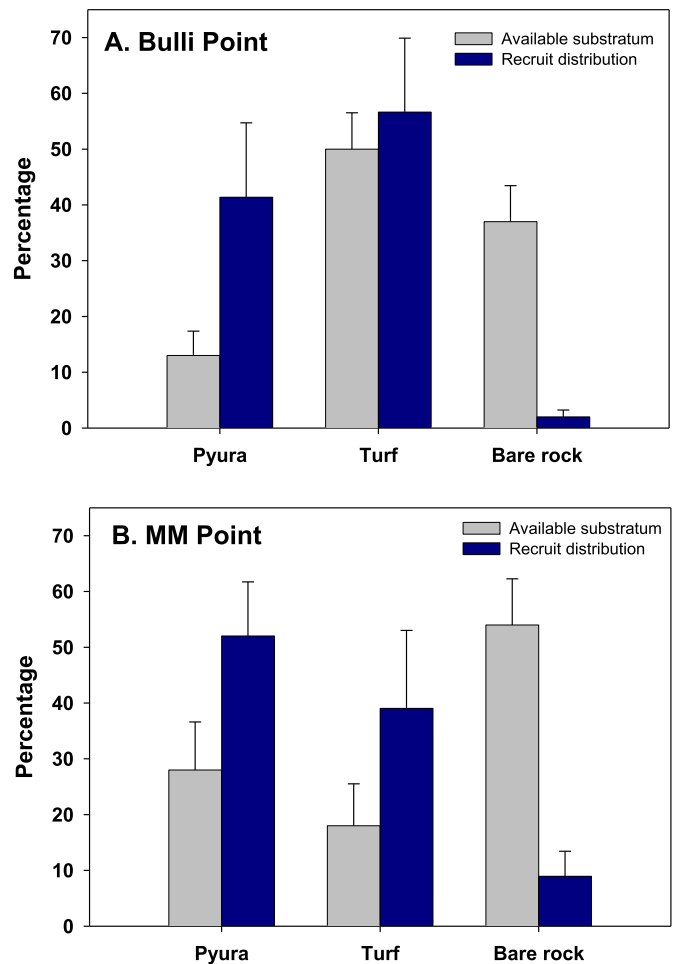


Fig. 3. Distribution of recruits of *Pyura praeputialis* in relation to the availability of key microhabitats at two locations A. Bulli Point and B. MM Point. Error bars are standard errors of the 5 replicate quadrats used in the sampling. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

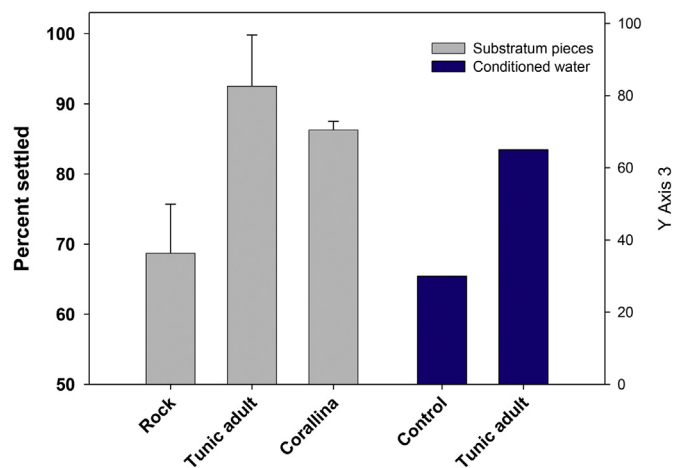


Fig. 4. Laboratory settlement responses of larvae of *Pyura praeputialis* in response to different substrata offered (grey bars, mean and SE of two runs) and to conditioned water (blue bars, only one run). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.2. Larval settlement choice

A high percentage of *Pyura* larvae had settled within 24 h on the tunic of adult conspecific ascidians (92.5%) and on frondose corallines (86.3%) (Fig. 4). In contrast, only 68.6% of larvae settled in treatments with rock; these differences were significant (G-test of the independence model, $G = 8.391$, $df = 2$, $p = 0.015$). Pairwise comparisons using G-tests confirmed that settlement in rock was significantly lower than in the two other treatments, which were not significantly different ($p = 0.677$). We then tested the potential existence of water-borne cues from the treatment showing the highest settlement (conspecifics). Settlement rates with conditioned water were lower, but showed a clear pattern. Namely, some 65% of larvae exposed to water in which adults of *Pyura* had been held for 24 h settled, while settlement in controls was significantly lower (ca. 30%, G-test of the independence model, $G = 5.019$, $df = 1$, $p = 0.025$).

3.3. Juvenile survivorship

We did not observe mortality in juveniles exposed to air when on adult conspecifics or turfing corallines over the course of our laboratory experiments. In stark contrast, survivorship was poor on rock with 100% mortality within 1 h. After this outcome, we did additional trials with the treatment showing high mortality (rock). Spraying individuals on rock with a mist of seawater improved survivorship, with 90% of recruits surviving the first hour (Fig. 5A). Fifty-day-old juveniles enjoyed slightly lower mortality than 20-day-old juveniles in the 30 min treatment, but there were no survivors on rock following 1 h and 2 h of aerial exposure (Fig. 5B).

Survivorship of recruits in the field was consistent with our laboratory findings (Fig. 6). There was a significant effect of substratum on the mortality of recruits fixed to surfaces with epoxy. We observed 100% mortality on rock while mortality on turf and conspecifics was lower than 50%. A *t*-test confirmed that survivorship on turf and conspecifics were not significantly different ($t = 0.916$, $df = 48$, $p = 0.364$). All treatments experienced losses due to wave action, although there was no significant substratum effect (Fig. 6, ANOVA: $F_{2,72} = 1.845$, $p = 0.165$).

3.4. Habitat associated amelioration of temperature

Our measures of temperature were markedly different among habitats (Fig. 7). The rugose *Pyura* tunics and turfing corallines were consistently cooler than the exposed rock surfaces. This was apparent at both sites and both sampling occasions – spring and summer. Two-way ANOVAs confirmed a locality effect (North Beach consistently warmer than the more southern MM Point) and a habitat effect (Table 2). The interaction term was not significant in spring but was so in summer, as a result of relatively higher readings in turf habitat at the North Beach locality. *Post-hoc* tests were therefore made for the main effect habitat in spring and separately for each locality in summer. The two ‘rugose’ microhabitats exhibited the coolest temperatures and did not differ significantly in temperature in the austral spring at both sites and in summer at MM Point. All other pairwise comparisons were significant.

4. Discussion

Our experiments reveal that habitat type exerts a significant influence on the settlement and subsequent survival of *Pyura praeputialis*. It appears that pre and post-settlement mechanisms reinforce each other as determinants of spatial pattern in this ecosystem engineer. Survivorship was much higher on the rugose substrata preferred by settling larvae, including clumps of turfing coralline algae or the tunic of adult conspecifics. We conclude that (i) the spatial distribution of *Pyura* is strongly clumped, (ii) juvenile distribution is correlated with the availability of biogenic habitats, (iii) larvae make choices that

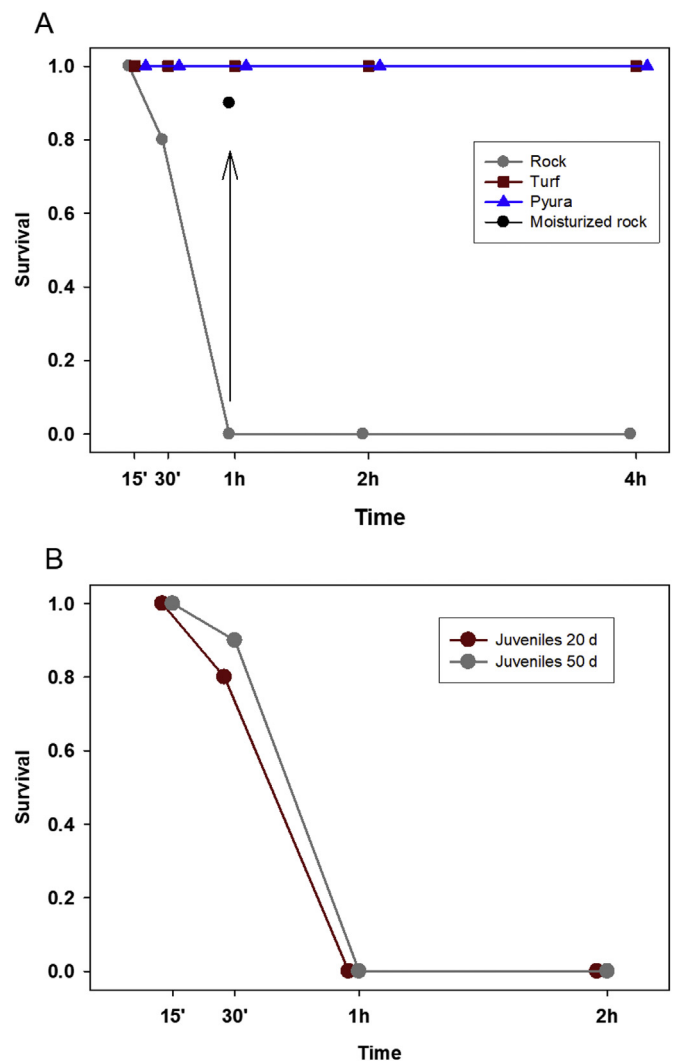


Fig. 5. Survivorship of juveniles of *Pyura praeputialis* in the laboratory. **A.** Twenty-day-old juveniles on three different microhabitats, note that a mist of seawater was sprayed onto dry rock for the moistened rock treatment (arrowed). Microhabitats were tunic of conspecific adults, turfing coralline algae and bare dry rock. **B.** Survivorship of 20- and 50-day-old juveniles on a single microhabitat – dry rock. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

enhance their likelihood of successful recruitment and (iv) pre and post-settlement mechanisms combine to ensure that *Pyura* is a dominant space occupier on rocky shores. Our results and the frequent presence of *Pyura* clumps in algal patches suggest that establishment on a new substratum can start by settlers on algal clumps acting as ‘bridgeheads’ from where the combined settlement preferences and post-settlement differential survivorship will rapidly amplify *Pyura* colonization.

A rich literature has focused on how settlement choices of larvae, particularly the larvae of solitary ascidians due to their relative large size and ease of culture, affect subsequent patterns of distribution. Pineda et al. (2012) showed the high sensitivity of early life-history stages of two ascidian species to abiotic stressors. Young and Chia (1984) confirmed the survival benefits of negative phototaxis for 6 species of solitary ascidian. The selection of predator- or grazer-free sites, via selective settlement, has also been observed (Young, 1989; Davis, 1996). Some larvae may actively avoid competitors by delaying metamorphosis in their presence (Young and Chia, 1981) or not settling near them (Grosberg, 1981; Ordóñez et al., 2013). Most experiments have confirmed the important role of larval choice in habitat selection, thereby enhancing the fitness of settlers, but there have been exceptions

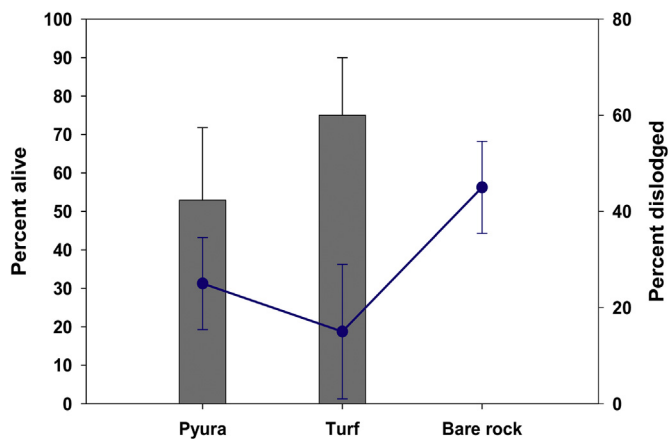


Fig. 6. Mean (\pm sem) survivorship (%) of juveniles of *Pyura praeputialis* in the field across three microhabitats (histogram bars). Microhabitats as in Fig. 5. Mean percentage of animals dislodged (\pm sem) (line) after three days of exposure. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

(Keough and Downes, 1982).

Community dominants, including members of the genus *Pyura*, can play a disproportionate role in the structure, stability and function of assemblages (Rius et al., 2017). Developing a mechanistic understanding of how members of this genus aggregate and come to dominate available space is of considerable ecological importance. The propensity of larvae to recruit to conspecific adults in the intertidal zone has been observed for *Pyura chilensis* (Davis, 1995) and *P. praeputialis* in Chile (Alvarado et al., 2001). In the absence of aggregations of adults at sites in Antofagasta, recruitment was not observed (Manríquez et al., 2016). Our laboratory settlement trials and those of Manríquez and Castilla (2007) highlight enhanced settlement and changes in larval behaviour associated with the presence of conspecifics. The inability of *Pyura praeputialis* to effect patch closure in experimental clearances in Chile (Alvarado et al., 2001) and the slow recovery of this species from experimental clearances in Australia (Fairweather, 1991) are consistent with the importance of recruitment to conspecifics or appropriate habitats. The strong preferences of larvae for adult conspecifics, including water-borne cues, revealed by our experiments and those of Manríquez and Castilla (2007) stand in contrast to the findings of Rius et al. (2010). They report no differences in settlement for larvae exposed to aqueous extracts of adults relative to filtered seawater controls for the south African *Pyura herdmani* and *P. stolonifera* in laboratory settlement trials.

The surprising element of our work was the apparent sensitivity to desiccation of juveniles of this intertidal animal. Mortality was apparent after just 30 min of aerial exposure for 20- and 50-day-old juveniles and after one hour there were no survivors. We speculate that the tunic of *Pyura* at these early developmental stages is vulnerable to water loss and the enhanced survivorship on 'rugose' habitats is due to their superior water-holding capacity. The marked reduction in mortality in juveniles following the spray of a mist of seawater adds further weight to the notion of the acute sensitivity of *Pyura praeputialis* to desiccation. On the other hand, it is possible that juveniles settling on bare rock in nature would choose small heterogeneities (crevices, pits) where survivorship could be higher. We could not test this effect in the laboratory, as the acetate strips with juveniles were laid on flat rock surfaces. Importantly, our laboratory survivorship experiment represents a conservative test of this hypothesis, as aerial exposure was in the confines of a laboratory at room temperature (18 °C) and in the absence of direct sunlight or UVR. Further, animals were affixed to acetate surfaces (Fig. 1B&C) which would have reduced the rugose nature of these natural habitats and presumably their ameliorating effects. Desiccation has been seen as a means of managing biofouling risk

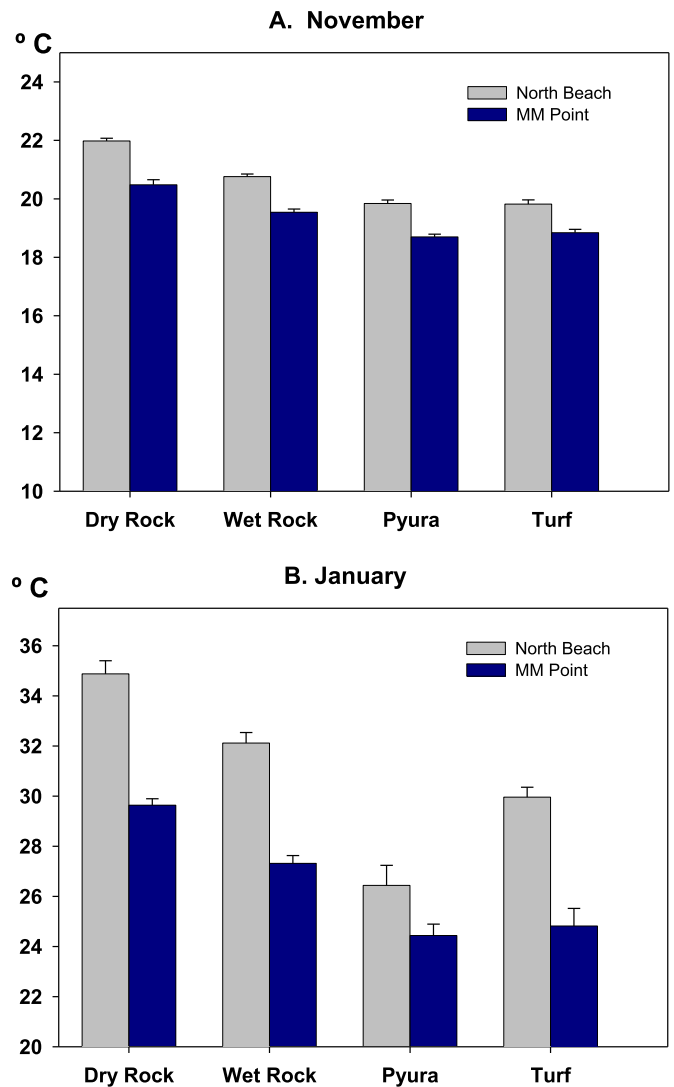


Fig. 7. Mean (\pm sem) temperature readings (°C) in four microhabitats at two sites in southern NSW. Microhabitats were the tunic of *Pyura praeputialis*, clumps of turfing coralline algae and rock (wet and dry). A. Late spring (November) B. Mid summer (January). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 2

Summary of two-way ANOVAs for November and January temperature readings for a range of microhabitats (Habitat factor: tunics of conspecifics, turfing coralline algae, dry rock and wet rock surfaces) in the two localities (Random factor) sampled.

	SS	DF	MS	F	P
November					
Locality	29.28	1	29.28	203.07	< 0.001
Habitat	50.37	3	16.79	116.44	< 0.001
Loc*Hab	0.68	3	0.23	1.57	0.203
Error	10.38	72	0.14		
January					
Locality	368.94	1	368.94	113.12	< 0.001
Habitat	521.15	3	173.72	53.30	< 0.001
Loc*Hab	35.64	3	11.88	3.65	0.017
Error	234.66	72	3.26		

in relation to solitary ascidians and direct exposure to incident radiation can further enhance its effectiveness (Hopkins et al., 2016).

The timing of reproduction and recruitment for *Pyura* coincides with the late Austral autumn, winter and early spring (Anderson et al., 1976, author's personal observations). This timing may minimise exposure of settled larvae and juveniles to challenging abiotic conditions. Similar predictions have been made for molluscs depositing egg masses on these shores, but were not supported (Przeslawski and Davis, 2007). Surprisingly, Manríquez et al. (2018) provide evidence that invasive *Pyura praeputialis* in Chile were reproductive throughout the year, although it remains unclear whether this equates to year-round recruitment. Australian and Chilean shores are dominated by heavy wave action and wave splash may act to ameliorate abiotic conditions (Harley and Helmuth, 2003). In Australia, the physical structure of these shores may also enhance the abiotic environment, as they usually are wave-cut sand-stone platforms with negligible slope and thus provide extensive habitat for *Pyura*. Field monitoring of recruitment using artificial substrata and cleared areas would further contribute to disentangle the effects of biotic and abiotic factors on the recruitment and survivorship of *P. praeputialis*, but these experiments are outside the scope of the present work.

As numerous authors have emphasised, determining likely outcomes in a future ocean is always going to be exceedingly difficult (eg Bertness et al., 1999). The close association between early life history phases of *Pyura* with turfing coralline algae may be telling. There is considerable published evidence that corallines, and by implication their associated species, will fare poorly in a future warm and 'acidified' ocean (Russell et al., 2009; Hepburn et al., 2011; Noiset et al., 2013). This may represent an issue for *Pyura* as it appears to rely heavily on turfing algae as habitat in its tidal range.

In conclusion, *Pyura* forms large aggregations on the lower shore, playing an important role in providing habitat for a suite of associated organisms. Aggregations provide structurally complex habitat as well as ameliorating abiotic stressors such as desiccation and wave shock. Our findings indicate that larval settlement choices as well as early post-settlement survival are key in establishing and maintaining the aggregations of this ecosystem engineer. Just how this species may respond in a future ocean is difficult to predict.

Competing interests statement

The authors confirm that they have no competing interests to report.

Declarations of interest

None.

Authorship

Collectively, all authors designed the study, collected and analysed the data as well as developed and edited the final manuscript.

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References

- Alvarado, J.L., Pinto, R., Marquet, P., Pacheco, C., Guíñez, R., Castilla, J.C., 2001. Patch recolonization by the tunicate *Pyura praeputialis* in the rocky intertidal of the Bay of Antofagasta, Chile: evidence for self-facilitation mechanisms. *Mar. Ecol. Prog. Ser.* 224, 93–101.
- Anderson, D.T., White, B.M., Egan, E.A., 1976. The larval development and metamorphosis of the ascidians *Pyura praeputialis* (Heller) and *Pyura pachydermatina* (Herdman)(Pleurogona, family Pyuridae). *Proc. Linn. Soc. N. S. W.* 100, 205–217.
- Bertness, M.D., Leonard, G.H., 1997. The role of positive interactions in communities: lessons from intertidal habitats. *Ecology* 78, 1976–1989.
- Bertness, M.D., Leonard, G.H., Levine, J.M., Bruno, J.F., 1999. Climate-driven interactions among rocky intertidal organisms caught between a rock and a hot place. *Oecologia* 120, 446–450.
- Branch, G.M., Griffiths, C.L., Branch, M.L., Beckley, L.E., 2016. Two Oceans: a Guide to the Marine Life of Southern Africa, Revised edn. Struik Nature, Cape Town.
- Brawley, S.H., Johnson, L.E., 1991. Survival of fucoid embryos in the intertidal zone depends upon developmental stage and microhabitat. *J. Phycol.* 27, 179–186.
- Castilla, J.C., Guíñez, R., Caro, A.U., Ortiz, V., 2004. Invasion of a rocky intertidal shore by the tunicate *Pyura praeputialis* in the Bay of Antofagasta, Chile. *Proc. Natl. Acad. Sci. U. S. A.* 101, 8517–8524.
- Connell, J.H., 1972. Community interactions on marine rocky shores. *Annu. Rev. Ecol. Systemat.* 3, 169–192.
- Davis, A.R., 1995. Over-exploitation of *Pyura chilensis* (Ascidacea) in southern Chile: the urgent need to establish marine reserves. *Rev. Chil. Hist. Nat.* 68, 107–116.
- Davis, A.R., 1996. Association among ascidians: facilitation of recruitment in *Pyura spinifera*. *Mar. Biol.* 126, 35–41.
- Elliot, J.M., 1977. Statistical analysis of samples of benthic invertebrates. *Freshw. Biol. Assoc. Sci. Publ.* 25, 1–159.
- Fairweather, P.G., 1991. A conceptual framework for ecological studies of coastal resources: an example of a tunicate collected for bait on Australian seashores. *Ocean Shores. Manag.* 15, 125–142. [https://doi.org/10.1016/0951-8312\(91\)90027-Y](https://doi.org/10.1016/0951-8312(91)90027-Y).
- Glasby, T.M., Gibson, P.T., Cruz-Motta, J.J., 2017. Differences in rocky reef habitats related to human disturbances across a latitudinal gradient. *Mar. Environ. Res.* 229, 291–303. <https://doi.org/10.1016/j.marenvres.2017.06.014>.
- Grosberg, R.K., 1981. Competitive ability influences habitat choice in marine invertebrates. *Nature* 290, 700–702.
- Harley, C.D., Helmuth, B.S., 2003. Local-and regional-scale effects of wave exposure, thermal stress, and absolute versus effective shore level on patterns of intertidal zonation. *Limnol. Oceanogr.* 48, 1498–1508.
- Heller, C., 1878. Beiträge zur näheren Kenntniss der Tunicaten. *Sitzungsberichte Acad. Wissenschaften Wien* 77, 83–109.
- Helmuth, B.S., Hofmann, G.E., 2001. Microhabitats, thermal heterogeneity, and patterns of physiological stress in the rocky intertidal zone. *Biol. Bull.* 201, 374–384.
- Hepburn, C.D., Pritchard, D.W., Cornwall, C.E., Mcleod, R.J., Beardall, J., Raven, J.A., Hurd, C.L., 2011. Diversity of carbon use strategies in a kelp forest community: implications for a high CO₂ ocean. *Global Change Biol.* 17, 2488–2497. <https://doi.org/10.1111/j.1365-2486.2011.02411.x>.
- Hopkins, G.A., Prince, M., Cahill, P.L., Fletcher, L.M., Atalah, J., 2016. Desiccation as a mitigation tool to manage biofouling risks: trials on temperate taxa to elucidate factors influencing mortality rates. *Biofouling* 32, 1–11.
- Keough, M.J., Downes, B.J., 1982. Recruitment of marine invertebrates: the role of active larval choices and early mortality. *Oecologia* 54, 348–352.
- Kott, P., 1985. The Australian Ascidacea, Part 1. Phlebobranchia and Stolidobranchia. *Memoir. Queensl. Mus.* 23, 1–438.
- Lathlean, J.A., 2014. Not all space is created equal: distribution of free space and its influence on heat-stress and the limpet *Patelloida latistrigata*. *J. Thermal Biol.* 46, 16–23.
- Lathlean, J.A., Ayre, D.J., Minchinton, T.E., 2013. Temperature variability at the larval scale affects early survival and growth of an intertidal barnacle. *Mar. Ecol. Prog. Ser.* 475, 155–166.
- Lathlean, J.A., Seuront, 2014. Infrared thermography in marine ecology: methods, previous applications and future challenges. *Mar. Ecol. Prog. Ser.* 514, 263–277.
- Lathlean, J.A., Seuront, L., McQuaid, C.D., Ng, T.P., Zardi, G.I., Nicastro, K.R., 2016. Size and position (sometimes) matter: small-scale patterns of heat stress associated with two co-occurring mussels with different thermoregulatory behaviour. *Mar. Biol.* 163, 189–200.
- Manríquez, P.H., Castilla, J.C., 2007. Roles of larval behaviour and microhabitat traits in determining spatial aggregations in the ascidian *Pyura chilensis*. *Mar. Ecol. Prog. Ser.* 332, 155–165.
- Manríquez, P.H., Castilla, J.C., Ortiz, V., Jara, M.E., 2016. Empirical evidence for large-scale human impact on intertidal aggregations, larval supply and recruitment of *Pyura praeputialis* around the Bay of Antofagasta, Chile. *Austral Ecol.* 41, 701–714.
- Manríquez, P.H., Guíñez, R., Olivares, A., Clarke, M., Castilla, J.C., 2018. Effects of inter-annual temperature variability, including ENSO and post-ENSO events, on reproductive traits in the tunicate *Pyura praeputialis*. *Mar. Biol. Res.* 23, 1–6.
- Marshall, D.J., 2002. *In situ* measures of spawning synchrony and fertilization success in an intertidal, free-spawning invertebrate. *Mar. Ecol. Prog. Ser.* 236, 113–119.
- Menge, B.A., 1978. Predation intensity in a rocky intertidal community: effect of an algal canopy, wave action and desiccation on predator feeding rates. *Oecologia* 34, 17–35.
- Monteiro, S.M., Chapman, M.G., Underwood, A.J., 2002. Patches of the ascidian *Pyura stolonifera* (Heller, 1878): structure of habitat and associated intertidal assemblages. *J. Exp. Mar. Biol. Ecol.* 270, 171–189.
- Narum, S.R., 2006. Beyond Bonferroni: less conservative analyses for conservation genetics. *Conserv. Genet.* 7, 783–787.

- Noisette, F., Egilsdottir, H., Dominique Davoult, D., Martin, S., 2013. Physiological responses of three temperate coralline algae from contrasting habitats to near-future ocean acidification. *J. Exp. Mar. Biol. Ecol.* 448, 179–187.
- Ordóñez, V., Rius, M., McQuaid, C.D., Pineda, M.C., Pascual, M., Turon, X., 2013. Early biotic interactions among introduced and native benthic species reveal cryptic predation and shifts in larval behavior. *Mar. Ecol. Prog. Ser.* 488, 65–79.
- Pineda, M.C., McQuaid, C.D., Turon, X., López-Legentil, S., Ordóñez, V., Rius, M., 2012. Tough adults, frail babies: an analysis of stress sensitivity across early life-history stages of widely introduced marine invertebrates. *PLoS One* 7 (10), e46672.
- Przeslawski, R., Davis, A.R., 2007. Does spawning behaviour minimize exposure to environmental stressors for encapsulated embryos on rocky shores? *Mar. Biol.* 152, 991–1002.
- R Core Team, 2016. *R: a Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.
- Raffaelli, D., Hawkins, S., 1996. *Intertidal Ecology*. Chapman & Hall, London.
- Rius, M., Branch, G.M., Griffiths, C.L., Turon, X., 2010. Larval settlement behaviour in six gregarious ascidians in relation to adult distribution. *Mar. Ecol. Prog. Ser.* 418, 151–163.
- Rius, M., Teske, P.R., 2013. Cryptic diversity in coastal Australasia: a morphological and mitonuclear genetic analysis of habitat-forming sibling species. *Zool. J. Linn. Soc.* 168, 597–611. <https://doi.org/10.1111/zoj.12036>.
- Rius, M., Teske, P.R., Manríquez, P.H., Suárez-Jiménez, R., McQuaid, C.D., Castilla, J.C., 2017. Ecological dominance along rocky shores, with a focus on intertidal ascidians. *Oceanogr. Mar. Biol. Annu. Rev.* 55, 55–84.
- Russell, B.D., Thompson, J.-A.I., Falkenberg, L.J., Connell, S.D., 2009. Synergistic effects of climate change and local stressors: CO₂ and nutrient-driven change in subtidal rocky habitats. *Global Change Biol.* 15, 2153–2162. <https://doi.org/10.1111/j.1365-2486.2009.01886.x>.
- Teske, P.R., Rius, M., McQuaid, C.D., Styan, C.A., Piggott, M.P., Benhissoune, S., Fuentes-Grünwald, C., Walls, K., Page, M., Attard, C.R.M., Cooke, G.M., McClusky, C.F., Banks, S.C., Barker, N.P., Beheregaray, L.B., 2011. “Nested” cryptic diversity in a widespread marine ecosystem engineer: a challenge for detecting biological invasions. *BMC Evol. Biol.* 11, 176–189. <https://doi.org/10.1186/1471-2148-11-176>.
- Underwood, A.J., Kingsford, M.J., Andrew, N.L., 1991. Patterns of abundance in shallow subtidal marine assemblages along the coast of New South Wales. *Aust. J. Ecol.* 16, 231–249.
- Young, C.M., 1989. Selection of predator-free settlement sites by larval ascidians. *Ophelia* 30, 131–140.
- Young, C.M., Chia, F.S., 1981. Laboratory evidence for delay of larval settlement in response to a dominant competitor. *Int. J. Invertebr. Reprod.* 3, 221–226.
- Young, C.M., Chia, F.S., 1984. Microhabitat-associated variability in survival and growth of subtidal solitary ascidians during the first 21 days after settlement. *Mar. Biol.* 81, 61–68.