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The effects of sub-lethal predation on the reproductive output of *Acanthaster planci* (crown-of-thorns starfish)

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ISP Ethics Review

(Note: Each AD must complete, sign, and submit this form for every student’s ISP.)

The ISP paper by Emily Mucha (student) does not* conform to the Human Subjects Review approval from the Local Review Board, the ethical standards of the local community, and the ethical and academic standards outlined in the SIT student and faculty handbooks.

*This paper does not conform to standards for the following reasons:

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Program: ASE- Australia: Rainforest, Reef, and Cultural Ecology

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ABSTRACT

Wide-scale declines in live coral cover have been observed throughout history. Modern day coral populations face a multitude of environmental disturbances, however one of the most devastating to the reefs in the Indo-Pacific is the crown-of-thorns starfish (*Acanthaster planci*), which feeds on the tissues of live corals. The rate at which these individuals feed combined with the explosion of populations in times of outbreak have been seen to exert some of the biggest pressures on coral reefs to date. Following recent episodes of crown-of-thorns starfish (COTS) outbreaks, research on the organism has become increasingly prevalent on the scientific agenda. The following study was conducted to assess the reproductive output of COTS with and without naturally damaged arms to further analyze the role of predation on the population dynamics of this species. Images of histological slides were utilized in doing so, allowing the egg sizes of individuals with damaged arms to be compared with the egg sizes of those with fully intact arms. Overall, it was found that while there was no noticeable trend between size of the COT individual and their respective egg sizes and the severity of injury that the individual experienced and the eggs they produced, however it appears there is a correlation between the individual’s overall state of health and their reproductive output. In short, fully intact individuals produced larger eggs than those that were predated upon; however, in looking closely at the data collected from four specific individuals (two being fully intact and the other two being injured), it became clear that this trend does not always hold true. Therefore, it is possible that other factors confounded the results of this study, and these are specifically outlined. Future research may include examining the effect of predation on the abundance of eggs produced, as this study focused solely on measuring egg size.
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1. INTRODUCTION

A vital area of scientific focus in this modern time is the effect of disturbance on the biological and ecological processes that occur within coral reef ecosystems. Between anthropogenic influences (including but not limited to: agricultural runoff, coastal development, pollution, oil spills, overfishing) and natural disturbances (e.g. tropical cyclones, crown-of-thorns outbreaks, parasites and diseases), 19% of the world’s coral reefs have been destroyed, with 35% of the remaining reefs threatened to experience the same fate over the next 10-40 years (De’ath et al. 2012, Hughes et al. 2014). Coral reefs are one of the most biodiverse ecosystems, exhibiting some of the highest rates of productivity on the planet (Hughes et al. 2014). Hard scleractinian corals contribute significantly to this
biodiversity, as they provide vital resources (food and shelter) for other reef-dwelling organisms, such as fish (Hughes et al. 2014). The abundance and diversity of fish species on any given reef is directly linked to the abundance and complexity of the coral cover that formulates these habitats, as exemplified in numerous studies (Jones et al. 2004, Pratchett et al. 2008, McCormick et al. 2010, Pratchett et al. 2012); The structural complexity provided by these corals creates an extensive amount of microhabitats, allowing fish to carry out niche partitioning in a fine-scale way (Hughes et al. 2014). Niche partitioning is a fundamental part of any ecosystem; individuals utilize resources in different ways, decreasing competition for these resources and therefore allowing the environment to support a greater amount of species (Bellwood et al. 2006). When abundance of living coral decreases, the density of individuals in areas of remaining cover increases, resulting in increased competition and consequently, higher rates of mortality among these organisms (McCormick et al. 2010). Evidently, a decline in live coral cover has subsequent effects on the ecosystem as a whole, as species at higher trophic levels are also negatively affected.

While an immense range of environmental disturbances have been seen to impact coral reefs, one of the most prevalent areas of research is the crown-of-thorns starfish (COTS): a predator of living coral found in the Indo-Pacific. The COTS is considered one of the biggest threats to coral reefs to date (“Crown-of-thorns Starfish on the Great Barrier Reef”, 2006, De’ath et al. 2012, Hughes et al. 2014); it consumes the tissues of scleractinian corals by boring through their skeleton with digestive enzymes, targeting the living tissue of these corals in particular. A large feeding scar covers the coral after it has been preyed upon, and the coral organism is left to die (“Crown-of-thorns Starfish on the Great Barrier Reef”, 2006). The COTS has been seen to feed at a devastating rate, and in outbreak events
(when populations boom), the coral cover within an area has the potential to decrease to a mere 1% of its original size ("Crown-of-thorns Starfish on the Great Barrier Reef", 2006).

In developing management efforts to controlling COTS populations, it is essential to first study the factors that influence the population dynamics of this species. Previous studies have looked into the response of COTS to various environmental conditions; for example, the effect of diet on the quality of their reproductive output (fecundity) (Caballes et al. 2016), or the effect of predation on their growth and fecundity (Green 2015). The latter study derived most of its background from something known as the predator removal hypothesis, which has been widely studied since the time of the first COTS outbreak in the 1960’s, after one of its primary predators-the giant triton (Charonia tritonis)-was severely hunted for its shell over previous years (Endean 1969). Scientists drew the connection between these two events, hypothesizing that COTS populations were highly regulated by the predation they experienced (McCallum 1987, 1990); these findings have been supported by studies since, which have demonstrated a direct correlation between overfishing of top predators and increased prevalence of COTS outbreaks (Dulvy et al. 2004, Sweatman 2008). To further investigate whether or not the encouragement of predation will prove successful in reducing COTS populations in times of outbreak, this study specifically examined whether reproductive output or regeneration (re-growth) was the priority in crown-of-thorns starfish that have been sub-lethally predated upon. It was hypothesized that the stress placed upon individuals that have been predated upon will cause a change in their energetic provisioning, resulting in a greater amount of energy allocated to regeneration rather than increased fitness of their eggs. If fecundity and recruitment are reduced in individuals that have been predated upon, then population
numbers will also decrease, thus reducing the severity of outbreak events. If so, these findings will support the predator removal hypothesis, in that predation does play a key role in determining the population dynamics of this species. The results of this study may be used to further the targeting of COTS populations in future management efforts.

2. METHODS

2.1 Study Site and Subjects

Data collection for this study took place November 7-18, 2016 at James Cook University in Townsville, Queensland, Australia (19.3276°S 146.7581°E). The study site consisted of a lab space in the university’s Coral Reef Studies department, where all measurements were taken. All subjects were of the crown-of-thorns species, however both individuals with all arms intact, as well as individuals with one or more arms fragmented were tested.

2.2 Data Collection

2.2.1 Egg Measurements

A total of 39 individual COTS were studied; for each individual, 6-7 of their arms were looked at, with approximately 7 different samples taken from each arm. To quantitatively measure the reproductive output of these individuals, images of histological slides were obtained and analyzed. Included in each image was a different sample of eggs taken from one of that individual's corresponding arms. The ImageJ software was used to measure egg sizes; this was achieved by tracing the ‘Straight Line’ tool over the egg to and from the egg wall, passing through the nucleus to measure the first length, and tracing a line perpendicular to the previous to obtain a second length. These two measurements
represented the minimum and maximum diameters of the egg. While it was intended to measure at least 50 eggs per arm per individual, the number of eggs measured in each sample image varied, as only eggs with a distinct nucleus, unbroken body, and generally circular shape were analyzed. This ensured that the way in which the egg laid on the slide did not play a role in influencing its apparent size; whole eggs that were laid flat were specifically targeted. These measurements were entered into a Microsoft Excel spreadsheet for further analysis, sorted by Female (individual) Number, Arm Number, Sample Letter, followed by Egg Number and its respective measurements.

### 2.3 Data Analysis

To begin, a document containing information on the total number of arms, number of short (fragmented) arms, list of which arms were short and long, and diameter of each COT individual was obtained. This allowed me to first look at the relationship between size of the COT individual and the size of the eggs it produces. Since both maximum and minimum diameters were taken as measurements of each egg, these diameters were analyzed separately to ensure that there would not be too much variation in the data to see a cohesive trend. Furthermore, only the measurements of eggs found in long arms were included in this section, as it is possible that regeneration could have played a role in determining egg size in short arms, adding another variable (besides COT diameter) and confounding the results. After calculating the average maximum egg diameter between these arms, the average value for each individual were then represented in a scatter plot, with one data set being fully intact individuals (no damaged arms) and the other being injured individuals (those with one or more fragmented arms). A line of best fit was included for each data set to indicate the prevalence of the trend, as well as an R-squared
value to exhibit variation in the data points. These methods were then repeated for the minimum egg diameters of each individual, represented in an additional scatter plot.

Continuing on, the possibility of severity of injury playing a role in determining egg size was analyzed. In doing so, another scatter plot was produced, representing the individual’s average maximum egg diameter against their severity of injury. Egg measurements in both long and short arms were factored into each individual’s average, as this section aimed to look at the individual as a whole. The severity index was calculated by dividing the individual’s number of short arms by their total number of arms and multiplying by 100, obtaining a percentage and therefore, allowing for comparison between individuals that had different numbers of total arms. A second scatter plot was then created, including the average minimum egg diameters. A line of best fit was similarly added to both plots in addition to an R-squared value for that line.

The following section looked at comparing the egg sizes of COTS in different states of health. In specific, the average egg diameter for each ‘Arm/Individual Type’ was represented in a bar graph: arm type being either short or long, and individual type being fully intact individuals versus injured. Individuals were split up into general categories for this, allowing me to see whether overall health played a factor in determining reproductive quality. Consequently, egg measurements were averaged across all long arms of intact individuals, then all long arms of injured individuals, and finally all short arms of injured individuals (for a total of three categories). Only the maximum egg diameters were taken into account and analyzed from this point forward. Standard error was calculated and error bars were included to account for any deviation in the average values.
Lastly, four individuals were chosen and analyzed in detail, two of which were fully intact individuals, and the other two being injured. This entailed comparing the average maximum egg diameter of each arm and categorizing these arms as either long or short in nature. Individuals with differing amounts of injured arms were chosen, in order to further examine whether severity of injury played a role. A total of four bar graphs were created to compare the reproductive output of each arm for each individual. Not all arms were measured for egg size, as there were not sample images of every arm for each individual. Therefore, only arms with corresponding histological slides were included and represented in this analysis. Standard error bars were again included as a means of exhibiting any possible deviation in the averages.

3. RESULTS

3.1 COT Diameter versus Egg Size

![Average Maximum Egg Diameter per COT Individual](image)

\[ y = -0.0028x + 105.41 \]
\[ R^2 = 0.00057 \]

\[ y = 0.0471x + 82.96 \]
\[ R^2 = 0.0385 \]
Figure 1. Average maximum egg diameter (um) among long arms for each of 39 crown-of-thorn (COT) individuals. COT diameter (mm) represents the maximum diameter of the individual’s entire body. Best fit lines for each of two data sets (‘Intact’ and ‘Injured’ individuals) are included, in addition to the R-squared value for these data sets.

![Average Minimum Egg Diameter per COT Individual](image)

Figure 2. Average minimum egg diameter (um) among long arms for each of 39 crown-of-thorn (COT) individuals. COT diameter (mm) represents the maximum diameter of the individual’s entire body. Best fit lines for each of two data sets (‘Intact’ and ‘Injured’ individuals) are included, in addition to the R-squared value for these data sets.

With R-squared values of less than 0.1 in both plots (Figure 1 and Figure 2), it was concluded that there was no relationship between the size of the individual and their respective egg sizes. There is more variation in egg sizes between injured individuals than there are between those intact, with individuals 14 and 15 being the most noticeable outliers in both plots. There is no significant difference between the results of the
maximum diameters and those of the minimum diameters, with a 0.85% difference in slope values and a 19.1% difference in R-squared values for injured individuals.

3.2 Severity of Injury versus Egg Size

![Graph showing average maximum egg diameter per severity of injury experienced by each individual.](image)

**Figure 3.** Average maximum egg diameter (um) among both long and short arms for each of 39 crown-of-thorn (COT) individuals. Severity of injury in each individual is represented by a percentage (%). A line of best fit for this data set is included, as well the R-squared value for these data.
Figure 4. Average minimum egg diameter (um) among both long and short arms for each of 39 crown-of-thorn (COT) individuals. Severity of injury in each individual is represented by a percentage (%). A line of best fit for this data set is included, as well the R-squared value for these data.

Both intact and injured individuals were included; evidently, those that experienced 0% severity of injury represent the fully intact individuals (Figure 3 and Figure 4). Looking at the R-squared values of both plots, it is clear that there is a very weak relationship between how relatively injured the individual is, and their reproductive output. Any significant variation in the data can be attributed to three individuals in particular, individuals 1, 14, and 15, which are shown as outliers in both plots. Two individuals exhibiting 19% injury severity show a noticeable difference in egg size (one
averaging 75.9 um while the other averages 91.7 um). Similarly, while individuals 1 and 15 experience approximately the same severity of injury (60% and 61%), individual 1 exhibits an average of 105.20 um eggs and individual 15 produced eggs of 70.15 um, showing a 35.05 um difference between one percentage point. This further indicates that there may not be a cohesive trend between amount of injury and the size of eggs produced. There is more a difference in the results found between the maximum and minimum diameters in these plots than in those previous (Figure 1 and Figure 2), however with a 14.1% difference in slope values and a 35.4% difference in R-squared values, there is still minimal difference between the data sets.

3.3 Arm/Individual Type versus Egg Size
Figure 5. Average maximum egg diameter (um) for the COTS individuals categorized by each arm and individual type. Error bars represent standard error.

With standard error taken into account, it is evident that both arm types (intact and injured) of injured individuals showed approximately the same average egg diameter; however, the arms of fully intact individuals put out larger eggs by an average of 7.40 um (Figure 5).

3.4 Egg Size in Specific Individuals

![Average Maximum Egg Diameter of an Injured Individual per Arm](image)

Figure 6. Average maximum egg diameter (um) of an injured COT individual (Female no. 3) per arm number and type. Error bars represent standard error.
Figure 7. Average maximum egg diameter (um) of an injured COT individual (Female no. 33) per arm number and type. Error bars represent standard error.
Figure 8. Average maximum egg diameter (um) of a fully intact COT individual (Female no. 31) per arm number and type. Error bars represent standard error.

![Average Maximum Egg Diameter of an Intact Individual per Arm](image)

Figure 9. Average maximum egg diameter (um) of a fully intact COT individual (Female no. 5) per arm number and type. Error bars represent standard error.

There is some variation in egg size between arms in all individuals (Figure 6-Figure 9); however, this doesn’t seem to be determined by whether the arm has been injured or not, as there are some instances where short arms exhibit larger egg sizes than long arms, and vice versa (Figure 6 and Figure 7). Looking at the egg sizes produced by Female no. 33 (Figure 7) with 3 injured arms as opposed to Female no. 3 (Figure 6) with two injured arms, it is also evident that the level of severity of injury is not a factor in determining egg size (agreeing with the results shown in Figure 3 and Figure 4), as Female no. 33 actually
produced generally larger eggs by an average of 5.80 um. In addition, there are instances where uninjured individuals produced smaller eggs than those that were injured; the findings presented in Figure 7 versus Figure 8 are an example of this, where Female no. 33’s largest maximum diameter was 110.40 um, and Female no. 31’s was 105.85 um.

4. DISCUSSION

While human intervention has proved more difficult in controlling the outcome of other disturbance events (e.g. the warming of waters due to climate change and the intensifying of tropical storms), management efforts across the Indo-Pacific have turned their focus toward controlling outbreaks of the COTS as a more direct method of recovery (De’ath et al. 2012). It is estimated that live coral cover would increase at a yearly rate of approximately 0.89% if pressures on coral reefs due to COTS were eliminated (De’ath et al. 2012); Therefore, the resilience of coral reef ecosystems in other disturbances will increase if scientific solutions to controlling COTS populations are evaluated and pursued (De’ath et al. 2012, Hughes et al. 2014). Understanding the biological processes of the COTS is considered a vital aspect of this type of research, as scientists become better informed of the factors that play a role in determining the population dynamics of this species. In multiple studies conducted previously, changes in energetic provisioning in response to sub-lethal predation or arm loss in Asteroidea have been observed, where growth of the individual, maturation, movement, foraging, and reproductive success have been compromised (Lawrence 1991, Lawrence 1996, Maginnis 2006). In this study, the effect of predation on the reproductive output of the COTS was specifically pursued. One might expect that if an individual is injured, it will retreat back into the reef matrix to rest and
divert its energy to healing and regeneration, due to the fact that being exposed to predators when injured makes the individual increasingly threatened (Lawrence 1991, Bernardo & Agosta 2005, Maginnis 2006, Bely & Nyberg 2010). Therefore, it was hypothesized that a greater amount of energy will be allocated to regeneration rather than reproduction in injured individuals.

The findings of this study appear to support this hypothesis from a general standpoint, however specific instances of variation in the findings suggest that there may be some extraneous variables factoring into these results. Overall, it was observed that while egg size varies between arms in all individuals, egg size is not necessarily determined by whether the arm itself is intact versus injured (Figure 6-9) or the amount of injured arms (Figure 3 and 4) that the individual has; Instead, the overall state of health of the individual demonstrates the biggest effect on determining the size of the eggs produced (Figure 5). Therefore, unharmed individuals exhibit greater reproductive quality in the form of larger eggs, than their counterparts. These findings are conclusive with those of other studies, in that diminished investment in each egg (reduction in egg size) has been exhibited in individuals that had fallen victim to sub-lethal predation (Bernardo & Agosta 2005, Maginnis 2006, Barrios et al. 2008); evidently, the idea that changes in energetic provisioning result from physical disturbance such as predation is also supported. However, in instances where specific individuals were evaluated in detail (Figure 6-9), these trends were not observed. There are instances where the injured individuals (Figure 6-7) produced larger eggs than those intact (Figure 8-9), which means that there may be other factors confounding these results, including (but not limited to) individuals’ proximity to spawning and access to food.
In a study conducted previously, it was found that egg quality in COTS was directly affected by the proximity of an individual to its spawning period. Individuals that were sampled before the spawning period demonstrated densely packed gonads with mature eggs of a generally uniform size, while individuals sampled after the spawning period exhibited a low density of broken eggs with a large variation in size and shape, and small, premature eggs along the gonad wall that appeared to be in the process of formation (Babcock & Mundy 1992). These observations coincide with the results in this study, in that qualitative observations of the gonads in some individuals appeared to be producing consistent egg sizes and shapes in a greater abundance, while others were filled with primarily broken material in a much lower density. Consequently, it is entirely possible that this factored into determining the quality of eggs analyzed in this study, and have therefore confounded the results.

Additionally, it has been found that decreased nutrient intake and reservation can result from events of sub-lethal predation. The loss of a limb in echinoderms means damage or loss of the pyloric caeca (organs that hold stored energy), which is a direct result of predation that can consequently cause decreased energy availability to the organism. This means that there is less energy to be utilized in bodily processes, including reproductive activity (Lawrence 1996, Barrios et al. 2008). Indirectly, as energy was allocated to regeneration, the investment of energy on feeding was reduced, as decreased amounts of energetic content such as proteins, carbohydrates, and lipids were found in intact pyloric caeca (Barrios et al. 2008). Therefore, adults became limited in their ability to store energy which then limited their reproductive potential, which is likely to be a factor confounding the results of this study. The role of diet on the reproductive quality of COTS
has additionally been examined in individuals that were not predated upon, where the
previously outlined trends remain. The findings of this particular study showed that
decreased access to food resulted in lightweight gonads and smaller eggs, meaning that
reproductive output and quality were compromised. As opposed to individuals that were
well-fed, starved individuals produced smaller larvae with smaller stomach areas. The
number of larvae that made it to developed larval stages after eight days was severely
decreased in starved individuals (Caballes et al. 2016). As larval development is
compromised, it is likely that recruitment will be negatively affected, having adverse effects
for COTS populations and therefore reducing the intensity of outbreaks. Future areas of
research may include looking into the settlement success of larvae that came from starved
individuals as opposed to those that were well-fed, to directly analyze the effect of diet on
the success of overall populations, giving insight to the origin of outbreaks.

Moving forward, it may be important in future research to consider a few different
aspects that were not included in this study. The likelihood of COT diameter playing a role
in determining the individual’s respective egg sizes was ruled out in this study, as a trend
was not demonstrated between the two (Figure 1-2). However, it is quite possible that
larger individuals are able to produce and carry a greater abundance of eggs rather than
producing larger ones; size may have an influence on population dynamics in this way.
Similarly, while this study considered the effect of predation on egg size, it did not consider
the abundance of eggs that the individuals produced under different circumstances. It is
therefore possible that there is a correlation between the loss of limbs on the amount of
viable eggs produced, which offers another route of future research in looking into the
biological processes of COTS in response to ecological pressures.
REFERENCES


