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BALANCING ANTI-PREDATION AND ENERGETIC NEEDS: COLOR POLYMORPHISM IN THE GIANT CLAM *TRIDACNA* *MAXIMA*

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Abstract. Color polymorphism has been implicated as an important component of cryptic coloration in organisms inhabiting complex environments. Recent studies have suggested that mantle color variation in Tridacnid clams may serve various functions, including as a mechanism to achieve background matching. The mantle color variation of *Tridacna maxima* was examined in a series of experiments, including a background-matching photo survey, a predation experiment, and a zooxanthellae count. The results of the photo survey showed a significant correlation between *T. maxima* mantle and background color. *T. maxima* which did not match their background experienced a significantly greater rate of predation. Finally, the population of zooxanthellae was seen to increase for the same size of mantle area with age. These results suggest that balancing photosynthetic efficiency and anti-predation needs can be addressed by mantle color variation throughout the lifetime of *T. maxima*.

Key words: bivalves; *Tridacna maxima*; cryptic coloration; Mo'orea, French Polynesia; iridophores

INTRODUCTION

Coral reefs are a natural laboratory for the study of complex ecological concepts because of their extraordinary biodiversity (Connell 1978). Despite supporting an abundance of species on par with the tropical rainforests, coral reefs exist in especially nutrient-poor oceans (Hubbell 1997). How such biodiversity can be supported by a nutrient-poor habitat is largely due to the symbiotic association of dinoflagellate single-celled algae of the *Gymnodinium* genus with anthozoan and other invertebrate inhabitants of the reef (Muscatine and Porter 1977). Scleractinian (reef-building) corals, anemones, and sponges all supplement their energy requirements through the products of photosynthetic symbionts to some degree (Van Oppen *et al.* 2005). This association allows for high photosynthetic efficiency, high nutrient flux and efficient

nutrient cycling, essentially allowing coral reefs to exist (Muscatine and Porter 1977).

Because coral reefs display such high complexity of structure in a small area, they pose difficult adaptive challenges (Todd *et al.* 2004). The complex web of predation that occurs with high biodiversity requires organisms to develop effective defense mechanisms (Strobel 2003). It has been experimentally observed by Merilaita and Lind (2005) that organisms whose coloration and pattern visually matches their background are at lower predation risk. This strategy is known as background matching or cryptic resemblance, and is defined by Endler (1978) as a color pattern that resembles a random sample of the background perceived by predators at the time and place where the prey is most vulnerable to predation. Thus, there is a fitness advantage gained by those

organisms which can best mimic the color and pattern of their background.

Among the most beautiful and conspicuous members of Indo-Pacific shallow water reef communities, Tridacnidae or 'giant clams' are the largest and fastest growing members of class Bivalvia (Pearson and Monro 1991). This fast growth rate and large size can be partially attributed to the metabolic contribution of the symbiotic algae *Gymnodinium microadriaticum*, (referred to hereafter as zooxanthellae), which exist within the tissues of the clam mantle (Sutton and Hoegh-Guldberg 1990). Though originally thought to host the same species of zooxanthellae found associated with scleractinian corals and other symbiotic reef members, recent genetic evidence indicates a high degree of specificity in host-symbiont species relations (Weis *et al.* 2001). The family Tridacnidae contains ten species belonging to the two genera *Hippopus* and *Tridacna* (Richter *et al.* 2008). All members of the family represent an important food resource for reef-dwelling predators like fish, clams and gastropods, as well as a food source for humans (Han *et al.* 2008, Green and Craig 1999).

Within genus *Tridacna*, the species *Tridacna maxima* is the most widespread and morphologically variable member (Rosewater 1965). Found in shallow reefs between one and 33 foot depths, and ranging from the East coast of Africa to Pitcairn Island in the Eastern Pacific, *T. maxima*'s high fecundity and burrowing lifestyle account for its great range (Lucas 1994). Perhaps the most striking characteristic of *T. maxima* is the remarkable coloration and patterning found on the clam mantle. These patterns are the result of their association with symbiotic zooxanthellae, which reside within tubular structures throughout the exposed mantle (Norton *et al.* 1992). Along with the pigments of the zooxanthellae themselves, iridophores, which are found chiefly along the mantle edge, produce iridescent colors ranging from deep blue to teal (Griffiths *et al.* 1992). Although

iridophores and zooxanthellae are common among all *T. maxima*, it is the unique placement of these features that gives rise to the large variation in color patterning seen among individuals within the same species.

Along with contributing to the color variation seen among individual *T. maxima*, iridophores have been hypothesized as playing an important role for all Tridacnids. Composed of aggregations of cells known as iridocytes, which themselves contain structures known as iridosomes, Griffiths *et al.* (1992) suggested several possible functions for iridophores. When the stacked platelets of iridosomes are parallel to the direction of incident light, they maximally interfere in the blue and ultraviolet wavelengths, thus possibly protecting the clam from ultraviolet light. When this stacking is instead perpendicular to the direction of incident light, Griffiths *et al.* predicted that iridophores could act as diffraction gratings, bending light towards zooxanthellae. Consequently, iridophores could also act to increase photosynthetic output of symbionts.

As sedentary members of the reef community, *T. maxima* are at constant risk of predation from a wide variety of marine organisms. Large triggerfish, octopuses, pufferfish and eagle rays are all known predators of adult *T. maxima*, while xanthid and portunid crabs, as well as the snail *Cymatium muricinum*, are known predators of juvenile clams (Chambers 2007). Consequently, *T. maxima* have developed several defense mechanisms designed to defend their sensitive mantle and visceral mass. Shell projections known as scutes increase the overall size of the shell, thus preventing crushing by crabs (Han *et al.* 2008). Also, observation of shadows or distant movement by their true eyes along the mantle edge will cause the clams to withdraw their mantle and close their shells (Wilkens 1986). However, each of these defense mechanisms is energetically expensive, and it would be evolutionarily advantageous for *T. maxima* to also utilize more passive strategies.

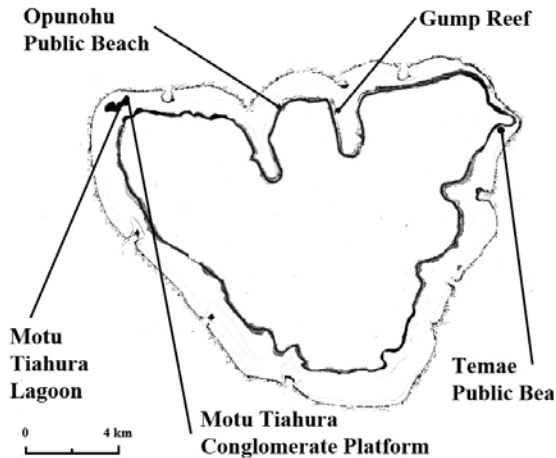


FIG. 1 Map of Mo'orea, French Polynesia, showing locations surveyed during the background matching photo study.

All research for the present study was conducted on the island of Mo'orea, French Polynesia (17°31' S, 149°50' W). Located in the south central Pacific, Mo'orea is a high island in the Society Islands chain (Fig. 1). The second youngest island in the chain, Mo'orea supports developed barrier and fringing reef around much of the island, separated by a shallow sandy lagoon. *T. maxima* has been observed between 2-15 feet in both fringing and barrier reef environments (personal observation). A source of gathered food for residents, *T. maxima* populations have been impacted by human fishing, with the largest individuals often being harvested for consumption (Wagner 2001).

Todd *et al.* (2009) recently examined the coloring of the closely related species *Tridacna crocea*, in an attempt to determine whether background matching is occurring in that species. They found a significant correlation between mantle color and background color. However, Todd *et al.* did not examine this connection for impacts on the risk of predation experienced by *T. crocea*. Therefore, the present study was undertaken to expand on this previous research to (1) determine whether similar background matching trends occur in other species of Tridacnidae,

specifically *T. maxima*; and (2) assess whether cryptic coloration, if present, reduces predation rates.

In addition, the present study was carried out to attempt to test the two hypotheses of Griffiths *et al.* Though it is difficult to examine the effect of iridophores on UV tolerance and symbiont photosynthetic output directly, proxy tests can be performed. Investigations into the distribution of color morphs with depth, and with age may support or refute Griffiths *et al.*'s hypotheses. Iridophores contribute greatly to mantle color, and their presence or absence may help to extend the understanding of how color polymorphism can serve multiple purposes in a population.

Therefore, the following study included these components: (1) Populations of *T. maxima* at five sites around the island of Mo'orea, French Polynesia were examined for evidence of background color matching, testing the hypothesis that *T. maxima* is utilizing cryptic coloration. The survey also included measurements of predation rates based on visible scars on the mantle in order to test whether background matching is in fact an effective defense mechanism. (2) An artificial reef experiment was undertaken. Using a controlled background color on an artificial reef, rates of predation were tracked to determine the importance of color contrast in predator avoidance. It was hypothesized that those *T. maxima* which poorly matched their artificial background would experience higher predation rates. (3) Finally, a zooxanthellae count was undertaken to determine whether an age discrepancy occurs in terms of the number of symbionts per the *T. maxima* mantle area. The goal of this was to determine a justification for the prevalence of non-matching color morphs observed at a younger age. It is hypothesized that non-matching color morphs are an adaptation to increase symbiont photosynthetic capacity, through an abundance of light refracting iridophores.

METHODS

Background matching

Methods for the photo study to test background matching were closely drawn from Todd *et al.* (2009). A photo survey was conducted at five sites around Mo'orea, French Polynesia in locations where high populations of *T. maxima* had been observed previously (Fig. 1). These sites included Gump Reef (17°29'22.75"S, 149°49'32.39"W), Opunohu Public Beach (17°29'25.31"S, 149°51'0.25"W), Temae Public Beach (17°29'56.23"S, 149°45'26.11"W) and the Tiahura Motu channel and conglomerate platform (17°29'7.71"S, 149°54'40.88"W). Photos were taken at high tide over six days in October, 2009. Over the course of two hours at each site, photographs were taken of each *T. maxima* encountered along the fringing reef between the beach and the lagoon.

All photos were taken using a Cannon PowerShot D10 digital camera, using a 3.0x zoom, no flash and the camera's 'auto' setting. An apparatus, consisting of two 60 cm steel pipes attached with an L-bend, was constructed to ensure a constant distance and angle for every picture taken. The camera was attached to the top of one pipe using zip-ties, and directed to focus at the end of the other pipe, thus ensuring that all photos were taken 84.85 cm from the clam. A white piece of underwater paper was attached to the end of the focus pipe, and therefore appeared in every photo. This paper was used as a reference for white balance. All photos were taken with the focus pipe horizontal, thus making all photos taken from a 45 degree angle.

Before taking the photo, light intensity measurements were taken in Klux, using an Exttech Instruments EasyView 30 light meter. To standardize light readings, the max function of the light meter was used. The meter was held as close to the clam as possible for ten seconds, while the meter recorded the greatest intensity of light received. The photos

were then taken within five seconds of taking the light measurements. If an obvious change in light conditions occurred in this time, the light reading was taken again.

After taking the photos, depth, and shell length measurements were recorded using a UWATEC 330m digital depth gage and a 20 centimeter metric ruler. Because the dive computer was only capable of measuring depth in whole foot integers, depth variations within a foot were not measured. The shell length was measured in centimeters, taken from the most distal points on each side. Additionally, rates of predation were recorded for each *T. maxima* by counting the number of scars on the mantle. Only obvious scars along the mantle edge were counted, as any on the mantle surface could be mistaken for natural patterning variation. Finally, the substrate immediately surrounding the clam was marked with colored chalk, to insure no repeat photos were mistakenly taken.

In all, 127 clams were photographed at the five different sites. *T. maxima* were grouped by size and color morph in order to aid statistical evaluation. According to Chambers (2007), *T. maxima* begin to reach sexual maturity as males around 5 cm, then about 50% become hermaphroditically mature around 10 cm, whereas 100% are hermaphroditically mature at 14 cm and larger. Thus, the surveyed clams were classified into three size groupings; 0-5 cm, 5.1-10 cm and > 10.1 cm.

Consistent with the methods of Todd *et al.*, descriptive names for the color morphs were developed. The color morphs were identified as 'white with green edge' (W), 'olive blue' (O), 'khaki' (K), 'green-blue' (GB), 'cloudy green' (C), 'dichromatic teal' (T), 'purple' (P), and 'electric blue' (E). All observed clams fit broadly into one of these categories, although there was considerable variation in pattern, if not color among each category.

Along with dividing all *T. maxima* into specific color morphs, they were also broadly categorized into generally 'brown' or 'blue.'

The brown category included all “white with green edge, ‘olive blue’, ‘khaki’ and “cloudy green’ clams, while the general blue category included the remaining four color morphs. These two general categories were then analyzed along with size category, using a chi-squared test for independence.

The photos of the *T. maxima* were analyzed in Adobe Photoshop CS2 (Adobe Systems), comparing the RGB values of the clam mantle tissue to the RGB values of the background. RGB values are a standard quantification for color tone and value. Each pixel of a digital color photo has three values between 0-255, one for each of the colors red, green and blue. When these three values are combined, the enormous possible variation can account for all known colors.

For the purpose of standardizing analysis, not all clams were used in the RGB value matching study. In order to reduce the impact of environmental variability on the photo analysis, light intensity levels were used to exclude some clams. Only photos taken in light levels between 10.0-100.0 Klux were analyzed. As a result, a total of 122 clams were used for statistical analysis.

In order to account for size deviation between different clams, the number of RGB points taken was kept proportional to the size of the clam mantle according to the formula:

$$[\sqrt{\text{area of mantle}}] \times 2$$

Mantle area was calculated using the software ImageJ. In order to accurately determine area, the recorded size of each clam’s mantle was input into the ‘set scale’ function. The lasso tool was then drawn around the mantle area of each photo, and subsequently measured for area. This area was then entered into the above formula and rounded up to determine the number of RGB points to take for each *T. maxima*.

In Photoshop, an overlain 5 mm grid was used along with a random number generator to produce coordinates for RGB sampling. The

same numbers of values were taken on the mantle as the background, with the background being defined as two mantle widths from the edge of each clam.

From the resulting data, the mean RGB values were calculated for the mantle of each *T. maxima*, along with the mean RGB values for each clam’s background. These points were plotted for each clam, and used to calculate Pearson’s correlation coefficients for each of the three colors, i.e., red, green and blue.

Tile predation study

The investigation of the impact of background color matching to predation was conducted at Opunohu Public Beach (17°29'25.31"S, 149°51'0.25"W) in October and November of 2009. An artificial background was created to attempt to control for natural variation in the background of a reef. Square, (15 cm per side) hard plastic tiles were spray-painted a color that closely matched the ‘khaki’ *T. maxima* color morph. These tiles were then cut in half diagonally, after which the resulting triangles were drilled through the center with a hand-held power drill.

At Opunohu Public Beach, ten *T. maxima* were selected for treatment. Five were identified as the ‘khaki’ color morph while the other five were from the ‘electric blue’ categories. In order to standardize for down-welling light and other variables, all *T. maxima* selected for treatment were at a depth of 10 feet. In order to standardize any effects from microhabitat variation, only clams that were located on the top of coral heads with a greatest width of 60-80 cm were used. Finally, to reduce variation, all *T. maxima* selected had a shell length between 7-10 cm.

After identification of individuals, the artificial background tiles were installed. This consisted of attaching one half-tile on either side of the clam into the surrounding dead coral using nails through the drilled central holes. Tiles were installed with the longest triangle side parallel to the clam mantle axis.

Both the tiles and the clam mantle were aligned in a flat plane.

Tiles were installed on October 23rd, and then visited every Friday for three weeks, until November 13th, 2009. Upon installation and every week after, photos were taken to document predation rates, as well as a visual evaluation and recording of predation scars. Original predation scars were recorded and measured in the same manner as the background matching study. These scars were kept track of between each week while they healed, with only fresh scars appearing from the previous week being counted towards the total number of scars gained per clam. Scars healed slowly enough that observation was only necessary once a week.

The resulting data were analyzed using a paired two sample for means t-test, to determine whether the differences between the numbers of predation scars gained over the study period were significantly different between those *T. maxima* matching their background (tile) color and those that did not. Individual clams were also evaluated for changes that were not quantifiable, such as color change to match new background color over three weeks.

Besides the tile predation experiment, results from the photo study were also used to evaluate other factors potentially influencing *T. maxima* predation rates. A paired two sample t-test was used to evaluate the relationship between the number of observed mantle scars and general morph category. The relationship between mantle size and the number of bite marks was also evaluated through Spearman's rank correlation analysis.

Zooxanthellae counts

Methods for the zooxanthellae counts were partially drawn from Griffiths and Klumpp (1996). All *T. maxima* used for this study were wild specimens collected at the barrier across from Gump Reef (17°28'57.48"S, 149°49'44.55"W). A total of seven clams were taken, ranging from 2.98 to 13.45 cm long. As

T. maxima is a CITES listed species, efforts were made to minimize the number of clams that had to be taken. Each was collected at depths between 6 to 10 feet, and placed in outdoor 3 m diameter, 0.5 m deep tanks supplied with filtered seawater. These clams were allowed to acclimate for a week before zooxanthellae counts were taken.

Mantle area was quantified as described above in the background matching experiment. Pictures were taken directly above the clams from a distance of 0.5 m, and only after clams were allowed ten minutes to acclimate to the disturbance of setting up the camera apparatus. All photos were taken on clear, sunny days, in order to maximize mantle extension.

To quantify zooxanthellae of a *T. maxima*, the shell was first cracked using a hammer and chisel, after which the visceral mass was sliced from the shell using a razor blade. The mantle was then cut from the visceral mass and separated into right and left regions. The left mantle lobe and the visceral mass were placed in separate dishes and dried.

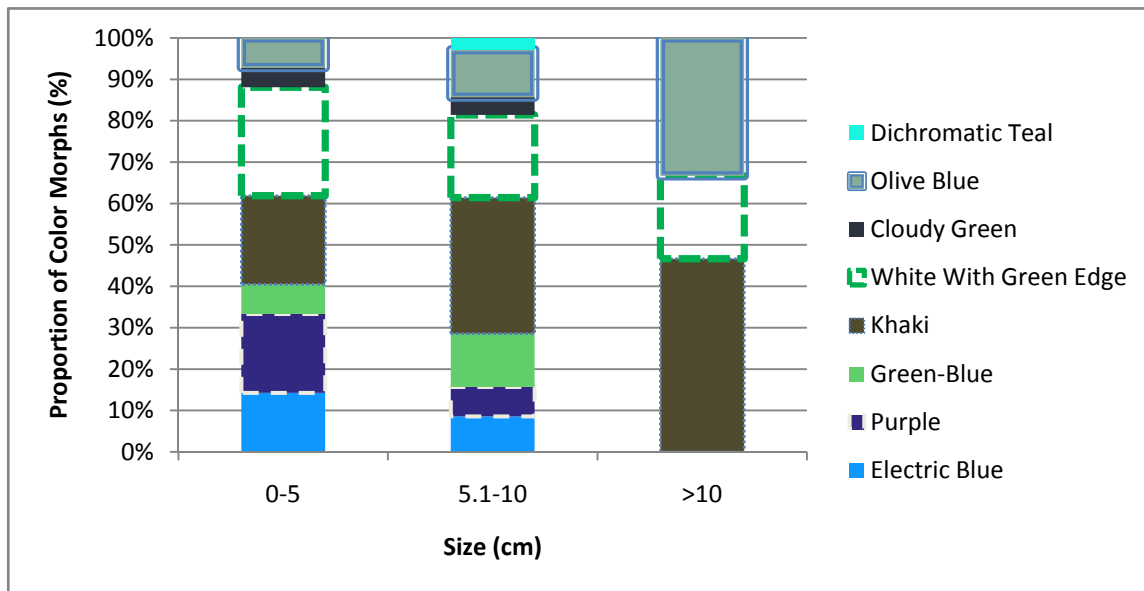
The right half of the mantle was cut into small pieces and homogenized in a kitchen blender for 5 minutes, after first being covered in 500 ml of filtered seawater. The resulting homogenate was filtered through a #4 coffee filter. One pipette drop of this zooxanthellae suspension was then examined using a compound light microscope at 100x magnification. Digital photographs were taken of the slides, with a focus on the area with the highest observed density of zooxanthellae. These photos were first made black and white in Photoshop, and then quantified using the count particles function in ImageJ.

RESULTS

Color morphs

The photo survey included 127 *T. maxima*; 42 with a shell length below 5 cm, 70 between 5.1 and 10 cm, and another 15 larger than 10 cm (Fig. 2). As described above, 8 color

FIG. 1 Distribution of the eight observed color morphs of all surveyed clams (n=127). Clams arranged by size category, based on reproductive maturity. Below 5 cm, *T. maxima* are non-reproductive. Between 5-10 cm clams are reproductive as males, and above 10 cm are hermaphroditic. A significant decrease in the proportion of blue morphs with age was observed, along with corresponding increase in brown morphs proportion (Chi-squared, df=2, $\chi^2=8.548$, p=0.0139). No blue morphs were observed above 10 cm.



morphs were identified, dividing evenly between generally 'blue' and generally 'brown' categories. The most common color morph observed was 'khaki', with 39 individuals. The least common was 'dichromatic teal', with 2 individuals observed. 'Dichromatic teal' was defined as a unique color morph, in that the mantle color would change between teal and electric blue, depending on the angle of the light.

The proportions of generally 'blue' and generally 'brown' morphs were significantly different (Chi-squared, df=2, $\chi^2=8.548$, p=0.0139) among the three different size categories. In the size range between 0-5 cm, 40.5% of the clams fell into the generally blue category. In the mid-size range, 31.4% of the clams were generally 'blue.' In the largest size, no blue color morphs were observed (Fig. 2). The proportion of generally 'brown' color morphs increased with size, until 'brown' color morphs were the only observed morphs above 10 cm.

The 127 clams were also evaluated for relationships between color morph and depth

(Fig. 3). Average 'blue' morph depths were significantly deeper than average 'brown' morph depths (t-test, df=3, t=2.4208, p= 0.0471). 'Dichromatic teal' morphs had the greatest mean depth at 5 ft, while the 'olive blue' morph had the shallowest mean depth at 2.86

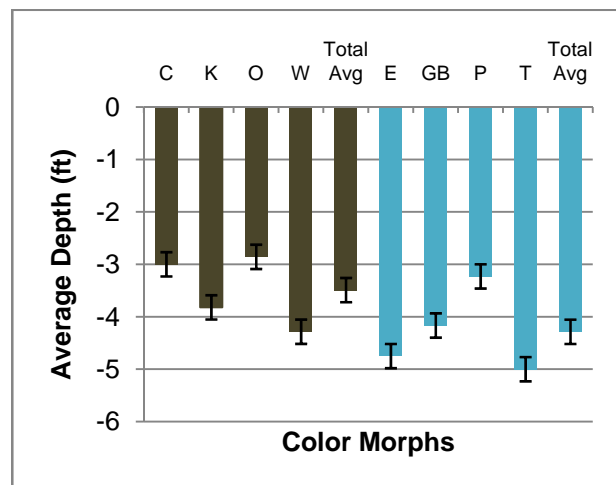


FIG. 3 Average depth of the eight *T. maxima* color morphs observed in the photo survey, along with total average for all brown and blue morphs. A paired one-tail t-test showed that blue morphs were found significantly deeper than brown morphs (t-test, df=3, t=2.4208, p= 0.0471).

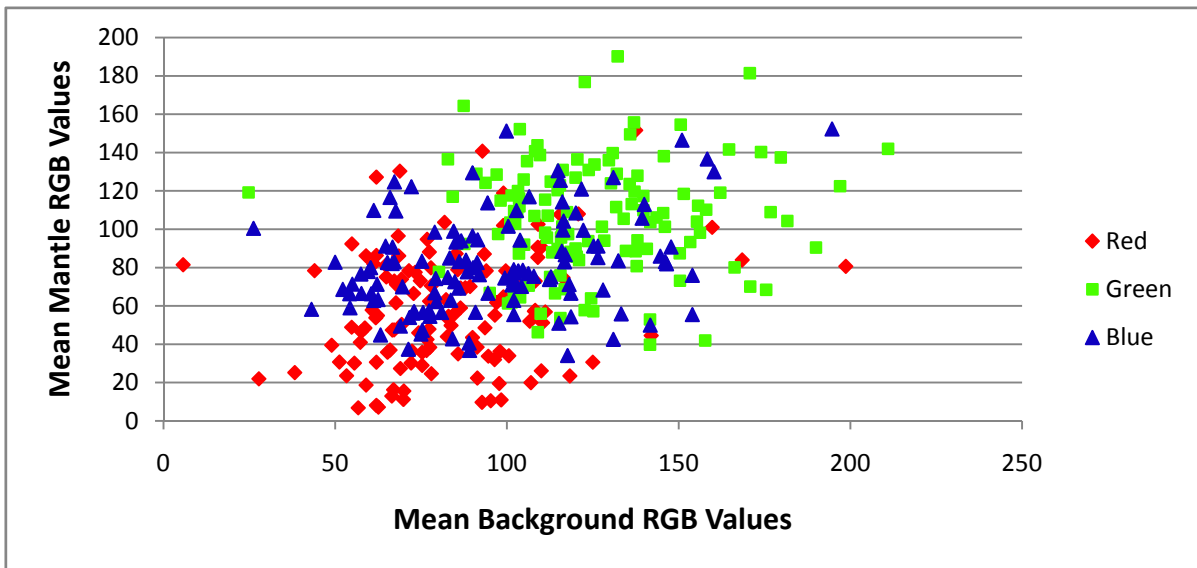


FIG. 4 Mean red, green, and blue (RGB) values for the mantle and immediate background of all clams photographed under ideal light conditions (n=122). Significant positive correlations were found to exist between background and clam mantle colors for red (Pearson’s correlation, df=120, r=0.2373, p=0.0085) and blue (df=120, r=0.3185, p=0.0003), but not green values (df=120, r=0.0770, p=0.3995).

RGB value analysis of mantle and background

The relationship between mantle and background RGB values were assessed for 122 *T. maxima* (Fig. 4). Significant correlations were observed between mantle and background data for red (Pearson’s Correlation, df=120, r=0.2373, p=0.0085) and blue (df=120, r=0.3185, p=0.0003), but not green values (df=120, r=0.0770, p=0.3995). The relationship between mantle and background values was greatest for blue, followed by red then green.

Photo study predation analysis

The number of mantle scars observed on all 127 *T. maxima* of the photo study was compared to their general ‘blue’ or ‘brown’ color morph categories (Fig. 5). No significant relationship was observed relating the mean number of mantle scars to general color morph category (t-test, df=3, p=0.4468). The 127 *T. maxima* were also evaluated for trends relating shell length to the number of mantle scars (Fig. 6). A significant positive

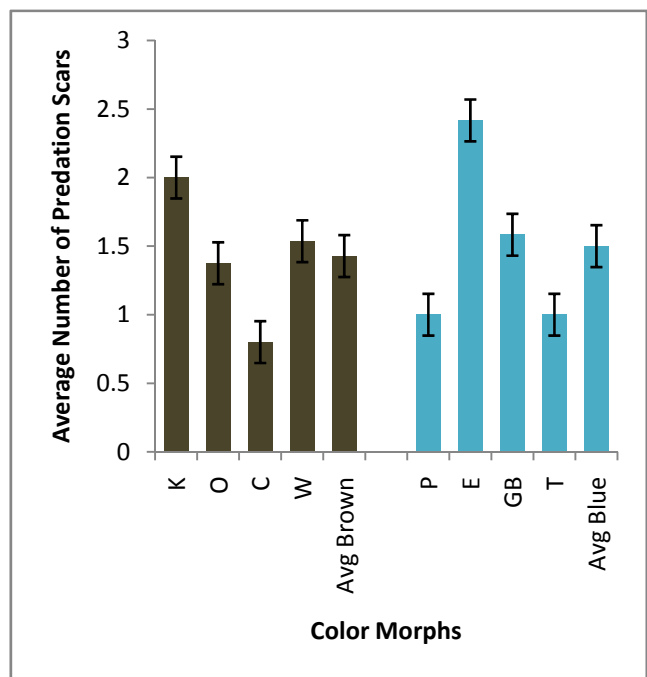


FIG. 5 Average number of predation scars for all *T. maxima* surveyed (n=127), organized by color morph and general blue versus brown category. A paired two sample t-test found no significant difference (t-test, df=3, p=0.4468) between the number of predation scars observed on the mantle of ‘blue’ versus ‘brown’ *T. maxima*.

relationship was observed with larger clams showing a greater number of mantle scars (Spearman's Rank Correlation, $df=125$, $\rho = 0.3108$, $p=0.0004$).

Tile predation study

Figure 7 shows the gain in mantle scars over four weeks between the *T. maxima* in the 'brown' color morph category, which matched the tile background, compared to the clams in the 'blue' color morph category, which did not match the tile background. The non-matching, 'blue,' *T. maxima* gained a significantly larger number of mantle scars compared to the matching individuals in the 'brown' category (t-test, $df=4$, $p=0.0445$). Matching clams gained an average of 0.8 predation scars in three weeks, while non-matching clams gained an average of 2.8.

Zooxanthellae counts

Seven *T. maxima* were evaluated for their average number of zooxanthellae (Table

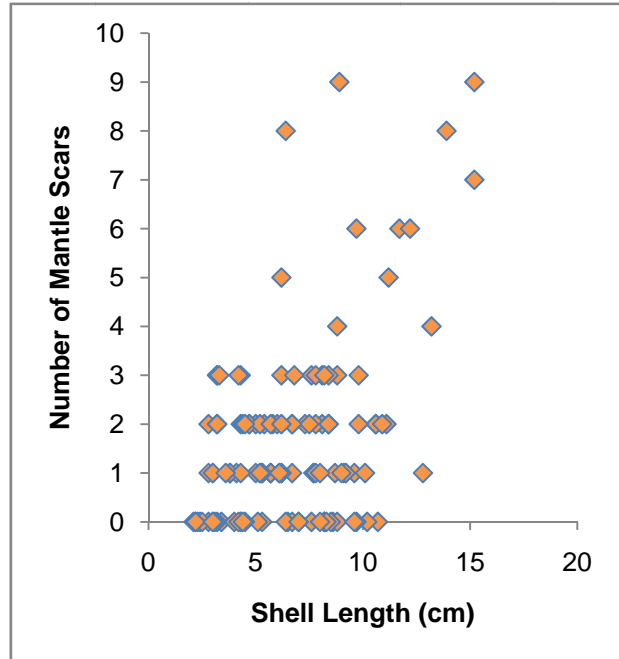


FIG. 6 The relationship between shell length and the number of mantle scars for *T. maxima* surveyed in the photo study. A significant positive relationship between shell length and the number of mantle scars was observed (Spearman's Rank Correlation, $df=125$, $\rho = 0.3108$, $p=0.0004$). Larger clams showed significantly more mantle scars than smaller.

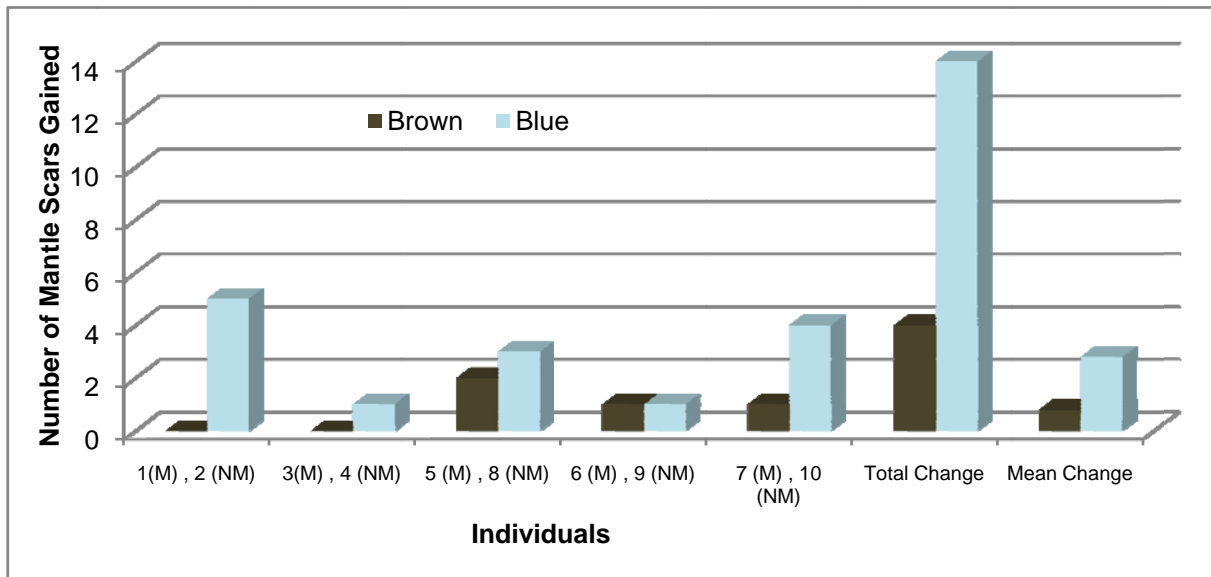


FIG. 7 Number of mantle scars gained over three weeks by 5 'electric blue' and 5 'khaki' color morphed *T. maxima*. The numbers on the x-axis indicate the individual clam used in the study, with M=matching and NM=non-matching. The final two columns show total number of scars gained and average number, for each general color morph category. The 'blue' morphs gained a significantly larger number of mantle scars over four weeks compared to the 'brown' morphs (t-test, $df=4$, $p=0.0445$).

TABLE 1. Gain in the number of zooxanthellae with an increase in mantle area. Smaller clams have approximately 3 fewer symbionts for the same mantle area

Clam Number	Color Morph	Mantle Area (cm ²)	Average Number Zooxanthellae Counted	Average Number of Zooxanthellae Per Same Area
1	Cloudy Green	2.728	2.75	1.0080
2	Dichromatic Teal	3.89	6.5	1.6709
3	Electric Blue	4.322	8.5	1.9667
4	Olive Brown	11.598	24.75	2.1340
5	Electric Blue	17.236	36.25	2.1032
6	White with Green	16.473	72	4.3708
7	White with Green	58.44	183	3.1314

1). The ratio of average number of zooxanthellae per mantle area did not stay constant. Instead, the smallest clams were found to have approximately 3 fewer symbionts for the same mantle area.

DISCUSSION

Cryptic coloration analysis

T. maxima have a need to balance two important requirements. Because they rely on the photosynthetic products of their symbionts for somewhere between 45-100% of their organic carbon needs, it is imperative that Tridacnids maximally expose their mantles to sunlight (Klumpp *et al.* 1992). However, mantle exposure renders the clam at risk for predation by small fast moving fish. Mantle exposure may also attract larger predators such as octopuses and rays, which consume the entire clam rather than only portions of the mantle. Although *T. maxima* is able to retract its mantle into its visceral mass, this defense mechanism prevents photosynthesis and requires energy to accomplish. The results of this study have indicated that *T. maxima* mantle color

polymorphism can act as a versatile strategy for balancing these two needs.

In order for an organism to be said to be utilizing cryptic coloration, its color pattern must resemble a random sample of the background perceived by predators, at the time when the prey is most at risk of being noticed (Endler 1978). Because *T. maxima* is sessile and generally motionless unless disturbed, it is at a relatively constant risk for predation during daylight hours. Significant positive correlations between mantle and background color values, drawn from random sampling of a moment of high predation risk (most daylight hours), should qualify *T. maxima* as utilizing cryptic coloration.

The photo survey undertaken found significant correlations between *red* and *blue* values of the mantle and background of 122 *T. maxima*. However, no significant correlation was found with *green* values. As the design of this portion of the study closely matches that of Todd *et al.* (2009), it is puzzling that the results did not replicate theirs. The most likely reason for this divergence is the greater sample size and finer control of depth and light in the previous study. Surveying

occurred only on one reef in Pulau Tioman, Malaysia, a site with little variation in depth (Todd *et al.* 2009). Surveying for this study occurred at five different reefs, each with a great deal of depth variation. Consequently, the observed correlations are not as significant as in the modeled study.

Because all three colors were not found to be significantly correlated with background colors, it is difficult to conclude whether *T. maxima* are utilizing cryptic coloration. Mantle coloring is the result of local variations in the orientation of iridophores, which produce interference colors in the blue wavelengths, as well as zooxanthellar and clam pigments, which are generally brown to black (Griffiths *et al.* 1992, Ishikura *et al.* 1997). This suggests that *T. maxima* may have the best control over the blue and brown coloring of its mantle, allowing it to better match its background in those colors. It is possible that *T. maxima* has no direct method for adjusting green mantle color, making its matching in this color imperfect. While zooxanthellae utilize the green pigments chlorophyll a and c for photosynthesis, the presence of other darker accessory pigments may obscure this green coloration (Jeffrey and Haxo, 1968). Though uncertain, the results of the study are suggestive that color matching between mantle and background qualifies as cryptic coloration.

Color morphs

At the most general level, there is an apparent loss of color diversity with increasing shell size, and therefore age. Results of the present study show that *T. maxima* are found in up to seven color categories when they are below 5 cm, and can reach up to eight different morphs between 5 and 10 cm. However, above 10 cm, this diversity drops to three color morphs, all of which belong to the generally brown category.

This reduction in the proportion of blue morphs with age can be explained by several hypotheses. Large blue *T. maxima* may be

preferentially predated upon because of their conspicuous coloring, and are therefore being removed from the population. However, preferential predation should lead to selective pressure to remove this morph from the population over evolutionary time. Consequently, a more likely explanation is that the clams are changing color as they age. Todd *et al.* (2009) observed a similar distribution of color morphs in *Tridacna crocea* and posited the same mechanism. The fact that a similar trend is observed in two closely related species suggests that the observed loss of blue morphs with age is a conserved evolutionary strategy. A similar investigation of the color morph distribution of the next most closely related Tridacnid, *Tridacna squamosa*, would help to determine how ancestral this trait is, thus helping to determine its relative importance to zooxanthellae clams (Maruyama *et al.* 1998).

However, it is important to note that this argument for color changing is predicated on the observed trends being the result of natural processes. It is well known that *T. maxima* are a favorite delicacy of the residents of French Polynesia, and that their gathering is not outlawed by current fishing laws (pers. obs.). Wagner (2001), in his investigation of the population of *T. maxima* on Mo'orea, found a significant trend of a decreased population of larger individuals near human population centers. Because large, 'blue' morphs are the most noticeable by humans, it is important to note that the observed trend of decreasing 'blue' morphs with age could potentially be an artifact of human predation.

The most interesting color morph observed for morphological study is the 'dichromatic teal' color morph. It is remarkable that this (color changing) morph was observed only in the size category between 5 and 10 cm, given that most 'blue' morphs observed were smaller. This is most likely due to the rarity of this color morph and the fact that the most clams found in this study were in this mid-range size category. Griffiths *et al.* (1992) predicted that if mantle

iridophores were oriented edge-on to the exposed light they could act as diffraction grating, possibly resulting in different colors depending on the angle of incident light. Observation of this phenomenon in *T. maxima* may indirectly support that hypothesis. Todd *et al.* (2009) made no mention of color changing morphs in *T. crocea*, indicating that it might be unique to *T. maxima*.

Predation rate analysis

Though the implications of the photo survey were somewhat ambiguous, the results of the predation study provided additional support for the hypothesis that *T. maxima* are utilizing cryptic coloration to avoid predation. The significantly larger number of predation scars gained by the blue morphs, when placed against a non-matching background, showed that these clams were experiencing a higher rate of predation than the brown color morphs with the same treatment.

While these results are compelling, they do not guarantee that it was the tile treatment that generates the observed effect. One possible argument against the tile treatment is that the 'blue' morphs generally experience a greater base level of predation. However, this possibility is contradicted by the findings of the photo survey, which showed no significant difference in the number of predation scars between the 'blue' and 'brown' color morphs. Clearly the introduction of the tiles in some way increases the threat of predation on the generally 'blue' clams. The *T. maxima* analyzed for mantle and background color matching were photographed in their unaltered reef habitat. These clams would have had their entire lives to adapt to their local microhabitat, matching not only in color, but also in pattern and shading. By contrast, after altering the microhabitats of the clams through the installation of the tiles, the 'blue' morph *T. maxima* used in the experiment definitely did not match their habitat in either color or texture. The fact that they experienced

significantly greater rates of predation than the 'brown' morphs, which retained some degree of background matching, suggests that the tiles did have an effect above and beyond natural predation rates.

There is an argument to be made that adding an artificial background to the habitat of the clams potentially attracted predators more than a regular background would. This effect was potentially observed in the field, where after the installation of tiles, Orange-Lined Triggerfish (*Balistapus undulates*) and Sixbar Wrasse (*Thalassoma hardwicke*) were often seen investigating. As these fish are known predators of *T. maxima*, it is possible that the recorded predation rates of *T. maxima* were higher than those experienced by clams in the wild (pers. obs.). However, this effect should be the same for both treatments. Therefore, the addition of the artificial background alone does not explain the higher predation rates for the 'blue' morphs compared to the 'brown'.

Despite the greater rates of predation observed for non-matching *T. maxima*, evidence from the photo study indicates that there are still morphs that are not matching their background coloration. As these clams are still present in the population, it is possible that for *T. maxima* that do not match their background, other factors are involved in keeping predation rate low. The significantly lower number of predation scars present on smaller clams would help to explain why a larger percentage of this size category can be made up of non-matching color morphs. If these clams gain an advantage from non-color matching, and can still avoid predation from size along, potentially they do not experience a large enough predation disadvantage to change color morph. This balance likely changes as *T. maxima* become larger.

Zooxanthellae count

The results of the tile experiment raise further questions about the purpose of mantle color polymorphism, especially in that blue

morphs appear to be at a disadvantage. This study did not evaluate the consequences of mantle predation, but even if it is not eventually fatal, there is a definite energy cost to repairing tissue and lost photosynthetic capacity. Why then, would such a disadvantageous form have evolved? Especially in that the blue morphs are most common at a younger age, a time when an individual is least able to recover from mantle predation?

Griffiths *et al.* (1992) have suggested that an abundance of iridophores might be an adaptation for ultraviolet (UV) tolerance. Evidence against this hypothesis can be found in the significantly greater depths observed for blue color morphs over brown. Ultraviolet light, though still biologically relevant at depths as deep as 200 m, is greatly filtered out in the first meters of water (Warrant and Locket, 2004). The present study found that on average most blue morphed *T. maxima* are located between 4-5 ft. If a greater number of iridophores is an adaptation to tolerate UV light, 'blue' morphs should instead be found at shallower depths.

Additionally, it is unreasonable to suggest that the need for UV protection is reduced in older clams, especially because older clams are reproductively active. Ultraviolet induced mutations in reproductively active clams could be passed on to offspring, thus decreasing the fitness of the whole species. Furthermore, a mechanism already exists for protection of zooxanthellae from UV light, through the production of mycosporine-like amino acids in the mantle of zooxanthellate clams (Ishikura *et al.* 1997). Though potentially useful at times of sun exposure during low tides, iridophores are not required for UV tolerance for most *T. maxima*, which do not experience daily exposure.

Another suggested explanation for blue coloring is the use of iridophores to redirect light towards zooxanthellae, thus increasing their photosynthetic output (Griffiths *et al.* 1992). Depending on the incident angle of light, iridophores can potentially act as a

diffraction grating, bending light towards symbionts. Clearly this would be advantageous to any *T. maxima* that is experiencing reduced photosynthetic capacity. Because water reduces light intensity with increasing depth, and light intensity is the primary indicator of photosynthetic rate, *T. maxima* found at deeper depths would gain an advantage with more iridophores, being able to capitalize on all incident light (Ryther 1956).

The fact that the number of zooxanthellae to mantle area ratio was found to increase with age (Table 1), this study suggests that younger clams may be able to produce less energy for the same area of mantle. The findings of Hirose *et al.* (2005) support this hypothesis, with the observation that the mantle tubular system becomes increasingly complex and branching with age. Younger *T. maxima* are therefore at a metabolic disadvantage to their older counterparts. Any trait that addresses this disadvantage would be selected for. This trend can potentially explain why a greater proportion of blue morphs are observed in younger clams. As a blue morphed *T. maxima* ages, eventually the advantages of the increased photosynthetic output would be counterbalanced by the deleterious effects of increased predation rate. If a mechanism exists to change color, and older clams are better able to utilize the same mantle area, it is possible that above 10 cm, no benefits can be gained from remaining a blue morph.

Future studies

Future research on *T. maxima* in general, and those on Mo'orea specifically, would be useful in determining if the observed trends are applicable to the rest of the population found around the island. Expanding the number of surveyed individuals to include other habitats where *T. maxima* can be found might show a greater ability for mantle color plasticity. The local variation observed in the photo study could be categorized in a more consistent manner, perhaps by associating

certain color morphs with other organisms making up the reef community. Examples might include more 'brown' morphs associated with certain types of algae, or more 'blue' morphs associated with different genera of coral. As the photo survey methodology was derived from the methods of Todd *et al.* (2009), the focus of the investigation was on background and mantle color matching. No system was established for evaluating differences in mantle patterning, which may also play a role in predator avoidance (Kiltie and Laine, 1992). The results of the predation study would be best extended with an increased sample size, along with an extended period of examination. Mantle color was not seen to change over the study period, but an extended observation time could potentially confirm or deny this hypothesis. Finally, an examination of the rates of photosynthesis between different color morphs would help to confirm the role of increased iridophores as a light gathering mechanism.

Conclusions

The role of mantle color polymorphism was examined in the giant clam *T. maxima*. Analysis of the RGB values of mantles and immediate backgrounds provides some support for the hypothesis that *T. maxima* is using cryptic coloration. Differential predation was observed on clams that did not match their backgrounds, through a habitat manipulation experiments. Finally, the hypothesis that blue morphs are an adaptation to boost photosynthetic output was supported through zooxanthellae counts. Mantle color polymorphism is likely a trait that fulfills many disparate needs for *T. maxima*.

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