Morphometrics Within a Cultured Cohort of Giant Clams (Tridacna gigas): An Observational Analysis of Gene Expression

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ABSTRACT

Tridacna gigas, the most threatened species of giant clam, is the source of a lot of attention in the Indo-Pacific region from researchers, to poachers, to tourists. Their numbers are declining because of the value of the fascinating shell and adductor muscle. This has sparked a rise in popularity for the mariculture of these giant clams, in an attempt to return abundance levels to their prior state. Researchers have conducted many studies on these large cohorts of clams, generally descendent from the same small group of parents. This study analyzes the morphological features and correlations among the cohort, as well as the influence of zooxanthellae on each feature. No statistically significant correlation was found between morphological traits and zooxanthellae density observed through mantle color. Frequency distributions for each morphological trait varied from normal, to sporadic with many gaps and jumps/dips in frequency. More studies focusing on the lifetime mantle color of each individual can help determine significance between the relationship of morphological size and zooxanthellae density.

Keywords: Tridacna gigas, morphometrics, symbiosis, cohort, endangered, mantle pattern
ISP Topic Codes: 606, 611, 616

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1.0 INTRODUCTION

1.1 Tridacna gigas

The giant clam (Tridacna gigas) is a staple organism of the Great Barrier Reef and of the Indo-Pacific region in general, drawing heavy attention from tourists across the world. Its enormity in size, coupled with its fascinatingly colorful mantle tissue, make it one of the must-see organisms on any trip to the area. Unfortunately, the distribution and abundance of the species is dramatically declining due to overexploitation by humans for its beautiful shell and tasty adductor muscle. In order to save these unique organisms from extinction, it is important to develop a thorough understanding of their specific requirements and processes associated with the species, in order to identify biologically and financially effective strategies to return to their natural patterns of distribution and abundance.

1.2 Taxonomy

T. gigas is the largest living bivalve species belonging to the family Cardiacea, and subfamily Tridacnidae, which contains all species of giant clams (Munro 1992). Within this subfamily, there are 9 recognized species of giant clams, seven belonging to the genus Tridacna and two belonging to the genus Hippopus. Giant clams within this subfamily are unique among their bivalve counterparts in their utilization of a symbiosis between the algal dinoflagellate zooxanthellae, enabling them to reach their distinctively large sizes (Munro 1992). The largest T. gigas individual ever recorded was 137 cm in length and 300 kg in weight (Rosewater 1965).

1.3 Distribution

The distribution of T. gigas is limited to the Indo-Pacific subtropical oceans, stretching from as far north as the South China Sea, down through Malaysia, Indonesia, and northern Australia, and up through Papa New Guinea and the Philippines. The clam used to be present in the waters of Taiwan, Japan, and Micronesia, but overexploitation in addition to influences from climate change due to human population increase has made them geographically extinct in these areas (Copland 1988). The distribution of the species is based off of its requirements for habitat selection, summarized by the clear, high-salinity waters of coralline tropical seas. They generally live in association with coral reefs, but the presence of coral is not an obligate requirement. T. gigas and other members of this subfamily are generally limited water depths less than 20 meters because of their dependence on the symbiotic photosynthetic algae (Munro 1992).

1.4 Reproduction/Growth

Because giant clams are sessile organisms, they reproduce through a method called broadcast spawning, in which sperm and eggs are released into the water and fertilization occurs in this water column (Alcazar 1988). All giant clams are hermaphrodites, making them capable of producing both eggs and sperm, allowing them to reproduce with any other individual of its species. During a spawning event, sperm is produced first, followed shortly by egg production. The release of sperm is dependent on a spawning inducer substance associated with the presence of ripe eggs (Wada 1952). Adult clams are capable of releasing up to 500 million eggs at a time (Knop 1996). It is estimated that T. gigas reaches sexual maturity at 25-35 cm (Nash, et al. 1988). A study on the variability of growth rates in both wild and cultured stocks of clams indicated a very high individual variability in growth, as well as a trend of a more rapid growth rate in wild stocks (Pearson 1991). This has been hypothesized to be a result of the strong influence of natural selection pressure on slow-growing clams in wild groups (Munro 1992).

1.5 The Symbiosis and Mantle Color

As stated previously, giant clams are unique among the bivalves in their presence of a symbiosis with the dinoflagellate algae zooxanthellae. T. gigas contain populations of these single-celled algae, the same algae present in the coral symbiosis, within its tissues. This photosynthetic alga uses the clam as a host, while the clam gains the excess products of photosynthesis, mainly glucose, making up a substantial portion of their daily energy requirements. Tubules stem from the gut of the clam, and the zooxanthellae concentrate near the mantle surface at the end of these tubules (Norton 1992). The algae are located in the mantle for maximum sun exposure when the clam is open.

The mantle coloration of these giant clams is greatly influenced by the amount of the zooxanthellae present within their tissues. The zooxanthellae are responsible for creating pigments as a result of photosynthesis, such as chlorophyll, while the clam creates pigments to protect its tissues from high levels of ultraviolet light (Yonge 1975). Zooxanthellae absorb visible light, needed for photosynthesis, in unequal amounts across the light spectrum. Because they don't absorb very much light in from the wavelengths corresponding with the colors red and green, they tend to have a brownish color in nature. Generally, the "browner" the mantle of a giant clam, the more zooxanthellae present, and the greater the benefits that host is receiving from the symbiosis. The iridescence of T. gigas mantles comes from these sunscreening pigments, known as iridiophores, that contain reflective platelets within them (Fatherree 2007). The mantle also contains up to thousands of eyespots, responsible for sensing the direction of the sunlight for maximum photosynthesis output, as well as detection of shadows as prevention from possible predation (Fatherree 2007).

1.6 Bleaching

Just as in corals, giant clams have the possibility of becoming bleached as a result of heightened environmental stress, characterized by a bare white mantle as opposed to the reflective green/brown color normally associated with the photosynthetic pigments in healthy clams. The clams achieve this pitch white color when they cut off the symbiosis between itself and the photosynthetic algae that they are so dependent on for their daily nutrition (Fatherree 2007). Bleaching usually occurs in patches across the mantle, but total bleaching can occur in extreme cases. When the clam expels this symbiont that they are so heavily dependent on, their metabolic intake drastically decreases, decreasing the fitness of the individual and slowing its growth rate (Leggat 2003). A number of environmental stresses have been identified as causes of bleaching, but the most prominent causes seem to be associated with increased water temperature and excessive ultraviolet exposure (Griffiths 1992). When the photosynthetic algae is exposed to such elevated levels of UV light, the byproducts of photosynthesis can actually cause damage to the host, leading to possible death if nothing is done to mitigate the stress. Because bleaching events are based mainly upon temperature and light, there are plenty of environmental conditions that play a part in the likelihood of an event. These include the length of day, cloud cover, rainfall, tide levels, water salinity and clarity, and air temperature. (Leggat 2003)

1.7 An Endangered Species

T. gigas is one of the most threatened giant clam species, commonly overexploited for its beautiful and valuable shell, as well as adductor muscle (Dawson & Philipson 1989). The adductor muscle is a common source of food throughout islands in the Indo-Pacific and has been connected to food sources of ancient groups inhabiting these islands many years ago. The International Union for Conservation of Nature identifies the giant clam as vulnerable, and that without proper active management strategies focusing on the recovery of the species, T. gigas will certainly become officially endangered in the near future (Munro 1992). As a result of their vulnerability, the mariculture of giant clams has become increasingly popular both in terms of studying the vulnerable species to gain a better understanding of its requirements as well as increasing their depleted numbers across the Indo-Pacific. Culturing cohorts, such as the one maintained by Richard Braley, is growing in popularity and may eventually prevent the species from becoming extinct.

1.8 Aims of the Study

The aims of this study are to:
1) Analyze the diversity in genetic expression within a cohort from the same 4 parent organisms through morphological observation
2) Identify morphometric correlations within a cohort
3) Identify morphological differences, if any, based on the amount of observed zooxanthellae from the mantle color
4) Identify morphological differences, if any, between bleached individuals and healthy individuals

2.0 METHODS

2.1 Study Site:

The study was conducted on Magnetic Island, located just 15 kilometers off of the coast of Townsville, in North Queensland, Australia. More specifically, the study was conducted at White Lady Bay (19° 6' 9.63" S, 146° 51' 45.52 E), a small fringing reef off of the north end of the island, right beside the much larger Horseshoe Bay. The site was located just a 20-minute hike around the coast of Horseshoe Bay, done at the start of low tide to ensure a safe commute to the site. All of the clams were located between the shoreline and a rock wall built by Keith Bryson, placed about 50 meters from the shore. This made it easy to locate each of the clams for data collection. White Lady Bay has been used as the site for many aquaculture projects on both oysters and giant clams. Three clams from the same cohort were located in Rick Braley's own laboratory aquarium on Magnetic Island and were also analyzed in the study.

2.2 Study Organisms:

The cohort of clams analyzed in this study was cultured and maintained by Dr. Braley himself and were some of the first clams to be cultured in Australia. This cohort of 54 clams are all products of the same spawning event between 4 parent clams, with 1 individual providing the eggs and 3 individuals providing the sperm. Because of this, all of the clams are exactly the same age (turning 27 years old in January 2013) and share the same genes. Because of their genetic similarity and identical ages, morphological observation will provide an analysis of the variance in genetic expression among the cohort.

2.3 Data Collection:

Data was collected between November 11th and November 18th, 2012, in association with the lowest tides during the study period to enable relatively simple collection. The time of collection shifted 50 minutes later each day in company with the time of the daily low tide. The tides ranged from .8 m to 1.2 m throughout the period of study. Morphological features of each of the 54 giant clam shells were measured in centimeters, using a large pair of calipers created by Rick Braley. Before any measurements were taken, it was important to ensure that the clam was as close to completely closed as possible to ensure a consistent means of attaining measurement values. This was done by gently rubbing the outer part of the mantle, in order to get the clam to close up, without providing an unnecessary amount of stress on the organism. If the clams differed in their degree of closure, the data collected would be inconsistent, as measurements such as width would be very skewed based on the degree of closure. Once this was done, measurements were taken of the shell length, width, depth, and scallop height to the nearest tenth of a centimeter:

- Length: measurement of the two outermost parts of the shell, parallel to the opening
- Width: measurement of the two outermost parts of the shell, perpendicular to the opening
- Depth: measurement from the base of the clam along the substrate to the highest point along the mantle
- Scallop Height: measurement of the curved projection of the shell, from the tip of the centermost ridge to the base of the opening

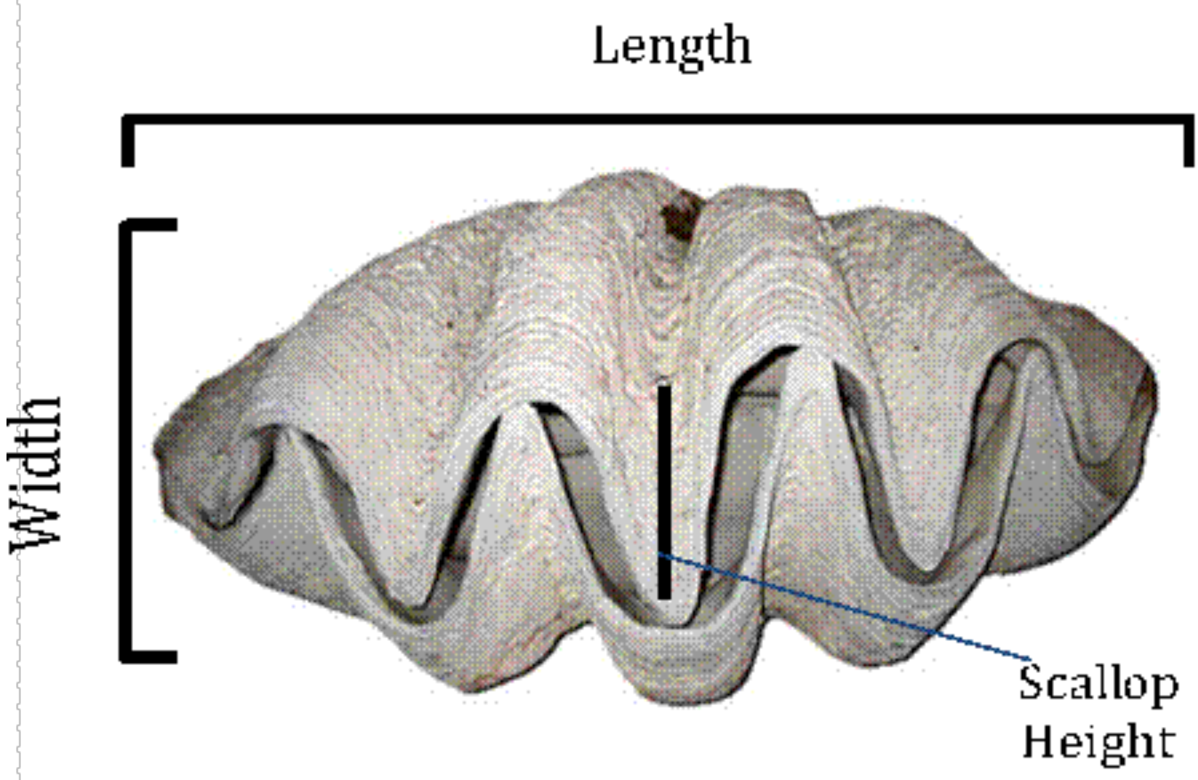


Figure 1: A photographic representation of each morphometric measurement

From the length, width, and depth measurements, total volume of the clam was calculated, assuming a three-dimensional rectangular shape. Cubic centimeters were converted to milliliters, and then to liters, to determine the size of the assumed "box" that the clam would fit inside of. While the volume measurement is not the true measure of volume for the clam because the clam is obviously not a rectangle, the values can still be compared in terms of relative volume across the cohort.

In addition to the morphological features measured with the giant calipers, observations on the color of the mantle were recorded, using a scale from 1 to 4, described by the table below (Table 1). This provided an observational account for the density of the symbiotic zooxanthellae present within the mantle of the clam.

Table 1 : A list of the 4 mantle color patterns used, with their associated descriptions

Mantle Scale	Description
1	Light brown, yellowish colored mantle
2	Dark brown colored mantle
3	Light brown mantle with dark spots
4	Dark brown mantle with light spots

2.4 Data Analysis:

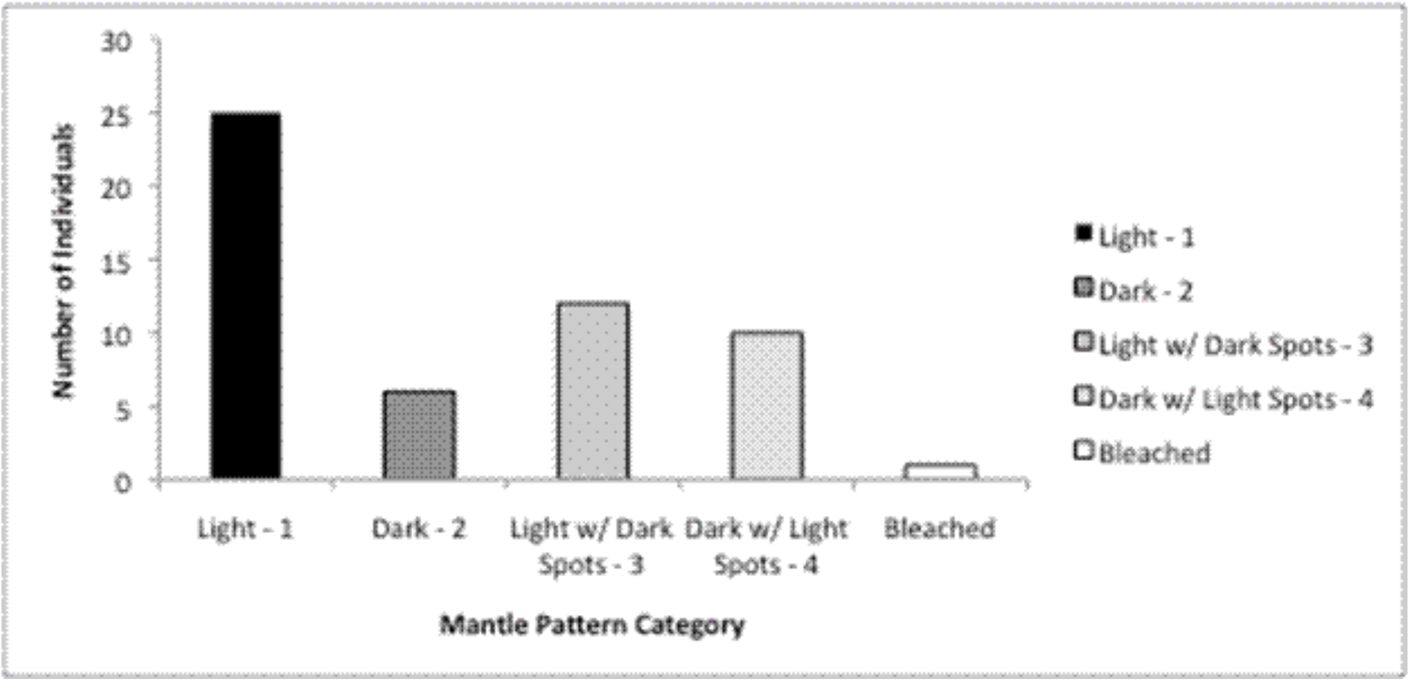
All of the data was then compiled and analyzed in Excel. Descriptive statistics were derived from the data for comparison across the four mantle color groups. With these descriptive statistics, T-Tests and analyses of variance (ANOVA) were completed to determine significance within the variance across the mantle scale. Linear regressions were completed to determine whether any other morphological feature had a correlative relationship to the scallop height. Histograms were completed for each morphological feature to determine the distribution of each physical trait among the cohort, enabling the analysis of individual groups within the cohort in terms of the parent clams they were derived from.

3.0 RESULTS

3.1 Descriptive Statistics

A total of 54 clams were measured and analyzed. Means for the length, width, depth, scallop height, and volume of the cohort were 64.44 cm (+/- 4.48 cm), 36.44 cm (+/- 3.33 cm), 38.31 cm (+/- 4.17 cm), 11.60 cm (+/- 2.58 cm), and 90.66 L (+/- 18.90 L), respectively. Mantle color pattern #1 (light) was by far the most abundant type (25 individuals), while pattern #2 (dark) was the least abundant type (6 individuals). Figure 2 shows the distribution of the mantle patterns across the individuals studied. One clam within the cohort was noticeably bleached, with about 70% of the mantle surface visibly free of algae and was 2.04 cm smaller in length, 1.36 cm greater in width, and 0.59 cm greater in depth when compared to the cohort averages. The mean values for the three clams analyzed in Rick Braley's aquarium, followed by a comparison to the mean of the cohort are as follows: Length: 59.86 cm (-4.48), Width: 35.06 (-1.37), Depth: 38.2 (-0.11), Scallop Height: 10.66 (-0.94), Volume: 81.00 (-9.66).

Figure 2: A graphical representation of the distribution of the 4 mantle pattern groups across the entire cohort



Mantle Number	Length (cm)	Width (cm)	Depth (cm)	Scallop Height (cm)	Volume (L)
Light - 1	65.76 (+1.32)	35.992 (-0.45)	38.44 (+0.13)	11.72 (+0.12)	90.99 (+0.32)
Dark - 2	63.5 (-0.94)	35.92 (-0.52)	39.62 (+1.30)	11.5 (-0.10)	90.35 (-0.31)
Light w/ Dark Spots - 3	63.1 (-1.34)	37.34 (+0.90)	38.56 (+0.24)	11.79 (+0.19)	90.85 (+0.19)
Dark w/ Light Spots - 4	63.54 (-0.90)	36.66 (+0.22)	36.87 (-1.45)	11.01 (-0.59)	85.88 (-4.78)
Bleached	62.4 (-2.04)	37.8 (+1.36)	38.9 (+0.58)	13.00 (+1.40)	91.75 (+1.09)

Table 2 shows the means for each of the morphological features among the mantle pattern groups, including the bleached individual.

Table 2: A summary of the morphological means for each mantle color, with their difference from the mean in parentheses.

3.2 ANOVA

An analysis of variance (ANOVA) between each of the four mantle pattern groups for each of the morphological measurements was conducted, yielding the following results: Length (p-value: 0.283), Width (p-value: 0.697), Depth (p-value: 0.624), Scallop Height (p-value: 0.894), Volume (p-value: 0.911). The means of the two lighter colored mantle patterns (1&3) were combined and compared with the combination of the means of the two darker colored mantle patterns (2&4) using a standard t-test, yielding the following results: Length (p-value: .335), Width (p-value: .964), Depth (p-value: .657), Scallop Height (p-value: .492), Volume (p-value: .557).

3.3 Regression

Width had the closest correlative relationship with scallop height in comparison with the rest of the morphological features but was not statistically significant in its relationship ($R2$ = .33691). The least correlative morphological feature related to scallop height was length ($R2$ = .006447). Figure 3 shows the correlations:

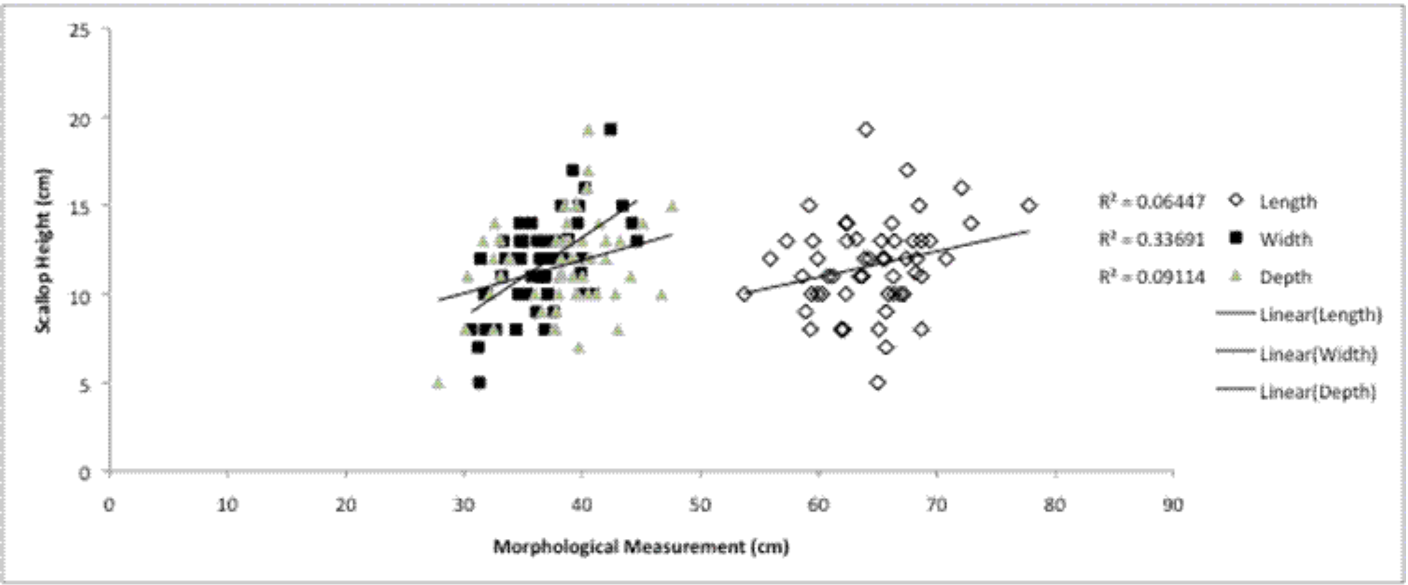


Figure 3: A graphical interpretation of the correlation between length, width, and depth on the scallop height across the cohort.

3.4 Morphological Frequency

3.4.1 Length: For the frequency graph regarding length, the peak frequency occurs at 64 cm (the average value for the cohort). The graph indicates a slight negative skew, with higher frequencies occurring to the right of the peak in comparison to the left of the peak. There is an interesting drop in frequency just below the mean at 62 cm, followed by an increase in frequency as the size decreases from the mean.

Figure 4: The frequency distribution of the length of the clams across the cohort

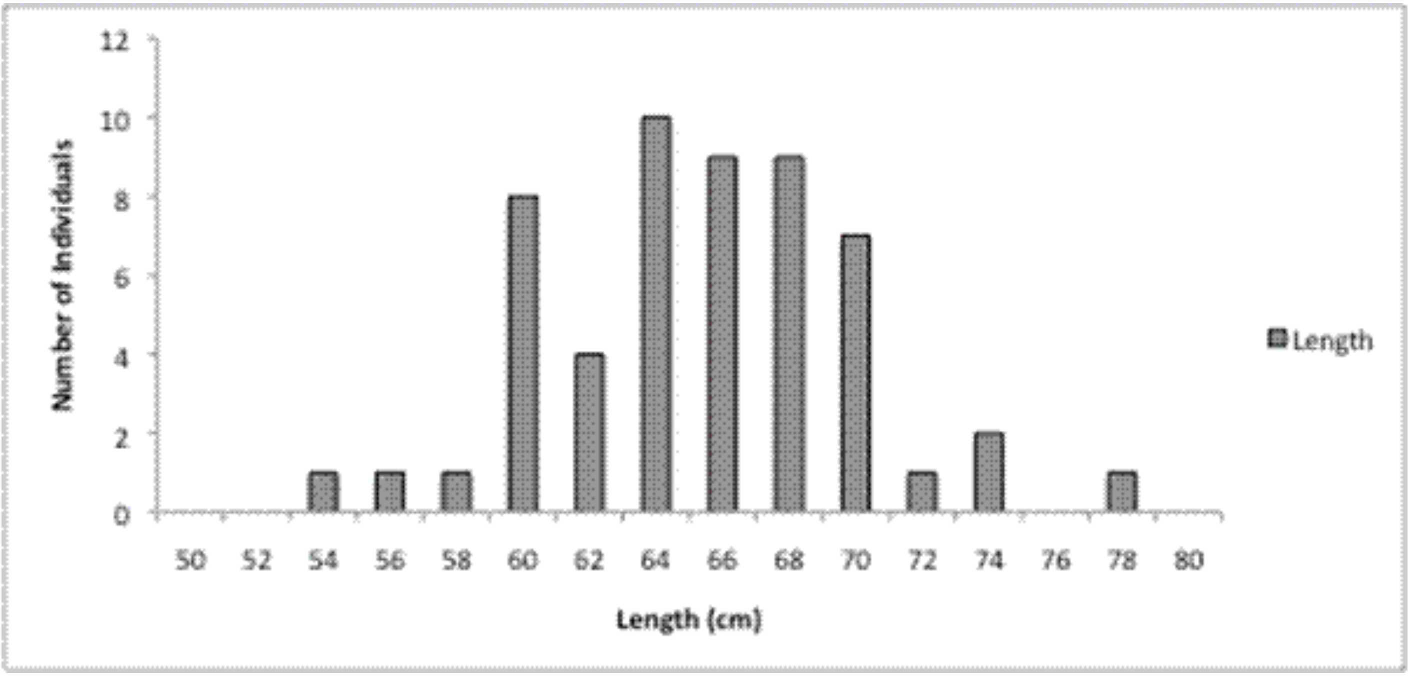
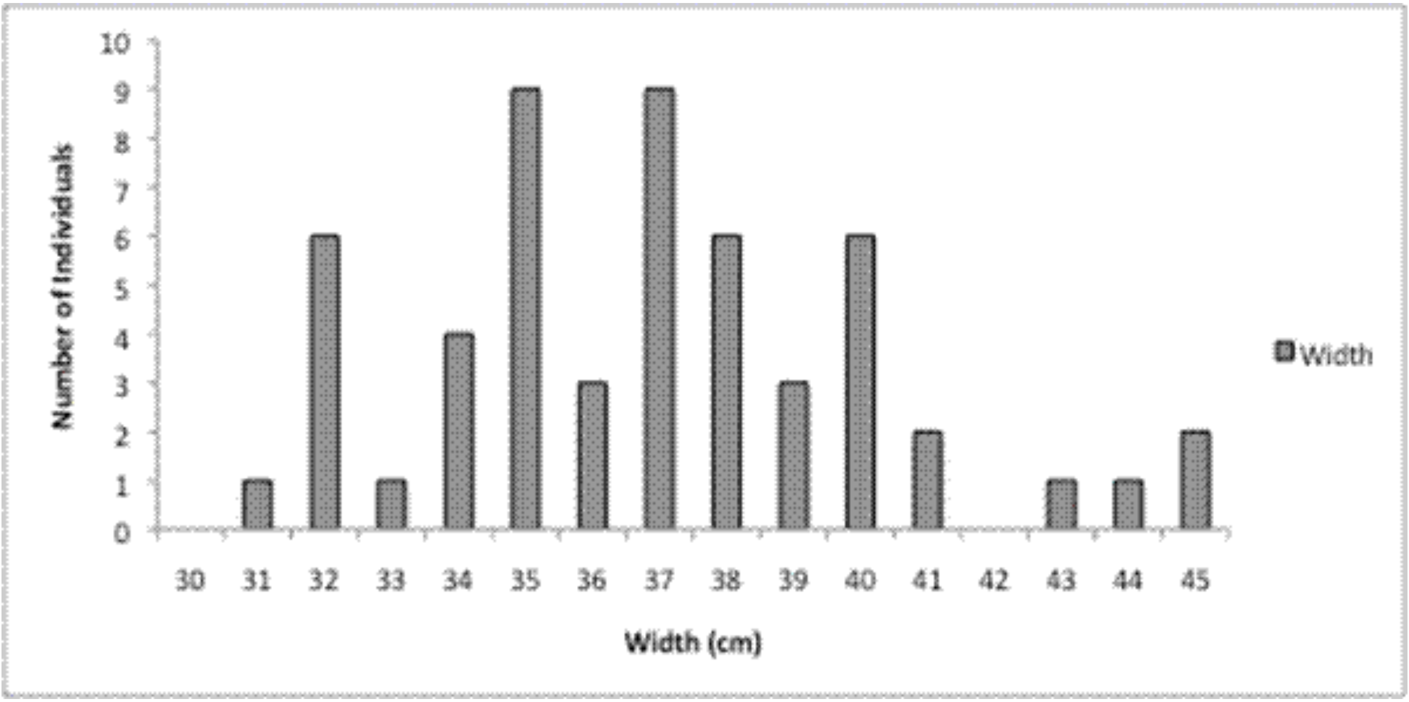
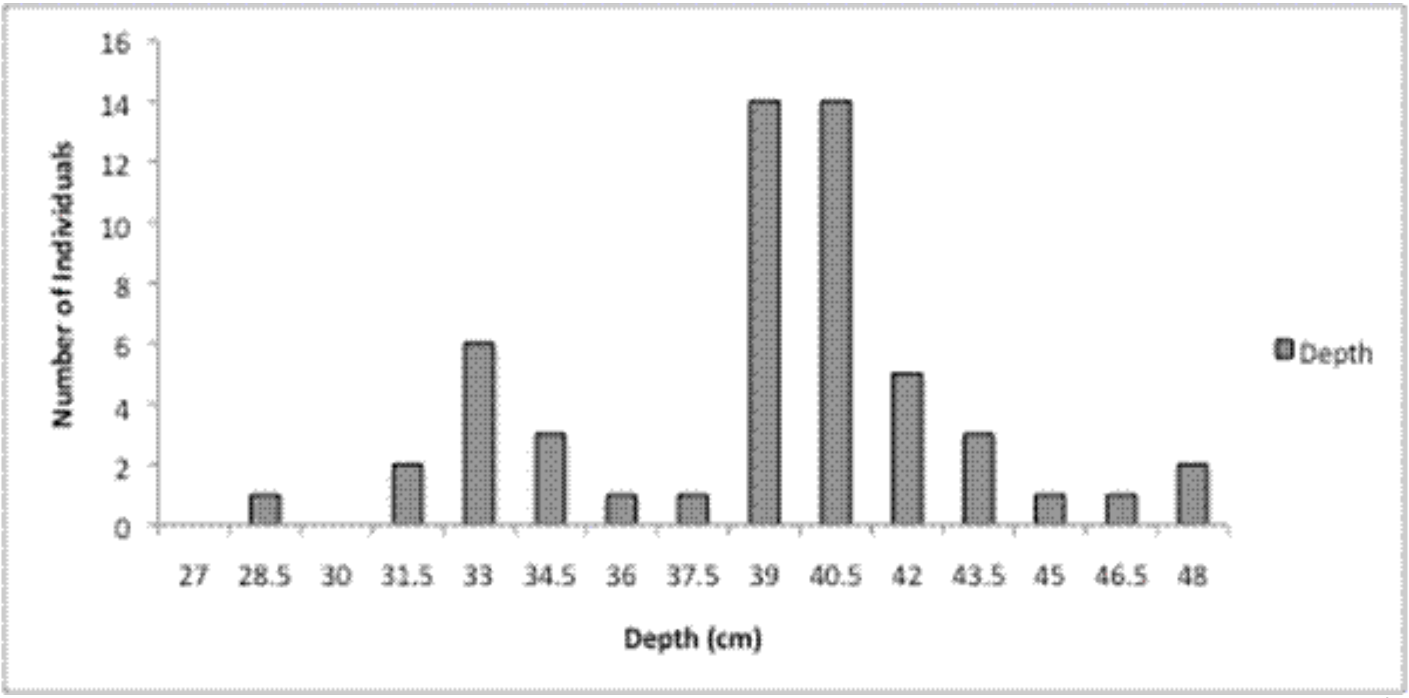


Figure 5: The frequency distribution of the width of the clams across the cohort



3.4.2 Width: For the histogram on width, there is much more variability in the frequencies corresponding to the associated widths. There are two peaks at 35 cm and 37 cm, with many gaps and varying degrees of frequency spread between each subsequent width bound. The graph, while having a peak near the average, is far from a normal distribution curve.



3.4.3 Depth: The histogram regarding depth shows an overwhelming peak between 39 cm and 40.5 cm, with over half of individuals registered within this small bound. The graph then thins out in either direction of the mean, but in opposite trends, with a frequency trend increasing as you deviate from the mean in the negative direction, and decreasing as you deviate from the mean in the positive direction.

Figure 6: The frequency distribution of the depth of the clams across the cohort

3.4.4 Scallop Height: This graph for the frequency of scallop height represents a near perfect normal distribution among the cohort, with a peak at 12 cm, and an equally gradual decline in frequency in either direction of the mean.

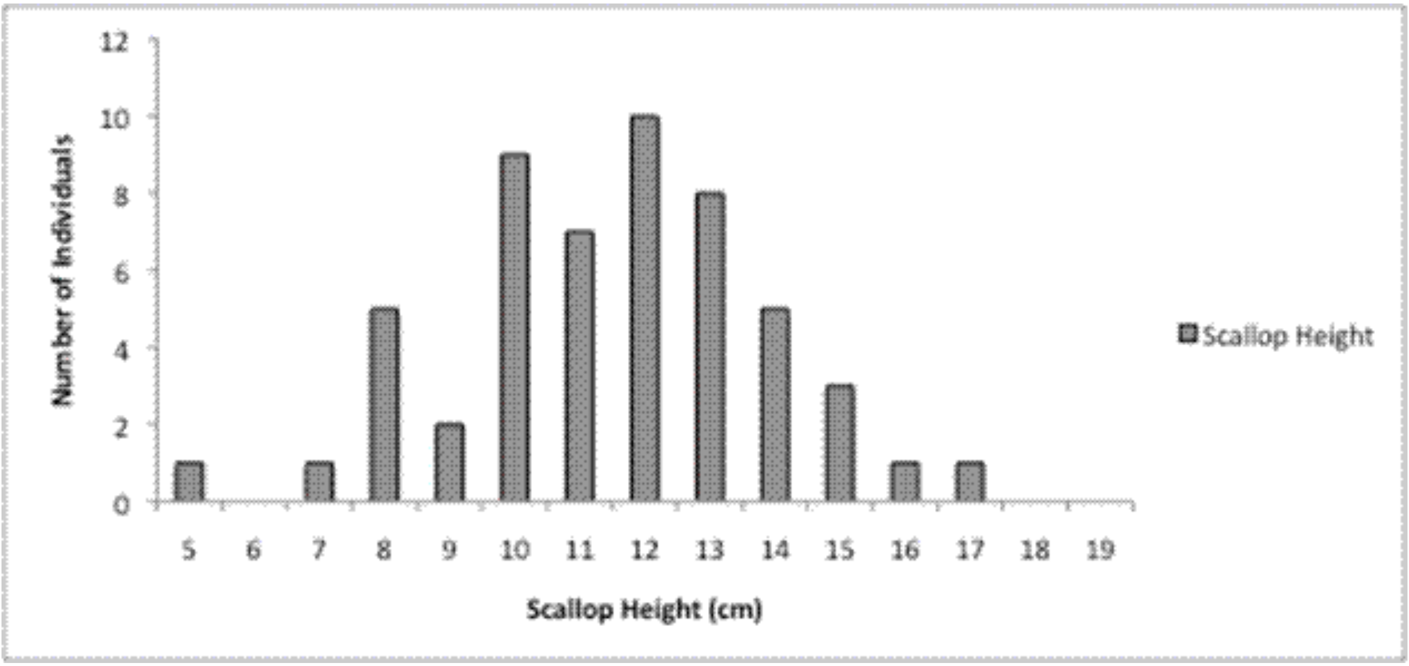
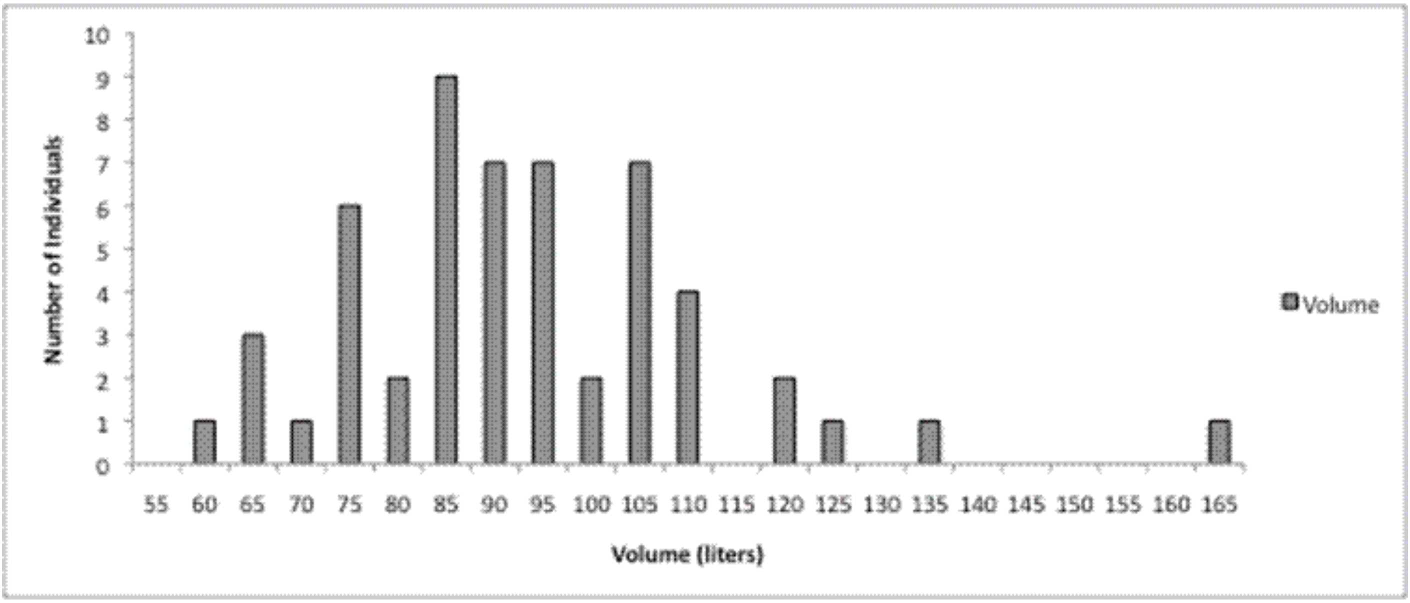


Figure 7: The frequency distribution of the scallop height of the clams across the cohort

Figure 8: The frequency distribution of the volume of the clams across the cohort



3.4.5 Volume: The histogram focusing on volume peaks at 85 liters, with a frequency of 9 individuals. Left of the peak, frequencies decline rather rapidly, while frequencies to the right of the peak remain quite high. This indicates a positively skewed graph, with more frequencies in the right tail of the curve than would be expected in a normal distribution. There is an obvious outlier at 165 L.

4.0 DISCUSSION

4.1 Zooxanthellae and Mantle Color

After conducting the experiment and analyzing the data, the p-values derived from the data (ranging from 0.283 to 0.911) indicate that there is no statistically significant difference between the means of each morphological feature across the four mantle patterns in the cohort. Even when the two light and dark mantle patterns were combined and compared with one another, the differences between the means lacked statistical significance. Basing a conclusion on this experiment alone, it would appear that the number of zooxanthellae within the mantle of T. gigas, through observational analysis, has no significant influence on the morphological features of giant clams. This disproves the originally postulated idea that the higher the apparent levels of zooxanthellae in the mantle, the larger and faster the clam will grow due to the resulting increase in metabolic efficiency.

A study completed in 2002 tried to determine a genetic link within mantle color but found nothing (Laurent et. Al). A study done in 2000 on the same cohort of clams used in this study concluded that the color of the mantle does in fact describe the number of zooxanthellae, with darker mantles having higher concentrations of the algae. However, the study also concluded that the mantle patterns can change over the course of a couple of years (Sullivan 2000). It may be that the mantle patterns change often enough to make any influence on growth rate indistinguishable from the rest of the cohort. This also seems to indicate that the giant clams have control over the number of zooxanthellae in their mantle, an important trait to have when combating certain environmental stresses. A long-term study on a cohort like this one, where the sizes and mantle colors of the clams are recorded repetitively and throughout the lifetime of the clams, could perhaps identify whether there is indeed a correlation between mantle color and morphological growth.

4.2 Morphological Correlation – Scallop Height

With R2 values ranging from 0.06 to 0.33, it can be concluded that the length, width, and depth of the giant clam have no linear correlation to the scallop height. In other words, whether the clam is very long or very wide has no influence on whether the scallop height will be longer or shorter. In the context of this study, the influence of zooxanthellae on the size (length, depth, and width) of the clam was disproven, so it can also be concluded that the algae has no influence on the scallop height. A future study that measures both scallop height and scallop width to provide a scallop area may yield a more significant correlation. If scallop height is a distinct genetic feature that it not as proportionally determined by environmental conditions, as other morphological features may be, it can be an accurate way of analyzing the diversity of genetic expression in a cohort of clams like this one.

4.3 Bleached Individual

Although there was only one individual identified with significant bleaching, it is worth comparing the means of its morphological features to the rest. Interestingly, while the clam was 2.04 cm smaller in length compared to the rest of the cohort, it was 1.36 cm greater in width and 0.59 cm greater in depth than the means. It is possible that bleaching has the largest influence on length, as opposed to the other morphological features, or it's possible that this clam hasn't been too affected by the bleaching, and the shorter length can be described by something other than the presence of zooxanthellae. A study done in 1998 on this same cohort also found that there was no significant difference in length between bleached and unbleached clams (Seilo 1998). This 1998 study was done following a large bleaching event, when more of the individuals of the cohort had apparent bleaching.

4.4 Morphological Frequencies

Although the histograms on morphological features do not provide us with 3 distinctly apparent groups, corresponding to the coupling of parents associated with this cohort, these graphs do help us analyze the distribution of morphological features across the cohort. While all of the graphs have peaks at the means, what the graphs do from there tends to vary with each feature. In the scenario of length, the graph represents a close to normal distribution. When comparing the frequency graph of width to length, it is easy to realize the increased variance present in the width frequency graph. With many gaps and sporadic increases in frequency far from the mean, it appears that the width is far more variable within a genetically similar cohort. The depth frequency graph shows much less variance, with more than half of the individuals ranking within a rather small bound. The graph of volume essentially combines these three features together, yielding a rather normal distribution pattern, yet still containing sporadic jumps and dips in frequency. Since scallop height has been proven to have no linear correlation with the other morphological features in the study, the observed normal distribution makes sense. If the scallop height is predetermined genetically and is not influenced by surrounding environmental conditions (like eye color, for example), then this graph can be used to analyze the gene expression variability within the cohort. It is possible that, had the number of individuals within the cohort been significantly higher, that certain groups could become much more apparent within the graph, corresponding to the parents of each group.

4.5 Management

As abundance numbers for T. gigas continue to decrease as a result of overexploitation, it is becoming increasingly more important to develop effective management plans to prevent the species from the possibility of extinction. Because they are sessile organisms that live in relatively shallow waters, they are extremely vulnerable to poachers, hunting them for the valuable adductor muscle and shell. The institution of an annual quota, in union with size restrictions, appears to be a valuable tool in terms of managing the long-term health of the species. It has also been postulated that establishing refugia for the species with greatly increase recruitment levels, helping the species bounce back to prior levels (Munro 1991).

The possibility of using mariculture as a means of returning population levels to where they once were across the Indo-Pacific is becoming a large topic on the management of the species. With continued success and rising efficiency in the culture of the giant clam, it is a potentially useful strategy for restocking these depleted reefs.

4.6 Conclusion

As an endangered species, it is important to understand everything there is to know about giant clams so that proper management techniques can be implemented to help T. gigas from extinction. Understanding the complexities between the clam and its symbiotic partner, zooxanthellae, may play an important role in the future success of the species, as we learn the conditions in which the species thrives. The continued study of cultured clams is vitally important in determining their potential to replenish depleted numbers across the Indo-Pacific. As a staple organism of the Great Barrier Reef and the Indo-Pacific region, the maintenance of species abundance and the prevention from extinction are important for maintaining the health of the associated ecosystems in general. If fascinating organisms such as the giant clam continue to exist, the argument for the preservation and protection of the ecosystems will increase, helping the continued success of all species in the area.

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APPENDIX

WHITE LADY BAY GIANT CLAM MEASUREMENTS							
Number	**Length**	**Width**	**Depth**	**Scallop Height**	**Mantle Color**	**Volume**	**Comments**
1	68.5	38.2	38.5	15	1	100.74295	
2	64	42.4	40.5	19.3	3	109.9008	
3	65.4	38.4	40.7	12	3	102.212352	
4	70.8	40	42	12	1	118.944	
5	66.4	34.7	38.4	13	3	88.476672	
6	68	44.6	40	13	4	121.312	
7	65.9	40.9	38	10	1	102.42178	
8	63.2	38.8	33	13.1	4	80.92128	
9	68.2	39.9	38.2	11.2	3	103.949076	
10	60.8	36.7	38.2	11	3	85.237952	
11	68.7	34.4	30.1	8	1	71.134728	
12	65.1	30.6	32.5	8	1	64.74195	
13	58.9	36.1	37.8	9	3	80.373762	
14	63.7	36.5	40	11	1	93.002	
15	62.1	31.8	37.8	8	1	74.646684	
16	59.9	37.3	33.9	12	4	75.741753	
17	62.3	34.7	32.6	14	1	70.475006	
18	68.7	36.9	33.1	11	3	83.909493	
19	65.3	33.3	31.6	13	4	68.713884	
20	63.9	34.1	32.5	12	4	70.817175	
21	65	31.3	27.8	5	1	56.5591	
22	62.4	37.8	38.9	13	BLEACHED	91.754208	
23	57.3	34.9	37.8	13	3	75.591306	
24	66.4	37.1	41.2	10	3	101.493728	
25	59.3	36.8	37.6	8	3	82.052224	
26	67.5	39.2	40.5	17	1	107.163	
27	59.9	39.9	39.6	10	4	94.644396	
28	69.4	35	43.2	13	1	104.9328	Green Tint
29	72.9	44.2	41.4	14	2	133.398252	
30	62.3	35.3	32.2	10	4	70.813918	
31	62.4	39.6	38.7	14	3	95.629248	
32	61.2	33.2	30.3	11	1	61.564752	
33	65.7	37.6	36.6	9	1	90.413712	
34	59.2	39.7	39.5	15	1	92.83448	
35	68.7	36.2	42	13	1	104.45148	
36	55.9	34.8	38.2	12	1	74.311224	
37	68.3	37.5	33.8	12	1	86.57025	
38	63.5	35.7	44.1	11	1	99.972495	
39	64.3	31.4	40.5	12	1	81.77031	
40	66.2	35.7	45.1	14	1	106.586634	
41	77.8	43.4	47.6	15	1	160.722352	
42	59.5	37	38.8	13	2	85.4182	
43	61.9	32.7	43	8	2	87.03759	
44	58.6	36.7	39.2	11	1	84.304304	
45	67.4	33.5	39.4	12	2	88.96126	
46	66.3	33.2	38	11	1	83.64408	
47	72.1	40.2	40.4	16	1	117.096168	
48	66.9	35	46.7	10	4	109.34805	
49	59.4	34.6	40.5	10	3	83.23722	
50	67.2	31.9	42.8	10	1	91.749504	
51	65.7	31.2	39.7	7	4	81.378648	
52	60.3	37.1	39.5	10	4	88.366635	Tank 7
53	53.7	31.7	36	10	2	61.28244	Tank 3
54	65.6	36.4	39.1	12	2	93.364544	Tank 2