Marine Biology (2020) 167:8
https://doi.org/10.1007/s00227-019-3615-0

SHORT NOTE

# Elevated temperature, but not acidification, reduces fertilization success in the small giant clam, *Tridacna maxima*

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Received: 9 May 2019 / Accepted: 30 October 2019


## Abstract

Elevated temperature and decreased ocean pH (ocean acidification) are associated with anthropogenic climate change and can adversely affect fertilization and development in marine invertebrates. However, the potential synergistic impact of these stressors on fertilization success remains unresolved for many ecologically and economically important species including giant clams of the genus *Tridacna*. Individual and interactive effects of warming and acidification on fertilization (successful first cleavage) were investigated in the small giant clam, *Tridacna maxima*. Experiments were performed on gametes of *T. maxima* (collected in October 2015 from the island of Moorea, French Polynesia; 17.54° S, 149.83° W) fertilized under ambient conditions (27 °C, pH 8.1) and under conditions congruent with temperature and pH projections for the coming century (31 °C, pH 7.6). Fertilization success was low, but within previously reported levels, under ambient conditions (47.7 ± 3.4%) and was significantly reduced at elevated temperature per se and in combination with lowered pH (18.5 ± 4.4% and 21.2 ± 4.6%, respectively). However, acidification alone had no effect on fertilization success in *T. maxima* (48.2 ± 3.1%). These results indicate that although fertilization in *T. maxima* is resilient to lowered pH, it is strongly inhibited by elevated temperature. Populations of *T. maxima* may, therefore, be at risk of low reproductive success over the coming century as a result of rising ocean temperature.

## Introduction

Elevated surface seawater temperature (ocean warming) and lowered ocean pH (ocean acidification) are products of increasing atmospheric $pCO_2$ and have been shown in many invertebrates to lead to altered developmental timelines (Stumpp et al. 2011; Armstrong et al. 2017), inhibition of growth (Stumpp et al. 2011; Dorey et al. 2013), malformations in calcitic exoskeletons (Gazeau et al. 2013), and reduced larval fitness (Kroeker et al. 2010; Armstrong et al. 2017). However, the magnitude of these effects can vary significantly both across taxa (Kroeker et al. 2010; Harvey et al. 2013) and across life stages within a species (Kurihara 2008; Harvey et al. 2013; Przeslawski et al. 2015).

Early life stages are often more susceptible to acute changes in the environment than juveniles or adults and require a narrower range of optimal conditions to successfully complete their development (Kurihara 2008; Byrne and Przeslawski 2013). Mortality in such stages can be extremely high (> 90%) even under ambient natural conditions (Kurihara 2008). Early life stages may, therefore, represent important bottlenecks for population growth and persistence under a changing climate (Byrne and

Responsible Editor: J. Grassle.

Reviewed by M. Mies and an undisclosed expert.

**Electronic supplementary material** The online version of this article (https://doi.org/10.1007/s00227-019-3615-0) contains supplementary material, which is available to authorized users.

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Published online: 03 December 2019

Przeslawski 2013). Recent studies have repeatedly demonstrated the heightened sensitivity of early developmental stages of marine organisms to increased temperature and lowered pH (Kurihara 2008; Przeslawski et al. 2015; Espinel-Velasco et al. 2018). However, for most species, we lack data on the sensitivity of zygotes and syngamy to temperature and pH (Przeslawski et al. 2015). Among these understudied taxa are the so-called giant clams of the genus *Tridacna* which are important both culturally and economically throughout their range (Neo et al. 2015a; Moorhead 2018).

Giant clams are highly fecund protandrous simultaneous hermaphrodites (Nash et al. 1988; Menoud et al. 2016) that inhabit tropical Indo-Pacific reefs (Lucas 1988) and display episodic broadcast spawning and sequential gamete release, first spawning sperm and then eggs (Nash et al. 1988; Mies et al. 2012). Populations of giant clams also exhibit synchronized spawning across wide stretches of reef habitat, although spawning cues in giant clams remain unresolved (Soo and Todd 2014). Seasonal and episodic deviations in temperature (often associated with alterations in flow across lagoonal reefs) may initiate spawning in some populations (Gilbert et al. 2006a; Van Wynsberge et al. 2017) and acute heat shock is a method commonly employed in giant clam aquaculture to promote gamete release (Braley 1992; Ellis 1997; Singh and Azam 2013). However, whereas elevated seawater temperatures may act as a spawning cue for adult tridacnines, the effects of warm water on syngamy and zygote viability are unresolved. Similarly, very little research has examined the potential negative effects of increasing oceanic $pCO_2$ on giant clam early development.

While much has been learned about tridacnine early development in mariculture (Beckvar 1981; Crawford et al. 1986; Lucas 1994; Toonen et al. 2011; Mies and Sumida 2012; Mies et al. 2012; Neo et al. 2015b; Southgate 2016, 2017; Militz et al. 2019), studies examining physiological responses to climate-related alterations in temperature and/or pH in these stages are few. In general, exposure to elevated temperature and/or lowered pH results in increased mortality in giant clams (Table 1). However, to date, only three studies have examined the effects of elevated temperature, per se, on giant clam early life stages (Neo et al. 2013; Mies et al. 2018; Enricuso et al. 2019), and none have investigated the potential for additive or synergistic effects of exposure to warming in tandem with acidification associated with increasing oceanic $pCO_2$ (Gunderson et al. 2015). Because giant clams are long-lived (Lucas 1988; Menoud et al. 2016) and slow to mature (~ 10 years to sexual maturity in *T. maxima*; Chambers 2007), they are likely to exhibit limited capacity for adaptation to the rapid environmental changes associated with the anthropocene. Thus, giant clams may be particularly vulnerable to the combined effects of ocean warming and acidification (Watson et al. 2012). With global temperature expected to rise by + 4 °C and average pH of Indo-Pacific reefs expected to decrease 0.5 units (to ca. 7.7) by the end of the century (IGBP et al. 2013; IPCC 2014), there is a need to examine the potential synergistic effects of these changes on early development in giant clams.

**Table 1** Summary of experimental studies manipulating temperature and/or $pCO_2$ in tridacnine species

| Stage | Species/study | Stressor | Effect summary |
|---|---|---|---|
| *Fertilization* | | | |
| | *Tridacna gigas*[a] | Elevated Temp | No effect |
| | *Tridacna squamosa*[b] | Elevated Temp; Reduced Salinity | Increased fertilization success under elevated temperature; No effect of salinity |
| *Larval* | | | |
| | *Tridacna gigas*[a] | Elevated Temp | Precocious development; Developmental abnormalities |
| | *Tridacna squamosa*[b] | Elevated Temp; Reduced Salinity | Reduced survival under elevated temperature; No effect of salinity |
| | *Tridacna crocea*[c] | Elevated Temp | Loss of algal symbionts (i.e., bleaching); Increased mortality |
| *Post-settlement* | | | |
| | *Tridacna gigas*[a] | Elevated Temp | Reduced survival |
| *Juvenile* | | | |
| | *Tridacna crocea*[d] | Elevated pCO2 | Shell dissolution; Increased *Symbionium* density |
| | *Tridacna squamosa*[e] | Low Irradiance; Elevated $pCO_2$ | Reduced survival under multistressors; Reduced growth under acidification |
| | *Tridacna squamosa*[f] | Elevated Temp; Elevated $pCO_2$ | Reduced survival |
| *Adult* | | | |
| | *Tridacna maxima*[g] | Elevated Temp | Change in lipid composition; Upregulation of lipid metabolism and ROS genes |
| | *Tridacna squamosa*[h] | Elevated Temp | Elevated respiration |
| | *Tridacna crocea*[i] | Elevated Temp | Oxidative stress; Apoptosis activation; Symbiosis disruption |

[a]Enricuso et al. (2019), [b]Neo et al. (2013), [c]Mies et al. (2018), [d]Kurihara and Shikota (2018), [e]Watson (2015), [f]Watson et al. (2012), [g]Dubousquet et al. (2016), [h]Elfwing et al. (2001) and [i]Zhou et al. (2019)

Our study is the first to examine the potential synergistic effects of simultaneous exposure to elevated temperature and lowered seawater pH on giant clam fertilization generally and also the first to investigate climate-related responses in a new species model, the economically and ecologically important giant clam, *Tridacna maxima*. We hypothesized that both stressors would result in significant decreases in fertilization success in *T. maxima*, with simultaneous exposure resulting in a larger, synergistic, reduction.

## Methods

### Broodstock acquisition and maintenance

Eight adult *Tridacna maxima*, mean shell length 12.2 ± 2 cm ($\bar{x} \pm$ SD), were collected from fringing reefs at depths of 1−3 m from the island of Mo'orea, French Polynesia in October 2015. The clams were immediately transferred to an onshore holding tank (~ 380 L) at the University of California, Richard B. Gump Field Station and maintained outdoors, under ambient insolation, in flowing seawater (26.9 ± 1 °C, salinity = 35.96 ± 0.2, pH = 8.14 ± 0. 10) for at least 2 day prior to use in the spawning experiment.

### Spawning induction

Spawning was carried out in two rounds (with four clams in each round) over a 2 day period in early November 2015 approximately 1 week after the previous full moon (i.e., during a waning half-moon). Spawning was induced in each clam by injection of dilute serotonin (5-hydroxytryptamine creatinine sulfate complex, Sigma-Aldrich CAS 153-98-0; prepared as 0.5 mL of 1 mM serotonin in 0.2 µm-filtered seawater) directly into the gonad (Crawford et al. 1986). Clams were placed in separate 1.5 L spawning tanks where they released sperm. Although all eight broodstock clams released sperm, only four individuals (two in each experimental round) also released eggs. Sperm from clams that produced only sperm were used to fertilize eggs from clams that had produced both gametes. Clams were paired on a 1:1 basis (i.e., four sperm-donor clams were paired with four egg-donor clams) with crosses proceeding in one direction only (i.e., sperm from a sperm-donor × eggs from an egg-donor). Sperm from clams that had released both eggs and sperm were not used in this study (i.e., no reciprocal crosses) and eggs from an egg-donor were never fertilized by more than one sperm-donor.

For each of the four sperm-donor clams, concentrated sperm stock was collected using a 50-mL syringe positioned above the exhalant siphon. In the four egg-donor clams, after sperm production had ceased, clams were rinsed thoroughly with fresh seawater and transferred to new 1.5-L egg collection tanks where gamete (i.e., egg) release continued. Clams were allowed to continue egg release until they were spent at which point, samples were collected for egg density counts and fertilization experiments as described below. No broodstock clam died as a result of serotonin injection and all were returned to the site of collection within 2 weeks of experiment completion.

### Fertilization and zygote culture

For each clam that released eggs ($n = 4$), one 50-mL sample of egg stock was aliquoted prior to fertilization and set aside for quantification of egg density. Eggs were immediately counted and imaged from ten, 0.1-mL aliquots of each stock solution using a Leica® dissecting microscope with optical light microscope camera (Leica MZ16; Leica DFC420). Images were also taken of eggs alongside scale references, and egg diameter was measured using the image analysis program ImageJ64 (Schneider et al. 2012; Fig. 1).

Culture water for fertilization was collected from offshore, and filtered through a 75-µm mesh before being delivered to four 80-L header tanks at a rate of ca. 50 L $h^{-1}$. Parameters in header tanks were maintained following a 2 × 2 temperature (28 ± 1 °C modern ambient, 31 ± 2 °C predicted future) by pH (7.6 ± 0.06 and 8.1 ± 0.04) design. Elevated temperature was achieved using 250-W aquarium heaters and low pH (i.e., high $pCO_2$) conditions were maintained as described in the Seawater Acidification section below. A full list of experimental seawater parameters is given in Table 2.

Fertilization took place under treatment conditions and was carried out in one direction (i.e., sperm from one clam with eggs from another, but not the inverse). Eggs were

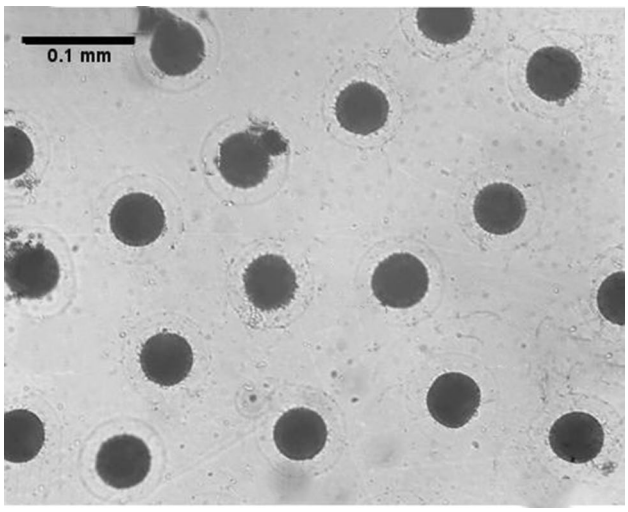


**Fig. 1** Newly spawned, unfertilized, *Tridacna maxima* eggs showing vitelline envelope surrounding plasma membrane

**Table 2** Measured and calculated seawater carbonate chemistry parameters ($\bar{x} \pm sd$) for all experimental treatments of $pCO_2$ and temperature (T)

| | Variable | Ambient $pCO_2$ | | High $pCO_2$ | |
|---|---|---|---|---|---|
| | | Ambient T | High T | Ambient T | High T |
| Measured | pH | 8.14 ± 0.10 | 8.00 ± 0.10 | 7.63 ± 0.10 | 7.61 ± 0.10 |
| | Temp (°C) | 27.08 ± 0.8 | 31.14 ± 2 | 27.08 ± 0.8 | 31.14 ± 2 |
| | Salinity | 36.0 ± 0.4 | 36.4 ± 0.7 | 36.3 ± 0.4 | 36.6 ± 0.7 |
| | $A_T$ (µmol kg$^{-1}$) | 2359.2 ± 83 | 2361.3 ± 93 | 2344.6 ± 97 | 2352.7 ± 104 |
| Calculated | $pCO_2$ (µatm) | 432.5 | 450.7 | 2023.4 | 2154.1 |
| | DIC (µmol kg$^{-1}$) | 2043.05 | 2016.05 | 2297.08 | 2293.05 |
| | $HCO_3^-$ (µmol kg$^{-1}$) | 1805.77 | 1759.76 | 2172.77 | 2162.92 |
| | $CO_3^{2-}$ (µmol kg$^{-1}$) | 225.71 | 245.34 | 70.28 | 77.85 |
| | $\Omega_{Aragonite}$ | 3.59 | 3.98 | 1.12 | 1.26 |
| | $\Omega_{Calcite}$ | 5.41 | 5.90 | 1.68 | 1.87 |

All parameters were calculated from salinity, temperature, total alkalinity ($A_T$) and pH (total scale) using the "carb" function of the R package seacarb (Lavigne et al. 2011)

washed twice with treatment water before 12, 50-mL aliquots of egg stock solution were transferred to individual 50-mL Falcon tubes (mean density of 222 ± 88 eggs mL$^{-1}$, $\bar{x} \pm ci$, $n = 40$ counts) which were modified with cap-mounted driplines and 75-µm mesh covered bottoms, permitting flow-through culturing, hereafter referred to as culture tubes. For each of the four clam pairs, 12 culture tubes were prepared yielding three replicates for each of the four temperature × pH treatments per pairing. Replicate culture tubes were placed in separate 1.7-L water baths containing respective treatment water. Fertilization was initiated by the addition of 1 mL of well-mixed, concentrated sperm stock from the paternal clam (i.e., an egg-sperm volumetric ratio of 1:50) to each tube, and tubes were sealed allowing fertilization to take place under treatment conditions. Immediately upon sealing of tubes, culture water began to be delivered from header tanks to culture tubes via cap-mounted driplines at a flow rate of 7.6 L h$^{-1}$ (ca. 150 volumetric turnovers per hour) and tubes were kept continuously immersed in treatment water in a semi-shaded location outdoors. Embryos were maintained in the flow-through culture tubes for 2 h prior to estimation of fertilization success.

## Assessment of fertilization success

Fertilization success was measured as the number of zygotes that had successfully undergone first cellular cleavage to the two-cell stage after 2 h. To count successfully divided embryos, inflow from header tanks was stopped and replicate culture tubes were gently agitated in their respective water baths to re-suspend eggs. A 1-mL aliquot was taken from each tube and transferred to a separate micro-centrifuge tube from which two subsequent 100-µL aliquots (i.e., technical replicates) were used in counting analyses. This resulted in the counting of 24 aliquots per clam pair (96 aliquots total): two 100-µL aliquots (technical replicates) from each of three replicate 50-mL culture tubes for each of the four treatment conditions. Aliquots were placed on a glass microscope slide for visual inspection of embryos. All embryos within a 100-µL aliquot were counted and the proportion of embryos which had successfully undergone the first cleavage was recorded. Aliquots were analyzed in a random order across all clam pairs/treatments to reduce the chance of time-dependent effects on any one treatment, and all counting was concluded within 1 h of collection (i.e., maximum of 3 h post-fertilization).

## Statistical analyses

Data were analyzed using the statistical software program R (v 3.2.5; R Development Core Team, 2008). Normality of data was checked using the shapiro.test() and test_normality() functions of the "stats" and "LambertW" packages (Goerg 2011, 2015). As we observed no significant differences in fertilization success within a given pairing across water baths or replicate culture tubes (i.e., no "tank effects"), we treated culture tubes as independent technical replicates and all data are therefore presented as pair-specific means (generated from the three replicate culture tubes per pairing per treatment). Fertilization success data were not normally distributed as a result of the low fertilization success of pairing 2. While removal of pairing 2 data did cause remaining data to meet normality criteria, it did not alter the results of statistical analyses (i.e., significant factors remained unchanged). Thus, a three-way factorial analysis of variance (ANOVA) was performed on all available data using the general linear model with pH, temperature, and clam pairing as explanatory factors.

### Seawater acidification and carbonate chemistry

Lowered pH treatments were maintained by the controlled bubbling of 80-L header tanks with pure $CO_2$ using an IKS Aquastar pH controller and solenoid-valve gas regulation system ($CO_2$ Art). Seawater $pH_T$ and salinity were measured every 15 min using a Professional Plus Multiparameter Instrument (YSI Quatro Dual, Model 1001 pH sensor) calibrated with four, spectrophotometrically determined, pH samples. Calibration standards were measured using *m*-cresol purple sodium salt dye using an Evolution 60S UV–Visible spectrophotometer against a Tris-buffered pH reference standard (Dickson Strand 13/Bottle 74) following modified best practice methods (Dickson et al. 2007). Seawater temperature was recorded using Thermochron iButton data loggers (±0.5 °C resolution; model DS192G-F5#; iButton Link Technology). Samples for determination of total alkalinity ($A_T$) were taken immediately prior to fertilization and were measured via open-cell potentiometric titration with an automatic titrator (T50, Mettler-Toledo). Measurements of $A_T$ were conducted on duplicate 50-mL samples at room temperature (~23 °C) and $A_T$ was calculated as described previously (Dickson et al. 2007). Titrations of certified reference materials (CRM) provided by A. G. Dickson (Dickson Batch 13/Bottle 74) yielded $A_T$ values within ±4 mol $kg^{-1}$ of the nominal value ($n=8$). Parameters of the seawater carbonate system were calculated from salinity, temperature, $A_T$ and $pH_T$ using the R package seacarb (Table 2; Lavigne et al. 2011).

## Results and discussion

### Fertilization success

Reported fertilization success estimates in tridacnine clams have spanned a wide range from highs of ~80–90% in *Tridacna gigas*, *T. noae* and *T. noae* ♀/*T. maxima* ♂ hybrids (Southgate 2017; Enricuso et al. 2019; Militz et al. 2019) to lows of ca. 30–60% in *T. squamosa* and *T. maxima* ♀/*T. noae* ♂ hybrids (Neo et al. 2011, 2013; Militz et al. 2019). Our estimate for *Tridacna maxima* in this study measured under similar, ambient, conditions (47.7 ± 3.4%, $\bar{x}$ ± 95% confidence interval; Fig. 2a) falls closer to these latter values.

Whereas polyspermy (resulting from high sperm:egg ratios ex vivo) can result in reduced fertilization success (Braley 1992) we did not observe any symptoms of polyspermy (e.g., malformed embryos or embryos surrounded by numerous sperm) during counts, and thus, some other factor must have caused these low fertilization success rates. One possible explanation for low fertilization success may be related to the reproductive condition of the broodstock clams. Although, average reproductive output was

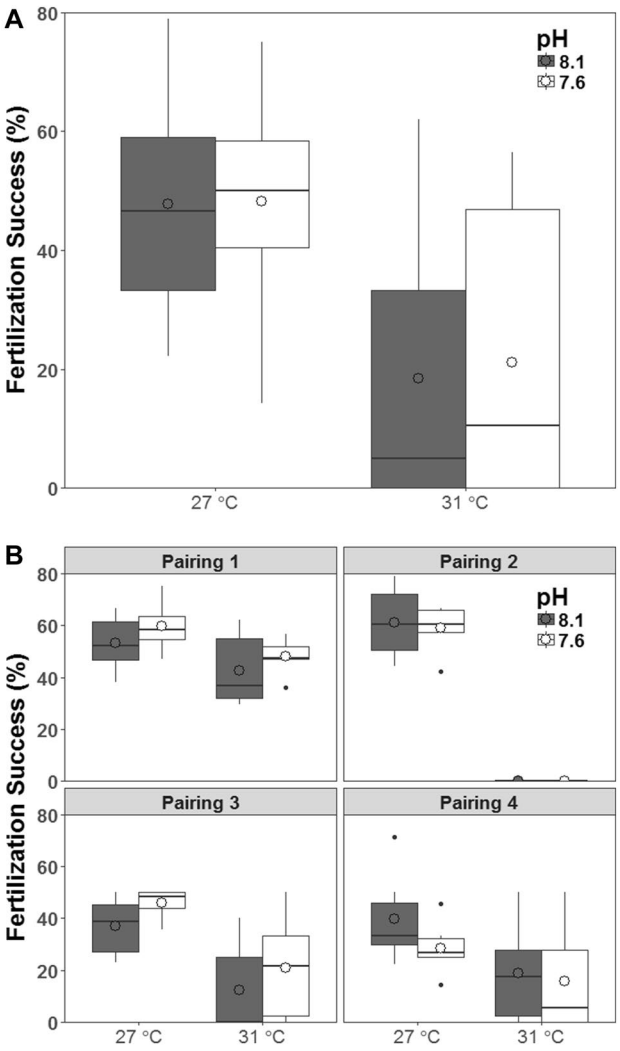


**Fig. 2** Box-plot of fertilization success in **a** all *Tridacna maxima* and **b** individual clam pairings under experimental temperature (x-axis) and pH (acidified in white, ambient in grey) conditions. Solid bars and open circles denote group median and mean fertilization success, respectively. Whiskers represent lower (Q1) and upper (Q3) quartile ranges

relatively high (377,469 ± 149,104 eggs $ind.^{-1}$; $\bar{x}$ ± ci, $n=40$ counts), individual output varied significantly, ranging from $1.00\times10^4$ eggs $ind.^{-1}$ in pair 2 to $1.38\times10^6$ eggs $ind.^{-1}$ in pair 1 (Appendix 1, $n=10$ counts per pairing). Most eggs appeared well formed with complete vitelline envelopes (Fig. 1), but released eggs were smaller, on average, than previously reported in tridacnine clams (84.0 ± 1.2 µm in diameter as compared to ~100 µm in diameter; Ellis 1997). The large variation in reproductive output and small average egg size may suggest that egg-donor clams were not fully ripe at the time of spawning. As reported in other bivalves (e.g., *Chlamys bifrons*, Styan and Butler 2000 and *Panopea zelandica,* Gribben et al. 2014) initiation of spawning via serotonin injection can result in the release of both ripe

and unripe oocytes. Given the significant effect of pairing (ANOVA, $F(3,90) = 20.44$, $p < 0.001$) and interactive effect between temperature and pairing (ANOVA, $F(3,90) = 18.85$, $p < 0.001$) on fertilization success observed in our study, it is possible that our estimates of fertilization success for *T. maxima* were artificially depressed as a result of release of some immature unviable eggs. This is particularly noteworthy for the pairing exhibiting the most extreme negative response to elevated temperature (i.e., 0% fertilization success in pairing 2, Fig. 2b) in which the egg-donor clam was the smallest (Appendix 1) and released the smallest eggs used in this study.

## Sensitivity to warming

We observed that even modest warming (+3 °C) significantly reduced fertilization success in *Tridacna maxima* (ANOVA, $F(1,90) = 115.85$, $p < 0.001$; Fig. 2a). Elevated temperature (alone and in combination with lowered pH) resulted in fertilization success rates of $18.5 \pm 4.4\%$ and $21.2 \pm 4.6\%$, respectively ($\bar{x} \pm$ CI; Fig. 2a). This high sensitivity of fertilization success in *Tridacna maxima* to increased temperature was surprising, especially given the narrow range of temperatures examined in this study (i.e., $28 \pm 1$ °C control versus $31 \pm 2$ °C treatment). This suggests a relatively high sensitivity of fertilization success to elevated temperature in *T. maxima* which stands in stark contrast to responses reported for other broadcast spawning organisms (e.g., corals, polychaetes, echinoderms) including several species of bivalve molluscs (*Crassostrea gigas*, *Mytilus galloprovinciallis*, *Saccostra glomerata*, *Spisula solidissima,* and *Laternula elliptica*) (Byrne 2011; Bylenga et al. 2015) and other closely related giant clam species, *Tridacna squamosa, T. gigas,* and *T. crocea* (Neo et al. 2013; Mies et al. 2018; Enricuso et al. 2019). In these species, high rates of fertilization were achieved over a broad range of temperatures including warming scenarios far in excess of predicted future ocean conditions (up to +6 °C above ambient; Byrne 2011). For example, in *Tridacna squamosa*, fertilization success was higher at 29.5 °C than at 22.5 °C suggesting a positive relationship between warming and early development (Neo et al. 2013). Similarly, in *Tridacna gigas*, fertilization success was unaffected by elevated temperature (up to +5 °C above ambient), although later developmental stages showed increased mortality under warming (Enricuso et al. 2019). In *T. crocea*, exposure of larvae to temperatures +3 °C above ambient (i.e., 29 °C) resulted in loss of most strains of symbiotic algae (i.e., bleaching), however, significant effects on larval survival were only observed under exposure to +6 °C above ambient (i.e., 32 °C; Mies et al. 2018). In commercial giant clam aquaculture, heat shock (exposure to temperatures up to +6 °C above ambient) is routinely used to obtain fertile gametes with little ill effect on larval yields (Braley 1992), and peak spawning in natural populations tends to occur during warm periods, particularly in the summer, when water temperatures may reach 31 °C (Jameson 1976; Beckvar 1981; Soo and Todd 2014).

For example, in French Polynesia, although giant clams are known to be reproductively active throughout the year (consistent, high spat collection; Remoissenet and Wabnitz 2012), peak gamete release often occurs during the warm austral summer (March–April; S. Van Wynsberge *pers. comm.*) presumably in response to elevated temperature. However, in some semi-closed, French Polynesian atolls, mass spawning in *Tridacna maxima* populations has been observed in the austral fall/winter, apparently in response to periods of lower ambient temperatures (~2 °C below the annual average; Gilbert et al. 2006b; Van Wynsberge et al. 2017) suggesting that some populations may have evolved temperature-specific gamete release strategies. If the *T. maxima* used in this study have evolved to reproduce under cooler conditions, this could explain, at least in part, the extreme sensitivity to the warming we observed. These potential differences among populations in reproductive timing relative to seasonal surface water temperature trends are interesting and could suggest the potential for adaptive differences in temperature-selective gamete release. Despite considerable research, no consensus has yet been reached regarding initiation of reproduction in tridacnine species (Soo and Todd 2014) and further research is certainly warranted to address the potential for temperature-selective spawning across giant clam species/populations.

## Resilience to acidification

In contrast to *T. maxima*'s demonstrated temperature-sensitivity, fertilization success in this species was insensitive to acidification. Exposure to acidification alone did not significantly affect fertilization success (ANOVA, $F(1,90) = 0.24$, $p = 0.6$), with eggs fertilized under lowered pH conditions showing a mean success rate of $48.2 \pm 3.1\%$ ($\bar{x} \pm$ CI). In addition and contrary to our initial hypothesis, simultaneous exposure to elevated temperature and lowered pH did not result in a significant synergistic reduction in fertilization success in *T. maxima* and there was no significant interactive effect between elevated temperature and lowered pH (ANOVA, $F(1,90) = 0.35$, $p = 0.6$) on fertilization success in this species. There was also no interactive effect observed between pH and pairing (ANOVA, $F(3,90) = 2.09$, $p = 0.1$) on fertilization success suggesting that, unlike for temperature, reproductive state or provisioning did not affect acidification tolerance in *T. maxima* zygotes. Similarly, we observed no significant three-way interactive effect among temperature, pH, and pairing on fertilization success in *T. maxima* (ANOVA, $F(3,90) = 0.18$, $p = 0.9$).

The relative insensitivity of *T. maxima* fertilization to lowered pH is surprising given that similar reductions in ambient pH have previously been shown to negatively impact fertilization success in many marine invertebrate species, most notably in echinoderms which have been studied extensively (Kurihara and Shirayama 2004; Dupont et al. 2008; Havenhand et al. 2008; Byrne et al. 2009), but also in other bivalve molluscs (Parker et al. 2009; Scanes et al. 2014; Wang et al. 2016; Shi et al. 2017; Świeżak et al. 2018). In the rock oyster *Saccostrea glomerata*, the clam *Limecola balthica,* and the scallop *Argopecten irradians*, exposure of gametes to elevated $pCO_2$ resulted in significant reductions in fertilization success (Parker et al. 2009; Wang et al. 2016; Świeżak et al. 2018). Similarly, in the broadcast spawning clam, *Tegillarca granosa*, a reduction in fertilization under elevated $pCO_2$ was directly linked to reduced sperm motility and impaired acrosomal fusion (Shi et al. 2017). No previous studies have examined responses to acidification in giant clam embryos or larvae, but exposure to increased $pCO_2$ resulted in reduced mass gain and increased mortality in juvenile *Tridacna squamosa* (Watson et al. 2012; Watson 2015) and decreased shell growth in juvenile *T. squamosa* and *T. crocea* (Watson 2015; Kurihara and Shikota 2018). This could imply that, contrary to our initial predictions, it is the later developmental stages (i.e., juvenile, adult) in giant clams that are most sensitive to lowered pH.

## Broader impacts

While the robust response of *T. maxima* fertilization to predicted future ocean pH is encouraging, the susceptibility of this process to elevated temperature suggests possible negative population responses to ocean warming. Even under ambient conditions, survival in tridacnine early life history stages is generally low, with reports of ~ 75% mortality 24-h post fertilization (Mies et al. 2012) and > 99% mortality over the course of development from zygote to juvenile metamorphosis (Beckvar 1981; Crawford et al. 1986). With populations of giant clams already severely overharvested over much of their range (Yamaguchi 1977; Van Wynsberge et al. 2016; Neo et al. 2017) and recent reports of massive broodstock losses as a result of climate-associated temperature variation (Andréfouët et al. 2013; Van Wynsberge et al. 2018), there are currently many efforts underway to utilize giant clam mariculture to restock populations on overexploited reefs (Neo and Todd 2013; Neo et al. 2017). However, these efforts may ultimately be hampered by gametic (this study) or larval (Neo et al. 2013; Enricuso et al. 2019) thermal sensitivity in restocked populations. Incorporating these "early stage" data into commercial giant clam mariculture practices could help to inform these restocking efforts. For example, current "best practices" for spawning of giant clam in mariculture suggest maintenance of temperature ranges (i.e., 28–30 °C; Oengpepa 2019) which, based on our data for *T. maxima*, may be too high to maximize fertilization success for some species or source populations. Determining to what extent observed thermal sensitivity depends on the reproductive state (e.g., insufficiently ripe reproductive tissues or low provisioning of eggs) or on other, potentially heritable, physiological factors is an important next step for tridacnine research. These data are vital for improving our understanding of the mechanisms underlying this variation in temperature sensitivity (as well as how quickly broodstock populations may respond after extreme thermal events) and are therefore of significant interest for improving mariculture and restoration outcomes in giant clams under a warming climate.

**Acknowledgements** We would like to thank Antoine Puisay and Benoît Le Marechal, for help in collecting mature broodstock clams, and Franck Lerouvreur, Pascale Ung, and Valentine Brotherson for their invaluable aid in construction and maintenance of the aquarium facilities and in securing $CO_2$ for use in this study. We would also like to thank Dr. Miguel Mies and another anonymous reviewer whose comments/suggestions helped improve and clarify this manuscript. This research was conducted with US Government support to EJ Armstrong under and awarded by the Department of Defense, Air Force Office of Scientific Research, National Defense Science and Engineering Graduate (NDSEG) Fellowship, 32 CFR 168a. A previous version of this article was published as a thesis chapter by Armstrong (2017) and is available at http://digitalassets.lib.berkeley.edu/etd/ucb/text/Armstrong_berkeley_0028E_17535.pdf.

## Compliance with ethical standards

Clams were collected under ordinance no. 88-184/AT of the French Polynesian Ministère de l'Économie, des Finances, du Travail et del'Emploi following all requirements laid out by the Plan de Gestion de l'Espace Maritime (PGEM) in French Polynesia and were maintained and studied in ways commensurate with all pertinent University of California guidelines. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

**Conflict of interest** The authors declare that they have no conflicts of interest.

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