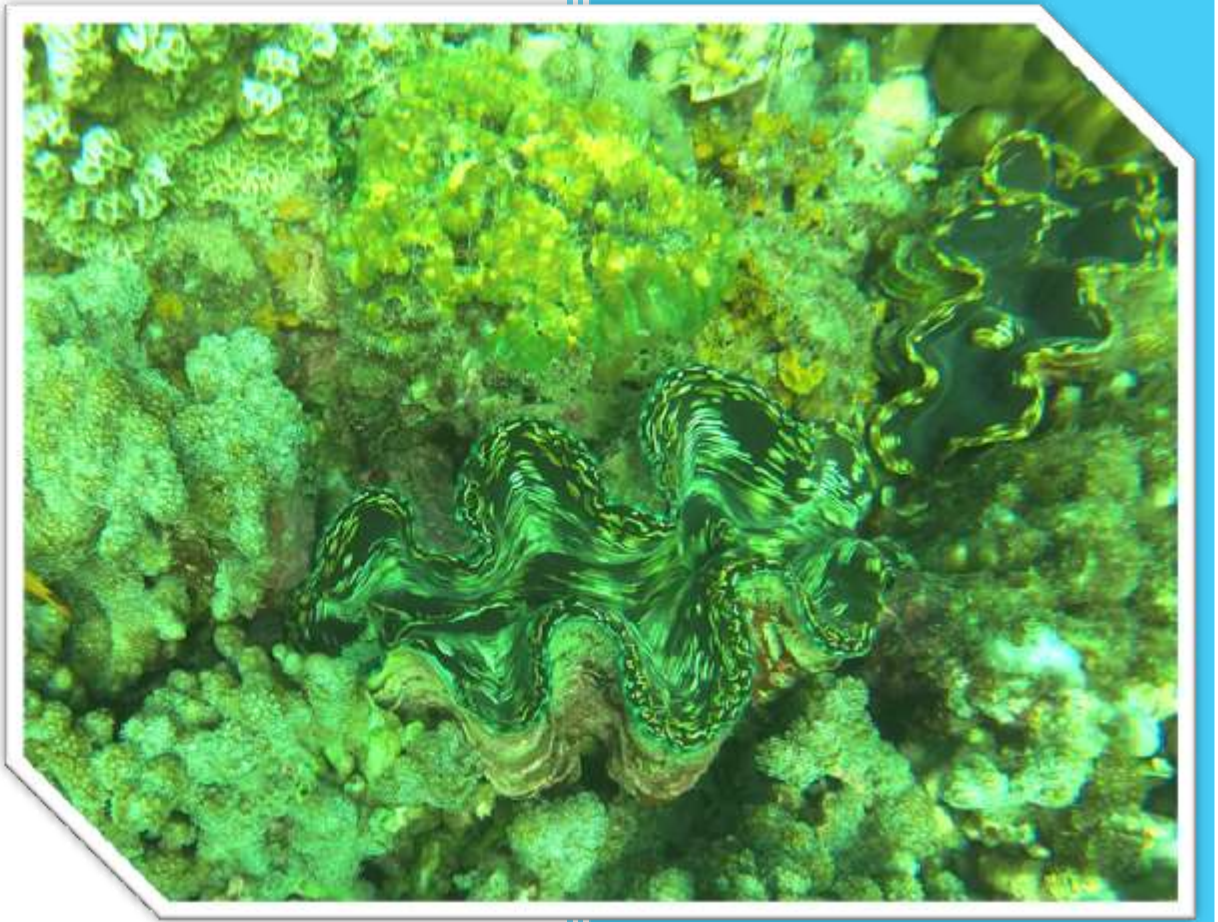
Problems occurring during the transplantation of marine cultured Tridacna squamosa



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Introduction

Coral reefs and their inhabitants are facing serious problems, threatening their very existence. One of the inhabitants with declining populations is *Tridacna*, the largest bivalve molluscs with the genus *Tridacna gigas* reaching lengths up to 1.4 meters being the largest and fastest growing in this family (Beckvar, 1981; Hawkins & Klumpp, 1995). A smaller genus is *Tridacna squamosa;* common name Fluted giant clam which grows up to 40 centimeters. This species is listed at lower risk, but conservation dependent by the UICN Redlist (Wells, 2015). *Tridacna sp.* are important habitants of the reef due to the fact that they provide shelter and food for other coral reef organisms, moreover their hard calcium carbonate shells are used by corals for substrate and are a way for the coral to venture out of the existing boundaries of the reef (JAMES R. GUESTa, y, PETER A. TODDb,*, EUGENE GOHa, z, 2008). Furthermore giant clams make the surrounding reef more resilient to climate change by providing zooxanthellae to the corals after major bleaching events (Maruyama & Heslinga, 1997). They are therefore important species within the coral reefs, being an addition to not only the biodiversity but also making the reef more resilient against problematic environmental issues such as climate or ocean warming.

Giant clams are overfished for their meat and shells, however marine culturing of the giant clams has brought a change in the giant clam fishing industry. Detailed recruitment and nursery protocols have been established, artificial spawning can be induced and marine-culturing as well as land-culturing of giant clams is successful nowadays (Ellis, 2000; Lola, Da, & Lola, 2006; V, Heslinga, & Perron, 1984; Waters, Story, & Costello, 2013). This reduced the pressure on natural population greatly. Moreover with the marine-culturing of giant clams new possibilities for the conservation opened up; the so called gardening concept can be implemented with giant clams. This concept involves the marine-culture of organisms to a suitable transportation and releasing size (Rinkevich, 2005).

This concept however has not been implemented successfully yet. Research for the restocking of Giant Clams started almost 20 years ago through the WorldFish Center and the Australian Centre For International Agricultural Research (ACIAR) (Gomez & Mingoa-Licuanan, 2006). Nevertheless a proper protocol is still lacking and much research is still needed to solve the occurring problems.

The main problem at the moment is the fact that high mortality rates are common while restocking areas with marine cultured *Tridacna squamosa*. Mortality rates range from 60 - 96% within the first 18 days (Scott, 2015; Waters et al., 2013). The study of Waters et al., 2013 shows that survival rates are as high as 100 percent when the giant clams are placed in an enclosure. The aim of Waters et al., 2013 was to make the giant clams less vulnerable to predators by enabling them to attach to the substrate before removing the cage. The reattachment of giant clams to substrate with byssal threads should take up to four days. However Scott, 2015 uses a method in which the relocated giant clams are still attached to tiles making it harder for predators to remove the giant clams and yet faces the same problem of high mortality rates. An escape size of 20 cm has been determined for *Tridacna squamosa* at which the giant clam has outgrown most natural predators (Gomez & Mingoa-Licuanan, 2006). However large individuals of *Tridacna squamosa* still face high mortality rates after transplantation (Scott, 2015). Due to the slow growth rate of 2-8 cm per year (Beckvar, 1981; Foyle, Bell, Gervis, & Lane, 1997) high mortality rates might make the restocking of reefs with adult clams too costly and are thus unacceptable.

One of the reasons for the high mortality rate could be the fact that small in situ differences could be at the basis of changed behavior. Although marine culturing of giant clams is based in the ocean the giant clams are placed in an enclosure. This enclosure is cleaned periodically which has been related to slower growth rates due to stress. Predator cues have been proven to induce changes in the shell morphology of *T. squamosa*, a difference as small as starving and well fed predators induced changes in shell parameters such as strength (Neo & Todd, 2011). The effects of excluding large predators on the defense of *T.* squamosa will therefore be researched in this study. Expected is that the lack of predators causes the giant clam to invest less energy in shell strength and scute density similarly with the study of Neo & Todd, 2011. Furthermore is expected that the giant clam does not respond to predators above the top of the cage and respond less intensive on predators as well.

A second reason for the high mortality rate could be the fact that the giant clams undergo a period of stress prior to be placed into the reef due to movement. High levels of stress are deadly (Gomez & Mingoa-Licuanan, 2006) and consequently the action of relocation is expected to have a temporarily negative effect on the responds towards predators making the giant clams more vulnerable in period directly after the transplantation.

Effective transplantation is not reached when relocated giant clams survive, for a sustainable population other parameters such as micro-scale distribution have to be considered as well. During spawning events 70% of the giant clams can signal a neighboring giant clam to a distance of 9 meters (Braley, 1984). Up to 20-30 meters the success rate of signaling and inducing spawning in neighboring clams is only 15% however (Waters et al., 2013). Additionally the fertilization rate drops down quickly with greater distances between giant clams (Babcock, Mundy, & Whitehead, 1994). A third section of this study will examine a healthy population and determine the natural distribution of giant clams to be able to make a recommendation for transplanting giant clams.

This study hence focusses on three different topics: firstly the impact of the enclosures on the constitutive and behavioral responds towards predators, secondly the impact of stress on the behavioral responds towards predators and lastly the natural distribution pattern of giant clams.

Methods

Methods is subdivided in three sections according the three research questions. The first section; impact of enclosures, includes the subsections constitutive defenses and behavioral defenses. The second section is the impact of stress. Lastly the third section; natural distribution, includes the subsections overall population size and clustering of giant clams. Figure 1 shows a diagram of the methods section layout.

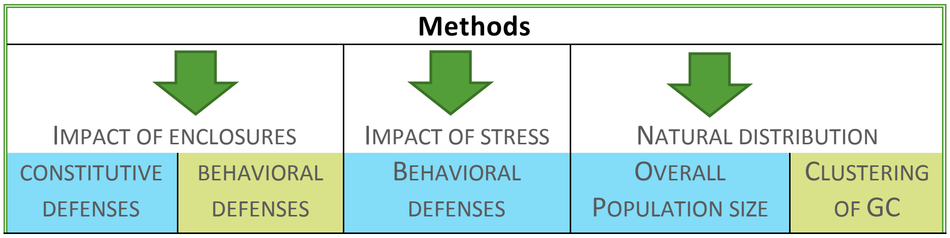


Figure 1 – The structure of Methods.

Impact of enclosures

Behavioral responds

Three behavioral responses have been measured for two types of attacks:

1) Closure of the valves after a simulated attack
2) The recovery time till the valves are opened to the initial state in seconds after an simulated attack
3) The height at which the giant clam initiated his responds at a simulated attack

Two types of attacks were simulated: A small predatory attack and a large predatory attack. The small predator has been simulated by lowering a plastic bulb of approximately 5 cm in width and filled with iron nails for additional weight. The large predator has been simulated by lowering a plastic slate of approximately 10 by 20 cm. Each experiment started with three consecutive large predator attacks followed by a rest period and subsequently three consecutive small predator attacks.

The setup of the experiment was as following: A thirty centimeter long ruler has been placed upwards perpendicular to the Giant Clam while a camera (Gopro hero 3+) has been placed at the opposite side of the ruler (See figure 2). Every simulated attack has been recorded, afterwards three screenshots were made from the movies:

- Initial distance between the valves before any attack
- Distance between the valves after a large predator attack
- Distance between the valves after a small predator attack

Figure 2 – Set up of the experiments to measure the behavioral responds

The distance between the valves on the three screenshots has been measured in pixels with Photoshop. The percentage the giant clam closes their valves has been calculated with the following formula:

Percentage closed = 100 – (Pixels between valves at moment of attack / (Pixels between valves when fully opened / 100))

The recovery time has been measured by replaying the film and thereby looking at the period between the last attack and no visible extension of either the valves or the mantle.

Lastly the height of responds has been measured by measuring the height of the object at the first visible sign of responds. The height of the object has been categorized into either >30cm or <30 cm.

Two groups; Wild GC (W-GC) and MC-GC have been made for this section. The groups are also compared with the groups made in the stress sub-section. Significant differences are determined with a One-Way-Anova. All test have been done with SPSS.

Constitutive responds

Two constitutive responses have been measured:

1) Shell density
2) Scute length

To measure the constitutive responds twenty shells of dead GC have been collected from the nurseries and 15 shells of dead GC from the coral reefs. All shells have been placed back on approximately the same place after the experiments.

The volume and weight of all shells have been measured. Afterwards the density has been calculated as following:

$$\text{Density} = \text{Weight} / \text{volume}$$

Secondly the scutes have been measured. From every shell the top scute, third scute from the top, fourth scute from the top and bottom scute have been measured at four folds, thus in total 16 scutes. If the scute was too worn out it was skipped. Lastly the height and width have been measured in cm.

Densities and Scutes of different groups have been compared with a One-Way-Anova and Bonferroni as post-hoc. A Pearson's correlation have been used to determine correlation all parameters of MC-GC and a Spearman's correlation has been used to determine a possible correlation between those facets of W-GC.

Impact of stress

To measure the impact of stress on the behavioral responses of marine cultured GC (MC-GC) the same experiment as explained in the subsection “behavioral responds”. However three different groups of MC-GC have been made.

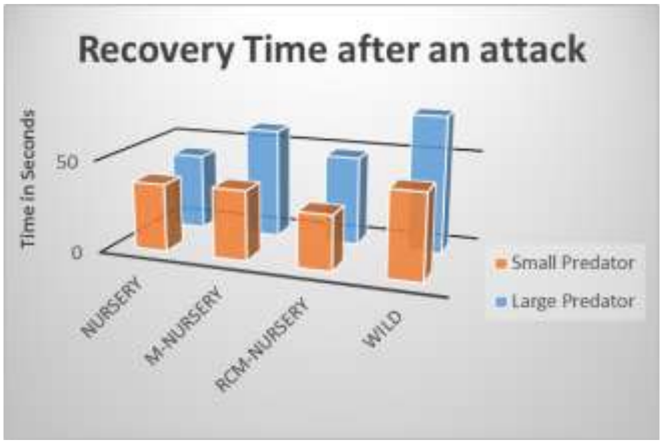
The first groups consist out of 24 MC-GC's and act as control group. The second group consist out 12 MC-GC which have been moved over 100 meters by a scuba diver prior to the experiment, the so called ReCently moved MC-GC (RC-MC-GC). The last groups consist out of the same 12 MC-GC's however with an additional rest period of two weeks from now on called (M-MC-GC).

A paired T-test has been used to determine significant difference between the groups RC-MC-GC and M-MC-GC. Differences between MC-GC and the other groups have been determined using a One-Way-Anova with Bonferroni as post-hoc test. Correlations have been determined with Pearson's correlation test. All tests have been done with SPSS.

Results

Behavioral responds

The height at which the responds initiated was for all groups >30 cm. The recovery time after an attack of a large predator is on average 20 seconds longer than the recovery time after an attack of a small predator. The category nursery (MC-GC) does not have a significant different recovery time from either attacker. Between the categories have Nursery // Wild; 31 seconds ($P < 0.0005$), and Wild // RCM-



Nursery; 25 seconds (P=0.008), of the large predator attack a significant difference.

The recovery time after a simulated attack from a small predator differs significantly between Nursery and RCM-Nursery; 7 seconds (P=0.032), Nursery and Wild; 8 seconds (P=0.002), RCM-Nursery and Wild; 8 seconds (P=0.015).

Figure 3 – Recovery time after an attack of a large and a small predator in seconds.

Tridacna sq. in the category nursery show no significant difference between the responds on small or large predators in terms of valve closure (P=0.262). The category moved similarly showed no difference (P=0.569). The category RCM and Wild both have a significant difference between the responds on small and large predators (RCM; P=0.006, Wild; P=0.039).

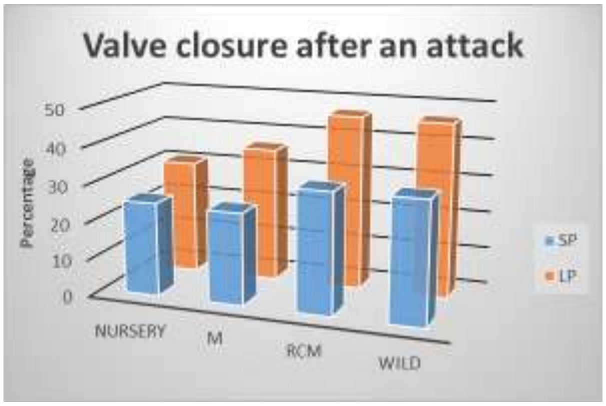


Figure 4 – Valve closure of categories Nursery, Moved Nursery, Recently moved nursery and wild after a simulated attack of a small and large predator.

All categories have indifferent responses to small predators. The large predators however do provoke different responses in comparison with the small predators in the four categories. The category wild GC is significantly higher than the category Nursery (P=0.001) and the category RCM Nursery is significantly higher than the category Nursery (P=0.005) and the category Moved Nursery (P=0.032).

All categories responds on either predator initiated above 30 cm and there was no significant difference between the groups.

Constitutive responds

The categories Wild and Marine Cultured GC show a slight but significant difference of 0.2444 g/cm^3 in shell density (P=0.044).

Marine cultured GC have a significant difference in the length of the scutes in all of the four positions (P>0.005) except from the upper middle and lower middle position. In wild GC only the bottom scute demonstrations a significant difference with all categories (P>0.005).

The wild and MC GC express an significant difference in scute length for all four categories (P>0.005)

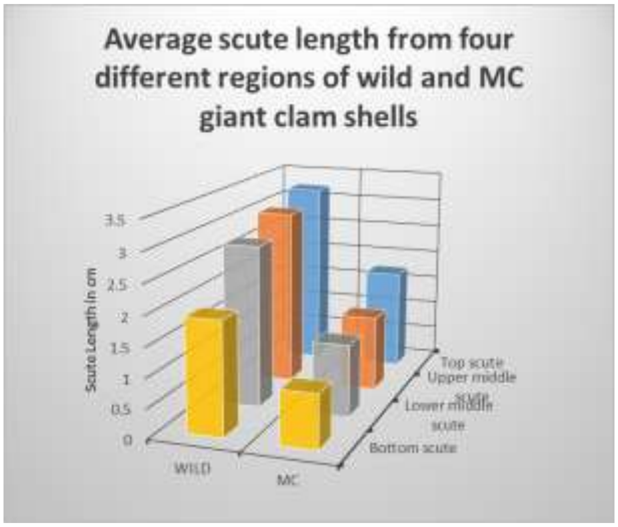


Figure 5 – Scute length of four different positions on the shell from wild and mc giant clams in cm

The width and height show a strong positive correlation in both categories (MC GC: R=0.0875; P>0.005, Wild GC: R=0.906, P>0.005). Wild GC display a less strong positive correlation between the width and scute length (R=0.730, P=0.002) and the height and scute length(R=0.596, P=0.019) as well.

Discussion

Impact of stress

Stress was in first instance expected to cause play a major role in the high mortality rate. However it turned out that small amount of stress caused by moved the giant clams over 100 meters contrarily has a positive effect on the manner of responds of giant clams towards predators which is intensified. After moving the marine cultured giant clams the behavioral defense appeared to be similar with those of wild individuals. Whereas it significant differs from the behavioral responds of other not-stressed marine cultured giant clams. This however is a temporary change as after two weeks the before stressed giant clams are responding similarly with other marine cultured giant clams again.

This changes the way transplantation of giant clams should be executed. If the giant clams have to be transported over longer distances it might be profitable to place them in a cage near the end location for two weeks before moving them. In that way the giant clams will have a temporary extra protection through the stress and are less vulnerable at the start. It is hard to determine when the stress starts to be detrimental due to the fact that unnecessary to increase the death rate of marine cultured giant clams for solely that purpose.

Impact of enclosures

The results of this study show that the shell density of marine cultured *Tridacna squamosa* is less then wild individuals. Moreover marine cultured individuals close their valves one third less then wild individuals after an attack of a large predator, additionally they open their valves up almost twice as quick after a large predator attack as well. Small predators however do not show a significant difference besides a slightly faster opening up of the valves after the attack.

As remarked by Neo & Todd, 2011 the presence of predators changes how the giant clam allocate his energy. The results of this study confirm this fact with a decreased shell density of marine cultured giant clams. Additionally the results imply that in the absence of large predators the reflex which causes the giant clam to close the valves decreases in intensity as well. It should be noted that although unlikely this result could also be caused by the fact that the giant clams are place in large groups of 15+ individuals. However since they cannot see sideward this is unlikely to be of influence since they sense with eye-like organs.

Interesting is that the giant clams apparently can separate large and small predators and respond differently according to the situation. Although this is not entirely unexpected since small fishes can hang around and nibble at giant clams for extended periods of time and closing their valves this period would mean a great loss of sunlight-energy.

This change in the giant clam's behavior can be regarded positive for the marine culturing of giant clams as it most likely increases the speed at which the giant clams grow. However for the transplantation of giant clams it is at first thought detrimental and causing a higher mortality rate. But as has been said in the paragraph "impact of stress" above recently moved giant clams have intensified responses towards predators, the mortality rate is still high indicating that something else is the major cause of the high mortality rate. Further research has to be done to prove if the process can be turned around and the giant clams can change their behavior in a positive way after transplanting them.

Although the scutes show an enormous difference between the categories this comes mainly due to difference in shell sizes.

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