Authors' response to reviewers' comments on the manuscript bg-2017-120 "Impact of diurnal temperature fluctuations on larval settlement and growth of the reef coral *Pocillopora damicornis*" by Lei Jiang et al.

To the Editor

Dear Dr. Christine Klaas,

We would express our sincerest gratitude for your help to correct some errors in the early version of this manuscript, and all the time and efforts it took to develop this manuscript and the review process. We appreciate the constructive comment from the two reviewers. We have carefully considered and incorporated the comments and suggestions from both reviewers and the point-by-point responses are attached as follows. Moreover, we have sent this manuscript for English editing service and amended all the potential grammar errors and wording changes. We are looking forward to receiving your response soon.

Best wishes,

Lei Jiang on behalf of all authors, jianglei12@mails.ucas.ac.cn

To Referee#1 Dr. D. Barshis

[*General comments*] *The authors present a comprehensive assessment of the role of diurnally fluctuating temperatures on growth, settlement, and bleaching response of larvae from the coral* Pocillopora damicornis. *The study is quite sound and represents an important contribution to the field. Most coral thermal stress studies use static temperature exposures, hence a movement in the field to more realistic natural thermal profiles is desperately needed. Yet we still lack a fundamental understanding of the different responses of corals to static or variable temperatures in the same study. This research begins to fill in that gap and the manuscript is technically sound and well-presented. There are a few minor comments that should be addressed prior to publication as well as an additional reference that should be integrated into the discussion on growth (see line-by-line comments below). Also, while the writing is generally sound, there are a few instances of misuse of the word "the" and singular/plural errors that may be resolved by additional editing of the language. All in all, I think this is a sound paper that makes an important and needed contribution to the literature.*

[Reply] Thanks for the positive comments regarding our manuscript and other insightful and helpful suggestions. We have integrated all the constructive suggestions and further resolved the mistakes about the wording and singular/plural errors through English editing service.

Reply to specific line-by-line comments:

[*Comment 1*] *Line 197. Siebeck found brightness and saturation to be indicative of bleaching, why was only saturation used?*

[Reply] Work by Siebeck *et al.*, 2006 suggested that for pictures of bleached *Pocillopora damicornis*, there were reduced saturation and elevated brightness values. Here, we measured the saturation and brightness values simultaneously and observed the reduction in saturation and increase in brightness (Fig S2). We only presented the saturation value to illustrate the paling of corals at elevated temperatures in the manuscript. We will further include the data on saturation and brightness in **Supplement (Fig. S2)**. Please refer to the Fig. S2 below for further details.

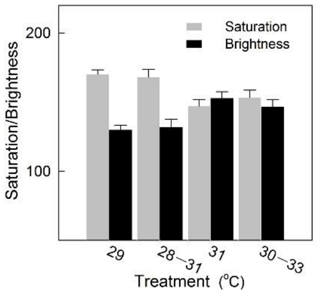

Fig. S2 Photographic metrics for *Pocillopora damicornis* recruits at different temperature treatments.

[*Comment 2*] *Section 2.7 Please specify the software used for statistical tests and copies of code (as supplementary information) if possible.*

[Reply] All statistical analyses were performed with STATISTICA version 12.0 (Statsoft). This will be clarified in the text and **Supplement**.

[*Comment 3*] *Line 250-252. Confusing wording. Please clarify that both the elevated 31 ℃ stable and 30-33 ℃ fluctuating treatments induced bleaching while the control and 28-31 ℃ fluctuating treatments did not.*

[Reply] Revised as suggested. Now it reads "Recruits at 31 ℃ exhibited a paler appearance than those at 29 ℃, as evidenced by the reduction in saturation and increase in brightness (Fig. S2). However, bleaching index which accounts for differences in recruit size, was unaffected by temperature level, regime, or their interaction (Fig. 3d)". Please see **[Reply]** to **[*Comment 4*]** below for explanations and details.

[*Comment 4*] *Lines 327-332. Would add discussion of the increased growth and survival in the higher temps. They may have decreased in color saturation but were not "stressed" according to the other metrics. There could also be a confound wherein a faster growing colony might pale simply because it's growing faster than the* Symbiodinium *are dividing so it's not losing cells, just diluting pigment. The photographic technique here does not allow for analysis of cell loss and it's unclear over how much area saturation was measured (i.e. how many pixels) and whether it was normalized to surface area or polyp number to account for size differences.*

[Reply] The discussion of increased growth and survival at higher temperatures will be added as follows, "Moreover, recruits with increased growth rates at elevated temperatures showed higher survivorship, consistent with previous field observations that survival in early stages of reef corals was strongly dependent on colony size and growth rates (Babcock and Mundy, 1996; Hughes and Jackson, 1985)".

After carefully examining our data, results totally supported the idea of the reviewer that coral recruits just became paling because of the faster growth and the resultant dilution of pigments. We are thankful to the reviewer for pointing out this puzzle and error. Generally, saturation and brightness of each recruit, were measured by taking the average value of 30 randomly placed quadrats (100×100 pixels each) on each coral picture using Photoshop's histogram function (Siebeck et al., 2006). The quantification of bleaching rates in juvenile corals was quite different from that was employed for adult branches in Siebeck et al., 2006. For adult branches, the mean saturation values can be taken as the proxy for symbiont density, however, for the new recruits here, only the saturation cannot totally reflect the change in symbiont content in coral holobiont. The bleaching index should consider the change in total content rather than the mean density, because all recruits came from a single coral larva and recruits had significantly different surface area after exposure to different temperature conditions. Therefore, to account for the size difference between different treatments, the total chlorophyll/symbiont content of each recruit was determined by multiplying the mean saturation by surface area (as measured in Section 2.6). Bleaching response can be further quantified as the reduction in chlorophyll/symbiont content of each recruit relative to the one yielding the maximum value. Since we got similar results from both saturation and brightness measurements, we only presented that results calculated from saturation in Fig. 3d.

Consequently, this would change our previous result about the bleaching response. In fact, recruits at 31 ℃ only exhibited a visible paling because of the faster growth rates and the resultant dilution of pigments, and there was no obvious bleaching either under elevated temperature or temperature fluctuations (Fig. 3d). We have amended this error in the whole manuscript.

References:

1. Babcock R, Mundy C. Coral recruitment: consequences of settlement choice for early growth and survivorship in two scleractinians. J Exp Mar Biol Ecol 206:179–201, 1996.
2. Hughes TP, Jackson JBC. Population dynamics and life histories of foliaceous corals. Ecol Monogr 55:141–66, 1985.
3. Siebeck, U., Marshall, N., Klüter, A., and Hoegh-Guldberg, O.: Monitoring coral bleaching using a colour reference card, Coral Reefs, 25, 453-460, 2006.

[*Comment 5*] *Section 4.4 Please see Buddemeier et al 2008 A modeling tool to evaluate regional coral reef responses to changes in climate and ocean chemistry. Limnology and Oceanography Methods. Particularly their meta-analysis in Figure 2. An alternative explanation may simply be a decreasing slope of the temperature x calcification relationship at higher temperatures as you approach the optimum (Buddemeier Fig. 2), wherein the corals are not calcifying linearly within the temperature fluctuation (i.e. at temperatures above the mean they're not growing much faster and they are growing slower at temperatures below the mean thus resulting in overall decreased calcification in comparison to 31 stable).*

[Reply] Thanks for the suggestion on reference and the idea about the non-linear relationship between calcification and temperature. The response of coral skeletal growth to temperature is non-linear and characterized by a parabola whose apogee indicates an optimum and threshold, beyond which the stimulatory impact of temperature will be reversed (Buddemeier et al., 2008; Castillo et al., 2014; Pratchett et al., 2015). Therefore, although the optimal temperature for calcification by *P. damicornis* recruits remains unknown here, it is possible that in the fluctuating 31 ℃, recruits may calcify at a slower rate when temperature was above 31 ℃ during daytime and below 31 ℃ during night, thus leading to an overall decrease in calcification compared to the constant 31 ℃. We have included this alternative explanation in the text as follows "The relationship between skeletal growth in corals and temperature is non-linear and characterized by a parabola whose apogee indicated an optimum and threshold, beyond which the stimulatory impact of temperature will be reversed (Buddemeier et al., 2008; Castillo et al., 2014; Inoue et al., 2012; W ŕum et al., 2007). Although the optimal temperature for calcification by P. damicornis recruits remains unknown, it is possible that the recruits exposed to the fluctuating 31 ℃ treatment calcified at a slower rate when the temperature was below 31 ℃ compared to those in the constant 31 ℃. However, given the well-established temperature performance curve for coral calcification (Buddemeier et al., 2008; W ŕum et al., 2007), daytime exposure to temperatures above 32 ℃ would have severely impaired the calcification process, thus leading to an overall decrease in calcification".

Reference:

1. Buddemeier R W, Jokiel P L, Zimmerman K M, et al. A modeling tool to evaluate regional coral reef responses to changes in climate and ocean chemistry [J]. Limnology & Oceanography Methods, 6(9):395-411, 2008.
2. Pratchett M S, Anderson K D, Hoogenboom M O, et al. Spatial, Temporal and Taxonomic Variation in Coral Growth-Implications for the Structure and Function of Coral Reef Ecosystems [J]. Oceanography & Marine Biology, 53:215-295, 2015.
3. Castillo K D, Ries J B, Bruno J F, et al. The reef-building coral *Siderastrea siderea* exhibits parabolic responses to ocean acidification and warming [J]. Proceedings Biological Sciences, 281(1797), 2014.

To Referee#2 Dr. E. Rivest

General comments: *In their manuscript titled "Impact of diurnal temperature fluctuations on larval settlement and growth of the reef coral* Pocillopora damicornis*," the authors present research on an exciting and timely topic – the effect of temperature variability on thermotolerance of two life history stages of a common reef-building coral. The topic is within the scope of the journal and the focus on effects of environmental variability is still novel within the coral field. Unfortunately, I find that this paper is not suitable for publication in its present form. There are several general ways in which this manuscript can be improved.*

[Reply] We are deeply grateful for the supreme and considerable efforts of the reviewer to give these valuable and helpful comments. We carefully considered the suggestions and corrections, and made the structure clearer and text more evident to the broad readership of Biogeosciences.

Reply to specific line-by-line comments:

[*Comment 1*] *The Introduction should include a description of the study species and of their reproduction (brooding) and the fact that the larvae contain symbionts upon release. These are critical pieces of information that the general readership of Biogeosciences will likely not know and are important for properly interpreting the results.*

[Reply] Thanks for the suggestion about providing the basic information about the reproductive biology and vertical transmission mode in this coral species. These facts will be added in Introduction. "*P. damicornis* is a widely distributed and major reef-building coral on reef flats in the Indo-Pacific region (Veron 1986). This species planulates almost every month and the release of free-swimming and zooxanthellate planula larvae follows a lunar cycle (Fan et al., 2002)"

References:

1. Veron, J.E.N.. Corals of Australia and the Indo-Pacific. University of Hawaii Press, Honolulu 644 pp, 1986.
2. Fan TY, Li JJ, Ie SX, Fang LS. Lunar periodicity of larval release by pocilloporid corals in southern Taiwan. Zool Stud 41:288–294, 2002

[*Comment 2*] *The Methods needs a much better overall description of the experimental design. It is difficult to tell if the spat were from the same or separate trials. Furthermore, the experimental design is flawed because it does not include replication of the treatments and the culturing techniques are not shown to avoid imposing artifacts on the responses of the corals.*

[Reply] Sorry for the confusion about the origin of coral spats in Methods. We have revised to make it clear about the two separate experiments. For the settlement assays, larvae were introduced to the petri-dishes with seawater and a CCA chip to test the effects of temperature treatments on larval settlement. Furthermore, another batch of larvae were transferred to petri-dishes and allowed to settle within 20 hours. Afterwards, these newly settled recruits were randomly assigned to treatment tanks to investigate the temperature effects on the early survival and growth of recruits. These important details will be included in the text.

It was a pity that the experimental design did not include replication and we have explicitly pointed out that limitation and problem in Methods. This problem was addressed by dispensing of larvae/recruits with randomization procedures and controlling other confounding factors such as salinity and light intensity which are of great importance to coral growth (Inoue et al., 2012; Dufault et al., 2013). Secondly, dishes were rotated daily to avoid the potential positional effects within each tank system. All these procedures were performed to ensure similar conditions across treatments except for temperatures during the experiment, and therefore the observed differences could be attributed to temperature treatments (Hurlbert 1984; Underwood 1997). Furthermore, this issue was also addressed by carefully examining the significance level of the treatment effects to make sure they were real (All the statistical results will be presented as Tables in Supplement).

References:

1. Dufault A M, Ninokawa A, Bramanti L, et al. The role of light in mediating the effects of ocean acidification on coral calcification [J]. Journal of Experimental Biology, 2013, 216(9):1570-7.
2. Inoue M, Shinmen K, Kawahata H, et al. Estimate of calcification responses to thermal and freshening stresses based on culture experiments with symbiotic and aposymbiotic primary polyps of a coral, *Acropora digitifera* [J]. Global & Planetary Change, 2012, 92-93(s 92–93):1-7.
3. Hurlbert S H. Pseudoreplication and the Design of Ecological Field Experiments [J]. Ecological Monographs, 1984, 54(2):187-211.
4. Underwood AJ. Experiments in ecology: Their logical design and interpretation using analysis of variance. Cambridge University Press, 1997.

[*Comment 3*] *The statistical tests and results need to be fully described. Posthoc analyses are not described. Table(s) with full results of all statistical models should be included, including results of* posthoc *analyses*

[Reply] In fact, the statistical results of *post-hoc* analyses have been displayed in the figures and in the text. In Line 235-238, Line 245-246, Line 250-252, Line 259-260 and Line 267-269, results of *post-hoc* analyses of settlement, budding, lateral growth and calcification were described. For instance, when describing the different effects temperature fluctuations on larval settlement and calcification at different mean temperatures, we were just depicting results from the post-hoc analyses. The detailed information of the *post-hoc* analyses will be included as Tables in **Supplement**.

[*Comment 4*] *More synthesis and integrative discussion is needed across all the responses measured to inform a broader picture of the implications for the ecology of this coral. The authors need to place their results in the broader context of biogeosciences and coral reef ecology.*

[Reply] Thanks for the suggestion about an integrative and broader discussion. However, we feel that results of this study may not be applied broadly, because the temperature variability in marine environment still cannot be accurately predicted by far. To do that, we changed the title of “Conclusions” to “Conclusions and implications”, and added a new paragraph after conclusions as follows: “The results of this study suggested that coral larvae subjected to diurnal temperature variations, especially at increased temperature, exhibit better settlement competence than those subjected to static thermal treatment. The fluctuating temperatures were favorable to the photo-physiology of endosymbionts and only had minor effects on post-settlement development of coral recruits. Therefore, for corals in highly fluctuating environments, they may have the potential to tolerate and acclimate to the changing seawater temperatures. These findings may also provide clues as to how diverse coral communities can persist and thrive in some thermally variable conditions (Craig et al., 2001; Richards et al., 2015). It is important to note that this study was technically limited to only one fluctuating amplitude, and the extent of thermal variance has as much of an impact on fitness as the changes in mean temperature (Vasseur et al., 2014). Given that there is currently still no consensus on the future temperature variability (Burroughs, 2007), it will be critical to study the impact of a broad range of thermal variations which corals may fare in a warming ocean.”

References:

1. Craig P, Birkeland C, Belliveau S. High temperatures tolerated by a diverse assemblage of shallow-water corals in American Samoa [J]. Coral Reefs, 20(2):185-189, 2001.
2. Richards Z T, Garcia R A, Wallace C C, et al. A Diverse Assemblage of Reef Corals Thriving in a Dynamic Intertidal Reef Setting (Bonaparte Archipelago, Kimberley, Australia) [J]. PLoS ONE, 10(2):e0117791, 2015.
3. Burroughs WJ (2007) Climate change: a multidisciplinary approach. Cambridge University Press
4. Vasseur DA, DeLong JP, Gilbert B, Greig HS, Harley CDG, McCann KS, Savage V, Tunney TD, O'Connor MI. Increased temperature variation poses a greater risk to species than climate warming. Proceedings of the Royal Society B: Biological Sciences 281, 2014.

[*Comment 5*] *L58-59 – “sea surface temperature have increased on average by 0.7 deg C”…since what date? A frame of reference is needed here.*

[Reply] Revised as suggested. Now it reads, **“**Sea surface temperatures have increased on average by 0.7 ℃ since preindustrial times (Feely et al., 2009)”.

Reference:

Feely R, Orr J, Fabry V, Kleypas J, Sabine C, Langdon C. Present and future changes in seawater chemistry due to ocean acidification. In: McPherson B, Sundquist E (eds) Carbon sequestration and its role in the global carbon cycle. Geophys Monogr Ser, Vol 183. AGU, p 175−188, 2009.

[*Comment 6*] *L65-70 – it would be good to cite studies that have quantitatively analyzed temperature variability for coral reefs here like Rivest and Gouhier, 2015 and Guadayol et al. 2014*

[Reply] Thanks for the suggestion on references. References have been included.

References:

1. Rivest E B, Gouhier T C. Complex environmental forcing across the biogeographical range of coral populations[J]. PLoS ONE, 10(3): e0121742, 2015.
2. Òscar Guadayol, Silbiger N J, Donahue M J, et al. Patterns in Temporal Variability of Temperature, Oxygen and pH along an Environmental Gradient in a Coral Reef[J]. PLoS ONE, 9(1): e85213, 2014.

[*Comment 7*] *L77-79 – actually, there are a handful of studies (at least 7) that have looked at the effects of temperature variability. I do see that the authors have described the results of a few of these studies in the next paragraph, but they should rephrase this sentence to better define the knowledge gap that their study aims to fill.*

[Reply] We have revised this sentence as suggested. Now it reads, “However, only a handful of studies have explored this thermodynamic effect on corals which routinely experience thermal oscillations in nature (e.g., Mayfield et al., 2012; Putnam et al., 2010)”

[*Comment 8*] *L83 – “more suited” is vague and confusing. Please be more specific here.*

[Reply] In Longman Dictionary of Contemporary English, “suit” means “be acceptable, suitable or convenient for a particular person or in a particular situation”. Therefore, we thought this word choice was proper.

[*Comment 9*] *L84 – “deleterious effects” of what? Diel temperature oscillations?*

[Reply] Revised as suggested. Now it reads, “Evidence for the deleterious effects of diel temperature oscillations includes the significant reductions in photochemical efficiency, symbiont density and aerobic respiration”.

[*Comment 10*] *L86 – “under diel temperature oscillations” compared to what?*

[Reply] We added the information as suggested. Now it reads, "exposed to fluctuating temperatures compared to those in constant temperatures".

[*Comment 11*] L90-93 – this statement needs references.

[Reply] References were added as required.

References:

1. Byrne M. Global change ecotoxicology: Identification of early life history bottlenecks in marine invertebrates, variable species responses and variable experimental approaches [J]. Marine Environmental Research, 2012, 76(2):3-15.
2. Keshavmurthy S, Fontana S, Mezaki T, et al. Doors are closing on early development in corals facing climate change [J]. Scientific Reports, 2014, 4:5633.

[*Comment 12*] *L126 – the date of collection of adult corals and the holding conditions of the corals prior to larval release need to be included. The temperature of the water at which the larvae were released should be included.*

[Reply] We have included information on date of collection and holding conditions. "Eight *P. damicornis* colonies were collected from 3 m depth on Luhuitou fringing reef on 20 August 2015. Colonies were transported to Tropical Marine Biological Research Station, and placed individually into 20 L flow-through tanks under partially shaded light conditions (noon irradiance, ~300 µmol photons m^{-2} s^{-1}) and ambient temperature (28.7 ± 0.5 ℃). The outflow of each tank was passed through a cup fitted with 180 µm mesh on the bottom to trap larvae"

[*Comment 13*] *L129 – "the recruit experiment" – is this the settlement or post-settlement experiment? This should be more clearly defined using a phrase like "to test the effects of xx on yy, larvae were transferred". This is confusing to the reader because the authors have not defined what settlers or recruits are. Remember – the audience is general and interdisciplinary. Or perhaps it would be clearer to describe more generally that the larvae and settlers are being tested in completely separate experiments?*

[Reply] Thanks for the suggestion. We revised the title of this section as "Collection and allocation of coral larvae", and this paragraph as well to clearly define the settlement and recruit experiments as follows "Larvae released from these colonies were collected at 07:00 on 22 August 2015, pooled and haphazardly assigned for the following experiments. For the settlement assays, larvae were transferred to 5.5-cm diameter plastic petri dishes as described below (see Section 2.4). To test the effects of temperature treatments on the photo-physiology and growth of recruits, another batch of larvae were transferred to 10-cm-diameter petri dishes which were left floating in a flow-through tank. Twenty hours later, 4 dishes with a total of 35–40 newly settled recruits were assigned to each treatment tank. Only recruits that settled individually and at least 1 cm apart from others were selected for the experiment to avoid possible contact between recruits through growth.”

[***Comment 14***] *L130 – were the dishes covered? Did the authors account for/measure effects of evaporation on salinity? Did the authors measure the temperature in the floating dishes during this time? Was there selection that could have influenced the performance of the spat? Again, “spat” is another new synonym used. Please choose one term for the juvenile corals, define it clearly for the reader, and use it consistently throughout the text.*

[Reply] All the dishes were covered with close-fitting lids to minimize evaporation while submerged in the tanks. Unfortunately, we did not measure the salinity of seawater within the dish after incubation. Preliminary measurements showed that the difference in seawater temperature between dishes and tanks was less than 0.4 ℃ and this information will be included in the text. The selection of recruits that were at least 1 cm apart from others was designed to make sure that they will not come into contact through lateral growth. Thanks for the suggestion of the wording for juvenile corals. We will use “recruits” consistently throughout the text.

[***Comment 15***] *L135 – “ambient temperature” where? At the collection site of the adult corals?*

[Reply] We revised this sentence to make it clear. Now it reads, **“**The 29 ℃ treatment, corresponding to the ambient temperature at the collection site of adult *P. damicornis*, was taken to represent the control treatment.”

[***Comment 16***] *L153-155 – these are results and should be moved to that section.*

[Reply] We agreed that these are also results. However, we feel that it is more suitable to present this in Section Materials and methods because they clearly illustrate detailed information on temperature treatments.

[***Comment 17***] *L155 – how was salinity checked?*

[Reply] **“**Salinity within each tank was measured using an Orion 013010MD conductivity probe twice a day”.

[***Comment 18***] *L159-162 – these are results and should be moved to that section.*

[Reply] Although this can also be regarded as results, information about the light conditions was a part of treatment conditions, and therefore we retained this in Materials and methods Section to show that the light conditions were precisely controlled and homogenous across tanks.

[*Comment 19*] *L162 – it is a significant limitation that the experiment has no true replication. I understand and empathize with the frustrations of facility and logistical constraints but more justification is needed for the validity of the results. Could the authors repeat the experiment to replicate the results in place of replication during the experiment?*

[Reply] We are sorry for this limitation and we have explicitly stated this problem in Methods. To try to eliminate other confounding effects, we randomly allocated coral recruits to each treatment and accurately controlled the salinity and light intensity between treatments. Furthermore, the dishes with recruits were rotated daily within each tank to minimize the potential positional effects. In fact, during the pilot study (as the results presented in Supplement), we failed to manipulate the fluctuating temperature treatments because of a technical problem, and therefore we only reported results of the constant temperature treatments in Supplement. The results of the pilot study were consistent with the later one on the aspect that the growth and development of *P. damicornis* recruits were accelerated at 31 ℃, therefore further consolidating our results.

[*Comment 20*] *L166 – the title "Settlement assay" makes me think that the authors are going to be testing effects on settlement and is confusing with "preparation of spat" in the title of the last section. Please revise.*

[Reply] We changed the structure in Methods and revised the title as suggested to make them clearer and easier to understand. Please see **[Reply]** to **[*Comment 2*] and [*Comment 13*]**.

[*Comment 21*] *L168 – is this species of CCA a natural settlement substrate for this species in your location? Please provide additional details here.*

[Reply] It has been shown that *P. damicornis* larvae have no specific discrimination for the settlement substrate and it can settle on plastic sheet without the presence of CCA (Hidaka 1985; Lei Jiang personal observation). *Hydrolithon reinboldii* is one of the most abundant CCA species in our study site and juvenile *P. damicornis* in the field are often found adjacent to *H. reinboldii* in our location. Our previous observation found that it was an effective settlement cue for *P. damicornis* larvae. These details are included in the text.

Reference:

Hidaka M. Tissue compatibility between colonies and between newly settled larvae of *Pocillopora damicornis* [J]. Coral Reefs, 1985, 4(2):111-116.

[*Comment 22*] *L170 – did the dishes have lids? Were they sealed in the treatment tank ("submerged")? What was the depth of the water in the dishes? It seems like a* *very high spat density in a small volume of water. Please provide justification that these are natural and representative settlement conditions for this species.*

[Reply] Yes, the petri dishes were covered with lids as they were submerged in the tanks. The depth of the water in each dish was approximately 7 mm. The volume of a single *P. damicornis* larvae ranged from 0.35-0.39 mm^3 (Isomura & Nishihira 2001; Edmunds et al., 2011), and the total volume of 15 larvae was approximately 5.3-5.8 mm^3, which only accounted for 0.04% of the total seawater volume (15 ml, 15,000 mm^3) in each dish. Furthermore, the larval density in the petri dishes here was 1 larva per ml, which is representative of that used in the literature (e.g., Heyward & Negri 2010; Putnam et al., 2008; Da-Anoy et al., 2017; Harii et al., 2010; Negri et al., 2005).

References:

1. Heyward A J, Negri A P. Plasticity of larval pre-competency in response to temperature: observations on multiple broadcast spawning coral species [J]. Coral Reefs, 2010, 29(3):631-636.
2. Putnam H M, Edmunds P J, Fan T Y. Effect of Temperature on the Settlement Choice and Photophysiology of Larvae From the Reef Coral *Stylophora pistillata* [J]. Biological Bulletin, 2008, 215(2):135-142.
3. Da-Anoy J P, Villanueva R D, Cabaitan P C, et al. Effects of coral extracts on survivorship, swimming behavior, and settlement of *Pocillopora damicornis*, larvae [J]. Journal of Experimental Marine Biology & Ecology, 2017, 486:93-97.
4. Harii S, Yamamoto M, Hoegh-Guldberg O. The relative contribution of dinoflagellate photosynthesis and stored lipids to the survivorship of symbiotic larvae of the reef-building corals [J]. Marine Biology, 2010, 157(6):1215-1224.
5. Isomura N, Nishihira M. Size variation of planulae and its effect on the lifetime of planulae in three Pocilloporid corals [J]. Coral Reefs, 2001, 20(3):309-315.
6. Edmunds P J, Cumbo V, Fan T Y. Effects of temperature on the respiration of brooded larvae from tropical reef corals [J]. Journal of Experimental Biology, 2011, 214(16):2783-90.
7. Negri A, Vollhardt C, Humphrey C, et al. Effects of the herbicide diuron on the early life history stages of coral [J]. Marine Pollution Bulletin, 2005, 51(1):370-383.

[*Comment 23*] *L180 – where did these spat come from? Were they from the “settlement assay” or from “preparation of spat”? Were they kept in the four treatments during this time? I can’t interpret the results of these tests without knowing these important details.*

[Reply] Sorry again for this structure problem and the confusion it caused. The larvae were mixed and randomly used for two separate experiments. Recruits for the post-settlement experiment were all from another batch of larvae which settled on 10-cm-diameter petri-dishes. Also refer to **[Reply]** to **[*Comment 2*], [*Comment 13*] and [*Comment 20*]**.

[*Comment 24*] *L194-195 – describe the settings for photography and illumination to allow others to replicate your measurements.*

[Reply] ISO setting of the camera was 12800 and the illumination provided while photographing was 35 μmol photons m^{-2} s^{-1}. This information will be added in the text.

[*Comment 25*] *L198 – the statistical comparison needs to be described here. What were the controls? Was the bleaching index assessed as relative to corals in the control treatment or was it a comparison of absolute values?*

[Reply] Saturation of each coral, a good proxy for chlorophyll/symbiont density (Siebeck et al. 2006), was measured by taking the average value of 30 randomly placed quadrats (100×100 pixels each) on each coral picture using Photoshop's histogram function. The total chlorophyll/symbiont content of each recruit was determined by multiplying the mean saturation by surface area (as measured in Section 2.6 below) to further account for the size difference. Bleaching response was quantified as the reduction in chlorophyll/symbiont content of each recruit relative to the one yielding the maximum value. Therefore, it was just a comparison of the relative values.

Reference:

Siebeck, U., Marshall, N., Klüter, A., and Hoegh-Guldberg, O.: Monitoring coral bleaching using a colour reference card, Coral Reefs, 25, 453-460, 2006.

[*Comment 26*] *L201 – which recruits? The ones assessed for bleaching? Different ones?*

[Reply] We are sorry for the unclear structure of Methods that made the reviewer feel perplexed. All the recruits in each treatment were checked daily for their survivorship. At the end of the experiment, recruits were also photographed to assess their surface area and bleaching response. They were the same batch throughout the recruit experiment. To make it clearer, we revised this sentence as "Throughout the recruit experiment, corals from each treatment were checked daily under a dissecting microscope and scored as alive or dead based on the presence of polyp tissue". Also refer to **[Reply]** to **[*Comment 2*], [*Comment 13*], [*Comment 20*] and [*Comment 23*]**.

[*Comment 27*] *L213 – details of post-hoc analyses need to be included.*

[Reply] When main effects were significant ($P < 0.05$), planned multiple comparisons following ANOVAs were conducted using Fisher's LSD tests (Day and Quinn, 1989). All the details of *post-hoc* analyses will be included in **Supplement**.

Reference:

Day, R. W., and Quinn, G. P.: Comparisons of treatments after an analysis of variance in ecology, Ecol Monogr, 59, 433-463, 1989.

[*Comment 28*] *L229-230 – is this 'normal' settlement behavior for this species? Could it be an artifact of the 'unnatural' settlement conditions?*

[Reply] It remains enigmatic whether it was "normal" settlement behavior or it was just an artifact of the "unnatural" settlement conditions. This phenomenon has been confirmed in a wide range of coral species in laboratory (Edmunds et al., 2001; Putnam et al., 2008; Vermeij, 2009; Mizrahi et al., 2014; Richmond, 1985; Denis et al., 2014). In the discussion part, we presented the possible ecological implications of this kind of larvae according to previous studies (Mizrahi et al., 2014; Richmond, 1985).

References:

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3. Vermeij, M. J. A.: Floating corallites: a new ecophenotype in scleractinian corals, Coral Reefs, 28, 987, 2009.
4. Mizrahi, D., Navarrete, S. A., and Flores, A. A. V.: Groups travel further: pelagic metamorphosis and polyp clustering allow higher dispersal potential in sun coral propagules, Coral Reefs, 33, 443-448, 2014.
5. Richmond, R. H.: Reversible metamorphosis in coral planula larvae, Mar. Ecol. Prog. Ser., 22, 181-185, 1985.
6. Denis V, Loubeyres M, Doo S S, et al. Can benthic algae mediate larval behavior and settlement of the coral *Acropora muricata*? [J]. Coral Reefs, 33(2):431-440, 2014.

[*Comment 29*] *L231-235 – since the results were not significant, there are no "distinct" differences. If the interaction is not significant, how can there be significant groupings stated on the figure (2c)?*

[Reply] It is certain that "despite a non-significant ANOVA F-test there are, in fact, significant differences between at least one set of means among the treatment groups tested which can be ultimately resolved using multiple comparison tests that have more power than the original ANOVA" (Underwood 1997; Dunne 2010; Lesser 2010). Therefore, it cannot exclude the possibility of significant groupings though the interaction term was not significant ($P < 0.05$). Firstly, the main effect of temperature on settlement was significant, and then the *post-hoc* analyses did show that the effects of temperature fluctuation were dependent on the mean temperature.

References:

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3. Lesser M P. Interactions between stressors on coral reefs: analytical approaches, re-analysis of old data, and different conclusions [J]. Coral Reefs, 2010, 29(3):615-619.

[*Comment 30*] *L237 – "greatly alleviated" is an interpretation and does not belong in the Results section. The phrase "in contrast" is inappropriate here because settlement success was not statistically distinct with that under the fluctuating and constant regimes at 29degC.*

[Reply] Revised as suggested. Now it reads "The settlement rate at fluctuating 31 ℃ was comparable to that in the control treatment, and significantly higher than that in the constant 31 ℃ treatment". About the phrase "in contrast", we thought it was appropriate. It was evident that the temperature fluctuations had different effect on settlement at different mean temperature levels. "Settlement was similar between fluctuating and constant regimes at 29 °C". However, it was not this case at 31 ℃. Hence, we used "in contrast" to make a comparison of the effects temperature fluctuations at 29 and 31 ℃.

[*Comment 31*] *L241 – what were the separate analyses?*

[Reply] Separate analyses meant separation of the results by timepoint, i.e., we analyzed the data separately for each timepoint. We revised this sentence as "Separation of the results by time showed that……"

[*Comment 32*] *L255 – replace "strongly" with "significantly." Also, the Chi-square test was not listed in the Results section. Please include.*

[Reply] The wording is changed as suggested. Moreover, the Chi-square test on the budding state among different treatments was included in Section 2.7 Data analyses in Methods. "Recruits were divided into 3 categories according to the number of polyps: 1-polyp, (2-4)-polyp and (5-6)-polyp. A Chi-square test was used to compare the differences in bud formation among treatments."

[*Comment 33*] *L264-267 – again how can the authors claim this if the model was not statistically significant?*

[Reply] Again, the reason was that the main effect of temperature was significant, and the temperature fluctuations had different effects on calcification at different mean temperatures as revealed by the *post-hoc* analyses. Please see the explanation in **[Reply]** to **[*Comment 29*]**.

[*Comment 34*] *L270 – survival of what?*

[Reply] Revised as suggested. Survival of recruits remained >86% in all treatments after 7 days.

[*Comment 35*] *L275 – this is the first time Q10 is mentioned. This needs to be included in the methods and defined carefully for the broad readership. Why was Q10 calculated for these results and not the others?*

[Reply] Thanks for the suggestion. The definition, calculation formula and the implications of temperature coefficient Q10 will be added in Methods. Q10 is widely used in temperature experiments to express the sensitivity of metabolism, development and growth to temperature changes (Hochachka & Somero 2002; Rivest & Hofmann 2014; Howe & Marshall 2001). Q10 was calculated using following equation: Q10 = (R2/R1) ^ (10/(T2-T1)), where R is the growth rate at temperature T2 or T1. Q10 values of enzyme-catalyzed reactions often double for the 10 ℃ increase in temperature. As Q10 is often calculated for respiration, growth and development, here it was calculated for changes in lateral growth, bud development and calcification at two temperatures.

Reference

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2. Rivest E B, Hofmann G E. Responses of the Metabolism of the Larvae of *Pocillopora damicornis* to Ocean Acidification and Warming[J]. PLoS ONE, 2014, 9(4): e96172.
3. Howe, S. A. and A. T. Marshall. Thermal compensation of metabolism in the temperate coral, *Plesiastrea versipora* (Lamarck, 1816). J. Exp. Mar. Biol. Ecol. 259: 231–248, 2001.

[*Comment 36*] *L279 –Based on my interpretation of the data, it was only lower at constant elevated temperatures.*

[Reply] Revised as suggested. Now it reads “The pronounced decline in successful settlement at constant 31 ℃”

[*Comment 37*] *L282 – “hardly impaired” – too qualitative*

[Reply] Revised as suggested. Now it reads, “Interestingly, the transient exposure to 33 ℃ in variable conditions did not produce the same negative response on larval settlement as constant exposure to 31 ℃; on the contrary, coral larvae experiencing diurnal shifts between 30 and 33 ℃ settled at a similar rate to those in the control”.

[*Comment 38*] *L283 – I am having difficulty with the phrase “greatly attenuated the thermal stress on settlement” throughout the manuscript (alleviated, mitigated, tempered….). Because of the lack of replication, it is hard to attribute the responses to thermal stress and constant vs. variable conditions. I think it would be better to say something like “did not produce the same negative response to high temperature as under exposure to constant high temperature.” Based on the experimental design, it is impossible to know whether the corals simply experienced less thermal stress overall because they spent some time at temperatures less than 31degC each day or if they responded differently to the high temperature. These mechanistic possibilities should be discussed and phrasing should be more careful.*

[Reply] Sorry for this confusion. We have revised the saying in Line 282-283 as suggested. Please see **[Reply]** to **[*Comment 37*]**. About the replication problem, please see **[Reply]** to **[*Comment 2*]** for explanations and details.

For the word choice of “mitigated, alleviated, tempered…….”, we should explain them one by one. For the first one in Line 32, since Q_m was lowered by temperature fluctuations, we revised it as “reduced the maximum excitation pressure”. In Line 236-237, we did agree with the reviewer’s comment that this was an interpretation and should be stated as facts. Therefore, we revised this sentence as “The settlement rate at fluctuating 31 ℃ was comparable to that in the control treatment and significantly higher than that in the constant 31 ℃ treatment”. For that in Line 282-283, we have revised following the reviewer’s suggestion. Please see **[Reply]** to **[*Comment 37*]**. For the one in Line 312, as stated before, there was a significant effect of temperature fluctuation on Q_m, and we revised it as “temperature oscillations could relieve the heat stress on corals”. For that in Line 423-424, we used the word “tempered” to state that the thermal stress caused by elevated mean temperature (31 ℃) on larval settlement was lessened by temperature fluctuations. This was consistent with the meaning of “temper” as “to make something less severe or extreme”,

Although, in the fluctuating treatment, corals spent some time at temperatures less than 31 ℃ each day compared to those in constant 31 ℃, that did not mean they experienced less thermal stress overall. Because the experiment was designed to create similar mean temperature values between constant and fluctuating temperature treatments. When determining thermal stress, there must be a reference level. Therefore, relative to control (29 ℃ in this study), the cumulative thermal stress, as assessed by degree heating days (Maynard et al., 2008), was equivalent for constant and fluctuating 31 ℃ treatments (corresponding to ~ 2 degree-heating day in the settlement assay). This index is useful in characterizing the experimental heating treatments and facilitating the comparison between temperature treatments (Oliver & Palumbi et al., 2011; Schoepf et al., 2015).

Moreover, we would like to thank the reviewer for the hint that larvae may respond differently to high temperatures. Previous studies have shown that short-term exposure (minutes to hours) of coral larvae to extremely high temperatures (33-37 ℃) would enhance the subsequent settlement at lower temperature, suggesting a strong latent effect (Coles 1985; Nozawa & Harrison, 2007). Therefore, another nonexclusive reason for the higher settlement in fluctuating 31 ℃ may be the 2-h exposure at 33 ℃ during daytime, thereby exerting a latent effect on settlement at night when the temperature was lowered. This possibility is added to the Discussion.

References:

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2. Schoepf, V., Stat, M., Falter, J. L., and McCulloch, M. T.: Limits to the thermal tolerance of corals adapted to a highly fluctuating, naturally extreme temperature environment. Sci. Rep., 5, 17639, 2015.
3. Oliver, T. A., and Palumbi, S. R.: Do fluctuating temperature environments elevate coral thermal tolerance?. Coral Reefs, 30, 429-440, , 2011
4. Coles SL. The effects of elevated temperature on reef coral planula settlement as related to power station entrainment. In: Proceedings of 5th international coral reef congress. 4:171–176, 1985.
5. Nozawa Y, Harrison P L. Effects of elevated temperature on larval settlement and post-settlement survival in scleractinian corals, *Acropora solitaryensis*, and *Favites chinensis* [J]. Marine Biology, 152(5):1181-1185, 2007.

[*Comment 39*] *L288 – I don't think the authors can say that fluctuating conditions favor settlement because the 29degC constant and fluctuating conditions produced statistically similar settlement rates. Furthermore, when did settlement happen? Did it happen during the daytime when temperatures were higher, or during the nighttime when temperatures were lower? These details could be important for appropriate interpretation of the results.*

[Reply] We totally agreed with the reviewer since fluctuating conditions did not impact settlement at 29 ℃. However, settlement rates at the mean temperature of 31 ℃ did differ between constant and fluctuating regimes. To address this, we revised this sentence to make it more specific. Now it reads, "whereas settlement may proceed as temperature descends to a more tolerable level at night (30 ℃ in this study). It is likely that the fluctuating temperature conditions may provide some respite for coral larvae, thereby favoring the settlement at elevated and fluctuating temperatures". For the purpose of not disturbing larvae while handling of petri dishes, we did not monitor settlement at multiple timepoints during incubation. Therefore, we added more discussion here to state this problem and further observations are clearly needed to confirm this hypothesis. The added discussion is as follows, "More precise assessment of settlement timing was not possible without disturbing larvae, given the use of small petri dishes. Future studies are needed to regularly observe and establish the dynamics of larval behavior under fluctuating temperatures to confirm this hypothesis.".

[*Comment 40*] *L298-301 – what about the desperate larval hypothesis?*

[Reply] The desperate larval hypothesis denotes that the non-feeding planktonic larvae become less discriminating in their selection of settlement substrate, i.e., more desperate to settle, as they age and energy reserves run low. The settlement assays only lasted 24 hours, and therefore the desperate larval hypothesis may not fit here.

[*Comment 41*] *L327 – both constant and fluctuating T treatments*

[Reply] The +2 ℃ treatment denoted both the constant and fluctuating 31 ℃ treatments.

[*Comment 42*] *L340-342 – this sentence needs to be better integrated with the paragraph*

[Reply] Thanks for this suggestion and we have revised this paragraph as follows: “Although juvenile P. damicornis at 31 ℃ exhibited apparent paling appearance compared to those in 29 ℃, loss of symbionts and bleaching were not indicated, as the faster lateral growth at 31 ℃ suggests that the paling is instead the result of pigment dilution due to a larger surface area. This outcome contrasts with previous work showing the sensitivity of endosymbionts within coral recruits to elevated temperatures (Anlauf et al., 2011; Inoue et al., 2012). The lack of bleaching response to elevated temperatures in the current study may be linked to the symbiont type. P. damicornis predominantly harbored Symbiodinium clade D in Luhuitou (Zhou, 2011), which has been found to be particularly thermally tolerant. In addition, the difference in treatment duration could also partially explain these contrasting sensitivities. Albeit ecologically relevant, the exposure duration in this study was much shorter than previous studies (Anlauf et al., 2011; Inoue et al., 2012), therefore resulting in less cumulative stress. It is possible that a loner exposure time may cause similar bleaching responses to those found by other studies.
Further, daytime exposure to high temperatures in fluctuating treatments did not induce significant symbiont loss in juvenile P. damicornis. This observation is in stark contrast to the observations of Putnam and Edmunds (2011) on adult corals. That study found that ephemeral exposure to 30 ℃ at noon in fluctuating conditions (26–30 ℃) elicited a 45% reduction in symbiont density of adult P. meandrina compared to corals at the steady 28 ℃ treatment, a larger effect than that was elicited by continuous exposure to 30 ℃ (36%). The flat structure of juvenile corals has been suggested to provide a higher mass transfer capacity to remove reactive oxygen species than the branching and three-dimensional adults (Loya et al., 2001). Hence, the discrepancy between our results and that of Putnam and Edmunds (2011) may, at least partially, be attributed to the morphology-specific difference in thermal tolerance of juvenile and adult corals”.

References:

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2. Putnam, H. M., and Edmunds, P. J.: The physiological response of reef corals to diel fluctuations in 570 seawater temperature, J. Exp. Mar. Biol. Ecol., 396, 216-223, 2011.
3. Zhou, G.W. Study on diversity of *Symbiodinium* and flexibility in scleractinian coral-algal symbiosis. Ph.D. thesis, Graduate School of Chinese Academy of Sciences, p 127.
4. Baker A C, Starger C J, Mcclanahan T R, et al. Coral reefs: corals' adaptive response to climate change [J]. Nature, 430(7001):741, 2004.

[*Comment 43*] *L344 – this section does not mesh well with the rest of the Discussion*

[Reply] In fact, section 4.3 was all about the higher growth rate and accelerated development at 31 ℃ compared to 29 ℃, which was an important and independent aspect of this study.

[*Comment 44*] *L407-410 – but calcification rates increased under the high temperature treatments…..?*

[Reply] Here we are only discussing about the possible explanation for the 20% reduction in calcification at fluctuating 31 ℃ relative to its constant counterpart. It did not contradict the fact that recruits calcified faster at higher temperatures.

[*Comment 45*] *L429 – but it was still elevated compared to the 29degC treatments... Figure S1. Panels a and b are not very relevant displays of temperature information for useful interpretation of the experimental design. A plot showing average seasonal daily temperature variability would be more useful. Plot d needs to have an x-axis label.*

[Reply] To make it clearer, now it reads “two hours’ exposure to 33 °C during the daytime apparently caused a reduction in calcification compared to constant exposure to 31 ℃”. For Fig S1., S1a did display the seasonal daily average temperatures and daily maximum and minimum values. The bold black line in Fig. s1a shows the daily average temperatures, and the shaded grey area illustrates the daily maximum and minimum temperatures Therefore, it did show the information about the seasonal daily temperature variability. The x-axial label “Date” for S1d was added.

[*Comment 46*] *L116 – Doesn’t the dataset go to 2016, not 2015?*

[Reply] Change was made as suggested in the text. Now it reads “Seawater temperatures at 3 m depth on Luhuitou fringing reef (18°12′N, 109°28′E) was recorded at 30 min intervals from 2012 to 2016.”

[*Comment 47*] *L123 – Should Fig. S1d be cited here instead of S1c?*

[Reply] Sorry for this mistake and correction was made accordingly in the text.

[*Comment 48*] *There are consistent errors in grammar and word choice throughout the manuscript. While it does not impede the reader from understanding the scientific content, I advise the authors to carefully copy edit the entire text.*

[Reply] We are truly sorry for this problem. With the help of Editor, reviewers and all authors, we have tried our best to correct errors in grammar and wording. We will further check the errors and improve the wording. After careful revision, the manuscript has been sent for English editing and we have carefully copy edited the entire text.

Impact of diurnal temperature fluctuations on larval settlement and growth of the reef coral *Pocillopora damicornis*

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Abstract

Diurnal fluctuations in seawater temperature are ubiquitous on tropical reef flats. However, the effects of such dynamic temperature variations on the ~~the~~ early stages of corals are poorly understood. ~~H~~In this study~~ere~~, we investigated the responses of larvae and new ~~spats~~recruits of *Pocillopora damicornis* to two constant temperature treatments (29 and 31 ℃), and two diurnally fluctuating treatments (28–31 and 30–33 ℃ with daily means of 29 and 31 ℃, respectively) simulating the 3 ℃ diel oscillations at 3 m depth on Luhuitou fringing reef (Sanya, China). Results showed that the thermal stress on settlement at 31 ℃ was almost negated by ~~the~~ the fluctuating treatment. Further, neither elevated temperature nor temperature fluctuations ~~did not exacerbate~~caused bleaching responses in recruits ~~but~~ , while ~~alleviated~~ the maximum excitation pressure over photosystem II (PSII) was reduced under fluctuating temperatures. Although early growth and development were highly stimulated at 31 ℃, oscillations of 3 ℃ had little effects on budding and lateral growth at either mean temperature. Nevertheless, daytime encounters with the maximum temperature of 33 ℃ in fluctuating 31 ℃ elicited a notable reduction in calcification compared to constant 31 ℃. These results underscore the complexity ~~in~~ of the effects caused by diel temperature fluctuations on early stages of corals, and suggest that ~~the~~ ecologically relevant temperature variability could buffer ~~the~~ warming stress on larval settlement and dampen the positive effects of increased temperatures on coral growth.

Keywords: temperature, diurnal fluctuation, *Pocillopora damicornis*, settlement, bleaching, calcification, budding

1 Introduction

Scleractinian corals and the reef ecosystems they construct are currently facing environmental changes at unprecedented rates of changes~~,~~. O~~o~~f ~~which the~~these changes, 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~~giving the affected colonies a pale appearance, 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).

On average~~Relative to preindustrial level~~, sea surface temperatures have increased ~~on average~~ by approximately 0.7 ℃ since preindustrial times (Feely et al., 2013) and a further increase of~~nother~~ 2–3 ℃ ~~increase are~~is expected by the end of this century

(Bopp et al., 2013), giving rise to increased concerns about effects on corals. T~~herefore, concerns about the devastating effects of rising temperatures on corals have escalated, and t~~he bulk of scientific work addressing the impact of ocean warming on corals ~~have~~ has ~~centered~~ focused on their tolerance ~~-~~and physiological responses to the predicted increases in mean temperature (Stambler, 2010). ~~Nevertheless~~However, seawater temperatures on coral reefs are characterized by striking fluctuations over timescales ranging from minutes to hours to months. ~~In particular~~Notably, temperature profiles from reef environments typically show diel oscillations of ~~up to~~ 4–10 ℃~~–~~ (Coles, 1997; Dandan et al., 2015; Guadayol et al., 2014; Oliver and Palumbi, 2011; Rivest and Gouhier, 2015)~~(Coles, 1997; Dandan et al., 2015; Mayfield et al., 2012; Oliver and Palumbi, 2011; Putnam and Edmunds, 2011)~~. A consistent daily cycle is commonly present, with temperature increasing after sunrise, peaking after noon and then gradually decreasing to the minimum (e.g., Zhang et al., 2013; Putnam and Edmunds, 2011).

It has been long ~~-~~established that the performance of organisms, including a diverse range of marine invertebrates, differs ~~in~~ between steady ~~versus~~ and variable thermal conditions of equivalent mean temperature~~, including a diverse range of marine invertebrates~~ (Bryars and Havenhand, 2006; Lucas and Costlow, 1979; Marshall and McQuaid, 2010; Orcutt and Porter, 1983; Pilditch and Grant, 1999; Sastry, 1979). These studies have demonstrated that temperature fluctuations can either speed up or retard early development and growth~~ can either be speeded up or retarded due to temperature fluctuations~~, depending upon the mean temperatures and amplitude

~~around the mean value~~of the fluctuations. However, few studies have explored this thermodynamic effect on corals which routinely experience temperature oscillations in nature (e.g., Mayfield et al., 2012; Putnam et al., 2010)~~ remains largely unstudied~~.

Recently, our understanding of the physiological responses of corals to diurnally fluctuating temperature has advanced, but results have been variable and even conflicting. For instance, the photo-physiology in larvale and adult~~s of~~ pocilloporid corals is more suited to the fluctuating than to the constant temperatures (Mayfield et al., 2012; Putnam et al., 2010). Conversely, evidence for the deleterious effects of diel temperature fluctuations includes the significant reductions in photochemical efficiency, symbiont density and aerobic respiration in corals exposed to fluctuating temperatures compared to those in constant temperatures ~~under diel temperature oscillations~~ (Putnam and Edmunds, 2011; Putnam and Edmunds, 2008). These contrasting results ~~reiterate~~ emphasize a clear need to further explore the impact of diurnally fluctuating temperatures, together with the projected increase in temperature on reef corals.

In the context of global deterioration of coral reefs and climate change, the early life history stages of corals have drawn increasing attention in recent decades, as they are more vulnerable to environmental changes than their adult counterparts, and more importantly, represent a bottleneck for the maintenance of populations (Byrne, 2012; Keshavmurthy et al., 2014). ~~The s~~Successful larval settlement, post-settlement survival and growth are of paramount importance to population persistence, as well as the recovery of degraded reefs (Ritson-Williams et al., 2009; Penin and Adjeroud, 2013). Mounting evidence suggests that ocean warming poses a serious threat to these early processes (reviewed in Keshavmurthy et al., 2014), but most~~ of those~~ previous experiments utilized steady temperature treatments, ~~therefore ~~neglecting the temporal variations of *in situ* temperature (but see Putnam et al., 2010). To date, there is a paucity of knowledge regarding the influence of dynamic temperatures on these crucial early stages ~~in ~~of reef corals. The risk imposed by ocean warming on fitness and development of corals ~~by ocean warming ~~can be best understood by integrating both~~such~~ diel thermocycles and changes in mean temperature (Boyd et al., 2016).

The present study aimed to investigate how the early stages of the reef coral *Pocillopora damicornis* will be affected by the diurnally oscillatory temperatures, together with ocean warming. *P. damicornis* is a widely distributed and major reef-building coral on reef flats in the Indo-Pacific region (Veron, 1993). This species planulates almost every month and the release of free-swimming and zooxanthellate planula larvae follows a lunar cycle (Fan et al., 2002). Brooded larvae and new ~~settlers~~recruits were exposed to ~~contrasts of ~~two temperature levels (29 and 31 ℃) ~~and ~~crossed with two temperatures regimes (constant and 3 ℃ diel fluctuations). Diurnal patterns of temperature fluctuations were ~~set according to~~based on~~ the~~ temperature records ~~in ~~from our study site, Luhuitou fringing reef in ~~,~~ Sanya, China. Larval condition and juvenile growth after incubation were assessed to compare their responses to constant and oscillatory temperatures.

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2 Materials and methods

2.1 Field seawater temperature monitoring

Seawater temperatures at 3 m depth on~~in~~ Luhuitou fringing reef (18°12′N, 109°28′E) were~~as~~ recorded at 30 min intervals from 2012 to ~~2015~~2016, using Hobo Pendant data loggers (Onset, USA). The temperature profiles showed large seasonal and diurnal fluctuations, with a~~the~~ maximum of 33.1 ℃ and a minimum of ~~33.1 ℃ and~~ 20.3 ℃ ~~respectively~~ (Fig. S1a). The mean annual temperature was 27 ℃ and the mean monthly ~~mean~~ temperature ranged from 22 ~~℃~~ to 30.2 ℃ (Fig. S1b). The d~~D~~iurnal range during summer (June–September) was between 0.6 and 5.4 ℃, with a mean value of 1.76 ℃ (Fig. S1c). ~~On a daily basis~~Each day, seawater temperature began to increase around 08:00, reached the maximum at 13:00, often remained constant for about two hours, and then gradually decreased (Fig. ~~S1c~~S1d).

2.2 ~~Larval c~~Collection and ~~preparation of spat~~allocation of coral larvae

Eight *P. damicornis* colonies were collected ~~from~~ at a depth of 3 m ~~depth in Luhuitou fringing reef~~on 20 August 2015. Colonies were transported to Tropical Marine Biological Research Station, and placed individually into 20 L flow-through tanks at ambient temperature (28.7 ± 0.5 ℃) under partially shaded light conditions (noon irradiance, ~300 µmol photons m^{-2} s^{-1}). The outflow of each tank was passed through a cup fitted with 180 µm mesh on the bottom to trap larvae. Larvae released from these colonies were collected at 07:00 on 22 August 2015, pooled and haphazardly assigned ~~for~~ to the following two experiments~~either the settlement or~~

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~~post-settlement experiments~~. For the settlement assays, larvae were introduced to 5.5-cm diameter plastic petri dishes as described below (see Section 2.4). To test the effects of temperature treatments on the photo-physiology and growth of recruits, another batch of larvae were transferred to 10-cm-diameter petri dishes which were left floating in a flow-through tank. Twenty hours later, 4 dishes with a total of 35–40 newly settled recruits were assigned to each treatment tank. Only recruits that settled individually and at least 1 cm apart from others were selected for the experiment in order to avoid possible contact between recruits during growth.~~For the recruit experiment, larvae were transferred to 10-cm-diameter petri dishes for settlement and dishes were left floating in a flow-through tank. Twenty hours later, 4 dishes with a total of 35–40 spats were assigned to each treatment tank. Only spat that settled individually and at least 1 cm apart from others were selected for the experiment.~~ Dishes were rotated daily to avoid the potential positional effects within each tank.

2.3 Experimental setup

The 29 ℃ treatment, corresponding to the ambient temperature ~~during this study~~at the collection site of adult *P. damicornis*, was ~~taken to represent~~used as the control treatment. The experimental temperature was 2 ℃ above the ambient and 1 ℃ above the bleaching threshold for coral communities on Luhuitou reef (30 ℃, Li et al., 2012), and within the range of projected increases (Bopp et al., 2013). Two temperature regimes, i.e., constant and fluctuating were set for each temperature level.

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The p~~P~~attern and range of temperatures in the two fluctuating ~~conditions ~~treatments were based on *in situ* records obtained during larval release of *P. damicornis* (Fig. S1d), and the assumption that the predicted 2 ℃ increase in mean temperature ~~will~~ would entail a 2 ℃ shift in the overall temperature ~~trajectory ~~time-course (Burroughs, 2007).

Four 40 L tanks were filled with sand-filtered seawater, which was ~~changed~~ partially changed (30%) with temperature-equilibrated seawater at 22:00 every day. Treatments were set using digital temperature regulators (Sieval, TC-05B, China) and 50 W heaters. The s~~S~~eawater was gently aerated and well mixed using submerged pumps (350 L h^{-1}). The water~~Water~~ temperature~~s~~ in each tank ~~were ~~was recorded with a Hobo Pendant logger~~s~~ at 15 min intervals throughout the experiment. In the two fluctuating treatments (Fig. 1), temperatures were programmed to increase from 28/30 ℃ at 08:00, reach the plateau of 31/33 ℃ around 13:00 and stabilize for 2 hours. A~~fterwards~~t 15:00, temperatures were allowed to decrease gradually to 28/30 ℃ around 22:00 and remained stable until 09:00 the next morning. Mean (± SD) daily temperature of the two stable treatments were 29 ± 0.2 and 30.8 ± 0.2 ℃, and the mean ~~values in~~temperatures of the two fluctuating treatments were 28.9 ± 1.3 and 30.7 ± 1.3 ℃ respectively. Salinity in each tank was checked using –an Orion 013010MD conductivity probe twice a day –and remained stable at 33 psu during the experiment.

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Each tank was illuminated by a LED lamp (Maxspect, 10,000K, China) on a 12:12 h light-dark cycle. Light was measured with a Li-Cor 4-π quantum sensor below the water surface. Light intensity was similar ~~among~~ in all tanks ($F_{3, 96} = 0.32$, $P = 0.81$), averaging at 183 ± 3 μmol photons m^{-2} s^{-1} (mean ± SE, n = 100), which was close to the irradiance in crevices where coral recruits were found at 3–4 m depths ~~in~~ at our study site (Lei Jiang, unpublished data). Facility and logistical constraints precluded the replication of treatments, but salinity and light were carefully controlled to eliminate any possible artefact (Underwood, 1997).

2.4 Settlement assay

The settlement experiments were conducted in 5.5-cm-diameter petri dishes on 22 August 2015 and began around 09:00. The c~~C~~rustose coralline algae (CCA), *Hydrolithon reinboldii*, one of the most abundant CCA species and an effective settlement cue for larval settlement of *P. damicornis* at our study site, ~~were~~ was collected at 2–3 m depths and cut into uniformly sized (5 × 5 × 3 mm) chips 4 days before the settlement experiment. Each dish contained 15 ml seawater and a CCA chip. Fifteen ~~15~~ actively swimming larvae were introduced into each dish, which was then ~~submerged~~ floated in the treatment tanks to ensure temperature control. Preliminary measurements showed that the difference in seawater temperature between dishes and tanks was less than 0.4 ℃. Four replicate dishes were used for each treatment. Larvae were allowed to settle for 24 hours, after which ~~the successful~~ settlement success was assessed under ~~the~~a dissecting microscope following the criteria of Heyward and Negri (1999). Larvae were categorized into four conditions: (i) dead, (ii) swimming, (iii) metamorphosed and floating in the water, i.e., premature metamorphosis (*sensu* Edmunds et al., 2001), and (iv) metamorphosed and firmly attached to CCA or dish, i.e., successful settlement.

2.5 Chlorophyll fluorescence and bleaching

Twenty 3-day-old ~~spat~~ recruits were randomly selected and marked in each treatment. ~~A~~ Diving-pulse-amplitude modulation (PAM) fluorometry (Walz, Germany) was used to measure the maximum quantum yield of PSII (F_v/F_m), a proxy for potential photochemical efficiency of symbionts (Genty et al., 1989). Measurements were conducted at 05:30 on four consecutive days to allow enough time for dark adaption. Both the measuring light and gain of PAM settings were adjusted to “7” to give optimal fluorescence signals ~~(specifically 7)~~.

To better assess the photo-physiological performance of symbionts, ~~At the last day of the experiment, e~~effective quantum yield ($\Delta F/F_m'$) was also measured for 15 recruits from each treatment ~~at~~ four time~~point~~s ~~(08:00, 11:00, 14:00, 17:00)~~on the last day of the experiment (08:00, 11:00, 14:00, 17:00). The maximum excitation pressure over PSII (Q_m) was calculated using the ~~following~~ equation: $Q_m = 1 - [(\Delta F/F_m'$ at 14:00)/(F_v/F_m)] (Iglesias-Prieto et al., 2004)~~, to better assess the photo-physiological performance of symbionts~~.

Bleaching response was assessed photographically following Siebeck et al. (2006) with some modifications. At the end of the experiment, ~~spat~~ recruits were photographed with a digital camera under the dissecting microscope and identical illumination (35 μmol photons m^{-2} s^{-1}). The camera was ~~adjusted to~~set on~~ the~~ manual mode with constant ISO settings (12800). Saturation of each coral picture, ~~Bleaching index was quantified as the reduction in saturation of each spat relative to the spat yielding the maximum saturation, because saturation in coral pictures is~~ a good proxy for symbiont or chlorophyll density during bleaching, was measured by taking the average value of 30 randomly placed quadrats (100×100 pixels each) on each coral picture using Photoshop's histogram function (Siebeck et al., 2006)~~.~~. The total chlorophyll/symbiont content of each recruit was determined by multiplying the mean saturation by surface area (as measured in Section 2.6 below) to account for differences in the size of recruits. Bleaching response was quantified as the reduction in chlorophyll/symbiont content of each recruit relative to the recruit yielding the maximum value.

2.6 Post-settlement survival and growth

Recruits were checked daily under a dissecting microscope throughout the experiment and scored as alive or dead based on the presence of polyp tissue. At each census, the number of living ~~spats~~ recruits ~~were~~ was recorded for each treatment. Digital images of recruits with scale calibration were also analyzed for lateral growth using ImageJ software (National Institutes of Health). The n~~N~~umber of polyps for each recruit ~~were~~ was ~~visually~~ counted visually. Juvenile growth was estimated as the rates of change in planar area and number of new polyps over time (Dufault et al., 2012; Jiang et al., 2015).

Calcification was calculated as the dry skeletal weight deposited per day (Dufault et al., 2012). Tissue of recruits was removed with a water-pick at the end of the experiment. Skeletons were weighed individually using an ultra-microbalance at an accuracy of ± 1 µg. Furthermore, the temperature coefficient (Q_{10}), which is widely used to express the sensitivity of metabolism, development and growth to temperature changes (Hochachka and Somero, 2002; Howe and Marshall, 2001; Rivest and Hofmann, 2014), was calculated using the equation: $Q_{10} = (R2/R1)$ ^ $(10/(T2-T1))$, where R is the growth rate at temperature T2 or T1. Q_{10} values of enzyme-catalyzed reactions often double for the 10 ℃ increase in temperature.

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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 ℃; constant and fluctuating regimes). When main effects were significant ($P < 0.05$), planned multiple comparisons were conducted using Fisher’s LSD tests, which are more powerful than ~~Fisher’s LSD test was performed as planned multiple comparisons following~~

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~~ANOVAs~~the original ANOVA~~–~~ (Day and Quinn, 1989; Lesser, 2010)~~(Day and Quinn, 1989)~~. Recruits were divided into 3 categories according to the number of polyps: 1-polyp, (2-4)-polyp and (5-6)-polyp. A Chi-square test was used to compare the differences in bud formation among treatments. ~~—~~Survivorship of coral ~~spats~~ recruits~~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. ~~–~~All statistical analyses were performed with STATISTICA version 12.0 (Statsoft).

3 Results

3.1~~–~~ Larval settlement

Larval mortality was only observed in the ~~the treatment with a~~ constant ~~temperature of~~ 31 ℃ treatment during the settlement assay (Fig. 2a). In all treatments, between 35 and ~~–~~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 the differences in these percentages among treatments were not significant ~~among treatments~~ (Table S1), there ~~was~~ were more metamorphosed and floating larvae ~~at~~ in the ~~a~~ constant ~~temperature~~ ~~of~~ 31 ℃ treatment than in ~~the~~ other treatments. Settlement was significantly affected by elevated temperature ~~($F_{1, 12}$ = 9.43, P = 0.01)~~ and marginally affected by the interaction between temperature level and regime~~Settlement success showed distinct different effects of temperature fluctuations vs. constant temperature~~

~~at 31 ℃~~ (~~Fig. 2c, interaction term: $F_{1, 12} = 4.00$, $P = 0.06$~~Table S1.). Specifically, percent s~~S~~ettlement was similar between the ~~fluctuating and constant~~two temperature regimes at 29 ℃~~; in contrast,~~ , but differed between the constant and ~~the thermal stress on settlement was greatly alleviated by diurnal~~ fluctuating treatments~~fluctuations~~ at 31 ℃. The settlement rate at fluctuating 31 ℃ was comparable to that in the control treatment~~,~~ and significantly higher than that in the constant 31 ℃ treatment (Fig. 2c, Table S2.).

3.2 Chlorophyll fluorescence and bleaching

A significant interaction between time, temperature level and regime was observed for maximum quantum yield F_v/F_m (~~$F_{3, 228} = 3.7$, $P = 0.01$~~Table S3., Fig. 3a). Separation of the results by time~~Separate analyses~~ showed that F_v/F_m was consistently lower at higher temperatures, but ~~this~~ the effect size was small, only amounting to a 3 % decrease (Table S4.). There was also a significant interaction between time, temperature level and temperature regime for effective quantum yield $\Delta F/F_m'$ (~~$F_{1, 56} = 21.5$, $P < 0.001$~~ Table S3.). Further separate analyses revealed that both temperature increase and fluctuations ~~showed~~ had strong effects except at 08:00 (Table S4.), with lower $\Delta F/F_m'$ at elevated temperature and higher $\Delta F/F_m'$ under fluctuating conditions (Fig. 3b).

Q_m, the maximum excitation pressure, was not influenced by elevated temperature (~~$F_{1, 56} = 0.14$, $P = 0.71$~~Table S5.). However, it was considerably reduced under fluctuating regimes (~~$F_{1, 56}$ = 10.4, *P* = 0.002,~~ Fig. 3c, Table S5.).~~–~~ Recruits at 31 ℃ exhibited a paler appearance than those at 29 ℃, as evidenced by the reduction in saturation and increase in brightness (Fig. S3). However, bleaching index which accounts for differences in recruit size, was unaffected by temperature level, regime, or their interaction (Fig. 3d, Table S5.)~~Bleaching rates increased by 40–65 % at elevated temperatures ($F_{1, 107}$ = 11.3, *P* = 0.001), while the fluctuating treatment showed little additional effect on bleaching rates ($F_{1, 107}$ = 0.02, *P* = 0.874, Fig. 3d).~~

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

The b~~B~~udding state of recruits differed ~~strongly~~ significantly among treatments (Chi-square test, χ^2 = 19.4, *df* = 6, *P* = 0.004). ~~After 7~~ Seven days after ~~post-~~settlement, approximately 70% of recruits at 31 ℃ produced at least one bud ~~at 31 ℃~~, compared to less than 50% of recruits at 29 ℃ (Fig. 4a). Budding rates at 31 ℃~~elevated temperature~~ were more than twice those at 29 ℃ (Fig. 4b, ~~$F_{1, 107}$ = 22.8, *P* < 0.001~~Table S6.). N~~At both temperatures, n~~o significant differences between the constant and fluctuating regimes were observed at either temperature (Table S6.~~$F_{1, 105}$ = 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, Table S6.). ~~S~~The skeletal weight deposited each day was ~~enhanced by~~ 56% higher at 31 ℃ ~~compared to~~than at 29 ℃ (Table S8.~~$F_{1, 96}$ = 36.7, *P* < 0.001~~). The effects of temperature fluctuations on calcification were dependent~~d~~ on the mean temperature (Fig. 4d), although the interaction between temperature level and regime was not statistically significant (Table S6.~~F$_{1, 96}$ = 2.07, *P* = 0.15~~). At 29 ℃, the fluctuating treatment had no discernible effect on calcification, while at 31 ℃ it caused a significant reduction (20%) in calcification compared to the constant regime (Table S7.).

Survival of recruits remained >86% in all treatments after 7 days, with the highest and lowest values at 31 ℃ (97%) and 29 ℃ (86%), respectively. Survivorship did not vary significantly across treatments (χ^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 respectively between 29 and 31 ℃, yielding a Q_{10} of 2.6, 36.8 and 17.8~~, respectively~~.

4 Discussion

4.1 Larval settlement under elevated and fluctuating temperatures

The pronounced declines in successful settlement at ~~elevated temperatures~~constant 31 ℃ ~~was~~ were consistent with ~~early~~ previous findings that reported the effects of thermal stress ~~effects~~ (>30 ℃) on coral larval settlement (Humanes et al., 2016; Randall and Szmant, 2009). Interestingly, transient exposure to 33 ℃ in variable conditions did not produce the same negative effect on larval settlement as constant exposure to 31 ℃~~the transient exposure to high temperatures in variable conditions~~

~~hardly impaired settlement~~; on the contrary, coral larvae experiencing ~~the~~ diurnal shifts ~~at~~ between 30 and ~~–~~33 ℃ ~~greatly attenuated the thermal stress on settlement~~settled at a similar rate to those in the control. During daytime ~~encounter with~~exposure to elevated and stressful temperatures, coral larvae may not initiate metamorphosis and settlement because larvae undergoing this complex stage are particularly susceptible to thermal perturbations (Randall and Szmant, 2009), ~~whereas~~ but settlement may proceed as temperature descends to a more tolerable level at night (~~28/~~30 ℃ in this study). It is likely that the fluctuating temperature conditions could provide some respite for coral larvae, thereby favoring settlement at elevated and fluctuating temperature conditions. More precise assessment of settlement timing was not possible without disturbing larvae, given the use of small petri dishes. Future studies are needed to regularly observe and establish the dynamics of larval behavior under fluctuating temperatures to confirm this hypothesis.

Another possible cause for the higher settlement of larvae in the fluctuating 31 ℃ treatment may be the brief exposure to extreme temperatures around noon. Previous studies have demonstrated that short-term exposure (minutes to hours) of coral larvae to extremely high temperatures (33-37 ℃) significantly enhanced the subsequent settlement at lower temperature, suggesting a strong latent effect (Coles, 1985; Nozawa and Harrison, 2007). Therefore, the 2-hour incubation at 33 ℃ during the daytime may have exerted a latent and stimulatory effect on settlement at night when the temperature was lower.

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Metamorphosed and floating larvae, previously noted in corals (Edmunds et al., 2001; Vermeij, 2009; Mizrahi et al., 2014; Richmond, 1985), were more frequent at elevated temperatures. One possible explanation is that ~~the~~ premature metamorphosis in coral larvae is a spontaneous response to increased temperatures (Edmunds et al., 2001). The floating polyps, as a result of pelagic metamorphosis, have been shown to have extended longevity, possibly because they can obtain energy from photosynthesis ~~of~~ by maternally derived symbionts and heterotrophic feeding using tentacles (Mizrahi et al., 2014; Richmond, 1985). ~~As a consequence~~Thus, ~~the~~ plasticity ~~in~~ of metamorphosis during the dispersive phase could be a strategy for coping with environmental stress in coral larvae, although it remains to be determined whether these floating polyps are capable of settling and contributing to recruitment in natural conditions.

4.2 Symbiont responses to elevated and fluctuating temperatures

The reduction in F_v/F_m at 31 ℃ does not indicate severe damage to the photosynthetic apparatus or chronic photoinhibition, as the values were still within the healthy range (Hill and Ralph, 2005). The fluctuating regime ~~showed~~ had positive effects on $\Delta F/F_m'$, suggesting a greater light use efficiency to drive photochemical processes. Q_m, an indicator of the excitation pressure over PSII, was ~~lessened at~~ reduced in fluctuating treatments, reflect~~ive of~~ing a stronger competitiveness of photochemical process for reaction centers over nonphotochemical quenching (Iglesias-Prieto et al., 2004). The higher $\Delta F/F_m'$ and lowered Q_m under fluctuating conditions suggest that the diel temperature oscillations could ~~mitigate~~ relieve ~~the~~ heat stress on corals and corroborate previous findings that temperature fluctuations are favorable to~~for~~ the photo-physiology of corals (Mayfield et al., 2012; Putnam et al., 2010). The positive effect of exposure to fluctuating temperatures on these photo-physiological metrics ~~from exposure to fluctuating temperatures~~ may be associated with the cooling ~~effect at~~ overnight and upregulation of the genes related to photosynthesis (Mayfield et al., 2012).

In contrast to the~~se~~ aforementioned studies, Putnam and Edmunds (2008) found that when incubated at fluctuating temperatures (26–32 ℃), F_v/F_m of *P. meandrina* and *Porites rus* nubbins were depressed by ~20% compared to those maintained at a constant temperature of 28 ℃. These contrasting results ~~are possibly~~may be due to methodological differences. Our study and Mayfield et al. (2012) mimicked ~~the~~ natural temperature fluctuations by progressively modulating temperatures over time ~~which closely resemble the natural settings~~, whereas Putnam and Edmunds (2008) directly transferred corals from low to high temperature in the morning ~~morning~~ and vice versa at night. This approach could cause instant heat-shock and prolonged exposure to extreme temperatures, thereby exaggerating~~overestimating~~ the stressful effects of diurnal thermal fluctuations.

Although juvenile *P. damicornis* at 31 ℃ exhibited apparent paling appearance compared to those in 29 ℃, loss of symbionts and bleaching were not indicated, as the faster lateral growth at 31 ℃ suggests that the paling is instead the result of pigment dilution due to a larger surface area. This outcome contrasts ~~The +2 ℃~~

~~treatment resulted in increased bleaching of juvenile *P. damicornis*, a result consistent~~ with previous work showing the sensitivity of endosymbionts within coral recruits to elevated temperatures (Anlauf et al., 2011; Inoue et al., 2012). The lack of bleaching response to elevated temperatures in the current study may be linked to the symbiont type. *P. damicornis* predominantly harbored *Symbiodinium* clade D in Luhuitou (Zhou, 2011), which has been found to be particularly thermally tolerant. In addition, the difference in treatment duration could also partially explain these contrasting sensitivities. Albeit ecologically relevant, the exposure duration in this study was much shorter than previous studies (Anlauf et al., 2011; Inoue et al., 2012)~~(Inoue et al., 2012)(Anlauf et al., 2011)~~, therefore resulting in less cumulative stress. It is possible that a loner exposure time may cause similar bleaching responses to those found by other studies. ~~Higher levels of symbiont expulsion will reduce the accumulation of reactive oxygen species (ROS) within *Symbiodinium* (Haryanti et al., 2015; Yakovleva et al., 2009), therefore resulting in high survival rates.~~

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Further, daytime exposure to high temperatures in fluctuating treatments did not ~~amplify the effects of elevated temperature on bleaching response~~induce significant symbiont loss ~~of~~ in juvenile *P. damicornis*. This observation is in stark contrast to the observations~~at~~ of Putnam and Edmunds (2011) ~~for~~ on adult corals. That study found that ephemeral exposure to 30 ℃ at noon in fluctuating conditions (26–30 ℃) elicited a 45% reduction in symbiont density of adult *P. meandrina* compared to corals at the~~those in~~ steady 28 ℃ treatment, a~~n effect size~~ larger effect than that ~~arising~~

~~from~~was elicited by continuous exposure to 30 ℃ (36%). The flat structure of juvenile corals has been suggested to provide a higher mass transfer capacity to remove reactive oxygen species than the branching and three-dimensional adults ~~The discrepancy between this study and our results may reflect of a greater thermal tolerance of juvenile corals. The flat structure of juvenile corals has been suggested to provide a higher mass transfer capacity to remove ROS, which would enhance thermal tolerance~~ (Loya et al., 2001)~~-~~. Hence, the discrepancy between our results and that of Putnam and Edmunds (2011) may, at least partially, be attributed to the morphology-specific difference in thermal tolerance of juvenile and adult corals.

4.3 Accelerated early development at elevated temperature

Early development of juvenile *P. damicornis*, including budding, lateral growth and calcification, was accelerated at 31 ℃, which is 2 ℃ above the local long-term summer mean and 1 ℃ above the local bleaching threshold (Li et al., 2012). Growth stimulation by temperature increase also occurred in a pilot study which showed that ~~,~~ ~~after two weeks at 31 ℃,~~ lateral growth and budding of *P. damicornis* after two weeks at 31 ℃ were~~was~~ 10% and 41% higher respectively than that of those at 29 ℃ (Fig. S4). Moreover, recruits with increased growth rates at elevated temperatures showed higher survivorship, consistent with previous field observations that survival in early stages of reef corals was strongly dependent on colony size and growth rates (Babcock and Mundy, 1996; Hughes and Jackson, 1985). In contrast to our study with a tropical coral, a previous study reported that calcification of symbiotic polyps of *Acropora digitifera* in subtropical Okinawa was highest at 29 ℃ (2 ℃ above the local summer mean), and was reduced at 31 ℃ (Inoue et al., 2012).

It has been widely accepted that warming is likely to be more deleterious to early stages of tropical corals than subtropical species (Woolsey et al., 2014). Clearly, thermal tolerance of corals is relative to the ambient temperature at a particular location. Given the ~~great~~ large seasonal temperature fluctuations and ranges in our study site (Fig. S1), it is not surprising that *P. damicornis* grew faster at 31 ℃. ~~However, the consistent positive effects of 2 ℃ increase on calcification by both *P. damicornis* and *A. digitifera* does not support the suggestion that warming is likely more deleterious to early stages of tropical corals as compared to subtropical species (Woolsey et al., 2014).~~ The positive effects of the 2 ℃ temperature increase on the early development of *P. damicornis* suggest that t~~T~~ropical corals dwelling in thermally dynamic habitats may also have the capacity to modify their thermal limits, thereby enhancing physiological performance and tolerance under increasing temperatures (Clausen and Roth, 1975; Dandan et al., 2015; Schoepf et al., 2015).

~~Unlike previous findings of the synchronized reductions in symbiont density and calcification in corals (Inoue et al., 2012; Tremblay et al., 2016), our study suggests symbiont loss and calcification are decoupled at increased temperatures.~~ There are two possible explanations for ~~this phenomenon~~ the increases in growth and development at elevated temperature in our study. Firstly, paling of recruits at elevated temperatures as a result of pigment dilution will ~~the enhanced~~enhance their internal light fields~~ during bleaching ~~, which could bring~~–~~ about 2- to 3-fold increase in symbiont specific productivity (Wangpraseurt et al., 2017), ~~, thereby~~and in turn support skeletal growth and asexual budding~~compensating for symbiont loss~~. ~~Unfortunately, the small size of juveniles and the variable temperatures here made it difficult to measure photosynthesis. However, given the dramatic increases in skeletal growth and new polyp formation at 31 ℃, it is plausible that photosynthesis was not compromised, or only minimally impacted, to support the observed growth.~~

Secondly, since coral calcification is positively correlated with carbon translocation between *Symbiodinium* and the host (Tremblay et al., 2016), the elevated calcification and growth at 31 ℃ indicates more efficient nutritional exchange, ~~thus~~ sustaining the metabolic expenditure of faster development. This interpretation is further ~~evidenced~~ supported by the excessive deviation of Q_{10} from the kinetic expectations (2–3): this signifies a strong amplifying effect through changes in fundamental biochemical systems along with the acceleration of functional enzyme activities at increased temperatures (Hochachka and Somero, 2002).

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~~ was 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 ℃, but resulted in a significant decline at 31 ℃. ~~This decline may be a result of inhibition by the maximum temperature (33 ℃) reached in this treatment.~~ In comparison, prior studies with corals did not find that ~~an influence of~~ temperature fluctuations ~~on~~ influenced 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 range~~s~~ encompasses the maximum thermal limits of the species (Vasseur et al., 2014).

The relationship between skeletal growth in corals and temperature is non-linear and characterized by a parabola whose apogee indicated an optimum and threshold, beyond which the stimulatory impact of temperature will be reversed (Buddemeier et al., 2008; Castillo et al., 2014; Inoue et al., 2012; W ŕum et al., 2007)~~{Castillo, 2014 #157;Inoue, 2012 #65}~~. Although the optimal temperature for calcification by *P. damicornis* recruits remains unknown, it is possible that the recruits exposed to the fluctuating 31 ℃ treatment calcified at a slower rate when the temperature was below 31 ℃ compared to those in the constant 31 ℃. However, given the well-established temperature performance curve for coral calcification (Buddemeier et al., 2008; W ŕum et al., 2007), daytime exposure to temperatures above 32 ℃ would have severely impaired the calcification process, thus leading to an overall decrease in calcification. At least two hypotheses from the literature can help explain this inhibitory effect~~The notable reduction in calcification under a fluctuating regime around 31 ℃ 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 explanation is that daytime exposure to extreme temperature could disturb the function and/or synthesis of skeletal organic matrix (OM) within the calcifying medium. The OM ~~plays~~ has critical roles in calcification such as calcium binding, providing carbonic anhydrase and the template for crystal nucleation (Allemand et al., 2011). Daytime temperatures of 33 ℃ may disrupt the function of carbonic anhydrases (Graham et al., 2015), ~~therefore~~ thereby severely~~greatly~~ inhibiting the conversion of respired CO_2 to bicarbonate for subsequent use in calcification.

Further, since the OM itself is also incorporated into the skeleton, the rate of OM synthesis is ~~also~~ a limiting factor for calcification (Puverel et al., 2005; Allemand et al., 2011). Extreme temperatures may impede the production of OM as it is highly sensitive and vulnerable to short-term thermal stress (Desalvo et al., 2010; Desalvo et al., 2008; Maor-Landaw et al., 2014). Although the exact mechanism has not yet been fully resolved, our study provides evidence that daytime exposure to extreme temperature in variable thermal conditions ~~would~~ adversely affects calcification, and dampens the stimulation of skeletal growth in *P. damicornis* at elevated temperature.

5 Conclusions and implications

This study was the first to examine the effects of both increased temperature and daily temperature variability on the early stages of a reef coral. We found that~~Using~~ realistic diurnal temperature ~~patterns, we found that~~fluctuations considerably tempered thermal stress on larval settlement~~ was greatly tempered by diurnal temperature fluctuations, whilst the fluctuating regime~~ , and had varied effects on the physiology and early development of *P. damicornis*. Diel oscillations in temperature did not ~~aggravate~~induce bleaching but ~~reduced~~ relieved ~~the~~ heat stress on photo-physiology. Further, temperature fluctuations had no obvious effects on budding and lateral growth, ~~but~~ although two hours' exposure to 33 ℃ during the daytime apparently caused a reduction in calcification compared to constant exposure to 31 ℃. Results reported here emphasize the distinction between the effects of constant and fluctuating temperature~~ effect~~s, ~~not only~~both for different mean temperatures ~~but also~~and on two successive life stages, and highlight the importance of incorporating diurnal ~~trends~~ fluctuations into research on ~~unravelling~~ the influence of ocean warming on coral biology.

The results of this study suggested that coral larvae subjected to diurnal temperature variations, especially at increased temperature, exhibit better settlement

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competence than those subjected to static thermal treatment. The fluctuating temperatures were favorable to the photo-physiology of endosymbionts and only had minor effects on post-settlement development of coral recruits. Therefore, for corals in highly fluctuating environments, they may have the potential to tolerate and acclimate to the changing seawater temperatures. These findings may also provide clues as to how diverse coral communities can persist and thrive in some thermally variable conditions (Craig et al., 2001; Richards et al., 2015). It is important to note that this study was technically limited to only one fluctuating amplitude, and the extent of thermal variance has as much of an impact on fitness as the changes in mean temperature (Vasseur et al., 2014). Given that there is currently still no consensus on the future temperature variability (Burroughs, 2007), it will be critical to study the impact of a broad range of thermal variations which corals may fare in a warming ocean.

Data availability

The d~~D~~ata associated with the present study is~~are~~ available from the corresponding author upon request.

Author contributions

L. J. and H. H. conceived and designed the experiments; L. J., Y. F. S., and Y. Y. Z. performed the experiments; X. B. L., L. J. M., J. S. L., X. M. L., G.W. Z., S. L., and P. Y. Q. contributed analysis and materials. L. J wrote the manuscript with comments from all co-authors.

Competing interests

The authors declare that they have no conflict of interest.

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Figures and captions

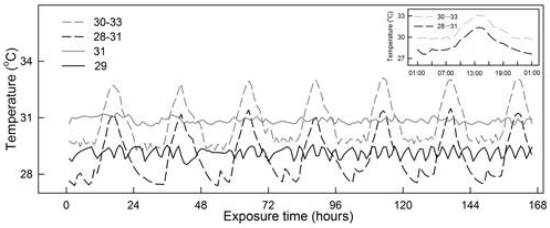

Fig 1. Temperature profiles for each treatment throughout the experiment. The inset shows the one-day temperature trajectory in the two oscillating treatments. Time course in fluctuating treatments was: 10 h at minimum temperature; 5 h of upward ramping; 2 h at maximum temperature; 7 h of downward ramping (passive).

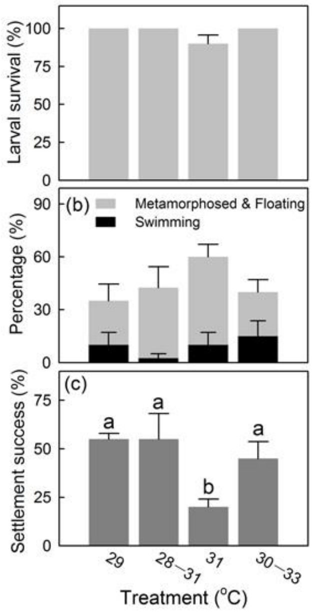

Fig 2. Percentage of *P. damicornis* larvae that (a) survived, (b) metamorphosed while floating and remained pear-shaped, and (c) successfully settled after 24 h exposure to temperature treatments. Error bars represent 1SEM. Different letters denote significant differences between treatments.

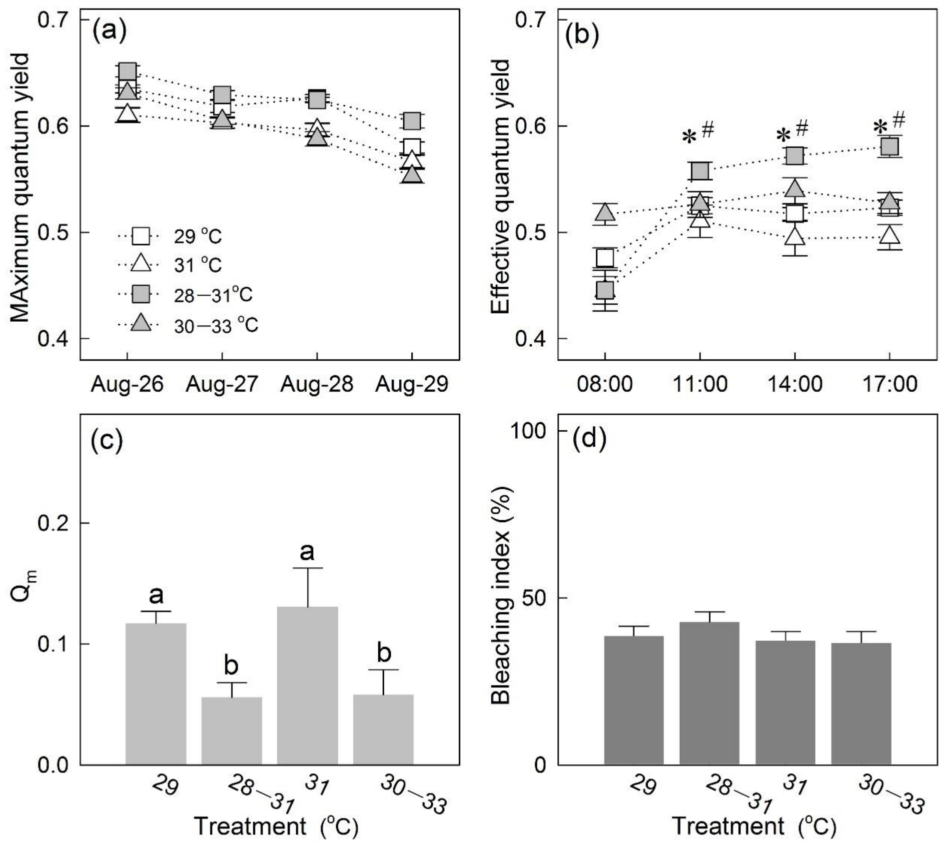

Fig 3. Photo-physiology and bleaching of *P. damicornis* recruits under constant and fluctuating conditions of two temperatures (29 and 31 ℃). (a) F_v/F_m over four consecutive days, (b) $\Delta F/F_m'$ throughout the last day of the experiment, (c) Q_m and (d) bleaching rates. Error bars represent 1SEM. Asterisks and hashes indicate significant effects of temperature increase and fluctuations at a specific time, respectively. Different letters represent significant differences between treatments.

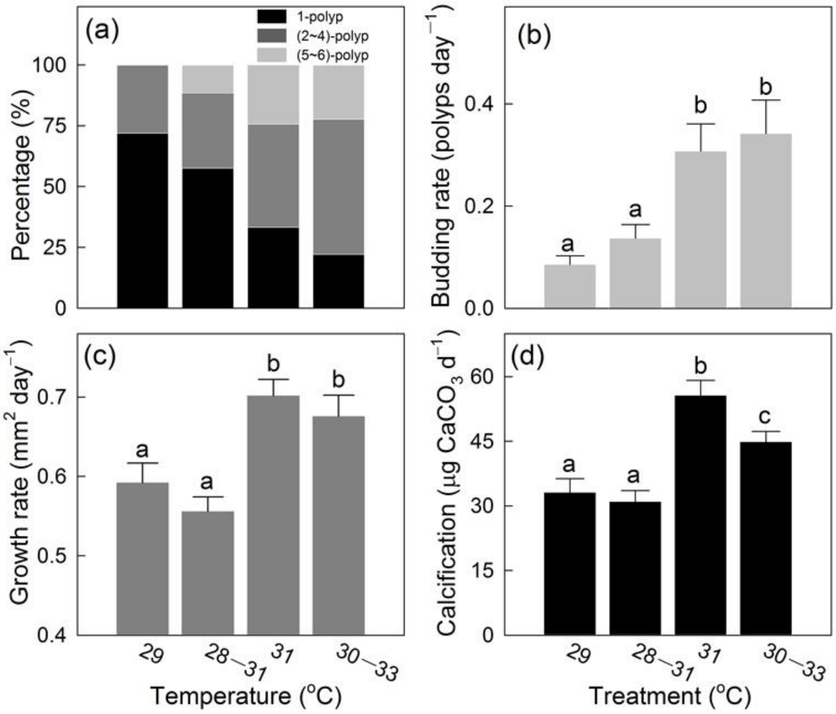

Fig 4. (a) Budding state, (b) polyp formation rate, (c) lateral growth and (d) calcification of *P. damicornis* recruits under constant and fluctuating conditions of two temperatures (29 and 31 ℃). Error bars represent 1SEM. Different letters denote significant differences between treatments.

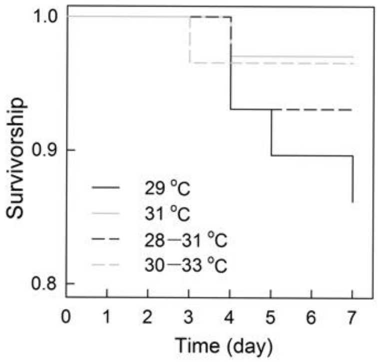

Fig 5. Survivorship of *P. damicornis* recruits estimated using Kplan-Meier analysis in each treatment over the 7-day experiment.