**INDO PAC J OCEAN LIFE**
Volume 7, Number 1, June 2023
Pages: 64-70

P-ISSN: 2775-1961
E-ISSN: 2775-1953
DOI: 10.13057/oceanlife/o070107

# Differential photo-physiological responses of two giant clam species to elevated temperature stress from Rodrigues Island, Western Indian Ocean

**SUNDY RAMAH[1,♥], DEEPEEKA KAULLYSING[1,2], MOUNESHWAR SOONDUR[1,2],**
**NAWSHEEN TALEB-HOSSENKHAN[1], RANJEET BHAGOOLI[1,2,3, ♥♥]**
[1]Department of Biosciences and Ocean Studies, Faculty of Science and Pole of Research Excellence, Sustainable Marine Biodiversity Research Group, University of Mauritius. Réduit 80837, Republic of Mauritius. Tel./fax.: +230-4037916, email: ♥sundy.ramah@gmail.com; ♥♥r.bhagooli@uom.ac.mu
[2]The Biodiversity and Environment Institute. Réduit, Republic of Mauritius
[3]The Society of Biology (Mauritius). Réduit, Republic of Mauritius.

Manuscript received: 25 December 2022. Revision accepted: 6 February 2023.

**Abstract.** *Ramah S, Kaullysing D, Soondur M, Taleb-Hossenkhan N, Bhagooli R. 2023. Differential photo-physiological responses of two giant clam species to elevated temperature stress from Rodrigues Island, Western Indian Ocean. Indo Pac J Ocean Life 7: 64-70.* Bleaching events leading to mass mortality of coral reef and its associated symbiotic organisms have become an alarming issue worldwide. However, as compared to corals, little has been documented regarding giant clams' (Tridacnines) thermal photo-physiological susceptibility. Triplicate specimens of the small giant clam *Tridacna maxima* and the fluted giant clam *T. squamosa* collected from the lagoon of Rodrigues Island were exposed at two temperatures, 29°C and 32°C, under a constant low light intensity of approximately 200 µmol quanta m$^{-2}$ s$^{-1}$ over a 12-hour duration. The photo-physiological parameters, namely, effective quantum yield of photosystem II ($\Phi_{PSII}$), relative maximum electron transport rate (rETR$_{max}$) and maximum non-photochemical quenching (NPQ$_{max}$) were determined using a diving Pulse-Amplitude-Modulated (D-PAM) fluorometer prior to and after 3 and 12 hours of exposure. At 29°C, the photo-physiological parameters did not vary significantly for both species. At 32°C, *T. squamosa* and *T. maxima* exhibited significant declines in $\Phi_{PSII}$ at 3 and 12 hours, respectively. The rETR$_{max}$ of *T. squamosa* showed a significant decrease at 3 hours, while both species showed a significant reduction in their NPQ$_{max}$ functioning after 3 hours. The experiment also recorded the disintegration of the mantle tissue in *T. squamosa* after 12 hours. These findings indicate that *T. squamosa* is thermally more susceptible than *T. maxima*. Further in-depth investigations on symbiont genetic types and antioxidant responses of both the *Tridacna* host and symbionts are required to thoroughly understand giant clams' variable heat stress responses in an era of ocean warming.

**Keywords:** D-PAM, Tridacnines, tropical island, thermal stress, photo-physiology

## INTRODUCTION

Coral bleaching, a general environmental stress response through the loss of symbionts and/or their photosynthetic pigments, has become an alarming issue resulting in mass coral mortality worldwide and not sparing Mauritian waters (Bhagooli and Sheppard 2012; Bhagooli and Kaullysing 2019; Bhagooli et al. 2021a,b,c). Many environmental factors such as elevated seawater temperatures (Gates et al. 1992; Brown et al. 1995, Bhagooli and Hidaka 2003, 2004; Hoegh-Guldberg and Bruno 2010), high light intensity (Hoegh-Guldberg and Smith 1989; Brown et al. 2000; Bhagooli and Hidaka 2004), salinity stress (True 2012; Gegner et al. 2017), cold shock (Gates et al. 1992; Kobluk and Lysenka 1994), and disease (Bhagooli et al. 2021d; Neely et al. 2021) are known to be responsible for coral bleaching. However, high sea surface temperature is believed to be the leading factor for coral bleaching. Many researchers have attempted to clarify the mechanism of coral bleaching, especially under conditions of elevated seawater temperature (e.g., Lesser 1997; Ralph et al. 2001; Bhagooli and Hidaka 2004; Downs et al. 2009; Bhagooli 2013). Experiments have demonstrated that elevated seawater temperature is a primary trigger of coral bleaching. However, many of these thermal stress experiments were performed at seawater temperatures greater than 32°C. Under such harsh thermal stress, a large number of Symbiodiniaceae were most likely expelled due to host cell detachment, and the subsequent loss of Symbiodiniaceae from coral tissues led to coral bleaching (Gates et al. 1992; Bhagooli and Hidaka 2004; Fujise 2013). Many natural coral bleaching events can occur even under conditions of moderate thermal stress, 1-2°C higher than the average ambient seawater temperature, for prolonged periods of time (Goreau and Hayes 1994; Winter et al. 1998; Lough 2000; Ward et al. 2002).

Climate change represents a challenge that may negatively affect the recovery of giant clams. It has been reported that the collective impact of ocean acidification and high seawater temperatures reduces calcification (Rodolfo-Metalpa et al. 2011; Mackenzie et al. 2015), fertilisation and development (Kurihara et al. 2007; Parker et al. 2009), and growth and metabolism (Talmage and Gobler 2011; Clark et al. 2013) in marine bivalve molluscs. Being a reef dwelling organism, giant clams will most likely face the impacts of climate change (Hoegh-Guldberg et al. 2007) causing a decline in their occurrence on reefs

(Ramah et al. 2019). Even though most studies have concentrated on temperature effects on corals, a recent focus has been the responses to heat stress in giant clams (Eckman et al. 2014; Pappas et al. 2017; Andrefouet et al. 2017). Unlike corals, giant clams host the symbiotic Symbiodiniaceae extra-cellularly, within zooxanthellal tubes in the mantle and stomach (Norton et al. 1992). Nonetheless, similar to corals, giant clams have also been affected by global ocean warming (Watson and Neo 2021) since they depend on symbiotic Symbiodiniaceae (Blidberg et al. 2000). Exposure of giant clams to elevated temperatures has led to their mass mortality around the world (Junchompoo et al. 2013).

The coastal waters of Rodrigues Island have not been spared by the ocean warming phenomenon. The effects of climate change on giant clams are still to be thoroughly understood, especially with respect to their photo-physiological responses (e.g. Bhagooli et al. 2021c,d) and their survival. This study therefore investigated the thermal susceptibility of the *in-hospite* Symbiodiniaceae of two giant clam species, *Tridacna maxima* and *T. squamosa*, from Rodrigues Island in a view to understand giant clams' survival and the process underpinning their declines worldwide.

## MATERIALS AND METHODS

### Study site and samples collection

Six specimens from each of the two species of giant clams, *Tridacna maxima* and *T. squamosa*, were morphologically identified *in situ* according to Ramah et al. (2017) and collected following a modified protocol of Trench et al. (1981) from the lagoon of the South-East Marine Protected Area (SEMPA) in Rodrigues (Figure 1). The sites were selected based on the availability of the two targeted giant clam species. The shell of both species collected ranged from 9.3 ± 0.23 cm to 8.2 ± 0.42 cm in length for *T maxima* and *T. squamosa*, respectively. Care was taken to minimize damage and trauma to the organisms when they were being removed from their substrate. The samples were allowed to acclimatize in a seawater tank at room temperature maintained with an air pump filter for 24 hours prior to experimental trials. The responsiveness of all specimens was checked, and their initial photo-physiological parameters were measured to assess their good health before placing them in the experimental tanks.

### Experimental design

Two seawater tanks were set up and maintained at an approximate temperature of 29ºC and 32ºC using heaters. The mean seawater temperature in both tanks were at 29.3 ± 0.18ºC and 31.9 ± 0.09ºC, respectively. Care was taken to keep a controlled light intensity of ~200 µmol photons $m^{-2}$ $s^{-1}$ using artificial light. Three specimens from the two giant clam species were placed in each experimental tank and were exposed for 12 hours (Figure 2). After 3 and 12 hours of treatment, the chlorophyll *a* fluorescence measurement for each sample was recorded.

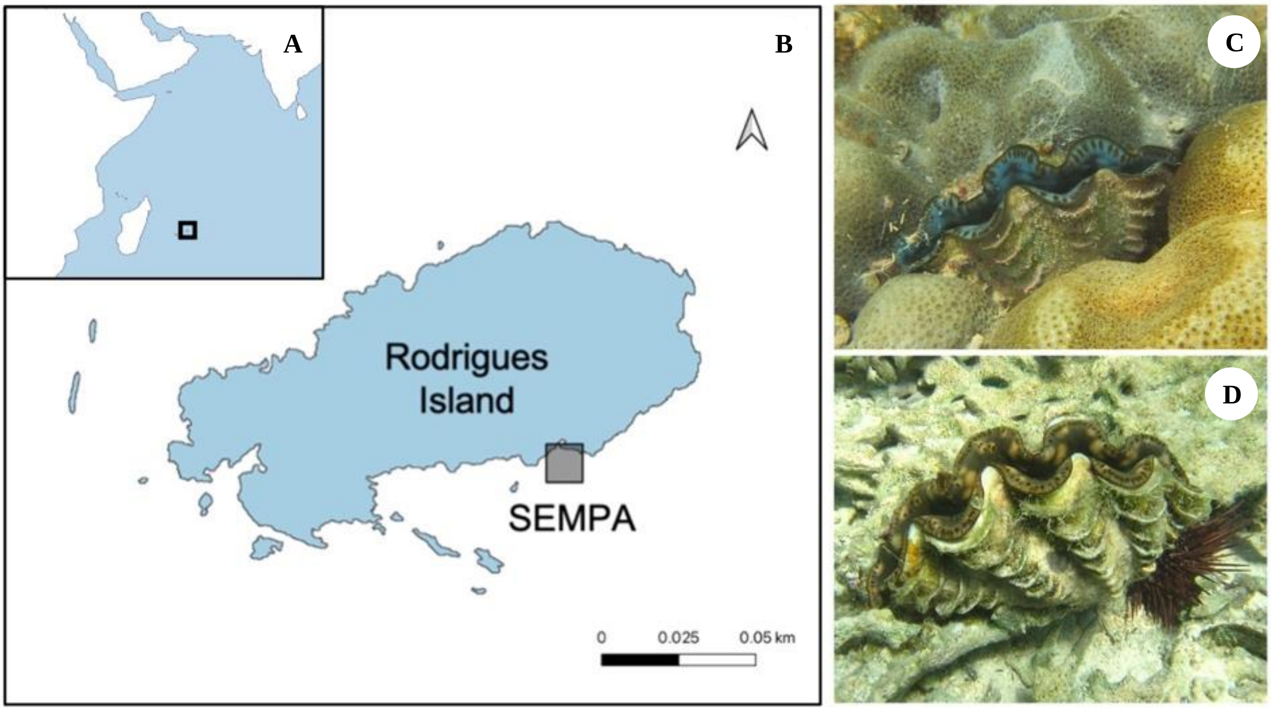

**Figure 1.** A. Western Indian Ocean Map showing location of Rodrigues Island (19.7245° S, 63.4272° E), B. South-East Marine Protected Area (SEMPA) location in Rodrigues Island, C. *Tridacna maxima* specimen collected for the experiment and D. *T. squamosa* specimens collected

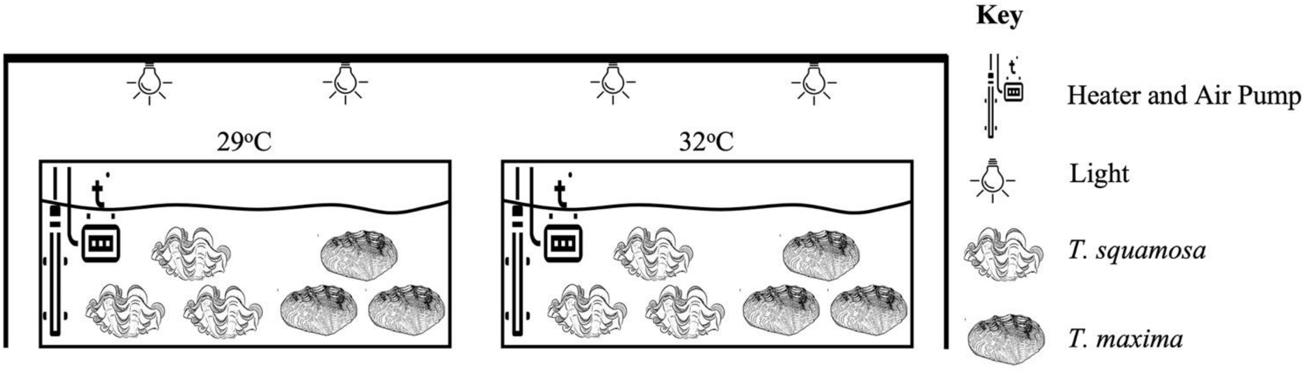

**Figure 2.** Experimental design for thermal experimentation of *T. maxima* and *T. squamosa* at 29°C and 32°C

### Chlorophyll *a* fluorescence measurement

Chlorophyll *a* fluorescence was measured using a Diving Pulse-Amplitude-Modulated (DIVING-PAM or D-PAM) fluorometer (Walz, Germany) as an indicator of the photo-physiological responses of the giant clams to thermal stress. To ensure consistency of measurements, readings were taken within the mantle of the giant clams only where the Symbiodiniaceae are hosted. The optical fibre of the D-PAM was placed at a distance of about 1 mm (probe to mantle). $F_o$ was measured by applying a weak pulsed red light (LED 650 nm, 0.6 kHz, 3 µs) followed by a saturating pulse of bright actinic light (4000 µmol photons $m^{-2}$ $s^{-1}$, width 800 ms) which was then applied to give the maximal fluorescence value ($F_m$). Three main chlorophyll *a* fluorescence parameters, namely, the effective quantum yield of Photosystem II ($\Phi_{PSII}$), the relative maximum electron transport rate ($rETR_{max}$) and the maximum Non-Photochemical Quenching ($NPQ_{max}$) were chosen as they are known to be widely used in photo-physiological stress studies in marine organisms (Bhagooli et al. 2021c). $\Phi_{PSII}$ is an indication of the photosynthetic efficiency of the PSII when all reaction centres are open. $rETR_{max}$ is used to measure the rate of electron transport through the reaction centres in the PSII, and $NPQ_{max}$ gives an indication of the ability of a photosynthetic organism to dissipate excess radiation through non-damaging heat emissions (Bhagooli et al. 2021c). The photo-physiological parameters were determined initially, i.e., prior to start of the experiment and after 3 and 12 hours of exposure.

### Data analysis

Data were transformed, if necessary, using arcsine-square root transformation to meet the assumptions of normality and equal variance for use of parametric statistical tests. The significant differences in the photo-physiology response ($\Phi_{PSII}$, $rETR_{max}$ and $NPQ_{max}$) of the two species of giant clams at 29°C and 32°C were analyzed using a two-way ANOVA. A Tukey's comparison of means was also run using the SPSS Software (Version 6.0). P-values less than 0.05 were considered statistically significant.

## RESULTS AND DISCUSSION

### Light and temperature variations

The seawater temperature and light intensity were monitored using a HOBO Pendant temperature and light data logger. The mean seawater temperature was 29.2°C ± 0.18 for the stress tank of 29°C and 31.9°C ± 0.07 for the stress tank set up at 32°C. The light intensity was maintained at a mean intensity of 197.9 ± 0.16 µmol photons $m^{-2}$ $s^{-1}$ for both set-ups.

### Photo-physiological responses of giant clams

The chlorophyll *a* fluorescence parameter did not vary significantly ($p$=0.086) during the exposure duration for both test species at 29°C (Figures 3.A, C, E). However, at 32°C, differential responses between the two studied species, both at the photo-physiological and survivorship levels, were notes. *T. squamosa* showed a significant drastic decline in the photo-physiological parameters after 3 hours of exposure and exhibited signs of disintegration, indicative of mortality after 12 hours of exposure. *T. maxima* started to show a significant drastic decline in its photo-physiological parameters only after 12 hours of exposure (Figures 3.B, D, F). The results demonstrated that $\Phi_{PSII}$, $rETR_{max}$ and $NPQ_{max}$ were highly influenced by the species of giant clam ($p$<0.01) (Table 1) at 32°C. That is, both *T. maxima* and *T. squamosa* showed a significant response in their photo-physiological response at exposed temperature of 32°C. A strong significant correlation (r=0.627, $p$=0.013) was also found between the photo-physiology responses and the temperature exposures at 32°C for both species.

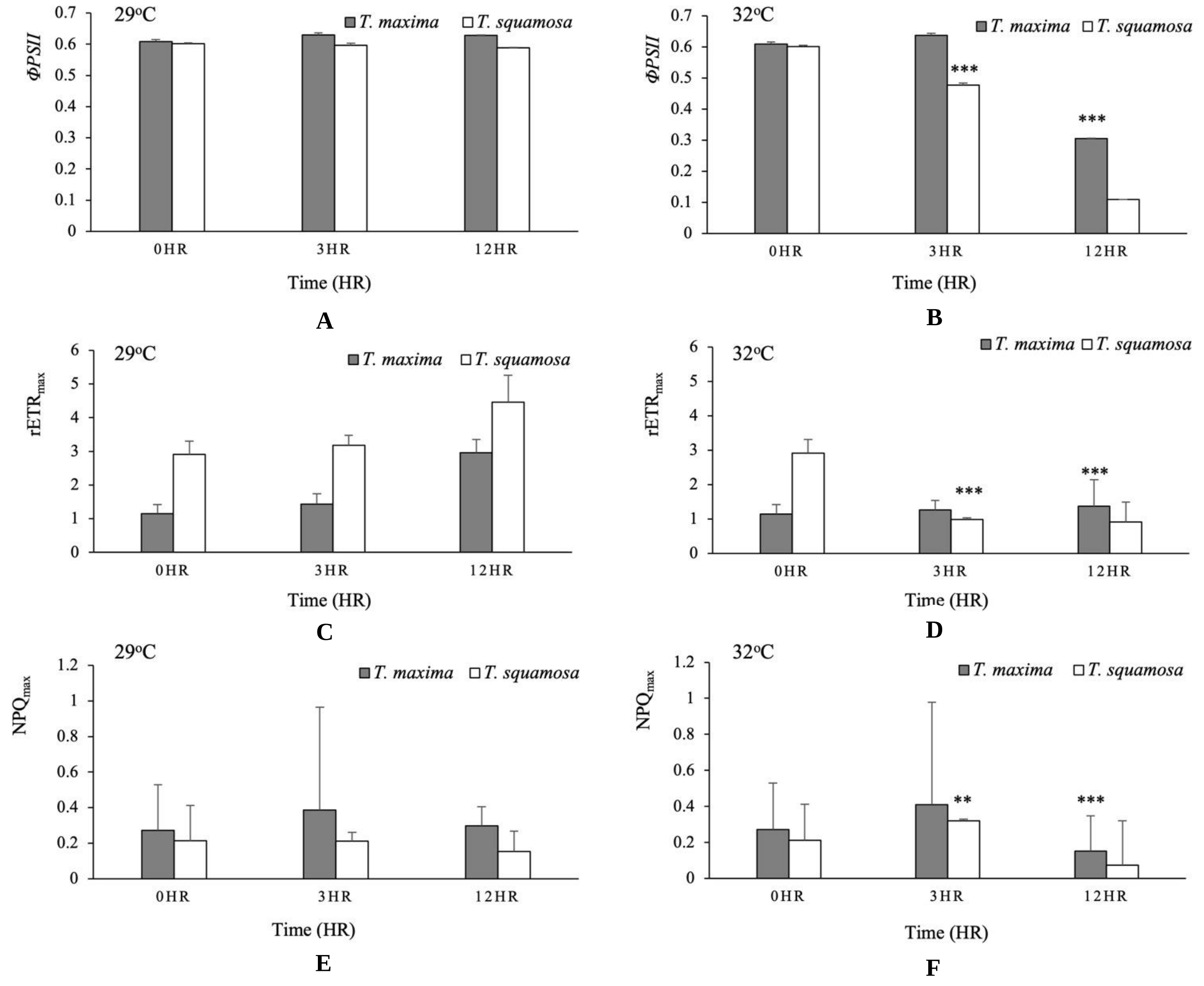

**Figure 3.** $\Phi_{PSII}$, $rETR_{max}$ and $NPQ_{max}$ responses of *T. maxima* and *T. squamosa* at 29°C and 32°C for 0, 3 and 12 hours. Bars represent Mean + SD (n=3)

**Table 1.** Two-way ANOVA on the effect of giant clam species on the photo-physiology responses ($\Phi_{PSII}$, $rETR_{max}$ and $NPQ_{max}$) to two temperatures namely 29°C and 32°C over two time points 3 and 12 hours (taking in consideration that at time 0 there will be no significant change recorded) . $p < 0.05$ = *, $p < 0.01$ = **, $p < 0.001$ = ***

| Parameters | Variation | df | MS | F | *p*-value |
|---|---|---|---|---|---|
| $\Phi_{PSII}$ | Species | 1 | 0.020 | 80.59 | ** |
| | Temperature | 1 | 0.001 | 200.15 | *** |
| | Species x Temperature | 1 | 0.089 | 30.25 | * |
| $rETR_{max}$ | Species | 1 | 0.039 | 168.25 | ** |
| | Temperature | 1 | 0.068 | 226.39 | ** |
| | Species x Temperature | 1 | 0.005 | 236.01 | * |
| $NPQ_{max}$ | Species | 1 | 0.090 | 14.23 | ** |
| | Temperature | 1 | 0.074 | 32.22 | *** |
| | Species x Temperature | 1 | 0.062 | 369.55 | * |

## Discussion

Thermal stress is known to be one of the leading causes of bleaching. Similar to corals, giant clams have been largely affected by global ocean warming (Watson and Neo 2021). An increase in seawater temperature can also negatively affect giant clams since they also host and strongly depend on symbiotic Symbiodiniaceae to survive (Blidberg et al. 2000). The sea surface temperature (SST) of the Republic of Mauritius waters is known to vary between 23°C (in winter) to 30°C (in summer) (Bhagooli and Kaullysing 2019). The temperature of 29ºC was, therefore, chosen to be closest to the normal summer maximal temperature in Mauritian waters. It was observed that both species of *Tridacna* could tolerate the temperature without being much affected in terms of their photo-physiological responses. The temperature of 32ºC, that is 3ºC above the normal temperature on a normal summer day (29ºC), was used to mimic the condition of the *El Niño* or an event of an extreme increase in water temperature as has been recorded previously in the waters of Mauritius (Bhagooli and Taleb-Hossenkhan 2012; Mattan-Moorgawa et al. 2012). The results of this study corroborate those of other studies such as Blidberg et al. (2000) who investigated the physiological responses of three species of giant clams by exposing them to different elevated temperature stress for 24 hours. It was observed that both respiration and primary production decreased when heat stress was increased. Junchompoo et al. (2013) reported altered physiological conditions of giant clams when seawater temperature fluctuated between 28.8 - 31.1ºC in Thailand coastal waters. The first bleaching event, characterised by loss of the micro-algal symbionts, was recorded with 60% of individuals showing faded coloration, 30% partly bleached and 10% bleached completely. Moreover, in response to a sustained increase in temperature, bleaching became more severe with 90% completely bleached, 8% partly bleached and just 2% remaining with faded colouring. Exposure to temperatures over 30ºC for longer than two weeks has been shown to result in the expulsion of the symbiotic living Symbiodiniaceae thus, depriving the giant clams of carbon (Junchompoo et al. 2013). These results concur with the results obtained from this study where the giant clam *T. maxima* showed sign of mild bleaching following 12 hours of consequent thermal stress, while *T. squamosa* showed sign of disintegration as compared to that of Brahmi et al. (2022) which demonstrated that at 30.7ºC, the symbiont's photosynthetic yield of *T. maxima* was highly impacted.

Dubousquet et al. (2016) demonstrated that when the temperature reached 31ºC and 32ºC, the giant clam *T. maxima*'s symbionts died and were degraded under thermal stress. They further put forward that the degraded symbiont's cells were used as an additional energy source for *T. maxima* to sustain the stress being encountered. It has been suggested in other studies that just like in some nudibranch (Burghardt and Wägele 2014; Norton et al. 1992; Soo and Todd 2014), sea anemones and corals (Dunn et al. 2004; Strychar and Sammarco 2009), the Symbiodiniaceae within giant clams are believed to be cultured by the host as a source of nutrition and energy supply as and when required by the host. Dubousquet et al. (2016) suggested that the degradation of Symbiodiniaceae cells observed during elevated temperature might offer *T. maxima* an alternative and rapid source of food which would delay the depletion of the lipid storage which goes in consistency with the energy budget model depicted by Brown et al. (2004) and Anthony et al. (2009) in their studies. However, this study revealed that, unlike *T. maxima*, *T. squamosa* was more susceptible to degradation after an elevated temperature stress. The $NPQ_{max}$ which is the protective mechanism (Slavova et al. 2016) showed rapid degradation. This may suggest that as compared to *T. maxima*, *T. squamosa* may not be able to use the degraded Symbiodiniaceae as an alternative and rapid source of food, an adaptive mechanism that may help the giant clams to delay the mortality process due to thermal stresses.

Genetic clades of Symbiodiniaceae have unique physiological characteristics (Rowan et al. 1997; Tchemov et al. 2004; Sampayo et al. 2008; Bhagooli 2009, 2010; Ghoora et al. 2018; Mattan-Moorgawa et al. 2020) which may play a big role in the host's survival affinity and its susceptibility to environmental stresses and changes (Baker 2001). Giant clams are known to host Symbiodiniaceae of clades A, C and D only or a mixture of these clades (Baillie et al. 2000 a,b; DeBoer et al. 2012). The choice of the clades and their composition is highly dependent on their surrounding environment such as seawater temperature such that giant clams that hosted the Symbiodiniaceae of clades C and D were known to be in area where mean seawater temperature was elevated (DeBoer et al. 2012). Though, Rowan (2004) showed that a temperature of 32°C decreased $\Phi_{PSII}$ in clade C, whereas clade D maintained an increased $\Phi_{PSII}$ which concurred with this study. One may be tempted to suggest that the survival of giant clams would be highly dependent on its Symbiodinaceae. Since the clade identity wasn't investigated in this study, further study is required in this direction.

The findings of this study showed that the giant clam *T. maxima* is more resistant to thermal stress as compared to *T. squamosa*. This could be explained by the fact that *T. maxima* may have the ability to delay its mortality process caused by thermal stress as compared to *T. squamosa*. However, further in-depth studies are warranted in this area to determine the survivorship and tolerance of giant clams to global warming along with more advanced studies on their Symbiodinaceae species susceptibility.

## ACKNOWLEDGEMENTS

The authors thank the Ministry of Blue Economy, Marine Resources, Fisheries and Shipping for granting the permits to carry out sampling and field studies in the Republic of Mauritius and the Rodrigues Regional Assembly (RRA) for granting the permit to carry out surveys and collect giant clam specimens from the South-East Marine Protected Area (SEMPA). The authors would also like to thank the University of Mauritius for logistics and partial financial support to undertake this research work. SR is thankful to the Mauritius Research and Innovation Council for a postgraduate award.

## REFERENCES

Andrefouet S, Van Wynsberge S, Kabbadi L, Wabnitz CCC, Menkes C, Tamata T, Pahuatini M, Tetairekie I, Teaka I, Ah Scha T, Teaka T, Remoissenet G. 2017. Adaptive management for the sustainable exploitation of lagoon resources in remote islands: Lessons from a massive *El Niño*-induced giant clam bleaching event in the Tuamotu atolls (French Polynesia). Environ Conserv 2017: 1-11. DOI: 10.1017/S0376892917000212.

Anthony KRN, Hoogenboom MO, Maynard JA, Grottoli AG, Middlebrook R. 2009. Energetics approach to predicting mortality risk from environmental stress: a case study of coral bleaching. Funct Ecol 23: 539-550. DOI: 10.1111/j.1365-2435.2008.01531.x.

Baillie BK, Belda-Baillie CA, Maruyama T. 2000b. Conspecificity and Indo-Pacific distribution of *Symbiodinium* genotypes (Dinophyceae) from giant clams. J Phycol 36: 1153-1161. DOI: 10.1046/j.1529-8817.2000.00010.x.

Baillie BK, Belda-Baillie CA, Silvestre V, Sison M, Gomez AV, Gomez ED, Monje V. 2000a. Genetic variation in *Symbiodinium* isolates from giant clams based on Random-Amplified-Polymorphic DNA (RAPD) patterns. Mar Biol 136: 829-836. DOI: 10.1007/s002270000290.

Baker AC. 2001. Ecosystems: Reef corals bleach to survive change. Nature 411: 765-766. DOI: 10.1038/35081151.

Bhagooli R, Hidaka M. 2003. Comparison of stress susceptibility of *in-hospit*e and isolated zooxanthellae among five coral species. J Exp Mar Biol Ecol 291: 181-197. DOI: 10.1016/S0022-0981(03)00121-7.

Bhagooli R, Jogee S, Kaullysing D, Ramah S. 2021d. First report of White Syndrome Disease on branching *Acropora* at Saya de Malha, Mascarene Plateau. West Indian Ocean J Mar Sci 2: 189-192. DOI: 10.4314/wiojms.si2021.2.15.

Bhagooli R, Kaullysing D. 2019. Seas of Mauritius - Chapter 12. In: CCR Sheppard (eds) World Seas: An Environmental Evaluation, 2nd Edition, Volume II: The Indian Ocean to the Pacific. Elsevier. Academic Press. DOI: 10.1016/B978-0-08-100853-9.00016-6.

Bhagooli R, Mattan-Moorgawa S, Kaullysing D, Chumun PK, Klaus R, Munbodhe V. 2021b. Status and sustainability of reefs and shorelines of the Republic of Mauritius. In: Gunputh RP (Eds). Sustainable Development Goals. Chapter 8. Star Publications Pvt. Ltd., New Delhi, India.

Bhagooli R, Mattan-Moorgawa S, Kaullysing D, Louis YD, Gopeechund A, Ramah S, Soondur M, Pilly SS, Beesoo R, Wijayawanti DP, Bachok ZB, Monrás VC, Casareto BE, Suzuki Y, Baker AC. 2021c. Chlorophyll fluorescence - a tool to assess photosynthetic performance and stress photo-physiology in symbiotic marine invertebrates and seaplants. Mar Pollut Bull 165: 112059. DOI: 10.1016/j.marpolbul.2021.112059.

Bhagooli R, Sheppard CCR. 2012. Prediction of recurrences of mass coral bleaching / mortality and vulnerability of reef-building corals to climate change in Mauritian and Japanese waters. Univ Maurit Res J 18A: 105-121. DOI: 10.1038/nature01987.

Bhagooli R, Soondur M, Ramah S, Gopeechund A, Jeetun S, Kaullysing D. 2021a. Photo-physiology of healthy and bleached corals from the Mascarene Plateau. SI - Studies on the Mascarene Plateau. West Indian Ocean J Mar Sci 2: 109-120. DOI: 10.4314/wiojms.si2021.2.8.

Bhagooli R, Taleb-Hossenkhan N. 2012. Thermal spatial heterogeneity and coral bleaching: implications for habitat refuges; Proceedings of the 12th International Coral Reef Symposium. Cairns 9-13 July 2012. [Australia]

Bhagooli R. 2009. Symbiont dependent thermal bleaching susceptibility in two reef-building corals, *Stylophora pistillata* and *Platygyra ryukyuensis*. Univ Maurit Res J 15: 608-625.

Bhagooli R. 2010. Photosystem II responses to thermal and/or light stresses of distinct endosymbiontic ITS2 *Symbiodinium* types isolated from marine animal hosts. J Environ Res Dev 5 (1): 117-133.

Bhagooli R. 2013. Inhibition of Calvin-Benson cycle suppresses the repair of photosystem II in *Symbiodinium*: Implications for coral bleaching. Hydrobiologia 714: 183-190. DOI 10.1007/s10750-013-1535-4.

Blidberg E, Elfwing T, Plantman P, Tedengren M. 2000. Water temperature influences on physiological behaviour in three species of giant clams (Tridacnidae). Proc 9th Intl Coral Reef Symp 1: 561-565.

Brahmi C, Chapron L, Le Moullac G, Soyez C, Beliaeff B, Lazareth CE, Gaertner-Mazouni N, Vidal-Dupiol J. 2021. Effects of elevated temperature and $pCO_2$ on the respiration, biomineralization and photophysiology of the giant clam *Tridacna maxima*. Conserv Physiol 9 (1): 1-17. DOI: 10.1093/conphys/coab041.

Brown B, Dunne R, Goodson M, Douglas AE. 2000. Bleaching patterns in reef corals. Nature 142-143. DOI: 10.1038/35004657.

Brown BE, Le Tissier MDA, Bythell JC. 1995. Mechanisms of bleaching deduced from histological studies of reef corals sampled during a natural bleaching event. Mar Biol 122: 655-663. DOI: 10.1007/BF00350687.

Brown JH, Gillooly JF, Allen AP, Savage VM, West GB. 2004. Toward a metabolic theory of ecology. Ecology 85: 1771-1789. DOI: 10.1890/03-9000.

Burghardt I, Wägele H. 2014. The symbiosis between the “solar-powered” nudibranch *Melibe engeli* Risbec, 1937 (Dendronotoidea) and *Symbiodinium* sp. (Dinophyceae). J Moll Stud 80: 508-517. DOI: 10.1093/mollus/eyu043.

Clark MS, Thorne MAS, Amaral A, Vieira F, Batista FM, Reis J, Power DM. 2013. Identification of molecular and physiological responses to chronic environmental challenge in an invasive species: The Pacific oyster, *Crassostrea gigas*. Ecol Evol 3 (10): 3283-3297. DOI: 10.1002/ece3.719.

Deboer T, Baker A, Erdmann M, Ambariyanto JP, Barber P. 2012. Patterns of *Symbiodinium* distribution in three giant clam species across the biodiverse Bird’s head region of Indonesia. Mar Ecol Prog Ser 444: 117-132. DOI: 10.3354/meps09413.

Downs CA, Kramarsky-Winter E, Martinez J, Kushmaro A, Woodley CM, Loya Y, Ostrander GK. 2009. Symbiophagy as a cellular mechanism for coral bleaching. Autophagy 5 (2): 211-6. DOI: 10.4161/auto.5.2.7405.

Dubousquet V, Gros E, Berteaux-Lecellier V, Viguier B, Raharivelomanana P, Bertrand C, Lecellier GJ. 2016. Changes in fatty acid composition in the giant clam *Tridacna maxima* in response to thermal stress. Biol Open 5: 1400-1407. DOI: 10.1242/bio.017921.

Dunn SR, Thomason JC, Le Tissier MDA, Bythell JC. 2004. Heat stress induces different forms of cell death in sea anemones and their endosymbiotic algae depending on temperature and duration. Cell Death Differ 11: 1213-1222. DOI: 10.1038/sj.cdd.4401484.

Eckman W, Vicentuan-Cabaitan K, Todd PA. 2014. Observations on the hyposalinity tolerance of fluted giant clam (*Tridacna squamosa*, Lamarck 1819) Larvae. Nat Singap 7: 111-116. DOI: 10.1007%2Fs00227-014-2545-0.

Fujise L, Yamashita H, Suzuki G, Koike K. 2013. Expulsion of zooxanthellae (*Symbiodinium*) from several species of scleractinian corals: Comparison under non-stress conditions and thermal stress conditions. Galaxea 15: 29-36. DOI: 10.3755/galaxea.15.29.

Gates RD, Baghdasarian G, Muscatine L. 1992. Temperature stress causes host cell detachment in symbiotic Cnidarians: Implications for coral bleaching. Biol Bull 182 (3): 324-332. DOI: 10.2307/1542252.

Gegner HM, Ziegler M, Rädecker N, Buitrago-Lópéz, Aranda M, Voolstra CR. 2017. High salinity conveys thermotolerance in the coral model Aiptasia. Biol Open 6: 1943-1948. DOI: 10.1242/bio.028878.

Ghoora MD, Pilly SS, Chumun PK, Jawaheer SJ, Bhagooli R. 2018. Short-term effects of heavy metal and temperature stresses on the photo-physiology of *Symbiodinium* isolated from the coral *Fungia repanda*. Ocean Life 2 (1): 11-20. DOI: 10.13057/oceanlife/o020102.

Goreau TJ, Hayes RL. 1994. Coral bleaching and ocean “Hot Spots”. Ambio 23: 176 - 180.

Hoegh-Guldberg O, Bruno JF. 2010. The impact of climate change on the world’s marine ecosystems. Science 328 (5985): 1523-1528. DOI: 10.1126/science.1189930.

Hoegh-Guldberg O, Mumby PJ, Hooten AJ, Steneck RS, Greenfield P, Gomez E, Harvell CD, Sale PF, Edwards AJ, Caldeira K, Knowlton N, Eakin CM, Iglesias-Prieto R, Muthiga N, Bradbury RH, Dubi A, Hatziolos ME. 2007. Coral reefs under rapid climate change and ocean acidification. Science 318 (5857): 1737-1742. DOI: 10.1126/science.1152509.

Junchompoo C, Sinrapasan N, Penpain C, Patsorn P. 2013. Changing seawater temperature effects on giant clams bleaching, Mannai Island, Rayong province, Thailand. Conference paper: Proceedings of the Design Symposium on Conservation of Ecosystem. The 12th SEASTAR2000 workshop, 71-76. DOI: 10.14989/176186. [Thailand]

Kobluk DR, Lysenko MA. 1994. "Ring" bleaching in southern Caribbean *Agaricia agaricites* during rapid water cooling. Bull Mar Sci 54 (1): 142-150.

Kurihara H, Kato S, Ishimatsu A. 2007. Effects of increased seawater $pCO_2$ on early development of the oyster *Crassostrea gigas*. Aquat Biol 1: 91-98. DOI: 10.3354/ab00009.

Lesser MP. 1997. Oxidative stress causes coral bleaching during exposure to elevated temperatures. Coral Reefs 16: 187-192. DOI: 10.1007/s003380050073.

Lough JM. 2000. 1997-98: Unprecedented thermal stress to coral reefs? Geophys Res Lett 27: 3901-3904. DOI: 10.1029/2000GL011715.

Mackenzie BW, Waite DW, Taylor MW. 2015. Evaluating variation in human gut microbiota profiles due to DNA extraction method and inter-subject differences. Front Microbiol 6: 1-11. DOI: 10.3389%2Ffmicb.2015.00130.

Mattan-Moorgawa S, Bhagooli R, Rughooputh SDDV. 2012. Thermal stress physiology and mortality responses in scleractinian corals of

Mauritius: Proceedings of the 12th International Coral Reef Symposium. Cairns, 9-13 July 2012. [Australia]

Mattan-Moorgawa S, Chumun PK, Taleb-Hossenkhan N, Rughooputh SDDV, Bhagooli R. 2020. Variable thermal and biochemical stress responses of tissue balls from *Lithophyllon repanda*, *Pocillopora damicornis* and *Acropora muricata*. Galaxea, J Coral Reefs Stud 22: 37-50. DOI: 10.3755/galaxea.22.1_37.

Neely KL, Shea CP, Macaulay KA, Hower EK, Dobler MA. 2021. Short- and long-term effectiveness of coral disease treatments. Front Mar Sci 8: 675349. DOI: 10.3389/fmars.2021.675349.

Norton JH, Shepherd MA, Long HM, Fitt WK. 1992. The zooxanthellar tubular system in the giant clam. Biol Bull 183: 503-506. DOI: 10.2307/1542028.

Pappas MK, He S, Hardenstine RS, Kanee H, Berumen ML. 2017. Genetic diversity of giant clams (*Tridacna* spp.) and their associated *Symbiodinium* in the central Red Sea. Mar Biodivers 47: 1-14. DOI: 10.1007/s12526-017-0715-2.

Parker LM, Ross PM, O'connor W. 2009. The effect of ocean acidification and temperature on the fertilization and embryonic development of the Sydney rock oyster *Saccostrea glomerata* (Gould 1850). Glob Change Biol 15 (9): 2123-2136. DOI: 10.1111/j.1365-2486.2009.01895.x.

Ralph PJ, Gademann R, Larku AWD. 2001. Zooxanthellae expelled from bleached corals at 33°C are photosynthetically competent. Mar Ecol Prog Ser 220: 163-168. DOI: 10.3354/meps220163.

Ramah S, Taleb-Hossenkhan N, Bhagooli R. 2017. Differential substrate affinity between two giant clam species, *Tridacna maxima* and *Tridacna squamosa,* around Mauritius. West Indian Ocean J Mar Sci 1: 13-20.

Ramah S, Taleb-Hossenkhan N, Todd P, Neo, ML, Bhagooli R. 2019. Drastic decline in giant clams (Bivalvia: Tridacninae) around Mauritius Island, Western Indian Ocean: Implications for conservation and management. Mar Biodivers 49: 815-823. DOI: 10.1007/s12526-018-0858-9.

Rodolfo-Metalpa R, Houlbrèque F, Tambutté E, Boisson F, Baggini C, Patti FP, Jeffree R, Fine M, Foggo A, Gattuso J-P, Hall-Spencer JM. 2011. Coral and mollusc resistance to ocean acidification adversely affected by warming. Nat Clim Change (Lett) 1: 308-312. DOI: 10.1038/nclimate1200.

Rowan R, Knowlton N, Baker A, Jara J. 1997. Landscape ecology of algal symbionts creates variation in episodes of coral bleaching. Nature 388: 265-269. DOI: 10.1038/40843.

Rowan R. 2004. Coral bleaching Thermal adaptation in reef coral symbionts. Nat (Brief Commun) 430: 742. DOI: 10.1038/430742a.

Sampayo EM, Ridgway T, Bongaerts P, Hoegh-Guldberg O. 2008. Bleaching susceptibility and mortality of corals are determined by fine-scale differences in symbiont type. Proc Natl Acad Sci 105: 10444-10449. DOI: 10.1073/pnas.0708049105.

Slavova C, Schrameyer V, Reusa M, Ralph PJ, Hill R, Bücheld C, Larkumb AWD, Holzwarth, AR. 2016. "Super-quenching" state protects *Symbiodinium* from thermal stress - Implications for coral bleaching. Biochim Biophys Acta 1857: 840-847. DOI: 10.1016/j.bbabio.2016.02.002.

Soo P, Todd PA. 2014. The behaviour of giant clams (Bivalvia: Cardiidae: Tridacninae). Mar Biol 161 (12): 2699-2717. DOI: 10.1007/s00227-014-2545-0.

Strychar KB, Sammarco PW. 2009. Exaptation in corals to high seawater temperatures: Low concentrations of apoptotic and necrotic cells in host coral tissue under bleaching conditions. J Exp Mar Biol Ecol 369: 31-42. DOI: 10.1016/j.jembe.2008.10.021.

Talmage SC, Gobler CJ. 2011. Effects of elevated temperature and carbon dioxide on the growth and survival of larvae and juveniles of three species of Northwest Atlantic bivalves. PLoS ONE 6 (10): e26941. DOI: 10.1371/journal.pone.0026941.

Tchernov D, Gorbunov MY, De Vargas C, Narayan YS, Milligan AJ Häggblom M. 2004. Membrane lipids of symbiotic algae are diagnostic of sensitivity to thermal bleaching in corals. Proc Natl Acad Sci 101: 13531-13535. DOI: 10.1073/pnas.0402907101.

Trench RK, Wethey DS, Porter JW. 1981. Observations on the symbiosis with zooxanthellae among the *Tridacnidae* (Mollusca, Bivalvia). Biol Bull 161 (1): 180-198. DOI: 10.2307/1541117.

True JD. 2012. Salinity as a structuring force for near shore coral communities; Proceedings of the 12th International Coral Reef Symposium. Cains, 9-13 July 2012. [Australia]

Ward S, Harrison P, Hoegh-Guldberg O. 2002. Coral bleaching reduces reproduction of scleractinian corals and increases susceptibility to future stress. Proc 9th Intl Coral Reef Symp 2: 1123-1128.

Watson SA, Neo ML. 2021. Conserving threatened species during rapid environmental change: Using biological responses to inform management strategies of giant clams. Conserv Physiol 9 (1): coab082. DOI:10.1093/conphys/coab082.

Winter A, Appeldoorn RS, Bruckner A, Williams EH, Goenaga C. 1998. Sea surface temperatures and coral reef bleaching off La Parguera, Puerto Rico (northeastern Caribbean Sea). Coral Reefs 17: 377-382. DOI: 10.1007/s003380050143.