1   **Climate change alters shellfish reef communities: a temperate mesocosm experiment**

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18 **Highlights**

19 • Climate change will cause significant changes to rocky shore diversity

20 • Outdoor mesocosms were used to test predictions of warming and ocean acidification

21 • Elevated carbon dioxide in the atmosphere reduced the growth of the native mussels

22 • Warming and carbon dioxide influenced the species that colonised the mussels

23

24 **Graphical Abstract**
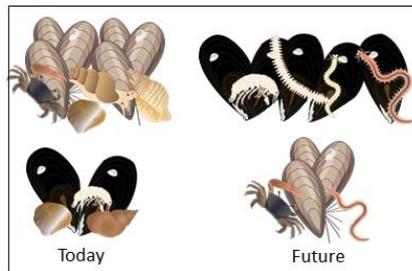
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29 **Abstract**

30 Climate change is expected to cause significant changes to rocky shore diversity. This study

31 used outdoor mesocosms to test the predictions that warming and ocean acidification will alter the

32 responses of two species of mussels (native *Trichomya hirsuta* and introduced *Mytilus*

33 *galloprovincialis*) and their associated communities of infauna. Experiments consisted of orthogonal

34 combinations of temperature (ambient 22°C or elevated 25°C), pCO$_2$ (ambient 400 µatm or elevated

35 1000 µatm), mussel species (*T. hirsuta* or *M. galloprovincialis*), and mussel configuration (native,

36   introduced, or both), with n = 3 replicates. Elevated pCO$_2$ reduced the growth of *T. hirsuta* but not

37   that of *M. galloprovincialis*, and warming and pCO$_2$ influenced the infauna that colonised both

38   species of mussels. There was a reduction in the overall abundance of infaunal molluscs and an

39   increase in polychaetes; there was, however, no effect on crustaceans. Results from this study

40   suggest that climate-driven changes from one mussel species to another can significantly influence

41   infaunal communities.

42

43   **Keywords:** Facilitation; Ocean acidification; Ocean warming; Non-indigenous species; Recruitment

44 **Introduction**

45   With global warming and ocean acidification, marine environments will undergo

46 unprecedented change (Walther et al. 2002). Predicting how this change will alter ecosystems

47 represents a major challenge for ecologists. Laboratory studies over the last decade have concluded

48 that ocean warming and acidification can affect the morphology (Parker et al. 2013), physiology

49 (Pörtner 2008), and behaviour (Munday et al. 2009) of marine species. The impact of combining

50 temperature and ocean acidification (Parker et al. 2010, Hiebenthal et al. 2013), and other multiple

51 stressors (Cole et al. 2016, Parker et al. 2017) has also been observed for many species. In some

52 cases, temperature exacerbates the impact of ocean acidification (Rodolfo-Metalpa et al. 2011), or

53 in other cases, elevated temperature completely removes the negative impacts of elevated pCO_2 (Ko

54 et al. 2014). This increasing list of single species studies has provided a good understanding of how

55 species will respond to climate change. How these single species studies translate to the "real world"

56 remains unknown. Species do not exist in isolation but rather in communities where they interact.

57 These interactions can be broadly grouped into interactions that reduce the overall abundance of

58 species i.e. "negative interactions" (e.g. competition and predation) or interactions that increase

59 their abundance i.e. "positive interactions" (Bertness et al. 1999). In order to predict the impacts of

60 climate change, models need to be built from species interactions within communities rather than

61 scaling up from individual responses (Gaylord et al. 2015, Kroeker et al. 2017).

62   Ongoing research has shown that warming and acidification may affect how marine

63 organisms interact. Ocean acidification can alter the outcome of negative interactions, e.g.

64 competition, shifting the dominance of species within the marine communities (Connell et al. 2013).

65 For example, under acidification, fleshy seaweeds outcompete calcareous species (Kroeker et al.

66 2013b). Positive interactions where an organism provides resources such as habitat (i.e. facilitation)

67 can also be affected by climate change. Organisms that form biogenic habitats that are exposed to

68 elevated pCO_2 can change their structure and complexity in a range of environmental contexts

69 (Sunday et al. 2017). The communities built around biogenic habitats are interlinked with the

70 attributes of the habitat itself (Cole 2009). Small changes in the biogenic habitat structure can

71 impact the diversity of the communities and have the greatest consequences for species that are

72 unique to specific habitats (Cole 2009, Cole et al. 2017).

73   The warming and acidification of the oceans, in many cases, causes a decline in the biogenic

74 habitat quality (Sunday et al. 2017). Not all change is, however, negative, and in some cases, the new

75 habitat can facilitate more abundant consumers (Nagelkerken et al. 2016, Goldenberg et al. 2018).

76 There is evidence that the loss of a biogenic habitat in an ecosystem can be functionally replaced (or

77 the loss of function is slowed to some extent) by another habitat forming organism (Nagelkerken et

78 al. 2016, Sunday et al. 2017). Specific examples of these shifts in relation to climate change are rare,

79 and more research is required on communities where biogenic habitat supports local diversity

80 (Sunday et al. 2017).

81   Shellfish reefs, particularly mussels, can form large areas of habitat that are vital to their

82 infaunal communities (Cole and McQuaid 2010), but past research has shown that as calcifying

83 organisms, they are the most vulnerable to warming and acidification (Kroeker et al. 2013a, Parker

84 et al. 2013). On temperate Australian rocky shores, habitats created by the native mussel *Trichomya*

85 *hirsuta*, and to a lesser extent, the invasive mussel *Mytilus galloprovincialis* support a local diversity

86 of annelids, crustaceans, molluscs, and echinoderms (People 2006, Cole 2010). Eastern Australia is a

87 climate change "hot-spot" with sea surface temperatures in this region increasing three times faster

88 than the global average (Wernberg et al. 2011, Hobday and Pecl 2014), and oceans are acidifying

89 worldwide (Collins et al. 2013). The invasive *M. galloprovincialis* is relatively tolerant to

90 environmental change (Hiebenthal et al. 2013); whereas little is known about the tolerance of *T.*

91 *hirsuta.* As the oceans warm and acidify, *M. galloprovincialis* may have the capacity to replace *T.*

92 *hirsuta* as the dominant biogenic habitat on the Australian rocky shores. Any changes in the biogenic

93 mussel habitat could alter the infaunal communities, with downstream consequences for dependent

94 organisms. Such consequences will have an impact not only on the natural communities but also on

95 the success outcomes of current and future shellfish reef restoration projects (Pereira et al. 2019).

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96   The aims of this study were twofold: the first was to compare the physiological responses of

97 the invasive *M. galloprovincialis* and the native *T. hirsuta* under ocean warming and acidification and

98 the second was to test whether the infaunal communities of the biogenic mussel habitat were

99 altered by warming and acidification and whether infauna were actively changing their preferences

100 to colonise either *T. hirsuta* or *M. galloprovincialis.* This was done in novel outdoor mesocosms,

101 which allowed us to test whether shifts in biogenic habitat caused by warming and acidification can

102 affect infaunal communities and "scale up" experiments beyond approaches used in laboratories.

103

104 **Methods**

105 *Outdoor experimental setup*

106   The outdoor experiment was performed in a purpose-built facility (Pereira et al. 2019) at the

107 Sydney Institute of Marine Science (SIMS), Chowder Bay, Sydney Harbour, New South Wales,

108 Australia. The experiment was performed during the summer peak recruitment period of marine

109 invertebrates in Sydney Harbour. Mussels were collected from Sydney Harbour, within a 10 km

110 radius of the experimental site at SIMS. Native hairy mussels, *Trichomya hirsuta*, were collected

111 during low tide from a rocky shore at Georges Head, Mosman (33°50'2''S, 151°15'37''E), and

112 Mediterranean mussels, *Mytilus galloprovincialis*, from a marina at Gladesville Bridge, Drummoyne

113 (33°50'35''S, 151°8'45''E). Although the two species of mussels do co-occur on the low intertidal on

114 the same shores, abundances of *M. galloprovincialis* are low where *T. hirsuta* occurs (Cole, pers.

115 obs.), it was not possible to collect them from the same site due to the need to collect large numbers

116 of each species.   Mussels were transported directly to the experimental setup at SIMS and left to

117 acclimate for 4 weeks.

118   Unfiltered seawater and larvae were pumped from the adjacent Chowder Bay into a flow-

119 through system, and the flow rate per header tank was set to 1 L min^{-1}, resulting in a flow of 78 min

120 header tank^{-1}. The experimental setup consisted of 52 L (645 mm length x 413 mm width x 276 mm

121 height) mesocosms that were gravity fed by 78 L (645 mm length x 413 mm width x 397 mm height)

122 header tanks. All the tanks were white and made of food grade, high-density polypropylene. The

123 header tanks had tightly fitting lids of the same material, but the mesocosms were left open to

124 experience natural environmental conditions. PVC fittings and hoses that were used were graded

125 safe for drinking water. There were 36 mesocosms in total, which consisted of 12 header tanks each

126 with three mesocosms.

127   The experiment consisted of orthogonal treatments of temperature (ambient mean = 22.5 ±

128 0.1 °C or elevated mean = 25.5 ± 0.4 °C) and pCO$_2$ (ambient pH 8.24 ± 0.1 and elevated pH 7.64 ±

129 0.1). Elevated temperature and pCO$_2$ are predicted for 2100 under a "business as usual" scenario for

130 Representative Concentration Pathways (RCP8.5) (Hoegh-Guldberg et al. 2014). In half of the header

131 tanks, the temperature was increased by 3°C above ambient with a 150 W heater (Aqua One

132 Thermosafe, suitable for tanks up to 150 L). There were two levels of pCO$_2$: ambient (399.6 ± 8.2 –

133 433.2 ± 8.9 µatm) and elevated (1151.7 ± 12.0 – 1241.0 ± 12.9 µatm). Elevated pCO$_2$ was

134 manipulated following Parker et al. (2011), by bubbling pure CO$_2$ into unfiltered seawater in each

135 header tank through a CO$_2$ reactor to ensure complete mixing. Ambient and elevated pCO$_2$, were

136 equivalent to pH 8.24 and pH 7.64 respectively. The pH was monitored with a pH probe connected

137 to a pH-negative feedback computer (Aqua Medic) set to an accuracy of ± 0.01 (Parker et al. 2011).

138   Stable pCO$_2$ and temperature conditions of the mesocosms were maintained throughout the

139 experimental period (Supplementary Figure 1). The temperature and pH of the header tanks and

140 mesocosms were measured daily (ambient pH 8.24 ± 0.1, elevated pH 7.64 ± 0.1, Supplementary

141 Table 1 and Supplementary Figure 1) with a pH meter with a temperature probe (Hanna HI 98160

142 pH/ORP Meter Calibration Check), and the total alkalinity (TA) was determined with an autotitrator

143 (Metrohm 888 Titrando). The pH value corresponding to the required pCO_2 level was determined by

144 quantifying TA (Table 1). Following titration, the TA and selected pCO_2 values were entered into a

145 CO_2 system calculation program (CO2sys, Lewis and Wallace 1998) using the dissociation constants

146 of Mehrbach et al. (1973).

147   After the 4-week acclimation period, the mussels were defaunated by carefully removing all

148 infauna and separating adult mussels (>1 cm) into 10-cm-diameter clumps (Cole 2010). Within each

149 replicate combination of temperature and pCO_2, the mussels were placed in three different

150 configurations consisting of clumps of 10-15 mussels. Mesocosms consisted of two clumps of *T.*

151 *hirusta* (hereafter *Trichomya* alone), two clumps of *M. galloprovincialis* (*Mytilus* alone), or one clump

152 each of *T. hirsuta* and *M. galloprovincialis* (mixed). This design allowed quantitative tests of active

153 choice behaviour of colonising infauna (based on Olabarria 2002, Underwood and Clarke 2005, and

154 adaped for field situations by von der Meden et al. 2015). Subsequently, there was n = 3 replicate

155 mesocosms for each combination of temperature, pCO_2, configuration and species.

156

157 *Determination of the effects on mussels*

158   To determine the effects of temperature, pCO_2, configuration and species, means of sub-

159 replicate individuals were used for each replicate mesocosm. Within each mesocosm, three

160 randomly selected mussels of each clump species were measured with Vernier callipers (to the

161 nearest 0.01 mm) and individually marked with shellfish tags. After the 8-week exposure period, the

162 mussels were remeasured and their growth was determined.

163   The standard metabolic rate (SMR), which is the oxygen consumption normalised for dry

164 tissue mass (mg O_2 g^{-1} dry tissue mass h^{-1}), was determined (Parker et al. 2012) for two randomly

165 chosen mussels of each species from each mesocosm. The SMR was calculated as follows:

166 $$SMR = \frac{V_r(L) \times \Delta C_w O_2 (mg\ O_2 L^{-1})}{\Delta t(h) \times bw\ (g)}$$

167 where V_r is the volume of the respiratory chamber minus the volume of the mussel, $\Delta C_w O_2$ is the

168 change in the concentration of oxygen, Δt is the duration of measurement, and bw is the dry tissue

169 mass (Parker et al. 2012). Measurements were done using a closed respiratory system, placed in a

170 darkened waterbath. Each mussel was placed in a 400 mL airtight chamber fitted with a fibre-optic

171 probe (PreSens dipping probe DP-PSt3, Regensberg, Germany). The temperature was set to the

172 relevant experimental temperature of either 22.5 °C or 25.5 °C, and the pH of the seawater was

173 either pH 8.24 or 7.64. The time taken to reduce the percentage oxygen saturation of the seawater

174 within the chamber by 20% was measured (Parker et al. 2012). Prior to measurements, the probes

175 were calibrated using two-point calibration. The SMR of six mussels from each combination of

176 treatments was determined (mean SMR of 2 mussels mesocosm^{-1}, n = 3).

177   The number of byssal threads were also enumerated for two randomly chosen adult mussels

178 from each combination of treatments (mean threads of 2 mussels mesocosm^{-1}, n = 3). The number

179 of byssal threads where they stem out from the mussel shells were counted using a Lecia M125

180 Stereo Microscope at 12.5x magnification.

181

182 *Determination of the effects on communities*

183   Infaunal larvae entered the mesocosms and defaunated clumps of mussels with the

184 unfiltered water during the 8 week exposure period. Each clump of mussels and the associated

185 infauna was carefully collected, placed into individual sample bags, and frozen in a -4°C freezer for

186 processing. The samples were defrosted and rinsed through a 500 μm sieve. All infauna (>500 μm)

187 were identified to species (or morphospecies) where necessary with the aid of a dissecting

188 microscope at 7.5x magnification.

189

190   *Data analysis*

191   Data were analysed with a 4-factor analysis of variance (ANOVA) for growth, SMR, byssal

192   threads, and abundances and numbers of taxa of infauna. The experimental design consisted of

193   orthogonal combinations of temperature (ambient or elevated), pCO$_2$ (ambient or elevated), mussel

194   species (native or invasive), and mussel presence (alone or together), with n = 3 replicates. For

195   assemblages of infauna, the same experimental design was used for multivariate analyses using

196   permutational multivariate analysis of variance (PERMANOVA+, PRIMER 6 add on). Separate

197   analyses were performed for each of the major taxa, namely Mollusca, Polychaeta, and Crustacea.

198   To test the hypotheses about active choice behaviour, a preference experiment enabled the

199   determination of active choice compared with what would be expected by random chance under no-

200   choice scenarios (Crowe and Underwood 1998, Olabarria et al. 2002, Underwood et al. 2004, Cole et

201   al. 2012). The analysis was modified for a field situation such as this, where known numbers of

202   individuals cannot be designated *a priori* (sensu von der Meden et al. 2015). In the present study,

203   the no-choice scenarios consisted of tanks with only *T. hirsuta* or *M. galloprovincialis*. To estimate

204   the number of individuals colonising a clump of mussels over the 8-week period when there was no

205   possibility of choice, i.e. "no choice" mesocosms with either *Trichomya* alone or *Mytilus* alone were

206   determined (stage 1 sampling). Estimations of preference were determined when colonisers were

207   given a "choice" between *T. hirsuta* or *M. galloprovincialis* (stage 2 sampling). Chi-square (χ^2)

208   contingency tests were performed to compare the observed data obtained from stage 2 sampling

209   with what was predicted under the null hypothesis of no preference from the stage 1 sampling

210   (Underwood and Clarke 2005). Contingency tables were constructed to determine whether

211   colonisation of *T. hirsuta* or *M. galloprovincialis* was dependent on the temperature of the seawater,

212   or in separate analyses dependent the level of pCO$_2$. Comparisons of temperature were done under

213   ambient pCO$_2$ and comparisons of pCO$_2$ were done under ambient temperature, with n = 3.

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214

Results

Responses of mussels

The two species of mussels responded differently, to warming and $p\text{CO}_2$. The native mussel *T. hirsuta* grew more under warming (Fig. 1a; ANOVA Species x Temperature $F_{1,32} = 6.13$, $P < 0.05$; Supplementary Table 2). In contrast, *M. galloprovincialis* grew the same at ambient and elevated temperatures (Fig. 1a; Supplementary Table 2). There was no effect of elevated $p\text{CO}_2$ on growth in either of the mussel species (ANOVA CO2 $F_{1,32} = 0.53$, $P > 0.05$; Supplementary Table 2).

The standard metabolic rate differed between species, with a higher rate for *T. hirsuta* than for *M. galloprovincialis* (Fig. 2; ANOVA Species $F_{1,32} = 65.75$, $P < 0.001$; Supplementary Table 2). Native *T. hirsuta* also showed a strong, but non-significant trend for a higher metabolic rate under elevated $p\text{CO}_2$ (Fig. 2).

The number of byssal threads of *M. galloprovincialis* was not affected by warming or elevated $p\text{CO}_2$ (Fig. 2). *M. galloprovincialis* had significantly more byssal threads than *T. hirsuta* (Fig. 2b; ANOVA $F_{1,32} = 4.42$, $P < 0.05$; Supplementary Table 2). When present in mesocosms together, both *T. hirsuta* and *M. galloprovincialis* had fewer byssal threads (Fig. 2b; ANOVA $F_{1,32} = 14.81$, $P < 0.05$; Supplementary Table 2.

Responses of communities

Different communities of infauna colonised mussel beds under elevated $p\text{CO}_2$ (Fig. 3). Communities (in terms of abundances of each species and the overall composition) of polychaetes and of crustaceans at ambient $p\text{CO}_2$ were significantly different from those under elevated $p\text{CO}_2$ when at ambient temperature, but this effect was lost under warming where there were no differences between $p\text{CO}_2$ levels (Fig. 3a,b; PERMANOVA Temp x CO₂, Polychaeta $F_{1,32} = 3.01$, Crustacea $F_{1,32} = 3.246$, $P < 0.05$, Supplementary Table 3). Under warming, different communities of molluscs colonised mussel beds than under ambient temperature (Fig. 3c; PERMANOVA Temp x

Species, $F_{1,32}$ = 2.985, $P < 0.05$, Supplementary Table 3). It is worth noting that the different groups of

infauna (i.e. Polychaeta, Crustacea, and Mollusca) had strikingly different responses to climate

stressors (Fig. 3).

Polychaetes generally had a positive response to climate change scenarios. Warming and

elevated pCO_2 interacted to increase the number of species of polychaetes in the elevated pCO_2

treatment at ambient temperature (Fig. 4; ANOVA Temp x CO$_2$, $F_{1,32}$ = 11.03, $P < 0.02$,

Supplementary Table 4). Under warming, there were fewer polychaete species recruiting to *T.*

hirsuta compared with that observed at ambient temperature*,* but there was no effect of warming

on the number of polychaete species that recruited to *M. galloprovincialis* (Supplementary Table 4)*.*

When *T. hirsuta* and *M. galloprovincialis* were present in the same mesocosms, there were

significantly more polychaetes under ambient temperature than under warming (Fig. 4; ANOVA

Temp x Presence, $F_{1,32}$ = 4.66, $P < 0.05$; Species x Presence, $F_{1,32}$ = 4.66, $P < 0.05$, Supplementary

Table 4). There were no significant effects of any treatments on the number of species, the number

of individuals of Crustacea and the number of species of Mollusca (Fig. 4; Supplementary Table 4).

Molluscs were negatively affected by elevated pCO_2 but unaffected by warming (Fig. 4). The elevated

pCO_2 treatment reduced the number of infauna mollusc individuals compared with ambient pCO_2

treatment (Fig. 4; ANOVA $F_{1,32}$ = 41.75, $P < 0.001$, Supplementary Table 4).

Active behaviour by infauna choosing to colonise either *T. hirsuta* or *M. galloprovincialis* was

affected by warming and pCO_2 treatments. Molluscs actively chose to colonise *T. hirsuta* and actively

avoided *M. galloprovincialis*, regardless of warming or pCO_2 levels (Table 1). Elevated pCO_2 caused

both polychaete species and crustacean species to actively choose *T. hirsuta*. This was a change from

the behavioural choices under ambient conditions where crustaceans showed no choice and

polychaetes chose *M. galloprovincalis* (Table 1). Warming caused crustaceans to actively avoid *M.*

galloprovincialis (Table 1). Conversely, at ambient temperature, polychaetes actively avoided *M.*

galloprovincialis but showed no behavioural preferences under warming. For molluscs, at ambient

265 temperature, they actively chose to colonise *T. hirsuta*, but under warming, their preference was

266 altered to actively choose *M. galloprovincialis* (Table 1).

267

268 **Discussion**

269   This study has shown that the invasive *M. galloprovincialis* was more tolerant of elevated

270 pCO$_2$ compared with the native *T. hirsuta.* We have also shown that the two mussel species possess

271 unique infaunal communities, which are also altered by climate change conditions. As the oceans

272 warm and acidify, *M. galloprovincialis* may have the capacity to replace *T. hirsuta* as the dominant

273 biogenic habitat on the Australian rocky shores. If such shifts in mussel habitat were to occur, it

274 would result in different infaunal communities, characterised by a loss of infaunal molluscs. These

275 findings suggest that climate change-related shifts in biogenic habitat can precipitate a "cascade of

276 consequences" triggering changes in the local biodiversity.

277

278 *The responses of ocean acidification and warming on native and invasive mussels*

279   There were clear differences in the responses of the two species of mussels to the 8-week

280 experimental exposure to elevated temperature. At the warmer temperature *T. hirsuta* grew faster,

281 and during summer low tides in Sydney Harbour, the temperature of the intertidal rocky shores

282 where *T. hirsuta* occur can reach as high as 30°C (Cole 2010). The +3°C increase in temperature to

283 25°C under the experimental warming scenario is within the range of thermal tolerance and is within

284 the optimal temperature range for growth of *T. hirsuta* (Wallis 1975, Wallis 1977). In contrast,

285 temperature did not affect any of the variables measured for *M. galloprovincilis*. Similarly, previous

286 studies on the genus *Mytilus* have found that there are very few effects of water temperature on its

287 physiology, potentially due to its rapid thermal acclimation (Widdows and Bayne 1971).

288   The two species of mussels responded differently to experimental ocean acidification.

289   Elevated pCO$_2$ resulted in *T. hirsuta* having a trend towards a greater standard metabolic rate.

290   Although metabolic rates are an indication of the amount of energy that is used by mussels

291   (Widdows and Bayne 1971), it may indicate the importance of increased food supply in enhancing

292   their ability to deal with stress (Pörtner and Farrell 2008, Melzner et al. 2011), thus reducing any

293   negative impacts of ocean acidification (Thomsen et al. 2013). Increased pCO$_2$ did, however, not

294   affect any of the physiological variables that were measured for *M. galloprovincialis*. In their native

295   range, exposure to comparable levels and duration of ocean acidification did not affect respiration

296   rates. Physiological tolerance to moderate increases in pCO$_2$ levels could be due the ability of *Mytilus*

297   spp. to acclimate to elevated pCO$_2$ within a few days (Berge et al. 2006).

298   Under elevated pCO$_2$, there was evidence of competition between the two species of

299   mussels such that they had fewer byssal threads. Furthermore, a trend towards a lower SMR was

300   observed in *T. hirsuta*, suggesting that it may be outcompeted by *M. galloprovincialis* under ocean

301   acidification. It is metabolically costly to produce byssal threads for attachment (Hawkins 1985), and

302   if fewer threads are produced when competing, additional stress, e.g. increased predation pressure

303   and wave activity (Cote 1995, Garner and Litvaitis 2013), may result in increased mortality of

304   mussels in the future. Warming and acidification of oceans will extend the range of some species,

305   including those that are considered invasive (King et al. 2021), further intensifying the competition

306   between native and invasive species (Hellmann et al. 2008). As *M. galloprovincialis* is currently listed

307   in the top 100 of the world's worst invaders by the International Union for Conservation of Nature,

308   its spread in Australia may be aided by a changing climate. This study suggests that the invasive *M.*

309   *galloprovincialis* may have an advantage over the native *T. hirsuta* in a future ocean.

310

311   *Effects of climate change on mussel bed communities*

Commented [A4]: The sentence beginning "Furthermore, a trend towards a lower SMR…" has been altered for clarity. Please check.

312   The effects of temperature and pCO$_2$ on the infauna associated with mussels were taxon-

313   specific. In general, elevated pCO$_2$ had a positive effect on polychaete communities, no effects on

314   numbers of species individuals of crustaceans, and a negative effect on molluscs. Any positive effects

315   of pCO$_2$ on polychaetes were lost with warming, and the effects of warming exceeding those of

316   elevated pCO$_2$ on the communities of molluscs. Many species of polychaetes (Cigliano et al. 2010)

317   and crustaceans have been shown to be relatively tolerant to ocean acidification (Kroeker et al.

318   2013a) including those observed here. It has been previously suggested that elevated pCO$_2$ allows

319   generalist species such as crustaceans and polychaetes to displace more specialist calcifiers such as

320   molluscs (Nagelkerken et al. 2017). Molluscs, particularly at their early life stages when they were

321   colonising the mussel beds, are generally sensitive to warming, and are among the species most

322   vulnerable to ocean acidification (Gazeau et al. 2013, Kroeker et al. 2013a, Przeslawski et al. 2015).

323   Changes in the community structure caused by ocean acidification have been reported

324   previously in other studies. Volcanic CO$_2$ vents are often characterised by an elevated abundance of

325   fleshy algae and reduced biomass of coralline algae as well as a decline in the abundance of

326   invertebrate calcifiers such as molluscs (Hall-Spencer et al. 2008). These findings have been

327   reinforced at other CO$_2$ vents around the globe including in tropical areas where the acidified vents

328   reduce hard coral abundance (Fabricius et al. 2014). In the present study, the observed differences

329   in the community structure may be due to delayed succession as a result of acidification (Brown et

330   al. 2018). Warming has also been shown to affect intertidal communities in mesocosm experiments,

331   for example in northern Germany, Pansch et al. (2018) found that the abundance and biomass of

332   communities was shifted by simulated heatwaves, including a decrease in the abundance of the

333   mussel *Mytilus edulis*. No changes in *Mytilus* biomass were reported in this study, but the

334   temperature regimes of northern Germany are not comparable to those of Eastern Australia (e.g.

335   Buschbaum et al. 2009, Cole 2010) where mussels are likely acclimated to warmer environments

336   (Widdows and Bayne 1971, Bayne et al. 1973).

Commented [A5]: In the sentence beginning "It has been previously suggested…" please check if the word "calcifiers" should be changed to "species".

337   Most importantly, elevated pCO$_2$ and temperature affected the behavioural choices of

338   infauna colonising native and invasive mussels. Elevated pCO$_2$ led to polychaetes switching from

339   choosing to colonise *M. galloprovincialis* under ambient conditions and crustaceans showing no

340   behavioural preference under ambient levels of pCO$_2$ to both choosing to colonise *T. hirsuta* under

341   elevated pCO$_2$. Temperature also affected the behavioural preferences of the infauna associated

342   with mussels. Polychaetes, crustaceans, and molluscs altered their behaviour to colonise the habitat

343   created by one species of mussel to another. This altered behavioural preference of infauna can be

344   driven by habitat-specific cues and the ability of infauna to make habitat choices (Cole et al. 2012,

345   von der Meden et al. 2015). For example, ocean acidification is known to affect neurotransmitter

346   function in fish, resulting in a lower capacity for homing and habitat choices (Munday et al. 2009). A

347   mesocosm study by Goldenberg et al. (2018) found that the olfactory and visual senses of fish and

348   shrimp are impaired by elevated pCO$_2$ when each of the senses are investigated separately. When

349   both senses were investigated together, there was no loss of sensory perception under elevated

350   pCO$_2$. Moreover, predation success in mesocosms was not affected by pCO$_2$ or warming (Goldenberg

351   et al. 2018). These findings are interesting in the context of our results, which indicated that

352   elevated pCO$_2$ and warming caused infauna to colonise different habitats, perhaps indicating that

353   the senses of larvae from recruiting organisms are more vulnerable to elevated pCO$_2$ than other

354   organisms such as adult fish and shrimp.

355   There were significantly different assemblages of molluscs associated with the habitat

356   formed by *T. hirsuta* compared with *M. galloprovincialis* under warming and acidification. This shows

357   that even a small change in biogenic habitat from one mussel species to another can have significant

358   effects on infaunal communities. In a study by Sunday et al. (2017), it was estimated that lost mussel

359   habitat would be replaced by filamentous algae and encrusting sponges because of ocean

360   acidification in the Pacific Northwest, USA. This new habitat would not have the structural

361   complexity of mussels, and this would result in a loss of local diversity. We have shown that less

17

362 drastic changes in habitat from a native to invasive mussel still has the capacity to alter the local

363 community structure.

364

365 *Conclusions*

366 This study has found that *T. hirsuta* may be displaced by *M. galloprovincialis* in a future

367 ocean, causing a shift in the biogenic habitat of the Australian shores. Such a shift in habitat may

368 affect the infauna; such conditions may cause infauna to prefer specific mussel habitats (either *T.*

369 *hirsuta* or *M. galloprovincialis*) and lead to an overall decline in infaunal molluscs. Previous studies

370 have shown that the loss of a biogenic habitat in an ecosystem can be functionally replaced (or the

371 loss of function is slowed to some extent) by another habitat-forming organism (Nagelkerken et al.

372 2016, Sunday et al. 2017). The results from the present study suggest that changes in biogenic

373 habitat from one shellfish reef species to another can affect infaunal communities.

374

Authors' contributions

376 VJC conceived and designed the study, did the field and laboratory work, analysed the data,

377 and drafted the manuscript; LMP conceived and designed the study; ES participated in the field and

378 laboratory work and drafted the manuscript; JW did the field and laboratory work; and PMR

379 conceived the study and drafted the manuscript. All authors gave final approval for publication.

380

> **Commented [A6]:** The sentence beginning "Such a shift in habitat…" has been altered for clarity. Please check.

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