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Temperature affects the reproductive outputs of coral-eating starfish *Acanthaster* spp. after adult exposure to near-future ocean warming and acidification

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Abstract

Outbreaks of the coral-eating crown-of-thorns starfish *Acanthaster* spp. (COTS) have become to be amongst the most severe threats to coral reefs worldwide. Although most research has focused on COTS early development, it remains unclear how COTS populations will keep pace with changing ocean conditions. Since reproduction is a key process contributing to outbreaks, we investigated the reproductive success of adult COTS acclimated for 3-4 months to different treatment combinations of ambient conditions, ocean warming (+2 °C) and acidification (-0.35 pH). Our results suggest that the optimal breeding season in New Caledonia is concentrated around the end of the calendar year, when water temperature reaches >26 °C. We found negative effects of temperature on egg metrics, fertilisation success, and GSI, conflicting with previously documented effects of temperature on echinoderm reproductive outputs. Fertilisation success dropped drastically (more than threefold) with elevated temperature during the late breeding season. In contrast, we detected no effects of near-future acidification conditions on fertilisation success nor GSI. This is the first time that COTS reproduction is compared among individuals acclimated to different conditions of warming and acidification. Our results highlight the importance of accounting for adult exposure to better understand how COTS reproduction may be impacted in the face of global change.

Keywords: Adult exposure, Ocean warming, Ocean acidification, Crown-of-thorns starfish, Reproductive outputs

1. Introduction

Coral reefs worldwide are severely impacted by large-scale disturbances and local stressors such as thermally induced bleaching events, cyclones, overfishing, water pollution, sedimentation, and increasingly, outbreaks of the coral-eating crown-of-thorns starfish *Acanthaster* spp. (COTS) (Bruno and Selig, 2007; Hoegh-Guldberg et al., 2007; Hughes et al., 2010, 2017a, 2017b; Kayal et al., 2012; Leray et al., 2012). Most Indo-Pacific reefs have already been impacted by COTS outbreaks, leading to growing concerns that they are becoming more frequent (Bellwood et al., 2004; Brodie et al., 2005; Dumas et al., 2015; Pratchett et al., 2014). For instance, the Great Barrier Reef (GBR) suffered a 21% decline in coral cover over 27 years due to recurrent COTS outbreaks alone (De'Ath et al., 2012). These outbreaks are also impacting coral reefs in New Caledonia where local densities of >10,000 ind.ha^{-1} and significant damages to coral communities have recently been reported, e.g. up to 92% loss in coral cover in some reefs (Adjeroud et al., 2018; Buttin, 2018; Sulu et al., 2002). Despite intensive research effort over the last two decades, triggers for COTS outbreaks remain mostly unresolved, making predictions of future events challenging (Pratchett et al., 2014). However, the most widely accepted hypothesis is currently the eutrophication hypothesis, stating that agricultural land runoff,

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making more phytoplankton available, would ultimately improve larvae survival (Fabricius et al., 2010). To further confound predictions, the Intergovernmental Panel on Climate Change estimates an ocean warming of 2 °C and a pH decrease to 7.75 by 2100 for the "business-as-usual" scenario (IPCC 2014). Recent studies suggest that these near-future ocean conditions could benefit COTS early life stages, potentially resulting in increased outbreaks as climate change continues. For example, a 2 °C warming of the sea surface temperature coupled with eutrophication may increase COTS larval survival by 240% (Uthicke et al., 2015), while ocean acidification to a pH_{NIST} of 7.6 could enhance juvenile growth (Kamya et al., 2016).

COTS development has been widely studied in the context of global change, from early embryonic stages to juveniles (Allen et al., 2017; Caballes et al., 2017b; Kamya et al., 2014, 2016, 2017, 2018; Lamare et al., 2014; Sparks et al., 2017; Uthicke et al., 2013a, 2015). For example, research conducted on the Great Barrier Reef on thermal tolerance of COTS fertilisation processes showed that 28 °C was the optimum temperature, with no significant decrease in fertilisation rates up to 31 °C (Rupp, 1973). These results were supported with high fertilisation rates (>80%) observed between 24 °C and 32 °C (Caballes et al., 2017b). In terms of ocean acidification effect on COTS fertilisation success, the results are varied. While some authors predicted negative effects on the fertilisation rates from a pH_{NBS} of 7.70 (Uthicke et al., 2013a), others found no effects of pH_{NIST} down to 7.60 (Caballes et al., 2017b). These discrepancies could be related to differences in experimental protocols, for example in terms of the number of adults used for the fertilisation trials, i.e. 4 versus 3 males and 5 versus 2 females in Uthicke et al. (2013a) and Caballes et al. (2017b) respectively. It has been suggested that moderate differences in protocols may lead to conflicting results, especially when parameters that exert a major influence on the outcome of fertilisation assays are considered (review in Byrne, 2012). Similarly, the environmental conditions experienced by adult COTS during gonad development could also affect the experimental outcomes. However, to date, deliberate exposure of broodstock to warming and acidification has not been conducted, which makes it difficult to accurately predict the potential impacts of altered ocean conditions on COTS reproductive processes.

Exposing broodstock to modified environmental conditions during gametogenesis and gonad maturation is an important step towards a more realistic experimental approach to global change studies (Byrne et al., 2020). This approach may allow for transgenerational plasticity, i.e. a phenotypic change in offspring in response to the environmental stress experienced by broodstock prior to fertilisation (reviewed in Ross et al., 2016). While no studies have performed such an approach on COTS yet, there are some examples of studies that have exposed adults of other echinoderms, to determine plasticity on reproductive outputs such as egg quality, quantity and fertilisation success of sea urchins. For example, female fecundity of the green sea urchin *Strongylocentrotus droebachiensis* decreased 4.5-fold after a 4 month exposure to an elevated pCO_2 during the reproductive conditioning period (Dupont et al., 2013). Moreover, this exposure had negative carry-over effects over subsequent life-history stages, with 5 to 9 times less offspring reaching the juvenile stage. Comparatively, Suckling et al. (2015) showed that sea urchins *Sterechius neumayeri* adult exposed to warming (+2 °C) and/or acidification (-0.3 and -0.5 units) for 6 months had higher fertilisation success (+17%) but on average ~12% smaller eggs than animals kept in control conditions. Nevertheless, a 6 week exposure of adult sea urchins *Echinometra mathaei* to either control or different elevated pCO_2 treatments (485-1770 µtam) did not make the progeny more resilient or sensitive to the treatments (Uthicke et al., 2013b). These few examples illustrate the potential importance of parental exposure as it could produce transgenerational plasticity responses and buffer the effects of ocean acidification on echinoderm populations (and different marine taxa, see Ross et al., 2016). Similarly, it appears that exposing broodstock to different temperatures during the reproductive conditioning phase could dramatically shift the thermotolerance of embryos and larvae (Byrne, 2012). This may be a consequence of temperatures in the ovary imprinting on eggs. Indeed, collected sea urchins *Heliocidaris tuberculata* have higher fertilization rates at the experimental temperature that aligns closest to their collection temperature, rather than any "optimal" breeding temperature for the population (O'Connor and Mulley, 1977). However, it has been showed that echinoderm fertilisation,

including COTS, seems resilient to moderate temperature increases as expected during the coming century (Rupp, 1973). Nevertheless, considering the combined effects of warming and acidification is critical, as they are expected to simultaneously impact marine organisms. Furthermore, it is currently unknown if transgenerational plasticity responses will be possible with multiple stressors, therefore making predictions difficult in the current context of global change studies.

The present study is the first to acclimate adult COTS to modified environmental conditions for several months prior to assessing potential transgenerational plasticity responses. An increase of maternal provisioning is one adaptive strategy associated with transgenerational plasticity responses: adults exposed to stressful environments would invest more energy per egg to the detriment of fecundity to increase the subsequent life stage fitness in this non-optimal environment (Allen et al., 2008). It has been suggested that egg size is directly linked to fertilisation success, as larger eggs would have more probability to encounter spermatozoids (reviewed in Levitan, 2006). Therefore, we focused on three proxies to evaluate COTS reproductive outputs: a) egg quality, assessed by a set of complementary criteria of size and shape (Ayukai et al., 1996; Caballes et al., 2016, 2017a), b) fertilisation success, and c) egg quantity estimated with the gonado-somatic index (GSI). To test for possible transgenerational plasticity effects, we exposed adult COTS to near-future ocean warming and acidification (2 °C warming, pH 7.75) following the "business-as-usual scenario" for 2100 (IPCC 2014), prior to investigate their reproductive outputs. We hypothesize that COTS in the experimental treatments of our study (higher temperature and/or lower pH) might have lower GSI but larger eggs compared to the control treatment, following a possible maternal provisioning effect. Moreover, from the known literature on COTS fertilisation, we predict that fertilisation success would not be affected by +2 °C neither by a pH target of 7.75, and would, if anything, be enhanced if COTS eggs are indeed larger. In the current context where COTS outbreaks constitute a major threat to coral reefs, this study attempts to better understand and forecast the future effects of climate change on COTS biology and dynamics. This study may also provide valuable information for local conservation and management efforts in New Caledonia, where 15,743 km^2 of reefs and lagoons were recently enlisted as a UNESCO World Heritage site.

2. **Material & Methods**

2.1 Study species, collection sites

About a hundred adult specimens of *Acanthaster* spp. were collected over two consecutive years, respectively in late October 2018 on the shallow fringing reefs near the Redika islet and in late August 2019 on the West benches, two mid-shelf reefs located in the southwestern lagoon of New Caledonia (22°30'S, 166°36'E, and 22°26'S, 166°29'E, respectively; Fig. S1). Within one hour of collection, COTS were transported to the Aquarium des Lagons facilities in coolers filled with seawater renewed every 20 minutes (Fig. S1). Individuals were then stored in flow-through 3000 l raceways for one week prior to the experiments. Several gonadic lobes were collected per specimen by incising the proximal end of one arm and sex was determined microscopically, depending on the presence of eggs or spermatozoid in the lobes (Conand, 1983). The individuals were distributed into 12 tanks of 500 l (2018: three males and three females per tank, n=72; 2019 four males and four females per tank, n= 96), as a compromise between maximizing genetic pool and avoiding overcrowded tanks (Ayukai et al., 1996; Kamya et al., 2017; Uthicke et al., 2015). A multiple parent approach was chosen to reflect a population of natural spawners and to avoid the potential maternal and paternal effects characteristics of single dam-sire crosses (Caballes et al., 2016; Palumbi, 1999; Sparks et al., 2017).

2.2 Experimental treatments

All tanks were supplied with flow-through seawater pumped from 5 m deep in nearshore waters facing the aquarium facility. Filtered Seawater was obtained by using 5 µm cartridge filters set at a flow rate of 1 $l.min^{-1}$. Tanks were haphazardly allocated to one of four different treatments (three replicates × four treatments; see details below and in Fig. S2). Each of the 12 tanks had temperature and pH controlled independently, in order to avoid pseudo-replication and follow appropriate design guidelines (Cornwall and Hurd, 2016). Depending on the desired treatment, seawater was manipulated to the target condition inside a technical compartment that acted as a header tank: water overflowed into this compartment, where it was warmed and/or acidified, and sent back to the tank by submersible Tetra aquarium pumps (1000 $l.h^{-1}$) to homogenise large quantities of water at the target conditions. COTS were exposed to one of the four following treatments: *Control*: ambient seawater parameters not modified. *Acidification* (elevated CO_2): pH_{NIST} target was a 0.35 decline from ambient to approximately 7.75. This treatment represents the upper values of ocean acidification projected for the end of the century (IPCC 2014). Water acidification was obtained by bubbling pure CO_2 through solenoid valves. In each tank, pH was monitored and regulated continuously using an IKS Aquastar pH-stat system (accuracy ± 0.05 pH units). Electrodes were calibrated using standardized NBS buffers and adjusted each day to the desired pH_{NIST} using a Mettler Toledo 1140 pH meter with an InLab Expert DIN temperature compensated probe; the probe was calibrated weekly using standardized NIST precision buffers. *Warming* (elevated temperature): temperature was increased by 2 °C compared to control conditions. This treatment represents the ocean warming projected by the "business as usual" scenario for the end of the century (IPCC 2014). Temperature was adjusted once a week to keep the +2 °C delta constant, while following the natural (seasonal) temperature changes. During the week, if the delta between ambient and elevated temperature treatments varied by >50% (i.e. ± 1 °C), it would be immediately readjusted to keep the +2 °C delta as stable as possible during the experiment. Temperature was controlled using 300 W titanium aquarium heaters (Tetra, HT) plugged with electronic temperature controllers (DR 983 Eliwell). *Acidification and Warming* (elevated CO_2 and elevated temperature): a combination of the two previous treatments, i.e ambient temperature +2 °C and pH 7.75.

Temperature and pH_{NIST} were progressively modified in the corresponding tanks, to reach target conditions over a week. Temperature and pH_{NIST} were measured twice a day in all tanks using a Mettler Toledo 1140 pH meter with an InLab Expert DIN temperature compensated probe. Total alkalinity was measured on water samples collected and filtered through 0.45 µm syringes and immediately analysed by titration. The pH_{NIST} was measured at 0.1 ml increments of 0.01 N HCl at 25 °C using a Schott Titroline Easy® titration system. Three replicates of 20 ml were analysed. Total alkalinity (A_T) was calculated from the Gran function applied to pH variations from 4.2 to 3.0 as mEq l^{-1} from the slope of the curve HCl volume versus pH. Parameters of the CO_2 system were calculated with the free access CO2SYS Systat package using the dissociation constants of Dickson & Millero (Dickson and Millero, 1987) (Table 1). It is worth noting that due to logistical constraints, we could not measure alkalinity for the experiment in 2018-2019, but we did it for the second one in 2019-2020 (Table 1). However, as the whole system was rigorously the same for both experimental periods, it is not expected to be an issue.

2.3 Adult exposure and spawning

COTS specimens were kept under the four treatment conditions for a 3-4 month exposure period, overlapping with the COTS natural spawning season in New Caledonia (October-November to February-March; Conand, 1983) (Fig. 1). To date, the only available data on COTS in New Caledonia gives a reproduction season extending from November to February (Conand, 1983). Therefore, we repeated our experiment two years in a row, to perform reproduction both during the early and late known season period, aiming to provide a more specific time frame for COTS natural peak spawning.

However, none of the animals were able to reproduce at the start of exposure showing that all of them spent part of their gametogenesis exposed to the treatments. Two fertilisation trials were performed each year using the same specimen: the first at the end of the exposure period, and the second one month later (Fig. 1; ocean temperatures for both experimental periods are provided in Fig. S3). During the first 3 months, COTS were fed twice a week with 50 g of seafood mix (squids, fishes, mussels) per tank. Feeding was stopped after the first fertilisation trial to minimize water pollution. Cumulative mortality was very low, i.e. <7% and 1% by the end of each year's experiments, respectively. Nevertheless, as 4 out of 6 animals died in a single tank (acidification treatment) the first year, we chose to exclude this tank from the analyses.

Spawning induction and *in vitro* reproduction were carried out using the methods described by Uthicke et al. (2015). For all steps, we used 1 µm filtered seawater from the corresponding tanks. In each tank, ovaries from each female COTS were collected and rinsed multiple times with sea water to eliminate loose eggs, then placed in maturation-inducing hormone (10^{-4} 1-méthyladenine diluted in ~200 ml of sea water). Testes were then collected from each male, and placed in covered well plates to prevent desiccation. After 60 minutes, eggs were washed through a 500 µm mesh to retrieve eggs without the gonadic envelopes and pooled together resulting in a stock solution of approximatively 350 eggs ml^{-1}. Two µl of sperm were collected from each male and diluted to 15 ml of seawater. One ml of this mix was then added in the egg stock solution achieving a sperm to egg ratio of approximately 50:1 (Kamya et al., 2014). Gametes of the males and the females were pooled to simulate a population of natural spawners.

2.4 Oocytes metrics, fertilisation success and Gonado-Somatic Index

We used oocyte metrics as quality criteria (Ayukai et al., 1996; Caballes et al., 2016, 2017a). After being released by the 1-methyladenine, 30-50 oocytes from each female were chosen haphazardly and photographed at x100 magnification with a camera integrated with a Leica DM750 microscope. Diameters of long and short axes were measured using ImageJ software (Schneider et al., 2012) (Fig. S4). Oocyte sphericity was calculated as the ratio of long and short axes and the oocyte volume was estimated using the following formula:

$$Oocyte\ Volume = {}^{4}/_{3}\,\pi \times (long\ axis\ radius)^2 \times short\ axis\ radius$$

Oocyte metric data were collected once a year, before mixing the gametes for the first fertilisation trial. After mixing the gametes, egg samples were collected every 5 minutes from t0 to t+20 minutes to account for potential delays in fertilisation (approximatively 200 eggs observed per assay). The eggs were examined under microscope (x100; Leica DM750) to determine their fertilisation status based on the presence or absence of a fertilisation envelope (Fig. S5). Fertilisation success was calculated every 5 minutes as the ratio of fertilised eggs out of all the eggs observed, resulting in 5 repeated measures for each fertilisation.

The Gonado-Somatic Index (GSI) was used as a proxy of sexual maturity; for every animal, it was estimated at the end of the second fertilisation trial from each year using the following formula (Dumas et al., 2016):

$$GSI\ (\%) = \left(\frac{\frac{Wg}{3} \times Na}{Wt}\right) \times 100$$

Where:

Wt: total weight of the animal

Wg: cumulated weight of the gonads from three consecutive, randomly chosen arms
Na: total number of arms

2.5 Statistical analyses

Normality and homogeneity of variances of data on oocyte metrics (maximum diameter, sphericity and volume) and GSI did not improve after transformations. Therefore, we used non-parametric 3-ways permutational ANOVA (PERMANOVA) tests to evaluate the effects of Year (two levels, fixed), Temperature (two levels, fixed) and pH (two levels, fixed) on these response variables. Analyses were conducted using the PERMANOVA+ add-on for PRIMER v.7.0.13 (Primer-E 2017) and used the Euclidean distance, Type III sums of squares, and 999 permutations of the residuals under a reduced model to calculate the significance of the pseudo-F statistics. Pair-wise comparisons were used to further analyse the significant results. Variability in COTS fertilization success across treatments (fixed effects of Year, Temperature, pH and their interactions) was tested using generalized linear mixed-effect models (GLMMs) in which the effects of repeated observations (longitudinal data) on multiple tanks within each experimental set was accounted for using random effects of Tank nested in Set. Data were arcsine transformed and model residuals were checked for normality and homoscedasticity. Autocorrelations in data structure were also tested, and GLMM parameters were corrected for where significant (Pinheiro et al., 2017). GLMMs were computed in R (R Development Core Team) complemented by the NLME package (Pinheiro et al., 2017).

3. Results

3.1 Oocyte metrics

Egg volumes and maximum diameters for the first year were significantly lower than the second (Pseudo-F = 136.74; p = 0.001 and Pseudo-F = 133.79; p = 0.001; respectively), independent of treatments (Fig. 2; Table 2). We found a significant effect of temperature on both the maximum diameter (Pseudo-F = 71.15; p = 0.001) and the egg volume (Pseudo-F = 77.81; p = 0.001), with smaller eggs for COTS exposed to elevated temperature compared to ambient, independent of the year (Table 2). More complex effects were observed with acidification, as pH alone had an overall negative effect on egg maximum diameters and volumes, though effects varied across years and temperature treatments (Table 2). Antagonistic effects of temperature and pH were detected for the three parameters studied, differing with the year studied (Table 2). For instance, egg volumes were smaller for COTS exposed to pH alone compared to control (mean ± SE: $Volume_{acidification} = 5.28 \pm 0.09 \times 10^{-3}$ mm^3 *vs* $Volume_{control} = 6.18 \pm 0.15 \times 10^{-3}$ mm^3) but larger in COTS exposed to pH and temperature combined compared to pH alone (mean ± SE: $Volume_{acidification\ \&\ warming} = 6.03 \pm 0.15 \times 10^{-3}$ *vs* $Volume_{acidification} = 5.28 \pm 0.09 \times 10^{-3}$ mm^3) and similar effects were detected for maximum diameters (Fig. 2; Table 2). These antagonistic effects were significant only the first year for the egg maximum diameter and volumes. Finally, egg sphericity was also affected by the combination of warming and acidification in the same way as described above, though the effect was only significant for the second year (Fig. 2; Table 2).

3.2 Fertilisation success

We observed lower fertilization success the first year compared to the second ($F = 84.083$; $p < 0.001$) (Table 3). Fertilisation success was also lower for COTS exposed to elevated temperatures ($F = 379.514$; $p < 0.001$), with effects that varied across years (interaction Year × Temperature, $F = 23.378$; $p < 0.001$; Table 3; Fig. 3). A three-fold decrease in fertilization success was found for COTS exposed to elevated temperature treatments (mean ± SE = 20.8 ± 7 %) compared to ambient temperature treatments the first year (mean = ± SE 77 ± 8%; Fig. 3). Despite also being significant the second year,

this effect was much less prevalent (mean ± SE = 93.6 ± 0.5 *vs* 96.5 ± 0.7 %). In contrast, no effects of pH on fertilization success were detected, whether alone or in interaction with temperature and/or year (Table 3).

3.3 Gonado-Somatic Index (GSI)

We observed significant differences in GSI between years (Pseudo-F = 4.06; p = 0.04), with lower values the first year regardless of the treatments considered (mean ± SE: 2018-2019 = 4.98 ± 0.7 %, 2019-2020 = 6.5 ± 0.5; Fig.4). GSI was significantly impacted by temperature (Pseudo-F = 8.69; p = 0.004; Table 4), with lower values for COTS exposed to elevated temperature (4.7 ± 0.6 %) treatments compared to ambient temperature treatments (7.2 ± 0.6 %). There was a non-significant trend that pH was associated with lower GSI values but the overall effect of pH over the two years was above the significance threshold (Pseudo-F = 3.63; p = 0.06; Table 4).

4. Discussion

Our study investigated the reproductive outputs of COTS after a 3-4 month adult exposure period to projected near-future ocean conditions. Following the "business-as-usual" scenario (IPCC 2014), animals were exposed to ocean warming (+2 °C) and ocean acidification (pH_{NIST} target 7.75) in a fully crossed design in two consecutive years. We used a combination of metrics, including egg size and shape (Ayukai et al., 1996; Caballes et al., 2016, 2017a), fertilisation success (Uthicke et al., 2013a), and GSI index (Dumas et al., 2016), to test for potential effects of adult exposure to modified seawater conditions. To our knowledge, this is the first study to keep COTS in modified conditions for several months prior to investigating reproductive outputs. We found that adult exposure to ocean warming (+2 °C above current) impacts all the reproductive outputs studied here, while ocean acidification (at 0.35 units below current; pH_{NIST} target 7.75) does not have unequivocal effect. We could associate lower GSI with high temperature treatments, but there was no unequivocal effect of pH. However, regarding our hypothesis, we could not demonstrate that eggs were larger nor fertilisation success higher in any experimental treatment. On the contrary, both these parameters were negatively impacted by the +2 °C adult exposure, therefore disagreeing with our main hypothesis. However, a moderate increase of temperature may have detrimental effects on egg quality, fertilisation and gonad index, which would become much prevalent in sub-optimal breeding season. Whether COTS are able to develop transgenerational plasticity responses after a longer period of exposure will require further investigation.

We observed marked differences among years across all parameters measured, though the experimental design did not change. All reproductive outputs of COTS were significantly higher the second year in comparison with the first, regardless of treatments considered. These differences could result from variable ambient temperatures, as we chose to retain the annual and seasonal natural variability. However, all fertilisation trials were done at similar temperatures in 2018-2019 and 2019-2020 (26.5-27 °C in ambient temperature treatments; 28.5-29 °C in elevated temperature treatments). Moreover, the variation patterns were globally similar during the exposure periods for both years, with the exception of slightly higher peak temperatures (> 29 °C) reached in 2018-2019 (Fig. 1). Another parameter that could generate discrepancies among years is the timing of the fertilisation trials within the spawning period. Due to logistical and technical constraints, fertilisation was performed at the end of the natural breeding season the first year (early February and March) but was moved to mid-December/mid-January the second year, closer to the beginning of the COTS natural spawning period (Conand, 1983). Our results showed that animals from the second year trials had better reproductive outputs as fertilisation were conducted early on the breeding season proposed by Conand (1983). These results allow us to propose a more specific time frame for the peak spawning of COTS in New Caledonia, which would indeed likely occurs around the end of the calendar year. COTS usually start spawning when temperature reaches a certain temperature threshold, dependent on the latitude (e.g. ~28 °C on the Great Barrier Reef; Lucas, 1973; Pratchett et al., 2014). Our results suggest that this

triggering temperature for COTS reproduction would likely be around 26.0-26.5 °C in New Caledonia, a temperature generally reached in early December. It is possible that COTS from the second year experiment had better reproductive success because their exposure period started at the onset of the gametogenesis and therefore the spawning was induced at the natural peak of reproduction when the gonad quality was the highest. Nevertheless, COTS in the late breeding season trial also had high fertilisation success, though it drastically fell under elevated temperature.

It has been documented that COTS grow and mature new gonads each year, following an annual cycle which is highly dependent on latitude, and therefore temperature (Mita et al., 2016; Pratchett et al., 2014). Here, the reproductive outputs of COTS were lower under elevated temperature (28.5-29 °C) compared to ambient conditions (26.5-27 °C). Nevertheless, the detrimental effects of temperature were much more prevalent the first year as compared to the second. For instance, animals exposed to elevated temperature had their fertilisation success reduced by more than threefold the first year (mean 77% in ambient temperature *vs* 20.8% in elevated temperature). The second year, although still significant, the effect was much less prevalent (mean 96.5% *vs* 93.6%, respectively). This suggests that, although fertilisation success was quite resilient at the natural peak spawning period, the negative effects of increased sea surface temperature may predominantly impact COTS fertilisation during other, sub-optimal periods. These results are particularly interesting as they contrast with current literature on COTS and, to a larger extent, on echinoderms (Conand, 1983; Rupp, 1973). Indeed, it is generally accepted that a moderate temperature rise enhances fertilisation, up to a certain physiological limit dependent on the species (Rupp, 1973). This may be attributed to several factors, such as increased motility, velocity and respiratory performances of spermatozoids, a decreased viscosity of warmed water that raises the probability of egg-spermatozoid encounter (Kupriyanova and Havenhand, 2005; Mita et al., 1984), and the loading of protective maternal factors (Hamdoun and Epel, 2007). Our results suggest an opposite, period-specific effect during the spawning cycle, where even a moderate increase in temperature may significantly alter COTS fertilisation success. This could result from a degraded quality of gonads/gametes in the late breeding season, which is supported by the lower eggs metrics measured the first year. Indeed, as the reproduction trials were later the first year compared to the second, animals spent a longer period of time at temperatures above their spawning trigger threshold (26-26.5°C), an impact that could explain why eggs were smaller and GSI lower the first year independent of treatments.

Another point that supports the thermal stress hypothesis is that all the reproductive outputs we studied here were systematically lower in elevated temperature treatments regardless of the year considered. Additionally, animals in elevated temperatures treatments spent 9-13 weeks in temperatures ranging from 28 to 30.5°C the first year (Fig. 1) against only 1-5 weeks in 28-29°C the second year. This reinforces our hypothesis, showing that animals facing higher and longer thermal stress similar to the first year would have less eggs of lower quality, eventually resulting in catastrophically low fertilisation success. Similarly, a recent study on the sea urchin *Evechinus chloroticus* showed that adult exposure to ambient temperature +3 °C for 3 months resulted in negative growth of gonad material entraining a complete loss of reproductive outputs (Delorme and Sewell, 2016). Experiments at elevated seawater temperature in different sea urchins species have shown similar patterns of negative gonad growth (Lawrence et al., 2009; Siikavuopio et al., 2008; Uthicke et al., 2014). For instance, Uthicke et al. (2014) showed that the decrease of gonad development at elevated temperatures is attributed to the high energy demands of increased metabolism at high temperatures for *Echinometra* sp.. There are no previous studies on the effects of future ocean conditions on COTS eggs with or without adult exposure, making these results difficult to compare. Only a few studies so far have tested the effects of parental exposure to both warming and acidification on echinoderm reproductive outputs (Morley et al., 2016; Suckling et al., 2015). Similar to our study, Suckling et al. (2015) found negative impacts of temperature on egg size when adult Antarctic sea urchins *Sterechinus neumayeri* were kept for 6 months in +2 °C treatments. Moreover, Karelitz et al. (2019) also found similar results, with eggs up to 21.8% smaller in female sea urchins *Tripneustes gratilla* reared for 7 weeks in +2 °C treatments. These results highlights the importance of including parental exposure in climate change studies, as the environmental history of broodstock may critically influence the resilience of offspring, i.e. the transgenerational plasticity. Further research on

parental exposure of adult COTS to increased temperatures will be needed to confirm these mechanisms.

In contrast with temperature, the effects of acidification on COTS reproductive outputs were less prevalent. Overall, we did not detect marked impacts of a moderate drop in pH on the measured parameters; acidification to a pH_{NIST} target of 7.75 had no significant effect on fertilisation success or GSI. The research on the effects of ocean acidification on COTS fertilisation has not yet reached a consensus, as multiple studies reveal inconsistent results. For instance, Uthicke et al. (2013a) reported a drop of ~25% in fertilisation at pH_{NBS} 7.70, while other research did not show any decrease down to a pH_{NIST} of 7.60 (Caballes et al., 2017a). In experimental conditions, acidification could reduce COTS spermatozoid performances, such as motility and velocity, in turn reducing fertilisation success at low sperm concentration (Gonzalez-Bernat et al., 2013; Uthicke et al., 2013a) and increasing polyspermy rates (Reuter et al., 2011). In the natural environment, however, COTS reproduction in outbreak densities involve massive synchronous spawning of highly fertile animals for which gametic activity is not likely to limit fertilisation (Pratchett et al., 2014).

We also did not observe univocal effects of pH on egg size or shape, instead demonstrating antagonistic effects of pH and temperature, with various levels of significance depending on the year and parameters considered. While smaller eggs were associated with acidification alone, egg size eventually increased under specific combinations of temperature, pH and periods. Amongst the studies that have investigated the combined effects of pH and temperature on echinoderms, it has been suggested that rising temperatures could buffer the detrimental effects of acidification (review in Byrne and Przeslawski, 2013), though not reverse it. Keeping in mind the intrinsic limitations of this study, e.g. the logistical constraints of a restricted number of replicates and only two years, our results suggest complex interactions of lower pH combined with rising temperature on COTS gametogenesis. Finally, it is important to underline that differences in timing relative to the reproduction period, as suggested by our study, along with differences in exposure period length, could affect study outcomes. Indeed, we show some detrimental effects on animal reproductive outputs after a 3-4 month exposure period which coincide with the exposure period and outcomes from Suckling et al. (2015). Nevertheless, longer periods of exposure could allow animals to acclimate their physiology to stressful conditions, resulting in less prevalent effects (Karelitz et al., 2020; Suckling et al., 2015). Indeed, a recent study from with a comparable design to ours found that future acidification and warming does not affect egg size of the coral reef sea urchin *Echinometra* sp. after 20 months of adult exposure (Karelitz et al., 2020). This underscores the necessity of including parental exposure periods of sufficient length to study the impact of projected near-future ocean conditions on the reproductive outputs of echinoderms and, to a larger extent, marine taxa.

Studies focusing on the impact of global change on echinoderms do not usually include a phase of adult exposure prior to experiments. In most cases, fertilisation is performed under standard (ambient) conditions, with eggs or larvae directly exposed to the target treatments (review in Byrne, 2012). This is partly due to logistical and technical constraints, which tend to increase exponentially with the size and number of species, conditions and replicates. However, it has been previously showed that acclimating echinoderms to ocean acidification with minimum losses is feasible (reviewed in Dupont et al., 2013), including a study focusing on a starfish species (Hernroth et al., 2011). Here, we demonstrated that keeping large adult COTS in warmed/acidified conditions for several months was achievable with minimal mortality, even in the absence of optimal food. We worried that the absence of fresh coral food would have detrimental effects on COTS reproductive outputs, as it is known that quantity and quality of food can substantially alter echinoderm reproduction (George, 1995). A single COTS of similar size than the ones in this study can consume up to 155-234 cm^2 of live coral per day (Keesing and Lucas, 1992). Feeding large numbers of adult COTS for a prolonged duration as in this study would have required collecting tremendous quantities of live corals, which is not allowed under current New Caledonia environmental regulation. Alternatively, COTS in our experiments were fed with a mix of seafood (see Methods section), considered to be the best alternative to keep starfish for prolonged periods in aquaria (Böttger et al.,

2004). However, very high reproductive success was eventually reached (e.g. >90%), consistent with reproduction trials on COTS fed with non-preferred corals species or even starved (Caballes et al., 2016). As these authors also emphasized potential carry over effects, from slowdowns to a complete stop in larval development, additional studies should also focus on subsequent biological stages. That being said, it would be interesting to extend the experiments performed in this study to the larval development phase, to test if larvae from COTS fed with sub-optimal food would develop at different rates depending on the treatments.

In conclusion, we suggest that COTS reproduction may be relatively resilient and would not be significantly affected by near-future changes in the ocean conditions (specifically warming and acidification), at least when fertilisation occurs during the natural peak spawning period. However, moving away from this specific time frame towards an increase in temperature, even moderate, entails detrimental impacts on egg quality, quantity, and fertilisation success. In New Caledonia, the breeding season is relatively condensed in the year compared to lower latitudes, like Guam (Conand, 1983). Temperature increases may shift the peak spawning period earlier in the year, as observed in the neighbouring archipelago of Vanuatu (Dumas et al., 2016). Ultimately, it might even extend the whole reproduction window, as is commonly observed at lower latitudes where reproduction extends throughout the year (e.g., Guam, Cheney, 1974 or Palau, Idip, 2003). Nevertheless, exceeding the upper limits of COTS thermal tolerance may counterbalance the process, given the detrimental effects on fertilisation and the subsequent stages of development (Caballes et al., 2017b; Kamya et al., 2014). COTS outbreaks are a rising threat across the Indo-Pacific, the patterns and processes of which are not fully understood. Further research will clarify whether the predicted effects are of sufficient magnitude to significantly alter current trends.

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Competing interests

The authors declare no competing interest.

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Temperature affects the reproductive outputs of coral-eating starfish *Acanthaster* spp. after adult exposure to near-future ocean warming and acidification

Table 1. Seawater physicochemical conditions for the 2019-2020 adult exposure experiment

Treatment	*pH_{NIST}*	*Temperature*	*DIC (µmol kg-1)*	*pCO_2 (µatm)*	*HCO^{3-} (µmol kg-1)*	*CO_3^{2-} (µmol kg-1)*	*A_T (µmol kg-1)*	*Ω Ca*	*Ω Ar*	*Salinity*
Control	8,01 ± 0,01	Ambient	2199,6 ± 7,2	548.4 ± 11.3	1981 ± 7,7	203,5 ± 2,05	2473.4 ± 6.9	4.91 ± 0.07	3.24 ± 0.04	34,83 ± 0,01
Acidification	7,74 ± 0,002	Ambient	2309,5 ± 7,1	989.2 ± 17.3	2149,3 ± 7,2	132,9 ± 1,4	2470.5 ± 6.8	3.20 ± 0.05	2.12 ± 0.03	34,81 ± 0,01
Warming	8,00 ± 0,006	Ambient +2°C	2183,8 ± 7	555.2 ± 6.1	1957,1 ± 7,1	212,1 ± 1,5	2470.2 ± 6.9	5.14 ± 0.03	3.42 ± 0.02	34,82 ± 0,01
Acidification & Warming	7,72 ± 0,01	Ambient +2°C	2298,4 ± 7,1	1022.7 ± 16.6	2133,3 ± 7,5	138,4 ± 1,9	2467.7 ± 6.6	3.35 ± 0.04	2.23 ± 0.03	34,82 ± 0,01

Values for DIC (Dissolved Inorganic Carbon), pCO_2, HCO^{3-}, CO_3^{2-}, Ω calcite and Ω aragonite were calculated from salinity, temperature, pH_{NIST} and total alkalinity using the CO2sys software (means ± SE). Temperature values are presented separately in Fig. 1, as they varied seasonally and could not be averaged. (n=30 except for pH where n=435). In 2018-2019, pH values were (mean ± SE): 8.08 ± 0.03 (Control); 7.75 ± 0.001 (Acidification); 8.06 ± 0.02 (Warming), 7.75 ± 0.03 (Acidification & Warming); n=415 for the 4 treatments.

Table 2. Results of PERMANOVA analyses performed on egg metrics (maximum diameter, volume and sphericity) of COTS subjected to different levels of temperature and pH on two experimental years.

	Maximum Diameter				***Volume***				***Sphericity***			
Source of variation	df	SS	Pseudo-F	p (perm)	df	SS	Pseudo-F	p (perm)	df	SS	Pseudo-F	p (perm)
Year	1	0.096	136.74	**0.001**	1	590.44	133.79	**0.001**	1	0.005	1.351	0.238
Temperature	1	0.05	71.156	**0.001**	1	343.38	77.81	**0.001**	1	0.001	0.366	0.546
pH	1	0.003	5.002	**0.034**	1	15.911	3.605	0.057	1	0.002	0.498	0.482
Year × Temperature	1	0.001	2.124	0.141	1	16.84	3.816	0.053	1	1.20E-05	0.002	0.963
Year × pH	1	0.007	10.635	**0.002**	1	40.201	9.109	**0.005**	1	2.76E-05	0.006	0.944
Temperature × pH	1	0.058	82.078	**0.001**	1	558.68	126.6	**0.001**	1	0.103	25.058	**0.001**
Temperature × pH ×Year	1	0.03	0.03	**0.001**	1	135.1	30.61	**0.001**	1	0.02	4.897	**0.02**
Residuals	3370	2.387			3370				3370	0.004		

Table 3: Results of generalised linear mixed-effect models (GLMMs) on the fertilisation success of COTS subjected to different levels of temperature and pH on two experimental years.

Fertilisation success				
Source of variation	numdf	denDF	F-value	p-value
Year	1	15	84.083	**<0.001**
Temperature	2	15	379.514	**<0.001**
pH	1	15	0.011	0.915
Year ×Temperature	1	15	23.378	**<0.001**
Year ×pH	1	15	<0.001	0.850
Temperature ×pH	1	15	0.036	0.993
Year ×Temperature × pH	1	15	0.005	0.941

Table 4: Results of the permutational ANOVA (PERMANOVA) performed on the Gonado-Somatic-Index (GSI) of COTS subjected to different levels of temperature and pH on two experimental years.

Gonado-Somatic Index (GSI)				
Source of variation	df	SS	Pseudo-F	p (perm)
Year	1	101.58	4.061	**0.047**
Temperature	1	217.39	8.691	**0.004**
pH	1	90.918	3.635	0.061
Year ×Temperature	1	1.254	5.014E-2	0.819
Year ×pH	1	55.198	2.207	0.148
Temperature ×pH	1	49.958	1.997	0.168
Year ×Temperature ×pH	1	4.711	0.188	0.646
Residuals	153			

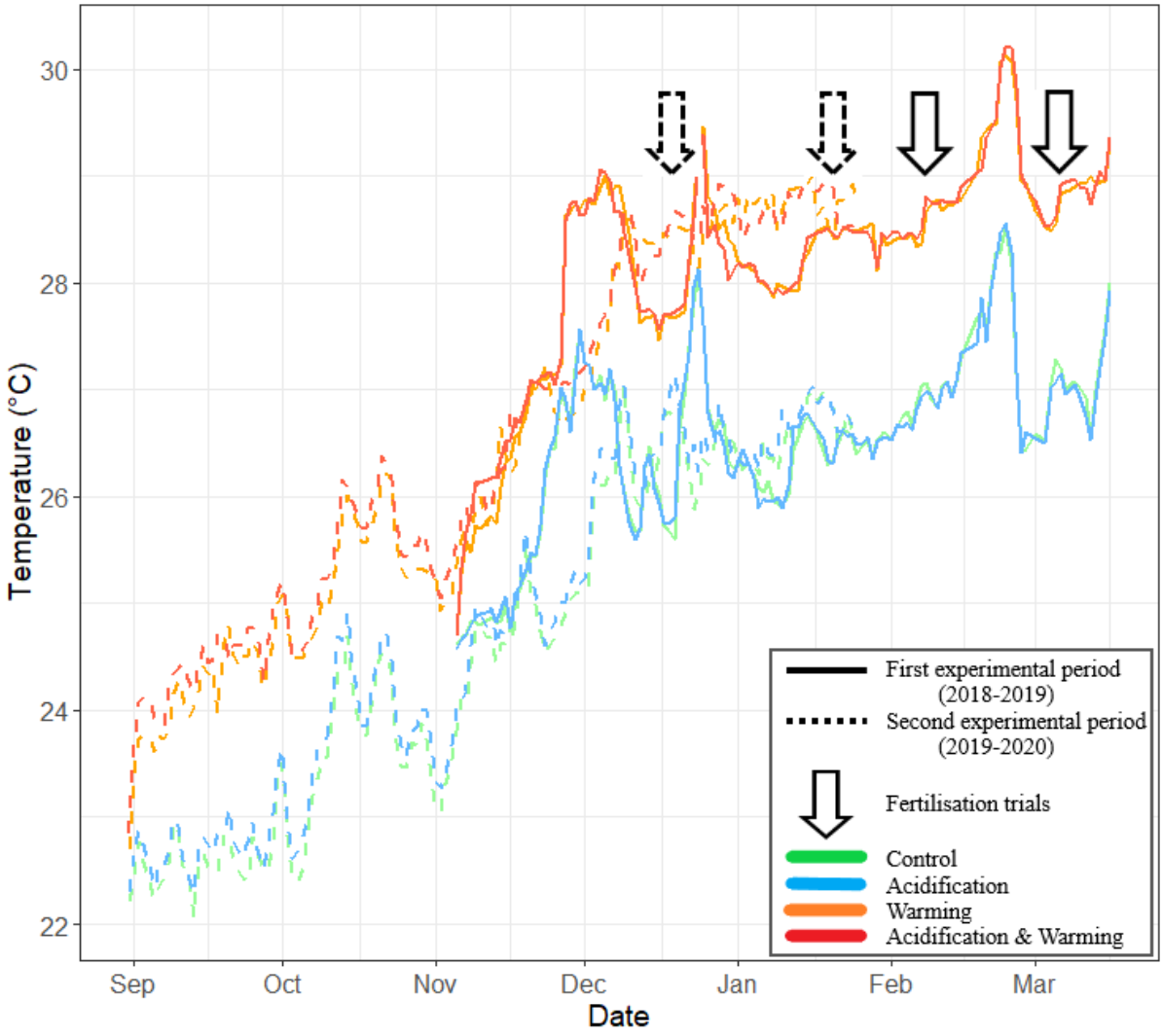


Figure 1. Variation of seawater temperatures over the experimental periods for the four treatments. Colour lines represent mean temperature for each treatment (n=3 replicates) through the whole exposure periods: solid line for the first experimental period 2018-2019, n=130 days and dashed lines for the second experimental period 2019-2020, n=145 days). The arrows mark indicate the fertilisation trials.

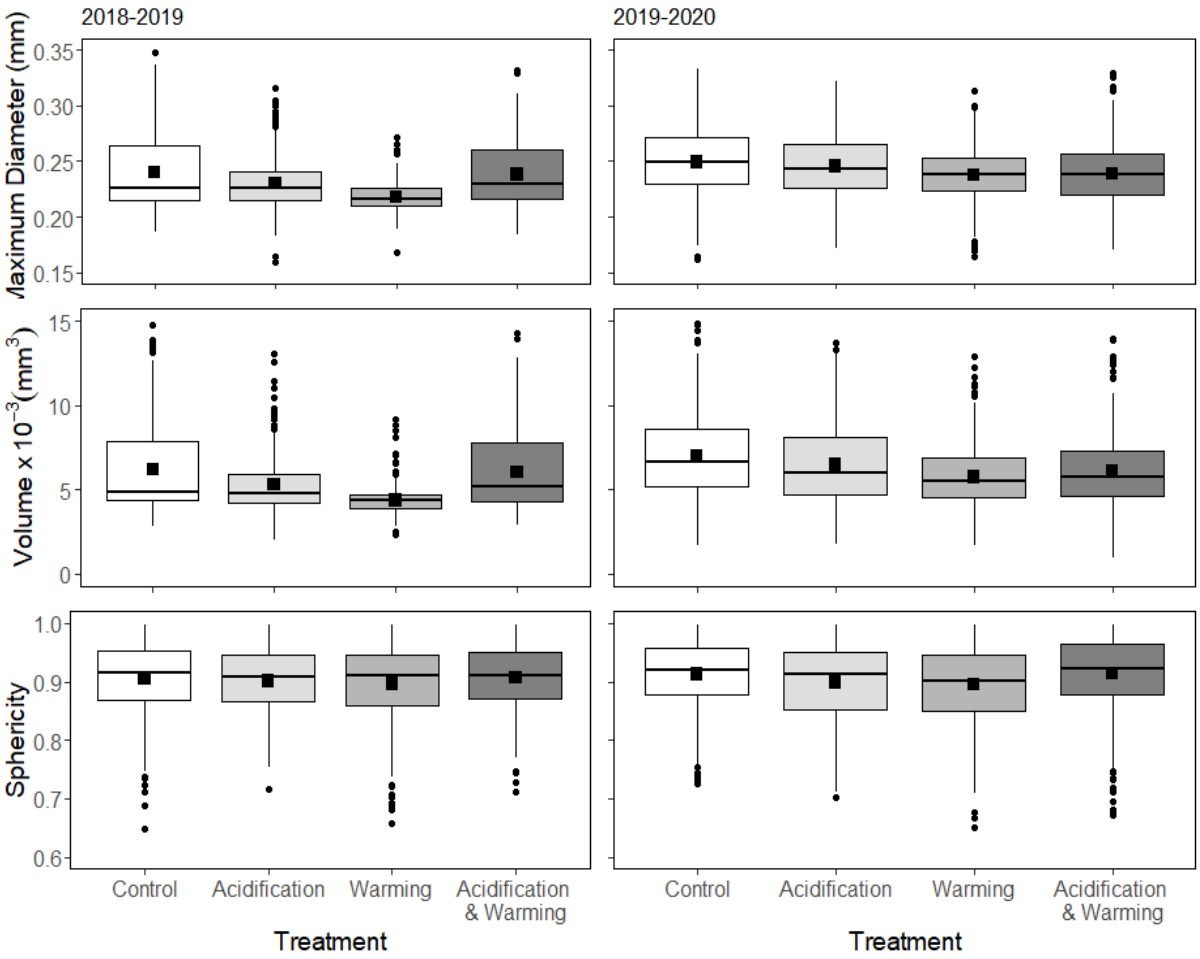


Figure 2. Effects of warming and acidification on maximum diameter, volume and sphericity of COTS eggs. Results of the 2018-2019 and 2019-2020 trials across the four experimental treatments. Bold horizontal lines indicate medians, boxes enclose the upper and lower quartiles of the data, whiskers mark the maximum and minimum values excluding outliers, and the dots show outliers. Bold squares indicate the mean values for each treatment. Sample sizes for 2018-2019 = 269-312. Sample sizes for 2019-2020: 543-565.

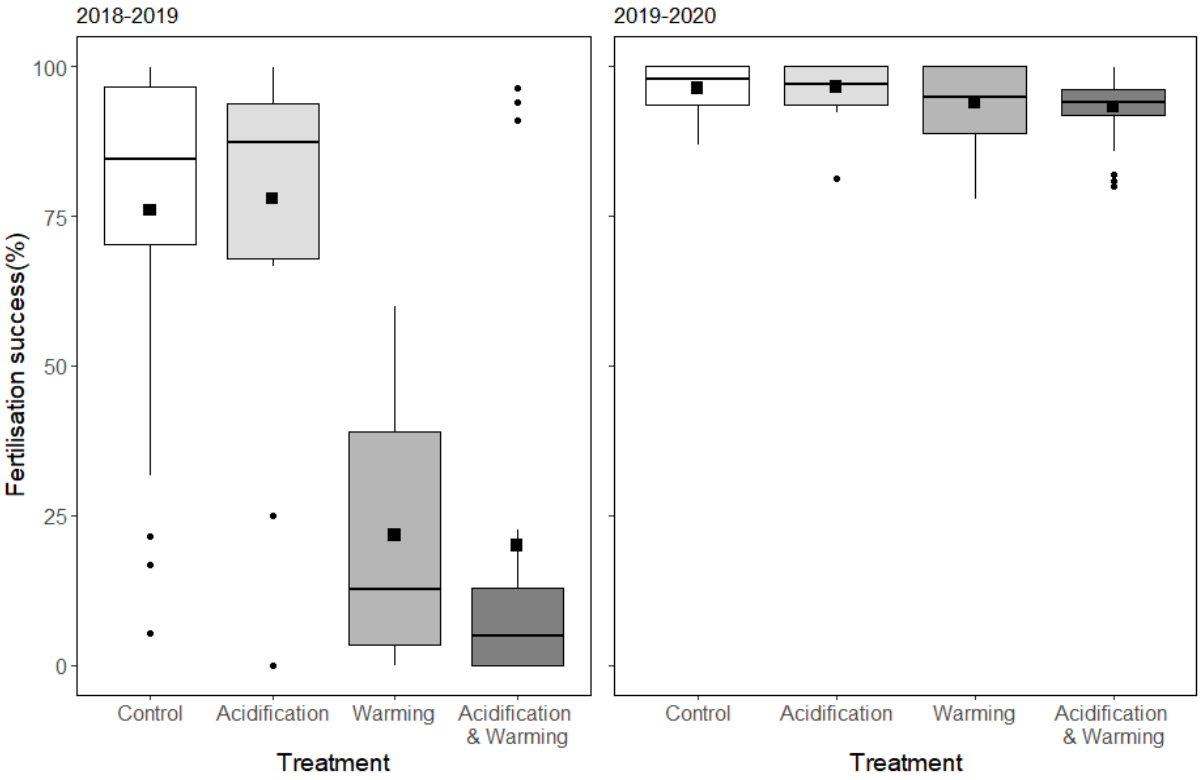


Figure 3. Effects of warming and acidification on COTS fertilisation success. Results of the 2018-2019 and 2019-2020 trials across the four experimental treatments. Bold horizontal lines indicate medians, boxes enclose the upper and lower quartiles of the data, whiskers mark the maximum and minimum values excluding outliers, and the dots show outliers. Bold squares indicate the mean values for each treatment. Sample sizes for 2018-2019 = 30 per treatment except for acidification treatment where n = 20. Sample size for 2019-2020: 30 for all treatments.

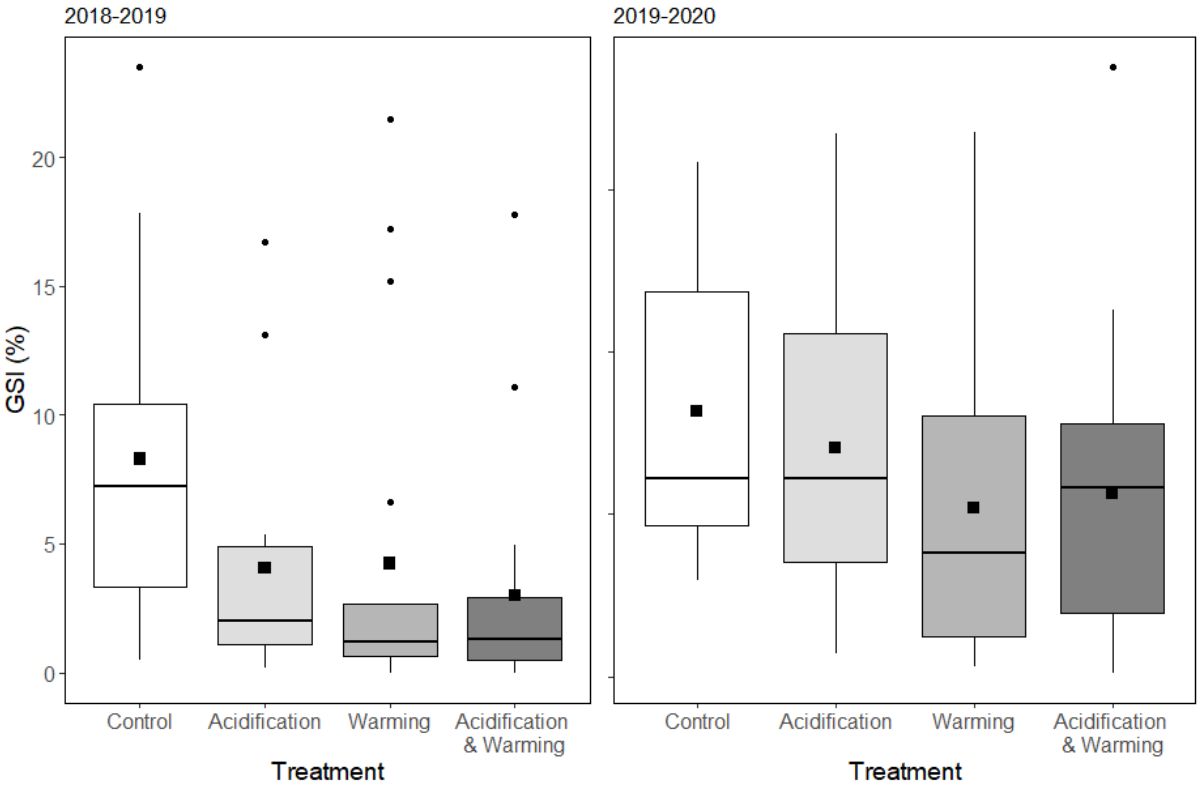


Figure 4: Effects of warming and acidification on COTS Gonado-Somatic-Index (GSI). Results of the 2018-2019 and 2019-2020 trials across the four experimental treatments. Bold horizontal lines indicate medians, boxes enclose the upper and lower quartiles of the data, whiskers mark the maximum and minimum values excluding outliers, and the dots show outliers. Bold squares indicate the mean values for each treatment. Sample sizes for 2018-2019 = 18 animals for control and warming treatments, 17 for acidification and warming and 13 for acidification. Sample size for 2019-2020: 24 animals for al treatments except acidification and warming 23.