

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25

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

Andrew R. Davis<sup>1</sup>, Mikel Becerro<sup>2</sup>, Xavier Turon<sup>2\*</sup>

<sup>1</sup> Centre for Sustainable Ecosystem Solutions &  
School of Biological Sciences  
University of Wollongong NSW 2522, AUSTRALIA  
adavis@uow.edu.au

<sup>2</sup> Centre for Advanced Studies of Blanes (CEAB, CSIC) Blanes, Girona, SPAIN  
m.becero@ceab.csic.es  
xturon@ceab.csic.es

\* corresponding author: [xturon@ceab.csic.es](mailto:xturon@ceab.csic.es) - phone: +34 972336101, fax:  
+34 972337806

Declarations of interest: none

26 ABSTRACT

27

28 The distribution of intertidal organisms can depend on processes operating early  
29 in their life history. The ascidian *Pyura praeputialis*, a mid- to low-intertidal  
30 habitat-forming ecosystem engineer, was strongly associated with specific  
31 microhabitats. Recruits were frequently found on the tunics of conspecifics and  
32 clumps of turfing coralline algae. Larvae preferred these same microhabitats in a  
33 series of laboratory settlement assays. Laboratory-reared juveniles (20 & 50  
34 days old) survived poorly on bare rock in the laboratory, while those on rugose  
35 surfaces - the tunic of adults and turfing corallines - showed high survivorship.  
36 Field-collected juveniles (<2cm) affixed to these rugose microhabitats also  
37 exhibited high survivorship in the field. We conclude that both pre and post-  
38 settlement processes determine spatial pattern in this important taxon. The  
39 acute sensitivity of juveniles to desiccating conditions was unexpected in an  
40 intertidal organism and how this space occupier will fare as the planet warms  
41 remains unclear.

42

43

44 **Key Words:** Benthic Ecology, Larvae, *Pyura praeputialis*, Reefs, Settlement,  
45 Southeastern Australia, Suspension feeder

46

47 **Authorship:** Collectively, all authors designed the study, collected and analysed  
48 the data as well as developed and edited the final manuscript.

49

50

51 INTRODUCTION

52 The intertidal zone is a demanding environment. It is characterised by strong  
53 patchiness as well as marked physical and biological gradients. The role of  
54 thermal stress and desiccation as structuring forces on rocky shores is well  
55 established in the marine ecological literature (see reviews by Connell 1972,  
56 Raffaelli and Hawkins 1996). Despite the strong abiotic gradients, small-scale  
57 variation in the spatial and temporal distribution of stressors is also apparent  
58 (e.g., Helmuth and Hoffman 2001, Lathlean et al. 2016) and may have important  
59 impacts on the survival and abundance of individuals (Brawley and Johnson  
60 1991).

61

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

74

75 Ascidians are group of chordates that are largely restricted to the subtidal-zone.  
76 Solitary ascidians in the genus *Pyura* represent an exception, with several taxa  
77 monopolising space and forming extensive bands on the lower intertidal-zone of  
78 the three main southern hemisphere continents; Australia, Africa and south  
79 America (Fig. 1A, Kott 1985, Davis 1995, Castilla et al. 2004, Branch et al. 2010).  
80 Members of this genus can form the highest biomass per square meter of any  
81 intertidal organisms (Rius et al. 2017). In addition, they are important ecosystem  
82 engineers, providing biogenic habitat that supports a diversity of organisms. The  
83 capacity of *Pyura* species to monopolize space is notorious, even beyond their  
84 natural distribution range, as some species have proven to be successful  
85 invaders. Recent molecular work has confirmed that at least three taxa have  
86 established large self-sustaining populations many thousands of km from their  
87 source (Teske et al. 2011). This includes the focus of our research, *P. praeputialis*,  
88 which has taken up residence in northern Chile (Castilla et al. 2004, Teske et al.  
89 2011).

90

91 For such an important group of organisms there has been little attention paid to  
92 the role of early life history phases in determining patterns of adult distribution  
93 and abundance. There are a series of largely anecdotal observations of larval  
94 retention (Marshall 2002) and of recruits associated with adults (Alvarado et al.  
95 2001, Monteiro et al 2002). The most compelling evidence for adult conspecifics  
96 affecting patterns of conspecific recruitment comes from an examination the  
97 invasive *Pyura praeputialis* in Chile; Manríquez et al. (2016) report strong positive  
98 relationships between adult cover and recruitment at four of their five study  
99 sites.

100

101 In this contribution, our focus was on a single species that dominates the lower  
102 shore of eastern and southeastern Australia, *Pyura praeputilis* (Heller, 1878).

103 Although there is some information on broad-scale spatial patterns for this  
104 species (Underwood et al. 1991, Glasby et al. 2017) the processes that underpin  
105 these patterns are poorly resolved. A clearer understanding of these processes  
106 may also contribute valuable knowledge applicable to invasive populations of  
107 this species and potential control options, particularly in a future ocean.

108

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

116

## 117 MATERIALS AND METHODS

### 118 *Study organism and study location*

119 *Pyura praeputialis* (Heller, 1878) is a member of the *Pyura stolonifera* species  
120 complex, a group of at least 5 closely related and morphologically very similar  
121 solitary ascidian species (Rius et al. 2017). Formerly known as *P. stolonifera*,  
122 molecular approaches have confirmed that this species is distributed from  
123 southern Queensland, down the Australian east coast to Victoria (Teske et al.  
124 2011, Rius & Teske 2013). Another species of the complex, *Pyura doppelgangera*,

125 has recently been transported across Bass Strait from Tasmania and occupies  
126 some sites in the Victorian portion of the range of *P. praeputialis* (Rius et al.  
127 2017). *Pyura praeputialis* has also invaded the coast of Chile, occupying 70km of  
128 an embayment near Antofagasta (Castilla et al. 2004).

129

130 We sampled and made collections from reefs in the vicinity of the city of  
131 Wollongong in southern New South Wales, Australia. This equates to the  
132 southern portion of the range of this species. We worked at four sites; Bulli Point  
133 (34.3302° S, 150.9289° E), Towradgi Point (34.3858° S, 150.9158° E), North  
134 Beach Rock platform (34.4181° S, 150.9030° E) and MM point (34.4771° S,  
135 150.9149° E). All of these sites are dominated by wave-cut sandstone platforms.  
136 *Pyura praeputialis* (*Pyura* henceforth) forms dense bands on the lower shore at  
137 each of these sites (Fig. 1A).

138

### 139 *Assessing spatial patterns in the field*

140 We assessed patterns of adult distribution at MM Point and Towradgi Point with  
141 5m long transects running parallel to the shore at each site. Five transects were  
142 placed at different tidal heights, dividing the distance from low to mid shore level  
143 into 5 evenly spaced levels. On each transect we counted the number of adult  
144 (>3cm diameter) *Pyura* in each of 5 randomly placed 0.25m<sup>2</sup> quadrats. We then  
145 calculated mean:variance ratios pooling across the two sites.

146

147 To measure the distribution of recruits in relation to the available substrata, we  
148 tossed 5 quadrats haphazardly in the band of distribution of *Pyura*, ranging from  
149 the low to mid shore at MM Point and Bulli Point. Each quadrat was subdivided

150 into a 10x10 grid, and we scored the type of substrate below 20 randomly  
151 selected intersections of the grid. Substratum was categorized as bare rock,  
152 *Pyura* and turfing algae; the main substrata at this shore level. In each of these  
153 quadrats, the number of recruits (<2cm in diameter) found on each of the  
154 substrata was counted.

155

#### 156 *Laboratory settlement trials*

157 We reared larvae of *Pyura* in the laboratory to provide a source of larvae for  
158 settlement experiments and juveniles for survival assessment (see below).

159 Mature adults were collected at Towradgi Point in August and gametes were  
160 obtained by dissection from 10 individuals and combined in filtered seawater  
161 (0.45 microns). Fertilised ova were washed five times with filtered seawater  
162 (FSW) in the first hour and then left to develop for the next 24 hours at 18°C in  
163 small glass fingerbowls. Only actively swimming larvae were used in the  
164 experiments.

165

166 In settlement trials, larvae were offered surfaces or 'conditioned' water (see  
167 below) from surfaces drawn from the common microhabitats present at the low  
168 shore level. Trials were done in 24 well polystyrene culture plates (Nunc). A  
169 single larva was placed in each well along with 1ml of FSW. Larvae were exposed  
170 to one of three treatments within the wells of culture plates; (i) small pieces of  
171 conspecific tunic (2x2mm), (ii) pieces of the same size of the dominant frondose  
172 coralline in turfs, *Corallina officinalis* and (iii) chips of rock of the same size.  
173 Twenty larvae were used for each treatment, which were interspersed in the  
174 plates, and the experiment was run twice. The number of larvae that had settled

175 24 hrs later was recorded, thereby ensuring that effects due to the degeneration  
176 of tissue were minimised.

177

178 In a second set of trials to examine the efficacy of water borne cues to elicit  
179 settlement, we exposed larvae to conditioned water from conspecifics.

180 Specimens of *Pyura* were steeped in FSW for 24hrs prior to placing 1ml of this  
181 water into each well and recording the number of settled larvae 24 hrs later.

182 Controls consisted of non-conditioned water, and 20 larvae were used per  
183 treatment as before. Only one run was conducted for this experiment.

184

#### 185 *Juvenile survival*

186 For survivorship trials with juveniles we allowed larvae to affix naturally onto  
187 pre-roughened acetate sheets laid on the bottom of petri dishes. Dishes were  
188 then maintained in aquaria with unfiltered seawater into which air was bubbled.

189 Regular water changes provided food for the juveniles and they were maintained  
190 in the laboratory for up to 50 days prior to being used in trials. We did two sets

191 of survivorship trials; the first at 20 days and the second at 50 days. Just prior to

192 survivorship trials, the acetate sheets were cut in strips with a juvenile affixed

193 near the tip (Fig. 1B). The strips were placed onto three microhabitats in air in

194 the laboratory: adult conspecifics, coralline algal turf and rock, with the tip of the

195 acetate sheet holding the juvenile inserted within the rugose surfaces of the first

196 two treatments (Fig 1C). For the rock treatment the acetate strips were laid flat

197 with the juvenile on the upward-facing side.

198



199 Time of exposure to air was a factor in the experiment, with sets of juveniles left  
200 for 15 minutes, 30 minutes, 1 hour, 2 hours, 4 hours. For each treatment and  
201 time, 10 different juveniles were employed (totalling 150 juveniles). After the  
202 respective exposure times, the juveniles were brought back to the aquaria and  
203 monitored regularly for the following 7 hours to determine if they were alive  
204 (relaxed with open siphons and reacting to touch with a probe) or dead (strongly  
205 contracted with closed siphons, no reaction to the probe). A further examination  
206 at 48 hours was made to confirm these assessments. Importantly, the laboratory  
207 in which all of these experiments took place was not air conditioned, and the  
208 laboratory temperature during the experiments was ca. 18°C. A second  
209 experiment consisted of repeating the rock treatment for 1 hour of exposure  
210 using another set of 10 juveniles. These juveniles were sprayed with a mist of  
211 seawater every 15 minutes. Post-experiment assessment was performed as  
212 above.

213

214 Finally, the assessment of survivorship was repeated for 50 day old juveniles.  
215 They were placed onto a single microhabitat – rock – again in the laboratory and  
216 exposed to the air for four periods of time (15 minutes, 30 minutes, one hour,  
217 two hours). Ten juveniles were used for each exposure period (for a total of 40  
218 juveniles). Survivorship was assessed as above, which allowed comparison with  
219 the 20-day old juvenile trial described earlier.

220

221 *Recruit survival*

222 We also assessed survivorship of individuals in the field. We collected recruits  
223 (<2cm diameter) from Towradgi Point in November. We then used epoxy

224 (Araldite™) to fix them to the three microhabitats of interest; the tunic of adult  
225 conspecifics, frondose coralline algae turf and rock at MM point (Fig. 1D). Five  
226 sets of five individuals were affixed, a total of 25 recruits for each microhabitat.  
227 We assessed survivorship at daily intervals for the following four days. At the  
228 conclusion of the experiment individuals were returned to the laboratory and  
229 placed in seawater aquaria for 48h to ensure that our assessments of  
230 survivorship in the field were correct.

231

### 232 *Microhabitat associated amelioration of temperature*

233 A handheld thermal IR gun (3M) was used to estimate temperature in the three  
234 microhabitats of interest – the external surface of *Pyura* tunics, coralline algae  
235 and bare rock, both wet and dry, with 10 measures each. This was done at two  
236 locations (North Beach rock platform and MM Point) on two occasions – late in  
237 the Austral spring (November) and mid-summer (January).

238

### 239 *Statistical analyses*

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

245

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

249

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

258

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

269

## 270 RESULTS

### 271 *Spatial patterns in the field*

272 As anticipated, adult *Pyura* increased in abundance with decreasing shore height.

273 There were three times as many individuals in quadrats at the low shore level,

274 relative to mid shore and this was consistent between sites (Fig. 2A). The ANOVA  
275 (Table 1) confirmed a significant effect of transect level on *Pyura* density, while  
276 neither the locality or interaction were significant. *Post-hoc* comparisons across  
277 transect levels showed that the low shore level had significantly higher  
278 abundances of *Pyura* than the two uppermost levels ( $P < 0.05$ , all other  
279 comparisons were not significant). We pooled both localities for the analysis of  
280 the spatial arrangement of individuals: the variance to mean ratio decreased  
281 drastically (from ca. 95 to ca. 7) as we moved towards lower shore levels (Fig.  
282 2B). However, in all cases this ratio corresponded to a clumped or aggregated  
283 distribution as tested with a  $\chi^2$  approximation. We noted that clumps were  
284 almost invariably centred on patches of coralline algal turf consisting mostly of  
285 *Corallina officinalis* and *Amphiroa anceps*.

286

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

294

#### 295 *Larval settlement choice*

296 More than 85% of *Pyura* larvae had settled within 24hrs on the tunic of adult  
297 congeneric ascidians and on frondose corallines (Fig. 4). In contrast, only ca.  
298 68.5% of larvae settled in controls; these differences were significant (G-test of

299 the independence model,  $G=8.391$ ,  $df=2$ ,  $p=0.015$ ). Pairwise G-tests confirmed  
300 that settlement in controls was significantly lower than in the two treatments,  
301 which were not significantly different ( $p=0.677$ ). Patterns of settlement with  
302 conditioned water were lower, but showed the same pattern. Namely, some 65%  
303 of larvae exposed to water in which adults *Pyura* had been held for 24hrs settled,  
304 while settlement in controls was significantly lower (ca. 30%, G-test of the  
305 independence model,  $G=5.019$ ,  $df=1$ ,  $p=0.025$ ).

306

### 307 *Juvenile survivorship*

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

316

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

324

325 *Microhabitat associated amelioration of temperature*

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

338

339 DISCUSSION

340 Our experiments reveal that microhabitat exerts a significant influence on the  
341 settlement and subsequent survival of *Pyura praeputialis*. It appears that pre and  
342 post-settlement mechanisms reinforce each other as determinants of spatial  
343 pattern in this ecosystem engineer. Survivorship was much higher on the rugose  
344 substrata preferred by settling larvae, including clumps of turfing coralline algae  
345 or the tunic of adult conspecifics. We conclude that (i) spatial arrangements of  
346 *Pyura* are strongly influenced by the availability of microhabitats, (ii) larvae  
347 make choices that enhance their likelihood of successful recruitment and (iii)  
348 these pre and post-settlement mechanisms combine to ensure that *Pyura* is a

349 dominant space occupier on rocky shores. Our results and the frequent presence  
350 of *Pyura* clumps in algal patches suggest that establishment on a new substrate  
351 can start by settlers on algal clumps, acting as bridgeheads from where the  
352 combined settlement preferences and post-settlement differential survivorship  
353 will rapidly amplify *Pyura* colonization.

354

355 A rich literature has focused on how settlement choices of larvae, particularly the  
356 larvae of solitary ascidians due to their relative large size and ease of culture,  
357 affect subsequent patterns of distribution. Pineda et al. (2012) showed the high  
358 sensitivity of early life-history stages of two ascidian species to abiotic stressors.  
359 Young and Chia (1984) confirmed the survival benefits of negative phototaxis for  
360 6 species of solitary ascidian. The selection of predator or grazer-free sites, via  
361 selective settlement, has also been observed (Young 1989, Davis 1996). Some  
362 larvae may actively avoid competitors by delaying metamorphosis in their  
363 presence (Young and Chia, 1981) or not settling near them (Grosberg 1981,  
364 Ordóñez et al. 2013). Most experiments have confirmed the important role of  
365 larval choice in habitat selection, thereby enhancing the fitness of settlers, but  
366 there have been exceptions (Keough and Downes, 1982).

367

368 Community dominants, including members of the genus *Pyura*, can play a  
369 disproportionate role in the structure, stability and function of assemblages  
370 (Rius et al. 2017). Developing a mechanistic understanding of how members of  
371 this genus aggregate and come to dominate available space is of considerable  
372 ecological importance. The propensity of larvae to recruit to conspecific adults in  
373 the intertidal zone has been observed for *Pyura chilensis* (Davis 1995) and *P.*

374 *praeputialis* in Chile (Alvarado et al. 2001). In the absence of aggregations of  
375 adults at sites in Antofagasta, recruitment was not observed (Manríquez et al.  
376 2016). Our laboratory settlement trials and those of Manríquez and Castilla  
377 (2007) highlight enhanced settlement and changes in larval behaviour  
378 associated with the presence of conspecifics. The inability of *Pyura praeputialis*  
379 to effect patch closure in experimental clearances in Chile (Alvarado et al. 2001)  
380 and the slow recovery of this species from experimental clearances in Australia  
381 (Fairweather 1991) are consistent with the importance of recruitment to  
382 conspecifics or appropriate microhabitats. The strong preferences of larvae for  
383 adult conspecifics, including water-borne cues, revealed by our experiments and  
384 those of Manríquez and Castilla (2007) stand in contrast to the findings of Rius et  
385 al. (2010). They report no differences in settlement for larvae exposed to  
386 aqueous extracts of adults relative to filtered seawater controls for the south  
387 African *Pyura herdmani* and *P. stolonifera* in laboratory settlement trials.

388

389 The surprising element of our work was the apparent sensitivity to desiccation  
390 of juveniles of this intertidal animal. Mortality was apparent after just 30 mins of  
391 aerial exposure for 20 and 50 day old juveniles and after one hour there were no  
392 survivors. We speculate that the tunic of *Pyura* at these early developmental  
393 stages is vulnerable to water loss and the enhanced survivorship on 'rugose'  
394 microhabitats is due to their superior water-holding capacity. The marked  
395 reduction in mortality in juveniles following the spray of a mist of seawater adds  
396 further weight to the notion of the acute sensitivity of *Pyura praeputialis* to  
397 desiccation. Importantly, our laboratory survivorship experiment represents a  
398 conservative test of this hypothesis, as aerial exposure was in the confines of a



399 laboratory at room temperature (18°C) and in the absence of direct sunlight or  
400 UVR. Further, animals were affixed to acetate surfaces (Fig. 1B&C) which would  
401 have reduced the rugose nature of these natural habitats and presumably their  
402 ameliorating effects. Desiccation has been seen as a means of managing  
403 biofouling risk in relation to solitary ascidians and direct exposure to incident  
404 radiation can further enhance its effectiveness (Hopkins et al. 2016).

405

406 The timing of reproduction and recruitment for *Pyura* coincides with the late  
407 Austral autumn, winter and early spring (Anderson et al. 1976, author's personal  
408 observations). This timing may minimise exposure of settled larvae and juveniles  
409 to challenging abiotic conditions. Similar predictions have been made for  
410 molluscs depositing egg masses on these shores, but were not supported  
411 (Przeslawski and Davis 2007). Surprisingly, Manríquez et al. (2018) provide  
412 evidence that invasive *Pyura praeputialis* in Chile were reproductive throughout  
413 the year, although it remains unclear whether this equates to year-round  
414 recruitment. Australian and Chilean shores are dominated by heavy wave action  
415 and wave splash may act to ameliorate abiotic conditions (Harley and Helmuth  
416 2003). In Australia, the physical structure of these shores may also enhance the  
417 abiotic environment, as they usually are wave-cut sand-stone platforms with  
418 negligible slope and thus provide extensive habitat for *Pyura*.

419

420 As numerous authors have emphasised, determining likely outcomes in a future  
421 ocean is always going to be exceedingly difficult (eg Bertness et al. 1999). The  
422 close association between early life history phases of *Pyura* with turfing coralline  
423 algae may be telling. There is considerable published evidence that corallines,

424 and by implication their associated species, will fare poorly in a future warm and  
425 'acidified' ocean (Russell et al. 2009, Hepburn et al. 2011, Noisette et al. 2013).  
426 This may represent an issue for *Pyura* as it appears to rely heavily on turfing  
427 algae as habitat at the upper limits of its tidal range.

428

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

436

437

438

439 COMPETING INTERESTS STATEMENT

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

441

442 ACKNOWLEDGEMENTS

443 One of us (ARD) drafted the initial version of this manuscript while on study  
444 leave at the Centre for Advanced Studies in Blanes (Catalunya, Spain) and wishes  
445 to acknowledge the staff and students that made his stay so pleasant. We  
446 acknowledge financial support from the Centre for Sustainable Ecosystem  
447 Solutions, University of Wollongong and from project CTM2017-88080 (AEI,  
448 FEDER-UE) from the Spanish Government. We thank Dr Alan Millar for  
449 identifying the coralline algae in our samples. This represents contribution no.  
450 xxx from the Ecology and Genetics Group at UOW.

451

452

453 REFERENCES

- 454 Alvarado JL, Pinto R, Marquet P, Pacheco C, Guiñez R, Castilla JC (2001) Patch  
455 recolonization by the tunicate *Pyura praeputialis* in the rocky intertidal of  
456 the Bay of Antofagasta, Chile: evidence for self-facilitation mechanisms.  
457 *Marine Ecology Progress Series* 224: 93-101.
- 458 Anderson DT, White BM, Egan EA (1976) The larval development and  
459 metamorphosis of the ascidians *Pyura praeputialis* (Heller) and *Pyura*  
460 *pachydermatina* (Herdman)(Pleurogona, family Pyuridae). *Proc. Linn. Soc.*  
461 *NSW* 100: 205-217.
- 462 Bertness MD, Leonard GH (1997) The role of positive interactions in  
463 communities: lessons from intertidal habitats. *Ecology* 78: 1976-1989.

464 Bertness MD, Leonard GH, Levine JM, Bruno JF (1999) Climate-driven  
465 interactions among rocky intertidal organisms caught between a rock and  
466 a hot place. *Oecologia* 120: 446-50.

467 Branch GM, Griffiths CL, Branch ML, Beckley LE (2010) *Two Oceans: A guide to*  
468 *the marine life of southern Africa*. Struik Publishers, Cape Town. Revised  
469 edn.

470 Brawley, S. H. and L. E. Johnson. 1991. Survival of furoid embryos in the  
471 intertidal zone depends upon developmental stage and microhabitat. *J.*  
472 *Phycol.* 27: 179–186.

473 Castilla JC, Guiñez R, Caro AU, Ortiz V (2004) Invasion of a rocky intertidal shore  
474 by the tunicate *Pyura praeputialis* in the Bay of Antofagasta, Chile.  
475 *Proceedings of the National Academy of Sciences of the United States of*  
476 *America* 101: 8517-8524.

477 Connell JH (1972) Community interactions on marine rocky shores. *Annu Rev*  
478 *Ecol Syst* 3: 169-192.

479 Davis AR (1995) Over-exploitation of *Pyura chilensis* (Ascidiacea) in southern  
480 Chile: the urgent need to establish marine reserves. *Revista Chilena de*  
481 *Historia Natural* 68: 107-116.

482 Davis AR (1996) Association among ascidians: facilitation of recruitment in  
483 *Pyura spinifera*. *Mar. Biol.* 126: 35-41.

484 Elliot JM (1977) Statistical analysis of samples of benthic invertebrates.  
485 *Freshwater Biological Association Scientific Publication* 25: 1 -159.

486 Fairweather PG (1991) A conceptual framework for ecological studies of coastal  
487 resources: an example of a tunicate collected for bait on Australian  
488 seashores. *Ocean and Shoreline Management* 15: 125-142.

489           doi.org/10.1016/0951-8312(91)90027-Y

490   Glasby TM, Gibson PT, Cruz-Motta JJ (2017) Differences in rocky reef habitats  
491           related to human disturbances across a latitudinal gradient. *Mar. Env. Res.*  
492           229: 291-303. <https://doi.org/10.1016/j.marenvres.2017.06.014>

493   Grosberg RK (1981) Competitive ability influences habitat choice in marine  
494           invertebrates. *Nature* 290: 700-702.

495   Harley CD, Helmuth BS (2003) Local-and regional-scale effects of wave exposure,  
496           thermal stress, and absolute versus effective shore level on patterns of  
497           intertidal zonation. *Limnology and Oceanography* 48: 1498-1508.

498   Heller C (1878) Beiträge zur näheren Kenntniss der Tunicaten. *Sitzungsberichte*  
499           *der Academie der Wissenschaften in Wien* 77: 83–109.

500   Helmuth BS, Hofmann GE (2001) Microhabitats, thermal heterogeneity, and  
501           patterns of physiological stress in the rocky intertidal zone. *Biol. Bull.* 201:  
502           374-384.

503   Hepburn CD, Pritchard DW, Cornwall CE, Mcleod RJ, Beardall J, Raven JA, Hurd CL  
504           (2011), Diversity of carbon use strategies in a kelp forest community:  
505           implications for a high CO<sub>2</sub> ocean. *Global Change Biology* 17: 2488–2497.  
506           doi:10.1111/j.1365-2486.2011.02411.x

507   Hopkins GA, Prince M, Cahill PL, Fletcher LM, Atalah J (2016) Desiccation as a  
508           mitigation tool to manage biofouling risks: trials on temperate taxa to  
509           elucidate factors influencing mortality rates. *Biofouling* 32: 1-11.

510   Keough MJ, Downes BJ (1982) Recruitment of marine invertebrates: the role of  
511           active larval choices and early mortality. *Oecologia*. 54: 348-352.

512   Kott P (1985) The Australian Ascidiacea, Part 1. Phlebobranchia and

513 Stolidobranchia. *Memoirs of the Queensland Museum* 23: 1-438.

514 Lathlean JA. (2014) Not all space is created equal: distribution of free space and  
515 its influence on heat-stress and the limpet *Patelloida latistrigata*. *J.*  
516 *Thermal biol.* 46: 16-23.

517 Lathlean JA, Ayre DJ, Minchinton TE (2013) Temperature variability at the larval  
518 scale affects early survival and growth of an intertidal barnacle. *Mar. Ecol.*  
519 *Prog. Ser.* 475: 155-166.

520 Lathlean JA, Seuront L, McQuaid CD, Ng TP, Zardi GI, Nicastro KR (2016) Size and  
521 position (sometimes) matter: small-scale patterns of heat stress  
522 associated with two co-occurring mussels with different  
523 thermoregulatory behaviour. *Mar. Biol.* 163: 189-200.

524 Manríquez PH, Castilla JC (2007) Roles of larval behaviour and microhabitat  
525 traits in determining spatial aggregations in the ascidian *Pyura chilensis*.  
526 *Marine Ecology Progress Series* 332: 155-165.

527 Manríquez PH, Castilla JC, Ortiz V, Jara ME (2016) Empirical evidence for large-  
528 scale human impact on intertidal aggregations, larval supply and  
529 recruitment of *Pyura praeputialis* around the Bay of Antofagasta, Chile.  
530 *Austral ecology.* 41: 701-14.

531 Manríquez PH, Guiñez R, Olivares A, Clarke M, Castilla JC (2018) Effects of inter-  
532 annual temperature variability, including ENSO and post-ENSO events, on  
533 reproductive traits in the tunicate *Pyura praeputialis*. *Marine Biology*  
534 *Research* 23: 1-6.

535 Marshall DJ (2002) *In situ* measures of spawning synchrony and fertilization  
536 success in an intertidal, free-spawning invertebrate. *Mar. Ecol. Prog. Ser.*  
537 236: 113-119.

538 Menge BA (1978) Predation intensity in a rocky intertidal community: effect of  
539 an algal canopy, wave action and desiccation on predator feeding rates.  
540 *Oecologia* 34: 17–35

541 Monteiro SM, Chapman MG, Underwood AJ (2002) Patches of the ascidian *Pyura*  
542 *stolonifera* (Heller,1878): structure of habitat and associated intertidal  
543 assemblages. *J. exp. Mar. Biol. Ecol.* 270: 171–189.

544 Narum SR (2006) Beyond Bonferroni: Less conservative analyses for  
545 conservation genetics. *Conservation Genetics* 7: 783-787

546 Noisette F, Egilsdottir H, Dominique Davoult D, Martin S (2013) Physiological  
547 responses of three temperate coralline algae from contrasting habitats to  
548 near-future ocean acidification. *J. exp. Mar. Biol. Ecol.* 448: 179-187.

549 Ordóñez V, Rius M, McQuaid CD, Pineda MC, Pascual M, Turon X (2013) Early  
550 biotic interactions among introduced and native benthic species reveal  
551 cryptic predation and shifts in larval behavior. *Marine Ecology Progress*  
552 *Series* 488: 65-79.

553 Pineda MC, McQuaid CD, Turon X, López-Legentil S, Ordóñez V, Rius M (2012)  
554 Tough adults, frail babies: an analysis of stress sensitivity across early life-  
555 history stages of widely introduced marine invertebrates. *PLoS ONE*  
556 7(10): e46672.

557 Przeslawski R, AR Davis (2007) Does spawning behaviour minimize exposure to  
558 environmental stressors for encapsulated embryos on rocky shores? *Mar.*  
559 *Biol.* 152: 991-1002.

560 R Core Team (2016). R: A language and environment for statistical computing. R  
561 Foundation for Statistical Computing, Vienna, Austria. URL [https://www.R-](https://www.R-project.org/)  
562 [project.org/](https://www.R-project.org/).

563 Rafaelli D, Hawkins S (1996) *Intertidal ecology*. Chapman & Hall, London

564 Rius M, Branch GM, Griffiths CL, Turon X (2010) Larval settlement behaviour in  
565 six gregarious ascidians in relation to adult distribution. *Marine Ecology*  
566 *Progress Series* 418: 151-163.

567 Rius M, Teske PR (2013) Cryptic diversity in coastal Australasia: a morphological  
568 and mitonuclear genetic analysis of habitat-forming sibling species. *Zool J*  
569 *Linn Soc* 168: 597–611. doi: 10.1111/zoj.12036

570 Rius M, Teske PR, Manríquez PH, Suárez-Jiménez R, McQuaid CD, Castilla JC  
571 (2017) Ecological dominance along rocky shores, with a focus on  
572 intertidal ascidians. *Oceanography and Marine Biology: An Annual Review*  
573 55: 55-84.

574 Russell BD, Thompson, J-AI, Falkenberg LJ, Connell SD (2009) Synergistic effects  
575 of climate change and local stressors: CO2 and nutrient-driven change in  
576 subtidal rocky habitats. *Global Change Biology* 15: 2153–2162.  
577 doi:10.1111/j.1365-2486.2009.01886.x

578 Teske PR, Rius M, McQuaid CD, Styan CA, Piggott MP, Benhissoune S, Fuentes-  
579 Grünewald C, Walls K, Page M, Attard CRM, Cooke GM, McClusky CF,  
580 Banks SC, Barker NP, Beheregaray LB (2011) “Nested” cryptic diversity in  
581 a widespread marine ecosystem engineer: A challenge for detecting  
582 biological invasions' *BMC Evolutionary Biology* 11: 176-189. doi:  
583 10.1186/1471-2148-11-176

584 Underwood AJ, Kingsford MJ, Andrew NL (1991) Patterns of abundance in  
585 shallow subtidal marine assemblages along the coast of New South Wales.  
586 *Aust. J. Ecol.* 16: 231-249.

587 Young CM (1989) Selection of predator-free settlement sites by larval ascidians.



588            *Ophelia* 30: 131-40.

589    Young CM, Chia FS (1981) Laboratory evidence for delay of larval settlement in  
590            response to a dominant competitor. *International Journal of Invertebrate*  
591            *Reproduction* 3: 221-226.

592    Young CM, Chia FS (1984) Microhabitat-associated variability in survival and  
593            growth of subtidal solitary ascidians during the first 21 days after  
594            settlement. *Marine Biology* 81: 61-68.

595

596

597 **Figure Captions**

598

599 Figure 1: A. a thick band of adult *Pyura praeputialis* at MM Point, near  
600 Wollongong. B. one of the 20 day old juvenile used in assessments of  
601 survivorship in the laboratory. It was settled at the tip of an acetate strip –  
602 note the open siphons (arrows in inset). C. assessment of lab survivorship on  
603 turfing coralline algae – note the acetate strips with juveniles at the tips  
604 placed inside the turf. D. Assessment of juvenile (<2cm) survivorship in the  
605 field. Four recruits (arrows) can be seen epoxied to adult conspecifics.

606 Figure 2: Patterns of spatial abundance for adult (>3cm diameter) *Pyura*  
607 *praeputialis* at two sites in southern NSW. **A.** Mean ( $\pm$ sem) density. **B.**  
608 Variance to mean ratios (pooled for the two sites).

609 Figure 3: Distribution of recruits of *Pyura praeputialis* in relation to the  
610 availability of key microhabitats at two locations **A.** Bulli Point and **B.** MM  
611 Point. Error bars are standard errors of the 5 replicate quadrats used in the  
612 sampling.

613 Figure 4: Laboratory settlement responses of larvae of *Pyura praeputialis* in  
614 response to different substrata offered (grey bars, mean and SE of two runs)  
615 and to conditioned water (blue bars, only one run).

616 Figure 5: Survivorship of juveniles of *Pyura praeputialis* in the laboratory **A.**  
617 twenty day old juveniles on three different microhabitats, note that a mist of  
618 seawater was sprayed onto dry rock for the moistened rock treatment  
619 (arrowed). Microhabitats were tunic of conspecific adults, turfing coralline  
620 algae and bare dry rock. **B.** survivorship of 20 and 50 day old juveniles on a  
621 single microhabitat – dry rock.

622 Figure 6: Mean ( $\pm$ sem) survivorship (%) of juveniles of *Pyura praeputialis* in the  
623 field across three microhabitats (histogram bars). Microhabitats as in Figure  
624 5. Mean percentage of animals dislodged ( $\pm$ sem) (line) after three days of  
625 exposure.

626 Figure 7: Mean ( $\pm$ sem) temperature readings ( $^{\circ}$ C) in four microhabitats at two  
627 sites in southern NSW. Microhabitats were the tunic of *Pyura praeputialis*,  
628 clumps of turfing coralline algae and rock (wet and dry). **A.** late spring  
629 (November) **B.** mid summer (January)



631

632 Table 1. Summary of two-way ANOVA for estimates of the mean number of adult

633 *Pyura praeputialis* found across five tidal heights (Transect factor) in the

634 two localities sampled in southeastern Australia.

635

636

	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		

637

638

639

640 Table 2. Summary of two-way ANOVAs for November and January temperature  
641 readings for a range of microhabitats (Habitat factor: tunics of  
642 conspecifics, turfing coralline algae, dry rock and wet rock surfaces) in the  
643 two localities (Random factor) sampled.

644

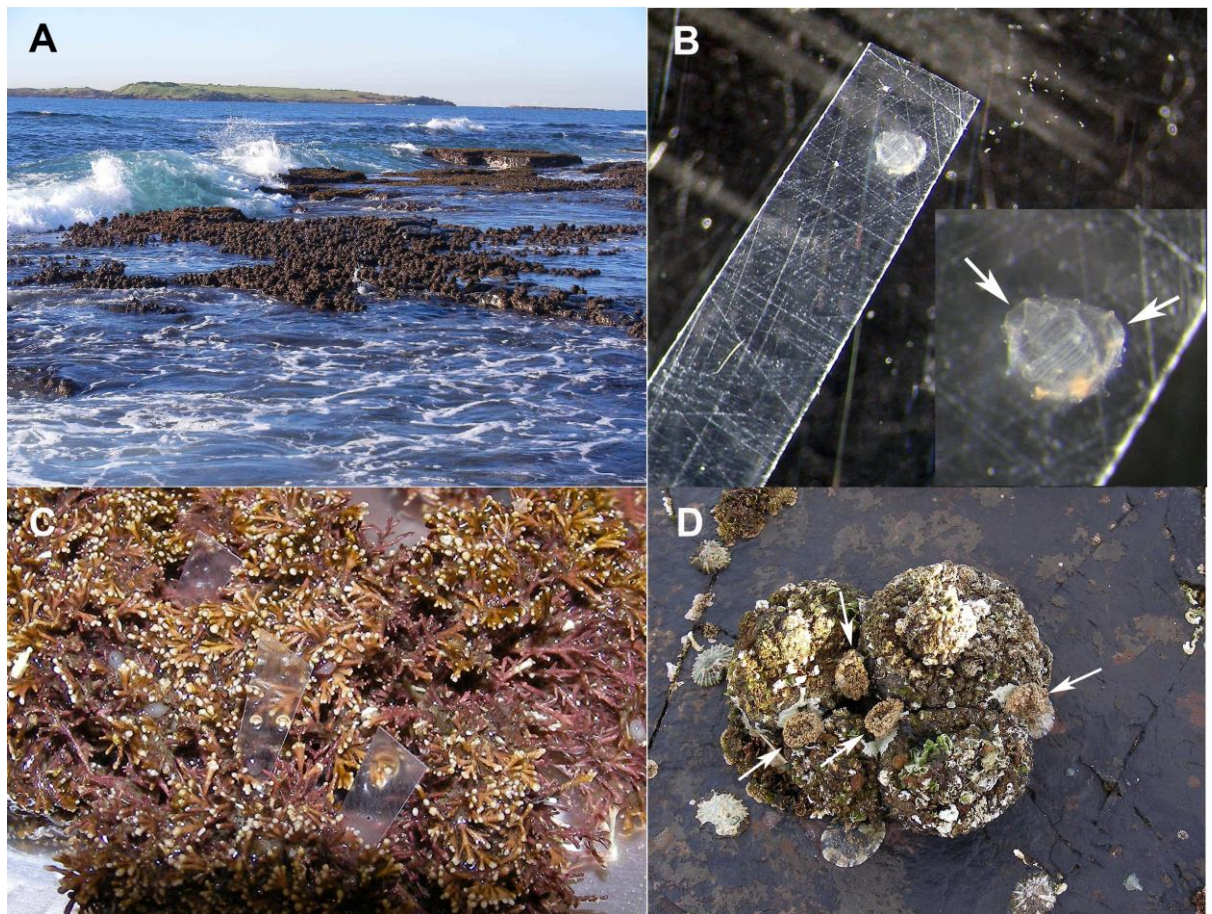
645

	SS	DF	MS	F	P
<b>November</b>					
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		
<b>January</b>					
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		

646

647

648

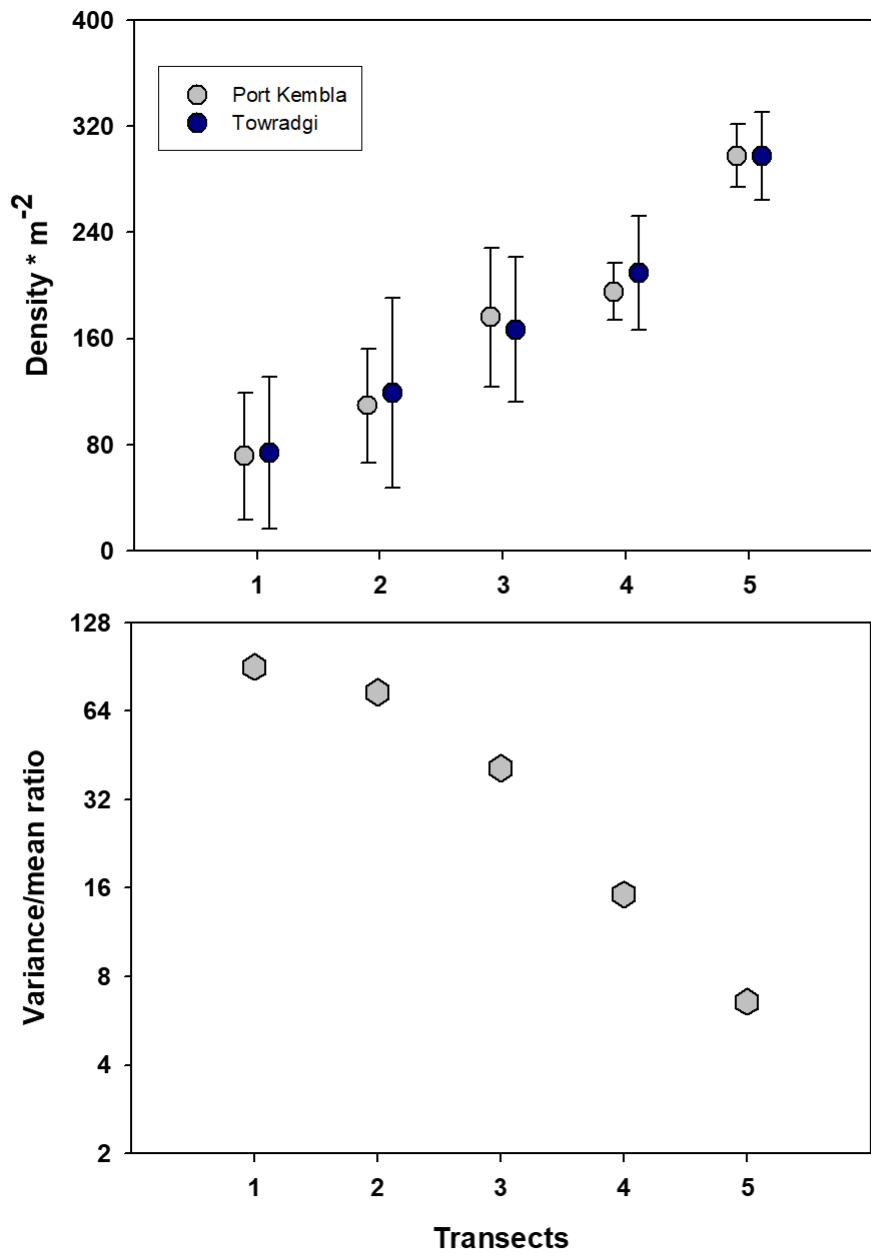


649

650

651 Fig. 1

652



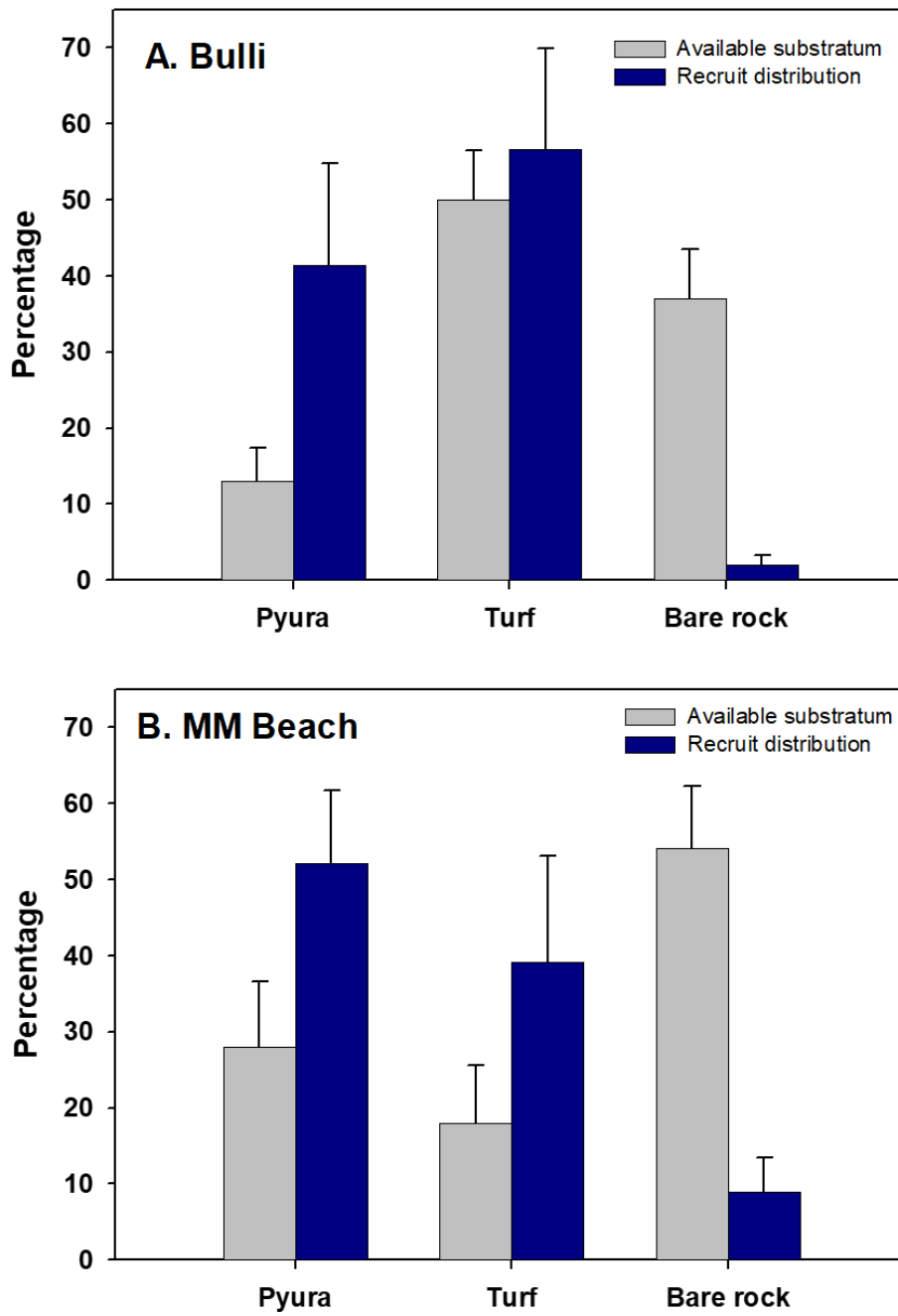
Mid-tide level → Low-tide level

653

654

655 Fig. 2

656



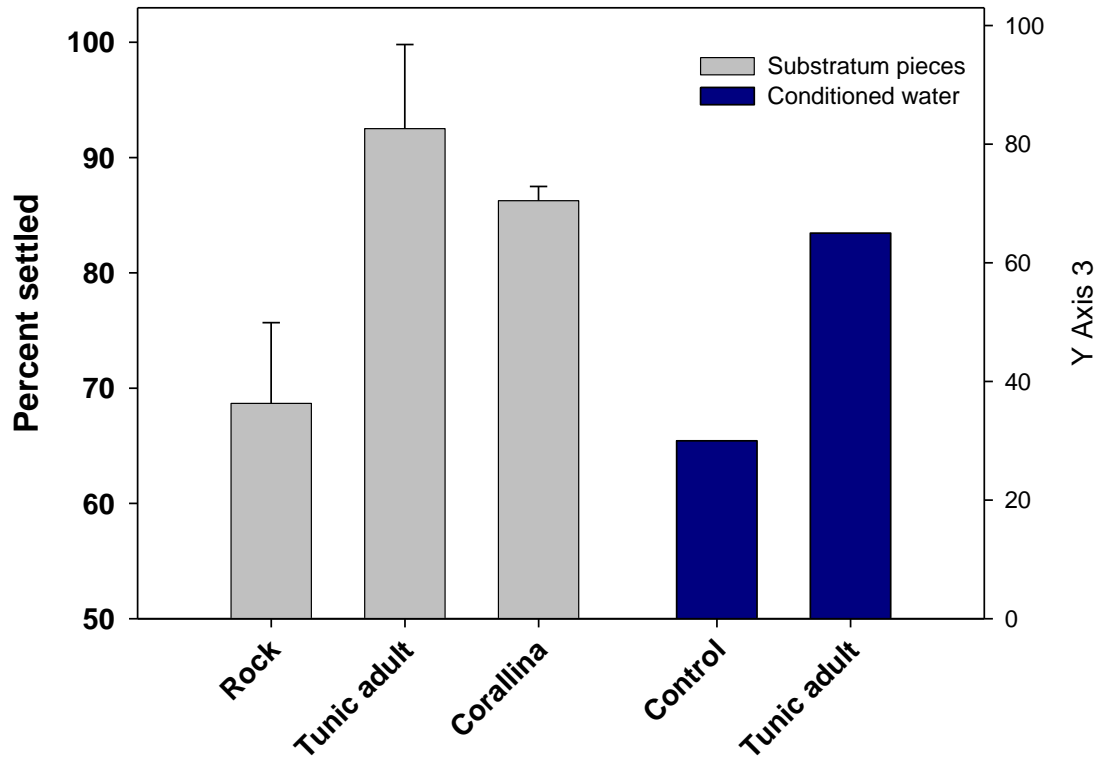
658

659 Fig. 3

660



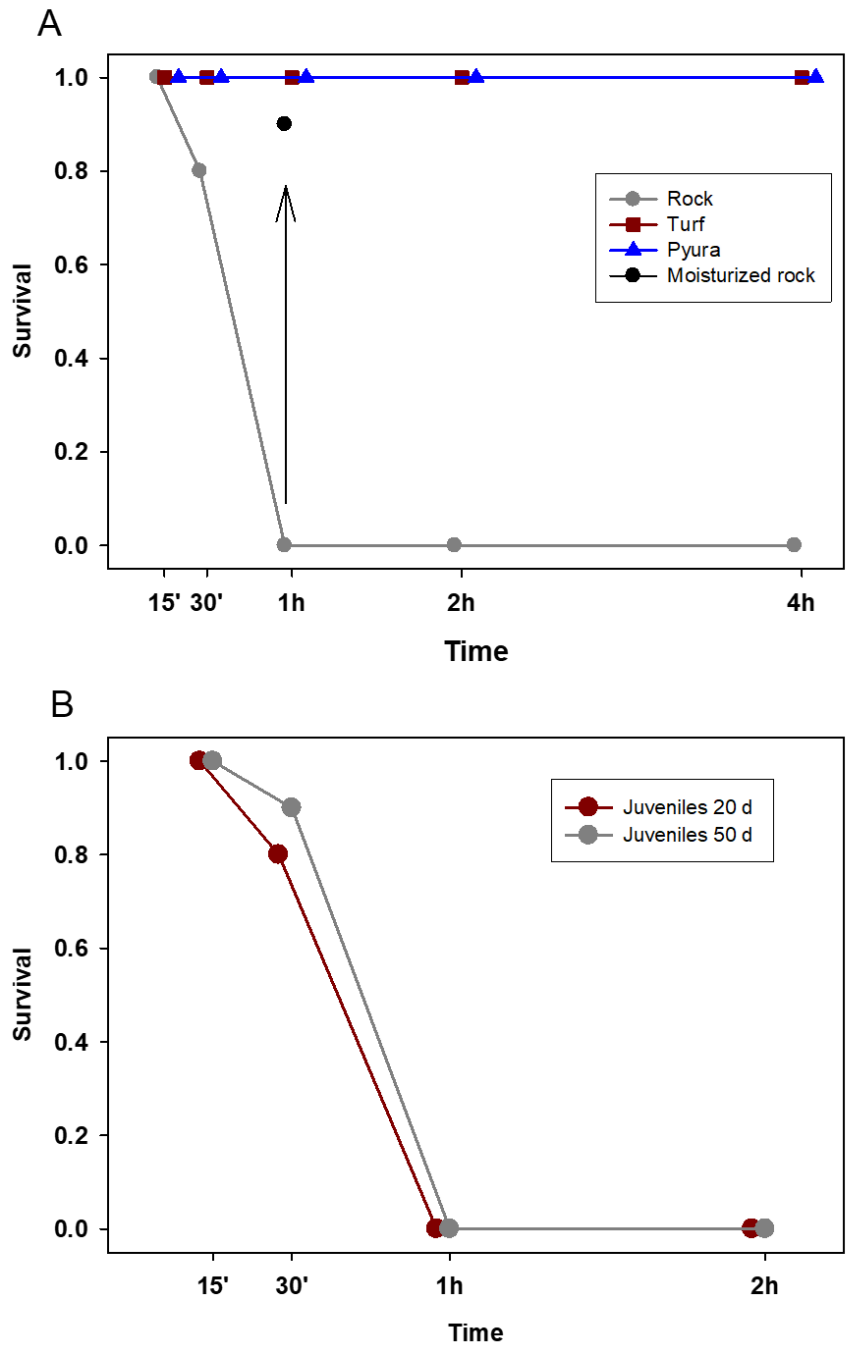
661



662

663 Fig. 4

664



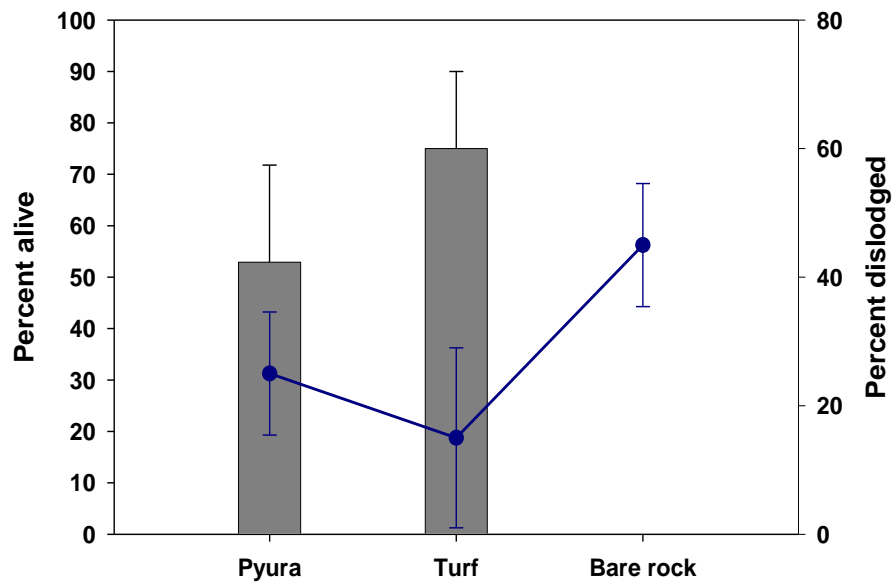
666

667 Fig. 5

668

669

670



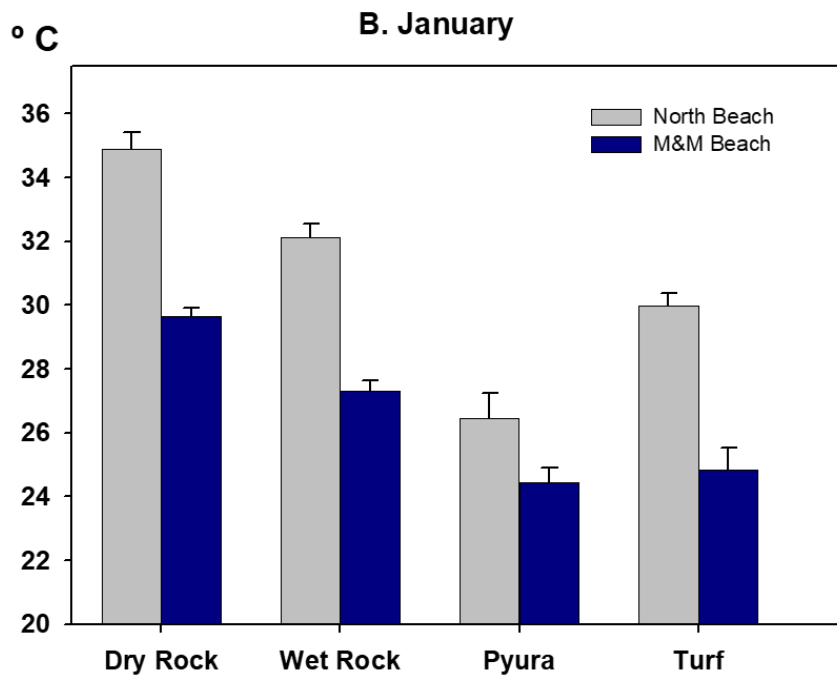
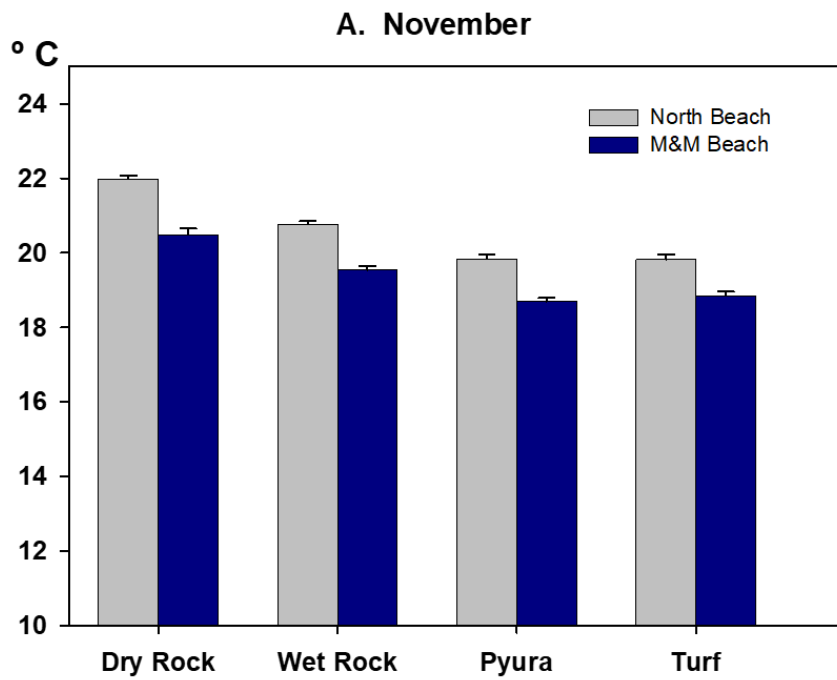
671

672 Fig. 6

673

674

675



676

677 Fig. 7