



Bleaching reduces reproduction in the giant clam *Tridacna gigas*

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ABSTRACT: Bleaching or the expulsion of the photosynthetic symbiont Symbiodiniaceae ('zooxanthellae') caused by the increase in seawater temperatures causes massive mortalities in giant clams. After a bleaching disturbance, population recovery is heavily dependent on reproduction and recruitment success. However, how bleaching directly affects the reproductive performance of giant clams is, to date, unknown. Here, we examined the direct impacts of bleaching on the reproduction of the giant clam *Tridacna gigas* between 2020 and 2021 in the Philippines. A continuous 6 mo monitoring of egg concentration, egg stage, egg size, recovery, and survival in prolonged bleached (moderate or severe) and non-bleached hatchery-bred *T. gigas* maintained in the wild was conducted. The probability of producing eggs and the proportion of intact eggs (developing and mature) were highest in healthy giant clams, followed by moderately bleached giant clams, and lowest in severely bleached giant clams in which >50 % individuals had empty gonads. The oocyte mean diameter was similar among giant clams, regardless of degree of bleaching. Only 5 severely bleached giant clams showed partial color recovery, whereas 2 healthy and 12 severely bleached giant clams died. This study shows that apart from the lethal effects of severe bleaching, the reproductive processes of surviving bleached *T. gigas* such as gametogenesis and egg production can be also negatively affected. Results suggest that the increasing frequency and intensity of bleaching due to thermal stress can lower the reproductive potential of giant clams and likely other zooxanthellate organisms, resulting in reduced recruitment and population decline.

KEY WORDS: Bleaching · Heat stress · Heatwaves · Reproduction · Gametogenesis · Giant clams

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1. INTRODUCTION

Bleaching, or the expulsion of the photosynthetic symbiont Symbiodiniaceae (also known as 'zooxanthellae'), caused by the increase in seawater temperatures causes massive mortalities in zooxanthellate reef organisms such as corals and giant clams, resulting in significant declines in their population worldwide (Gomez & Mingoa-Licuanan 1998, Baker et al. 2008, Junchompoo et al. 2013, DeCarlo et al. 2017, Apte et al. 2019, Raymundo et al. 2019). After a bleaching disturbance, the recovery of their population is heavily dependent on successful reproduction

and recruitment. Several studies have shown that bleaching negatively affects reproduction in corals. Bleaching was observed to reduce the number of reproductive polyps and fecundity in many species of *Acropora* and *Pocillopora* corals (Hirose & Hidaka 2000, Ward et al. 2000), and effects were observed to persist even after recovery from bleaching (Johnston et al. 2020, Ward et al. 2000, Leinbach et al. 2021). Colonies of *Montastrea annularis* that remained bleached for 7 mo failed to complete gametogenesis during their reproductive season, but colonies that had recovered from bleaching showed normal gametogenesis (Szmant & Gassman 1990).

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Many bleached and even recovered species of *Acropora* were observed to produce smaller eggs (Ward et al. 2000), although some species such as *A. millepora* and *P. verrucosa* were able to maintain the size of their eggs after bleaching events (Hirose & Hidaka 2000, Jones & Berkelmans 2011). Also, spawning rates in *P. verrucosa* and *Orbicella* spp. were reduced by bleaching (Hirose & Hidaka 2000, Levitan et al. 2014). All these studies demonstrate that bleaching can impede reproductive success and reduce recruitment in corals, which can be a challenge to the sustainability and recovery of their population.

The effects of bleaching on reproduction in other zooxanthellate organisms such as giant clams are not well known; however, it is likely that their reproductive performance will also be affected by bleaching as, like many species of corals, they also rely on zooxanthellae as their main energy source (Goreau et al. 1973, Fisher et al. 1985, Ishikura et al. 1999, Klumpp et al. 1992, Lucas 1994). Considering that successful reproduction and recruitment are crucial to the sustainability and recovery of a population after a disturbance, and also that rapid climate change will increase the intensity and frequency of bleaching events, there is an urgent need to understand how bleaching affects the reproductive processes and population dynamics of other zooxanthellate organisms, aside from corals, under future oceans.

Here, we focus on giant clams (Bivalvia: Cardiidae: Tridacninae), the largest living bivalves worldwide, found in shallow coral reefs and seagrass areas throughout the Indo-Pacific region (Lucas 1988, Jantzen et al. 2008, Neo et al. 2017). Giant clams play a key role in coral reef ecosystem functioning by providing habitat and food to various marine fauna (Cabaitan et al. 2008, Neo et al. 2015). Giant clams host millions of zooxanthellae in the tubular systems of their mantle tissues (Norton et al. 1992), which can provide them with majority of the essential nutrients such as glucose, amino acids, and carbon, which they need for maintenance, growth, and reproduction (Goreau et al. 1973, Fisher et al. 1985, Klumpp et al. 1992, Lucas 1994, Ishikura et al. 1999). Although giant clams are mixotrophs, heterotrophy through filter feeding only contributes a small amount of energy for their daily metabolic needs (Klumpp et al. 1992, Jantzen et al. 2008). For instance, giant clams can only acquire up to 65% of their carbon requirements from filter feeding, whereas autotrophy through photosynthesis of zooxanthellae can provide the giant clams with up to 126% of their carbon requirements (Klumpp et al. 1992). Thus, the increase in seawater temperatures that causes bleach-

ing can also be detrimental to giant clams by reducing their nutrient resource, which consequently may affect their survival and physiological processes such as reproduction.

Bleaching in giant clams was reported across their distribution during times when corals were also bleaching (Gomez & Mingoa-Licuanan 1998, Addessi 2001, Buck et al. 2002, Leggat et al. 2003, Junchompoo et al. 2013, Apte et al. 2019, Mies 2019). Aside from the observed mass mortalities in giant clam populations (Gomez & Mingoa-Licuanan 1998, Junchompoo et al. 2013, Apte et al. 2019), their recruitment was also observed to be reduced after bleaching events, which has been suggested to be the consequence of mortalities in their reproducing adult individuals (Andréfouët et al. 2013, Van Wynsberge & Andréfouët 2017, Apte et al. 2019). However, failure in the reproduction of the surviving adult individuals can also be a possible reason for the reduced recruitment. Thermal stress was also found to cause bleaching and mortality in the larvae of the giant clam *T. crocea* (Mies et al. 2018). Additionally, Enricuso et al. (2019) reported that post-settlement survival of *T. gigas* juveniles was reduced at a bleaching temperature of 33°C, suggesting potential thermal stress effects on the early life stages of giant clams.

Giant clams are protandric simultaneous hermaphrodites that reproduce through broadcast spawning of their eggs and sperm (Braley 1984). Their gametogenesis can be completed in as little as 3 mo (Murakoshi & Kawaguti 1986, Braley 1988), and spawning peaks during the summer months (Braley 1984, Nash et al. 1988, Shelley & Southgate 1988, Tan & Yasin 2000), although some species such as *T. gigas* have been observed to contain mature gametes throughout the year (Bell & Pernetta 1988, Nash et al. 1988).

To better understand how bleaching affects the reproductive performance of giant clams, we evaluated the direct impacts of bleaching on the reproduction of *T. gigas*, the largest and most vulnerable species of giant clams. To date, wild *T. gigas* individuals can only be found in protected sites such as in the Great Barrier reef in Australia (Barrier Reef Australia 2021), whereas the species was reported to be locally extinct in many regions such as in the Philippines, mainly due to overfishing (Wells 1996, Gomez & Mingoa-Licuanan 2006). However, through artificial spawning and aquaculture, *T. gigas* has been successfully restocked in many sites around the Philippines (Gomez & Mingoa-Licuanan 2006). The Bolinao Marine Laboratory (BML) of the Marine Science Institute, University of the Philippines (UP-MSI) has

been successful in culturing *T. gigas* since the 1980s and to date, ~34,000 *T. gigas* individuals are found in the Giant Clam Ocean Nursery of the BML, the largest giant clam nursery in the country.

In 2019 and 2020, giant clam bleaching was observed in the Giant Clam Ocean Nursery of BML, coincident with high temperatures and coral bleaching in nearby reefs. Hence, a continuous 6 mo monitoring of egg concentration, egg stage, egg size, and the progress of bleaching and survival in prolonged bleached (moderate or severe) and non-bleached giant clams *T. gigas* was conducted. This information is crucial to understanding how thermal stress and bleaching may directly affect the reproduction and population dynamics of zooxanthellate reef organisms, particularly for vulnerable and endangered species such as the giant clam *T. gigas*.

2. MATERIALS AND METHODS

2.1. Study site and organism

This study was conducted in the Giant Clam Ocean Nursery of BML located off Silaqui Island, Bolinao, Pangasinan, northwestern Philippines (16° 26' 40" N, 119° 55' 15" E). The nursery is located in a reef flat at a depth range of 2–3 m, with sandy and coral rubble substrates, and patches of seagrass. The nursery is approximately 5 ha in size, and has ~35 000 giant clams from different species (*Tridacna gigas*, *T. derasa*, *T. squamosa*, *T. maxima*, *T. crocea*, *Hippopus hippopus*, and *H. porcellanus*), which are reared for conservation and restocking efforts. These giant clams are artificially spawned in the ocean nursery and brought to a land-based hatchery of the BML for culture and rearing until juvenile stage (at least 3 cm in shell length, approximately 3 to 5 mo old). The juveniles are then placed back in the ocean nursery where they are exposed to regular fluctuations of natural conditions up to adult stage (e.g. approximately 10 yr for the giant clams used in this study).

Bleaching prevalence in giant clams in the ocean nursery was surveyed, particularly during periods of high temperatures and when corals were observed bleaching in nearby reefs. The giant clam bleaching surveys were conducted in July and December 2019, and in March, May, and August 2020 (Fig. 1). The monitoring focused on *T. gigas*, the largest species of giant clams and the most abundant in the giant clam nursery since it is the main species cultured for restocking efforts in the country (Gomez & Mingoa-Licuanan 2006). Each bleached *T. gigas* observed

during the monitoring periods was tagged with a unique number using a steel plate attached to a rod and fixed in the sand beside the bleached giant clam. All tagged bleached giant clams were photographed with their mantle fully extended and with scale and color references on their side (Fig. 2). The percentage of bleaching per giant clam individual was measured by dividing the giant clam mantle into 4 quadrants by single horizontal and vertical imaginary lines, and the bleaching status was scored into 5 categories: 0, 1–25, 26–50, 51–75, and 76–100 %.

The bleaching events observed in 2019 and 2020 provided us with an opportunity to examine the direct effects of bleaching on reproduction and survival of *T. gigas*. Based on the percentage of bleached mantle, 3 different condition states (hereafter 'types') of *T. gigas* were selected: (1) healthy: individuals with no signs of bleaching between July 2019 and August 2020; (2) moderately bleached: individuals with 26–50 % bleached mantle; and (3) severely bleached: individuals with 76–100 % bleached mantle (Fig. 2, Table S1 in the Supplement at www.int-res.com/articles/suppl/m706p047_suppl.pdf). Thirty individuals of each bleaching type were randomly chosen in the nursery, and 30 healthy individuals that were approximately 1 to 3 m away from these bleached giant clams were chosen as controls. All *T. gigas* used in this study were already at reproductive age (≥ 11 yr old; Gomez & Mingoa-Licuanan 2006), and the shell lengths (50.63 ± 0.45 cm, mean \pm SE) were not significantly different among types (1-way ANOVA: $F_{2,87} = 1.78$, $p > 0.05$).

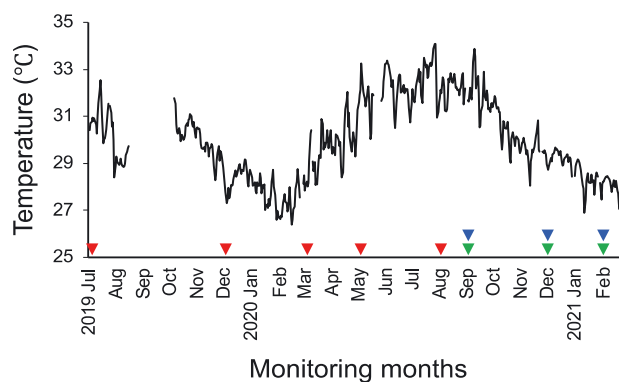


Fig. 1. Daily average seawater temperatures (black line) from July 2019 to February 2021 in the Silaqui Giant Clam Ocean Nursery. Red triangles represent months when bleaching prevalence in all *T. gigas* giant clams in the ocean nursery was monitored, green triangles represent months when bleaching and survival was monitored in giant clams *T. gigas* used in this study only, and blue triangles represent months when *T. gigas* egg production was monitored

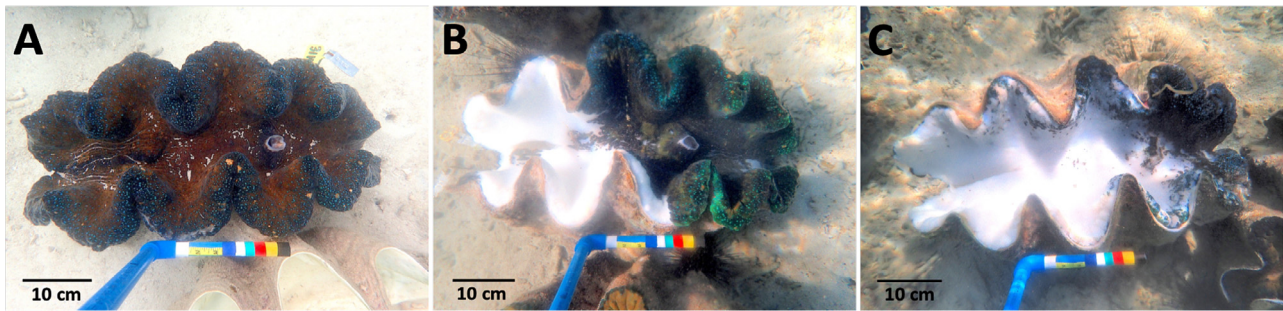


Fig. 2. The 3 giant clam types: (A) healthy, (B) moderately bleached with 26 to 50 % bleached mantle, and (C) severely bleached with 76 to 100 % bleached mantle

In September 2020, December 2020, and February 2021 (Fig. 1), the progress of bleaching and survival was evaluated in all 90 *T. gigas* individuals in the ocean nursery. Additionally, egg condition including egg concentration, egg stage, and egg size, was examined in each giant clam according to the following methods.

2.2. Gonad biopsy technique

To examine the egg condition, all 90 *T. gigas* individuals were biopsied in September and December 2020, and February 2021 using an 18G needle and a 5 ml syringe (Fig. 3a). The needle was inserted about 2.5 cm below the excurrent siphon of the giant clam until it hit the gonad wall. Subsequently, the needle was further pushed through the gonad for a few mm (Braley 1984), and <1 ml gonad, containing eggs, was extracted. When no visible eggs were observed in the syringe, we biopsied the clams twice to be certain

of lack of eggs. The gonad extract was immediately transferred to a 5 ml plastic vial, and 70 % ethanol, at least twice of the gonad volume, was added. The syringe and the needle were washed and disinfected with 70 % ethanol after every use.

2.3. Egg concentration, stage, and size

Egg concentrations were evaluated under a compound microscope (Motic Zeiss) and categorized as (1) empty (no visible eggs in extract), (2) few (loosely suspended eggs in extract), and (3) concentrated (many suspended and settled eggs or white tissue clump in extract) following the descriptions of Roa-Quiaoit (2005) with some modifications.

To examine the gamete stage and size, the first 100 eggs encountered in each extract were photographed using a microscope with built-in Motic camera (Motic Zeiss) and images at 10× magnification were captured using Zeiss Labscope imaging soft-

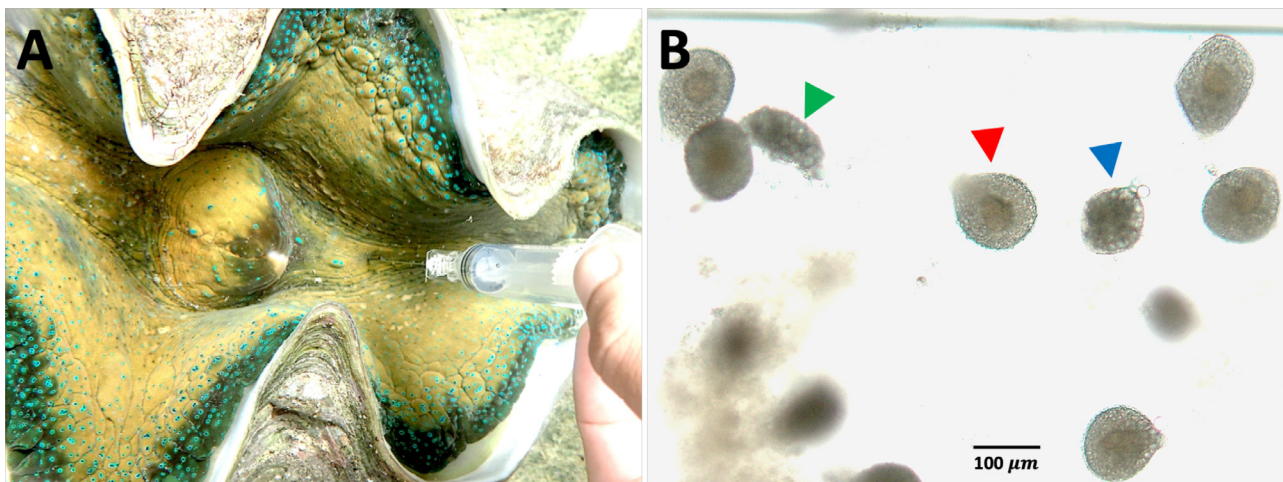


Fig. 3. The gonad biopsy procedure: (A) extraction of eggs using 18G needle and 5 ml syringe, and (B) eggs from the biopsy extract under the compound microscope. Arrows indicate the different egg stages — red arrowhead: intact mature egg; blue arrowhead: intact developing egg; green arrowhead: regressive egg

ware. Eggs were assigned into 2 categories: intact (developing or mature egg with spherical to polygonal in shape and chorion layers are present and not damaged) or regressive (eggs at any size and shape but degenerative, chorion layers are damaged or not present and usually surrounded with phagocytic amoebocytes) (Braley 1984, 1988; Fig. 3b). Only the intact eggs were measured for their longest and shortest diameter using the Image J software. The oocyte mean diameter (OMD) of intact eggs was calculated using the following formula: (longest + shortest diameter)/2 (Menoud et al. 2016).

2.4. Statistical analyses

All statistical analyses were conducted in R version 4.1.1 software using RStudio version 1.4.1717 (R Core Team 2021). All data were tested for normality using Shapiro-Wilk's test and for homogeneity using Levene's test. Aligned rank transform (ART) was used to examine differences in egg concentrations, a generalized linear mixed model (GLMM) with a negative binomial distribution was used to examine differences in the proportion of intact eggs, whereas linear mixed models (LMMs) were used to examine differences in OMD. The model for ART, GLMM, or LMM includes giant clam bleaching type and sampling time as fixed effects, and a random effect of giant clam individuals to account for repeated measures. Pairwise comparison analysis using aligned rank transform contrasts (ART-C) test procedure, corrected with Holm's sequential Bonferroni procedure, was conducted on significant ART results, whereas Tukey's HSD test was conducted on significant GLMM and LMM results. Survival was analyzed using Kaplan-Meier survival analysis and a log-rank test was performed to compare differences in survival between giant clam bleaching types. A significance level of $\alpha = 0.05$ was used in all analyses.

3. RESULTS

3.1. Egg concentration

Egg concentration was significantly different between giant clam type (ART: $F_{(2, 86.57)} = 64.34$, $p < 0.0001$), and across time (ART: $F_{(2, 160.33)} = 11.46$, $p < 0.0001$), and there was an interaction between giant clam type and time (ART: $F_{(4, 159.54)} = 3.76$, $p < 0.01$, Fig. 4). The egg concentration was highest in healthy, followed by moderately bleached type, and lowest in

the severely bleached type, except in September 2020 when the healthy and moderately bleached types were not significantly different (ART-C: $p = 0.44$). The egg concentration of the healthy type was significantly higher in February 2021 compared with September 2020 (ART-C: $p < 0.01$), while there was no significant change in the moderately and severely bleached types across time.

3.2. Egg stage

While the majority of eggs were intact, comprising developing and mature stages, there was a significant difference in their proportions between giant clam type (GLMM: $X^2 = 49.70$, $p < 0.0001$), with the highest number in healthy and lowest in heavily bleached type (healthy > moderate > severe; Tukey's HSD test: $p < 0.01$). Additionally, there was a significant difference across time (GLMM: $X^2 = 7.09$, $p < 0.05$; September < December = February; Tukey's HSD, $p < 0.05$), and there was no interaction between giant clam type and time (GLMM: $X^2 = 0.44$, $p = 0.98$; Fig. 5).

3.3. Egg size

OMD (range: 76–111 μm) was not significantly different among giant clam type (LMM: $X^2 = 0.33$,

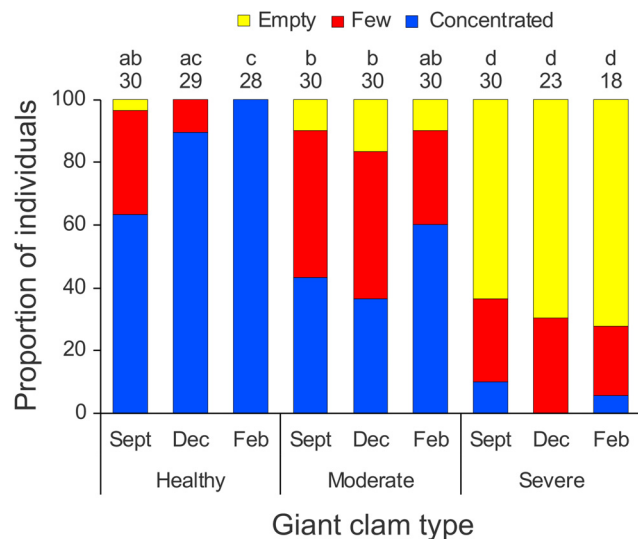


Fig. 4. Proportion of different egg concentrations from each giant clam type across sampling months. The number of giant clam individuals examined for each giant clam type across sampling months is shown at the top of each bar. Bars with the same letter superscripts are not significantly different from each other based on the results of Tukey's HSD tests

$p = 0.85$), but significantly different across time (LMM: $X^2 = 15.84$, $p < 0.001$), and there was no interaction between giant clam type and time (LMM: $X^2 = 4.96$, $p = 0.29$, Fig. 6). OMD was larger during December 2020 (Tukey's HSD: $p < 0.001$) and February 2021 (Tukey's HSD: $p < 0.01$) compared with September 2020, whereas there was no significant difference between December 2020 and February 2021 (Tukey's HSD: $p = 0.33$).

3.4. Bleaching recovery and mortality

No bleached giant clams fully recovered their colors, but partial color recovery was observed within 6 mo of monitoring between September 2020 and February 2021. Five individuals from the severely bleached types regained $\geq 25\%$ colored mantle starting December 2020 (3 mo after the baseline bleaching survey), whereas all moderately bleached giant clams remained with 26 to 50% bleached mantles until the end of the experiment (Table S1). Survival probability was significantly different between giant clam types (log-rank test: $X^2 = 21.6$, $p < 0.0001$; Fig. 7). Survival of the giant clams at the end of monitoring was not significantly different between healthy (93.33%) and moderately bleached type (100%) (Tukey's HSD: $p = 0.15$), but the survival of severely bleached type (60%) was significantly lower compared with both healthy (Tukey's HSD: $p < 0.01$) and moderate bleached type (Tukey's HSD: $p < 0.001$).

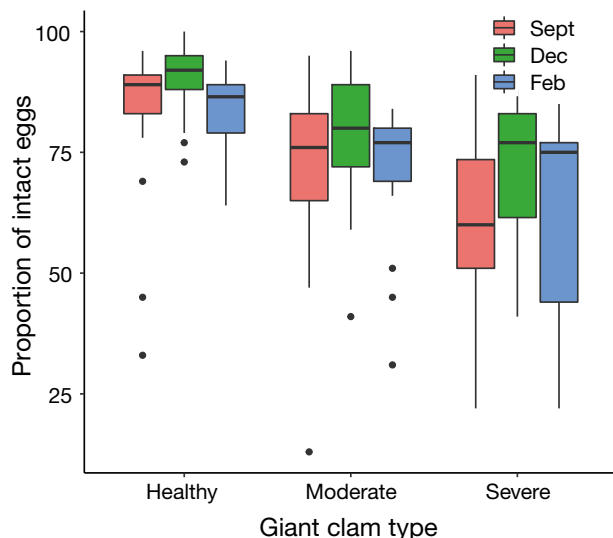


Fig. 5. Proportion of intact eggs from each giant clam type across sampling months. Boxes contain values within the interquartile range, with medians indicated by a solid line across the box. Whiskers extend to the most extreme values. Dots represent the outlier values

4. DISCUSSION

In this study, we found that bleaching due to elevated seawater temperatures reduced the reproductive output and caused mortality in the giant clam *Tridacna gigas*, particularly in severely bleached individuals. This suggests that sustainability and recovery of its population is likely to be very challenging after bleaching disturbance and consequently may result in population decline, even without mortalities in reproducing adult individuals during bleaching events.

T. gigas are suggested to have a continuous gametogenesis, since they have been found to contain developing to mature eggs in most months in Australia and Papua New Guinea (Bell & Pernetta 1988, Nash et al. 1988). Although there is no information so far on the reproductive periodicity of *T. gigas* in the Philippines, induced spawning in *T. gigas* for aquaculture in Silaqui Giant Clam Ocean Nursery in the country, which has been conducted by the BML, has been successful year-round, although the spawning success is known to be higher during warmer months, similar to other regions across the Indo-Pacific (Bralley 1984, Nash et al. 1988, Shelley & Southgate 1988, Tan & Yasin 2000). The present study shows that healthy giant clams contained concentrated eggs—mostly in developing to mature stages—at least between September and February, suggesting their potential for spawning, whereas several individuals of the moderately bleached giant clams had fewer or

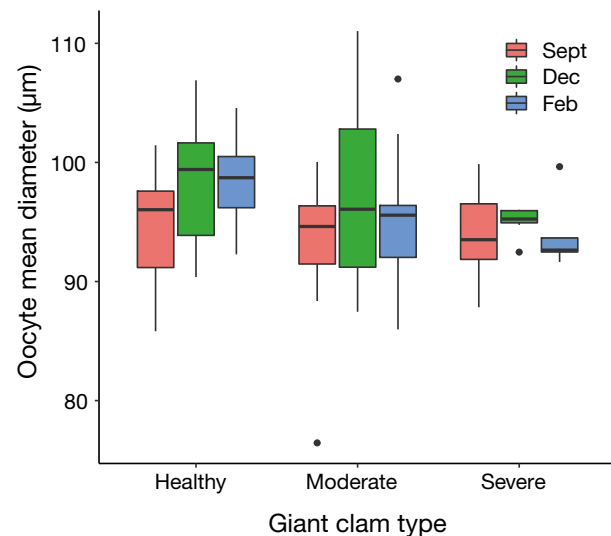


Fig. 6. Oocyte mean diameter from each giant clam type across sampling months. Boxes contain values within the interquartile range, with medians indicated by a solid line across the box. Whiskers extend to the most extreme values. Dots represent the outlier values

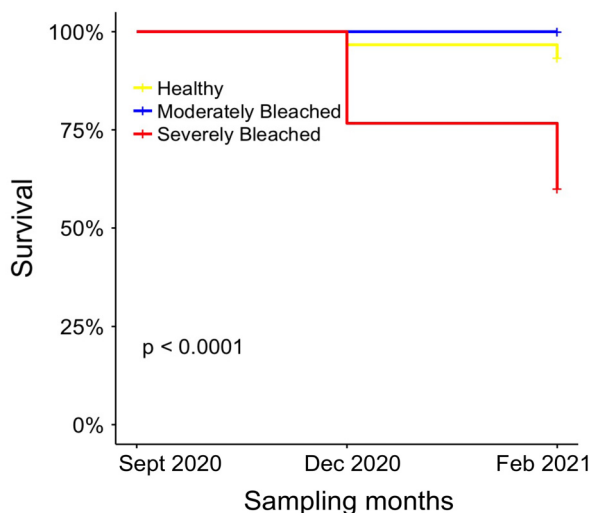


Fig. 7. Survival probability of different giant clam types across sampling months

no eggs, and the majority of the severely bleached giant clams had empty gonads, suggesting that production of eggs is impaired during bleaching (Table S2). This reduction of egg production relative to bleaching severity was also observed in the coral *Acropora hyacinthus*, wherein only 60 and 20 % of the moderately and severely bleached colonies, respectively, produced gametes (Baird & Marshall 2002). Similarly, reduction of eggs due to bleaching was also observed in some *Montipora* spp. and *Pocillopora* spp. corals (Hirose & Hidaka 2000, Ward et al. 2000, Jones & Berkelmans 2011). Considering that spawning in giant clams is known to be higher during warmer months (Braley 1984, Nash et al. 1988, Shelley & Southgate 1988, Tan & Yasin 2000), which may coincide with bleaching events, the lower probability of bleached giant clams producing eggs and participating in spawning will likely reduce their reproductive success and consequently recruitment.

During stressful events, organisms may allocate their energy towards essential processes to ensure survival and sacrifice costly processes such as reproduction. Indeed, energy investments toward reproduction completely ceased in the coral *M. digitata* when nutrient resource was severely limited due to low light (Leuzinger et al. 2012), whereas the sea urchin *Tripneustes gratilla*, exposed to high wave exposure and relative storminess, allocated more of its energy towards protection and maintenance and limited its energy towards production of gonads (Bangi & Junio-Meñez 2019). Therefore, during bleaching stress, when giant clams suffered from limited energy resources due to reduced autotrophy (Leggat et al. 2003) and from higher respiration due to temperature

stress (Blidberg et al. 2000), *T. gigas* likely allocated their limited energy towards maintenance (to meet the high metabolic demands of these environmental conditions and to ensure their survival) at the expense of reproduction, thus reducing their egg production.

Organisms may also resorb their eggs during stress, as observed in the corals *Lobophytum compactum* and *A. formosa* (Michalek-Wagner & Willis 2001, Okubo et al. 2007), and it was suggested that nutrients from these resorbed eggs can serve as an immediate source of energy during stress. However, resorption of eggs resulted in incomplete gametogenesis in bleached coral *Montastrea annularis* (Szmant & Gassman 1990). Although we found that both healthy and bleached giant clams contained resorbed eggs, the proportion was higher in bleached individuals, resulting in them having lower proportions of intact eggs compared to healthy individuals, and suggesting interruption in their gametogenesis. The observed resorption of eggs in healthy giant clams may suggest reutilization of nutrients from the resorbed eggs that can be used to produce new gametes (Braley 1988).

Interestingly, the egg size was not significantly different between the healthy and bleached giant clams. Maintenance of the egg size during bleaching events was also observed in corals *A. millepora* and *P. verrucosa* (Hirose & Hidaka 2000, Jones & Berkelmans 2011). Similarly, the recovered coral *A. hyacinthus* had a similar egg size to those in resistant conspecific colonies (i.e. colonies that did not experience bleaching) (Leinbach et al. 2021). Jones & Berkelmans (2011) posited that the maintenance of egg size during bleaching stress may suggest that organisms can still supply sufficient nutrients for the normal development of their eggs, which are now in lower numbers, to ensure normal growth and survival of their larvae. However, Hagedorn et al. (2016) found that fertilization was reduced and larval development delayed in *Funaria scutaria* during bleaching events, resulting in a high percentage of larval abnormalities and lower survival. While development was accelerated in *A. palmata* (Randall & Szmant 2009), they still showed a higher percentage of abnormalities, and lower survival compared to non-bleached corals (Randall & Szmant 2009, Hagedorn et al. 2016). In comparison, although we found that bleached giant clams maintained their egg size, there is no assurance that subsequent processes such as fertilization, larval development, and survival would proceed as normal, as thermal stress may directly affect the early life stages. Indeed, previous studies have shown that when gametes of giant clams are exposed even to mild ther-

mal stress, fertilization success (*T. maxima*; Armstrong et al. 2020) and larval survival (*T. squamosa*; Neo et al. 2013) were reduced.

Partial color recovery was observed in giant clams (5 out of 60 bleached individuals only) after December 2020, when temperatures were approximately 3 to 4°C lower than in warmer months (Fig. 1, Table S1). However, the severity of bleaching was maintained in most *T. gigas*, even during periods of lowest temperature, and bleaching severity was either maintained or intensified during warmer months (Table S1). Giant clams can recover from bleaching through the reestablishment of their zooxanthellae when environmental conditions are favorable. However, severe bleaching can cause atrophy of the tubular systems of their mantle tissues where zooxanthellae are located, thereby making the reestablishment of symbiosis difficult (Norton et al. 1992, 1995). These results may suggest that, particularly in tropical reefs such as in the Philippines where warm seawater temperatures are sustained, the recovery of giant clams from bleaching may take longer, resulting in prolonged bleaching; a further increase of temperature due to ocean warming and marine heatwaves can have detrimental effects on giant clams, resulting in severe bleaching or even mortality.

While a very low number of the giant clams died from the healthy and moderately bleached types (2 and 0 out of 30 individuals, respectively), around half (12 out of 30 individuals) of the severely bleached *T. gigas* died. During the 1998 bleaching event, almost 40% of the *T. gigas* broodstock died in the Giant Clam Ocean Nursery of BML (Gomez & Mingoa-Licuanan 1998) which can be attributed to starvation and physiological stress during unusual high temperatures (Blidberg et al. 2000, Leggat et al. 2003). This suggests that severe bleaching can cause large-scale mortality in giant clams, which is likely to reduce their recruitment due to significant reductions in the number of reproductive adult individuals, especially for *T. gigas*, which has lower numbers in the wild. Even though the moderately bleached giant clams suffered from 26 to 50% bleaching for more than a year, no individuals died (Table S1), suggesting that the nutrients they acquire both from autotrophy and heterotrophy are sufficient for self-maintenance to ensure survival. Although moderate bleaching did not cause mortality, the observed reduction in the production of their eggs will likely reduce their recruitment, highlighting the impacts of bleaching stress on giant clam reproduction. On the other hand, the death of 2 healthy giant clam individuals was likely due to poaching as empty shells

looked very clean, although predation cannot be ruled out. Although it is illegal to collect giant clams (CITES 2021), poaching has been reported in the Silaqui Giant Clam Ocean Nursery, since fishing activity is allowed in the area, and giant clams are accessible to poachers because of the shallow waters of the nursery (~2 to 3 m depth).

We show that bleaching reduced the reproductive output and caused mortality in *T. gigas*, particularly in severely bleached individuals. These results suggest that after a bleaching disturbance, even without high mortality in adult individuals, the ability of *T. gigas* to sustain and recover their population likely will be reduced due to the lower probability that surviving bleached individuals complete their gametogenesis and produce eggs. This is especially so in tropical reefs such as those in the Philippines, where relatively high sea water temperatures can persist for long periods, and may cause prolonged bleaching in giant clams. However, differential bleaching susceptibility observed among *T. gigas* individuals, along with the ability of some bleached individuals to reproduce even when severely affected, may be an indication of their capacity to adapt and persist under future oceanic regimes.

Giant clam populations are already vulnerable to natural and anthropogenic disturbances, such as thermal stress and overharvesting, which have led to local extinction in many regions (Wells 1996, CITES 2021). Thus, the intensification and increasing frequency and duration of bleaching events will further threaten giant clams through mortality and reductions in their reproductive capability. Considering that responses to thermal stress may differ across taxa or species, it is recommended that similar studies are conducted on other giant clam species and other zooxanthellate reef organisms, to better assess their resilience and to predict their future population dynamics in a warming ocean.

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