# The effects of temperature on embryonic development and larval survival in two scleractinian corals

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**Abstract** Raised temperatures are deleterious to early life stages in many organisms, however, the biological effects of lowered temperatures are rarely explored. For example, the tolerance of marine invertebrate larvae to temperatures lower than ambient might affect the capacity of species to disperse from tropical to sub-tropical locations. In addition, reduced rates of development are likely to affect the proportion of larvae retained on natal reefs. Here, we explore the relationship between temperature, embryonic development and larval survival over an 8°C temperature range (-4 to +4°C around the ambient temperature at the time of spawning of 24ºC) in two reef-building corals, *Goniastrea favulus* and *Acropora spathulata* from One Tree Island (OTI) in the southern Great Barrier Reef (GBR). Rates of development were generally slower at lower temperatures: embryos of both species took longer to complete gastrulation and to become motile at temperatures below ambient. In contrast, temperatures below ambient did not affect larval survivorship in either species. *A. spathulata* larvae were more sensitive to raised temperatures than *G. favulus*, which also had higher survivorship than *A. spathulata* at all temperatures except 20°C. These results suggest that fluctuations in temperature at the time of spawning will influence patterns of coral larval dispersal. Furthermore, cold water is unlikely to prevent the dispersal of tropical corals to sub-tropical locations.

Keywords**:** coral reefs**,** larval ecology, thermal tolerance, dispersal, development, cold tolerance

## INTRODUCTION

The earth's environment is changing rapidly as a consequence of global warming. Rising temperatures are affecting terrestrial, marine and freshwater populations by altering processes such as growth and reproduction (Parmesan & Yohe 2003; Root et al. 2003; Poloczanska 2007). However, climate change will not necessarily result in all locations becoming hotter. For example, the effects of climate change are expected to alter ocean currents, including the East Australian Current, which delivers warm waters from the tropics to higher latitudes in eastern Australia (Poloczanska et al. 2007). Such changes in circulation patterns may result in some sub-tropical locations, such as Lord Howe Island, becoming colder than at present. Consequently, it is important to investigate the effects of both raised and lowered temperatures in order to accurately predict the consequences of global warming (Addo-Bediako et al. 2000; Pörtner 2001).

The effects of raised temperature on coral larval biology are well known. Deleterious effects, such as an increase in the proportion of abnormal embryos and a decrease in larval survivorship are evident as little as 2ºC above ambient (Bassim et al. 2002). Raised temperatures also increase rates of coral larval development (Chua et al. 2012) and coral larvae become competent to settle more quickly at higher temperatures (Nozawa & Harrison 2007; Heyward & Negri 2010). Given a strong association between rates of development and levels of self-recruitment in corals (Figueiredo et al. 2013), rising sea surface temperatures are likely to affect patterns of dispersal by reducing the levels of connectivity among populations (O'Conner et al. 2007). The effects of colder temperatures on coral larval biology are less well known. Edmondson (1946) demonstrated that coral larvae were robust to short term exposures to temperatures as low as 0.5°C. In contrast, metamorphosis to CCA by *Stylophora*

*pistillata* was 5 times lower at 2ºC below ambient (Putnam et al. 2008). Similarly, settlement was approximately 50% lower in *Acropora solitaryensis* larvae at 3ºC below ambient (Nozawa & Harrison 2007).

Climate-driven changes in ocean circulation are altering dispersal patterns in many marine organisms (O'Conner et al. 2007; Przeslawski et al. 2008). For example, the mussel *Mytilus edulis* (Jones et al. 2009), many reef fish species (Feary et al. 2013) and some corals (Yamano et al. 2011; Baird et al. 2012) have recently shifted their ranges pole-ward. Similarly, the fossil record indicates that scleractinian corals have been tracking climate on geological timescales (Veron 1992; Precht & Aronson 2004; Greenstein & Pandolfi 2008). This tendency of marine organisms to track changing climates strongly suggests there are environmental barriers to dispersal, although geographical ranges could also be limited indirectly, for example, by changes in competitive interactions among species (Cahill et al. 2013). Nonetheless, one potential factor limiting the dispersal of corals south from the Great Barrier Reef (GBR) into sub-tropical areas may be the capacity of coral larvae to withstand the colder waters they encounter en route.

In this study, we compared the response of the early life history stages of two species of scleractinian corals, *Goniastrea favulus* and *Acropora spathulata* to an 8ºC temperature range from -4 to +4ºC around the ambient experienced at the natal location, One Tree Island, around the time of spawning. In addition to comparing the temperature response, we aimed to test whether cool water is a barrier to the dispersal of larvae of these species to higher latitudes from this location. Both *G. favulus* and *A. spathulata* are common at One Tree Island (OTI), however, while OTI is the southern latitudinal limit for *A. spathulata* (Wallace 1999), *G. favulus* occurs as far south as Lord Howe Island (Veron 1993).

## MATERIALS AND METHODS

*Coral collection and culture of propagules*

Six colonies of *Acropora spathulata* and five colonies of *Goniastrea favulus* were collected from the reef flat of the first lagoon at One Tree Island (23°30'S, 152°05'E) in the southern GBR, a few days prior to the predicted spawning period in 2010. Colonies were maintained in flow-through filtered seawater (FSW) in shaded outdoor aquaria. Just prior to spawning, species were placed in separate aquaria and water flow was stopped to prevent gametes being washed away. *G. favulus* spawned on the afternoon of 26 November 2010 and *A. spathulata* spawned on the night of 30 November 2010. *A. spathulata* egg and sperm bundles were collected and broken apart with gentle agitation and the density of sperm diluted to ca. $10^6$ sperm $ml^{-1}$ in order to maximize the fertilization success (Oliver & Babcock 1992). Once cleavage was observed approximately 2 h post-fertilization (hpf), embryos were washed three times in 0.2 micron FSW to remove excess sperm which can cause cultures to deteriorate. In contrast to the positively bouyant egg/sperm bundles released by *A. spathulata*, *G. favulus* releases eggs and sperm separately, with the negatively buoyant eggs released approximately 30 min before sperm. Consequently, the eggs of *G. favulus* were collected from the base of parent colonies approximately 30 min after spawning was complete. The time that eggs were spawned was considered to be the time of fertilization in *G. favulus*.

*Experimental design*

To test for the effects of raised and lowered temperature on larval development and survivorship, water baths were set up in a temperature-controlled room at five

temperatures (20°C, 22°C, 24ºC, 26°C, 28°C i.e. -4°C, -2°C, ambient, +2°C, +4°C). Aquarium heaters, coolers, and pumps kept treatment baths stable and within 0.5°C of the target temperatures (monitored with HOBO data loggers). Ambient average SST for the month prior to spawning (24.2°C) was determined from on-reef sensors (GBROOS, http://data.aims.gov.au/gbroos/).

*The effect of temperature on embryonic development*

To test the effect of temperature on embryonic development, washed embryos were transferred to 20 ml glass vials filled with 0.2 µm FSW and distributed among temperature treatments at 2 hpf (ca. 30 embryos per vial; 3 vials per treatment). The stage of development of the first 20 embryos in each vial was assessed at 8 or 9 time points depending on the species: 18, 24, 30, 36, 48, 72, 96, 120 and 144 hpf (6 days). The following five development stages were identified (following Ball et al. 2002): 4-cell blastula, multiple cell blastula, early gastrula, gastrula and planulae (motile stage). To test for differences in development time between treatments, the average time for propagules to reach gastrulation and motility was estimated following Chua et al. (2012):

Average time to reach stage, $\overline{X} = \Sigma$ [time (hours) x number of propagules to reach stage]/Total number of propagules

*Effect of temperature on larval survival*

To test the effect of temperature on coral larval survival, 50 washed embryos were placed in 50 ml glass vials filled with 0.2 µm FSW and distributed among temperature treatments 2 hpf (50 embryos x 3 vials per treatment). Survival was measured by

135 counting the number of embryos remaining at each of the above time points. Coral
136 larvae lyse within 24 h of death (Baird et al. 2006) so all larvae counted were
137 considered to be alive at the time of census.
138
139 *Data Analysis*
140 Differences in mean time to complete gastrulation and to reach the planula stage (for
141 *G. favulus* only) among temperature treatments (fixed, 5 levels: 20, 22, 24, 26 and
142 28ºC) were tested using a 1-way ANOVA for each species separately. Data were log-
143 transformed and homogeneity of variance was confirmed by Levene's Test. Tukey's
144 HSD post-hoc tests were used to identify which treatment levels differed. Non-
145 parametric Kaplan-Meier product limit analyses were used to test for differences in
146 median survivorship among temperatures for each species separately. Median
147 survivorship (in hours) was considered significantly different when the 95%
148 confidence intervals did not overlap. All analyses were performed using SPSS v19®.
149
150 **RESULTS**
151 Temperature had a significant effect on rates of propagule development in both
152 species. In general, the slowest rates of development occurred at the lowest
153 temperatures (Fig. 1 & 2). Temperature had a significant effect on the mean time to
154 complete gastrulation in both *A. spathulata* ($F_{4, 10} = 71.53$, $p < 0.001$) and *G. favulus*
155 ($F_{4, 10} = 11.84$, $p = 0.001$) (Fig. 1). *A. spathulata* embryos at 28ºC took 23.1 ± 0.9 h to
156 complete gastrulation compared to 37.7 ± 2.1 h at 20ºC. Similarly, *G. favulus*
157 embryos required 30.4 ± 4.0 h to complete gastrulation at 20ºC compared with 20 ±
158 1.0 h at 28°C. In addition, *G. favulus* developed more rapidly than *A. spathulata* at all
159 temperatures (Fig. 1). Over all temperatures pooled, the mean time to complete

gastrulation was 21.6 ± 1.4 h in *G. favulus* and 28.4 ± 1.3 h in *A. spathulata*. Similarly, temperature had a significant effect on the mean time to reach the planula stage in *G. favulus* ($F_{4,10}$=15.62, p=<0.001; Fig. 2). The mean time to reach the planula stage was greatest at 20ºC (129.7 ± 6.3 h) and lowest at 26ºC & 28ºC (Fig. 2).

Only raised temperatures had a significant effect on larval survival (Fig. 3). In *A. spathulata,* survival was reduced at both temperatures above ambient (Fig. 3a). In contrast, *G. favulus* survival was reduced only at the highest temperature (Fig. 3b). In addition, *G. favulus* larval had higher survivorship than *A. spathulata* larvae at all temperatures, with the exception of 20ºC (Fig. 3).

## DISCUSSION

Embryonic development was strongly affected by temperature. In general, the lower the temperature, the longer it took to complete gastrulation and for larvae to become motile. In contrast, larval survival was only reduced at temperatures above ambient. While the response of both species to temperature was broadly similar, there were, nonetheless, differences between the species in development rate, larval survivorship and thermal tolerance.

The effect of temperature on development rates in these coral embryos is typical of most marine invertebrates (Pechenik 1987). For example, embryos of *Goniastrea australensis* in the Solitary Islands (30ºS) developed more slowly at 22ºC than at 26 and 28ºC (Wilson & Harrison 1998). This suggests that rates of embryonic development are likely to depend on the temperature conditions prevailing shortly after the time of spawning. Given that rates of self-recruitment are typically higher in larvae that develop more rapidly (Figueiredo et al. 2013), patterns of dispersal are likely to vary among years if ambient temperatures vary. In addition, patterns of

dispersal might vary predictably among locations at different latitudes. In particular, high latitude locations, are likely to have lower levels of self-recruitment than tropical locations because larvae take longer to develop. In addition, rates of predation are likely to increase the longer larvae remain in the plankton. For example, reduced levels of self-recruitment might help explain low numbers of juvenile corals at Lord Howe Island (LHI) (Latitude 33°S) when compared to many tropical locations (Hoey et al. 2011). However, the effect of low temperatures on rates of recruitment can not be discounted (Putnam et al. 2008).

Rates of embryonic development were also influenced by the size of the propagules. Across all temperatures, *G. favulus* embryos (mean diameter of 320 µm) developed more rapidly than *A. spathulata* embryos (mean diameter of 500µm; Fig. 1 & 2), which can most likely be attributed to faster rates of cell division in species with smaller eggs (Berrill 1935; Marshall & Keough 2008). Similarly, in eighteen species of broadcast spawning corals, egg size was strongly and positively correlated with time to motility (Figueiredo et al. 2013). The more rapid rate of development in *G. favulus* embryos did not come at the cost of reduced larval survival: *G. favulus* larvae survived longer that *A. spathulata* at all temperatures, except at 20°C where there was no difference between the species (Fig. 3).

In contrast to the relationship between development and temperature, larval survival was only reduced at temperatures 2-4ºC (Fig. 3). These upper thermal limits (2-4ºC above ambient: Fig. 3) appear to be consistent over a very large geographical scale and among many different species (Bassim et al. 2002; Randall & Szmant 2009; Heyward & Negri 2010), supporting the hypothesis that many corals live very close to their upper thermal limits, In contrast, temperatures up to 4°C below ambient had no effect on larval survival (Fig. 3). Projections based on the speed and direction of the

East Australia Current suggest that the time taken to disperse from OTI in the southern GBR to LHI takes approximately 16-33 days. Given that spawning occurs at OTI in November, larvae will arrive at LHI between late November and early January. In the course of this journey water temperatures can be as low as 19ºC (AIMS 2012). Consequently, it is unlikely that temperature is a barrier to dispersal from the southern GBR to higher latitudes for either of these species and therefore other factors must determine why *A. spathulata* is not found on LHI.

Thermal tolerance differed between the species. In particular, larval survival was reduced at 26 °C in *A. spathulata* and 28°C in *G. favulus* (Fig. 3). A similar difference in thermal tolerance was also observed between acroporid and merulinid embryos by Negri et al. (2007). Consistent differences in stress tolerance are also apparent between adult colonies of these two families: adult acroporids are much more susceptible to bleaching and disease when compared to adult merulids (Hughes & Connell 1999; Marshall & Baird 2000; Diaz & Madin 2011).

In conclusion, temperature has important effects on many aspects of coral larval biology. In particular, development rates varied predictably with temperature, suggesting that patterns of dispersal are likely to change in response to global warming. In addition, coral larvae appear to be tolerant of temperatures 2-4ºC below ambient, suggesting that cold water is unlikely to limit the dispersal of tropical species to sub-tropical locations.

## ACKNOWLEDGEMENTS

We thank S. & A. Schmidt-Roach and onacloV for field assistance, K. Miller, P. Davies, and staff of One Tree Island Research Station. This project was funded by ARC Grants to AHB.

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236 **REFERENCES**


237 Addo-Bediako A, Chown SL, Gaston KJ (2000) Thermal tolerance, climatic
238 variability and latitude. Proc R Soc Lond B 267:739-745

239 AIMS (2012) Marine Observation Map. Australian Institute of Marine Science:
240 data.aims.gov.au/aimsrtds/map.xhtml

241 Baird AH, Gilmour JP, Kamiki TM, Nonaka M, Pratchett MS, Yamamoto HH,
242 Yamasaki H (2006) Temperature tolerance of symbiotic and non-symbiotic
243 coral larvae. Proceedings of 10th International Coral Reef Symposium:38-42

244 Baird AH, Sommer B, Madin JS (2012) Pole-ward range expansion of Acropora spp.
245 along the east coast of Australia. Coral Reefs 31:1063

246 Ball EE, Hayward DC, Reece-Hoyes JS, Hislop NR and others (2002) Coral
247 development: from classical embryology to molecular control. Int J Dev Biol
248 46:671−678

249 Bassim KM, Sammarco PW, Snell TL (2002) Effects of temperature on success of
250 (self and non-self) fertilization and embryogenesis in *Diplora strigosa*
251 (Cnidaria, Scleractinia). Mar Biol 140:479-488

252 Berrill NJ (1935) Studies in tunicate development part III: differential retardation and
253 acceleration. Philos Trans R Soc Lond B Biol Sci 225:255-326

254 Cahill AE, Aiello-Lammens ME, Fisher-Reid MC, Hua X, Karanewsky CJ, Yeong
255 Ryu H, Sbeglia GC, Spagnolo F, Waldron JB, Warsi O, Wiens JJ (2013) How
256 does climate change cause extinction? Proceedings of the Royal Society B:
257 Biological Sciences 280

258 Chua CM, Leggat W, Moya A, Baird AH (2013) Temperature affects the early life
259 history stages of corals more than near future ocean acidification. Marine

Ecology Progress Series 475:85-92

Diaz M, Madin JS (2011) Macroecological relationships between coral species traits and disease potential. Coral Reefs 30:73-84

Edmondson CH (1946) Behavior of coral planulae under altered saline and thermal conditions. Bernice P Bishop Museum- Occassional Papers 18:283-304

Feary DA, Pratchett MS, J Emslie M, Fowler AM, Figueira WF, Luiz OJ, Nakamura Y, Booth DJ (2013) Latitudinal shifts in coral reef fishes: why some species do and others do not shift. Fish and Fisheries: doi: 10.1111/faf.12036

Figueiredo J, Baird AH, Connolly SR (2013) Synthesizing larval competence dynamics and reef-scale retention reveals a high potential for self-recruitment in corals. Ecology 94:650-659

GBROOS (2012). Great Barrier Reef Ocean Observing System. Australian Institute of Marine Science: data.aims.gov.au/gbroos/

Greenstein BJ, Pandolfi JM (2008) Escaping the heat: range shifts of reef coral taxa in coastal Western Australia. Global Change Biology 14:1-16

Heyward AJ, Negri AP (2010) Plasticity of larval pre-competency in response to temperature: observations on multiple broadcast spawning coral species. Coral Reefs 29:631-636

Hoey AS, Pratchett MS, Cvitanovic C (2011) High macroalgal cover and low coral recruitment undermines the potential resilience of the world's southernmost coral reef assemblages. Plos One 6:e25824

Hughes TP, Connell JH (1999) Multiple stressors on coral reefs: a long-term perspective. Limnol Oceanogr 44:932-940

283 Jones SJ, Mieszkowska N, Wethe DS (2009) Linking thermal tolerances and
284 biogeography: *Mytilus edulis* (L.) at its southern limit on the east coast of the
285 United States. Biol Bull 217:73-85
286 Marshall PA, Baird AH (2000) Bleaching of corals on the Great Barrier Reef:
287 differential susceptibilities among taxa. Coral Reefs 19:155-163
288 Marshall DJ, Keough MJ (2008) The relationship between offspring size and
289 performance in the sea. Am Nat 171:214-224
290 Negri A, Marshall P, Heyward A (2007) Differing effects of thermal stress on coral
291 fertilization and early embryogenesis in four Indo Pacific species. Coral Reefs
292 26:759-763
293 Nozawa Y, Harrison PL (2007) Effects of elevated temperature on larval settlement
294 and post-settlement survival in scleractinian corals, *Acropora solitaryensis* and
295 *Favites chinensis*. Mar Biol 152:1181-1185
296 O'Connor MI, Bruno JF, Gaines SD, Halpern BS, Lester SE, Kinlan BP, Weiss JM
297 (2007) Temperature control of larval dispersal and implications for marine
298 ecology. Proc Natl Acad Sci USA 104:1266-1271
299 Oliver J, Babcock R (1992) Aspects of the fertilization ecology of broadcast spawning
300 corals: sperm dilution effects and *in situ* measurements of fertilizations.
301 Biological Bulletin 183:409-417
302 Parmesan C, Yohe G (2003) A globally coherent fingerprint of climate impacts across
303 natural systems. Nature 421:37-42
304 Pechenik JA (1987) Environmental influences on larval survival and development. In:
305 Giese AC, Pearse JS (eds) Reproduction of Marine Invertebrates. Academic
306 Press, New York, pp551-608

307 Poloczanska ES, Babcock RC, Butler A, Hobday AJ, Hoegh-Guldberg O, Kunz TJ,
308 Matear R, Milton DA, Okey TA, Richardson AJ (2007) Climate Change and
309 Australian Marine Life. Oceanography and Marine Biology: An Annual Review
310 45:407-478
311 Pörtner HO (2001) Climate change and temperature-dependent biogeography: oxygen
312 limitation of thermal tolerance in animals. Naturwissenschaften 88:137-146
313 Precht WF, Aronson RB (2004) Climate flickers and range shifts of reef corals. Front
314 Ecol Environ 2:307-314
315 Przeslawski R, Ahyong S, Byrne M, Worheide G, Hutchings P (2008) Beyond corals
316 and fish: the effects of climate change on non-coral benthic invertebrates of
317 tropical reefs. Global Change Biol 14:2273-2795
318 Putnam HM, Edmunds PJ, Fan TY (2008) Effect of temperature on the settlement
319 choice and photophysiology of larvae from the reef coral *Stylophora pistillata*.
320 Biol Bull 215:135
321 Randall CJ, Szmant AM (2009) Elevated temperature affects development,
322 survivorship, and settlement of the elkhorn coral, *Acropora palmata* (Lamarck
323 1816). Biol Bull 217:269
324 Root TL, Price JT, Hall KR, H. SS, Rosenzweig C, Pounds JA (2003) Fingerprints of
325 global warming on wild plants and animals. Nature 421:57-60
326 Veron JEN (1992) Environmental control of holocene changes to the world's most
327 northern hermatypic coral outcrop. Pac Sci 46:405-425
328 Veron JEN (1993) A biogeographic database of hermatypic corals. Australian
329 Institute of Marine Science, Townsville
330 Wallace CC (1999) Staghorn Corals of the World. CSIRO, Collingwood

331 Wilson JR, Harrison PL (1998) Settlement-competency periods of larvae of three
332 species of scleractinian corals. Mar Biol 131:339-345
333 Yamano H, Sugihara K, Nomura K (2011) Rapid poleward range expansion of
334 tropical reef corals in response to rising sea surface temperatures. Geophys Res
335 Lett 38:L04601, doi:04610.01029/02010GL046474
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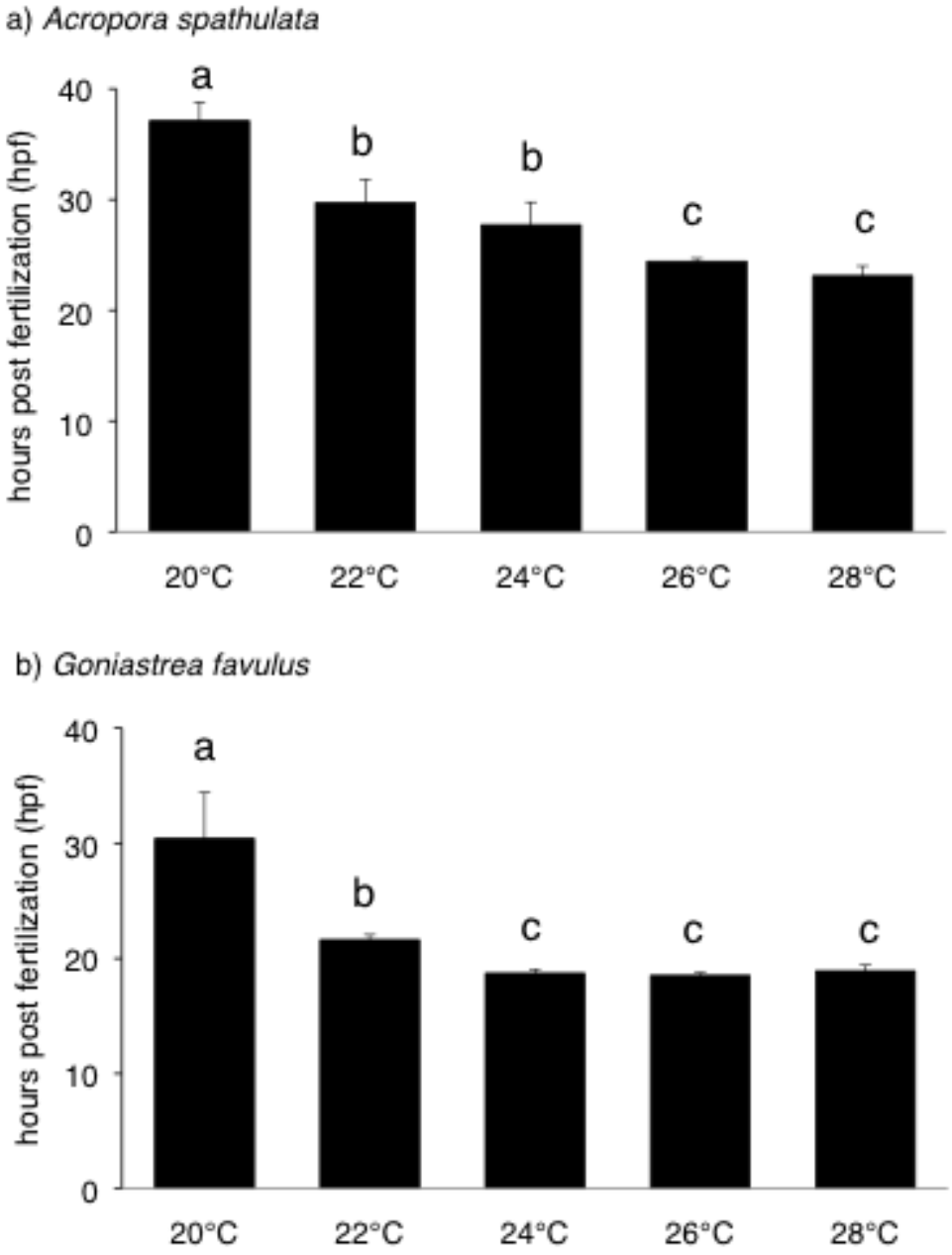
337 FIGURES




338
339 **Fig. 1** Mean time to gastrulation (hours post-fertilization ± one SE) of a)
340 *Acropora spathulata* and b) *Goniastrea favulus* at five temperatures (n= 60,
341 ambient = 24°C). Letters above the error bars indicate homogenous groups
342 identified by Tukey HSD post-hoc analysis ($p<0.05$).

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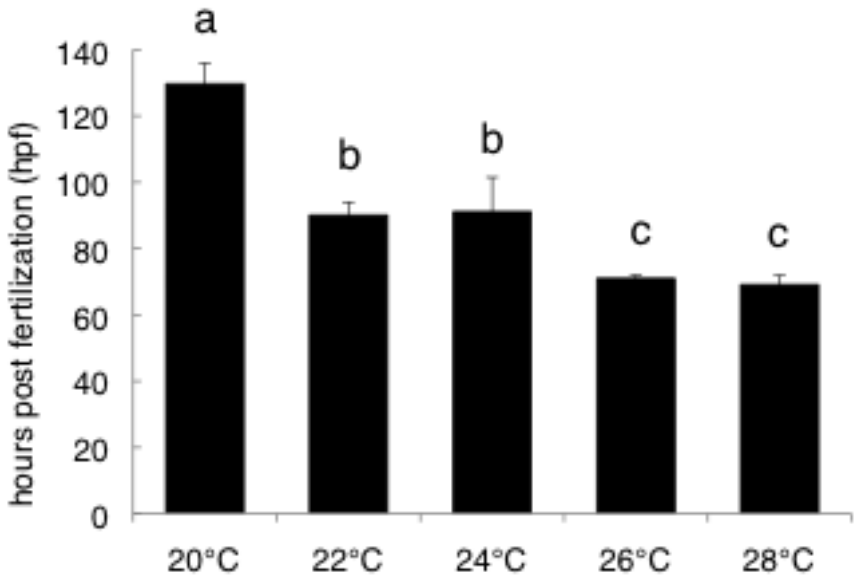


**Fig. 2.** Mean time to the planula stage (hours post-fertilization ± one SE) in *Goniastrea favulus* at five temperatures (n=60, ambient = 24°C). Letters above the error bars indicate homogenous groups identified by Tukey HSD post-hoc analysis ($p<0.05$).

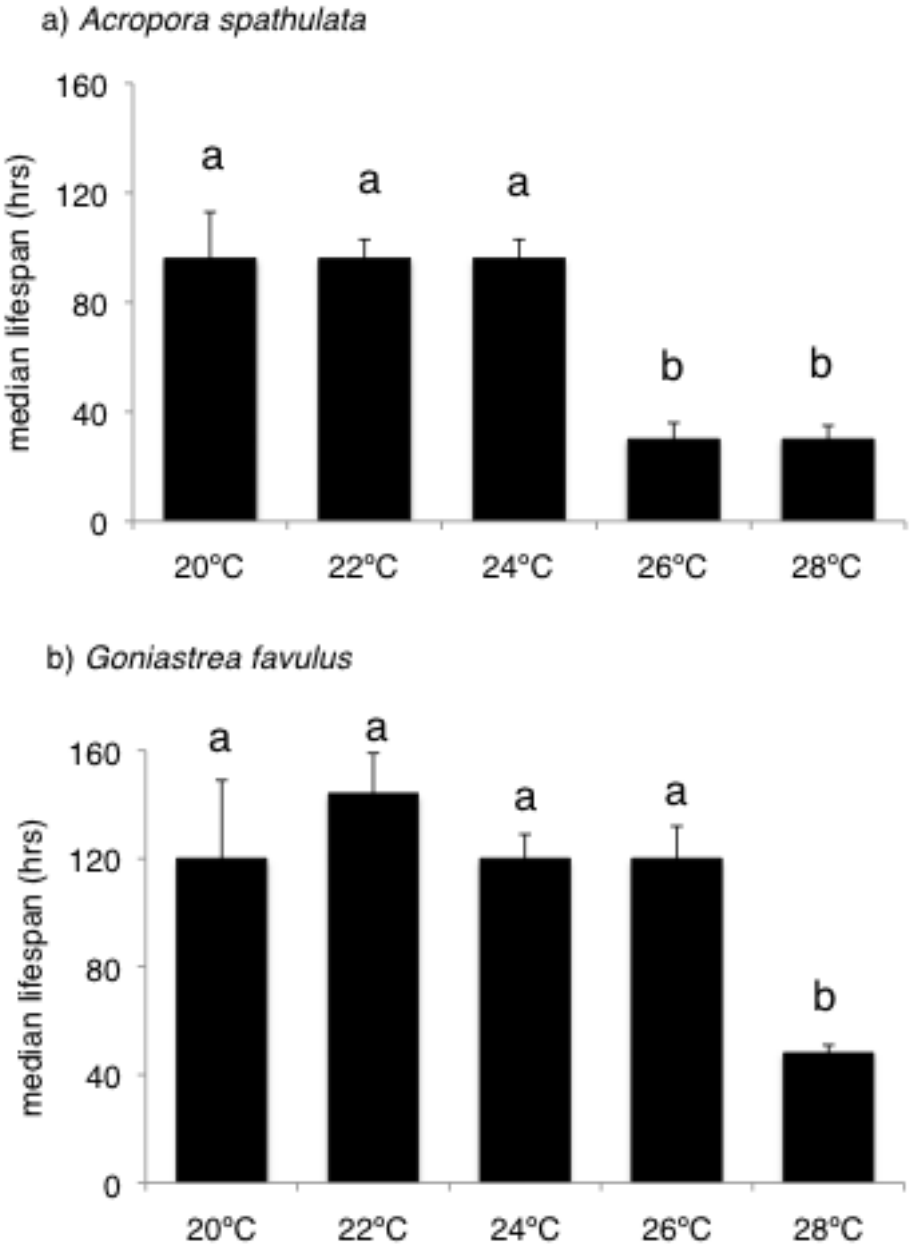


**Fig. 3** Kaplan-Meier median survivorship estimates for a) *Acropora spathulata* and b) *Goniastrea favulus* at five temperatures (n=150, ambient = 24°C). Error bars show 95% confidence intervals and letters indicate homogenous groups determined by the overlap of confidence intervals.