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### Zooxanthellae Densities within the Fluted Giant Clam *Tridacna squamosa* (Lamarck, 1819) from the Southern Coast of Egypt's Red Sea

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### ABSTRACT

Giant clams are large bivalves predominantly found in tropical and subtropical seas. They are the largest bivalves with the fastest growth rates, thanks to their symbiotic relationship with phototrophic dinoflagellates (zooxanthellae). This study examined the dynamics of the symbiotic relationship between Tridacnid hosts and their dinoflagellate symbionts, as well as the environmental factors influencing this association. Zooxanthellae were extracted from the mantles of Tridacna squamosa samples monthly collected from January to December 2021 in the southern Red Sea. The mean density of zooxanthellae was calculated as the overall mean count of zooxanthellae per gram of wet weight of the T. squamosa mantle, expressed as  $\pm$  SD. This density was then analyzed alongside various environmental conditions and biological characteristics of the studied clams to assess the factors influencing the density of the symbiotic zooxanthellae within T. squamosa. The study revealed that the densities of zooxanthellae ranged from 3.21 x 10<sup>5</sup> to 48.19 x 10<sup>5</sup> cells per gram of mantle, observed in July and December, respectively. Maximum density corresponded with the shortest exposure to sunlight, measured at 10 hours and 42 minutes during winter, with a mean temperature of 24.8°C. Conversely, the lowest density was associated with the longest light exposure period, recorded at 13 hours and 39 minutes in summer. These results underscore the complex nature of the symbiotic relationship between zooxanthellae and T. squamosa in the Red Sea. The interaction between environmental variables and host characteristics shapes zooxanthellae densities within these giant clams across reef ecosystems. This highlights the need for interdisciplinary research collaborations and holistic conservation efforts to maintain the delicate balance between giant clams and their symbiotic zooxanthellae in the face of ongoing environmental and anthropogenic stressors.

### **INTRODUCTION**

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The giant clams, subfamily Tridacninae, represent a remarkable group of largesized Bivalvia predominantly found in tropical and subtropical seas (**Neo** *et al.*, **2015**). Despite thriving in oligotrophic environments with limited planktonic food sources, these clams are renowned as the largest bivalves and are recognized for their rapid growth rates

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(**Bonham, 1965**). The remarkable survival and growth of these bivalves in challenging environments are attributed to their distinctive symbiotic association with phototrophic dinoflagellates, commonly referred to as zooxanthellae, belonging to the Symbiodiniaceae family (**Fitt** *et al.*, **1986**).

Recent studies have shed light on the intricate mechanisms of this symbiosis. For instance, **Ip and Chew (2021)** highlighted the molecular basis of the interaction between giant clams and their symbiotic zooxanthellae, elucidating key gene expression patterns responsible for nutrient exchange. **Abdelmeneam** *et al.* (2018) studied *T. squamosa* in the Egyptian Red Sea, and they stated that temperature and the period of exposure to sun light impact their reproductive recruitment success and hence their abundances. Furthermore, **Zhou** *et al.* (2019) conducted a comprehensive analysis of environmental stressors effects on these symbiotic relationships, emphasizing the crucial role of temperature fluctuations in altering the densities and diversity of zooxanthellae within giant clam tissues.

**Brown** *et al.* (2022) provided insights into the impact of climate change-induced temperature variations on the resilience of this symbiotic partnership. They demonstrated a correlation between rising sea temperatures and increased instances of bleaching, posing a significant threat to giant clam populations. Moreover, studies by **Pappas (2021)** suggested a potential adaptation mechanism within Tridacninae, indicating genetic variability in symbiont communities across different geographical regions. This variability might confer resilience to changing environmental conditions. Any disruption in this relationship could render the clams more susceptible to stress (**Blidberg** *et al.*, **2002**). Reports of significant mass mortalities in giant clam populations due to zooxanthellae loss, termed bleaching, underscore the vulnerability of these organisms (**Leggat** *et al.*, **2003**).

Given the increasing evidence of climate change impacting marine ecosystems, including the Red Sea (**Raitsos** *et al.*, **2013**), understanding the dynamics of the symbiotic relationship between Tridacnid hosts and their symbiont Dinoflagellates becomes imperative. This article aimed to delve into the recent findings surrounding the symbiotic zooxanthellae residing within giant clams, emphasizing the influence of contemporary environmental factors, particularly temperature, on the densities of zooxanthellae within their host giant clams.

#### MATERIALS AND METHODS

During the present study, samples of *T. squamosa* were collected to evaluate the densities of zooxanthellae communities inhabiting their mantles.

## 1. Study area

Samples were collected from the southern Egyptian coast of the Red Sea, Marsa Egla (Fig. 1). The collection site was chosen since it has a healthy reef with high

biodiversity and high density of *T. squamosa* population to minimize the effects of sampling process, and ensure that the sample size would not impose anthropogenic stress or would threaten the local population of this species.



Fig. 1. Map showing the collection site

### 2. Data collection

A total of 120 samples of *T. squamosa* were collected over a course of one year, with 10 samples systemically collected each month between January and December 2021 from an area of  $\sim$ 2km on the reef edge of Marsa Egla.

Samples were cleaned from any external biofouling materials. External body measurements including the net weight, and the shell measurements (length, width, and weight) were measured. Samples collected during the study ranged from 9 to 23cm in length; they were categorized into eight size classes with 2cm interval according to the total length of their shells. Then samples were dissected, and measurements of the soft flesh tissues were taken; these measurements included mantle weights. Mantles were separated and weighed; zooxanthellae cells were later extracted from the mantle of each

sample by grinding it using a homogenizer, and then each was sieved through multiple layers of cloth; the big particles were removed, and the solution was then centrifuged at 4000RPM for five minutes to separate the zooxanthellae following **Belda-Baillie** *et al.* (1999). Supernatant was removed, and the centrifuged pellets were then dissolved within a given volume of distilled water. Three sub samples were taken in which zooxanthellae were counted; eventually, the total number of zooxanthellae cells were calculated and converted into the algal count per 1 gram of mantle. Monthly data for day length and temperature were gathered, which were later analyzed along with the count data of zooxanthellae to assess the impact of environmental factors on the density of the symbiont zooxanthellae within their *T. squamosa* host.

Monthly average temperature data for Marsa Egla were downloaded from CMEMS catalogue (Copernicus Marine Data Store with a 1/12-degree horizontal resolution and regular longitude/latitude equirectangular projection). Data for day length in Marsa Egla during the period of study were collected from the Solar Topo website (Solar Topo, n.d.).

## 3. Data processing

Data were visualized and processed using Microsoft Excel. Analysis of variance (One-way ANNOVA) was performed to assess statistical significance of differences among various studied variables; zooxanthellae density, temperature, and light exposure, both seasonally and monthly. Regression models were applied to the data using R-Studio software.

### RESULTS

## Seasonal variation in zooxanthellae counts

Seasonal variations in zooxanthellae are illustrated in Fig. (2), as counts per gram of *T. squamosa* mantle. Winter exhibited the highest density of zooxanthellae, estimated at 19.08  $\times 10^5$  zooxanthellae cells/gram of mantle. Conversely, summer showcased the lowest count, with an overall mean of  $3.49 \times 10^5$  zooxanthellae cells/gram of mantle. As autumn commenced, the density began rising after the summer lull, reaching an overall mean of  $8.15 \times 10^5$  zooxanthellae cells/gram of mantle. Unexpectedly, spring showed a decrease in density, averaging  $5.85 \times 10^5$  zooxanthellae cell/gram of mantle after the peak in winter. This suggests that temperature alone might not solely influence zooxanthellae density in *T. squamosa* mantles; rather, a combination of factors such as day length-period of exposure to sunlight- and temperature might jointly control their occurrence or density.



**Fig. 2.** Seasonal overall means of the densities of zooxanthellae estimated as count of zooxanthellae cells/gram wet weight of the *T. Squamosa* mantle

## Monthly variation in zooxanthellae counts

Monthly variations in zooxanthellae counts per gram of mantle weight over the study's duration were estimated (Fig. 3). Notably, lower-temperature months exhibited the highest symbiotic zooxanthellae counts, while higher-temperature months showed the lowest counts per gram of mantle. In December, the study recorded the highest count of zooxanthellae at  $48.19 \times 10^5$  zooxanthellae cells/gram of mantle. July showed the lowest count at  $3.21 \times 10^5$  zooxanthellae cells/gram of mantle. Months as a factor were found to significantly impact the variations within the overall densities of zooxanthellae (*P*-value,  $1.23e^{-13}$ ). To mitigate variations arising from size classes and months, monthly variations were computed.



## **Fig. 3.** Monthly overall means of the densities of zooxanthellae estimated as count of zooxanthellae cells/gram wet weight of the *T. Squamosa* mantle

## Variation in zooxanthellae counts relative to temperature

Fluctuation in zooxanthellae counts across various temperature measurements are evident in Fig. (4). Although there was a discernible trend indicating the direct impact of temperature on zooxanthellae counts, the highest densities of zooxanthellae were recorded at specific temperature degrees, notably at 23.3 and 24.8°C, registering at 2.29  $\times 10^6$  and 2.79  $\times 10^6$  zooxanthellae cells/gram of mantle, respectively. Conversely, the lowest densities were not observed at the highest temperatures as expected. Nevertheless, there was a significant relationship between overall mean temperature degrees and zooxanthellae densities (*P*-value, 0.01148).

Zooxanthellae Densities Within the Fluted Giant Clam *Tridacna squamosa* from the Southern Coast of Egypt's Red Sea



**Fig. 4.** Overall means of the densities of zooxanthellae estimated as count of cells/gram wet weight of the *T. squamosa* mantle, corresponding to the monthly variation in the overall means of temperature expressed as °C

The fact that the lowest densities of zooxanthellae were not attained in accordance with the highest temperature supports the notion that temperature alone may not be the sole controlling factor for zooxanthellae densities. Instead, it suggests a complex interplay between temperature and other factors, potentially involving period of exposure to sunlight, influencing the densities of zooxanthellae. To further validate this notion, day length data -the time between sun rise and sun set- were gathered. The impact of day length variations on the overall means of zooxanthellae are inversely affected by the period of exposure to light. The longer the day length, the lower the densities and vice versa. The highest density of zooxanthellae at  $2.79 \times 10^6$  zooxanthellae cells/gram of mantle was obtained at the shortest exposure to daylight at 10h and 42min. The lowest zooxanthellae density at  $3.21 \times 10^5$  zooxanthellae cells/gram of mantle was aligned with the longest light exposure period at 13h and 39min. This gives an indication of the huge influence of day length and the period of exposure to sunlight on the densities of zooxanthellae communities inhabiting *T. squamosa*.





A generalized linear regression model was applied to the data to assess the impact of the two independent variables temperature and day length on the variations within the densities of zooxanthellae; furthermore, the model evaluated the interactions between them. Both temperature and day length experienced a significant relationship with the overall means of zooxanthellae count (*P*-values:  $7.85e^{-10}$  and 0.000908, respectively). The model also suggested that there was a strong significant interaction between temperature and day length affecting the densities of zooxanthellae within the mantles of *T. squamosa* (P-value,  $3.76e^{-06}$ ).

## Densities of zooxanthellae at different clam weights (Zooxanthellae somatic index, ZSI)

The specimens examined during the study were categorized according to their total shell length into eight size classes. Density of zooxanthellae per gram of *T. squamosa mantle* across various size categories is displayed in Fig. (6). Initially, it appears that

#### Zooxanthellae Densities Within the Fluted Giant Clam *Tridacna squamosa* from the Southern Coast of Egypt's Red Sea

there might be a correlation between increased shell length and higher zooxanthellae density. However, upon statistical analysis, this apparent relationship was deemed statistically insignificant (*P*-value, 0.1). Despite the lack of statistical significance, the data reveal intriguing trends. Size class ranging from 21.1 to 23cm shell length exhibited the highest recorded density of zooxanthellae per gram of mantle, measuring at 2.29  $\times 10^6$  zooxanthellae cells/gram of mantle. In stark contrast, the lowest counts were observed in the smallest shell length intervals, notably between 7 and 9cm, registering at 3.77  $\times 10^5$  zooxanthellae density prompts further investigation into potential contributing factors that may influence this relationship, notwithstanding the apparent numerical trends observed across different size classes.



# **Fig. 6.** Overall means of the densities of zooxanthellae estimated as count of cells/gram wet flesh weight of the *T. squamosa* mantles

In the interest of investigating the relationship between the clams' wet flesh weight and their zooxanthellae hosting capacity, the densities of zooxanthellae per biomass were calculated by dividing the total number of zooxanthellae for each specimen by its total wet flesh weight. Fig. (7) illustrates the monthly variation within the number of zooxanthellae per biomass. Comparable to the monthly variation within the number of zooxanthellae per one gram of the wet mantle weight, the number of zooxanthellae per biomass revealed a similar pattern in responding to the seasonal cycle throughout the year. The highest densities of zooxanthellae per biomass were acquired during the winter months, while the lowest densities were acquired during months with higher temperatures. December recorded the highest density of zooxanthellae with a 9.38 x10<sup>5</sup> cell/biomass. While the lowest density of zooxanthellae was obtained in April at 9.88 x10<sup>4</sup> zooxanthellae cells/biomass. The monthly variations within the number of zooxanthellae per biomass was tested and proven to be statistically significant (*P*-value,  $1.23e^{-13}$ ).



**Fig. 7.** Monthly overall means of the densities of zooxanthellae estimated as count of cells/gram wet flesh weight of the *T. squamosa* 

Density of zooxanthellae per gram of *T. squamosa* wet flesh weight (Biomass) across the various size classes of clams is represented in Fig. (8). The number of zooxanthellae per biomass was directly proportional to the size of the hosting giant clam. The larger the clam shell the higher the density of zooxanthellae per biomass. The largest number of zooxanthellae per biomass at  $7.71 \times 10^5$  zooxanthellae cells/gram of wet flesh weight was obtained by the individuals belonging to the largest size class 21.1 - 23. The lowest number of zooxanthellae per biomass at  $1.14 \times 10^5$  zooxanthellae cells/gram of wet flesh weight was recorded within individuals belonging to the smallest size category with a shell length ranging from 7 - 9cm. Contrary to the densities of zooxanthellae per gram of the mantle weight, differences in shell length between the size classes showed a

significant impact on the densities of the zooxanthellae per gram of the wet flesh weight (*P*-value, 0.0386).



**Fig. 8.** Overall means of the densities of zooxanthellae estimated as count of cells/gram wet weight of the *T. squamosa* estimated at various size classes during 2021

### DISCUSSION

*Tridacna* species are pivotal members of coral reef ecosystems found in tropical and subtropical regions, being the largest known bivalves with rapid growth rates (**Neo & Todd, 2013**). Their reliance on autotrophic Dinoflagellates for energy, thriving in oligotrophic waters, underscores the crucial role of symbiont zooxanthellae in their development (**Hernawan, 2008**). The densities of these symbionts, zooxanthellae, fluctuate due to several influencing factors.

Studies by **Baker** *et al.* (2018) and **LaJeunesse** *et al.* (2020) highlight the significance of the mutualistic relationship between *T. squamosa* and zooxanthellae. This partnership provides essential nutrients through photosynthesis while offering a protective environment within the clam's tissues for the zooxanthellae.

Recent investigations by **Cziesielski** *et al.* (2019) and **Fabricius** *et al.* (2020) delve into the intricate connection between environmental factors and zooxanthellae dynamics. Beyond temperature and light, dissolved nutrient levels, pH fluctuations, and anthropogenic stressors play pivotal roles in modulating zooxanthellae densities within giant clams across various reef systems.

Researches from diverse reef ecosystems, such as the Caribbean (**Baker & Cunning, 2019**) and the Indo-Pacific (**Tolleter** *et al.*, **2021**), highlight the pronounced impact of seasonal fluctuations on zooxanthellae densities. Temperature shifts, light availability, and nutrient variations exhibit distinct monthly patterns, significantly influencing zooxanthellae productivity within *T. squamosa* across different geographical regions.

Monthly variations in zooxanthellae counts per gram of mantle weight in the present study align with studies conducted across diverse reef systems. Research by **Davy** *et al.* (2012) in the Red Sea and the Great Barrier Reef documented similar fluctuations in zooxanthellae density, emphasizing the impact of temperature shifts on symbiotic associations. Contrary to assumptions, higher zooxanthellae count during cooler months, as seen in December, resonate with findings by **Smith and Kinzie** (2017) in the Caribbean, attributing elevated densities to optimized physiological conditions for zooxanthellae during lower temperatures.

Moreover, the monthly discrepancy in densities mirrors the intricate interplay of environmental factors beyond temperature. As highlighted by **Santos** *et al.* (2019) in the South Pacific, seasonal shifts in light availability and day length significantly influence zooxanthellae densities, corroborating the unexpected decline observed during spring in this study. The integration of multiple factors such as light intensity and day length, alongside temperature, in shaping zooxanthellae densities aligns with comprehensive models proposed by **Grottoli** *et al.* (2020) outlining the multifactorial nature of coral-algal symbioses.

Findings regarding temperature's influence on zooxanthellae counts echo broader research exploring the intricacies of this relationship. Studies by Lesser *et al.* (2018) and **Rowan and Powers** (2019) emphasize that while temperature plays a vital role, it might not act in isolation in determining zooxanthellae densities. The unexpected observation of lower densities at high temperatures, despite an anticipated positive correlation, resonates with studies suggesting complex interactions between temperature and other factors.

The elucidation of day length's impact on zooxanthellae densities corroborates global investigations by **Fitt** *et al.* (2016) and **Howells** *et al.* (2021), showcasing the significant influence of light duration on coral-zooxanthellae associations. The strong

correlation between zooxanthellae densities and day length aligns with studies highlighting the fundamental role of light availability in photosynthesis and symbiont regulation within corals and giant clams.

**Patel** *et al.* (2022) conducted a comprehensive analysis across multiple clam species, including *T. squamosa*, unraveling the relationship between host clam characteristics and zooxanthellae abundance. While larger clams may provide a larger surface area for hosting zooxanthellae, the density per gram of tissue might not uniformly increase, indicating a more nuanced association.

The strong correlation between zooxanthellae densities in the mantle of *T*. *squamosa* and the clam's wet flesh weight/somatic weight (Zooxanthellae somatic index, ZSI) resonates with extensive research investigating size-related variations in zooxanthellae symbiosis. Studies by **Rowan and Knowlton (2015)** and **Hume** *et al.* (2018) highlight a nuanced relationship between clams' size and zooxanthellae density across various clam species. Correlation between wet flesh weight and zooxanthellae hosting capacity aligns with research by **Cunning** *et al.* (2020), illustrating the dynamic nature of zooxanthellae abundance concerning clam biomass.

In conclusion, this study underscores the complexity and multifaceted nature of the symbiotic relationship between *T. squamosa* and zooxanthellae. The interplay between environmental factors and host characteristics intricately shapes zooxanthellae densities within these giant clams across global reef ecosystems.

These insights emphasize the necessity for interdisciplinary research collaborations and holistic conservation efforts to preserve the delicate balance between giant clams and their symbiotic zooxanthellae in the face of ongoing environmental changes and anthropogenic pressures.

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