1 **Impact of diurnal temperature fluctuations on larval settlement and growth of the**

2 **reef coral *Pocillopora damicornis***

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23   **Abstract**

24   Diurnal fluctuations in seawater temperature are ubiquitous on tropical reef flats.

25   However, effects of such dynamic temperature variations on the early stages of corals

26   are poorly understood. Here, we investigated the responses of larvae and new spats of

27   *Pocillopora damicornis* to two constant temperature treatments (29 and 31 °C), and two

28   diurnally fluctuating treatments (28–31 and 30–33 °C with daily means of 29 and 31 °C,

29   respectively) simulating the 3 °C diel oscillations at 3 m depth on Luhuitou fringing

30   reef (Sanya, China). Results showed that the thermal stress on settlement at 31 °C was

31   almost negated by the fluctuating treatment. Further, temperature fluctuations did not

32   exacerbate bleaching responses but alleviated the maximum excitation pressure over

33   photosystem II (PSII). Although early growth and development were highly stimulated

34   at 31 °C, oscillations of 3 °C had little effects on budding and lateral growth.

35   Nevertheless, daytime encounters with the maximum temperature of 33 °C elicited a

36   notable reduction in calcification. These results underscore the complexity in the effects

37   caused by diel temperature fluctuations on early stages of corals, and suggest that the

38   ecologically relevant temperature variability could buffer the warming stress on larval

39   settlement and dampen the positive effects of increased temperatures on coral growth.

40

41   **Keywords**: temperature, diurnal fluctuation, *Pocillopora damicornis*, settlement,

42   bleaching, calcification, budding

43

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1 Introduction

Scleractinian corals and the reef ecosystems they construct are currently facing
unprecedented rates of changes, of which the rising seawater temperature is generally
recognized as one of the most immediate and widespread threats (Hoegh-Guldberg,
1999; Hughes et al., 2003). The most conspicuous response of corals to elevated
temperatures is to expel their endosymbiotic dinoflagellates and/or photosynthetic
pigments, resulting in the paling of the affected colonies, a process known as coral
bleaching (Hoegh-Guldberg, 1999). Due to the loss of zooxanthellae, bleached corals
usually fail to obtain their key metabolic requirements from the photosynthetically fixed
carbon (Grottoli et al., 2006). As a result, massive mortality of corals has been
frequently observed following bleaching, leading to serious decline and impaired
ecosystem functionality due to bleaching events (Hoegh-Guldberg, 2011; Graham et al.,
2006).

Relative to preindustrial level, sea surface temperatures have increased on average
by 0.7 °C and another 3 °C increase are expected by the end of this century (Bopp et
al., 2013). Therefore, concerns about the devastating effects of rising temperatures on
corals have escalated, and the bulk of work addressing the impact of ocean warming on
corals have centered on their tolerance and physiological responses to the predicted
increases in mean temperature (Stambler, 2010). Nevertheless, seawater temperatures
on coral reefs are characterized by striking fluctuations over timescales from minutes
to hours to months. In particular, temperature profiles from reef environments typically
show diel oscillations of up to 4–10 °C (Coles, 1997; Dandan et al., 2015; Mayfield et

67   al., 2012; Oliver and Palumbi, 2011; Putnam and Edmunds, 2011). A consistent daily

68   cycle is commonly present, with temperature increasing after sunrise, peaking after

69   noon and then gradually decreasing to the minimum (e.g., Zhang et al., 2013; Putnam

70   and Edmunds, 2011).

71   It has been long-established that performance of organisms differs in steady versus

72   variable thermal conditions of equivalent mean temperature, including a diverse range

73   of marine invertebrates (Bryars and Havenhand, 2006; Lucas and Costlow, 1979;

74   Marshall and McQuaid, 2010; Orcutt and Porter, 1983; Pilditch and Grant, 1999; Sastry,

75   1979). These studies have demonstrated that early development and growth can either

76   be speeded up or retarded due to temperature fluctuations, depending upon the mean

77   temperatures and amplitude around the mean value. However, this thermodynamic

78   effect on corals which routinely experience temperature oscillations in nature remains

79   largely unstudied.

80   Recently, our understanding of the physiological responses of corals to diurnally

81   fluctuating temperature has advanced, but results have been variable and even

82   conflicting. For instance, photo-physiology in larvae and adults of pocilloporid corals

83   is more suited to the fluctuating than to the constant temperatures (Mayfield et al., 2012;

84   Putnam et al., 2010). Conversely, evidence for the deleterious effects includes the

85   significant reductions in photochemical efficiency, symbiont density and aerobic

86   respiration under diel temperature oscillations (Putnam and Edmunds, 2011; Putnam

87   and Edmunds, 2008). These contrasting results reiterate a clear need to further explore

88   the impact of diurnally fluctuating temperatures, together with the projected increase in

89   temperature on reef corals.

90   In the context of global deterioration of coral reefs and climate change, the early life

91   history stages of corals have drawn increasing attention in recent decades, as they are

92   more vulnerable to environmental changes than their adult counterparts, and more

93   importantly, represent a bottleneck for the maintenance of populations. The successful

94   larval settlement, post-settlement survival and growth are of paramount importance to

95   population persistence, as well as the recovery of degraded reefs (Ritson-Williams et

96   al., 2009; Penin and Adjeroud, 2013). Mounting evidence suggests that ocean warming

97   poses a serious threat to these early processes (reviewed in Keshavmurthy et al., 2014),

98   but most of those experiments utilized steady temperature treatments, therefore

99   neglecting the temporal variations of *in situ* temperature (but see Putnam et al., 2010).

100   To date, there is a paucity of knowledge regarding the influence of dynamic

101   temperatures on these crucial stages in reef corals. The risk imposed on fitness and

102   development of corals by ocean warming can be best understood by integrating such

103   diel thermocycles and changes in mean temperature (Boyd et al., 2016).

104   The present study aimed to investigate how the early stages of the reef coral

105   *Pocillopora damicornis* will be affected by the diurnally oscillatory temperatures,

106   together with warming. Brooded larvae and new settlers were exposed to contrasts of

107   two temperature levels (29 and 31 °C) and two temperatures regimes (constant and 3 °C

108   diel fluctuations). Diurnal patterns of temperature fluctuations were set according to the

109   temperature records in our study site, Luhuitou fringing reef, Sanya, China. Larval

110   condition and juvenile growth after incubation were assessed to compare their

111   responses to constant and oscillatory temperatures.

112

113   **2 Materials and methods**

114   **2.1 Field seawater temperature monitoring**

115   Seawater temperatures at 3 m depth in Luhuitou fringing reef (18°12′N, 109°28′E) was

116   recorded at 30 min intervals from 2012 to 2015, using Hobo Pendant data loggers

117   (Onset, USA). The temperature profiles showed large seasonal and diurnal fluctuations,

118   with the maximum and minimum of 33.1 °C and 20.3 °C respectively (Fig. S1a). The

119   mean annual temperature was 27 °C and monthly mean temperature ranged from 22 °C

120   to 30.2 °C (Fig. S1b). Diurnal range during summer (June−September) was between

121   0.6 and 5.4 °C, with a mean value of 1.76 °C (Fig. S1c). On a daily basis, seawater

122   temperature began to increase around 08:00, reached the maximum at 13:00, often

123   remained constant for about two hours, and then gradually decreased (Fig. S1c).

124

125   **2.2 Larval collection and preparation of spat**

126   Eight *P. damicornis* colonies were collected from 3 m depth in Luhuitou fringing reef.

127   Larvae released from these colonies were collected at 07:00 on 22 August 2015, pooled

128   and haphazardly assigned for either the settlement or post-settlement experiments. For

129   the recruit experiment, larvae were transferred to 10-cm-diameter petri dishes for

130   settlement and dishes were left floating in a flow-through tank. Twenty hours later, 4

131   dishes with a total of 35–40 spats were assigned to each treatment tank. Only spat that

132   settled individually and at least 1 cm apart from others were selected for the experiment.

133

134   **2.3 Experimental setup**

135   The 29 °C treatment, corresponding to the ambient temperature during this study, was

136   taken to represent the control treatment. The experimental temperature was 2 °C above

137   the ambient and 1 °C above the bleaching threshold for coral communities on Luhuitou

138   reef (30 °C, Li et al., 2012), and within the range of projected increases (Bopp et al.,

139   2013). Two temperature regimes, i.e., constant and fluctuating were set for each

140   temperature level. Pattern and range of temperatures in the two fluctuating conditions

141   were based on *in situ* records during larval release of *P. damicornis* (Fig. S1d), and the

142   assumption that the 2 °C increase in mean temperature will entail a 2 °C shift in the

143   overall temperature trajectory (Burroughs, 2007).

144   Four 40 L tanks were filled with sand-filtered seawater, which was changed partially

145   (30%) with temperature-equilibrated seawater at 22:00 every day. Treatments were set

146   using digital temperature regulators (Sieval, TC-05B, China) and 50 W heaters.

147   Seawater was gently aerated and well mixed using submerged pumps (350 L h^{-1}). Water

148   temperatures in each tank were recorded with Hobo Pendant loggers at 15 min intervals

149   throughout the experiment. In the two fluctuating treatments (Fig. 1), temperatures were

150   programmed to increase from 28/30 °C at 08:00, reach the plateau of 31/33 °C around

151   13:00 and stabilize for 2 hours. Afterwards, temperatures were allowed to decrease

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152   gradually to 28/30 °C around 22:00 and remained stable until 09:00 next morning.

153   Mean (± SD) daily temperature of the two stable treatments were 29 ± 0.2 and 30.8 ±

154   0.2 °C, and the mean values in two fluctuating treatments were 28.9 ± 1.3 and 30.7 ±

155   1.3 °C respectively. Salinity in each tank was checked twice a day and remained stable

156   at 33 psu during the experiment.

157   Each tank was illuminated by a LED lamp (Maxspect, 10,000K, China) on a 12:12 h

158   light-dark cycle. Light was measured with a Li-Cor 4-π quantum sensor below the water

159   surface. Light intensity was similar among tanks ($F_{3,\,96} = 0.32$, $P = 0.81$), averaging at

160    $183 \pm 3\ \mu$mol photons m^{-2} s^{-1} (mean $\pm$ SE, n = 100), which was close to the irradiance

161   in crevices where coral recruits were found at 3−4 m depths in our study site (Lei Jiang,

162   unpublished data). Facility and logistical constraints precluded the replication of

163   treatments, but salinity and light were carefully controlled to eliminate any possible

164   artefact (Underwood, 1997).

165

166   **2.4 Settlement assay**

167   The settlement experiments were conducted in 5.5-cm-diameter petri dishes on 22

168   August and began around 09:00. Crustose coralline algae (CCA), *Hydrolithon*

169   *reinboldii*, were collected at 2–3 m depths and cut into uniformly sized (5 × 5 × 3 mm)

170   chips 4 days before the settlement experiment. Each dish contained 15 ml seawater and

171   a CCA chip. Fifteen 15 actively swimming larvae were introduced into each dish, which

172   was then submerged in the treatment tanks to ensure temperature control. Four replicate

173   dishes were used for each treatment. Larvae were allowed to settle for 24 hours, after

174   which the successful settlement was assessed under the dissecting microscope

175   following the criteria of Heyward and Negri (1999). Larvae were categorized into four

176   conditions: (i) dead, (ii) swimming, (iii) metamorphosed and floating in the water, i.e.,

177   premature metamorphosis (*sensu* Edmunds et al., 2001), and (iv) metamorphosed and

178   firmly attached to CCA or dish, i.e., successful settlement.

179   **2.5 Chlorophyll fluorescence and bleaching**

180   Twenty 3-day-old spat were randomly selected and marked in each treatment. A Diving-

181   pulse-amplitude modulation (PAM) fluorometry (Walz, Germany) was used to measure

182   the maximum quantum yield of PSII (F_v/F_m), a proxy for potential photochemical

183   efficiency of symbionts (Genty et al., 1989). Measurements were conducted at 05:30

184   on four consecutive days to allow enough time for dark adaption. Both the measuring

185   light and gain of PAM settings were adjusted to give optimal fluorescence signals

186   (specifically 7).

187   At the last day of the experiment, effective quantum yield ($\Delta F/F_m'$) was also

188   measured for 15 recruits from each treatment at four timepoints (08:00, 11:00, 14:00,

189   17:00). The maximum excitation pressure over PSII (Q_m) was calculated using the

190   following equation: $Q_m = 1 - [(\Delta F/F_m'$ at 14:00)$/(F_v/F_m)]$ (Iglesias-Prieto et al., 2004),

191   to better assess the photo-physiological performance of symbionts.

192   Bleaching response was assessed photographically following Siebeck et al. (2006).

193   At the end of the experiment, spat were photographed with a digital camera under the

194 dissecting microscope and identical illumination. The camera was adjusted to the

195 manual mode with constant ISO settings. Bleaching index was quantified as the

196 reduction in saturation of each spat relative to the spat yielding the maximum saturation,

197 because saturation in coral pictures is a good proxy for symbiont or chlorophyll density

198 during bleaching (Siebeck et al., 2006).

199

200 **2.6 Post-settlement survival and growth**

201 Recruits were checked daily under a dissecting microscope and scored as alive or dead

202 based on the presence of polyp tissue. At each census, the number of living spats were

203 recorded for each treatment. Digital images of recruits with scale calibration were also

204 analyzed for lateral growth using ImageJ software (National Institutes of Health).

205 Number of polyps for each recruit were visually counted. Juvenile growth was

206 estimated as the rates of change in planar area and number of new polyps over time

207 (Dufault et al., 2012; Jiang et al., 2015).

208 Calcification was calculated as the dry skeletal weight deposited per day (Dufault et

209 al., 2012). Tissue of recruits was removed with a water-pick at the end of the experiment.

210 Skeletons were weighed individually using an ultra-microbalance at an accuracy of ± 1

211 µg.

212

2.7 Data analyses

Data were tested for homogeneity of variances, using Cochran's test, and normality was assessed using Q−Q plots. Percent data in settlement assays and budding rates were square root transformed to meet the requirements of homogeneity of variances. Larval settlement, Q_m and growth parameters were compared among treatments using two-way analyses of variances (ANOVAs) with mean temperature and temperature variability as fixed factors, each with two levels (29 and 31 °C; constant and fluctuating regimes). Fisher's LSD test was performed as planned multiple comparisons following ANOVAs (Day and Quinn, 1989). Survivorship of coral spats during the experiment was analyzed using a Kaplan-Meier (KM) log-rank analysis. Two-way ANOVAs with repeated measures were used to test for the effects of temperature treatments on F_v/F_m or $\Delta F/F_m{}'$ over the sampling time points.

3 Results

3.1 Larval settlement

Larval mortality was only observed in the treatment with a constant temperature of 31 °C during settlement assay (Fig. 2a). In all treatments, 35−60% of larvae metamorphosed whilst in a free floating polyp state (Fig. S2), and between 2.5 and 15% were swimming actively (Fig. 2b). Although differences in these percentages were not significant among treatments, there was more metamorphosed and floating larvae at a

233   constant temperature of 31 °C than in the other treatments. Settlement success showed

234   distinct different effects of temperature fluctuations vs. constant temperature at 31 °C

235   (Fig. 2c, interaction term: $F_{1, 12}$ = 4.00, P = 0.06). Settlement was similar between

236   fluctuating and constant regimes at 29 °C; in contrast, the thermal stress on settlement

237   was greatly alleviated by diurnal fluctuations at 31 °C (Fig. 2c).

238

239   **3.2 Chlorophyll fluorescence and bleaching**

240   A significant interaction between time, temperature level and regime was observed for

241   maximum quantum yield F_v/F_m ($F_{3, 228}$ = 3.7, P = 0.01, Fig. 3a). Separate analyses

242   showed that F_v/F_m was consistently lower at higher temperatures, but this effect size

243   was small, only amounting to a 3 % decrease. There was also a significant interaction

244   between time, temperature level and temperature regime for effective quantum yield

245     $\Delta F/F_m'$ ($F_{1, 56}$ = 21.5, P < 0.001). Further analyses revealed that both temperature

246   increase and fluctuations showed strong effects except at 08:00, with lower $\Delta F/F_m'$ at

247   elevated temperature and higher $\Delta F/F_m'$ under fluctuating conditions (Fig. 3b).

248   Q_m, the maximum excitation pressure, was not influenced by elevated temperature

249   ($F_{1, 56}$ = 0.14, P = 0.71). However, it was considerably reduced under fluctuating

250   regimes ($F_{1, 56}$ = 10.4, P = 0.002, Fig. 3c). Bleaching rates increased by 40−65 % at

251   elevated temperatures ($F_{1, 107}$ = 11.3, P = 0.001), while the fluctuating treatment showed

252   little additional effect on bleaching rates ($F_{1, 107}$ = 0.02, P = 0.874, Fig. 3d).

253

3.3 Growth, survival and temperature coefficient (Q_{10})

Budding state of recruits differed strongly among treatments (Chi-square test, $\chi^2 = 19.4$, $df = 6$, $P = 0.004$). After 7 days post-settlement, approximately 70% of recruits produced at least one bud at 31 °C, compared to less than 50% at 29 °C (Fig. 4a). Budding rates at elevated temperature were more than twice those at 29 °C (Fig. 4b, $F_{1, 107} = 22.8$, $P < 0.001$). At both temperatures, no significant differences between constant and fluctuating regime were observed ($F_{1, 105} = 1.25$, $P = 0.26$).

Lateral growth rates increased significantly with elevated temperature ($F_{1, 107} = 25.4$, $P < 0.001$), but were not affected by temperature fluctuations ($F_{1, 107} = 2.12$, $P = 0.15$, Fig. 4c). Skeletal weight deposited each day was enhanced by 56% at 31 °C compared to 29 °C ($F_{1, 96} = 36.7$, $P < 0.001$). The effects of temperature fluctuations on calcification depended on the mean temperature (Fig. 4d), although the interaction between temperature level and regime was not statistically significant ($F_{1, 96} = 2.07$, $P = 0.15$). At 29 °C, the fluctuating treatment had no discernible effect on calcification, while at 31 °C it caused a significant reduction (20%) in calcification compared to constant regime.

Survival remained >86% in all treatments after 7 days, with the highest and lowest values at 31°C (97%) and 29°C (86%), respectively. Survivorship did not vary significantly across treatments ($\chi^2 = 4.49$, $df = 3$, $P = 0.21$, Fig. 5), although it was 6–13% higher at elevated temperature. For juvenile *P. damicornis*, lateral growth, budding and calcification increased by 1.19-, 1.91- and 1.68-fold between 29 and 31 °C, yielding a Q_{10} of 2.6, 36.8 and 17.8, respectively.

276

4 Discussion

4.1 Larval settlement under elevated and fluctuating temperatures

279 The pronounced declines in successful settlement at elevated temperatures was

280 consistent with early findings that reported the thermal stress effects (>30 °C) on coral

281 larval settlement (Humanes et al., 2016; Randall and Szmant, 2009). Interestingly, the

282 transient exposure to high temperatures in variable conditions hardly impaired

283 settlement; on the contrary, the diurnal shifts at 30–33 °C greatly attenuated the thermal

284 stress on settlement. During daytime encounter with elevated and stressful temperatures,

285 coral larvae may not initiate metamorphosis and settlement because larvae undergoing

286 this complex stage are particularly susceptible to thermal perturbations (Randall and

287 Szmant, 2009), whereas settlement may proceed as temperature descends to a more

288 tolerable level at night (28/30 °C in this study). It is likely that the fluctuating

289 temperature conditions could provide some respite for coral larvae, thereby favoring

290 settlement.

291   Metamorphosed and floating larvae, previously noted in corals (Edmunds et al., 2001;

292 Vermeij, 2009; Mizrahi et al., 2014; Richmond, 1985), were more frequent at elevated

293 temperatures. One possible explanation is that the premature metamorphosis in coral

294 larvae is a spontaneous response to increased temperatures (Edmunds et al., 2001). The

295 floating polyps, as a result of pelagic metamorphosis, have been shown to have

296 extended longevity, possibly because they can obtain energy from photosynthesis of

297   maternally derived symbionts and heterotrophic feeding using tentacles (Mizrahi et al.,

298   2014; Richmond, 1985). As a consequence, the plasticity in metamorphosis during the

299   dispersive phase could be a strategy for coping with environmental stress in coral larvae,

300   although it remains to be determined whether these floating polyps are capable of

301   settling and contributing to recruitment in natural conditions.

302

303   **4.2 Symbiont responses to elevated and fluctuating temperatures**

304   The reduction in F_v/F_m at 31 °C does not indicate severe damage to the photosynthetic

305   apparatus or chronic photoinhibition as values were still within the healthy range (Hill

306   and Ralph, 2005). The fluctuating regime showed positive effects on $\Delta F/F_m'$, suggesting

307   a greater light use efficiency to drive photochemical process. Q_m, an indicator of the

308   excitation pressure over PSII, was lessened at fluctuating treatments, reflective of a

309   stronger competitiveness of photochemical process for reaction centers over

310   nonphotochemical quenching (Iglesias-Prieto et al., 2004). The higher $\Delta F/F_m'$ and

311   lowered Q_m under fluctuating conditions suggest that the diel temperature oscillations

312   could mitigate the heat stress on corals and corroborate previous findings that

313   temperature fluctuations are favorable for the photo-physiology of corals (Mayfield et

314   al., 2012; Putnam et al., 2010). The positive effect on these photo-physiological metrics

315   from exposure to fluctuating temperatures may be associated with the cooling effect at

316   night and upregulation of the genes related to photosynthesis (Mayfield et al., 2012).

317   In contrast to these aforementioned studies, Putnam and Edmunds (2008) found that

318   when incubated at fluctuating temperatures (26–32 °C), F_v/F_m of *P. meandrina* and

319   *Porites rus* nubbins were depressed by ~20% compared to those maintained at a

320   constant temperature of 28 °C. These contrasting results are possibly due to

321   methodological differences. Our study and Mayfield et al. (2012) mimicked the

322   fluctuations by progressively modulating temperatures over time which closely

323   resemble the natural settings, whereas Putnam and Edmunds (2008) directly transferred

324   corals from low to high temperature in the morning morning and vice versa at night.

325   This approach could cause instant heat-shock and prolonged exposure to extreme

326   temperatures, thereby overestimating the effects of diurnal thermal fluctuations.

327   The +2 °C treatment resulted in increased bleaching of juvenile *P. damicornis*, a

328   result consistent with previous work showing the sensitivity of endosymbionts within

329   coral recruits to elevated temperatures (Anlauf et al., 2011; Inoue et al., 2012). Higher

330   levels of symbiont expulsion will reduce the accumulation of reactive oxygen species

331   (ROS) within *Symbiodinium* (Haryanti et al., 2015; Yakovleva et al., 2009), therefore

332   resulting in high survival rates.

333   Further, daytime exposure to high temperatures in fluctuating treatments did not

334   amplify the effects of elevated temperature on bleaching response of *P. damicornis*.

335   This observation is in stark contrast to that of Putnam and Edmunds (2011) for adult

336   corals. That study found that ephemeral exposure to 30 °C at noon in fluctuating

337   conditions (26–30 °C) elicited a 45% reduction in symbiont density of adult *P.*

338   *meandrina* compared to those in steady 28 °C, an effect size larger than that arising

339   from continuous exposure to 30 °C (36%). The discrepancy between this study and our

340   results may reflect of a greater thermal tolerance of juvenile corals. The flat structure

341   of juvenile corals has been suggested to provide a higher mass transfer capacity to

342   remove ROS, which would enhance thermal tolerance (Loya et al., 2001) .

343

344   **4.3 Accelerated early development at elevated temperature**

345   Early development of juvenile *P. damicornis*, including budding, lateral growth and

346   calcification, was accelerated at 31°C, which is 2°C above the local long-term summer

347   mean and 1°C above bleaching threshold (Li et al., 2012). Growth stimulation by

348   temperature increase also occurred in a pilot study which showed that, after two weeks

349   at 31 °C, lateral growth and budding of *P. damicornis* was 10% and 41% higher than

350   those at 29 °C (Fig. S3). In contrast to our study with a tropical coral, a previous study

351   reported that calcification of symbiotic polyps of *Acropora digitifera* in subtropical

352   Okinawa was highest at 29 °C (2 °C above the local summer mean), and was reduced

353   at 31 °C (Inoue et al., 2012).

354   Given the great seasonal temperature fluctuations and ranges in our study site (Fig.

355   S1), it is not surprising that *P. damicornis* grew faster at 31 °C. However, the consistent

356   positive effects of 2 °C increase on calcification by both *P. damicornis* and *A. digitifera*

357   does not support the suggestion that warming is likely more deleterious to early stages

358   of tropical corals as compared to subtropical species (Woolsey et al., 2014). Tropical

359   corals dwelling in thermally dynamic habitats may also have the capacity to modify

360   their thermal limits, thereby enhancing physiological performance and tolerance under

361   increasing temperatures (Clausen and Roth, 1975; Dandan et al., 2015; Schoepf et al.,

362   2015).

363   Unlike previous findings of the synchronized reductions in symbiont density and

364   calcification in corals (Inoue et al., 2012; Tremblay et al., 2016), our study suggests

365   symbiont loss and calcification are decoupled at increased temperatures. There are two

366   possible explanation for this phenomenon. Firstly, the enhanced internal light fields

367   during bleaching could bring about 2- to 3-fold increase in symbiont specific

368   productivity (Wangpraseurt et al., 2017), thereby compensating for symbiont loss.

369   Unfortunately, the small size of juveniles and the variable temperatures here made it

370   difficult to measure photosynthesis. However, given the dramatic increases in skeletal

371   growth and new polyp formation at 31 °C, it is plausible that photosynthesis was not

372   compromised, or only minimally impacted, to support the observed growth.

373   Secondly, since coral calcification is positively correlated with carbon translocation

374   between *Symbiodinium* and host (Tremblay et al., 2016), the elevated calcification and

375   growth at 31 °C indicates more efficient nutritional exchange, thus sustaining the

376   metabolic expenditure of faster development. This interpretation is further evidenced

377   by the excessive deviation of Q_{10} from the kinetic expectations (2–3): this signifies a

378   strong amplifying effect through changes in fundamental biochemical systems along

379   with the acceleration of functional enzyme activities at increased temperatures

380   (Hochachka and Somero, 2002).

381

4.4 Differing effects of temperature fluctuations on growth

The growth-related processes, including budding, lateral growth and calcification differ

in their responses to temperature fluctuations, with calcification being more responsive.

The lack of statistically significant effects of temperature fluctuations on budding and

lateral growth suggests that either these processes were not affected by fluctuating

temperatures, or the length of exposure to the peak temperatures may not be long

enough to trigger a detectable effect (Lucas and Costlow, 1979).

The impact of fluctuating temperatures on calcification was different at ambient and

elevated temperatures: the fluctuating treatment did not affect calcification at 29 °C,

but resulted in a significant decline at 31 °C. This decline may be a result of inhibition

by the maximum temperature (33 °C) reached in this treatment. In comparison, prior

studies with corals did not find an influence of temperature fluctuations on skeletal

growth (Mayfield et al., 2012; Putnam and Edmunds, 2011). It is likely that the impact

of temperature fluctuations depends critically on whether the temperature ranges

encompass the maximum thermal limits of the species (Vasseur et al., 2014).

The notable reduction in calcification under a fluctuating regime around 31 °C may

be explained by two processes. First, during the hottest part of a daily temperature cycle,

metabolic rates will usually be depressed to improve energy conservation (Marshall and

McQuaid, 2010; Putnam and Edmunds, 2008; Sastry, 1979). Depression in metabolism

and ATP production in this specific "quiescent" period may impose constraints on

daytime calcification, as calcification is energetically costly, consuming up to 30% of

the coral's energy budget (Allemand et al., 2011). An alternative and nonexclusive

404 explanation is that daytime exposure to extreme temperature could disturb the function

405 and/or synthesis of skeletal organic matrix (OM) within the calcifying medium. The

406 OM plays critical roles in calcification such as calcium binding, providing carbonic

407 anhydrase and the template for crystal nucleation (Allemand et al., 2011). Daytime

408 temperatures of 33 °C may disrupt the function carbonic anhydrases (Graham et al.,

409 2015), therefore greatly inhibiting the conversion of respired CO_2 to bicarbonate for

410 subsequent use in calcification.

411 Further, since the OM itself is also incorporated into the skeleton, the rate of OM

412 synthesis is also a limiting factor for calcification (Puverel et al., 2005; Allemand et al.,

413 2011). Extreme temperatures may impede the production of OM as it is highly sensitive

414 and vulnerable to short-term thermal stress (Desalvo et al., 2010; Desalvo et al., 2008;

415 Maor-Landaw et al., 2014). Although the exact mechanism has not yet been fully

416 resolved, our study provides evidence that daytime exposure to extreme temperature in

417 variable thermal conditions would adversely affect calcification, and dampen the

418 stimulation of skeletal growth in *P. damicornis* at elevated temperature.

419

420 **5 Conclusions**

421 This study was the first to examine the effects of both increased temperature and daily

422 temperature variability on the early stages of a reef coral. Using realistic diurnal

423 temperature patterns, we found that thermal stress on larval settlement was greatly

424 tempered by diurnal temperature fluctuations, whilst the fluctuating regime had varied

425 effects on the physiology and early development of *P. damicornis*. Diel oscillations in

426 temperature did not aggravate bleaching but reduced the heat stress on photo-

427 physiology. Further, temperature fluctuations had no obvious effects on budding and

428 lateral growth, but two hours' exposure to 33 °C during daytime apparently caused a

429 reduction in calcification. Results reported here emphasize the distinction between

430 constant and fluctuating temperature effects, not only for different mean temperatures

431 but also on two successive life stages, and highlight the importance of incorporating

432 diurnal trends in unravelling the influence of ocean warming on coral biology.

433

434 **Data availability**

435 Data associated with the present study are available from the corresponding author upon

436 request.

437

438 **Author contributions**

439 L. J. and H. H. conceived and designed the experiments; L. J., Y. F. S., and Y. Y. Z.

440 performed the experiments; X. B. L., L. J. M., J. S. L., X. M. L., G.W. Z., S. L., and P.

441 Y. Q. contributed analysis and materials. L. J wrote the manuscript with comments from

442 all co-authors.

443

444 **Competing interests**

445 The authors declare that they have no conflict of interest.

446

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613   **Figures and captions**

614
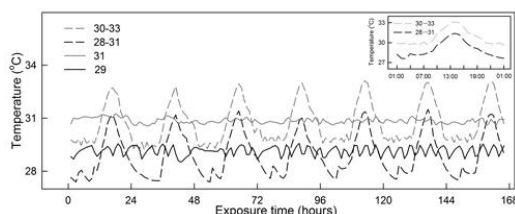
Fig 1. Temperature profiles for each treatment throughout the experiment. The inset

shows the one-day temperature trajectory in the two oscillating treatments. Time course

in fluctuating treatments was: 10 h at minimum temperature; 5 h of upward ramping; 2

h at maximum temperature; 7 h of downward ramping (passive).

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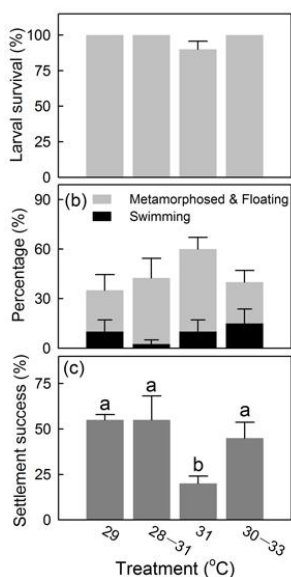
Fig 2. Percentage of *P. damicornis* larvae that (a) survived, (b) metamorphosed while

floating and remained pear-shaped, and (c) successfully settled after 24 h exposure to

temperature treatments. Error bars represent 1SEM. Different letters denote significant

differences between treatments.

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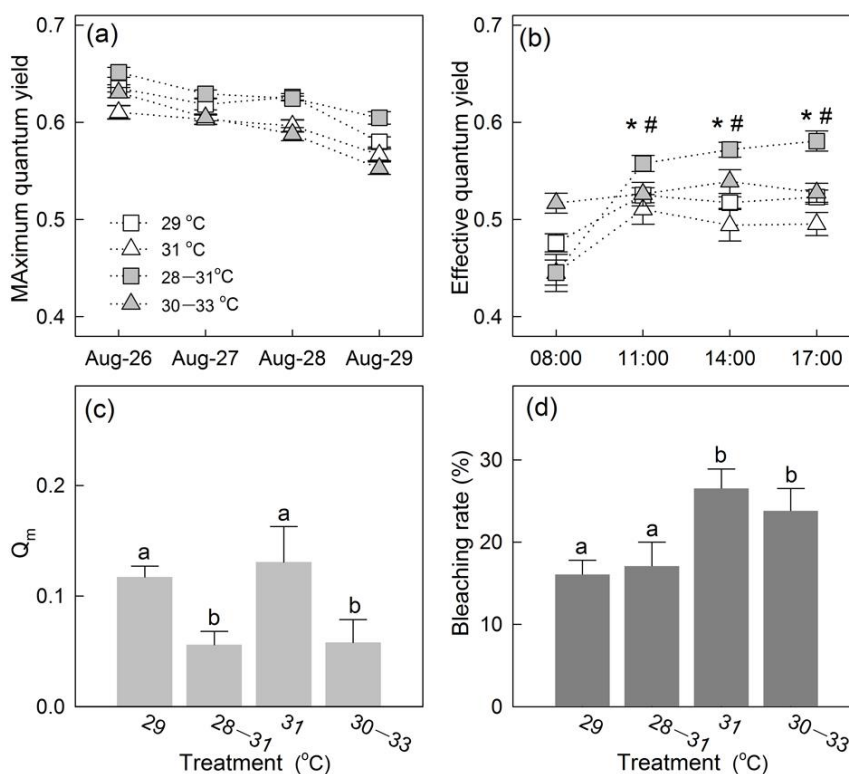
Fig 3. Photo-physiology and bleaching of *P. damicornis* recruits under constant and

fluctuating conditions of two temperatures (29 and 31 °C). (a) F_v/F_m over four

consecutive days, (b) $\Delta F/F_m'$ throughout the last day of the experiment, (c) Q_m and (d)

bleaching rates. Error bars represent 1SEM. Asterisks and hashes indicate significant

effects of temperature increase and fluctuations at a specific time, respectively.

Different letters represent significant differences between treatments.

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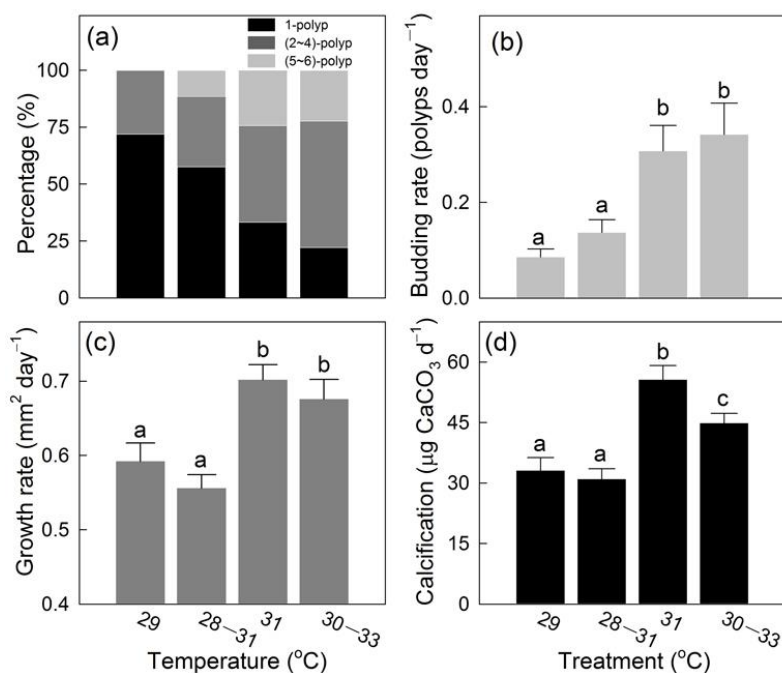
Fig 4. (a) Budding state, (b) polyp formation rate, (c) lateral growth and (d) calcification

of *P. damicornis* recruits under constant and fluctuating conditions of two temperatures

(29 and 31 °C). Error bars represent 1SEM. Different letters denote significant

differences between treatments.
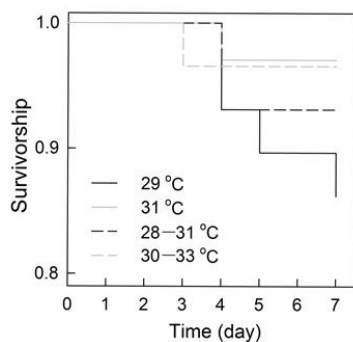
Fig 5. Survivorship of *P. damicornis* recruits estimated using Kplan-Meier analysis in

each treatment over the 7-day experiment.