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Correspondence and requests for materials should be addressed to C.A.C. (cac@gate.sinica.edu.tw)

* These authors contributed equally to this work.

Doors are closing on early development in corals facing climate change

Shashank Keshavmurthy[1]*, Silvia Fontana[1,2,3]*, Takuma Mezaki[4], Laura del Caño González[1,5] & Chaolun Allen Chen[1,2,6]

[1]Biodiversity Research Center, Academia Sinica, Taipei, 11529, Taiwan, [2]Taiwan International Graduate Program - Biodiversity, Academia Sinica, Taipei, 11529, Taiwan, [3]National Taiwan Normal University, Taipei, 106, Taiwan, [4]Biological Institute on Kuroshio, Kochi, Otsuki, Nishidomari, 788-0333, Japan, [5]Pierre and Marie Curie University, 4 Palace Jussieu, 75005, Paris, France, [6]Institute of Oceanography, National Taiwan University, Taipei, 106, Taiwan.

Marine invertebrates are particularly vulnerable to climatic anomalies in early life history stages because of the time spent in the water column. Studies have focused on the effect of seawater temperature on fertilization, development, and larval stages in corals; however, none of them show comparative results along an environmental gradient. In this study, we show that temperatures in the range of 15–33°C have strong effects on fertilization rates and embryonic stages of two coral species, *Acropora muricata* in the subtropical environment and *Acropora hyacinthus* in subtropical and temperate environments. Deformations after the first cleavage stages were observed at low (15°C) and high (33°C) temperatures. Development was delayed by 6–7 h in the slightly non-optimal temperature of 20°C. We found significant differences in fertilization rates and responses of embryos from different latitudes, with temperate corals being more sensitive to extremely hot temperatures and vice versa. We hypothesize that the coral development is restricted to a narrow temperature range and deviation outside this window could inhibit a species' continuance and ecological success. Thus, it would have significant negative effects on adult populations and communities, playing a role in future of coral reef survival.

Abiotic factors such as seawater temperature fluctuations, acidification, salinity, typhoons, changing weather patterns, and habitat characteristics have been considered primary factors affecting the survival/physiological performance, fitness, and distribution of marine organisms[1–6]. Broadcast-spawning marine invertebrates fertilize their gametes externally and have motile larval stages that spend days to months in the water column, making them vulnerable to various environmental perturbations[4,7]. Thus, it is essential to consider multiple life stages when assessing a species' ability to tolerate stress. It is important for them to succeed at every stage of their early life cycle, including the fertilization of gametes, morula stage (also called the prawn-chip stage due to its irregular concave shape), gastrulation (the so-called donut shape), motile larval stage, settlement, and growth[4,5,8]. These are critical life history phases for many organisms, especially when exposed to anthropogenic stressors. The importance of successful early stages is critical as it determines the long-term viability of local populations[9]. In case of corals, this in turn can have community level consequences because they act as ecosystem engineers, providing habitat for myriad associated organisms while often excluding competitors for space[9]. Novel strategies or behaviors that increase overall reproductive success might be responsible for ensuring population survival[4].

Studies conducted on scleractinian corals have shown conflicting results on fertilization rates when seawater temperature is a stress factor. While some studies observed no differences in fertilization rates with increasing temperatures[10,11], an increase in cleavage rates were found in the corals *Favites abdita, F. chinensis*, and *Mycedium elephantotus*[12], and *A. millepora* showed reduced fertilization[12]. Studies on other non- coral marine invertebrates have shown that when seawater temperature is used as a stress factor, fertilization is not affected but subsequent life stages are vulnerable[8].

Like other marine invertebrates, corals are facing their threshold limits as they respond to seawater temperature fluctuations and other ocean changes[13–16]. Studies have shown the presence of interspecific differences in their response to seawater temperature stress during their early stages[10,12,17]. At higher temperatures there is increased mortality, low rates of early stage development, increased embryonic abnormalities, reduced survival of larvae, shorter pre-settlement times[10,12,18–23], and the bleaching and death of adult corals[24–36]. The sensitivity of developmental stages (low fertilization and high juvenile mortality rates) in corals to increasing seawater temperatures might result in a bottleneck for species persistence and ecological success, thereby having a negative effect on adult

populations and communities[4]. Therefore, documenting the effects of temperature on early life stages is fundamental for understanding the potential for long-term resistance in corals to changing environmental conditions. Numerous studies (laboratory-based aquarium tank experiments and field-based sampling and observations) have been conducted to observe the responses of corals to seawater temperature stress in adults[24–36] as well as larvae and pre-motility (fertilization and embryonic stages) stages[11,37–41] (see Table 1 and references cited therein). However, there is a lack of information on how seawater temperatures that parent colonies are exposed to over the long term affect fertilization and development and whether the effects are the same at different latitudes. Furthermore, only a single study[42] used gametes from known parent colonies and thus considered the genotypic effect on the response to temperature stress.

Studies have reported that the ideal temperature for fertilization and early development is 25–28°C[23,43], whereas in cases of adult corals bleaching is observed at sea water temperatures around 31°C (1–2°C higher than the average summer seawater temperature)[44–46] in tropical areas. However, the range of temperatures in which adult coral can thrive is between ~15°C[47] (temperate) to ~35°C (in Persian Gulf)[48]. This suggests early developmental stages might be particularly sensitive to temperature stress. Hence, it is important to understand the effects of temperature on pre-settlement in order to understand how recruitment is influenced by external factors.

Here we report the effects of various seawater temperatures (at 15, 20, 25, 28, and 33°C) on the fertilization success, survival ratio, and development in two coral species: *Acropora hyacinthus*, at Penghu Marine Biology Research Center (PMBRC), Penghu, Taiwan (subtropical) and at the Biological Institute of Kuroshio (BIK), Kochi, Japan (temperate); and *Acropora muricata* at PMBRC (subtropical) (Fig. 1). We performed three different trials, one for *A. muricata* and two for *A. hyacinthus*. In each trial we exposed unfertilized gametes and subsequent embryo stages to five temperature treatments (15, 20, 25, 28, and 33°C). We crossed the gametes from three different colonies (for a total of six crosses, since we separated the maternal and paternal gametes from each hermaphrodite colony), with three replications for each cross, for a total of 18 repetitions per treatment (Fig. 2). Furthermore, we included three replicates for self-fertilization and controls (eggs cultivated in sperm-free sea water) (Fig. 2). We hypothesized that there are inter- and intraspecies differences in their responses to seawater temperature stress and that the variations between the two locations were due to local adaptations to latitudinal differences. In addition, we tested for variability between colony crosses due to the genotypic effect on survival rate.

Results

Seawater temperatures at two study locations. Data loggers deposited at Penghu Kochi recorded hourly seawater temperature for a period of 14 months (Fig. 3). The minimum, maximum, and mean winter (January) temperatures were 14.5°C, 28.0°C, and 17.2 ± 3.5°C, respectively, in Penghu and 12.2°C, 18.7°C, and 16.9 ± 1.0°C, respectively, in Kochi. Minimum, maximum, and mean summer (August) temperatures were 25.8°C, 29.1°C, and 27.1 ± 0.6°C, respectively, in Penghu and 25.1°C, 29.6°C, and 28.0 ± 0.6°C, respectively, in Kochi. The experiment in Kochi was conducted in August 2012. In Penghu, the experiment was conducted in May 2012 when minimum and maximum seawater temperatures were 23.8°C and 30.8°C and the mean temperature was 25.8 ± 0.9°C. The transition in seawater temperatures from winter to summer was sharper in Penghu than Kochi (Fig. 3).

Fertilization and development time. Development in both *A. muricata* and *A. hyacinthus* (Fig. 4) was consistent with previous observations for other congeneric species[49–52] until 24 h when they reached the "donut stage." Although not completely overlapping, a similar pattern was observed in our study in the time required to achieve motility and develop into spindle planular larvae. *A. muricata* became motile after 41 h and *A. hyacinthus* after 38 h in both localities.

Effect of temperature on fertilization and development. There were differences in fertilization and development rates between temperature treatments in all three trials (Fig. 5). In all experiments, optimum fertilization and development were observed to occur at 25°C (Fig. 5), although the control temperature (ambient seawater temperature at the time of sampling and experimentation) was around 28°C at both locations. Each stage of the embryonic cycle (prawn chip, donut, and pear) took the same amount of time for completion under 25 and 28°C conditions (Fig. 6, 7, 8). At 20°C there was a delay in developmental time, and at 15°C fertilization was delayed by 6–7 h (Fig. 6, 7, 8). At the last observed stage (motile larvae), no survivors were observed at the more extreme temperatures (15°C and 33°C), the exceptions being in both species at PMBRC at 33°C with few survivors. Healthy larvae were observed to have high survival percentages at optimal temperatures (25°C and 28°C) (Figs. 5, 6, 7, 8). Treatments at 15, 20, and 33°C showed large variations (5–100%) in the number of eggs fertilized and those that underwent development. Although high mortality was observed at 20°C, a small percentage of the survivors reached the last stage (motile larvae) successfully (Figs. 5, 6, 7, 8). Responses to the 20°C treatment showed high variability (Fig. 5).

Inter- and intra-location variation in fertilization and development of *A. hyacinthus* at PMBRC and BIK. Fertilization success rates showed differences between the two localities at 33°C (Fig. 5, central and right columns). Although PMBRC (subtropical) fertilization success rates were highly variable at 15 and 20°C, and 100% at 33°C (Fig. 5), at BIK (temperate) we observed the opposite, with almost 100% fertilization at 15 and 20°C and a sharp drop to <30% at 33°C (Fig. 5). In the PMBRC trial, no survival was observed after fertilization at 15°C, while at 33°C an average of 60 and 40% of individuals reached the prawn-chip and donut stages, respectively. Conversely, no survivorship was observed in post-fertilization stages at 15 and 33°C in the BIK trial (Figs. 5, 7, 8). Development only rarely reached the motile phase at lower and higher temperatures, with only a few individuals still alive and functional at 33°C at PMBRC (Fig 5). At the sub-optimal temperature of 20°C, better performance was observed in BIK with respect to PMBRC, with a higher average percentage of survival at every stage (Figs. 5, 7, 8). Multiple comparison results showed significant differences ($p = 0.0001$) in fertilization between locations at 33°C, with *A. hyacinthus* in BIK having lower rates compared to PMBRC (Fig. 5, Table 2). Similarly, there was a significant difference ($p = 0.001$) in prawn chip stage development at 33°C, with *A. hyacinthus* at BIK being more sensitive compared to PMBRC. There were no significant differences between the species in all other stages and temperature treatments (Fig. 5, Table 2).

Inter- and Intraspecies variation in fertilization and development of gametes at PMBRC. The general trends were similar for *A. muricata* and *A. hyacinthus* at PMBRC (Fig 5, left and central columns). Difference was found between the two species only in fertilization at 15°C, and the slight differences generally observed can be explained by inter-colony variation (see below). There were no differences in the effects of temperature stress on developmental stages between the two species. However, in the 33°C treatments, an average of 30% of *A. muricata* did reach the motile stage (Figs. 5, 6) while a few individuals of *A. hyacinthus* survived later than the donut stage (Figs. 5, 7). Treatments at 15, 20, and 33°C showed a large variation (5–100%) in the number of eggs fertilized and those that underwent development. There was more variation within *A. muricata*, which showed significant differences between temperature treatments ($p \leq$

Table 1 | Summary of available information on experiments conducted to observe the effects of different stresses on early development stages in corals. **Fertilization *Pre larval stages

Coral Species	Development stage observed	Type of stress	Main Results	Reference
**Acropora digitifera*	Fertilization, embryo development, larval survivorship and settlement	Sediment	Decreased fertilization, larval survivorship and settlement. Embryo development not affected	Gilmour, 1999 (74)
Porites astreoides	Larvae survivorships and metamorphosis	Temperature	Elevated temperature increases mortality and metamorphosis	Edmunds et al, 2001 (18)
**Diploria strigosa*	Fertilization, development, and larval survivorship	Temperature	No significant differences in fertilization at 30,31,32°C; aberration in development at 31°C and 32°C	Bassim et al, 2002 (10)
Diploria strigosa	Effect on larvae, larval survivorship, and metamorphosis	Combined effects of ammonium concentration and temperature	Ammonium and increased temperature cause a decrease in motility and rate of settlement	Bassim et al, 2003 (20)
Acropora palmata, Montastrea annularis, M. franksi	Larval survivorship	UV radiation	Larvae from deep water parents have lower survivorship than conspecifics from shallow water parents	Wellington and Fitt, 2003 (75)
Acropora muricata	Larval survivorship	Temperature, light condition, and presence of zooxanthellae	Presence of zooxanthellae did not affect survivorship; at 36°C all larvae die within 40 hours	Baird et al, 2006 (21)
**Favites abdita, F. chinensis, Mycedium elephantotus, Acropora millepora.*	Fertilization and early embryo development	Temperature	Reduced fertilization and more embryonic abnormalities with increasing temperatures in *A. millepora*; high level of fertilization in other corals	Negri et al, 2007 (12)
Acropora solitaryensis and Favites chinesis	Larval settlement and post-settlement survivorship	Temperature	Larval settlement is increased at higher temperatures, while post-settlement mortality increases with long exposure	Nozawa and Harrison, 2007 (22)
**Acropora palmata*	Development, survivorship, and settlement	Temperature	Development is accelerated at higher temperatures and rate of mortality and presence of abnormalities are increased	Randall and Szmant, 2009 (23)
**Fungia repanda, Acropora millepora, A. spathulata, Symphyllia recta*	Larval pre-competency	Temperature	Higher % of larval metamorphosis in higher temperatures	Heyward and Negri, 2010 (38)
Porites panamensis	Larval survivorship and settlement, and growth into primary polyps	Combined effect of temperature and CO_2	Survival and settlement unaffected by increasing CO_2 and 1°C, polyp growth reduced by the combined effect	Anlauf et al, 2011 (39)
**Montastraea faveolata*	Early embryo stages sensitivity	UV radiation	Low sensitivity during early development; susceptibility in the motile planula stage	Aranda et al, 2011 (76)
Pocillopora damicornis, Seriatopora hystrix, and *Stylophora pistillata*	Larval respiration	Temperature	Respiration rate was parabolic in relation to temperature, peaking at 28°C	Edmunds et al, 2011 (37)
Acropora millepora, A. tenuis	Larval metamorphosis	Combined effects of copper contamination and temperature	Synergic interactions: reducing Cu concentration prevents negative effect of 2–3°C increase	Negri and Hoogeneboom, 2011 (40)
Goniastrea retiformis and *Leptastrea cf transversa*	Larval metamorphosis and settlement	CO_2	No direct effects of acidification	Chua et al, 2012 (77)
**Acropora palmata*	Fertilization and development	Temperature and effect of different genotypes	Genotypic diversity affects the response of fertilization and developmental success	Baums et al, 2013 (42)
**Acropora tenuis, Acropora millepora*	Fertilization, development, survivorship, and settlement	Combined effects of CO_2 and temperature	No effect of CO_2; no effect of 2°C difference on fertilization, survivorship, and metamorphosis, increases rate of development	Chua, 2013 (11)
**Goniastrea favulus, Acropora spathulata*	Embryo and larval survivorship	Temperature	Slower development at 20°C. Temperature above ambient lower survival. *A. spathualata* is more affected that *G. favulus*	Woolsey et al, 2013 (17)

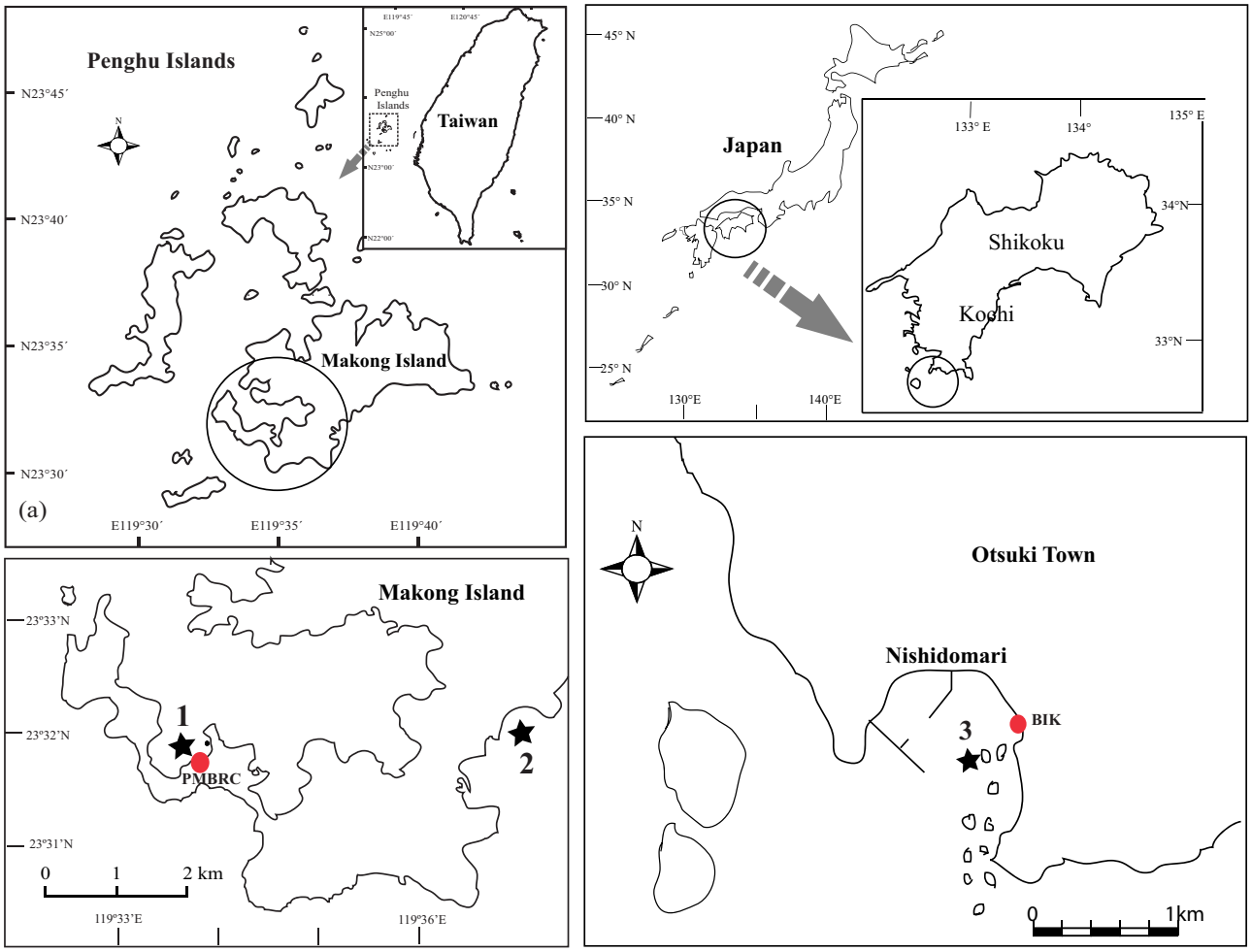

Figure 1 | Map of sampling locations and crossing experiments in subtropical Penghu, Taiwan (PMBRC) and temperate Kochi, Japan (BIK). Location of PMBRC and BIK are shown as red filled circles and sampling locations are shown as black stars. The maps were drawn using the software Magic Maps ver. 1.4.3 and Adobe Illustrator CS5 (Macintosh version).

0.001 for the fertilization, donut, and pear stages, and $p \geq 0.01$ for the prawn chip stage) (Fig. 5). However, *A. hyacinthus* had lower and very few significant differences to temperature stress ($p \leq 0.01$ in the pear stage and $p \leq 0.05$ in the donut stage). Multiple interspecific comparisons between *A. muricata* and *A. hyacinthus* from PMBRC showed that the fertilization rate was significantly different between the two species only at 15°C ($p = 0.01$) (Table 3).

Inter- and intra-cross (between repetitions) variation. High variability was observed in many cases within each treatment, especially at non-optimal temperatures such as 15, 20, and 33°C (Fig. 5, high standard deviations are shown in the bar, especially at 15 and 20°C). For example, in the 15°C treatment with *A. hyacinthus* at PMBRC, a 50% fertilization average was observed, while the range of fertilization among repetitions was 0–100%. We observed the maximum variability in the 20°C treatment. We therefore performed a statistical analysis on the response of each cross to verify whether the variability was due to genotypic differences among the colonies used in the experiment (Figs. 9 A, B, C). We found inter-cross differences in fertilization at 15 and 20°C treatments for both species at PMBRC and subsequently at 20°C for prawn chip, donut, and pear stages (Fig. 9 A, B, Table 4). A multiple comparison analysis showed that *A. muricata* crosses at PMBRC (Fig. 9 A) exhibited significant differences in fertilization among some crosses at 15 and 20°C ($p = 0.001$ and $p = 0.002$; Table 4) and less significant differences in other stages ($p = 0.02$; Table 4). However, there were no significant differences among crosses at higher temperatures (25, 28, and 33°C). In the case of *A. hyacinthus* from PMBRC (Fig. 9 B), the difference was less significant ($p = 0.02$) between the cross at 15 and 20°C for fertilization but showed greater significance in other stages ($p = 0.001$) (Table 4). Unlike *A. muricata*, a significant difference ($p = 0.001$) was seen between crosses at 33°C in the donut stage (Table 4). *A. hyacinthus* from BIK (Fig. 9 C) showed significant differences ($p = 0.001$) between crosses only for fertilization, prawn chip and donut stages at 20°C (Table 4).

Aberrant development of the embryos. Aberrations in embryo development were detected at low (15°C) and high (33°C) temperatures. At 15°C, developmental deformations were observed immediately after fertilization, which was delayed by 2–3 h. Irregular and disproportional cell divisions were observed at ~9 h after fusion of fertilized eggs. For this reason, embryos never reached the prawn-chip stage, instead continuing to fuse and degrade until the end of the experiment. This pattern was consistent for all three trials (two at PMBRC and one at BIK).

At 33°C, fertilization was faster than optimal conditions (25°C and 28°C treatment) by ~1 hour and was often followed by rapid and irregular cell division. At ~9 h, cell division had stopped and aberrant embryos started to degrade. Although all fertilized *A. hyacinthus* embryos at BIK underwent degradation, some fertilized embryos in both PMBRC trials were non-aberrant and survived till the end of the

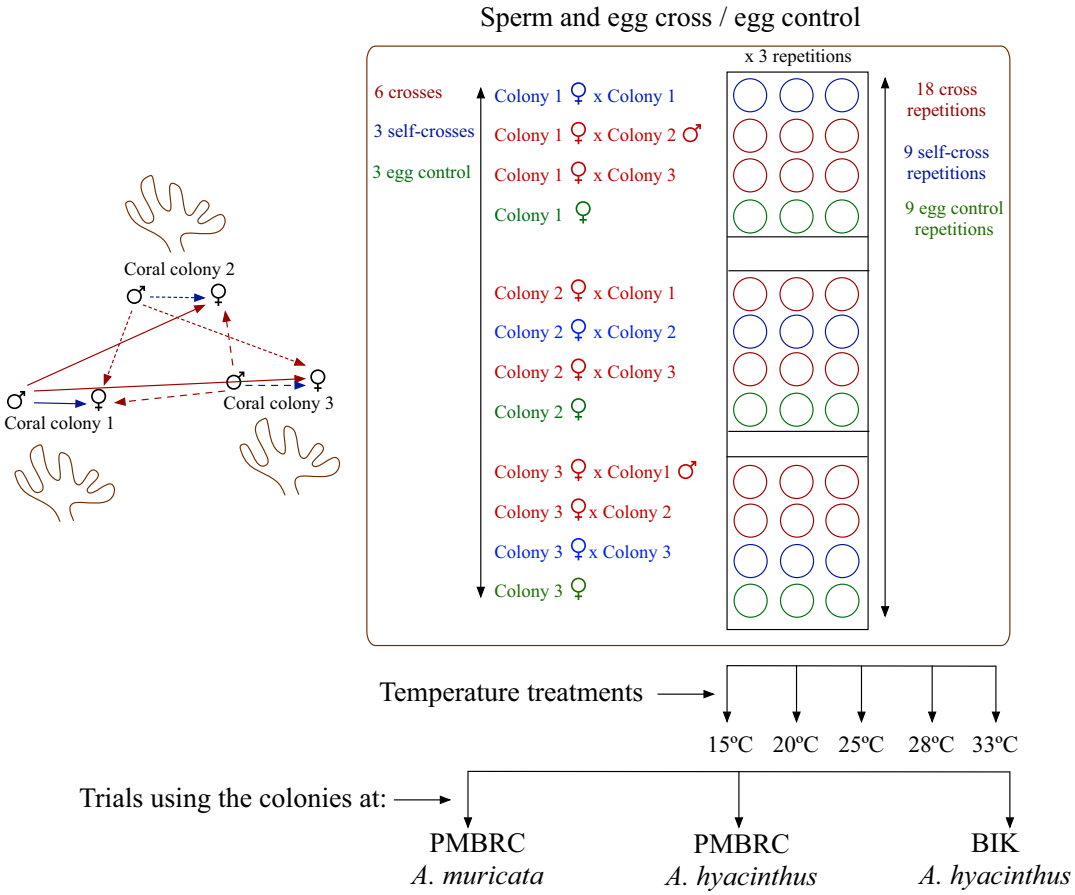

Figure 2 | **Schematic representation of the experimental design.**

experiment, and attained motility. The percentages of prawn chip, donut, and pear stages reflect only visually healthy and non-aberrant embryos (Fig. 5).

The above-described aberrations were not observed in control and self-fertilization treatments where non-fertilized eggs exhibited the round shape that gradually dissolves.

Discussion

This study is the first to address the effects of seawater temperature on the fertilization and early development in identical coral species living at two latitudes (subtropical and temperate) and during the same spawning year (2012) by conducting separate parental trials. The optimal temperature for survival after fertilization and before motility was 25°C irrespective of latitudinal differences in the seawater temperatures that corals are exposed to during gametogenesis and spawning. Although survival was highly affected by temperature of 15 and 33°C, this effect was different at the two latitudes. We posit that a narrow range of suitable seawater temperatures for early development in corals will create bottlenecks when seawater temperatures rise 2.0–3.0°C by 2050[4,53]. This study is first to show in detail the effects of temperature on different stages of coral development as a function of latitude and species.

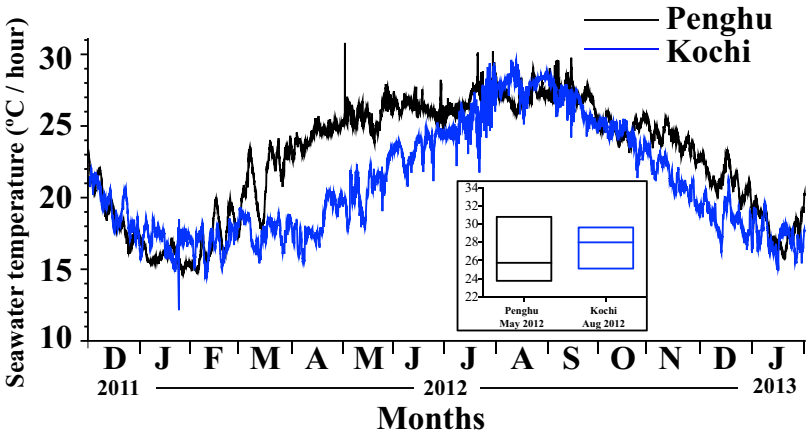

Figure 3 | **Seawater temperature plot at Penghu (subtropical) and Kochi (temperate).** Values represent hourly seawater temperatures plotted from December 2011–January 2013. The inset box plot represents seawater temperatures for those months in which the experiments were carried out at two locations.

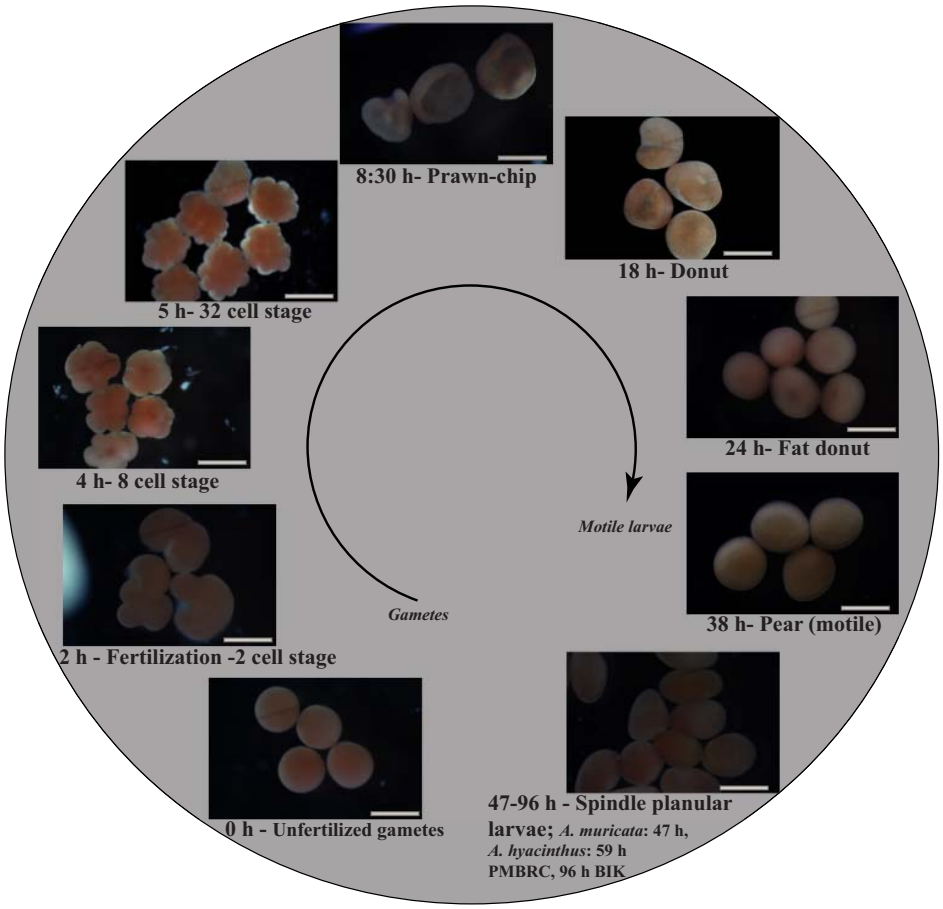

Figure 4 | Embryo stages at ambient temperature from unfertilized gametes (0 h) to planular larval stage (46–96 h). Scale bar = 500 μm.

The timing of developmental stages was similar for *A. muricata* and *A. hyacinthus* at both localities (Fig. 4) and congruent with the developmental cycles of other *Acropora* species[49–52] for the first 24 h. Although not completely overlapping with the previous study[51] a similar pattern was observed in the time required to achieve motility and develop into spindle planular larvae. *A. muricata* became motile after 41 h in this study, whereas it was previously observed[52] to attain motility after 47 h in the same location (PMBRC). Similarly, *A. hyacinthus* became motile after 38 h in both localities, whereas[50] found that it gained motility after only 36 h in concomitance with three other *Acropora* species. These slight differences may be due to different observation times during the experiments. Major differences were observed in this study between species and localities and with respect to previous studies in the time it takes to develop into spindle planular larvae. This last stage can be influenced by external conditions[51].

Our latitudinal comparison showed that gametes from temperate Japan were susceptible to the high temperature of 33°C, but gametes of the same species from sub-tropical Taiwan were less sensitive (Fig. 5). In a similar study[54], it was observed that the effect of temperature on the development of crown-of-thorns starfish depends on the geographic source of its larvae and the recent history of adult temperature exposures. Above or below the predicted temperature range (25–28°C), development was either delayed with a high frequency of subsequent death (20°C) or deformations in developmental stages occurred (15 and 33°C) (Figs. 6–8). Aberrant development was observed at lower and higher temperatures after the first cell division (Figs. 6–8). Aberrant embryos showed irregular cell division and fusion, always followed by degradation. Similarly, *A. millepora* showed deformed development at 32°C after the first cleavage stage[12], while abnormalities in *Diploria strigosa* were observed later in their development[10]. However, gametes were able to fertilize at all temperatures they were exposed to, but at a significantly lower rate in the 33°C treatment for *A. hyacinthus* at BIK (Fig. 5). This might be because all ontogenic stages of a life cycle are exposed to environmental conditions, so population persistence depends on the performance of adults and offspring. However, if seawater temperatures become non-conducive for coral developmental stages, corals would have to shift their spawning away from summer by either advancing or delaying gametogenesis to cooler months[8]. Less affected fertilization rates can be attributed to stress resistant traits passed on directly to gametes from parents and genes for stress resistance in fertilized gametes not being expressed until after fertilization[4]. However, during the late stages of embryonic development, the differential expression of genes affects their ability to respond to particular stresses.

Studies on the fertilization of other marine invertebrates such as echinoids, polychaetes, mollusks, and echinoderms also indicate that gametes have the ability to fertilize over a wide temperature range (4–15°C) above local ambient fertilization temperatures[4]. Similarly, found[10] no significant differences in fertilization success rates at 30, 31, and 32°C in *Diploria strigosa*, but developmental aberrations were observed at 31 and 32°C. At elevated temperatures (+5°C, 26–32°C), high levels of abnormalities and reduced fertilization rates occur in *Acropora millepora* and increased cleavage rates occur in *Favites chinensis*, *F. abdita*, and *Mycedium elephantotus*[12]. The developmental sequence in *Acropora palmata* was faster at 30 and 31.5°C than 28°C, but at higher temperatures greater rates of abnormal embryo development occur[23]. A similar response was observed in other marine invertebrates (*e.g.*, oyster, sea urchin) tested for fertilization and development success at high temperatures[4,55].

Our observations lead us to believe that the present increase in seawater temperatures will affect embryonic development and might

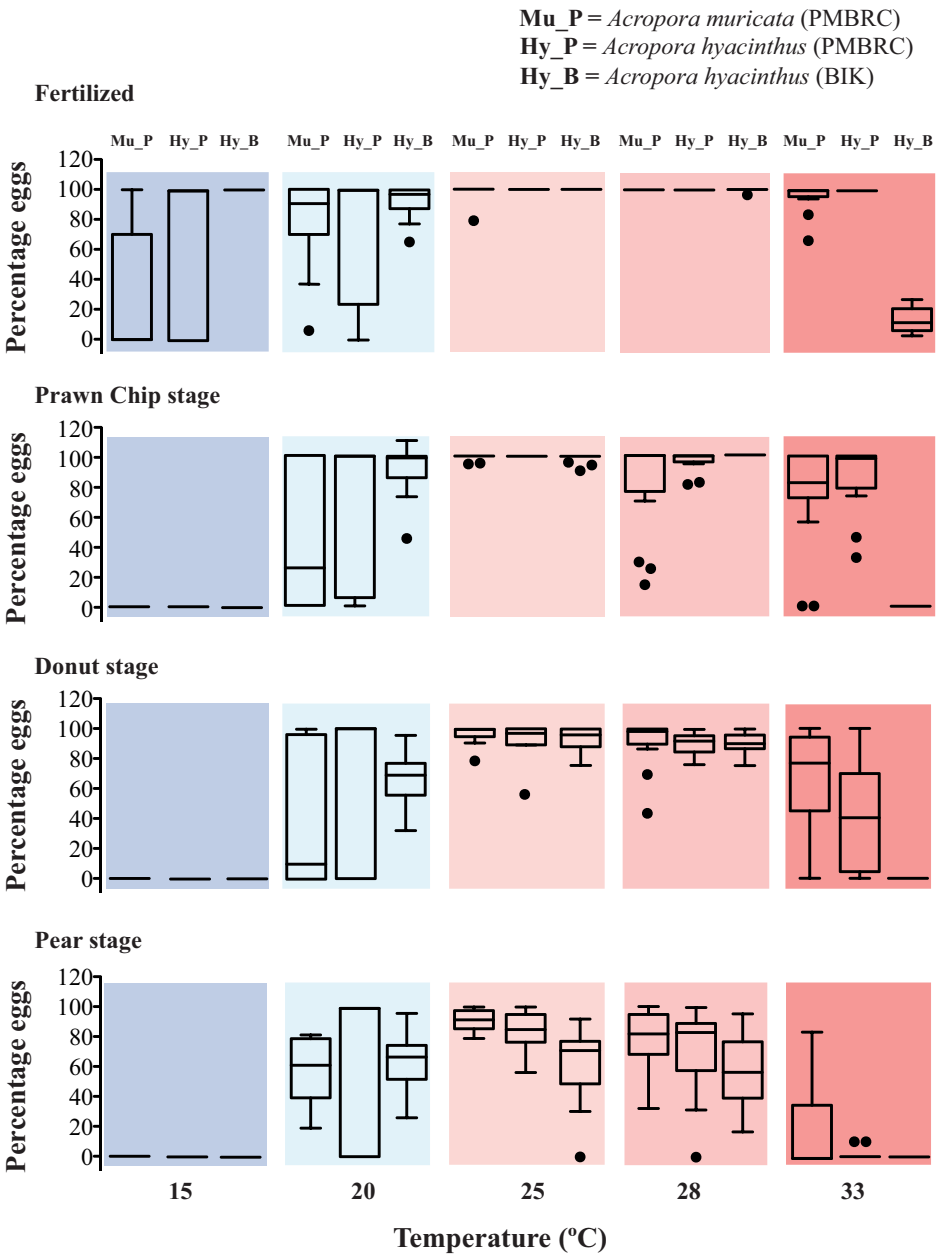

Figure 5 | Quantitative data for fertilization; prawn chip, donut, and pear stages at different temperature treatments (15, 20, 25, 28, and 33°C columns are in different colors). The percentages of individuals reaching each stage that appeared healthy and non-aberrant are plotted. Three bars are depicted for each trial, which correspond to treatments performed with *A. muricata* in PMBRC (left bar), *A. hyacinthus* in PMBRC (central bar), and *A. hyacinthus* in BIK (right bar). Mu_P = *A. muricata* in PMBRC, Penghu; Hy_P = *A. hyacinthus* in PMBRC, Penghu, and Hy_B = *A. hyacinthus* in BIK, Kochi. The box indicates the 25th and 75th percentiles, and the line within the box marks the median. Whiskers below and above the box indicate the 10th and 90th percentiles.

influence fertilization rates under more extreme conditions. This outcome might influence the timing of gametogenesis and spawning[56]. In fact, water temperature affects the timing of reproduction for many invertebrates[41,57,58], including corals[56]. It will also result in a decrease in the survival of corals at later life stages[10,23], thus determining the long-term viability of local populations[9]. Our results show that temperatures deviating ±5°C from the optimal development temperatures of 25–28°C affected gamete development. This implies that a temperature fluctuation of ±5°C will severely affect coral developmental stages in spite of their gametes being fertile. Corals will face greater seawater temperature fluctuations in the ocean change scenario when tropical corals are hypothesized to migrate to higher latitudes[59,60]. In this event, it might result in the early life stages of corals being more impacted than adult corals since the latter are known to thrive in seawater temperatures above 35°C in places like the Persian Gulf[48] or also tolerate daily fluctuations in seawater temperature (up to 10°C) as a result of internal waves and upwelling[61].

Our study also showed how parental lineage genotypes had a certain degree of influence on organism survival, especially genotype under high selective pressure at sub-optimal conditions (such as 20°C, see figs. 5, 9 A, B, C, Table 4). We performed separate trials in this study for each parent cross (which was previously done only by[41]), applying a series of different seawater temperatures to coral embryo stages to determine the role of genotype in reproductive success. We observed differences between crosses at different stages

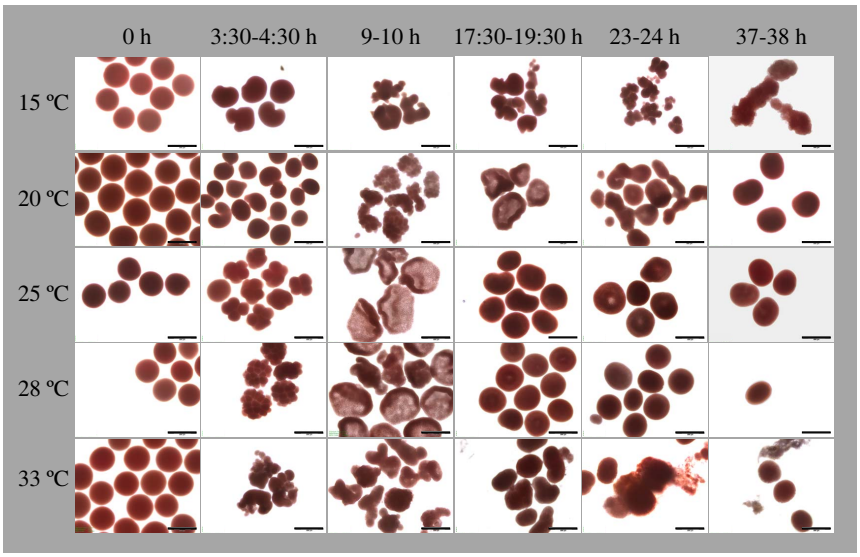

Figure 6 | Time series photos showing the effect of temperature on embryonic development in *A. muricata* at PMBRC, Penghu, Taiwan. Columns represent the different times that elapsed from fertilization (0 h) to motility. Rows represent temperature treatments of 15, 20, 25, 28, and 33°C. Scale bar = 500 μm.

of development, particularly at lower temperatures (15 and 20°C; Fig. 9 A, B, C, Table 4). Such variability across different crosses might also be due to phenotype. For example, there were higher success rates in certain parent crosses when fertilizing *Acropora palmata* under stressful conditions[42]. Future studies on this aspect will give important insight as to how genotypic and phenotypic characters influence the tolerance of corals to varying temperatures during their early life stages.

Due to their broad latitudinal distribution and associated temperature ranges, some reef communities may have a built-in adaptive capacity to accommodate temperature increases[8]. The water temperature increases slowly in Kochi from winter to summer compared to Penghu (Fig. 3). Although the summer average seawater temperature is similar (~26–28°C) at both locations, it might be that since adults are more accustomed to longer periods of low seawater temperatures, early coral life stages tolerate colder temperatures much better in temperate areas like Kochi. At 15°C, *A. hyacinthus* at BIK had a 100% fertilization rate (Fig. 5, Fig. 9 C), and at 20°C the variation between crosses was lower in comparison to *A. hyacinthus* from PMBRC (Fig. 5, Fig. 9 B). Upon exposure to high temperature (33°C), early stages of *A. hyacinthus* from PMBRC fared better than those from BIK (Fig. 5, Fig. 9 A, B). The physiological and life history traits[62] and exposure of adult corals to historical temperature[63,64] could play a role in determining the thermal limits of early life stages[2,4,9]. Nevertheless, organisms will often be concurrently exposed to multiple stresses in addition to fluctuations in seawater temperature, including anthropogenic stresses[8]. This will add to the challenges that corals must endure in their early stages of development, and only time will tell if they can overcome this challenge through adaptive and acclimative mechanisms such as changes in larval dispersal and recruitment success, shifts in community structure, and range extensions through migration[8].

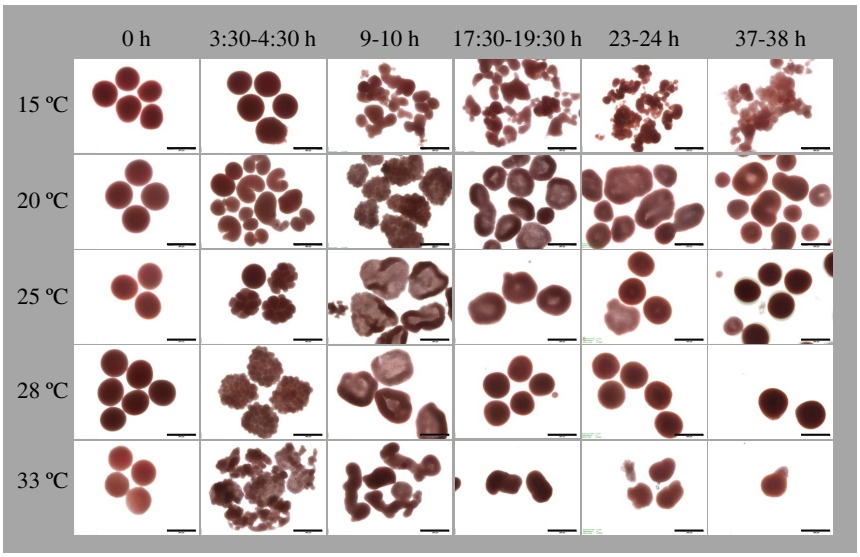

Figure 7 | Time series photos showing the effects of temperature on embryonic development in *A. hyacinthus* at PMBRC, Penghu, Taiwan. Columns represent the different times elapsing from fertilization (0 h) to motility. Rows represent temperature treatments of 15, 20, 25, 28, and 33°C. Scale bar = 500 μm.

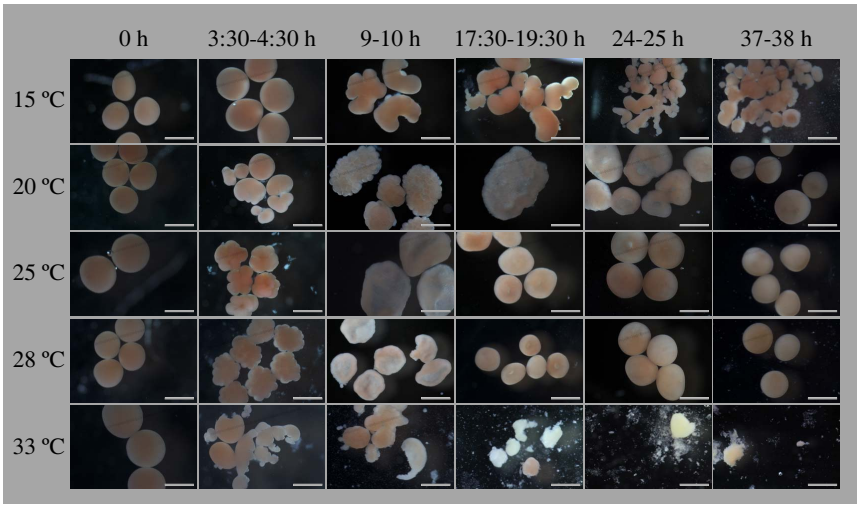

Figure 8 | Time series photos showing the effects of temperature on embryonic development in *A. hyacinthus* at BIK, Nishidomari, Otsuki, Japan. Columns represent the different times elapsing from fertilization (0 h) to motility. Rows represent temperature treatments of 15, 20, 25, 28, and 33°C. Scale bar = 500 μm.

Our study clearly shows that corals, like other marine invertebrates, do have a wide temperature tolerance range (15–33°C in the present study) for successful gamete fertilization. Also, there is a latitudinal difference in response to temperature, so local adaptations to prevailing seawater temperatures might play an important role[36]. Recent studies on climate change-induced ocean warming have indicated seasonal abnormal seawater temperature fluctuations leading to episodic high or low seawater temperatures in summer and winter[33,65,66]. Moreover, the average seawater temperature is getting warmer in the summer months in tropical, sub-tropical, and temperate seas[67–69]. As a result of this, we predict that the problem will be in the response of pre-motile developmental stages to seawater temperature stress. This is particularly true for broadcast-spawning corals, because egg fertilization occurs in the water column followed by larval development that occurs in direct contact with warm water[41]. It is believed that stressors like ocean warming will have carryover effects from one life history stage to another, creating bottlenecks for populations that have sub-lethal or lethal consequences[41]. Recent studies have shown that temperature stress can also influence circadian rhythms, thereby affecting the behavior, physiology, and metabolism of bacteria, fungi, plants, and animals[70,71]. Such impacts on circadian rhythms during early developmental stages in corals could also hamper their survival. The implication that stresses like high seawater temperatures may play a role in future coral survival, plus the fact that the narrow window of opportunity for successful development is fast closing due to the rapidity of the changes in seawater temperature, means that corals will have to find effective adaptive strategies quickly, as time is running out for their continued survival.

Methods

Study site and environmental conditions. The experiments were conducted at Penghu Marine Biology Research Centre (PMBRC), Penghu, Taiwan (subtropical, N023.31, E119.33) in May 2012 and at the Biological Institute on Kuroshio (BIK), Otsuki, Kochi-Japan (temperate, N032.46, E132.43) in August 2012 (Fig. 1). The locations from which the coral colonies were collected at PMBRC (*Acropora hyacinthus* and *A. muricata*), Aiman (N023.33, E119.38), and Chinwan Inner Bay (N023.31, E119.33) are characterized by shallow patchy coral communities at a distance of ~10 m from shore. The distance between Aiman and Chinawan Inner Bay is 5 km. At BIK, experimental colonies (*Acropora hyacinthus*) were collected from the adjacent coral community at Nishidomari, where coral communities are located on patch rocks at a distance of ~10 m from the shore. Coral colonies were collected from 3–4 m depths at all locations. Temperature loggers (HOBO© pendant, Onset Corp, USA) were maintained at the two locations from December 2011–January 2013 at a depth of 4 m near the coral colonies to document the differences in seawater temperatures.

Sample collection. Approximately one week prior to predicted spawning dates[72] at PMBRC, colonies were collected and deposited in tagged individual buckets in tanks with continuous flows of seawater and aeration. Seawater flow in the tanks was stopped daily after sunset (*ca.* 1830 hrs) throughout the spawning period. If no spawning was observed on any particular day, seawater flow was restored after 2230 hrs. At BIK, spawning was observed *in situ* by SCUBA diving every night from 1900 hrs during predicted spawning dates[73]. On the peak spawning day, colonies with maximum egg-sperm bundles (as seen visually underwater) were collected 1 h before spawning, transported to BIK, and placed in individual 100 L containers with aeration. *A. muricata* spawning was observed and their bundles collected at PMBRC on 11 May, five days after the full moon, and on 13 May for *A. hyacinthus*, seven days after the full moon. Collections at BIK for *A. hyacinthus* occurred on 7 August, five

Table 2 | Inter-location comparison of the responses of *A. hyacinthus* to seawater temperature stress at PMBRC and BIK. Hy_P = *A. hyacinthus* from PMBRC, Hy_B = *A. hyacinthus* from BIK

	15°C	20°C	25°C	28°C	33°C
Fertilization					
Hy_P vs. Hy_B	ns	ns	ns	ns	**0.0001**
Prawn chip					
Hy_P vs. Hy_B	ns	ns	ns	ns	**0.001**
Donut					
Hy_P vs. Hy_B	ns	ns	ns	ns	ns
Pear					
Hy_P vs. Hy_B	ns	ns	ns	ns	ns

Table 3 | Intra-location comparison of the responses by *A. muricata* and *A. hyacinthus* to seawater temperature stress at PMBRC. Mu_P = *A muricata* from PMBRC, Hy_P = *A. hyacinthus* from PMBRC

	15°C	20°C	25°C	28°C	33°C
Fertilization					
Mu_P vs. Hy_P	**0.01**	ns	ns	ns	ns
Prawn chip					
Mu_P vs. Hy_P	ns	ns	ns	ns	ns
Donut					
Mu_P vs. Hy_P	ns	ns	ns	ns	ns
Pear					
Mu_P vs. Hy_P	ns	ns	ns	ns	ns

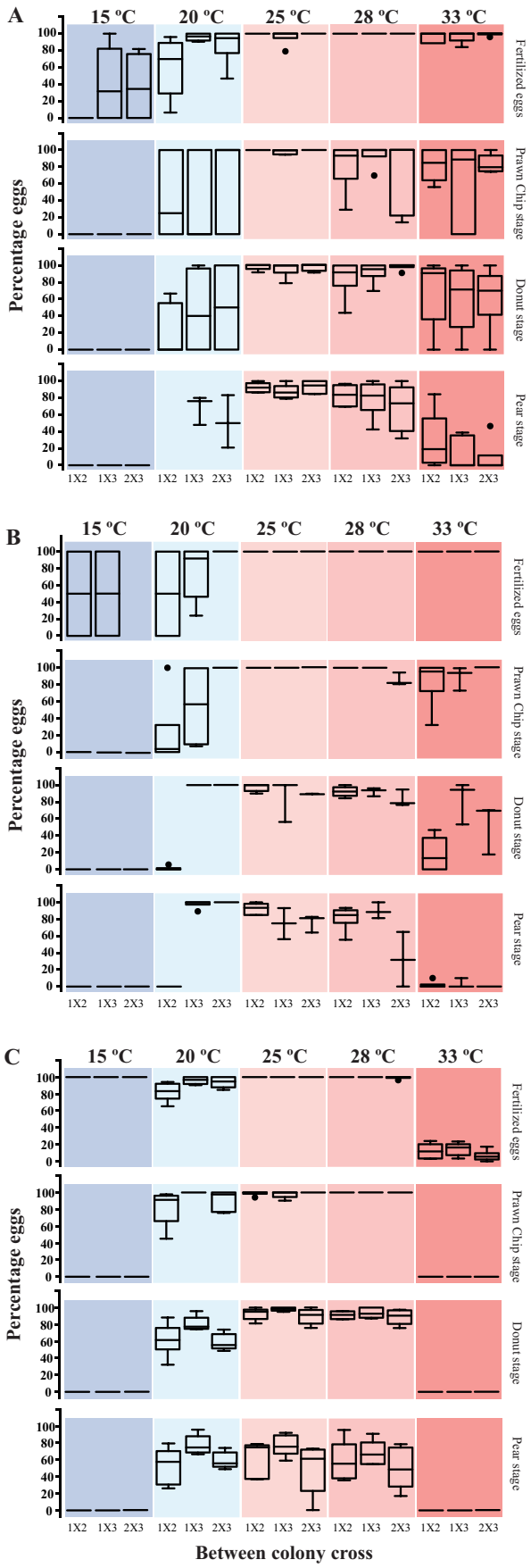

Figure 9 | Quantitative data for inter-cross fertilization for the three trials separately: (A) *A. muricata* in PMBRC, (B) *A. hyacinthus* in PMBRC, and (C) *A. hyacinthus* in BIK at different temperature treatments (15, 20, 25, 28, and 33°C columns are depicted in different colors). The crosses are: 1X2 = cross between colonies 1 and 2, 1X3 = cross between colonies 1 and 3 and 2X3 = cross between colonies 2 and 3. The box indicates the 25th and 75th percentiles, and the line within the box marks the median. Whiskers above and below the box indicate the 10th and 90th percentiles.

days after the full moon. *A. muricata* spawning was not observed during the survey period at BIK. Gamete bundles released from three colonies of each species onto the surface of the water in the buckets/containers were separately scooped up using tagged plastic cups and brought back to the laboratory for crossing experiments.

It was not possible to perform the same type of trial for *A. muricata* in both localities due to its overlap in spawning times in 2012. We tried to carry out the same experiment for *A. muricata* in 2013, but the experiment was unsuccessful due to gamete fertilization failure during two successive trials in June of that year.

Experimental design and crossing experiments. Crossing experiments for the three trials were performed by the same individuals (1st and 2nd authors) at both locations using the same experimental setup by shipping it to BIK after experiments were completed at PMBRC. Three colonies from each species were chosen for crossing and temperature stress experiment (Fig. 2). Bundles were filtered through a 120 μm plankton mesh to separate eggs and sperm, and aliquots of eggs and sperm were collected for density counts. A 9 μl aliquot of sperm was fixed with 1 μl of 2% formalin and the sperm counted in a haemocytometer using an Olympus microscope

Table 4 | Inter-cross comparison of the responses between *A. muricata* and *A. hyacinthus* to seawater temperature stress at PMBRC and BIK. Values in black = *A. muricata* (PMBRC), brown = *A. hyacinthus* (PMBRC), green = *A. hyacinthus* (BIK)

	15°C	20°C	25°C	28°C	33°C
Fertilization					
Cross 1X2 vs. Cross 1X3	0.001	0.002	ns	ns	ns
	ns	ns	ns	ns	ns
	ns	0.001	ns	ns	ns
Cross 1X2 vs. Cross 2X3	0.001	0.02	ns	ns	ns
	0.02	0.02	ns	ns	ns
	ns	0.001	ns	ns	0.04
Cross 1X3 vs. Cross 2X3	ns	ns	ns	ns	ns
	0.02	ns	ns	ns	ns
	ns	ns	ns	ns	0.004
Prawn chip					
Cross 1X2 vs. Cross 1X3	ns	ns	ns	ns	ns
	ns	0.005	ns	ns	ns
	ns	0.001	ns	ns	ns
Cross 1X2 vs. Cross 2X3	ns	ns	ns	ns	ns
	ns	0.001	ns	ns	ns
	ns	0.01	ns	ns	ns
Cross 1X3 vs. Cross 2X3	ns	0.05	ns	ns	ns
	ns	0.005	ns	ns	ns
	ns	0.01	ns	ns	ns
Donut					
Cross 1X2 vs. Cross 1X3	ns	ns	ns	ns	ns
	ns	0.001	ns	ns	0.001
	ns	0.001	ns	ns	ns
Cross 1X2 vs. Cross 2X3	ns	0.06	ns	ns	ns
	ns	0.001	ns	ns	0.001
	ns	ns	ns	ns	ns
Cross 1X3 vs. Cross 2X3	ns	ns	ns	ns	ns
	ns	ns	ns	ns	0.003
	ns	0.001	0.03	ns	ns
Pear					
Cross 1X2 vs. Cross 1X3	ns	ns	ns	ns	ns
	ns	0.001	0.01	ns	ns
	ns	ns	ns	ns	ns
Cross 1X2 vs. Cross 2X3	ns	ns	ns	0.1	0.03
	ns	0.001	0.01	0.001	ns
	ns	ns	ns	ns	ns
Cross 1X3 vs. Cross 2X3	ns	ns	ns	ns	ns
	ns	ns	ns	0.001	ns
	ns	0.03	0.003	0.03	ns

BX43 (Olympus Corporation, Tokyo, Japan) to obtain the correct concentration. Sperm density was diluted (10^5–10^6 ml^{-1}) for the crossing experiment[72]. Eggs were washed ten times in sperm-free filtered seawater in order to prevent self-fertilization. This water was collected some hours before spawning to guarantee the absence of sperm from early spawning corals. Three colonies from each species were crossed to obtain a total of nine separate crosses, including self-fertilization crosses (*e.g.*, egg colony 1 × sperm colony 2, egg colony 1 × sperm colony 3) (Fig. 2). The control treatment contained only eggs in filtered seawater (Fig. 2). Approximately 20 washed eggs were counted using a glass Pasteur pipette and placed in separate wells of a 12-well culture plate with 4 ml of diluted sperm (10^6 ml^{-1}). Three replicates were established for all egg-sperm crosses, with a total of 18 repetitions for each trial (*i.e.*, for each species and location). Three replicates of the self-fertilization treatments and controls were established for each colony.

After mixing the different egg-sperm combinations, culture plates were placed in plastic tanks with different seawater temperatures: 15, 20, 25, 28, and 33°C. The temperature in each tank was controlled with aquarium coolers/heaters (HAILEA HC-150A, Guangdong Hailea Group Co., Ltd, China) and metal-halide lamps with 12 h night/day cycles providing light. The temperature in each tank was recorded throughout the experimental period with temperature loggers (HOBO, Pendant™, USA) set to record water temperature at 10 min intervals. Gametes from separate colonies were also crossed in cylindrical glass jars at ambient temperature to detect the timing of the developmental cycle without any temperature treatment (Fig. 4). To prevent decreases in water quality due to degrading unfertilized gametes, water was changed in each well using a plastic Pasteur pipette after the fertilization stage and subsequently at 5, 10, 24, and 48 h of the developmental cycle.

Fertilization and development. Developmental stages were observed from the crossings at 0, 2, 8, 17, 24, and 37 h following the developmental cycle[51,52]. Photos were taken at each stage using an Olympus microscope with an Olympus DP72 CCD camera (Olympus Corporation, Tokyo, Japan) and a Canon 7D camera (Figs. 6, 7, 8), and survival rates were observed under an Olympus CX31 microscope (Olympus Corporation, Tokyo, Japan). Fertilization success, the percentage of cells clearly reaching every step of the normal cycle in non-aberrant shape (prawn chip, donuts, and pear, per[51]) (Fig. 4), and the rates of aberrant development (Fig. 6, 7, 8) were recorded. Cycle time was established by performing fertilizations at ambient temperature (Fig. 4). The experiment was conducted until the appearance of the first motile stage (pear-shaped larvae).

Data analysis. The quantitative data comprising the percentages of eggs fertilized and their subsequent development into prawn chips, donut, and pear stages were plotted for each temperature treatment as Tukey box plots. For each developmental stage, the data from six crosses (crossing between three eggs and three sperms), with a total of 18 replicate values (self-cross and control were excluded since they were not fertilized) in each temperature column were compared to each other by a one-way ANOVA to see if there were any significant differences between column values. A nonparametric Kruskal-Wallis test without assuming Gaussian distribution was carried out with a Dunn's post-test to compare all pairs of columns. This analysis was carried out for inter- and intraspecies differences during different stages of development. Furthermore, we carried out a similar analysis for the inter-treatment comparison for the trials between the two locations. An analysis of the repetition effect on the variability within each trial was performed as well to detect possible genotypic effects on performance. All the graphs and statistical analyses were performed using GraphPad Prism version 6.0 for Mac OS X, GraphPad Software, San Diego California USA.

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Author contributions

S.K. and C.A.C. designed the project. S.K., S.F., T.M. and L.C.G. performed the experiments. S.K., S.F. and L.C.G. analyzed the results. S.K., S.F. and C.A.C. wrote the manuscript.

Additional information

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