Answers to Reviewers Interactive comment on "Light-dependent calcification in Red Sea giant clam Tridacna maxima" by Susann Rossbach et al.

Anonymous Referee #1

The manuscript investigates a common yet little known question on the light dependency of iconic giant clams. Using experiments, Authors were able to show interesting results that showed congruence to the species' natural depth distribution. Some explanations were provided regarding their results, but I think there is scope to expand their Discussion (e.g. how symbiont species may play a role in affecting depth distributions and affect calcification). The current manuscript depth peer review, as well as to provide more details on the mechanism of light-induced calcification and why there is a maximum light threshold before calcification rates drop could be further expounded.

We thank the reviewer for the comments, which were very constructive and helpful. Below, we provide a "Response to reviewer" document detailing, point-by-point, the actions taken to address each comment. The original comments are shown in black with our responses in blue and refer to line numbers of the revised manuscript whenever possible, so that the changes can be easily assessed.

Specific comments:

Abstract, Line 8: Tridacninae is the subfamily for giant clams. Please amend the first sentence.

Abstract, Line 8 was changed to:
'Tropical giant clams of the subfamily Tridacninae, including the species Tridacna maxima, are unique among bivalves as they live in a symbiotic relationship with unicellular algae and generally function as net photoautotrophic.'

Introduction, Line 15: Suggest to use Neo et al., 2017 - a review of species status, instead of Neo et al., 2015 that looks at ecological roles.
Reference citation: Neo ML, CCC Wabnitz, RD Braley, GA Heslinga, C Fauvelot, S Van Wynsberge, S Andréfouët, C Waters, AS-H Tan, ED Gomez, MJ Costello & PA Todd (2017) Chapter 4. Giant clams (Bivalvia: Cardiidae: Tridacninae): A comprehensive update of species and their distribution, current threats and conservation status.

In: Hawkins SJ, Evans AJ, Dale AC, Firth LB, Hughes DJ, Smith IP (eds.), Oceanography and Marine Biology: An Annual Review, Volume 55. Pp. 87–388. CRC Press: Boca Raton, FL.

We agree with the reviewer and changed the text accordingly:

Page 1, Line 13-16:

'Currently, all giant clam species are listed in the IUCN Red List of Threatened Species (IUCN, 2016) and protected under Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). Most of them are considered under a lower risk / *conservation dependent status, however the IUCN status of tridacnine species, is in need of updating according to Neo et al. (2017).*

Discussion, Section 4.1: Suggest Authors to look at the following papers to compare how locations of giant clams (sheltered versus exposed sites) can affect distribution.

References: Militz TA, J Kinch & PC Southgate (2016) Population Demographics of Tridacna noae (Röding, 1798) in New Ireland, Papua New Guinea. Journal of Shellfish Research 34(2): 329-335.
Neo ML, L-L Liu, D Huang & K Soong (2018) Thriving populations with low genetic diversity in giant clam species, Tridacna maxima and T. noae, at Dongsha Atoll, South China Sea. Regional Studies in Marine Science 24: 278–287.

We thank the reviewer for this comment and added the following paragraph to the discussion:

Page 10, Line 17-33:

'Explanations for the observed contrasts in numbers of clams per m^2 at both reefs could lay in the probable differences in abiotic environmental conditions at the surveyed sites. For instances, giant clams at the exposed reef are potentially more at risk from high wave action than at the sheltered reef site, which could impact the initial settlement (Jameson, 1976) as well as the survival of juveniles (Foyle et al., 1997), as both have been shown to be influenced by geographical factors (Foyle et al., 1997). While a previous study (Militz et al., 2015), in which abundances of giant clam species in French Polynesia were examined, report similar patterns for T. crocea, opposite patterns were observed for abundances of T. maxima in that region. In the reefs surveyed by Militz and collegues (2015), T. maxima showed higher abundances at reef sites with a high exposure, in comparison to those with low exposure levels. However, additional factors such as temperature and local geomorphology might also have an influence on giant clam densities. Therefore,

it is not possible to confidently identify the underlying causes for the observed differences by considering exposure alone. For example, T. maxima specimens from our study, which were located at the more southern reef, could be possibly also exposed to higher surface water temperatures due to location of this reef at lower latitudes. Mean seasurface annual temperature of the Red Sea have been shown to increase towards lower latitudes and can be as high as 33 °C in the Central and Southern Red Sea (Chaidez et al., 2017). Further, the local geomorphological features of each reef could influence the light availability of benthic habitats. Consequently, differences in the local topography could have led to different angles of incident light and shading conditions, which would then result in differences between reefs even though the examined depths are identical.

Discussion, Section 4.2: Suggest Authors to refer to LaJeunesse et al., 2018 (Systematic Revision of Symbiodiniaceae Highlights the Antiquity and Diversity of Coral Endosymbionts) and symbiont-related papers on giant clam(e.g. DeBoer et al., 2012; Ikeda et al., 2017; Lim et al., 2019), and make inferences on how symbiont species may affect depth distribution with respect to light.

References: DeBoer TS, AC Baker, MV Erdmann, Ambariyanto, PR Jones & PH Barber (2012) Patterns of Symbiodinium distribution in three giant clam species across the biodiverse Bird's Head region of Indonesia. Marine Ecology Progress Series 444: 117-132. Ikeda S, Yamashita H, Kondo S-n, Inoue K, Morishima S-y, Koike K (2017) Zooxanthellal genetic varieties in giant clams are partially determined by species-intrinsic and growth-related characteristics.PLoS ONE 12(2): e0172285. Lim SSQ, D Huang, K Soong & ML Neo (2019) Diversityof endosymbiotic Symbiodiniaceae in giant clams at Dongsha Atoll, northern South China Sea. Symbiosis.

We thank the reviewer for this valuable comment and added the following paragraph to the manuscript:

Page 12, Line 11-25:

'Giant clams, including T. maxima can potentially harbour multiple genera of Symbiodiniaceae simultaneously (DeBoer et al., 2012;Ikeda et al., 2017), including Symbiodinium, Cladocopium and Durusdinium (previously referred to as clade A, C and D (LaJeunesse et al., 2018)) (DeBoer et al., 2012). The composition of these associated algal symbionts might therefore also impact the susceptibility to (high) light levels, as different genera of Symbiodiniaceae (in symbiosis) exhibit different physiological and ecological patterns, including sensitivity to light and temperature (Rowan et al., 1997;Berkelmans and Van Oppen, 2006). However, a previous study on Red Sea giant clams and their associated Symbiodiniaceae (Pappas et al., 2017), report that T. maxima in the region exclusively associated with Symbiodinium spp.

(previously clade A) which was thus assumed to represent an optimal group for the local environmental conditions.

Yet, the reliance of calcification of host organisms (e.g. T. maxima) on their relationship with symbiotic algae could provide an explanation for the significant decrease in net calcification rates at the highest light treatment (1061 µmol photons m-2 s-1). These diminished rates could be the result of photoinhibition and even photodamage of the associated unicellular algae, when exposed to these high incident light levels. This would be also supported by the pronounced decrease in gross primary production rates at this light treatment. High incident light level, especially high level of UV radiation in shallow waters, have been previously shown to be correlated with decreased calcification rates in other marine calcifiers such as stony corals, e.g. Porites compressa (Kuffner, 2001).

Anonymous Referee #2

Rossbach et al. demonstrated depth-dependent abundances of Tridacna maxima in natural reefs and experimentally examined short term net calcification rates of T. maxima in different light conditions. Tridacna is abundant bivalves in coral reefs and has demand as fishery resources and environmental proxies. However, the knowledge about their calcification rates are scarce. While calcification rates of tridacna shells seem to be also strongly related to temperature conditions (Warter et al., 2018), this study provide new insight of the relationship between their calcification and light. I recommend this paper published in "Biogeosciences" after some revisions.

I hope my comments below will be useful to improve the manuscript.

We thank the Reviewer for the comments, they were very constructive and helpful. The journal shared the recommendations from the reviewer previously (as a quick report) and we amended the manuscript following the reviewer's suggestions before its publication online in Biogeosciences Discussions. We provide a "Response to reviewer" document detailing, point-by-point, the actions taken to address each comment. The original comments are shown in black with our responses in blue and refer to line numbers of the revised manuscript whenever possible, so that the changes can be easily assessed.

P.3/L7: please check reference style. Probably you can write like "Ip et al., 2006, 2015, 2017".

The references have been changed accordingly.

P.5/L28: …following Dickson et al. (2007).

Changed to ***'following Dickson et al. (2007).'***

2.1. Clam abundance surveys: How many belt transects were conducted in each depth?

At the sheltered reef, we conducted six transects at each depth, at the exposed reef three at each depth. We added this information to the manuscript which now reads:

Page 4, Line 8 -10:

'At the sheltered reef, a total area of 1,000 m^2 was covered and we conducted six transects at each depth. At the exposed reef 560 m^2 were covered, with three transects at each depth.'

2.2. Clam incubations: How many clams incubate in each condition?

For the incubations at moderate light levels (530, 358, 244 and 197 µmol quanta m^{-2} s^{-1}) we used 20 clams which were set in pairs (2 clams each) in the ten, independent incubation chambers (e.g. ten replicates). Net calcification was then later normalized for mantle surface area (cm^2) or tissue dry-mass (g) of the clams. Each pair (n=10) was incubated at all four light levels.

For the incubations at high light levels (561, 959 and 1061 µmol quanta m^{-2} s^{-1}), 8 clams were set in individual incubations chambers (e.g. 8 replicates). All 8 clams were incubated at 561 and 959 µmol quanta m^{-2} s^{-1}, while at 1061 µmol quanta m^{-2} s^{-1} only six clams were incubated as two died after the incubations at the second highest treatment.

The following has been changed in the manuscript:

Page 4, Line 22-23**:**

'The experimental setup consisted of ten flow-through independent LDPE (low density polyethylene) outdoor aquaria (30 L). Each aquaria contained two clams (in total 20), cleaned with a brush from epibionts prior to the experiment.'

Page 4, Line 31-33:

'We conducted short-term incubations of 6 hours (from approx. 09:30 to 15:30 mean solar time) under four different shadings and one dark incubation (at night) (n = 10), allowing 3 days acclimatization period to the clams, prior to each incubation.'

P.2/L32: I think, after the flow-through system turned off, the incubation tanks should be completely closed to measure carbonate chemistry. This description is needed here.

The aim of the first set of incubations (moderate light level) was to measure the calcification rate of the clams using the TA anomaly method. We are providing DIC, omega and the rest of the carbonate system only to show that the incubation did not generated an artifact of extreme low or high omega aragonite due to TA reduction, combined with net DIC emission or uptake by photosynthesis and respiration. It is right that in the event that we would have wanted to evaluate primary production / respiration rate through the variation of DIC, the tanks should have been hermetically closed to avoid air-sea CO_2 exchange. However, our goal was to maintain ambient pCO_2 in our incubations by allowing air-water gas exchange, thereby preventing extreme variations of omega that would have affected the calcification rates.

And, how did you sample seawater during the experiments?

Seawater was sampled using gas tight 100 mL borosilicate bottles.

Changes:

Page 5, Line 28-29:

'At the start, after three and after six hours of incubation, seawater was sampled from each experimental aquaria in gas tight 100 mL borosilicate bottles (Schott Duran, Germany) and poisoned with mercury chloride, following Dickson et al., 2007.'

P.8/L2: Please refer to "Fig.2" here.

I added the reference to Figure 2.

P.10/L3 "In the Red Sea, T. maxima shows a significant increase in net calcification rates with increasing incident light.". In your results, strong light conditions over 900 µmol photons m-2 s-1 made decreasing net calcification rate. So, net calcification rates were not always increasing with light. Is it right?

Yes, we agree. In fact, net calcification rates show a significant dependence of incident light levels. We changed the sentence and it now reads:

Page 10, Line 3:

'In the Red Sea, T. maxima shows a significant dependence of net calcification rates with incident light.'

4.1. Depth-dependent abundances: I think that local geomorphological feature can also change light availability of benthic habitats. Even at same depth, the angle of incident light and local topography makes different shade conditions for each clam. In connection with the matter, please add the detail description of geomorphology at each site in 2.1 and Figure 1.

We thank the reviewer for this comment and agree that the local geomorphology of the reefs could influence the incident light levels as well.

Unfortunately, we did not conduct a more detailed survey on the overall topography of the observed reefs. Thus, the only information that can be shared is what is already part of the manuscript: Page 4, Line 4-7: 'The first station was Abu Shosha (22.303833 N, 39.048278 E), a small inshore reef, were abundances were examined at the sheltered, leeward side (Southeast) of the reef, which are relatively protected from wave action and currents (Khalil et al., 2013). Additionally, abundances were assessed at a second station (20.753764 N, 39.442561 E), a fringing reef close to Almojermah, were we conducted transects at the exposed, windward side (Northwest) of the reef.'

However, we added the following sentence to the discussion:

Page 10, Line 17-33:

'Explanations for the observed contrasts in numbers of clams per m^2 at both reefs could lay in the probable differences in abiotic environmental conditions at the surveyed sites. For instances, giant clams at the exposed reef are potentially more at risk from high wave action than at the sheltered reef site, which could impact the initial settlement (Jameson, 1976) as well as the survival of juveniles (Foyle et al., 1997), as both have been shown to be influenced by geographical factors (Foyle et al., 1997). While a previous study (Militz et al., 2015), in which abundances of giant clam species in French Polynesia were examined, report similar patterns for T. crocea, opposite patterns were observed for abundances of T. maxima in that region. In the reefs surveyed by Militz and collegues (2015), T. maxima showed higher abundances at reef sites with a high exposure, in comparison to those with low exposure levels. However, additional factors such as temperature and local geomorphology might also have an influence on giant clam densities. Therefore, it is not possible to confidently identify the underlying causes for the observed differences by considering exposure alone. For example, T. maxima specimens from our study, which were located at the more southern reef, could be possibly also exposed to higher surface water temperatures due to location of this reef at lower latitudes. Mean seasurface annual temperature of the Red Sea have been shown to increase towards lower latitudes and can be as high as 33 °C in the Central and Southern Red Sea (Chaidez et al., 2017). Further, the local geomorphological features of each reef could influence the light availability of

benthic habitats. Consequently, differences in the local topography could have led to different angles of incident light and shading conditions, which would then result in differences between reefs even though the examined depths are identical.

A more detailed satellite photo of the reefs is unfortunately not available.

4.2./P.11/L19: Not only photosynthetic activity, but also the efficiency of photosynthesis and the density of symbionts might intervene between light availability and calcification. Increased light could be also stressor for zooxanthellae (e.g. Weis, 2018). Additional discussion about the influence of light to algal-tridacna holobiont and its calcification processes could persuade the readers of the results in this study.

Agreed.
Thus we added the following paragraph to the discussion:

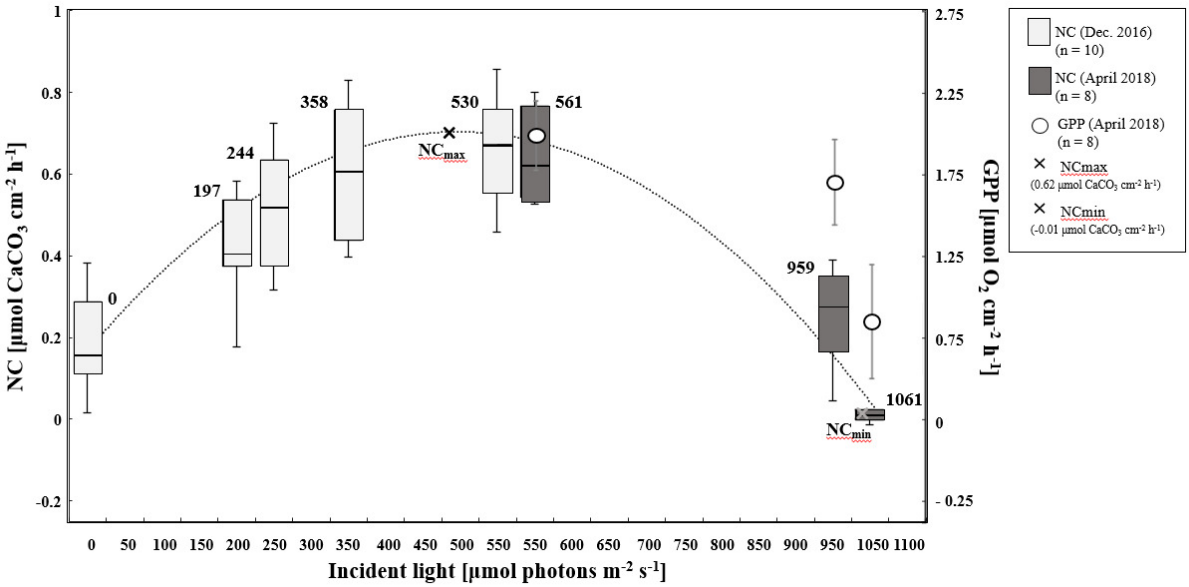
Page 12, Line 3-9:
'The reliance of calcification of calcifying host organism (e.g. T. maxima) on their relationship with symbiotic algae could also provide an explanation for the significant decrease in net calcification rates at the highest light treatment (1061 µmol photons m^{-2} s^{-1}). These diminished rates could be the result of photoinhibition and even photodamage of the associated Symbiodinium algae, when exposed to these high incident light levels. This would be also supported by the pronounced decrease in gross primary production rates at this light treatment. High incident light level, especially high level of UV radiation in shallow waters, have been previously shown to be correlated with decreased calcification rates in other marine calcifiers such as stony corals, e.g. Porites compressa (Kuffner, 2001).'

Fig.1 (b) and (c): Please zoom up the map and point the area of study sites to see topographical differences among two reefs.

Please see reply to previous question.

Fig.2 and Fig.3: How many specimens did you use for each condition?

I added the number of replicates to the legend of Figure 3



and changed the description to:

Figure 3:

'Box plots showing net calcification rates [µmol $CaCO_3$ cm^{-2} h^{-1}] of T. maxima under seven different light regimes (197, 244, 358, 530, 561, 959 and 1061 µmol quanta m^{-2} s^{-1}) (n = 10 in Dec. 2016 and n = 8 in April 2018) and in the dark as well as gross primary production [µmol O_2 cm^{-2} h^{-1}] (n = 8) as dots (±SE), under three high light regimes (561, 959 and 1061 µmol quanta m^{-2} s^{-1}).'

As well as the description of Figure 4:

Figure 4:

'Net calcification (µmol CaCO3 ind-1 h-1) (n = 10) in T. maxima at four different incident light levels (197, 244, 358 and 531 µmol photons cm-2 h-1) and in the dark, plotted against tissue dry-mass (g). Data are shown with polynomial trendlines.'

S2.1 and S2.2: Legends for each parameter are needed. I couldn't clealy understand the meaning of this table.

We changed the table legends to:

Table S1.1

'Seawater carbonate chemistry conditions at start of incubations under moderate light conditions (530,

358, 244, 197 µmol photons m^{-2} s^{-1}) and during the dark. Total alkalinity (TA) and dissolved inorganic carbon (DIC) were measured, while the inorganic carbon speciation , including pH, partial pressure of carbon dioxide ($_pCO_2$), carbon dioxideaq ($CO_{2(aq)}$), bicarbonate (HCO_3^-), carbonate (CO_3^{2-}) as well as the aragonite (Ω_{Arag}) and calcite (Ω_{Calc}) saturation state were calculated using R package Seacarb. Values are means ± SD (n = 10).'

Table S1.2

'Total alkalinity (TA) at start of each incubation under high light conditions (1061, 959, 561 µmol photons m-2 s-1) and during the dark. Values are means ± SD (n = 8).'

Table S2.2.2: Why are the values in the column of "diff" all zero?

The values were < 0.0001 and since we only reported them up to the 4th decimal place they appear in the table as 0. Respective values in the table are now changed to < 0.0001

Light-dependent calcification in Red Sea giant clam *Tridacna maxima*

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Abstract. Tropical giant clams of the subfamily Tridacninae, including the species *Tridacna maxima*, are unique among bivalves as they live in a symbiotic relationship with unicellular algae and generally function as net photoautotrophic. Light is therefore crucial for these species to thrive. Here we examine the light-dependency of calcification rates of *T. maxima* in the central Red Sea as well as the patterns of its abundance with depth in the field. Red Sea *T. maxima* show highest densities in a depth of 3 m with 0.82 ± 0.21 and 0.11 ± 0.03 individuals m^{-2} (mean ± SE) at sheltered and exposed sites, respectively. Experimental assessment of net calcification (µmol $CaCO_3$ cm^{-2} h^{-1}) and gross primary production (µmol O_2 cm^{-2} h^{-1}) under seven light levels (1061, 959, 561, 530, 358, 244 and 197 µmol quanta m^{-2} s^{-1}) showed net calcification rates to be significantly enhanced under light intensities corresponding to a water depth of 4 m (0.65 ± 0.03 µmol $CaCO_3$ cm^{-2} h^{-1}; mean ± SE), while gross primary production was 2.06 ± 0.24 µmol O_2 cm^{-2} h^{-1} (mean ± SE). We found a quadratic relationship between net calcification and tissue dry-mass (DM in gram), with clams of an intermediate size (about 15 g DM), showing the highest calcification. Our results show that the Red Sea giant clam *T. maxima* stands out among bivalves as a remarkable calcifier, displaying calcification rates comparable to other tropical photosymbiotic reef organism, such as corals.

1 Introduction

Giant clams (Family Cardiidae, Subfamily Tridacninae) are among the largest and fastest growing bivalves on earth, reaching up to one meter in size (Rosewater, 1965) and growth rates of up to 8 – 12 cm yr^{-1} in the largest species, *Tridacna gigas* (Beckvar, 1981). In the Indo-Pacific, giant clams are considered ecosystem engineering species (Neo et al., 2015), playing multiple roles in the framework of coral reef communities, such as providing food for a number of predators and scavengers (Alcazar, 1986), shelter for commensal organisms (De Grave, 1999) and substrate for epibionts (Vicentuan-Cabaitan et al., 2014). By producing calcium carbonate shell material they can occasionally even form reef-like structures (Andréfouët et al., 2005). However, due to their specific habitat preference (Yonge, 1975;Hart et al., 1998) and their presumed longevity (Chambers, 2007) Tridacninae are exceedingly vulnerable to exploitation and environmental degradation (Ashworth et al., 2004;Van Wynsberge et al., 2016). In Southeast Asia, giant clams are harvested for human consumption (adductor muscle and mantle meat) and for their shells (Lucas, 1994), already since pre-historic times (Hviding, 1993). Giant clams are also reared in aquaculture farms for the fishkeeping market (Bell et al., 1997), and in an effort of restocking the natural population (Gomez and Mingoa-Licuanan, 2006). Currently, all giant clam species are listed in the IUCN Red List of Threatened Species (IUCN, 2016) and protected under Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). Most of them are considered under a lower risk / conservation dependent status, however the IUCN status of tridacnine species, is in need of updating according to (Neo et al., 2017). Besides the pressure of fishing on natural stocks, giant clams are also predicted to be vulnerable to the effects of climate change, including heat waves which have been associated with mass die-off events of Tridacninae in French Polynesia (Andréfouët et al., 2013).

Giant clams are one of the few molluscan groups living in symbiotic relationship with dinoflagellates of the genus *Symbiodinium* (Yonge, 1936;Taylor, 1969;LaJeunesse et al., 2018), likewise to as corals and sea anemones. They are generally described as being mixotrophic (Klumpp et al., 1992), obtaining their energy both from filter-feeding and photosynthesis, however some species appear to be even functionally autotrophic (Beckvar, 1981;Jantzen et al., 2008). This dual capacity is assumed to support their fast calcification and growth rates, exceeding those of most other bivalves (Klumpp and Griffiths, 1994). Thus, the availability of light seems to be a critical factor affecting the growth and overall performance of giant clams (Lucas et al., 1989). To date, several studies have examined long-term growth rates of giant clams in response to different environmental factors, such as nutrient enrichment (Hastie et al., 1992;Hoegh-Guldberg, 1997;Belda-Baillie et al., 1998), water temperature (Hart et al., 1998;Schwartzmann et al., 2011) and wave exposure (Hart et al., 1998). Only a few studies assessed net calcification of Tridacninae as a short-term process and how environmental factors, especially light, are influencing calcification, physiology and general metabolic rates of Tridacninae.

A positive correlation between light and calcification has been observed in several photosynthetic calcifying organisms, symbiotic (e.g. scleractinian corals) or not (e.g. coccolithophorids and calcifying algae) (Allemand et al., 2011). For corals, the term Light Enhanced Calcification (LEC) has been coined (Yonge, 1931), however the underlying mechanisms remain poorly understood and various hypotheses have been proposed: (1) The photosynthetic uptake of carbon dioxide by the symbionts lowers CO_2 levels, while increasing pH and the concentration of carbonate ions at the calcification site, which eventually could favour calcium carbonate precipitation (McConnaughey and Whelan, 1997), (2) the removal of inhibiting substances (such as phosphates) by the symbionts during photosynthesis (Simkiss, 1964) or (3) the light-induced production of signalling molecules by the symbionts, could lead to an increase in enzymatic activity, essential for the calcification of the host (Ip et al., 2015). Only within the last years it has been possible to investigate LEC mechanisms at the molecular level (Moya et al., 2008;Bertucci et al., 2015) leading to an increasing number of publications reporting light-enhanced expression of enzymes, such as carbonic anhydrase, supporting shell formation in giant clams (Ip et al., 2006, 2015, 2017; Hiong et al., 2017a, 2017b; Chan et al., 2018; Chew et al.,

2019). There is also evidence for the light enhanced expression of genes encoding for those transporters / enzymes needed for calcification within the inner mantle and ctenidium of *Tridacna squamosa* (Hiong et al., 2017a;Hiong et al., 2017b;Ip et al., 2017;Chew et al., 2019). As both tissues are lacking the presence of symbiotic algae, it has been supposed that light could also directly affect the giant clam host. Despite recent progress in understanding LEC processes in *Tridacninae*, much remains unknown to date. Previous studies mostly focussed on molecular processes or long-term (several months) effects of light on growth rates, assessed either as increase in shell length (Lucas et al., 1989;Adams et al., 2013) or total weight (Adams et al., 2013) and did not differentiate between different light intensities. Only a small number of studies actually reported short-term (hours to few days) effect of light on calcification. They either focused on the development of proxies (Strontium / Calcium ratio) for parameters of the daily light cycle (Sano et al., 2012) through tracer (Strontium) incorporation or aimed to understand environmental and physiological parameters controlling daily trace element incorporation, using the total alkalinity (TA) anomaly technique (Warter et al., 2018). As growth and calcification rates in calcifying organisms are considered to be controlled by the corresponding light intensities (Barnes and Taylor, 1973) and as the penetration of light decreases with depth, so is the calcification rate expected to decrease (Goreau, 1963).

Tridacna maxima, the most abundant giant clam species in the Red Sea, can be found on shallow reef flats and edges, usually shallower than 10 m, where light intensity is high due to these transparent waters of tropical, oligotrophic oceans (Van Wynsberge et al., 2016). Although tridacnid clams are one of the most dominant and charismatic molluscan taxa in the Red Sea (Zuschin et al., 2000) little is known about their ecology in this area. In addition, the majority of studies on *Tridacninae* in the region exclusively focused on the Gulf of Aqaba in the Northern Red Sea (Roa-Quiaoit, 2005;Jantzen et al., 2008;Richter et al., 2008), which represents less than 2% of the entire basin of the Red Sea (Berumen et al., 2013).

In the present study, we assessed the net calcification rates (as µmol calcium carbonate per hour) of *T. maxima* in two short incubation experiments under seven different incident light levels (corresponding to a water depth of 0 – 14m) and in the dark, as well as photosynthetic rates at three experimental light level corresponding to the high light conditions in shallow waters (0 – 4m). Further, we assessed *in situ* abundances of *T. maxima* in different depth zones (0.5 – 11m) at a sheltered and an exposed reef in the Central Red Sea. To our knowledge, this is the first study quantifying the light-dependence of short-term net calcification rates of tridacnid clams of the Red Sea, interrelating these rates with their abundances in the field.

2 Material and Methods

2.1 Clam abundance surveys

Abundance surveys on *T. maxima* were conducted either via snorkelling or SCUBA diving at two reefs in the eastern central Red Sea (Fig.1). The first station was Abu Shosha (22.303833 N, 39.048278 E), a small inshore reef, were abundances were examined at the sheltered, leeward side (Southeast) of the reef, which are relatively protected from wave action and currents (Khalil et al., 2013). Additionally, abundances were assessed at a second station (20.753764 N, 39.442561 E), a fringing reef close to Almojermah, were we conducted transects at the exposed, windward side (Northwest) of the reef. At both stations, belt transects were conducted in six different depths (0.5, 1.5, 3, 5, 8 and 11 m). At the sheltered reef, a total area of 1,000 m^2 was covered and we conducted six transects at each depth. At the exposed reef 560 m^2 were covered, with three transects at each depth. Transect lines of 25 m were deployed and all *T. maxima* specimen within 2 meters of the transect where counted (e.g., 50m^2 area was covered on each transect). In addition, their length (maximum anterior to posterior distance) was recorded at the sheltered reef, using a measuring tape to the nearest cm.

2.2 Clam incubations to obtain net calcification rates

We determined net calcification (see section 2.4 below) in *T. maxima* during two consecutive incubation experiments. During the first incubations, conducted in December 2016, we assessed net calcification of *T. maxima* under four different, moderate experimental light level, mimicking light intensities at different water depths ranging from 4 to 14 m and during a dark incubation. In November 2016, 20 specimen of *T. maxima* (shell length of 17 ± 2 cm; mean ± SD) were collected in a water depth of about 4 m at a sheltered reef site (Station 1) (Fig. 2). As *T. maxima* is often embedded in the substrate, specimens were removed by carefully cutting their byssus with a knife. The incubations took place in December 2016 at the Coastal and Marine Resources Core Lab (CMOR) of King Abdullah University of Science and Technology (KAUST) in Thuwal, Saudi Arabia. The experimental setup consisted of ten flow-through independent LDPE (low density polyethylene) outdoor aquaria (30 L). Each aquaria contained two clams (in total 20), cleaned with a brush from epibionts prior to the experiment. Aquaria were supplied with water by gravity through an intermediate PVC (polyvinylchloride) tank of 77 L, itself receiving water pumped from the adjacent Red Sea surface water at a flow of 0.22 m^3 h^{-1}, leading to a complete water exchange in each single aquaria every 80 minutes. To maintain ambient Red Sea surface water temperatures, all aquaria were immerged to the last top cm in a large flow-through pool of 12 m^3, receiving the overflowing water from the intermediate tank and the 10 experimental tanks. An Exo1 probe (YSI Incorporated, Yellow Springs, USA) was used to log water temperature and salinity at 30 min frequency. Both remained constant during the experimental period with an average temperature of 27.2 ± 0.8 °C (mean ± SD, n = 672) and salinity of 38.4 ± 0.8 (mean ± SD, n = 672). Experimental aquaria were shaded with nets to reproduce light levels that mimicked natural conditions at different depths on the reef. We conducted short-term incubations of 6 hours (from approx. 09:30 to 15:30 mean solar time) under four different shadings and one dark incubation (at night) (n = 10), allowing 3 days acclimatization period to the clams, prior to each incubation. During the incubations, the flow-through system was turned off in order to determine changes in seawater carbon chemistry over time as a measure for calcification processes. Photosynthetically active radiation (PAR) was recorded with a light logger (Odyssey Logger, Dataflow Systems Ltd., New Zealand) as µmol quanta m^{-2} s^{-1} and averaged over the incubation period, as natural light conditions fluctuated over the course of the day. Experimental light levels comprised 530, 358, 244 and 197 µmol quanta m^{-2} s^{-1}. Using data on depth-dependent decrease of light levels (Dishon et al., 2012), we calculated the extinction of light with water depth. The experimental irradiation levels therefore correspond to incident light conditions at about 4, 8, 12, and 14 m water depth. No additional food was provided, as natural and unfiltered seawater was flowing into the tanks.

During the subsequent incubation, conducted in April 2018, we examined net calcification and primary production of *T. maxima* under three additional experimental high light level, addressing light effects encountered in very shallow waters (between 0 and 4 m). We collected eight specimen of *T. maxima* (shell length of 17 ± 1 cm; mean ± SD) in a water depth of about 4 m at an exposed, fringing reef close to Almojermah (Station 2) (Fig. 2). The incubation experiment was conducted on board of R/V Thuwal in a setup consisting of two big PVC flow-through tanks (350 L each), containing 9 individual PVC tanks (10 L), eight of them containing one clam each (cleaned from epibionts) and one serving as a control tank. To maintain ambient Red Sea surface water temperatures, all aquaria were immerged into the flow-through pool and water was constantly pumped (0.36 m^3 h^{-1}), assuring a constant water exchange and movement in the individual tanks. Temperature and salinity were checked four times a day using a handheld CTD probe (CastAway-CTD, SonTek, USA). Both remained constant during the experimental period with an average temperature of 31.5 ± 0.3 °C (mean ± SD, n = 16) and salinity of 38.2 ± 0.1(mean ± SD, n = 16). During the incubations, the individual tanks were closed airtight with see-through PVC lids and water movement was generated with battery-driven motors (Underwater motor, Playmobil, Germany). Nets were used for shading and therefore to reproduce light levels that mimicked natural conditions at different depths on the reef. We conducted closed short-term incubations of 3 hours (from approx. 11:00 to 14:00 mean solar time) under three different shadings and one dark incubation (at night), allowing one day acclimatization to the clams

prior to each incubation. Measurements of PAR intensities were identical to the first round of incubations. Experimental light levels comprised 561, 959 and 1061 µmol quanta m^{-2} s^{-1}. The amount of light received by the highest experimental light level was identical to light received directly at the water surface in the reef of collection at the same time of the day. Experimental irradiation levels correspond to incident light conditions at 0 m, 0.5 m and at 4 m. No additional food was provided, as raw unfiltered seawater was used.

2.3 Carbonate chemistry

At the start, after three and after six hours of incubation, seawater was sampled from each experimental aquaria in gas tight 100 mL borosilicate bottles (Schott Duran, Germany) and poisoned with mercury chloride, following (Dickson et al., 2011). Each sample was analysed for TA by open-cell titration with an AS-ALK2 titrator (Apollo SciTech,USA) using certified seawater reference material (CRM) (Andrew Dickson, Scripps Institution of Oceanography). During the incubations at moderate light levels (530, 358, 244 and 197 µmol quanta m^{-2} s^{-1}), additional samples for dissolved inorganic carbon (DIC) were analysed using an AS-C3 infrared DIC analyser (Apollo SciTech, USA). Further components of the carbonate system were calculated with the R package Seacarb (Lavigne and Gattuso, 2013) using first and second carbonate system dissociation constants of (Millero, 2010) as well as the dissociations of HF and HSO_4^- (Dickson, 1990;Dickson and Goyet, 1994) respectively. Carbonate chemistry at the beginning of each incubation and in all experimental aquaria were comparable with mean (± SD) TA of 2324 ± 83 and Ω_{Ara} of 3.44 ± 0.33 (n = 50) during the moderate light incubations and a TA of 2489 ± 38 (n = 4) during the high light incubations (Supplementary Material_S1).

2.4 Net calcification

Net calcification (G in µmol $CaCO_3$ h^{-1}) was estimated from changes in total alkalinity (TA) using the alkalinity anomaly technique (Smith and Key, 1975) using the following equation (Eq. 1):

$$G = -\frac{\Delta TA}{2} \times \frac{1}{\Delta t} \quad (1)$$

Where ΔTA is the variation of TA during the time (t) of the incubations and the factor 2 accounts for a decrease of TA by 2 equivalents per $CaCO_3$ precipitated (Zeebe and Wolf-Gladrow, 2001). Calcification rates were expressed relative to either mantle surface area (cm^2) or tissue dry-mass (g). For mantle surface area, the power relationship between standard length in cm (L) and mantle area (cm^2) (Jantzen et al., 2008) was used to calculate mantle surface in cm^2. For tissue dry-mass (DM in gram) of clams, all clams were dissected, and their biomass was determined subsequently to the incubation experiment. Clams were opened by cutting the adductor muscle with a scalpel, the mantle and other tissues were separated from the shells and dried at 60 °C for 24 to 48 hours to determine tissue DM to the nearest 0.01 g.

Experimentally determined net calcification rates (µmol $CaCO_3$ h^{-1}) of *T. maxima* for different DM under four moderate light level (197, 244, 358 and 530 µmol quanta m^{-2} s^{-1}) were used to create a multiple regression modelling net calcification for any given light level and DM.

2.5 Primary production

Primary production was assessed during the high light incubations (561, 959 and 1061 µmol quanta m^{-2} s^{-1}), only. Therefore, oxygen (µmol L^{-1}) content in the incubation chambers was automatically logged (miniDOT, Precision Measurement Engineering, Inc., USA) in 15 minute intervals over the three-hour incubation period. Net photosynthesis (NPP) was calculated from the

variation of oxygen concentration over time and normalized for clam mantle surface area (μmol O_2 cm^{-2} h^{-1}). Dark respiration rates (R), also given in μmol O_2 cm^{-2} h^{-1} were used to calculate gross primary production (GPP) as Eq. (2):

$$GPP = NPP + R \quad (2)$$

2.6 Statistical analyses

For assessing the comparisons of clam abundance at the six survey depths, an analysis of variance (ANOVA) and pairwise post-hoc Tukey analysis (Tukey HSC) were performed. A statistical model was built to explain calcification rates from the combination of PAR and clam tissue dry-mass. The model chosen was a multiple non-linear relationship built as the combination of a linear dependency between PAR and calcification rates and a quadratic dependency net calcification rates and clam tissue mass. This model was selected against other concurrent models by using Akaike information criterion (AIC) (Burnham and Anderson 2003). Statistical analyses were performed using R (Foundation for Statistical Computing, Vienna, Austria, Version 3.4.2) and Statistica (Dell Software).

3 Results

3.1 Depth-dependent abundances

At the sheltered reef site, significantly highest abundances of *T. maxima* (0.82 ± 0.21 individuals m^{-2}; mean ± SE) were observed in a water depth of 3 m (ANOVA, $p < 0.001$, $F = 35.6$; Post-hoc Tukey test $p < 0.001$; Supplementary Material_S2_1), being twice as high as in shallower waters (between 0.41 ± 0.02 and 0.44 ± 0.01 individuals m^{-2} mean ± SE; at 0.5 and 1.5 m, respectively) (Fig. 2). No clams were found at the deepest survey depth of 11 m and abundances at 8 m were low with 0.04 ± 0.01 individual m^{-2} (mean ± SE). Giant clams were significantly less abundant in deeper water when compared to shallow reef areas ($p < 0.001$ for both 0.5 and 1.5 m when compared to 8 and 11 m). In average, the density of *T. maxima* at the sheltered reef (0.5 – 11 m depth) was 0.32 ± 0.05 individuals m^{-2}; mean ± SE). The average size of clams was 16.6 ± 5.1 cm (mean ± SD, n = 422) and their calculated mantle surface area 140.4 ± 90.4 cm^2 (mean ± SD, n = 422) respectively.

At the exposed reef, abundances of *T. maxima* were overall lower with 0.04 ± 0.01 individuals m^{-2} (mean ± SE), however we also found highest densities of clams at a water depth of 3 m (0.11 ± 0.03 individuals m^{-2}; mean ± SE)(ANOVA, $p = 0.027$, $F = 3.813$; Supplementary Material_S2_2), however they were only significantly higher than those found at 8 and 11 m (with mean ± SE of 0.02 ± 0.01 and 0.01 ± 0.01, respectively) (Post-hoc Tukey test; Electronic Supplementary Material_S2_2)(Fig. 2).

3.2 Net calcification and primary production

We combined observed net calcification (as the balance between calcification and dissolution) at all seven experimental incident light level and the dark incubation and identified a polynomial relationship ($R^2 = 0.77$) between net calcification (NC, µmol $CaCO_3$ cm^{-2} h^{-1}) and incident light (I, µmol quanta m^{-2} s^{-1}) (Eq. 3) (Fig. 3),

$$NC = -2e^{-6} \times I^2 + 0.0019 \times I + 0.1643 \quad (3)$$

Among all light incubations, net calcification rates of *T. maxima* were highest (mean ± SE 0.65 ± 0.03 µmol $CaCO_3$ cm^{-2} h^{-1}) at experimental incident light levels of 530 to 561 µmol quanta m^{-2} s^{-1} (Fig. 3). *T. maxima* still showed positive, but low net calcification during the night (0.18 ± 0.02 µmol $CaCO_3$ cm^{-2} h^{-1}; mean ± SE). The lowest NC rates (mean ± SE of 0.01 ± 0.01 µmol $CaCO_3$ cm^{-2} h^{-1}) were observed at the highest incident irradiance of 1061 µmol quanta m^{-2} s^{-1}. Overall, we observed a decline in net calcification with both, decreasing and increasing light intensities (Table 1), with polynomial regression indicating the maximum calcification (NC_{max}) to be reached at an incident light level of 475 µmol quanta m^{-2} s^{-1}. From an incident light level of 1033 µmol quanta m^{-2} s^{-1} on, we expect to see dissolution processes outweighing calcification (NC_{min}, -0.01 µmol $CaCO_3$ cm^{-2} h^{-1}).

Gross primary production (GPP) under the high light incubations (561, 959 and 1061 µmol quanta m^{-2} s^{-1}) showed an identical decreasing trend with increase in incident light as observed for net calcification. At 561 µmol quanta m^{-2} s^{-1}, GPP was highest (2.06 ± 0.24 µmol O_2 cm^{-2} h^{-1}; mean ± SE) and production rates were significantly lower (ANOVA, $p = 0.039$, $F = 4.982$; Supplementary Material_S3), during the incubations at 959 and 1061 µmol quanta m^{-2} s^{-1} (Table 1, Fig. 3), with mean ± SE of 1.76 ± 0.28 and 0.87 ± 0.37 µmol O_2 cm^{-2} h^{-1}, respectively. Two specimens died after the second highest light treatment of 959 µmol quanta m^{-2} s^{-1}.

We identified a quadratic relationship between net calcification and tissue dry-mass, with clams of an intermediate size (DM of about 15 g), showing the highest calcification rates at the four incubations at moderate light level (197, 244, 358, 530 µmol quanta m^{-2} s^{-1}) (Fig. 4). Therefore, we combined the influence of light and dry-mass into a statistical model, explaining 77% of the

variance in observed calcification rates (all parameters $p < 0.05$, Table 2, Fig. 5). Based on this model, we identify maximum rates on clams of an intermediate size (DM of about 15 g), showing the highest calcification rates at the four light level incubations.

5

4 Discussion

4.1 Depth-dependent abundances

In the Red Sea, *T. maxima* shows a significant dependence of net calcification rates with incident light. This light-dependency is consistent with significantly higher abundances of this species in shallow, sunlit reef flats. Globally, densities of *T. maxima* range between 0.1 to 0.0001 individuals m^{-2} (Van Wynsberge et al., 2016), with some exceptions such as at the Ningaloo Marine Park in Western Australia with 0.86 clams m^{-2} (Black et al., 2011), the Egyptian Sinai peninsula with peak values of 0.80 clams m^{-2} (Roa-Quiaoit, 2005) and 0.42 clams m^{-2} in Kiribati (Chambers, 2007).

In water depths between 0.5 and 11 m, we found averaged (± SD) abundances of *T. maxima* of 0.04 ± 0.01 individuals m^{-2} and 0.32 ± 0.05 individual per m^{-2} (mean ± SE) at an exposed and sheltered reef, respectively. Abundances at the sheltered reef are ranking amongst the highest abundances reported world-wide, representing a 50% higher abundance than previously reported for a local reef (Bodoy, 1984) with 0.22 clams m^{-2}. This difference in average abundances between the two reefs observed in this study could be explained by the leeward and windward (sheltered or exposed, respectively) character of the examined sites. As reviewed by Van Wynsberge (2016), the 'reef type' can influence Tridacna abundances, as it potentially affects the water exchange (and thus water temperature and nutrient availability) as well as the exposure to waves. Similar to Roa-Quiaoit (2005), we found that *T. maxima* abundances in the Red Sea seems to display great differences between locations, as we found significant lower numbers of giant clams at the exposed reef, with an average of 0.04 ± 0.01 individuals m^{-2}; (mean ± SE) in water depths between 0.5 and 11 m. Explanations for the observed contrasts in numbers of clams per m^2 at both reefs could lay in the probable differences in abiotic environmental conditions at the surveyed sites. For instances, giant clams at the exposed reef are potentially more at risk from high wave action than at the sheltered reef site, which could impact the initial settlement (Jameson, 1976) as well as the survival of juveniles (Foyle et al., 1997), as both have been shown to be influenced by geographical factors (Foyle et al., 1997). While a previous study (Militz et al., 2015), in which abundances of giant clam species in French Polynesia were examined, report similar patterns for *T. crocea*, opposite patterns were observed for abundances of *T. maxima* in that region. In the reefs surveyed by Militz and collegues (2015), *T. maxima* showed higher abundances at reef sites with a high exposure, in comparison to those with low exposure levels. However, additional factors such as temperature and local geomorphology might also have an influence on giant clam densities. Therefore, it is not possible to confidently identify the underlying causes for the observed differences by considering exposure alone. For example, *T. maxima* specimens from our study, which were located at the more southern reef, could be possibly also exposed to higher surface water temperatures due to location of this reef at lower latitudes. Mean seasurface annual temperature of the Red Sea have been shown to increase towards lower latitudes and can be as high as 33 °C in the Central and Southern Red Sea (Chaidez et al., 2017). Further, the local geomorphological features of each reef could influence the light availability of benthic habitats. Consequently, differences in the local topography could have led to different angles of incident light and shading conditions, which would then result in differences between reefs even though the examined depths are identical.

Contrasting findings to previously reported *Tridacninae* abundances in the Central Red Sea could be further a result of differences in sampling depths in the respective studies, as e.g. Bodoy et al., (1984) only accounted for clams in water depth of maximum 2 m, while we assessed abundances of *T. maxima* in six different depths (0.5, 1.5, 3, 5, 8 and 11 m). Previous studies have shown that the depth of abundance surveys significantly impact the estimates (Van Wynsberge et al., 2016), even though generally, highest densities of *T. maxima* are always reported for shallow reefs (0 – 5 m) (Jantzen et al., 2008;Andréfouët et al., 2009). This is also reflected in the results of previous studies in the Red Sea (Roa-Quiaoit, 2005) showing highest abundances of *T. maxima* in shallow water (< 3 m). However, Roa-Quiaoit (2005) accumulated abundances at all depths less than 3 m, while we differentiated even between the 0.5 m, 1.5 m and 3 m depth level and thereby found that although *T. maxima* shows the highest

density at 3 m, abundances in shallower depths are significantly reduced. Furthermore, we found only few specimens of *T. maxima* in water depths between 5 and 11 m. This finding is similar to previous studies, describing *T. maxima* as being mostly restricted to reefs shallower than 10 m, principally reef flats and edges (Van Wynsberge et al., 2016). This depth-distribution is most likely a result from a trade-off between maximizing light dependent photosynthesis while minimizing temperature stress, UV irradiation, wave exposure and / or emersion stress. All these stressors have been previously reported to lead to massive bleaching and mass die-off events in *T. maxima* (Addessi, 2001) and preventing settlement and recruitment in the shallow waters of the reef flat (Watson et al., 2012). The average size of *T. maxima* specimens at the sheltered reef was 16.6 ± 5.1 cm (± SD), similar to previous studies on this species in the Red Sea (Roa-Quiaoit, 2005), corresponding, according to the size classification by (Manu and Sone, 1995) to broodstock (i.e., sexually mature individuals) hermaphrodites. However, the number of small, juvenile specimens (< 4 cm) is potentially underestimated, as they are extremely cryptic (Munro and Heslinga, 1983).

4.2 Light-dependent calcification and production in Red Sea giant clam *T. maxima*

Overall, we found significantly enhanced net calcification rates in Red Sea *T. maxima* during light incubations compared to the dark incubation. Net calcification rates also significantly increased with light intensity up to 475 µmol photons m^{-2} s^{-1} (incident light level corresponding to a water depth of approximately 5 m, at the same time of the day and season, when the incubations were conducted), thenceforward decrease until an eventual dominance of dissolution over calcification at approximately 1033 µmol photons m^{-2} s^{-1} (corresponding to light conditions received directly at the at the air-water interface in the reef of collection). Likewise to net calcification in *T. maxima*, we observed gross primary production (GPP) to be highest at intermediate light levels of around 560 µmol photons m^{-2} s^{-1} (corresponding to a water depth of about 4 m) and to decrease with increasing light intensities (at 959 µmol quanta m^{-2} s^{-1} and 1061 µmol quanta m^{-2} s^{-1}, corresponding to 1.5 and 0.5 m water depth, respectively). We conclude that net calcification in the Red Sea giant clam *T. maxima* is not only enhanced by light, but is likely coupled to the photosynthetic activity of their algal symbionts. Further, our results show that both, net calcification and primary production in Red Sea *T. maxima* are highest at incident light level received in water depths between 5 and 3 m at Red Sea reefs. This is especially noteworthy as these findings correlate with the observed depth-related abundances of *T. maxima*, displaying highest densities in intermediate water depths around 3 m in the Central Red Sea. The observed irradiance optima for both, net calcification and primary production of *T. maxima* could therefore provide an explanation for the maximum in abundances in intermediate waters (3 – 5 m) and the decreasing numbers of observed clams at both, shallower and deeper reef sites.

Overall, our finding of enhanced calcification rates under light are consistent with reports on the related species *Tridacna gigas* (Lucas et al., 1989), *Tridacna derasa* (Sano et al., 2012) and *Tridacna squamosa* (Adams et al., 2013). The mechanisms of light-enhanced calcification (LEC) have been intensely studied in zooxanthellate scleractinian corals, leading to several hypotheses proposed to explain LEC (Tambutté et al. 2011). The majority of these refer to mechanisms that are influenced by the symbiotic relationship of host and *Symbiodinium*, with the most supported hypothesis relating photosynthetic CO_2 uptake by the algal symbionts to increase pH and the concentration of carbonate ions, thereby favouring calcification through the corresponding elevated saturation state for carbonate minerals (McConnaughey and Whelan, 1997).

Giant clams, including *T. maxima* can potentially harbour multiple genera of Symbiodiniaceae simultaneously (DeBoer et al., 2012;Ikeda et al., 2017), including Symbiodinium, Cladocopium and Durusdinium (previously referred to as clade A, C and D (LaJeunesse et al., 2018)) (DeBoer et al., 2012). The composition of these associated algal symbionts might therefore also impact the susceptibility to (high) light levels, as different genera of Symbiodiniaceae (in symbiosis) exhibit different physiological and ecological patterns, including sensitivity to light and temperature (Rowan et al., 1997;Berkelmans and Van Oppen, 2006). However, a previous study on Red Sea giant clams and their associated Symbiodiniaceae (Pappas et al., 2017), report that *T.*

maxima in the region exclusively associated with *Symbiodinium* spp. (previously clade A) which was thus assumed to represent an optimal group for the local environmental conditions. Yet, the reliance of calcification of host organisms (e.g. *T. maxima*) on their relationship with symbiotic algae could provide an explanation for the significant decrease in net calcification rates at the highest light treatment (1061 µmol photons m^{-2} s^{-1}). These diminished rates could be the result of photoinhibition and even photodamage of the associated unicellular algae, when exposed to these high incident light levels. This would be also supported by the pronounced decrease in gross primary production rates at this light treatment. High incident light level, especially high level of UV radiation in shallow waters, have been previously shown to be correlated with decreased calcification rates in other marine calcifiers such as stony corals, e.g. *Porites compressa* (Kuffner, 2001). However, recent findings for hermatypic corals also report that the contribution by the symbionts might not be the primary or sole driver for LEC, but the blue light spectrum could trigger the light sensors of the host itself, leading to higher calcification rates (Cohen et al., 2016). It is suggested that blue light photoreceptors in coral tissues of *Porites lutea* and *Acropora variabilis,* could potentially sense the light which is ultimately activating a cascade of processes involved in blue light-enhanced calcification (Cohen et al., 2016). However, our experimental light level, produced by different layers of neutral screen shading, only differed in light intensities but not in the wavelength that *T. maxima* would receive in the respective water depth. In a previous study on *Tridacna crocea*, short-term calcification rates were also reported to be strongly light-dependent (Warter et al., 2018). However, in this their experiment, Warter et al. (2018) exposed the clams not only to artificial light but also light level that were not comparable to actual conditions in the environment, as the average treatment comprised only 162 ± 7 µmol quanta m^{-2} s^{-2} (corresponding to a water depth of approximately 16 m in an oligotrophic ocean such as the Red Sea).

4.2.1 Allometric relationship between calcification and biomass

We determined a non-linear relationship between net calcification and biomass (as tissue DM) in *T. maxima*. Clams of an intermediate DM of approximately 15 g showed the highest net calcification throughout the four incubations as moderate light levels (530, 358, 244 and 197 µmol quanta m^{-2} s^{-1}). Specimens of a smaller or higher biomass calcified less during the incubations. A similar allometric relationship has been previously described for the photosynthetic metabolic performance of the zooxanthellae in *T. maxima* (Yau and Fan, 2012). This allometric pattern is most likely due to an optimal ratio of symbionts to clam body-mass at intermediate sizes. As the clam grows, its mantle tissue increases in thickness and thus the three dimensional tubular system, bearing the utmost of symbionts (Fisher et al., 1985). However, as the mantle thickens, impinging light must penetrate through more tissue before reaching the stacked zooxanthellae (Trench et al., 1981) and there is evidence for increased shading of the symbionts in the mantles of bigger clams (Fisher et al., 1985). With further increasing size, the number of symbionts per unit clam biomass also decreases (Fisher et al., 1985;Fitt et al., 1993;Griffiths and Klumpp, 1996). In general, growth rates giant clams seem to decrease with age once they reached the threshold for maturity and become broodstock hermaphrodites (Van Wynsberge et al., 2016). Past this age, a growing portion of their energy is invested in reproduction (Romanek and Grossman, 1989;Van Wynsberge et al., 2016), especially since there is an exponentially increase of produced egg numbers with increasing shell size (Jameson, 1976).

4.2.2 Comparison with other calcifiers

We compared net calcification rates of *T. maxima* with those of other benthic phototrophic and mixotrophic calcifiers (Table 3). In most calcifying organisms that live in symbiotic relationship with zooxanthellae (such as corals), metabolic rates and calcification are normalized by surface area. In contrast to corals however, which host their symbiotic algae intracellularly in their endodermal cell layer, the symbionts of Tridacninae are located in delicately branching and specialized channels within the mantle, which extend from the stomach (Trench et al. 1981; Norton et al. 1992). Although this difference makes the comparison to other

calcifiers conceptually difficult, normalisation of calcification rates per mantle surface area would be also appropriate, as *Symbiodinium* cells in giant clams are mostly found in the upper 5 mm of the mantle (Ishikura et al. 1997).

The Red Sea giant clam *T. maxima* shows averaged net calcification rates of 0.47 ± 0.03 µmol $CaCO_3$ cm^{-2} h^{-1} (mean ± SE), which are comparable than those reported for hermatypic corals (0.42 ± 0.08 µmol $CaCO_3$ cm^{-2} h^{-1}; mean ± SE) and those for calcifying macroalgae (0.43 ± 0.38 µmol $CaCO_3$ cm^{-2} h^{-1}; mean ± SE). However, in comparison with averaged rates of other heterotrophic, temperate bivalve species, such as *Mytilus edulis*, *Argopecten purpuratus* and *Crassostrea gigas* with 0.08 ± 0.07 µmol $CaCO_3$ g DM^{-1} h^{-1} (mean; ± SD), calcification in *T. maxima* is about 70 times higher (5.38 ± 0.42 µmol $CaCO_3$ g DM^{-1} h^{-1}; mean ± SE) (Table 4). When compared to heterotrophic cold-water coral species, such as *Lophelia pertusa* and *Madrepora oculata*, net calcification rates of *T. maxima* are more than 7 times higher (0.80 ± 0.70 µmol $CaCO_3$ g DM^{-1} h^{-1}; mean ± SD); Table 4). Our comparative assessment of the net calcification rates of giant clams with temperate / azooanthellate species show that rates in the Red Sea *T. maxima* tested here comparable to other photosymbiotic organisms (such as corals) and calcifying algae.

5 Conclusion

The present study shows that net calcification and photosynthetic rates of Red Sea *T. maxima* are light-dependent, but show a maximum at intermediate irradiance, suggesting strong inhibition at the highest incident light levels received in very shallow (0 – 1.5 m) waters. This is consistent with the depth-related distribution of this species in the Red Sea, and elsewhere, which showed maximum abundances in shallow (3 m), sunlit coral reefs, but a decrease in abundance from 3 m towards the surface and below. Albeit enhanced calcification is consequently beneficial for *T. maxima*, the light-dependency of both calcification and production restricts them to shallow waters, which also makes them more vulnerable to potentially harmful environmental changes, such as predicted increasing water temperatures associated to global warming (Hughes et al., 2003) as well as high levels of incident light, including high levels of UV radiation (Shick et al., 1995). The present study provides an important baseline for future studies examining the impact of wavelength specific responses of calcification and metabolic rates on giant clams as well as for a better overall understanding of light enhanced calcification in Red Sea Tridacninae and their relationship with the symbiotic algae.

Author contributions

C.M.D, V.S., and S.R. conceptualized the research, V.S. and S.R. collected the animals and performed the abundance surveys. Experimental execution was carried out by S.R., A.A. and V.S. conducted the data curation and ran formal analyses. S.R. prepared the first draft of the manuscript and all co-authors contributed substantially to subsequent versions including the final draft.

Competing interests

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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References

Adams, A. L., Needham, E. W., and Knauer, J.: The effect of shade on water quality parameters and survival and growth of juvenile fluted giant clams, *Tridacna squamosa*, cultured in a land-based growth trial, Aquaculture international, 21, 1311-1324, doi:10.1007/s10499-013-9634-9, 2013.

Addessi, L.: Giant clam bleaching in the lagoon of Takapoto atoll (French Polynesia), Coral reefs, 19, 220-220, doi:10.1007/PL00006957, 2001.

Alcazar, S. N.: Observations on predators of giant clams (Bivalvia: Family Tridacnidae), Silliman Journal, 33, 54-57, 1986.

Allemand, D., Tambutté, É., Zoccola, D., and Tambutté, S.: Coral calcification, cells to reefs, in: Coral reefs: an ecosystem in transition, Springer, 119-150, 2011.

Andréfouët, S., Gilbert, A., Yan, L., Remoissenet, G., Payri, C., and Chancerelle, Y.: The remarkable population size of the endangered clam *Tridacna maxima* assessed in Fangatau Atoll (Eastern Tuamotu, French Polynesia) using in situ and remote sensing data, ICES Journal of Marine Science, 62, 1037-1048, doi:10.1016/j.icesjms.2005.04.006, 2005.

Andréfouët, S., Friedman, K., Gilbert, A., and Remoissenet, G.: A comparison of two surveys of invertebrates at Pacific Ocean islands: the giant clam at Raivavae Island, Australes Archipelago, French Polynesia, ICES Journal of Marine Science, 66, 1825-1836, doi:10.1093/icesjms/fsp148, 2009.

Andréfouët, S., Van Wynsberge, S., Gaertner-Mazouni, N., Menkes, C., Gilbert, A., and Remoissenet, G.: Climate variability and massive mortalities challenge giant clam conservation and management efforts in French Polynesia atolls, Biological conservation, 160, 190-199, doi:10.1016/j.biocon.2013.01.017, 2013.

Ashworth, J. S., Ormond, R. F., and Sturrock, H. T.: Effects of reef-top gathering and fishing on invertebrate abundance across take and no-take zones, Journal of Experimental Marine Biology and Ecology, 303, 221-242, doi:10.1016/j.jembe.2003.11.017, 2004.

Barnes, D., and Taylor, D.: In situ studies of calcification and photosynthetic carbon fixation in the coral *Montastrea annularis*, Helgoländer wissenschaftliche Meeresuntersuchungen, 24, 284, 1973.

Beckvar, N.: Cultivation, spawning, and growth of the giant clams *Tridacna gigas*, *T. derasa*, and *T. squamosa* in Palau, Caroline Islands, Aquaculture, 24, 21-30, doi:10.1016/0044-8486(81)90040-5, 1981.

Belda-Baillie, C., Leggat, W., and Yellowlees, D.: Growth and metabolic responses of the giant clam-zooxanthellae symbiosis in a reef-fertilisation experiment, Marine Ecology Progress Series, 131-141, doi:10.3354/meps170131, 1998.

Bell, J., Lane, I., Gervis, M., Soule, S., and Tafea, H.: Village‐based farming of the giant clam, *Tridacna gigas* (L.), for the aquarium market: initial trials in Solomon Islands, Aquaculture Research, 28, 121-128, doi:10.1046/j.1365-2109.1997.t01-1-00834.x, 1997.

Berkelmans, R., and Van Oppen, M. J.: The role of zooxanthellae in the thermal tolerance of corals: a 'nugget of hope'for coral reefs in an era of climate change, Proceedings of the Royal Society of London B: Biological Sciences, 273, 2305-2312, 2006.

Bertucci, A., Foret, S., Ball, E., and Miller, D. J.: Transcriptomic differences between day and night in *Acropora millepora* provide new insights into metabolite exchange and light‐enhanced calcification in corals, Molecular ecology, 24, 4489-4504, doi:10.1111/mec.13328, 2015.

Berumen, M. L., Hoey, A., Bass, W. H., Bouwmeester, J., Catania, D., Cochran, J. E., Khalil, M. T., Miyake, S., Mughal, M., and Spät, J. L.: The status of coral reef ecology research in the Red Sea, Coral Reefs, 32, 737-748, doi:10.1007/s00338-013-1055-8, 2013.

Black, R., Johnson, M. S., Prince, J., Brearley, A., and Bond, T.: Evidence of large, local variations in recruitment and mortality in the small giant clam, *Tridacna maxima*, at Ningaloo Marine Park, Western Australia, Marine and Freshwater Research, 62, 1318-1326, doi:10.1071/MF11093, 2011.

Bodoy, A.: Assessment of human impact on giant clams, *Tridacna maxima*, near Jeddah, Saudi Arabia, Symposium on coral reefs environment of the Red Sea, 1984,

Chaidez, V., Dreano, D., Agusti, S., Duarte, C. M., and Hoteit, I.: Decadal trends in Red Sea maximum surface temperature, Scientific Reports, 7, 8144, 2017.

Chambers, C. N.: Pasua (*Tridacna maxima*) size and abundance in Tongareva Lagoon, Cook Islands, SPC Trochus Information Bulletin, 13, 7-12, 2007.

Chew, S. F., Koh, C. Z., Hiong, K. C., Choo, C. Y., Wong, W. P., Neo, M. L., and Ip, Y. K.: Light-enhanced expression of Carbonic Anhydrase 4-like supports shell formation in the fluted giant clam *Tridacna squamosa*, Gene, 683, 101-112, doi: 10.1016/j.gene.2018.10.023, 2019.

Cohen, I., Dubinsky, Z., and Erez, J.: Light Enhanced Calcification in Hermatypic Corals: New Insights from Light Spectral Responses, Frontiers in Marine Science, 2, 10.3389/fmars.2015.00122, 2016.

De Grave, S.: Pontoniinae (Crustacea: Decapoda: Palaemonidea) associated with bivalve molluscs from Hansa Bay, Papua New Guinea, Bulletin van het Koninklijk Belgisch Instituut voor Natuurwetenschappen. Biologie= Bulletin de l'Institut Royal des Sciences Naturelles de Belgique. Biologie, 1999.

DeBoer, T. S., Baker, A. C., Erdmann, M. V., Jones, P. R., and Barber, P. H.: Patterns of *Symbiodinium* distribution in three giant clam species across the biodiverse Bird's Head region of Indonesia, Marine Ecology Progress Series, 444, 117-132, 2012.

Dickson, A.: The oceanic carbon dioxide system: planning for quality data, US JGOFS News, 2, 2, 1990.

Dickson, A. G., and Goyet, C.: Handbook of methods for the analysis of the various parameters of the carbon dioxide system in sea water, ORNL/CDIAC-74, 107, 1994.

Dickson, A. G., Sabine, C. L., and Christian, J. R.: Guide to best practices for ocean CO2 measurements, 2011.

Dishon, G., Dubinsky, Z., Fine, M., and Iluz, D.: Underwater light field patterns in subtropical coastal waters: A case study from the Gulf of Eilat (Aqaba), Israel Journal of Plant Sciences, 60, 265-275, doi:10.1560/ijps.60.1-2.265, 2012.

Fisher, C. R., Fitt, W. K., and Trench, R. K.: Photosynthesis and respiration in Tridacna gigas as a function of irradiance and size, The Biological Bulletin, 169, 230-245, doi:10.2307/1541400, 1985.

Fitt, W., Rees, T., Braley, R., Lucas, J., and Yellowlees, D.: Nitrogen flux in giant clams: size-dependency and relationship to zooxanthellae density and clam biomass in the uptake of dissolved inorganic nitrogen, Marine Biology, 117, 381-386, doi:10.1007/BF00349313, 1993.

Foyle, T. P., Bell, J. D., Gervis, M., and Lane, I.: Survival and growth of juvenile fluted giant clams, *Tridacna squamosa*, in large-scale grow-out trials in the Solomon Islands, Aquaculture, 148, 85-104, 1997.
Gomez, E. D., and Mingoa-Licuanan, S. S.: Achievements and lessons learned in restocking giant clams in the Philippines, Fisheries Research, 80, 46-52, doi:10.1016/j.fishres.2006.03.017, 2006.
Goreau, T. F.: Calcium carbonate deposition by coralline algae and corals in relation to their roles as reef‐builders, Annals of the New York Academy of Sciences, 109, 127-167, doi:10.1111/j.1749-6632.1963.tb13465.x, 1963.
Griffiths, C., and Klumpp, D.: Relationships between size, mantle area and zooxanthellae numbers in five species of giant clam (Tridacnidae), Marine Ecology Progress Series, 139-147, doi:10.3354/meps137139, 1996.
Hart, A. M., Bell, J. D., and Foyle, T. P.: Growth and survival of the giant clams, *Tridacna derasa*, *T. maxima* and *T. crocea*, at village farms in the Solomon Islands, Aquaculture, 165, 203-220, doi:10.1016/S0044-8486(98)00255-5, 1998.
Hastie, L., Watson, T., Isamu, T., and Heslinga, G.: Effect of nutrient enrichment on *Tridacna derasa* seed: dissolved inorganic nitrogen increases growth rate, Aquaculture, 106, 41-49, doi:10.1016/0044-8486(92)90248-J, 1992.
Hiong, K. C., Cao‐Pham, A. H., Choo, C. Y., Boo, M. V., Wong, W. P., Chew, S. F., and Ip, Y. K.: Light‐dependent expression of a Na^+/H^+ exchanger 3‐like transporter in the ctenidium of the giant clam, *Tridacna squamosa*, can be related to increased H^+ excretion during light‐enhanced calcification, Physiological reports, 5, e13209, doi:10.14814/phy2.13209, 2017a.
Hiong, K. C., Choo, C. Y., Boo, M. V., Ching, B., Wong, W. P., Chew, S. F., and Ip, Y. K.: A light-dependent ammonia-assimilating mechanism in the ctenidia of a giant clam, Coral Reefs, 36, 311-323, doi:10.1007/s00338-016-1502-4, 2017b.
Hoegh-Guldberg, O.: Effect of nutrient enrichment in the field on the biomass, growth and calcification of the giant clam *Tridacna maxima*, Marine Biology, 129, 635-642, doi:10.1007/s002270050206, 1997.
Hughes, T. P., Baird, A. H., Bellwood, D. R., Card, M., Connolly, S. R., Folke, C., Grosberg, R., Hoegh-Guldberg, O., Jackson, J., and Kleypas, J.: Climate change, human impacts, and the resilience of coral reefs, science, 301, 929-933, 2003.
Hviding, E.: The rural context of giant clam mariculture in Solomon Islands: an anthropological study, WorldFish, 1993.
Ikeda, S., Yamashita, H., Kondo, S.-n., Inoue, K., Morishima, S.-y., and Koike, K.: Zooxanthellal genetic varieties in giant clams are partially determined by species-intrinsic and growth-related characteristics, PloS one, 12, e0172285, 2017.
Ip, Y. K., Ching, B., Hiong, K. C., Choo, C. Y., Boo, M. V., Wong, W. P., and Chew, S. F.: Light induces changes in activities of Na^+/K^+-ATPase, H^+/K^+-ATPase and glutamine synthetase in tissues involved directly or indirectly in light-enhanced calcification in the giant clam, *Tridacna squamosa*, Frontiers in physiology, 6, doi:10.3389/fphys.2015.00068, 2015.
Ip, Y. K., Hiong, K. C., Goh, E. J. K., Boo, M. V., Choo, C. Y. L., Ching, B., Wong, W. P., and Chew, S. F.: The Whitish Inner Mantle of the Giant Clam, *Tridacna squamosa*, Expresses an Apical Plasma Membrane Ca^{2+}-ATPase (PMCA) Which Displays Light-Dependent Gene and Protein Expressions, Frontiers in Physiology, 8, doi:10.3389/fphys.2017.00781, 2017.
IUCN: Red List of Threatened Species, www.iucnredlist.org Version 2016-3, 2016.
Jameson, S. C.: Early life history of the giant clams *Tridacna crocea* Lamarck, *Tridacna maxima* (Roding), and *Hippopus hippopus* (Linnaeus), 1976.
Jantzen, C., Wild, C., El-Zibdah, M., Roa-Quiaoit, H. A., Haacke, C., and Richter, C.: Photosynthetic performance of giant clams, *Tridacna maxima* and *T. squamosa*, Red Sea, Marine Biology, 155, 211-221, doi:10.1007/s00227-008-1019-7, 2008.
Khalil, M. T., Cochran, J. E., and Berumen, M. L.: The abundance of herbivorous fish on an inshore Red Sea reef following a mass coral bleaching event, Environmental biology of fishes, 96, 1065-1072, doi:10.1007/s10641-012-0103-5, 2013.
Klumpp, D., Bayne, B., and Hawkins, A.: Nutrition of the giant clam *Tridacna gigas* (L.) I. Contribution of filter feeding and photosynthates to respiration and growth, Journal of Experimental Marine Biology and Ecology, 155, 105-122, doi:10.1016/0022-0981(92)90030-E, 1992.
Klumpp, D., and Griffiths, C.: Contributions of phototrophic and heterotrophic nutrition to the metabolic and growth requirements of four species of giant clam (Tridacnidae), Marine Ecology Progress Series, 103-115, 1994.
Kuffner, I.: Effects of ultraviolet radiation and water motion on the reef coral *Porites compressa* Dana: a flume experiment, Marine Biology, 138, 467-476, 2001.
LaJeunesse, T. C., Parkinson, J. E., Gabrielson, P. W., Jeong, H. J., Reimer, J. D., Voolstra, C. R., and Santos, S. R.: Systematic Revision of Symbiodiniaceae Highlights the Antiquity and Diversity of Coral Endosymbionts, Current Biology, 10.1016/j.cub.2018.07.008, 2018.
Lavigne, H., and Gattuso, J.: Package 'seacarb': seawater carbonate chemistry with R, v. 2.4. 8 (ed. R Development Core Team). See http.cran.r-project.org/web/packages/seacarb/index.html., 2013.
Lucas, J., Nash, W., Crawford, C., and Braley, R.: Environmental influences on growth and survival during the ocean-nursery rearing of giant clams, *Tridacna gigas* (L.), Aquaculture, 80, 45-61, doi:10.1016/0044-8486(89)90272-X, 1989.
Lucas, J. S.: The biology, exploitation, and mariculture of giant clams (Tridacnidae), Reviews in Fisheries science, 2, 181-223, doi:10.1080/10641269409388557, 1994.
Manu, N., and Sone, S.: Breeding season of the Tongan shellfish. 3. Elongated giant clam, *Tridacna maxima*, Fish Res Bull Tonga, 3, 25-33, 1995.
McConnaughey, T., and Whelan, J.: Calcification generates protons for nutrient and bicarbonate uptake, Earth-Science Reviews, 42, 95-117, doi:10.1016/S0012-8252(96)00036-0, 1997.
Militz, T. A., Kinch, J., and Southgate, P. C.: Population demographics of Tridacna noae (Röding, 1798) in New Ireland, Papua New Guinea, Journal of Shellfish Research, 34, 329-336, 2015.
Millero, F. J.: Carbonate constants for estuarine waters, Marine and Freshwater Research, 61, 139-142, doi:10.1071/MF09254, 2010.
Moya, A., Tambutté, S., Bertucci, A., Tambutté, E., Lotto, S., Vullo, D., Supuran, C. T., Allemand, D., and Zoccola, D.: Carbonic anhydrase in the scleractinian coral *Stylophora pistillata* characterization, localization, and role in biomineralization, Journal of Biological Chemistry, 283, 25475-25484, doi:10.1074/jbc.M804726200, 2008.
Munro, J., and Heslinga, G.: Prospects for the commercial cultivation of Giant Clams, 1983.
Neo, M. L., Eckman, W., Vicentuan, K., Teo, S. L.-M., and Todd, P. A.: The ecological significance of giant clams in coral reef ecosystems, Biological Conservation, 181, 111-123, doi:10.1016/j.biocon.2014.11.004, 2015.

Neo, M. L., Wabnitz, C. C., Braley, R. D., Heslinga, G. A., Fauvelot, C., Van Wynsberge, S., Andrefouet, S., Waters, C., Tan, A. S.-H., and Gomez, E. D.: Giant clams (Bivalvia: Cardiidae: Tridacninae): a comprehensive update of species and their distribution, current threats and conservation status, Oceanography and Marine Biology: An Annual Review, 55, 87-388, 2017.
Pappas, M. K., He, S., Hardenstine, R. S., Kanee, H., and Berumen, M. L.: Genetic diversity of giant clams (Tridacna spp.) and their associated Symbiodinium in the central Red Sea, Marine Biodiversity, 1-14, 2017.
Richter, C., Roa-Quiaoit, H., Jantzen, C., Al-Zibdah, M., and Kochzius, M.: Collapse of a new living species of giant clam in the Red Sea, Current Biology, 18, 1349-1354, doi:10.1016/j.cub.2008.07.060, 2008.
Roa-Quiaoit, H. A. F.: The ecology and culture of giant clams (Tridacnidae) in the Jordanian sector of the Gulf of Aqaba, Red Sea, PhD, Universitat Bremen, 2005.
Romanek, C. S., and Grossman, E. L.: Stable isotope profiles of *Tridacna maxima* as environmental indicators, Palaios, 402-413, doi:10.2307/3514585 1989.
Rosewater, J.: The family Tridacnidae in the Indo-Pacific, Department of Mollusks, Academy of Natural Sciences of Philadelphia, 1965.
Rowan, R., Knowlton, N., Baker, A., and Jara, J.: Landscape ecology of algal symbionts creates variation in episodes of coral bleaching, Nature, 388, 265, 1997.
Sano, Y., Kobayashi, S., Shirai, K., Takahata, N., Matsumoto, K., Watanabe, T., Sowa, K., and Iwai, K.: Past daily light cycle recorded in the strontium/calcium ratios of giant clam shells, Nature Communications, 3, 761, doi:10.1038/ncomms1763, 2012.
Schwartzmann, C., Durrieu, G., Sow, M., Ciret, P., Lazareth, C. E., and Massabuau, J.-C.: *In situ* giant clam growth rate behavior in relation to temperature: A one‐year coupled study of high‐frequency noninvasive valvometry and sclerochronology, Limnology and oceanography, 56, 1940-1951, doi:10.4319/lo.2011.56.5.1940, 2011.
Shick, J., Lesser, M., Dunlap, W., Stochaj, W., Chalker, B., and Won, J. W.: Depth-dependent responses to solar ultraviolet radiation and oxidative stress in the zooxanthellate coral Acropora microphthalma, Marine Biology, 122, 41-51, 1995.
Simkiss, K.: Phosphates as crystal poisons of calcification, Biological Reviews, 39, 487-504, doi:10.1111/j.1469-185X.1964.tb01166.x, 1964.
Smith, S., and Key, G.: Carbon dioxide and metabolism in marine environments, Limnology and Oceanography, 20, 493-495, doi:10.4319/lo.1975.20.3.0493, 1975.
Taylor, D. L.: Identity of zooxanthellae isolated from some Pacific Tridacnidae, Journal of phycology, 5, 336-340, doi:10.1111/j.1529-8817.1969.tb02623.x, 1969.
Trench, R., Wethey, D., and Porter, J.: Observations on the symbiosis with zooxanthellae among the Tridacnidae (Mollusca, Bivalvia), The Biological Bulletin, 161, 180-198, doi:10.2307/1541117, 1981.
Van Wynsberge, S., Andréfouët, S., Gaertner-Mazouni, N., Wabnitz, C. C. C., Gilbert, A., Remoissenet, G., Payri, C., and Fauvelot, C.: Drivers of density for the exploited giant clam *Tridacna maxima* : a meta-analysis, Fish and Fisheries, 17, 567-584, doi:10.1111/faf.12127, 2016.
Vicentuan-Cabaitan, K., Neo, M. L., Eckman, W., Teo, S. L., and Todd, P. A.: Giant clam shells host a multitude of epibionts, Bulletin of Marine Science, 90, doi:10.5343/bms.2014.1010, 2014.
Warter, V., Erez, J., and Müller, W.: Environmental and physiological controls on daily trace element incorporation in *Tridacna crocea* from combined laboratory culturing and ultra-high resolution LA-ICP-MS analysis, Palaeogeography, Palaeoclimatology, Palaeoecology, 496, 32-47, doi:10.1016/j.palaeo.2017.12.038, 2018.
Watson, S.-A., Southgate, P. C., Miller, G. M., Moorhead, J. A., and Knauer, J.: Ocean acidification and warming reduce juvenile survival of the fluted giant clam, *Tridacna squamosa*, Molluscan Research, 32, 177-180, 2012.
Yau, A. J.-Y., and Fan, T.-Y.: Size-dependent photosynthetic performance in the giant clam *Tridacna maxima*, a mixotrophic marine bivalve, Marine biology, 159, 65-75, doi:10.1007/s00227-011-1790-8, 2012.
Yonge, C.: Giant clams, Scientific American, 232, 96-105, 1975.
Yonge, C. M.: Mode of life, feeding, digesting and symbiosis with zooxanthellae, Scientific Reports / Great Barrier Reef Expedition, 1, 283-321, 1936.
Zeebe, R. E., and Wolf-Gladrow, D. A.: CO_2 in seawater: equilibrium, kinetics, isotopes, Elsevier Oceanography Series, 65, edited by: Halpern, D., Gulf Professional Publishing, Amsterdam, The Netherlands, 2001.
Zuschin, M., Hohenegger, J., and Steininger, F. F.: A comparison of living and dead molluscs on coral reef associated hard substrata in the northern Red Sea—implications for the fossil record, Palaeogeography, Palaeoclimatology, Palaeoecology, 159, 167-190, doi.org/10.1016/S0031-0182(00)00045-6, 2000.

Tables

Table 1: Net calcification [µmol $CaCO_3$ cm^{-2} h^{-1}; ± SE] and gross primary production [µmol O_2 cm^{-2} h^{-1}; ± SE] under the seven experimental incident light level [µmol quanta cm^{-2} h^{-1}] and during the dark.

Incident light [µmol quanta cm^{-2} h^{-1}]	**Net calcification** [µmol $CaCO_3$ cm^{-2} h^{-1}]	**Gross primary production** [µmol O_2 cm^{-2} h^{-1}]
0	0.18 ± 0.02	-
197	0.43 ± 0.04	N/A
244	0.51 ± 0.04	N/A
358	0.60 ± 0.04	N/A
530	0.66 ± 0.05	N/A
561	0.65 ± 0.04	2.06 ± 0.24
959	0.25 ± 0.04	1.76 ± 0.28
1061	0.01 ± 0.01	0.87 ± 0.37

Table 2: Description of the statistical model parameters (Fig.5) combining the influence of irradiance (E, µmol m^{-2} s^{-1}) and clam dry-mass (DM; g) on calcification (G; µmol $CaCO_3$ ind^{-1} h^{-1}), where d is the fitted intercept:

$G = a \cdot E + b \cdot DM^2 + c \cdot DM + d$						
	Estimate	**SE**	**t – value** df = 46	**p - value**	**Lower confidence limit** [95% CI]	**Upper confidence limit** [95% CI]
a	0.126	0.011	11.420	<0.0001	0.104	0.148
b	-0.298	0.070	-4.242	<0.0001	-0.439	-0.156
c	9.115	2.006	4.545	<0.0001	5.079	13.152
d	-29.085	11.955	-2.433	0.019	-53.148	-5.022

R^2: 0.77

Table 3: Comparison of net calcification rates in relation to light conditions in different marine phototrophic and mixotrophic calcifiers. Values are given as average value mean (± SE) or [a] (± SD). Experimental light incubation levels are given in µmol photons m^{-2} s^{-1}. Net calcification values were converted to µmol $CaCO_3$ cm^{-2} h^{-1} from: [b] mg $CaCO_3$ cm^{-2} d^{-1}.

Organism	Species	Nutrition	Region	Light incubation [µmol photons m^{-2} s^{-1}]	Net calcification [µmol $CaCO_3$ cm^{-2} h^{-1}]	Method	Study
Coral	*Acropora variabilis*	mixotroph	Northern Red Sea	800	0.1 [a]	TA anomaly method	**Cohen et al. 2016**
Coral	*Porites lutea*	mixotroph	Northern Red Sea	800	0.28 [a]	TA anomaly method	**Cohen et al. 2016**
Coral	*Porites spp.*	mixotroph	Japan	700	0.79 [b]	Buoyant weighing	**Comeau et al. 2014b**
Coral	*Pocillophora damicornis*	mixotroph	Japan	700	0.49 [b]	Buoyant weighing	**Comeau et al. 2014b**
Coral	*Porites compressa*	mixotroph	Hawaii	698	0.81 ± 0.02 [b]	Buoyant weighing	**Marubini et al. 2001**
Coral	*Acropora pulchra*	mixotroph	French Polynesia	640 ± 30	0.42 ± 0.02 [b]	Buoyant weighing	**Comeau et al. 2014a**
Coral	*Madracis auretenra*	mixotroph	Caribbean	200	0.36 ± 0.4 [a]	TA anomaly method	**Jury et al. 2010**
Coral	*Porites compressa*	mixotroph	Hawaii	150	0.43 ± 0.03 [b]	Buoyant weighing	**Marubini et al. 2001**
Coral	*Acropora pulchra*	mixotroph	French Polynesia	149.2 ± 0.1	0.11 ± 0.01 [b]	Buoyant weighing	**Comeau et al. 2014a**
Average Net calcification corals					**0.42 ± 0.08**		
Algae	*Porilithion onkodes*	phototroph	Hawaii	700	0.80 [ab]	Buoyant weighing	**Comeau et al. 2014b**
Algae	*Hydrolithion reinboldii*	phototroph	French Polynesia	640 ± 30	0.05 ± 0.00 [b]	Buoyant weighing	**Comeau et al. 2014b**
Average Net calcification calcifying algae				0.25 ± 0.11	**0.43 ± 0.38**		
Mollusk	*Tridacna maxima*	mixotroph	Central Red Sea	1061	0.01 ± 0.01	TA anomaly method	**This study**
Mollusk	*Tridacna maxima*	mixotroph	Central Red Sea	959	0.25 ± 0.04	TA anomaly method	**This study**
Mollusk	*Tridacna maxima*	mixotroph	Central Red Sea	530 – 561	0.65 ± 0.03	TA anomaly method	**This study**
Mollusk	*Tridacna maxima*	mixotroph	Central Red Sea	358	0.60 ± 0.04	TA anomaly method	**This study**
Mollusk	*Tridacna maxima*	mixotroph	Central Red Sea	244	0.51 ± 0.04	TA anomaly method	**This study**
Mollusk	*Tridacna maxima*	mixotroph	Central Red Sea	197	0.43 ± 0.02	TA anomaly method	**This study**
Average Net calcification *T. maxima*					**0.47 ± 0.03**		

Table 4: Comparison of net calcification rates of different marine, heterotrophic calcifiers. Values are given as average value mean (± SE) or [a] (± SD). All net calcification values were normalized for gram dry-mass (DM), rates given in fresh-weight were converted to DM [b] after Ricciardi and Bourget (1998) or [c] Dame (1972). Net calcification values were converted to µmol $CaCO_3$ g DM^{-1} h^{-1} from d µg $CaCO_3$ g DM^{-1} d^{-1}.

Organism	Species	Nutrition	Region	Net calcification [µmol $CaCO_3$ g DM^{-1} h^{-1}]	Method	Study
Coral	*Lophelia pertusa*	heterotroph	North Atlantic	1.5 [a]	TA anomaly method	**Hennige et al. 2014**
Coral	*Madrepora oculata*	heterotroph	Mediterranean Sea	0.091 ± 0.027	TA anomaly method	**Maier et al. 2016**
Averaged Net calcification for heterotrophic corals				**0.80 ± 0.70**		
Mollusk	*Mytilus edulis*	heterotroph	North Sea	0.0244 [ab]	TA anomaly method	**Gazeau et al. 2007**
Mollusk	*Crassostrea gigas*	heterotroph	North Sea	0.219 [ab]	TA anomaly method	**Gazeau et al. 2007**
Mollusk	*Argopecten purpuratus*	heterotroph	Southern Pacific	0.004 ± 0.001 [d]	Buoyant weighing	**Ramajo et al. 2016**
Averaged Net calcification for heterotrophic mollusks				**0.08 ± 0.07**		
Mollusk	*Tridacna maxima*	mixotroph	Central Red Sea	5.38 ± 0.42	TA anomaly method	**This study**
Averaged Net calcification in *T. maxima*				**5.38 ± 0.42**		

Figures

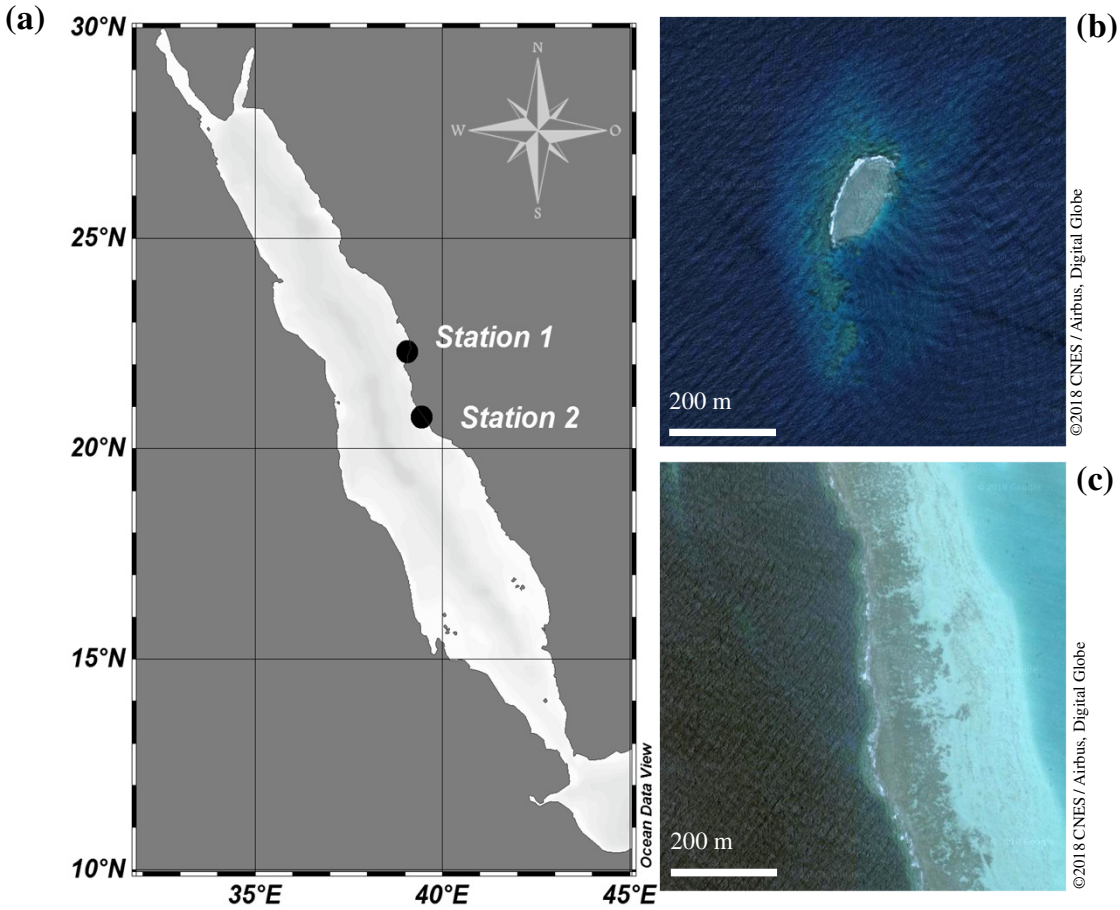

Figure 1: (a) Map of the Red Sea. Abundance surveys and sampling of clams for incubation experiments where conducted at both, a sheltered reef (Station 1; 22.303833 N, 39.048278 E) and an exposed reef (Station 2; 20.753764 N, 39.442561 E), (b) satellite image of sheltered reef (Station 1), (c) satellite image of exposed, fringing reef (Station 2).

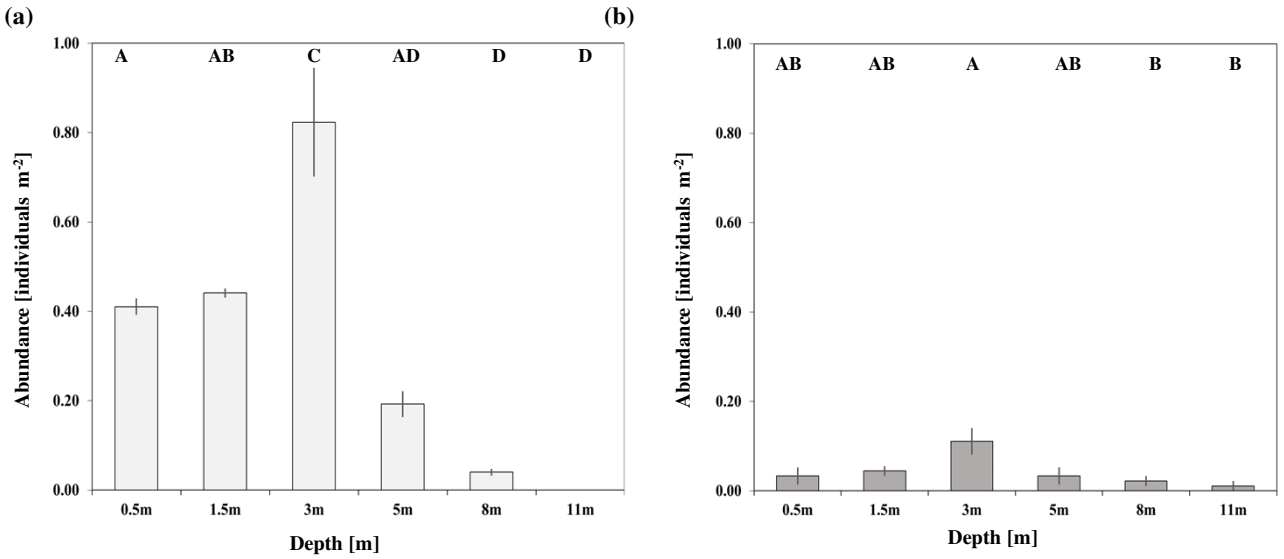

Figure 2: Changes in the abundance [individuals $m^{-2} \pm$ SD] of *T. maxima* with depth at a sheltered reef (a) and an exposed reef (b) in the central Red Sea. Different capital letters describe statistically significant differences in abundance between survey depths.

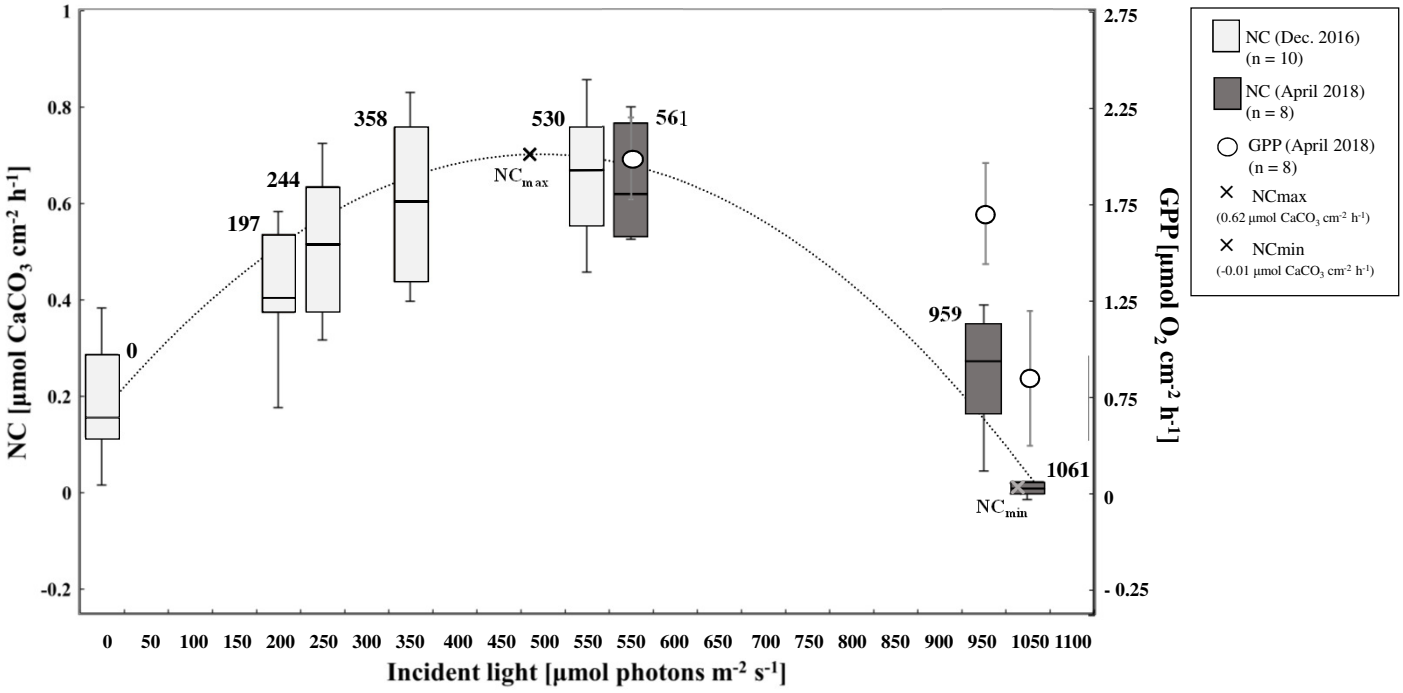

Figure 3: Box plots showing net calcification rates [µmol $CaCO_3$ cm^{-2} h^{-1}] of *T. maxima* under seven different light regimes (197, 244, 358, 530, 561, 959 and 1061 µmol quanta m^{-2} s^{-1}) (n = 10 in December 2016 and n = 8 in April 2018) and in the dark, as well as gross primary production [µmol O_2 cm^{-2} h^{-1}] (n = 8) as dots (±SE), under three high light regimes (561, 959 and 1061 µmol quanta m^{-2} s^{-1}). Calculated maximum net calcification (NC_{max}) at 475 µmol quanta m^{-2} s^{-1} and incident light level where dissolution outweighs calcification processes (NC_{min}) are symbolized by a cross ×. Net calcification rates obtained during incubations under moderate light conditions are symbolised by light grey boxplots, those from the high light incubations by dark grey boxplots, the central line represents the median, the boxes encompass the central 50% of the data and the lines extend to the 95% quartiles.

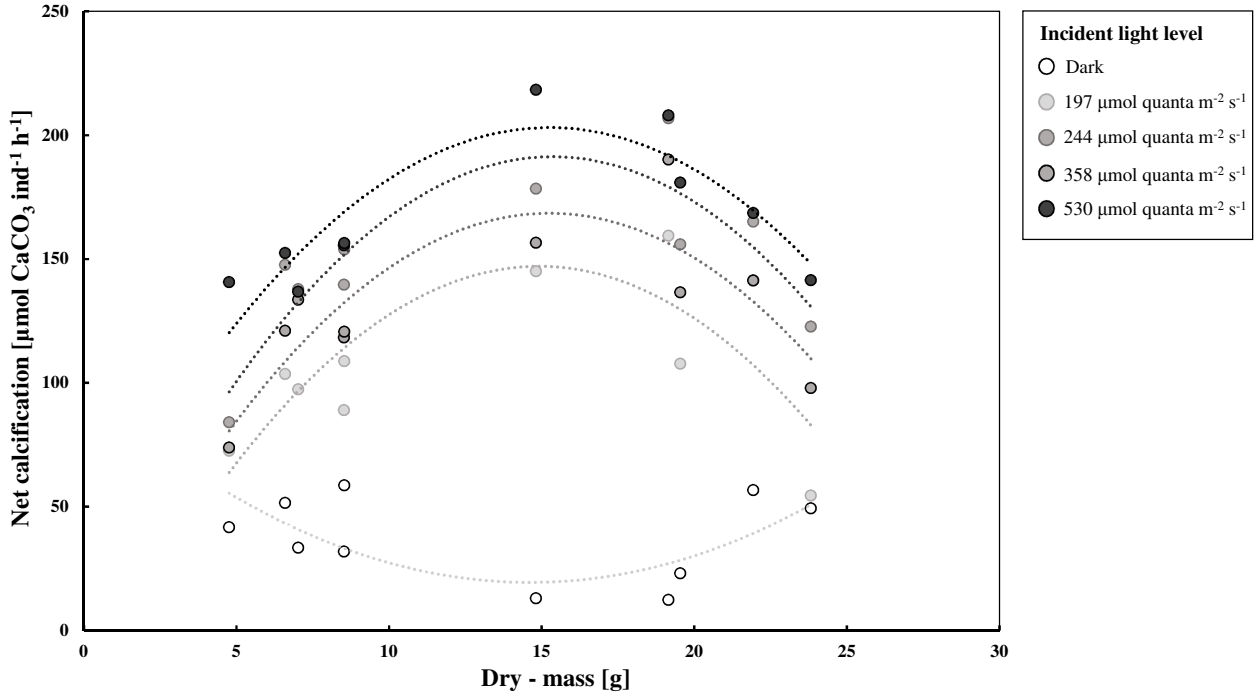

Figure 4: Net calcification (µmol $CaCO_3$ ind^{-1} h^{-1}) (n = 10) in *T. maxima* at four different incident light levels (197, 244, 358 and 531 µmol photons cm^{-2} h^{-1}) and in the dark, plotted against tissue dry-mass (g). Data are shown with polynomial trendlines.

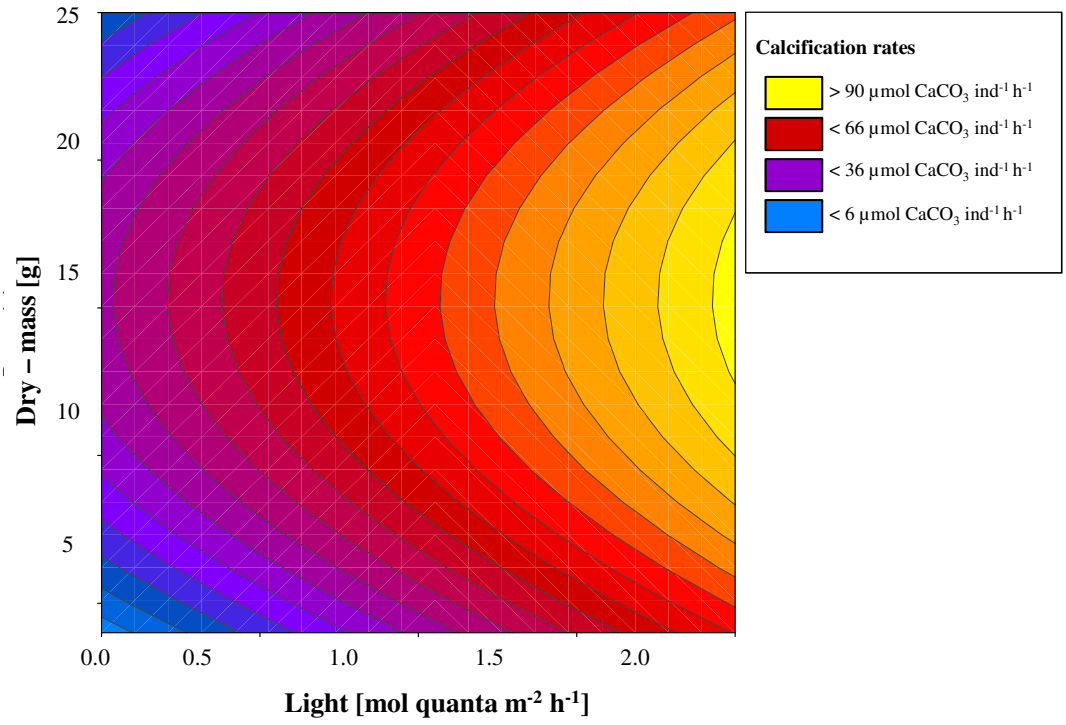

Figure 5: Model considering a linear relationship between light and calcification and a first order polynomial relationship between dry-mass (DM) and net calcification (NC) explaining 77 % of the variance.