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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

Andrew R. Davis

University of Wollongong, adavis@uow.edu.au

Mikel Becerro

Centre for Advanced Studies of Blanes

Xavier Turon

Centre for Advanced Studies of Blanes

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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

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 (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.

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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

Andrew R. Davis¹, Mikel Becerro², Xavier Turon^{2*}

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

² 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 - phone: +34 972336101, fax:
+34 972337806

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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 types
31 of habitat (biogenic vs. bare rock). We examined field patterns and performed
32 laboratory and field experiments to assess the nature of this association. Recruits
33 were frequently found on the tunics of conspecifics and clumps of turfing
34 coralline algae. Larvae preferred these same habitats in a series of laboratory
35 settlement assays. Laboratory-reared juveniles (20- & 50-days-old) survived
36 poorly on bare rock in the laboratory, while those on rugose surfaces - the tunic
37 of adults and turfing corallines - showed high survivorship. Field-collected
38 juveniles (<2cm) affixed to these rugose habitats also exhibited high
39 survivorship in the field. We conclude that both pre and post-settlement
40 processes determine spatial pattern in this important taxon. The acute
41 sensitivity of juveniles to desiccating conditions was unexpected in an intertidal
42 organism.

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44

45 **Key Words:** Benthic Ecology, Larvae, *Pyura praeputialis*, Reefs, Settlement,

46 Southeastern Australia, Suspension feeder

47

48 **Authorship:** Collectively, all authors designed the study, collected and analysed

49 the data as well as developed and edited the final manuscript.

50

51

52 INTRODUCTION

53 The intertidal zone is a demanding environment. It is characterised by strong
54 patchiness as well as marked physical and biological gradients. The role of
55 thermal stress and desiccation as structuring forces on rocky shores is well
56 established in the marine ecological literature (see reviews by Connell 1972,
57 Raffaelli and Hawkins 1996). Despite the strong abiotic gradients, small-scale
58 variation in the spatial and temporal distribution of stressors is also apparent
59 (e.g., Helmuth and Hoffman 2001, Lathlean et al. 2016) and may have important
60 impacts on the survival and abundance of individuals (Brawley and Johnson
61 1991).

62

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

75

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

91

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

101

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

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

109

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

118

119 MATERIALS AND METHODS

120 *Study organism and study location*

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

126 2011, Rius & Teske 2013). Another species of the complex, *Pyura doppelgangera*,
127 has recently been transported across Bass Strait from Tasmania and occupies
128 some sites in the Victorian portion of the range of *P. praeputialis* (Rius et al.
129 2017). *Pyura praeputialis* has also invaded the coast of Chile, occupying 70km of
130 an embayment near Antofagasta (Castilla et al. 2004).

131

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

140

141 *Assessing spatial patterns in the field*

142 We assessed patterns of adult distribution at MM Point and Towradgi Point with
143 5m long transects running parallel to the shore at each site. Five transects were
144 placed at different tidal heights, dividing the distance from low to mid shore level
145 into 5 evenly spaced levels. On each transect we counted the number of adult
146 (>3cm diameter) *Pyura* in each of 5 randomly placed 0.25m² quadrats. We then
147 calculated mean:variance ratios pooling across the two sites.

148

149 To measure the distribution of recruits in relation to the available substrata, we
150 tossed 5 quadrats haphazardly in the band of distribution of *Pyura*, ranging from

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

157

158 *Laboratory settlement trials*

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

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

167

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

176 plates, and the experiment was run twice. The number of larvae that had settled
177 24 hrs later was recorded, thereby ensuring that effects due to the degeneration
178 of tissue were minimised.

179

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

187

188 *Juvenile survival*

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

201

202 Time of exposure to air was a factor in the experiment, with sets of juveniles left
203 for 15 minutes, 30 minutes, 1 hour, 2 hours, 4 hours. For each treatment and
204 time, 10 different juveniles were employed (totalling 150 juveniles). After the
205 respective exposure times, the juveniles were brought back to the aquaria and
206 monitored regularly for the following 7 hours to determine if they were alive
207 (relaxed with open siphons and reacting to touch with a probe) or dead (strongly
208 contracted with closed siphons, no reaction to the probe). A further examination
209 at 48 hours was made to confirm these assessments. Importantly, the laboratory
210 in which all of these experiments took place was not air conditioned, and the
211 laboratory temperature during the experiments was ca. 18°C. After recording the
212 outcome this trial (see Results), we decided to perform a second experiment
213 consisting of repeating the rock treatment for 1 hour of exposure using another
214 set of 10 juveniles. These juveniles were sprayed with a mist of seawater every
215 15 minutes. Post-experiment assessment was performed as above.

216

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

224

225 *Recruit survival*

226 We also assessed survivorship of individuals in the field. We collected recruits
227 (<2cm diameter) from Towradgi Point in November. These recruits were
228 collected amid *P. praeputialis* bed during low tide at the mid-tidal height. We
229 then used epoxy (Araldite™) to fix them to the three habitats of interest; the tunic
230 of adult conspecifics, frondose coralline algae turf and rock at MM Point (Fig. 1D).
231 The same tidal height (mid-point between high and low tide) was selected to
232 deploy the experiments. Five sets of five individuals were affixed, a total of 25
233 recruits for each microhabitat. We assessed survivorship at daily intervals for
234 the following four days. At the conclusion of the experiment individuals were
235 returned to the laboratory and placed in seawater aquaria for 48h to ensure that
236 our assessments of survivorship in the field were correct.

237

238 *Microhabitat associated amelioration of temperature*

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

244

245 *Statistical analyses*

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

251

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

255

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

264

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

275

276 RESULTS

277 *Spatial patterns in the field*

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

293

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

301

302 *Larval settlement choice*

303 A high percentage of *Pyura* larvae had settled within 24hrs on the tunic of adult
304 conspecific ascidians (92.5%) and on frondose corallines (86.3%) (Fig. 4). In
305 contrast, only 68.6% of larvae settled in treatments with rock; these differences
306 were significant (G-test of the independence model, $G=8.391$, $df=2$, $p=0.015$).
307 Pairwise comparisons using G-tests confirmed that settlement in rock was
308 significantly lower ($p<0.026$) than in the two other treatments, which were not
309 significantly different ($p=0.677$). We then tested the potential existence of water-
310 borne cues from the treatment showing the highest settlement (conspecifics).
311 Settlement rates with conditioned water were lower, but showed a clear pattern.
312 Namely, some 65% of larvae exposed to water in which adults of *Pyura* had been
313 held for 24hrs settled, while settlement in controls was significantly lower (ca.
314 30%, G-test of the independence model, $G=5.019$, $df=1$, $p=0.025$).

315

316 *Juvenile survivorship*

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

326

327 Survivorship of recruits in the field was consistent with our laboratory findings
328 (Fig. 6). There was a significant effect of substratum on the mortality of recruits
329 fixed to surfaces with epoxy. We observed 100% mortality on rock while
330 mortality on turf and conspecifics was lower than 50%. A *t* test confirmed that
331 survivorship on turf and conspecifics were not significantly different ($t=0.916$,
332 $df=48$, $p=0.364$). All treatments experienced losses due to wave action, although
333 there was no significant substratum effect (Fig. 6, ANOVA: $F_{2,72} = 1.845$, $p=0.165$).

334

335 *Microhabitat associated amelioration of temperature*

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

348

349 DISCUSSION

350 Our experiments reveal that habitat type exerts a significant influence on the
351 settlement and subsequent survival of *Pyura praeputialis*. It appears that pre and
352 post-settlement mechanisms reinforce each other as determinants of spatial
353 pattern in this ecosystem engineer. Survivorship was much higher on the rugose
354 substrata preferred by settling larvae, including clumps of turfing coralline algae
355 or the tunic of adult conspecifics. We conclude that (i) the spatial distribution of
356 *Pyura* is strongly clumped, (ii) juvenile distribution is correlated with the
357 availability of biogenic habitats, (iii) larvae make choices that enhance their
358 likelihood of successful recruitment and (iv) pre and post-settlement
359 mechanisms combine to ensure that *Pyura* is a dominant space occupier on rocky
360 shores. Our results and the frequent presence of *Pyura* clumps in algal patches
361 suggest that establishment on a new substrate can start by settlers on algal
362 clumps, acting as bridgeheads from where the combined settlement preferences
363 and post-settlement differential survivorship will rapidly amplify *Pyura*
364 colonization.

365

366 A rich literature has focused on how settlement choices of larvae, particularly the
367 larvae of solitary ascidians due to their relative large size and ease of culture,
368 affect subsequent patterns of distribution. Pineda et al. (2012) showed the high
369 sensitivity of early life-history stages of two ascidian species to abiotic stressors.
370 Young and Chia (1984) confirmed the survival benefits of negative phototaxis for
371 6 species of solitary ascidian. The selection of predator- or grazer-free sites, via
372 selective settlement, has also been observed (Young 1989, Davis 1996). Some
373 larvae may actively avoid competitors by delaying metamorphosis in their
374 presence (Young and Chia, 1981) or not settling near them (Grosberg 1981,

375 Ordóñez et al. 2013). Most experiments have confirmed the important role of
376 larval choice in habitat selection, thereby enhancing the fitness of settlers, but
377 there have been exceptions (Keough and Downes, 1982).
378
379 Community dominants, including members of the genus *Pyura*, can play a
380 disproportionate role in the structure, stability and function of assemblages
381 (Rius et al. 2017). Developing a mechanistic understanding of how members of
382 this genus aggregate and come to dominate available space is of considerable
383 ecological importance. The propensity of larvae to recruit to conspecific adults in
384 the intertidal zone has been observed for *Pyura chilensis* (Davis 1995) and *P.*
385 *praeputialis* in Chile (Alvarado et al. 2001). In the absence of aggregations of
386 adults at sites in Antofagasta, recruitment was not observed (Manríquez et al.
387 2016). Our laboratory settlement trials and those of Manríquez and Castilla
388 (2007) highlight enhanced settlement and changes in larval behaviour
389 associated with the presence of conspecifics. The inability of *Pyura praeputialis*
390 to effect patch closure in experimental clearances in Chile (Alvarado et al. 2001)
391 and the slow recovery of this species from experimental clearances in Australia
392 (Fairweather 1991) are consistent with the importance of recruitment to
393 conspecifics or appropriate microhabitats. The strong preferences of larvae for
394 adult conspecifics, including water-borne cues, revealed by our experiments and
395 those of Manríquez and Castilla (2007) stand in contrast to the findings of Rius et
396 al. (2010). They report no differences in settlement for larvae exposed to
397 aqueous extracts of adults relative to filtered seawater controls for the south
398 African *Pyura herdmani* and *P. stolonifera* in laboratory settlement trials.
399

400 The surprising element of our work was the apparent sensitivity to desiccation
401 of juveniles of this intertidal animal. Mortality was apparent after just 30 mins of
402 aerial exposure for 20- and 50-day-old juveniles and after one hour there were
403 no survivors. We speculate that the tunic of *Pyura* at these early developmental
404 stages is vulnerable to water loss and the enhanced survivorship on 'rugose'
405 microhabitats is due to their superior water-holding capacity. The marked
406 reduction in mortality in juveniles following the spray of a mist of seawater adds
407 further weight to the notion of the acute sensitivity of *Pyura praeputialis* to
408 desiccation. On the other hand, it is possible that juveniles settling on bare rock
409 in nature would choose small heterogeneities (crevices, pits) where their survival
410 could be higher. We could not test this effect in the laboratory, as the strips with
411 juveniles were laid on flat rock surfaces. Importantly, our laboratory
412 survivorship experiment represents a conservative test of this hypothesis, as
413 aerial exposure was in the confines of a laboratory at room temperature (18°C)
414 and in the absence of direct sunlight or UVR. Further, animals were affixed to
415 acetate surfaces (Fig. 1B&C) which would have reduced the rugose nature of
416 these natural habitats and presumably their ameliorating effects. Desiccation has
417 been seen as a means of managing biofouling risk in relation to solitary ascidians
418 and direct exposure to incident radiation can further enhance its effectiveness
419 (Hopkins et al. 2016).

420

421 The timing of reproduction and recruitment for *Pyura* coincides with the late
422 Austral autumn, winter and early spring (Anderson et al. 1976, author's personal
423 observations). This timing may minimise exposure of settled larvae and juveniles
424 to challenging abiotic conditions. Similar predictions have been made for

425 molluscs depositing egg masses on these shores, but were not supported
426 (Przeslawski and Davis 2007). Surprisingly, Manríquez et al. (2018) provide
427 evidence that invasive *Pyura praeputialis* in Chile were reproductive throughout
428 the year, although it remains unclear whether this equates to year-round
429 recruitment. Australian and Chilean shores are dominated by heavy wave action
430 and wave splash may act to ameliorate abiotic conditions (Harley and Helmuth
431 2003). In Australia, the physical structure of these shores may also enhance the
432 abiotic environment, as they usually are wave-cut sand-stone platforms with
433 negligible slope and thus provide extensive habitat for *Pyura*. Field monitoring of
434 recruitment using artificial substrates and cleared areas would further
435 contribute to disentangle the effects of biotic and abiotic factors on the
436 recruitment and survivorship of *P. praeputialis*, but these experiments are
437 outside the scope of the present work.

438

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

447

448 In conclusion, *Pyura* forms large aggregations on the lower shore, playing an
449 important role in providing habitat for a suite of associated organisms.

450 Aggregations provide structurally complex habitat as well as ameliorating abiotic
451 stressors such as desiccation and wave shock. Our findings indicate that larval
452 settlement choices as well as early post-settlement survival are key in
453 establishing and maintaining the aggregations of this ecosystem engineer. Just
454 how this species may respond in a future ocean is difficult to predict.

455

456

457

458 COMPETING INTERESTS STATEMENT

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

460

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470

471

472 REFERENCES

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

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

486 Branch GM, Griffiths CL, Branch ML, Beckley LE (2016) *Two Oceans: A guide to*
487 *the marine life of southern Africa*. Struik Nature, Cape Town. Revised edn.

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

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

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

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

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

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

504 Fairweather PG (1991) A conceptual framework for ecological studies of coastal
505 resources: an example of a tunicate collected for bait on Australian
506 seashores. *Ocean and Shoreline Management* 15: 125-142.
507 [doi.org/10.1016/0951-8312\(91\)90027-Y](https://doi.org/10.1016/0951-8312(91)90027-Y)

508 Glasby TM, Gibson PT, Cruz-Motta JJ (2017) Differences in rocky reef habitats
509 related to human disturbances across a latitudinal gradient. *Mar. Env. Res.*
510 229: 291-303. <https://doi.org/10.1016/j.marenvres.2017.06.014>

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

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

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

518 Helmuth BS, Hofmann GE (2001) Microhabitats, thermal heterogeneity, and
519 patterns of physiological stress in the rocky intertidal zone. *Biol. Bull.* 201:
520 374-384.

521 Hepburn CD, Pritchard DW, Cornwall CE, Mcleod RJ, Beardall J, Raven JA, Hurd CL
522 (2011), Diversity of carbon use strategies in a kelp forest community:
523 implications for a high CO₂ ocean. *Global Change Biology* 17: 2488–2497.
524 doi:10.1111/j.1365-2486.2011.02411.x

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

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

530 Kott P (1985) The Australian Ascidiacea, Part 1. Phlebobranchia and
531 Stolidobranchia. *Memoirs of the Queensland Museum* 23: 1-438.

- 532 Lathlean JA. (2014) Not all space is created equal: distribution of free space and
533 its influence on heat-stress and the limpet *Patelloida latistrigata*. *J.*
534 *Thermal biol.* 46: 16-23.
- 535 Lathlean JA, Ayre DJ, Minchinton TE (2013) Temperature variability at the larval
536 scale affects early survival and growth of an intertidal barnacle. *Mar. Ecol.*
537 *Prog. Ser.* 475: 155-166.
- 538 Lathlean JA, Seuront L, McQuaid CD, Ng TP, Zardi GI, Nicastro KR (2016) Size and
539 position (sometimes) matter: small-scale patterns of heat stress
540 associated with two co-occurring mussels with different
541 thermoregulatory behaviour. *Mar. Biol.* 163: 189-200.
- 542 Manríquez PH, Castilla JC (2007) Roles of larval behaviour and microhabitat
543 traits in determining spatial aggregations in the ascidian *Pyura chilensis*.
544 *Marine Ecology Progress Series* 332: 155-165.
- 545 Manríquez PH, Castilla JC, Ortiz V, Jara ME (2016) Empirical evidence for large-
546 scale human impact on intertidal aggregations, larval supply and
547 recruitment of *Pyura praeputialis* around the Bay of Antofagasta, Chile.
548 *Austral ecology.* 41: 701-14.
- 549 Manríquez PH, Guiñez R, Olivares A, Clarke M, Castilla JC (2018) Effects of inter-
550 annual temperature variability, including ENSO and post-ENSO events, on
551 reproductive traits in the tunicate *Pyura praeputialis*. *Marine Biology*
552 *Research* 23: 1-6.
- 553 Marshall DJ (2002) *In situ* measures of spawning synchrony and fertilization
554 success in an intertidal, free-spawning invertebrate. *Mar. Ecol. Prog. Ser.*
555 236: 113-119.
- 556 Menge BA (1978) Predation intensity in a rocky intertidal community: effect of

557 an algal canopy, wave action and desiccation on predator feeding rates.
558 *Oecologia* 34: 17–35

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

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

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

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

571 Pineda MC, McQuaid CD, Turon X, López-Legentil S, Ordóñez V, Rius M (2012)
572 Tough adults, frail babies: an analysis of stress sensitivity across early life-
573 history stages of widely introduced marine invertebrates. *PLoS ONE*
574 7(10): e46672.

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

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

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

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

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

588 Rius M, Teske PR, Manríquez PH, Suárez-Jiménez R, McQuaid CD, Castilla JC
589 (2017) Ecological dominance along rocky shores, with a focus on
590 intertidal ascidians. *Oceanography and Marine Biology: An Annual Review*
591 55: 55-84.

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

596 Teske PR, Rius M, McQuaid CD, Styan CA, Piggott MP, Benhissoune S, Fuentes-
597 Grünewald C, Walls K, Page M, Attard CRM, Cooke GM, McClusky CF,
598 Banks SC, Barker NP, Beheregaray LB (2011) “Nested” cryptic diversity in
599 a widespread marine ecosystem engineer: A challenge for detecting
600 biological invasions' *BMC Evolutionary Biology* 11: 176-189. doi:
601 10.1186/1471-2148-11-176

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

605 Young CM (1989) Selection of predator-free settlement sites by larval ascidians.
606 *Ophelia* 30: 131-40.

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

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

613

614

615 **Figure Captions**

616

617 Figure 1: **A.** A thick band of adult *Pyura praeputialis* at MM Point, near
618 Wollongong. **B.** One of the 20-day-old juvenile used in assessments of
619 survivorship in the laboratory. It was settled at the tip of an acetate strip –
620 note the open siphons (arrows in inset). **C.** Assessment of lab survivorship
621 on turfing coralline algae – note the acetate strips (arrows) with juveniles at
622 the tips placed inside the turf. **D.** Assessment of juvenile (<2cm) survivorship
623 in the field. Four recruits (arrows) can be seen epoxied to adult conspecifics.

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

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

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

634 Figure 5: Survivorship of juveniles of *Pyura praeputialis* in the laboratory **A.**
635 Twenty-day-old juveniles on three different microhabitats, note that a mist
636 of seawater was sprayed onto dry rock for the moistened rock treatment
637 (arrowed). Microhabitats were tunic of conspecific adults, turfing coralline
638 algae and bare dry rock. **B.** Survivorship of 20- and 50-day-old juveniles on a
639 single microhabitat – dry rock.

640 Figure 6: Mean (\pm sem) survivorship (%) of juveniles of *Pyura praeputialis* in the
641 field across three microhabitats (histogram bars). Microhabitats as in Figure
642 5. Mean percentage of animals dislodged (\pm sem) (line) after three days of
643 exposure.

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

649

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

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

652 two localities sampled in southeastern Australia.

653

654

	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		

655

656

657

658 Table 2. Summary of two-way ANOVAs for November and January temperature

659 readings for a range of microhabitats (Habitat factor: tunics of

660 conspecifics, turfing coralline algae, dry rock and wet rock surfaces) in the

661 two localities (Random factor) sampled.

662

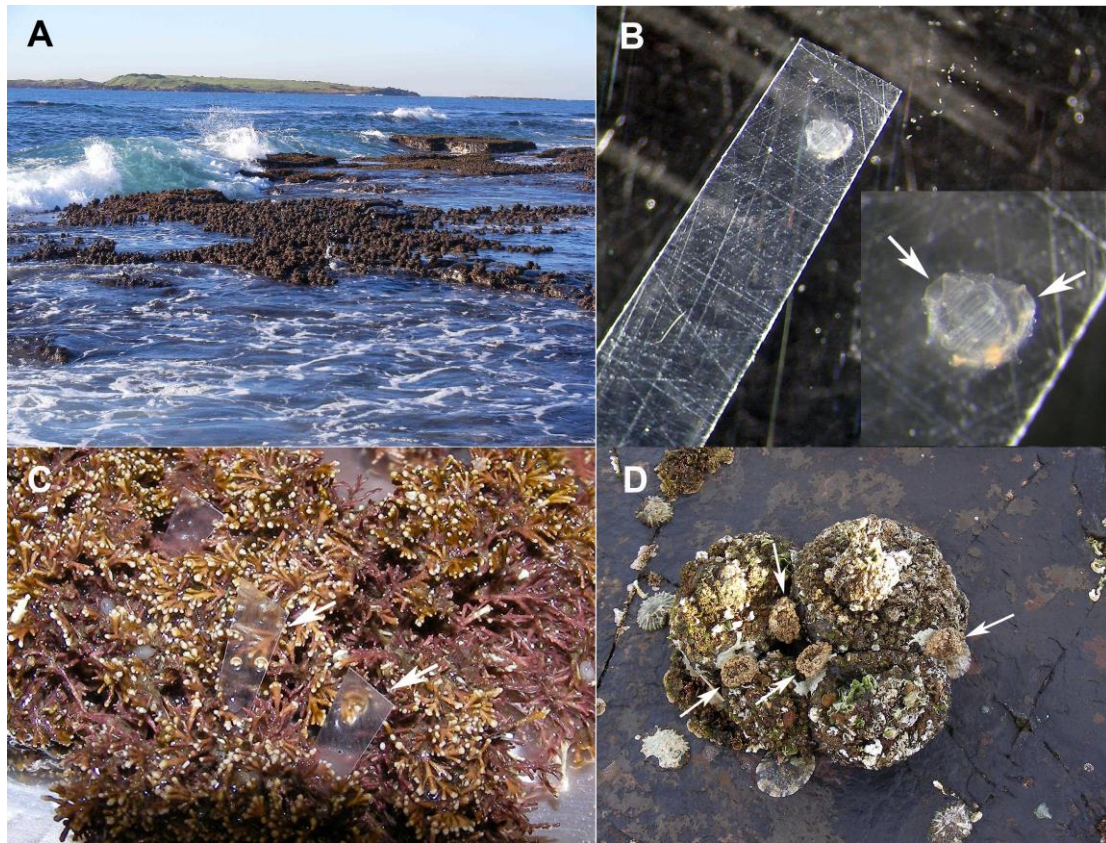
663

	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		

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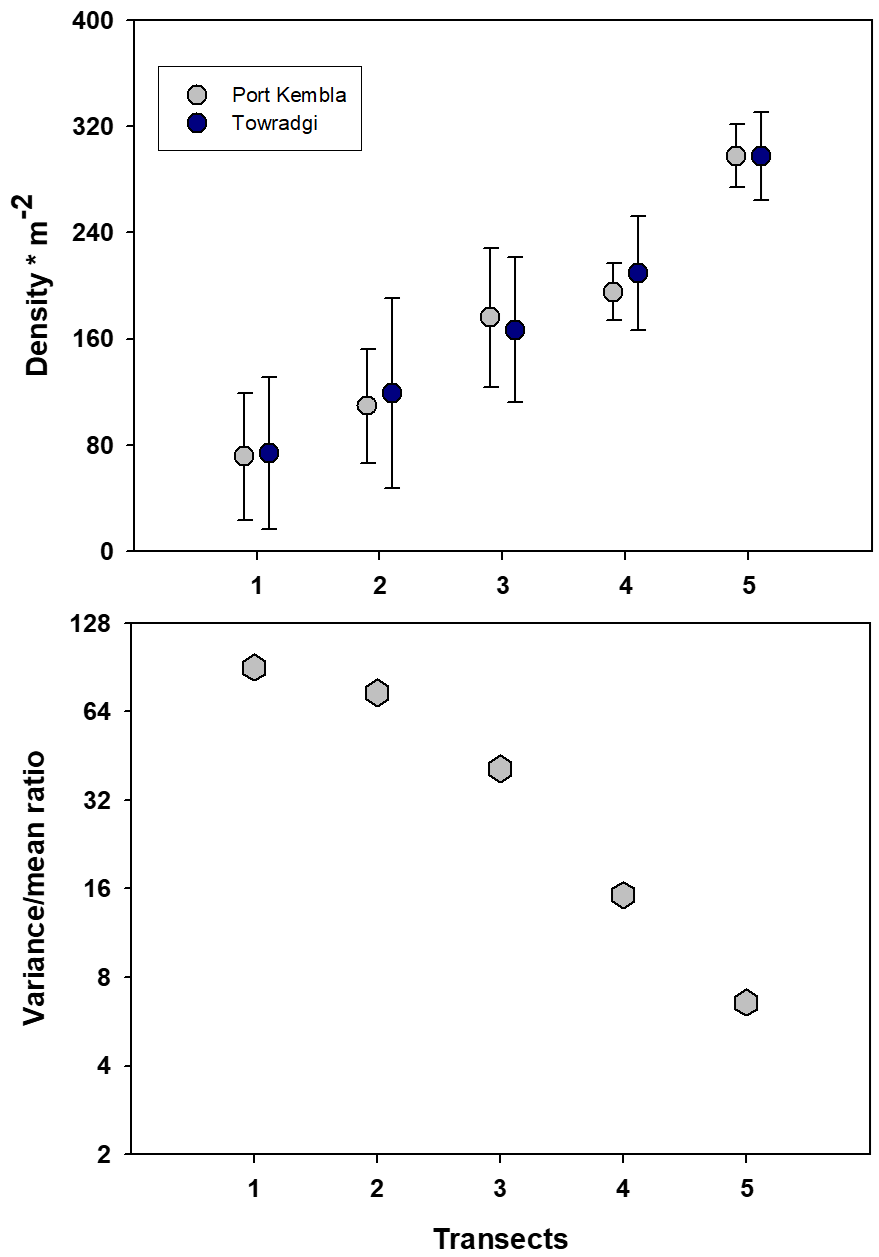


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669 Fig. 1

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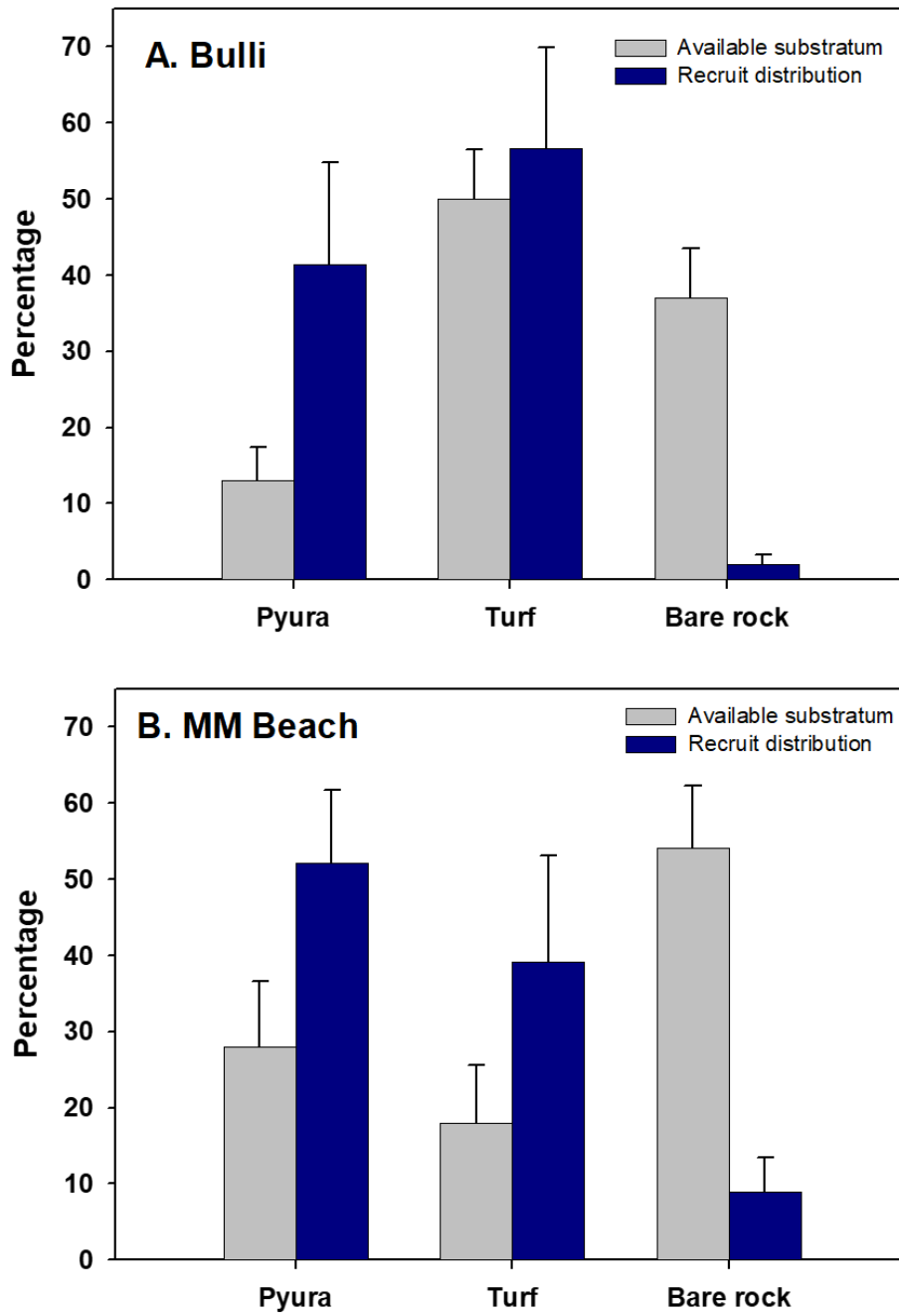
Mid-tide level → Low-tide level

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673 Fig. 2

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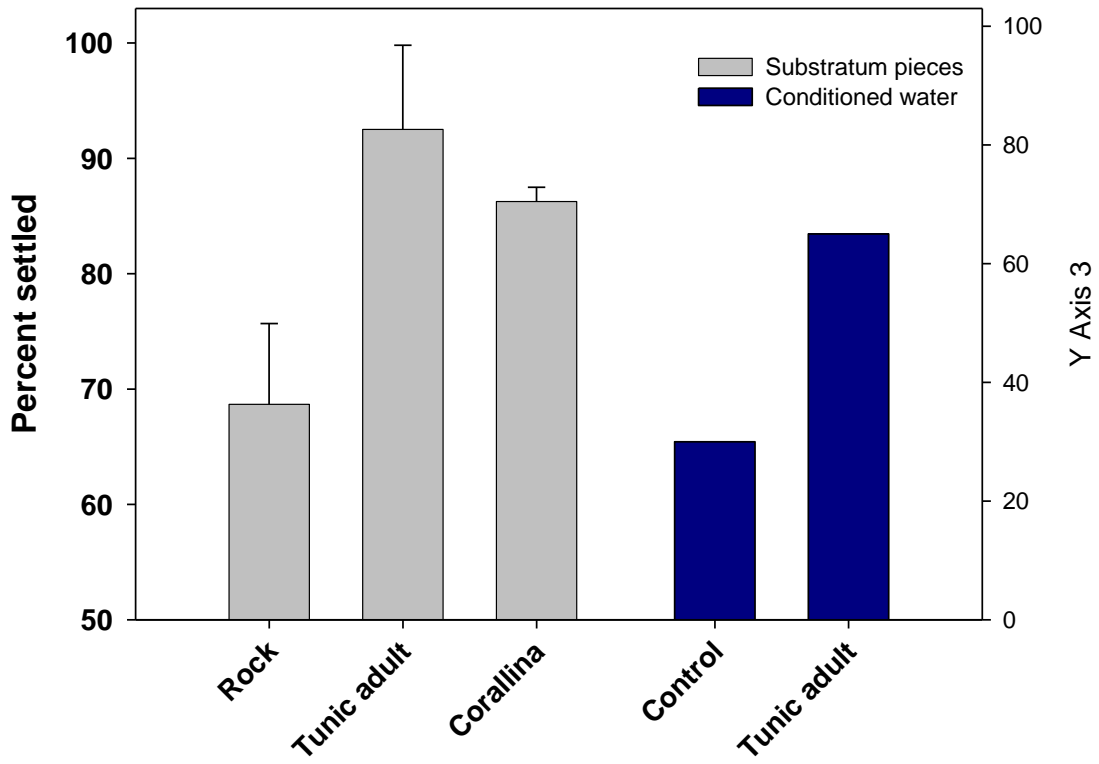


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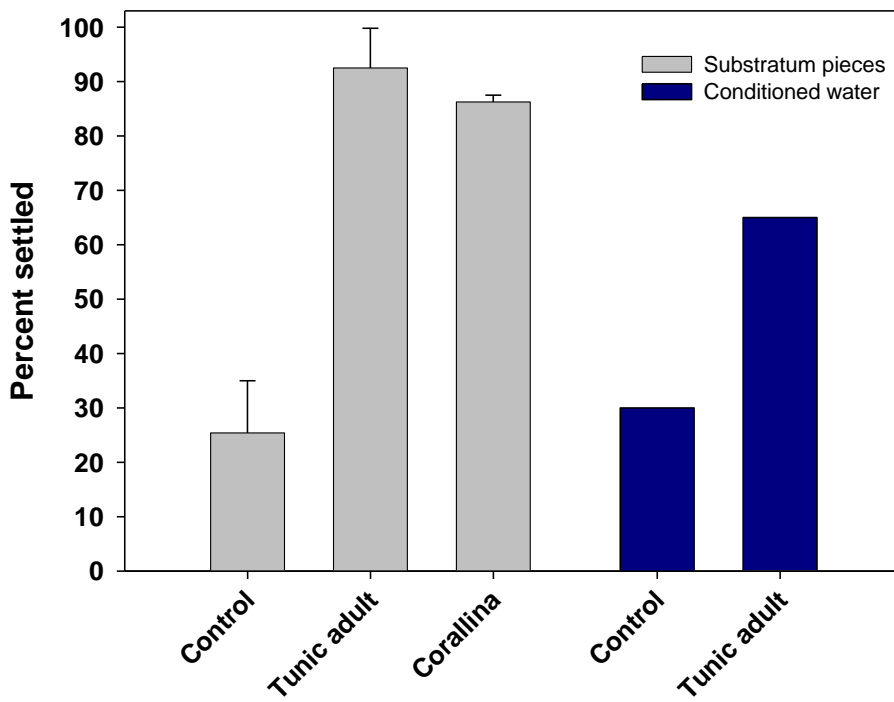
677 Fig. 3

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679



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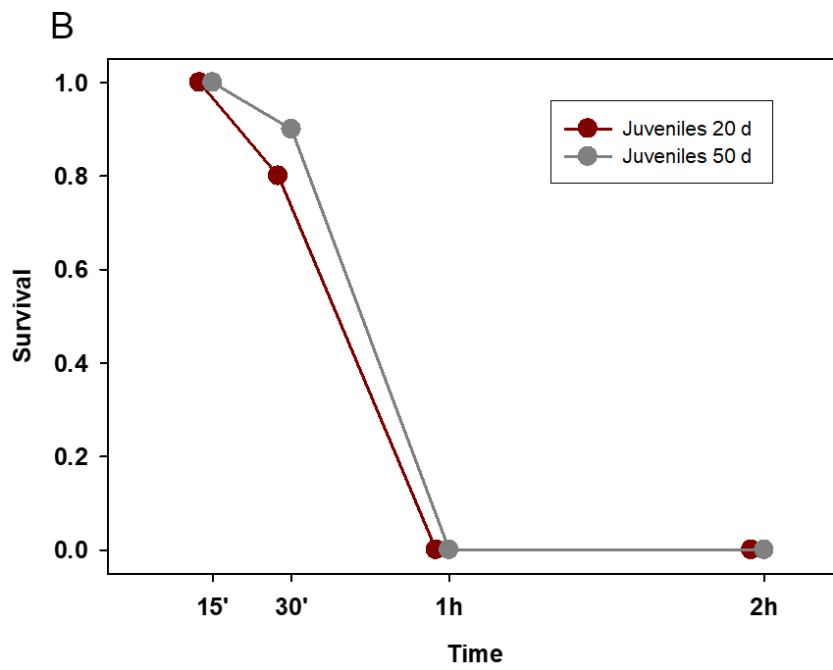
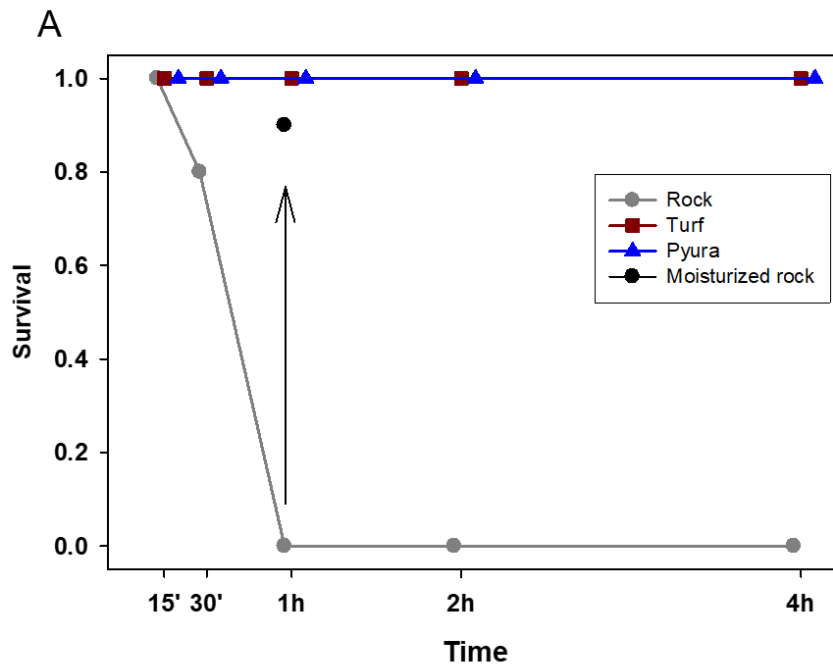


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682 Fig. 4

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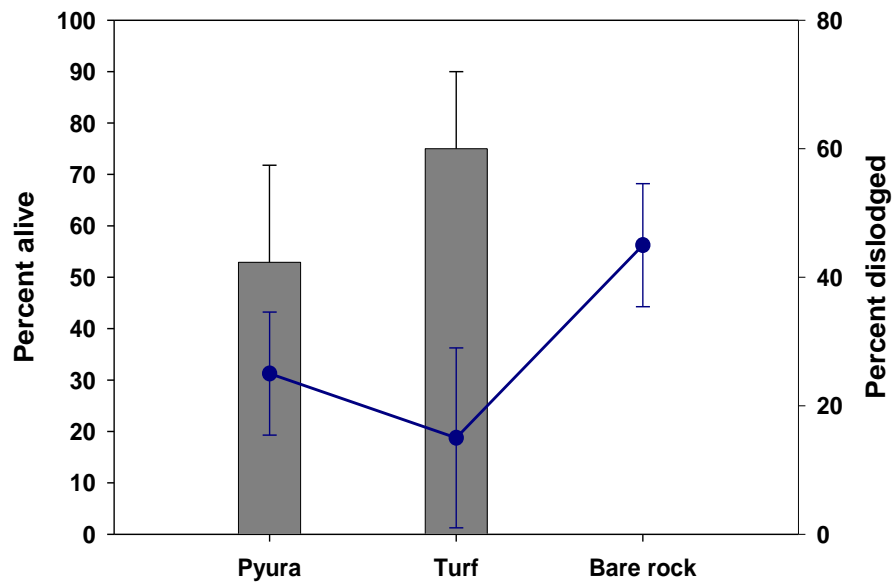
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686 Fig. 5

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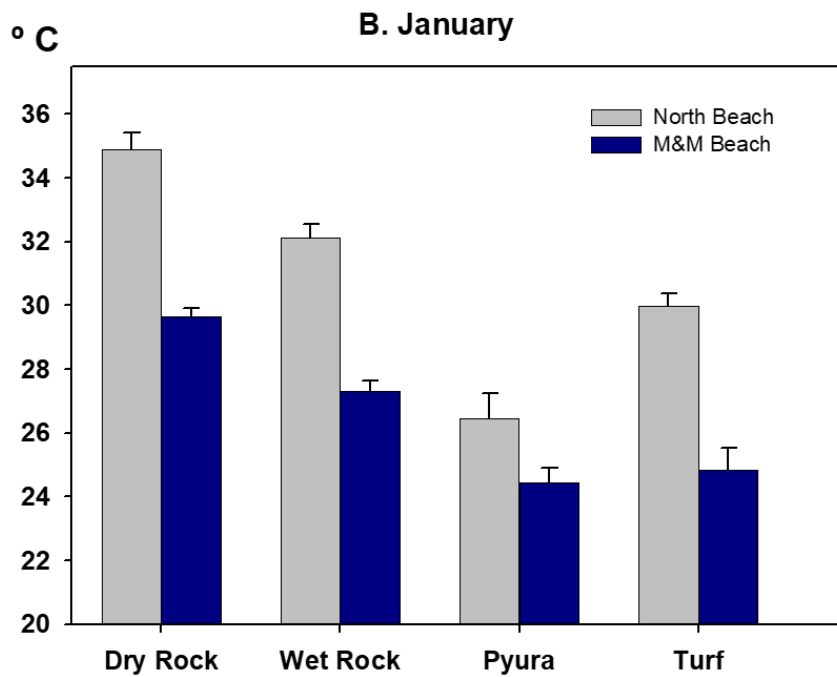
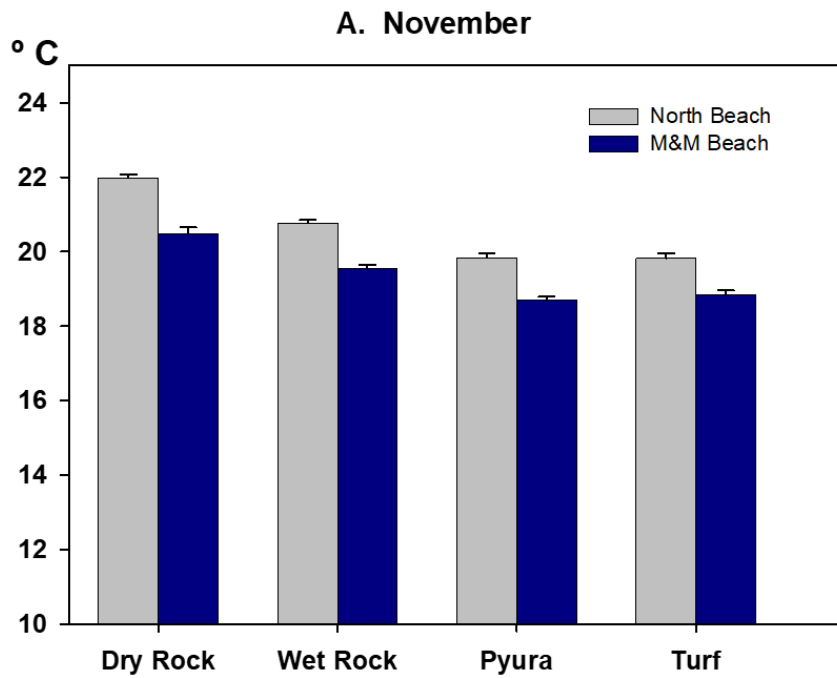


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691 Fig. 6

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696 Fig. 7