Contemporary climate change hinders hybrid performance of ecologically dominant marine invertebrates

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Running title: Climate change and hybrid performance

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Abstract

Human activities alter patterns of biodiversity, particularly through species extinctions and range contractions. Two of these activities are human mediated transfer of species and contemporary climate change, and both allow previously isolated genotypes to come into contact and hybridise, potentially altering speciation rates. Hybrids have been shown to survive environmental conditions not tolerated by either parent, suggesting that, under some circumstances, hybrids may be able to expand their ranges and perform well under rapidly changing conditions. However, studies assessing how hybridisation influences contemporary range shifts are scarce. We performed crosses on *Pyura herdmani* and *Pyura stolonifera* (Chordata, Tunicata), two closely related marine invertebrate species that are ecologically dominant and can hybridise. These sister species live in sympatry along the coasts of southern Africa, but one has a disjunct distribution that includes northern hemisphere sites. We experimentally assessed the performance of hybrid and parental crosses using different temperature regimes, including temperatures predicted under future climate change scenarios. We found that hybrids showed lower success than parental crosses at the experimental temperatures, suggesting that hybrids are unlikely to expand their ranges to new environments. In turn, we found that the more widespread species performed better at a wide array of temperatures, indicating that this parental species may cope better with future conditions. This study illustrates how offspring fitness may provide key insights to predict range expansions, and how contemporary climate change may mediate both the ability of hybrids to expand their ranges and the occurrence of speciation as a result of hybridisation.

Keywords: Early life history stages, intertidal ecology, post-metamorph, pre-metamorph, *Pyura stolonifera* species complex, recruitment, settlement, thermal sensitivity.

Introduction

Changes in gene flow between genetically distinct populations or species are known to alter speciation rates. On one hand, increased gene flow can accelerate speciation through reinforcement (Hoskin *et al.*, 2005; Abbott *et al.*, 2013) or lead to the formation of novel genetic entities via hybrid speciation (Mallet, 2007). On the other hand, hybridisation may slow speciation by allowing gene flow among diverging populations (Abbott *et al.*, 2013). Consequently, hybridisation can have a variety of effects on populations experiencing increased gene flow. Other effects of hybridisation include an increase in genetic variation within populations, creating a larger pool of genotypes on which natural selection can act (Hegarty, 2012), or the purge of deleterious recessive alleles that have accumulated in parental populations (Keller & Waller, 2002). Hybridisation can also be detrimental to parental populations, either directly, by leading to the extirpation of one or both parental populations via introgression (Rhymer & Simberloff, 1996; Arcella *et al.*, 2014; Muhlfeld *et al.*, 2014), or indirectly, by providing first-generation hybrids with phenotypic superiority over their parents (i.e. heterosis). Hybridisation may also lead to the genesis of new phenotypes as a result of transgressive segregation, offering later generations a selective advantage (Lexer *et al.*, 2003). For example, studies on cichlid fish, sunflowers, copepods, and water fleas have shown the generation of hybrid phenotypes with traits that are extreme compared to the parental phenotypes (Rieseberg, 2003; Stelkens *et al.*, 2009; Pritchard *et al.*, 2013; Griebel *et al.*, 2015). Finally, hybridisation can erode accumulated genetic differentiation when reproductive barriers are eventually removed (Taylor *et al.*, 2006). Taken together, this evidence indicates that hybridisation can have several genetic and phenotypic effects with outcomes that are difficult to predict, especially at a time when

74 contemporary climate change (CCC) is extensively reshaping species distributions and
75 abiotic conditions (Potts *et al.*, 2014).
76
77 It is well established that range shifts allow previously isolated populations to come into
78 contact and hybridise. Natural range expansions may arise due to changes in both biotic and
79 abiotic factors (e.g. sudden removal of predators or gradual changes in temperatures)
80 occurring over a wide range of temporal and/or spatial scales (Sexton *et al.*, 2009). For
81 example, historic changes in climate led to the expansion of the European common frog,
82 *Rana temporaria*, due to glacial retreat across Europe ca. 20,000 years ago (Vences *et al.*,
83 2013). Another example of natural range expansions occurs when two habitats that were
84 previously isolated because of a physical barrier come into contact, such as the formation of
85 the Isthmus of Panama ca. 3×10^6 years ago, which led to the Great American Interchange
86 (Marshall, 1988). Similarly, CCC greatly affects species distributions and often leads to range
87 shifts. Indeed, warming winter temperatures associated with CCC has promoted a range
88 expansion in the butterfly *Atalopedes campestris* across western North America (Crozier,
89 2004). In addition to major changes in abiotic factors, species interactions such as predation
90 (Huang *et al.*, 2012) and competition (van der Knaap *et al.*, 2005) can also shape the nature
91 of range shifts (Svenning *et al.*, 2014). Range expansion of the Humboldt squid *Dosidicus*
92 *gigas* in the eastern Pacific has been attributed in part to the removal of competing top
93 predators, which prey on juvenile *D. gigas* (Zeidberg & Robison, 2007). Other types of range
94 shifts are those associated with human activities, such as anthropogenic transport of species
95 that has resulted in unprecedented increases in the speed and magnitude of species
96 translocations (Carlton & Ruiz, 2015; Seebens *et al.*, 2016). Anthropogenic transport is
97 particularly problematic in the marine environment, where shipping provides an

unparalleled intercontinental vector for the translocation of organisms from their native ranges to new locations, mainly because it allows the transport of large numbers of propagules to distant regions (Carlton & Geller, 1993). Range expansions as a result of anthropogenic transport may be exacerbated by CCC (Occhipinti-Ambrogi, 2007; Hellmann *et al.*, 2008; Sorte *et al.*, 2010; Rius *et al.*, 2014; Canning-Clode & Carlton, 2017; Chan *et al.*, 2019), with rising temperatures enhancing settlement success (Raitsos *et al.*, 2010) or causing phenological shifts that favour range shifting species (Stachowicz *et al.*, 2002; Wolkovich & Cleland, 2011; Chefaoui *et al.*, 2019).

As CCC and human-mediated transport of species bring previously isolated genotypes into contact, unprecedented levels of hybridisation have been reported in recent times (Vallejo-Marín & Hiscock, 2016; Canestrelli *et al.*, 2017; Makino *et al.*, 2018). As some hybrids have been reported to survive conditions that the parents cannot, hybridisation is often associated with recent range expansions (Hegarty, 2012; Hovick *et al.*, 2012; Rius & Darling, 2014). Consequently, complex interactions among CCC, hybridisation, anthropogenic transport of species and natural range shifts are potentially shaping speciation patterns. A clear example of this is the release of *Ambystoma tigrinum* (the barred tiger salamander), which was intentionally released from southern to western regions of North America (Riley *et al.*, 2003) where it has now become invasive. Part of the range of *A. tigrinum* now overlaps with that of the native *Ambystoma californiense* (the California tiger salamander), allowing interspecific hybridisation to occur. Experimental crosses have shown that temperature has a positive effect on juvenile salamander dispersal distances, suggesting that CCC may facilitate a rapid range expansion of the hybrid swarm and hence the spread of non-native genotypes (Johnson *et al.*, 2010).

Research to date has assessed how CCC affects the potential for hybridisation, which can alter both pre-mating and post-mating reproductive barriers (Chunco, 2014). CCC has been shown to promote maladaptive hybrids, altering life-history traits in toads (*Bufo* spp.) (Canestrelli *et al.*, 2017). Similarly, CCC can foster range shifts and increase the likelihood of hybridisation in insects (Sánchez-Guillén *et al.*, 2013). In line with this, studies have shown alteration of spatiotemporal patterns of hybridisation between the salmonids *Oncorhynchus clarkii lewisi* and *Oncorhynchus mykiss* (Muhlfeld *et al.*, 2014). Despite all this research, little is known about how hybridisation facilitate range expansions and how hybrids react to rapidly changing climatic conditions. Anthropogenic transport of species and CCC drive range shifts and species invasions, involving both hybrids or parental genotypes (Hegarty, 2012), potentially altering ecosystem structure and function across large geographic areas (Miehls *et al.*, 2009; Katsanevakis *et al.*, 2014). Thus, understanding how hybridisation influences range expansions is an important aspect for predicting the effects of CCC on species distributions.

Here, we examined how conditions expected under CCC affect the performance of a range of ontogenetic stages of both hybrids and parental individuals of sympatric marine ascidian species, and how hybridisation shapes the probability of future range shifts and speciation. Our objectives were to: i) Quantify the performance of hybrid and parental crosses; ii) Assess differences in performance at different temperatures between hybrid and parental crosses; iii) Find links between offspring performance and species distributions. We hypothesised firstly that hybrids would perform similarly to parental species under conditions matching the area where their species distributions overlap, and secondly

hypothesised that under extreme temperatures, hybrids would show different performance than parental crosses. To achieve this, we tested the effects of different temperature regimes across multiple life history stages, as each ontogenetic stage has the potential to act as a bottleneck for species persistence (Byrne, 2011). We predicted that later life history stages would be tolerant to a wider range of temperatures than earlier stages, in line with previous studies (Pineda *et al.*, 2012).

Methods

Study species

We selected two closely related bioengineer marine invertebrates [*Pyura herdmani* (Drasche, 1884) and *Pyura stolonifera* (Heller, 1878), Chordata, Tunicata, Ascidiacea] that coexist along extensive stretches of coastline in southern Africa (Rius *et al.*, 2017). *Pyura herdmani* has also been described in north Africa (Monniot & Bitar, 1983; Lafargue & Wahl, 1986 - though identified in both references as *Pyura stolonifera*) and has recently been reported in southwestern Europe (X. Turon, personal communication). *Pyura* spp. are solitary ascidians that are broadcast spawners with a very short pelagic larval duration of <24 hours (Svane & Young, 1989). This, combined with its disjunct distribution, suggests that *P. herdmani* is either undergoing current, or has previously undergone historic, range expansion due to anthropogenic transport. In southern Africa, the two species inhabit the lower intertidal and subtidal zones, where they form dense aggregates (Rius & Teske, 2011). A phylogenetic study based on mitonuclear and nuclear loci revealed that *P. herdmani* consists of two presumably temperature-defined lineages in South Africa: a temperate lineage inhabiting the southwest and south coasts, and a tropical/ subtropical lineage

inhabiting the east coast (Teske *et al.*, 2011). The temperate lineage of *P. herdmani* lives in sympatry with *P. stolonifera* along the south and southeast coasts of southern Africa (Figure 1) and thus there is potential for hybridisation in the field. Previous work suggested that *P. herdmani* and *P. stolonifera* can hybridise in the laboratory (Rius & Teske, 2013), but no empirical data are available on how these hybrids perform compared to parental crosses or how they are affected by temperature.

Field sampling

Individuals of *P. herdmani* and *P. stolonifera* were collected from natural hard substrata at spring low tides between September and November 2017 from Shark Rock Pier (only *P. stolonifera*; 33° 59' 28" S, 25° 40' 37" E) and Summerstrand Lighthouse (both *P. herdmani* and *P. stolonifera*; 33° 58' 47" S, 25° 39' 29" E; Figure 1) on the south coast of South Africa. Care was taken not to damage the inner body of the ascidians during collection, and to remove any damaged epibionts from the tunic as dead tissue leads to bacterial infection and causes causalities amongst the collected individuals (Monniot, 1990). Sampled ascidians were placed inside insulated cooler boxes filled with seawater and returned to the laboratory as soon as possible (within approximately two hours).

Sea surface temperature data

We obtained daily sea surface temperature (SST) data for the study site from the JPL MUR MEaSUREs Project (2015) for the years between 2003 and 2017, at a 0.01 (latitude) x 0.01 (longitude) spatial resolution. From this, we calculated monthly and yearly average SST temperatures. All data extraction and analyses were performed in R version 3.3.1 (R Core Team, 2016).

193

194 *Laboratory housing of animals*

195 Individuals were maintained in 50 litre aquaria in a constant temperature room, with a
196 twelve-hour light/dark cycle. The aquaria were oxygenated using air pumps and seawater
197 was replaced every other day using water from either the Swartkops River estuary or from
198 Kenton-on-Sea (33° 41' 1.71" S, 26° 41' 8.52" E). Each day, the ascidians were fed 200ml of
199 either *Isochrysis galbana*, *Dunaliella primolecta*, or a mixture of the two algae. The ascidians
200 were checked daily for signs of bacterial infection, and any individual showing signs of
201 infection (identified by either the presence of a white bacterial mat growing on the tunic, or
202 a reduced response of the siphons to gentle physical stimuli) were immediately removed.

203

204 *Fertilisation methods*

205 Two individuals of each species were used for each cross (Figure 2). The tunics of all
206 individuals were first removed, revealing the inner soft body. The dorsal tubercle was used
207 to confirm the identity of each species (Rius & Teske, 2011). The strip-spawning method of
208 Marshall *et al.* (2000) was followed to dissect out gametes. The mixture of sperm and ova
209 was poured through a 160 µm mesh filter so the ova were retained on the mesh but the
210 sperm were allowed to wash through to another Petri dish. The ova were then washed off
211 the mesh using 15 ml filtered seawater (FSW) into a final Petri dish. This process was then
212 repeated for the remaining individuals. Filtered seawater was obtained using a vacuum
213 pump to filter the seawater collected from the field through a 0.7 µm filter. In all crosses,
214 sperm concentration was kept as high as possible to limit the effect of sperm ageing
215 (Marshall *et al.*, 2000) and the time between gamete extraction and gamete mixing was
216 kept to a minimum. The ova of each individual were then aliquoted into two Petri dishes,

with the ova in one dish receiving conspecific sperm and the ova in the second dish receiving interspecific sperm (Figure 2). A total volume of 1 ml of sperm (either from one individual for control crosses, or multiple individuals for hybrid crosses) was added to each Petri dish with ova. The sperm and eggs were kept at 20°C to allow fertilisation to occur as this temperature has previously been shown to promote successful egg development in both parental species (Rius *et al.*, 2014). Once egg cleavage was observed, one more round of filtering ensured excess sperm would be washed away, reducing the probability of polyspermy. Fertilised eggs were then grouped by cross and kept in 500 ml of aerated FSW in total darkness at 20°C (Figure 2).

Once motile larvae had hatched (after c. 12 hours), 20 randomly selected larvae from each cross were transferred into one of five pre-roughened Petri dishes with 15 ml FSW (Figure 2). The Petri dishes had been left in unfiltered seawater for a few days to promote the formation of a biofilm, which is known to facilitate larval settlement (Wieczorek & Todd, 1997). Each of the five Petri dishes was then randomly allocated to one of five temperature-controlled rooms set at 12, 16, 20, 24, or 28°C (Figure 2). Due to different numbers of hatched larvae in the various crosses, the number of replicates for testing settlement success and post-metamorph performance at each temperature varied, ranging from four to eight (Table S1).

After 24, 72, and 120 hours, the larvae in each temperature-controlled room were examined under a microscope and the stage of development of each individual was noted. These stages of development comprised floating tadpole larvae, attached tadpole larvae, attached settlers, non-attached settlers, settlers with obvious tail reabsorption, pre-metamorphs, and

post-metamorphs (Figure S1). As the duration of metamorphosis determines the length of time individuals are exposed to sources of mortality however (O'Connor *et al.*, 2007), it was assumed that if individuals had not reached the post-metamorph stage within 120 hours in the laboratory, they would be unlikely to survive in the field due to the pressures of smothering and predation. Therefore, the percentage of post-metamorphs at 120 hours was used as a proxy for species performance under the different temperature treatments.

Data analysis and statistics

Due to the proportional nature of our datasets, we analysed the data using generalised linear models (GLM) with a binomial error distribution and a logit link function. We tested for overdispersion in our data, and where present we included a random factor (individual Petri dish) using the *glmer* package (Bates *et al.*, 2015). In order to determine whether there were any interactive effects of temperature and cross on development or not, we first investigated the effect of cross under *in situ* temperatures (i.e. 20°C, Fig. S2) and then assessed pre-metamorph and post-metamorph performance at 120 hours. As performance values were zero at certain temperatures, we removed 28°C from the pre-metamorph analysis at 120 hours, and similarly removed 12°C and 28°C from the post-metamorph analysis at 120 hours. Post-hoc Tukey tests were used to determine the pairwise comparisons that drove significant differences. Repeated-measured analyses could not be performed as offspring performance through time was measured at the level of the Petri dish rather than the individual. All statistical analyses were performed in R (R Core Team, 2016).

Results

Temperature results

Sea surface temperature records indicate that the average SST for Port Elizabeth waters was 19.23 ± 0.13°C (SE) between 2003 to 2017, with average summer and winter fluctuating around 22°C and 17°C respectively (Figure S2).

Development at in situ temperature

All reciprocal crosses at 20°C produced well-developed motile larvae (see Figure S1), with hatching occurring ~12 hours after fertilisation. There were significant differences in settlement success (Table 1) between crosses under *in situ* temperatures (i.e. 20°C), with control *P. stolonifera* crosses being more successful than control *P. herdmani* ($P<0.05$, Tukey HSD test; Table 1, Figure 3). However, there was no significant difference in pre-metamorph success after 72 or 120 hours, or in post-metamorph success after 72 hours (Table 1B-D). Despite this, we found that after 120 hours there was a significant difference between the proportion of larvae that developed into post-metamorphs from control *P. stolonifera* crosses and control *P. herdmani* crosses ($P<0.05$, Tukey HSD test; Table 1E, Figure 3E).

Development at experimental treatment temperatures

Pre-metamorphs developed under a wide range of temperatures for all crosses, only failing to develop after 120 hours at 28°C (all crosses), 12°C (hybrids crosses only) and 16°C (hybrid cross with eggs from *P. stolonifera* only; Figures 4, 5). Post-metamorphs developed in all crosses after 120 hours, with this development only apparent at 16°C, 20°C and 24°C.

Effects of temperature and cross on pre-metamorphic development

There was no significant interaction between the effects of temperature and cross on pre-metamorph development after 120 hours (chi-square = 8.429, d.f. = 6, P = 0.208; Table 2A), but there were significant effects of both temperature and cross individually on pre-metamorph development (temperature: chi-square = 17.433, d.f. = 3, $P < 0.001$; cross: chi-square = 8.368, d.f. = 3, $P < 0.05$). The percentages of control *P. herdmani* and control *P. stolonifera* pre-metamorphs after 120 hours were similar at each temperature treatment except for 24°C (Tukey post-hoc test, $P < 0.05$, Figure 5). For all crosses, the lowest percentage of pre-metamorphs developed at 12°C (except 28°C, where no pre-metamorphs developed for any cross).

Effects of temperature and cross on post-metamorphic development

There was a low percentage of post-metamorphs at all temperatures (mean <30%). In contrast to pre-metamorphs, there was a statistically significant interaction between the effects of temperature and cross on post-metamorphic development after 120 hours (chi-square = 13.384, d.f. = 4, $P \leq 0.01$; Table 2B). The highest percentage of post-metamorphs for control *P. herdmani* crosses was found at 24°C, whereas for control *P. stolonifera* this was found at 20°C (Figure 6). Interestingly, maximum values for hybrid post-metamorphs were recorded at 20°C and were intermediate between the values for the two control crosses at that temperature (Figure 6). Although some post-hoc comparisons were unable to detect significant differences (Figure 6), the overall pattern showed no survival of the post-metamorph stage at the lowest and highest temperatures, with limited differences among crosses at intermediate temperatures.

Discussion

Our results showed that ontogenetic stages of hybrids survived a narrower range of temperatures than either parental species, suggesting that hybrids are unlikely to both expand their ranges to dissimilar environments and perform better under future conditions. We also found that the more widespread parental species performed better at higher temperatures. Thus, our results indicate that the more tolerant parental species (*P. herdmani*) may perform better under warming conditions. Finally, our study provides insights into how CCC may inhibit both the ability of hybrids to expand their ranges and the occurrence of speciation due to hybridisation.

We confirmed that reciprocal fertilisation between *P. herdmani* and *P. stolonifera* produces viable offspring that can develop to the post-metamorph stage. We hypothesised that hybrids would perform differently from parental species at extreme temperatures (e.g. Welch & Rieseberg, 2002) and found that neither hybrid cross showed broader temperature tolerances than the parental crosses. In southern Africa, evidence of hybridisation between *P. herdmani* and *P. stolonifera* had, until now, been anecdotal (Rius & Teske, 2013). Our study confirms that fertilisation can occur between these species and provides empirical evidence of the relative success of hybrids in a laboratory setting. However, future studies are needed to study the fertility of these hybrids and their viability in the field. Even though the study species live in sympatry (Figure 1), whether hybrids occur naturally remains unclear. *Pyura stolonifera* is common along rocky shores with high wave-exposure, whereas *P. herdmani* often inhabits more sheltered regions (Rius & Teske, 2011). Consequently, opportunities for hybridisation at locations where both species are found may be fewer than initially expected. Mosaic-style hybrid zones have been reported along the European

335 Atlantic coast, where salinity, wave exposure, and tidal height explain the spatial
336 distribution of alleles within the hybrid zones of the mussels *Mytilus edulis* and *Mytilus*
337 *galloprovincialis* (Gardner, 1994). Niche segregation may therefore contribute to a low
338 prevalence of hybrids, minimising or even preventing gene flow between species. Gene flow
339 can also be reduced by spawning asynchrony, something that could occur among individuals
340 separated by as little as 10s of metres (Marshall, 2002).
341
342 Hybrids performed generally well but in a narrower range of temperatures than either
343 parent species. Considering the direction of predicted global SST changes, it appears unlikely
344 that hybrids of *P. herdmani* and *P. stolonifera* will expand their range to locations with novel
345 environmental conditions. Whilst hybridisation has been suggested to contribute to range
346 shifts (Chown *et al.*, 2015; Pfennig *et al.*, 2016), especially considering expected CCC
347 conditions (Chunco, 2014), our results suggest that this pattern may not be as general as
348 previously reported, as many failed hybridisation events occur in the field and remain
349 unreported.
350
351 Irrespective of parental source, all crosses showed a wider range of thermal tolerance at the
352 pre-metamorph stage than the post-metamorph stage (Figures 4, 5, 6). This contrasts other
353 studies using ascidians that found that later life history stages are less sensitive to
354 environmental stress than earlier ones (Pineda *et al.*, 2012), but is in line with studies
355 showing that ontogenetic stages of copepods and gastropods are more tolerant to heat
356 stress than later developmental stages (Diederich & Pechenik, 2013; Tangwancharoen &
357 Burton, 2014). A possible explanation to this pattern is that early developmental stages of
358 intertidal organisms experience a high variability of conditions and selection favours larvae

359 with high thermal tolerance (Tangwancharoen & Burton, 2014). Both *P. herdmani* and *P.*
360 *stolonifera* can be found in the low intertidal, where ontogenetic stages have to survive
361 highly variable temperatures, whereas previous studies of ascidians have focussed on
362 subtidal species (Pineda *et al.*, 2012) that experience more stable conditions than in the
363 intertidal zone.
364
365 Whilst hybrid crosses did not outperform both parental crosses at any temperature (Figures
366 5, 6), the percentage of post-metamorph hybrids at the temperature treatment matching *in*
367 *situ* conditions at our sampling sites (i.e. 20°C, approximate yearly mean SST of sample sites,
368 Figure S2) was intermediate to values for the parental crosses at this temperature (Figure
369 6). This is in line with previous studies that have reported hybrids possessing similar fitness
370 to parent species (Arnold & Hodges, 1995). This suggests that whilst temperature does not
371 preclude the ability of hybrids and parental species to live in an area of sympatry, hybrids
372 are unlikely to undergo spread to locations with an environmental (temperature) mismatch.
373 There are two caveats to our findings though. First, we based our characterisation of *in situ*
374 temperature on satellite data rather than field measurements. In situ temperature readings
375 are clearly the best way to assess the effect of temperature in the intertidal zone. However,
376 when field and satellite SST data have been compared in the study area, studies have found
377 an almost complete matching [see. Fig. 4 in Smit *et al.* (2013)]. A second caveat is that we
378 only used 20°C to perform fertilisation and thus poor performance in some treatments
379 could be a result of thermal shock from fertilisation to when the petri dishes were placed at
380 the different temperature treatments. Performing the crosses at a range of temperatures
381 would have tackled this and allowed testing the effects of temperature from fertilisation to
382 subsequent development stages.

The ability of species to develop successfully under conditions dissimilar to their native environment suggests the potential for future range expansions (Sorte *et al.*, 2010; Rius *et al.*, 2014). Although some of the differences among crosses were not significant, the highest survival of post-metamorphs at 24°C were from *P. herdmani* control crosses. Interestingly, the areas to which *P. herdmani* has supposedly expanded to (northern Africa and southern Europe) exhibit temperatures that are either similar to, or cooler than those in Port Elizabeth. It has been suggested that the current disjunct range of *P. herdmani* is a relic of a historical Gondwanan distribution (Kott, 1985, 2006), but another possibility is that this distribution is due to a combination of modern anthropogenic transport and an ancient long-distance dispersal event (Teske *et al.*, 2011; Rius *et al.*, 2017). Given the close geographical proximity between a possible Moroccan source and the recent southern European population, and the fact that our results indicate that *P. herdmani* can survive at lower temperatures, it seems likely that these regions are in gene flow contact. As seen in *P. herdmani*, the ability of *P. stolonifera* to develop to the post-metamorph stage successfully at 16, 20, and 24°C implies a wide range of temperature tolerance. Indeed, *P. stolonifera* has been recorded along an extensive stretch of the southern Africa coastline (Monniot *et al.*, 2001), encompassing a wide range of temperature conditions (Rius *et al.*, 2014).

Anthropogenically induced CCC is causing major alterations in the marine environment at an unprecedented rate. Mean global SST has increased at 0.07°C per decade since 1960 and at 0.11 ± 0.02°C per decade since the 1970s (Burrows *et al.*, 2011). This upwards trend is predicted to continue, with mean global SST projected to increase by up to 2°C by 2060 (Kirtman *et al.*, 2013). Despite this global trend of SST warming, there is increasing evidence

that SST values are becoming more extreme in certain regions (Taboada & Anadón, 2012). Between 1960 and 2010, coastal waters around South Africa exhibited both warming and cooling of SST (Rius *et al.*, 2014). The SST of our study area, the Port Elizabeth region, cooled between 1982 - 2009, with especially strong cooling during austral winter months, whilst farther east, the coast experienced strong warming of SST over the same period (Rouault *et al.*, 2010). The sampled region of this study is ~300km away from the documented eastern-most limit of the temperate lineage and the western limit of the subtropical lineage of *P. herdmani* (Teske *et al.*, 2011). If the subtropical lineage had expanded southwards since the collection of the samples analysed in Teske *et al.* (2011), either naturally or through human-mediated transport, then it means that this lineage is now present in Port Elizabeth. As a result, the possibility exist that subtropical individuals were collected and crosses, which may explain the highest success of *P. herdmani* post-metamorphs at 24°C.

Previous studies on marine invertebrates inhabiting the south-east coast of Africa have suggested the presence of a biogeographic break reflecting oceanographic conditions and dispersal rather than physiological tolerance (Teske *et al.*, 2008; Zardi *et al.*, 2011). For example, the invasive mussel *M. galloprovincialis* is prevented from further spread towards the east of the South African coastline by a steep transition between cool-temperate and subtropical waters along the south-eastern coast (Assis *et al.*, 2015). Therefore, it appears unlikely that either lineage of *P. herdmani* could have spread across this biogeographic break naturally. Nevertheless, our results suggest that divergence between these divergent lineages is driven by prezygotic barriers (e.g. oceanography and dispersal) rather than thermal tolerance. It is likely that the dispersal of *P. stolonifera* to the east is similarly limited, despite the ability of its larvae to develop at the higher temperatures exhibited

there (Figure 5, 6). Extreme temperature treatments (12°C and 28°C) precluded post-metamorph development in all crosses, indicating that these temperatures are outside the thermal thresholds of both species and all hybrids and that the species' ranges are unlikely to expand to cooler temperate or tropical regions (or at least regions where these temperatures coincide with spawning periods). Two points temper this interpretation. First, we sampled animals from a single area and thus we did not consider that individual responses may vary throughout a species range (Neuheimer et al., 2011), and secondly, future temperature change is likely to be gradual, raising the possibility of rapid adaptation helping to cope with CCC in both species.

Early ontogenetic stages are often particularly sensitive in marine invertebrates (Verween *et al.*, 2007; Pineda *et al.*, 2012), and consideration of multiple life-history stages is key when exploring thermal tolerances, as these can vary considerably across the life-cycle (Rius *et al.*, 2010). Changing SST will render some previously inhospitable environments habitable (Poloczanska et al., 2016), and this could occur in our study system.. Mass mortalities of *P. stolonifera* have occurred along the southern coast of Africa in both 1991 (Hanekom *et al.*, 1999) and 2012 (Hanekom, 2013) and have been attributed to rapidly changing air and sea temperatures. The coastline where these mass mortalities took place is within the area of sympatry for *P. herdmani* and *P. stolonifera* (Figure 1). It is unknown whether the species identified as *P. stolonifera* in Hanekom *et al.* (1999) and Hanekom (2013) were *P. stolonifera*, *P. herdmani* or hybrids. If only *P. stolonifera* is affected by these mortalities, there may be the potential for *P. herdmani* or hybrids to quickly occupy new available substratum (Rius *et al.*, 2017). Rates of change in SST are not consistent throughout the year in southern Africa (Rouault *et al.*, 2010), and fluctuations in maximum and minimum SST in

455 the study region have become more extreme in recent years (Rius *et al.*, 2014). Therefore,
456 whilst fluctuating climatic conditions may not promote the expansion of hybrids to novel
457 locations *per se*, the opening of an ecological niche may ultimately affect community
458 composition (Sagarin et al., 1999).
459
460 To conclude, we found that: i) *In situ* temperature conditions did not preclude hybridisation
461 between *P. herdmani* and *P. stolonifera* and subsequent development from larvae to the
462 post-metamorph stage; ii) Hybridisation did not enhance survival under a wider range of
463 temperatures; iii) Changes in SST as a result of CCC may enhance range expansions of the
464 parental species but not the hybrids. Our results indicate that offspring performance at a
465 variety of temperatures may be a good predictor of range expansions, and that ongoing CCC
466 may inhibit, rather than promote range expansions by hybrids.
467
468 **Author Contributions**
469 M.R. conceived the study; J.H., C.D.M., M.R., designed the study; J.H. collected data; J.H.,
470 M.R. analysed data; J.H., C.D.M., M.R. wrote the manuscript
471
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714

715 **Table 1.** Results of generalised linear models with a binomial error distribution and a logit
716 link function testing the effects of cross, at 20°C, on the proportion of (A) settlers after 24
717 hours, (B) pre-metamorphs after 72 hours, (C) post-metamorphs after 72 hours, (D) pre-
718 metamorphs after 120 hours, and (E) post-metamorphs after 120 hours.
719

Source	**Chi-square**	**d.f.**	**P value**
(A) Proportion of settlers at 24 hours			
Cross	19.481	3	**<0.001**
(B) Proportion of pre-metamorphs at 72 hours			
Cross	5.540	3	0.063
(C) Proportion of post-metamorphs at 72 hours			
Cross	2.663	3	0.264
(D) Proportion of pre-metamorphs at 120 hours			
Cross	0.348	3	0.951
(E) Proportion of post-metamorphs at 120 hours			
Cross	9.857	3	**0.020**

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Table 2. Results of generalised linear models with binomial error distributions and a logit link function testing the effects of temperature and cross on the proportion of (A) pre-metamorphs and (B) post-metamorphs.

Source	Chi-square	d.f.	P value
(A) Proportion of pre-metamorphs			
Temp	17.433	3	**<0.001**
Cross	8.368	3	**0.039**
Temp x Cross	8.429	6	0.208
(B) Proportion of post-metamorphs			
Temp	4.130	2	0.127
Cross	4.699	3	0.195
Temp x Cross	13.384	4	**0.010**

738 ***Figure legends***

739 **Figure 1.** A) The distribution of two *Pyura herdmani* lineages and *Pyura stolonifera* along the
740 coasts of southern Africa, based on Teske *et al.* (2011) and Rius and Teske (2011). The two
741 sample sites used for this study were SRP (Shark Rock Pier) and SLH (Summerstrand
742 Lighthouse) along the south coast of South Africa. B) *P. herdmani* has spread its range to
743 include northwest Africa and southwest Europe (see details in main text). C) Three *P.*
744 *herdmani* temperate lineage individuals collected in Port Elizabeth harbour (PEH). D) One *P.*
745 *herdmani* subtropical lineage individual collected in Park Rynie (north east coast of South
746 Africa) and housed in an aquarium at Rhodes University with an open siphon. E) Three *P.*
747 *stolonifera* individuals collected at SRP in an aquarium at Rhodes University.

748

749 **Figure 2.** Experimental design used to test the effects of temperature on inter/intraspecific
750 crosses. A) Two individuals of species were used per cross. B) Surgical collection of sperm
751 (♂) and eggs (♀). C) Eggs from each individual were outcrossed with sperm from either one
752 (control cross- same species) or two (hybrid cross- different species) individuals in 5ml of
753 filtered seawater. The symbols above each dish represent the source individual for eggs (♀)
754 and sperm (♂) in each dish. D) Fertilised eggs from each dish from C were washed and
755 grouped into 500 ml beakers of filtered seawater and incubated at 20°C in darkness to
756 hatch. E) Hatched larvae were pipetted into Petri dishes and designated a control
757 environment room (either 12°C, 16°C, 20°C, 24°C, or 28°C) where they were assessed after
758 24, 72 and 120 hours. Twenty larvae were pipetted into each Petri dish, and the number of
759 Petri dishes at each temperature for each cross is shown in Table S1.

760

Figure 3. Violin plots depicting the development of control and hybrid crosses at 20°C. Percentage of (A) settlers after 24 hours, (B) pre-metamorphs after 72 hours, (C) post-metamorphs after 72 hours, (D) pre-metamorphs after 120 hours, and (E) post-metamorphs after 120 hours. Percentage values are means, error bars denote standard error (SE). Dots represent raw data points. Letters indicate homogenous groups identified by post-hoc Tukey tests.

Figure 4. Stacked bar plots showing the percentage of post-metamorphs, pre-metamorphs, and other/dead stages of larvae at different times across temperature treatments. First letter of cross abbreviation represents mother species and second letter represents father species.

Figure 5. Effects of temperature on the pre-metamorph development after 120 hours of control and hybrid crosses. Percentage values are means, error bars denote standard error (SE). Letters indicate homogenous groups identified by post-hoc Tukey tests.

Figure 6. Effect of temperature on the development of different control and hybrid crosses on post-metamorphic development after 120 hours. Error bars denote standard error (SE). Letters indicate homogenous groups identified by post-hoc Tukey tests. Note absence of post-metamorphs at 12 and 28°C.

Figure 1.

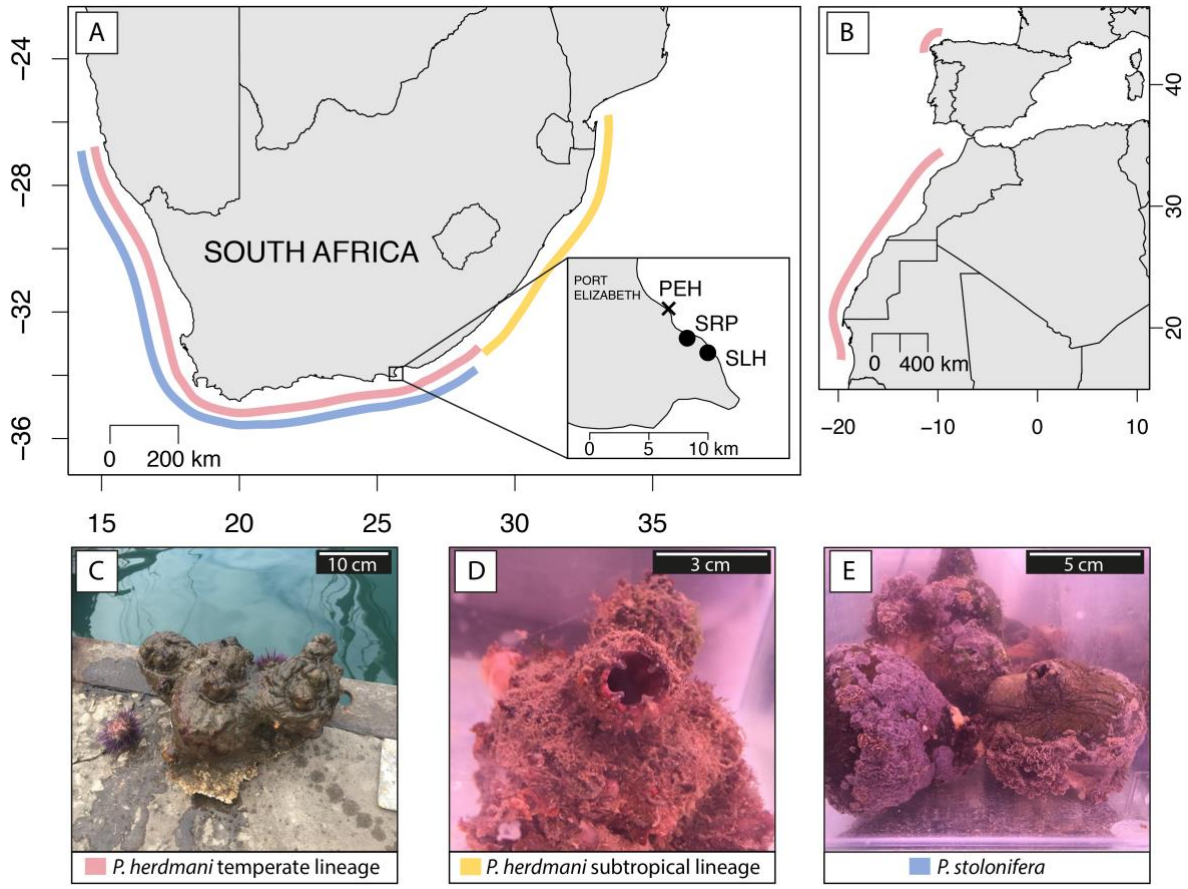

A
SOUTH AFRICA
PORT ELIZABETH
PEH
SRP
SLH
0 200 km
0 5 10 km
−24
−28
−32
−36
15
20
25
30
35
B
0 400 km
−20
−10
0
10
40
30
20
C
10 cm
P. herdmani temperate lineage
D
3 cm
P. herdmani subtropical lineage
E
5 cm
P. stolonifera

Figure 2.

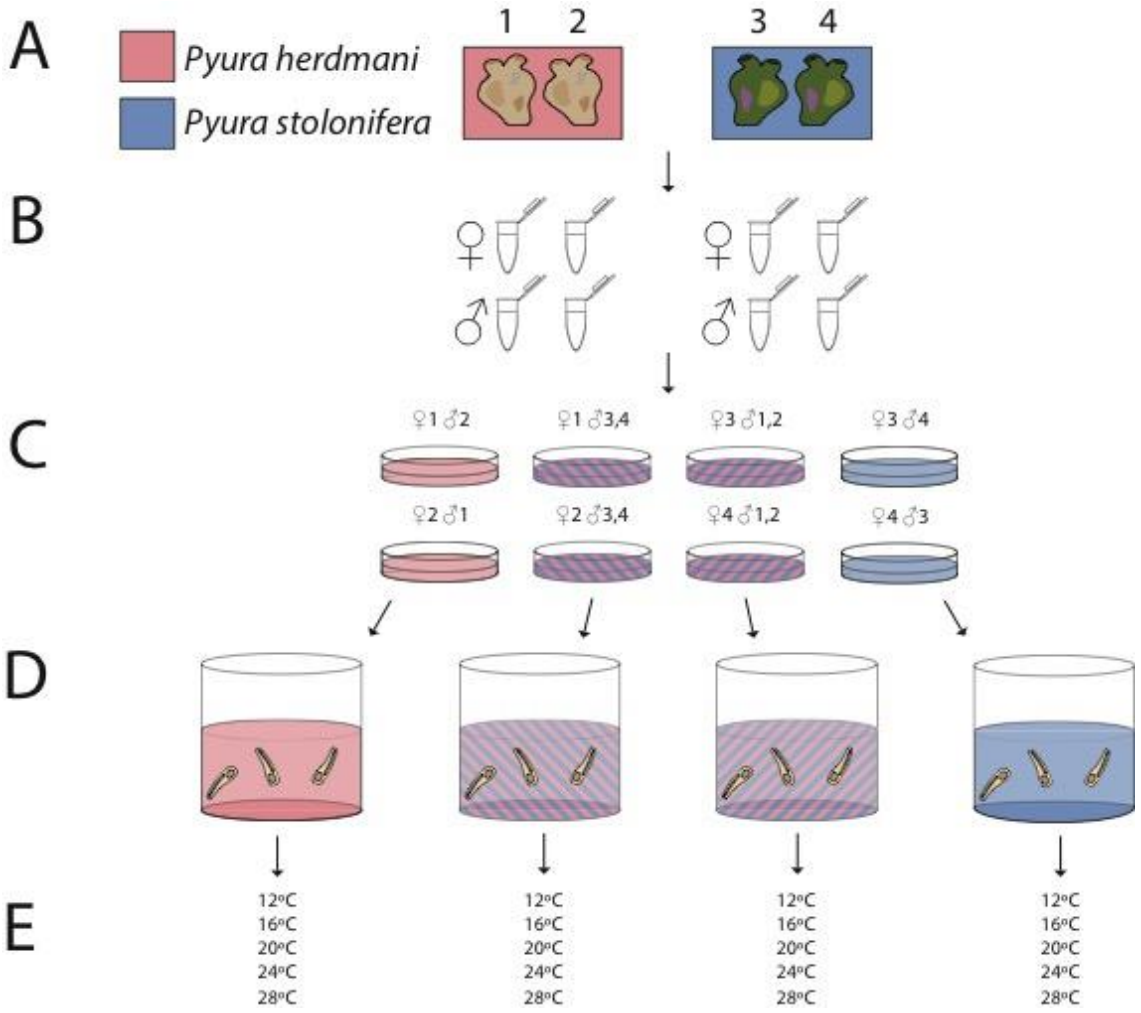

A
Pyura herdmani
Pyura stolonifera
1 2
3 4
B
C
♀1 ♂2
♀1 ♂3,4
♀3 ♂1,2
♀3 ♂4
♀2 ♂1
♀2 ♂3,4
♀4 ♂1,2
♀4 ♂3
D
E
12ºC
16ºC
20ºC
24ºC
28ºC

Figure 3.

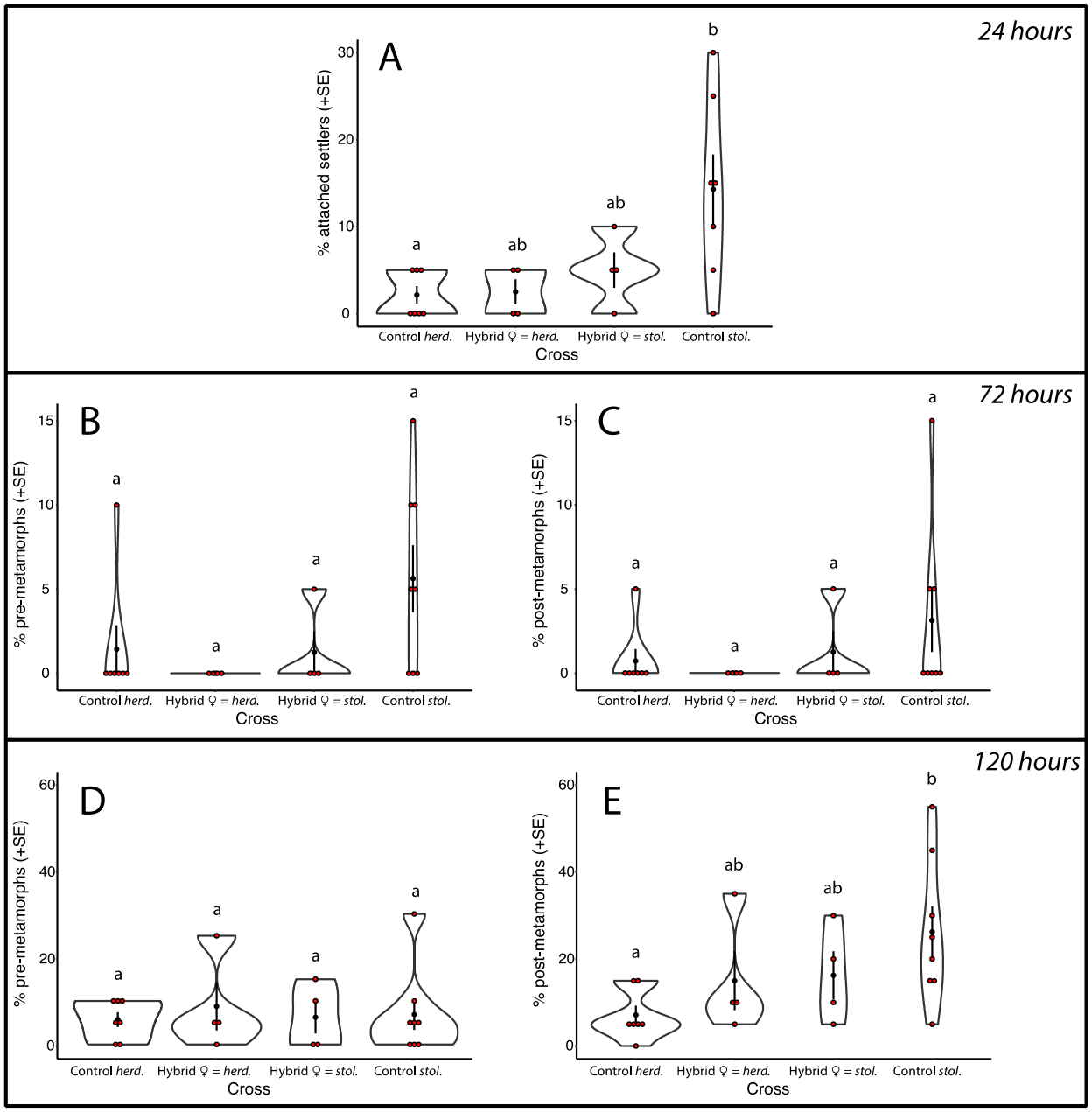

24 hours
A
% attached settlers (+SE)
30
20
10
0
a
ab
ab
b
Control herd.
Hybrid ♀ = herd.
Hybrid ♀ = stol.
Control stol.
Cross
72 hours
B
% pre-metamorphs (+SE)
15
10
5
0
a
a
a
a
Control herd.
Hybrid ♀ = herd.
Hybrid ♀ = stol.
Control stol.
Cross
C
% post-metamorphs (+SE)
15
10
5
0
a
a
a
a
Control herd.
Hybrid ♀ = herd.
Hybrid ♀ = stol.
Control stol.
Cross
120 hours
D
% pre-metamorphs (+SE)
60
40
20
0
a
a
a
a
Control herd.
Hybrid ♀ = herd.
Hybrid ♀ = stol.
Control stol.
Cross
E
% post-metamorphs (+SE)
60
40
20
0
a
ab
ab
b
Control herd.
Hybrid ♀ = herd.
Hybrid ♀ = stol.
Control stol.
Cross

Figure 4.

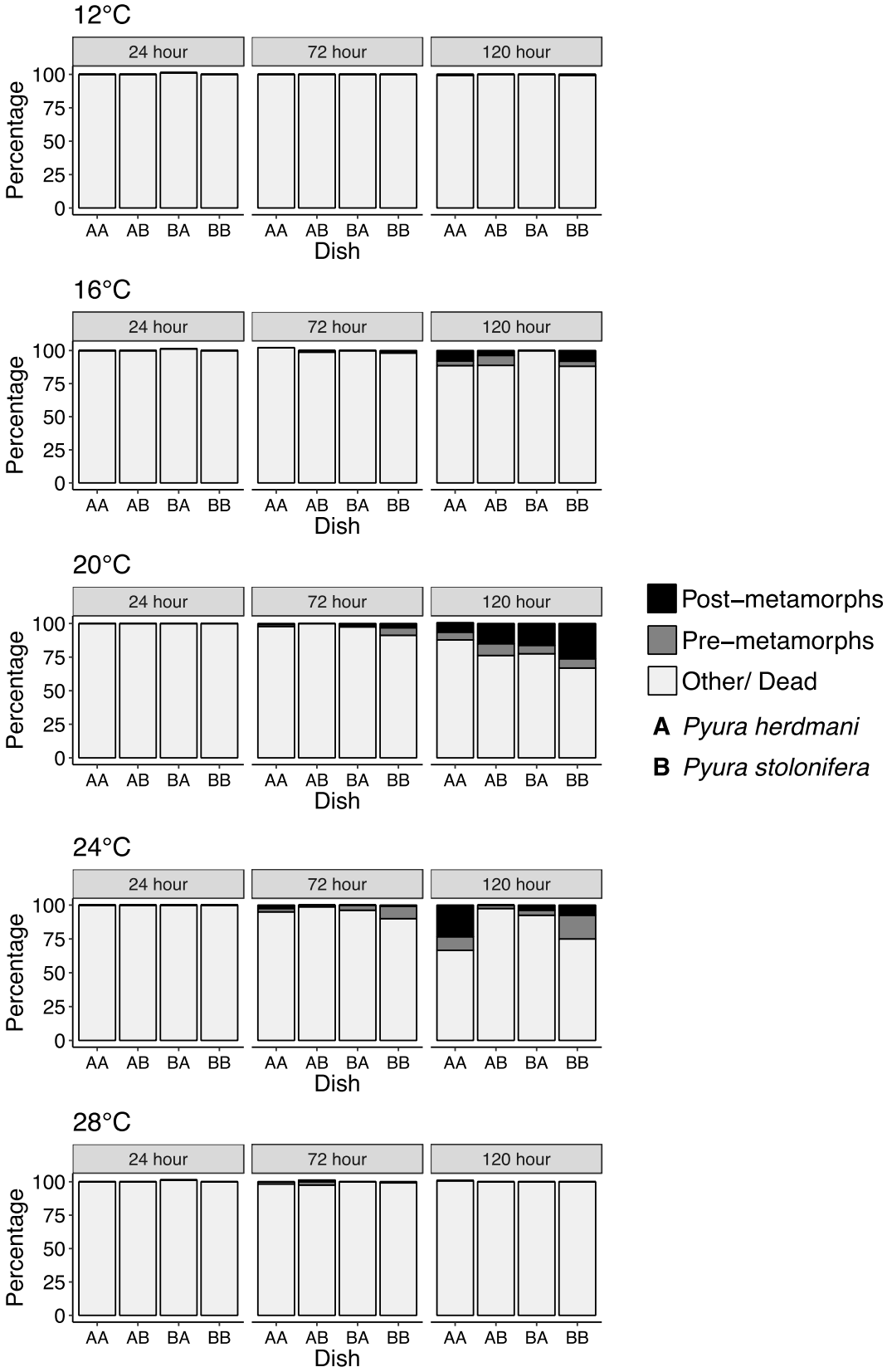
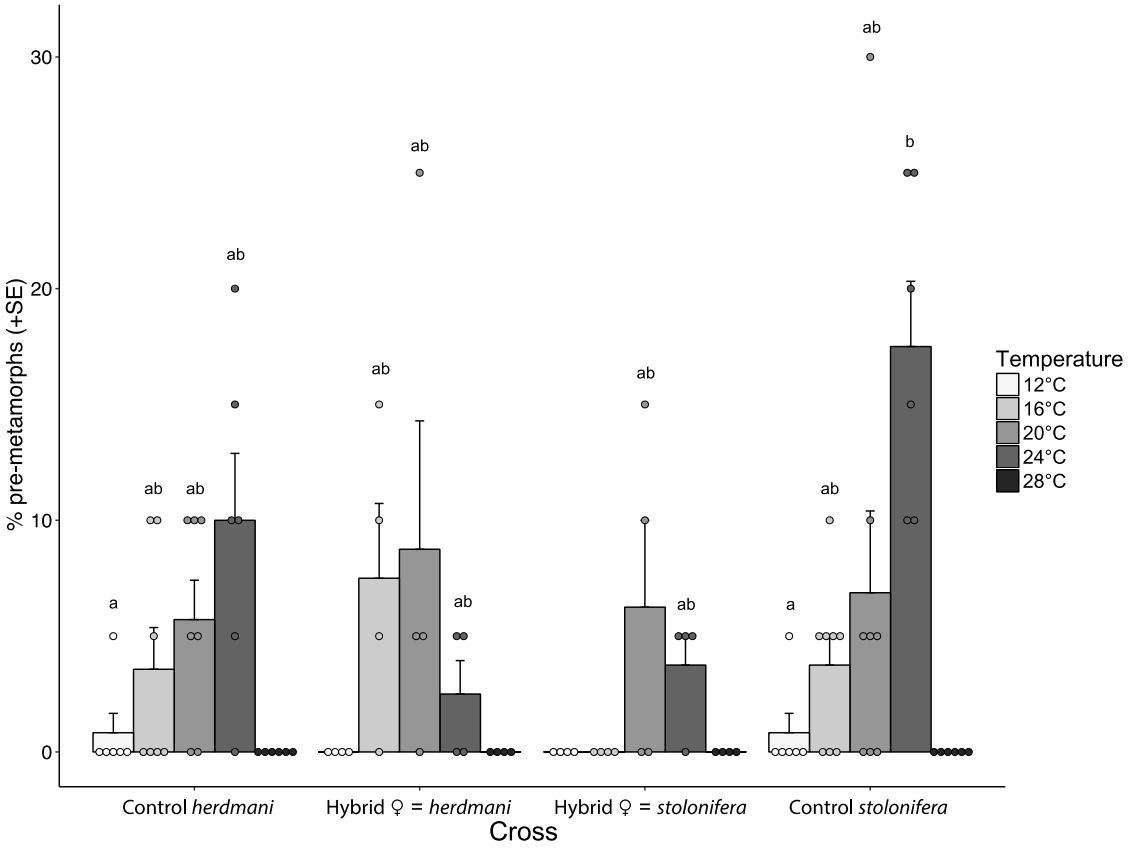
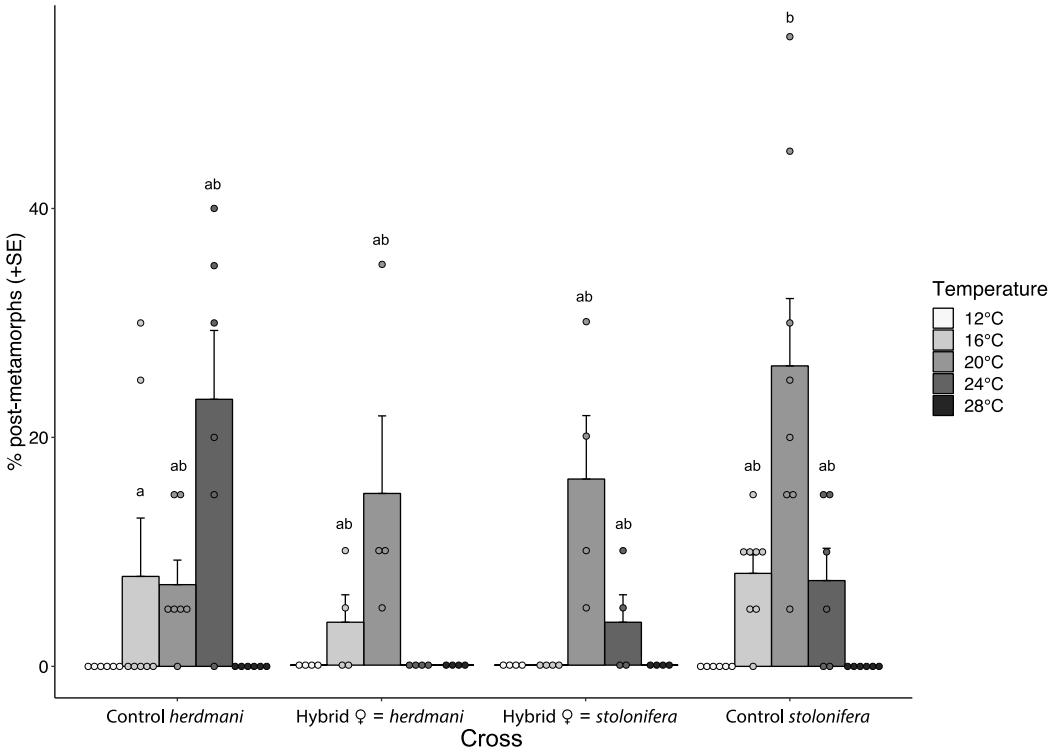

12°C
16°C
20°C
24°C
28°C
24 hour
72 hour
120 hour
Percentage
100
75
50
25
0
AA AB BA BB
Dish
Post–metamorphs
Pre–metamorphs
Other/ Dead
A Pyura herdmani
B Pyura stolonifera

Figure 5.


% pre-metamorphs (+SE)
30
20
10
0
a
ab
ab
ab
ab
ab
ab
ab
ab
ab
a
ab
ab
b
Control herdmani
Hybrid ♀ = herdmani
Hybrid ♀ = stolonifera
Control stolonifera
Cross
Temperature
12°C
16°C
20°C
24°C
28°C

832 **Figure 6.**


% post-metamorphs (+SE)
0
20
40
a
ab
ab
ab
ab
ab
ab
ab
b
Control herdmani
Hybrid ♀ = herdmani
Hybrid ♀ = stolonifera
Control stolonifera
Cross
Temperature
12°C
16°C
20°C
24°C
28°C