Tactics To Combat Visual Predators: Cryptic Coloration in Christmas Tree Worms

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ABSTRACT

Color polymorphism is widespread in many species living in highly complex environments, including coral reefs such as those found on the island of Moʻorea, French Polynesia, but its ecological function in marine invertebrates remains understudied. This research investigates the role of color polymorphism in Christmas tree worms (Spirobranchus giganteus), which display diverse color morphs. Specifically, determining if Christmas tree worms are cryptic and if coloration of the worm affects predation rate. Using RGB analysis of worm and coral substrate colors, this study found a significant correlation between certain worm color morphs and their substrate, indicating successful background matching. Predation experiments using mimic worms revealed that cryptic color morphs that blended well with their immediate background were marginally less targeted by predators compared to colors that stuck out from their immediate background (non-cryptic). However, predation differences between cryptic and non-cryptic color morphs were not statistically significant. These findings suggest that Christmas tree worms exhibit crypsis and blend in well with their immediate coral background. This finding reveals some of the functions of visible polymorphisms in Christmas tree worms and contributes to scientific knowledge of how organisms enhance their survival through crypsis.

Introduction

The existence of multiple unique color variations within the same population is referred to as color polymorphism.[1] Color polymorphisms in marine invertebrates can arise through a variety of evolutionary, ecological, and physiological mechanisms. These mechanisms include genetic control, physiological processes, and selective pressures.[2] Some organisms that exhibit color polymorphism use cryptic coloration, in which prey blend into their substrate to reduce vulnerability to visually searching predators.[3,4,5] Color polymorphism is widespread in species living in highly complex environments, including marine systems.[6] Coral reefs such as those found on the island of Moʻorea, French Polynesia, have high rates of color polymorphisms due to their unpredictability in both space and time, making them challenging environments for organisms to adapt to.[6] As a result, marine invertebrates may employ a bet-hedging strategy by producing polymorphic offspring, increasing the likelihood that at least some individuals will match the substrate in which they settle.[7]

One species that is highly polymorphic and found within Moʻorea is Spirobranchus giganteus, also known as the Christmas tree worm. The species has been identified to have nine different color morphs and pattern variations such as, yellow, blue, orange, green, white, striped brown, and purple.[8, 9] The worm's branchial crown, which emerges from its burrow for feeding and breathing, is composed of two feather-like, spiraling stalks.[10] Although the worm's branchial crown is its only visible portion, its abdomen, which makes up more than half of its body weight, is hidden inside its calcareous tube.[11] They feed by drawing water and nutrients up from the base of their branchial crown to the tip of the worm.[10] The worms have an operculum that, when they retract, closes the entrance of their tube, acting as a protective plug that seals off its branchial crown in reaction to threats or disruptions.[12] For the worms this trade off causes them to sacrifice their ability to eat and breathe when they are retracted into their tubes.[13]

These worms are sessile invertebrates that inhabit coral and are obligate symbionts of coral.[14] Christmas tree worms improve water circulation across coral polyps, influencing coral nutrition, growth, and recovery while also receiving support and protection from their host coral substrates.[15] This means that they have a mutualistic relationship with their host coral substrates.[15] Their symbiont coral, Porites, has dominant color morphs of brown and purple.[16]

Christmas tree worms also have many predators, including wrasses (Family Labridae), butterflyfish (Family Chaetodontidae), surgeonfish, tang, unicorn fish (Family Acanthuridae), and triggerfish (Family Balistidae).[12] All of these fish species are abundant within the coral reefs in Moʻorea, French Polynesia.[17,18] These fish have been found to use visual cues to aid in foraging for prey.[19] Most of these predators have trichromatic color vision and are sensitive to all colors perceived within the visible spectrum of light, which are the same colors perceived by the human eye (400-700 nm).[20,21,22,23] Triggerfish showed preferential foraging response bias to red stimuli followed by green, yellow, and then blue.[20] These many visual searching predator's point to the fact that despite their wide range of vibrant, outstanding colors, Christmas tree worms could be at least to some degree cryptic and have a coloration that resembles that of its background and thus providing protection from these visual predators.

This study aims to understand the function of the Christmas tree worm's visible color polymorphism. Specifically, does cryptic coloration of Christmas tree worms deter visual predators? My hypotheses are that: (1) Christmas tree worm branchial crown colors will match the colors of their substrate more often than what would be expected from random color morph distributions, (2) different color morphs will have different degrees of correlation to their coral substrate because specific worm color morphs could overlap more with the dominant coral color and could exhibit more successful cryptic coloration, and (3) worms that exhibit cryptic coloration will be less likely to be predated than worms with non-cryptic color morphs because fish looking for visual cues will struggle to notice cryptic worms that look like their coral substrate.

Methods

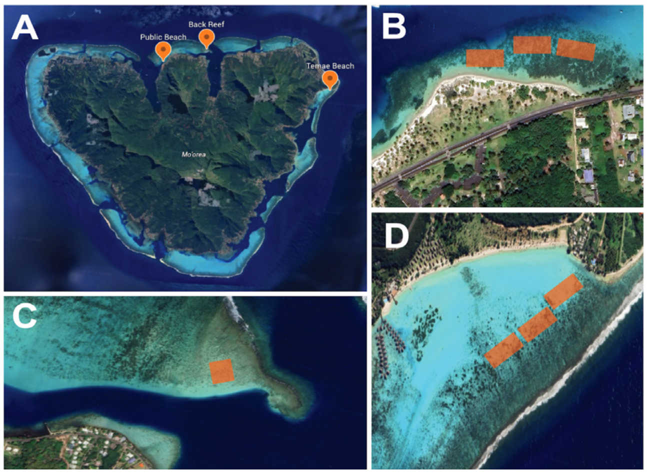

Figure 1. A) Map of Moʻorea, French Polynesia depicting the location of the 3 sites used in the study (Google Earth 2024). B) Sampling locations at Taʻahiamanu Beach (Google Earth 2024). C) Sampling location at the UC Berkeley Gump Station Back Reef (Google Earth 2024). D) Sampling locations at Temae Beach (Google Earth 2024).

A. Study Site

All data was collected in Moʻorea, French Polynesia which is a fringing reef and therefore is a prime site to study Christmas tree worms as the species is regionally found in the Indo-Pacific and lives only within coral reefs.[24] On Moʻorea, data was collected at Taʻahiamanu Public Beach, Temae Beach, and the UC Berkeley Gump Station Back Reef (Fig. 1). These three study sites were chosen based on their abundance of Christmas tree worms, predator species, and coral symbiont species. The sites were also easily accessible, as most were public beaches, which allowed safe and easy data collection.

B. Background Matching as Evidence of Crypsis

All data was collected during October and November 2024. A total of 168 photos were taken and included in this study with an underwater camera with a total of 249 worms captured in these photos. Each photo was taken and included in the study if the worm could easily be identified by having the branchial crown be completely in frame. In each photo, a white and black standard was held within the frame to be used later during the manual color correction photo processing. To avoid repeats in data collection, the location of every survey conducted was recorded based on physical landmarks and marked on a map on the same day that the data was collected (Fig. 1). All photos were manually color corrected in Adobe Photoshop.[25] After this, a RGB value was collected using the Eyedropper Tool in Adobe Photoshop taking a 31x31 pixel average sample from the branchial crown of the worm for the worm RGB value. This method was repeated to get the RGB value of the coral substrate directly adjacent to the worm's branchial crown.

All statistics were performed in R version 4.4.1.[26] Data was checked for normality and variance. All data was normally distributed, and all assumptions were met for performing all statistical tests. Three Pearson correlation tests were used to analyze the RGB values of the worm in comparison to the RGB values of the substrate to determine if certain color morphs exhibit cryptic coloration. Three trend lines were created based on three linear regressions between how the RGB values of the substrate affected the RGB values of the worms. The RGB values were also analyzed further by using the CIEDE2000 color difference formula to calculate CIEDE2000 values which represent the color differences perceived by organisms with trichromatic color vision.[27] I then did a Chi-Squared Goodness-of-Fit Test to test how the observed frequencies of the CIEDE2000 values fit an expected distribution of CIEDE2000 values that are categorized as above 10 or not. This value is based on the thresholds for perceptibility which is any CIEDE2000 value above 10.[27] This means that if the CIEDE2000 value is above 10 an organism with trichromatic color vision can perceive the difference between the two colors. This allowed for further analysis on how visual fish predators, which have trichromatic color vision, perceive noticeable color differences between the worm and its substrate.

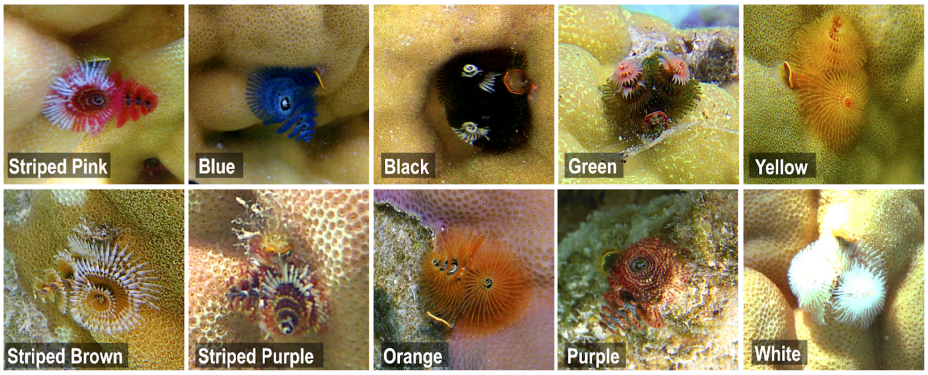

Figure 2. All Christmas Tree Worm color morphs identified across Temae Public Beach, Taʻahiamanu Beach, and the UC Berkeley Gump Station Back Reef in Moʻorea, French Polynesia.

C. Most Successful Color Morph

The RGB values of the worms were sorted into 10 color morph categories based on their color to the naked eye: purple, white, orange, striped purple, yellow, green, blue, striped pink, striped brown, and black (Fig. 2). In order to determine which color morphs exhibit the most successful cryptic coloration, Pearson correlation tests were run in accordance with each color category and the RGB values of the substrate (Fig. 2). Medians of CIEDE2000 values were then calculated within each color morph and cryptic categories. Further, CIEDE2000 values between color morphs were analyzed for statistical significance with a one-way ANOVA.

D. Predation Experiment

Six artificial Christmas tree worm mimics were made from painted frayed rope (Fig. 3).

To determine the least and most cryptic worm colors to the substrate color, preliminary Pearson Correlation tests were conducted. Preliminary results suggested that striped purple ($R^2 = 0.63$), purple ($R^2 = 0.51$), and orange ($R^2 = 0.36$) were the most positively correlated with Porites substrate

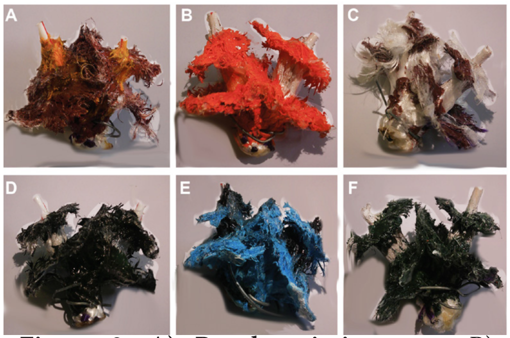

Figure 3. A) Purple mimic worm. B) Orange mimic worm. C) Striped purple mimic worm. D) Black mimic worm. E) Blue mimic worm. F) Green mimic worm

color, and green ($R^2 = 0.46$), black ($R^2 = 0.01$), and blue ($R^2 = 0.08$) were the least positively correlated; thus, three mimic worms were painted striped purple, purple, and orange in order to be considered cryptic and three mimic worms were painted green, black, and blue to be considered non-cryptic.

Experiments took place at Taʻahiamanu Public Beach and 6 trials were conducted. Each trial tested all 6 mimic worms in the same location. A unique coral head with two or more real Christmas tree worms was selected as the experimental site for each trial. The coral head also had to be Porites coral to account for the substrate specialization that the worms have for Porites coral. Shrimp were attached to each mimic worm to possibly mimic the chemical cues of real Christmas tree worms. Within a trial each mimic worm color was placed in the same spot on a coral head until 4 fish came within a 0.5m radius from the mimic worm. This was done to only study fish interactions with the mimic worm and to standardize the number of fish that were interacting with each model. The duration that a fish stayed in the 0.5m area around the mimic worm was measured. The type of interaction the fish had with the mimic worm during the time period was recorded: whether the fish noticed the mimic worm or not, if the fish touched/bit the worm or not and how many times the fish touched/bit the worm was collected. All trials were recorded on video to confirm observations recorded during the live experiment. I filtered the data to categorize fish behavior based on whether strikes occurred within a designated radius around the mimic worm. A strike was defined as a fish biting or touching the mimic worm within this radius, while a non-strike occurred when no such behavior was observed (i.e., the number of strikes = 0). This filtering process allowed me to create two categories: (1) predator-prey interaction observed, where a fish struck the mimic worm within the radius, and (2) no predator-prey interaction observed, where the fish entered the radius but did not strike the mimic worm. For the predator-prey interaction observed category, I calculated the ratio of the number of strikes to the time spent within the radius. This was done to account for differences in the time each fish spent in the designated area.

A square root transformation and the removal of outliers was conducted on the strike ratios to create a normal distribution. These ratios were then analyzed for statistical significance with a one-way ANOVA analysis of variance test to test the effect of mimic worm color on the square root of the strike ratios. The category of no predator-prey interaction (if the strike ratio was equal to 0) was then analyzed separately with a Kruskal-Wallis test (due to a non-normal distribution) to test the effect of mimic worm color on the total count of strike ratios that were 0 within each color category.

Results

A. Background Matching as Evidence of Crypsis

The RGB values of Christmas tree worms were positively correlated with their substrate coral RGB values (Fig. 4). Red, green, and blue values were all highly correlated with substrate color ($R^2 = 0.66$, 0.54, and 0.34, t = 21.98, 16.90, and 11.23, df = 247, 247, and 247, and $p < 0.001$, 0.001, and 0.001 respectively). Red colors of the worms and the substrates were most positively correlated, with a slope of 0.91. The largest percentage of CIEDE2000 values fell above 10, meaning that the majority of the worm colors were perceivably different from their coral substrate. The predicted and observed values of CIEDE2000 differed significantly for the two categories (CIEDE2000 <10 and >10; $p < 0.001$, $X^2 = 77.02$).

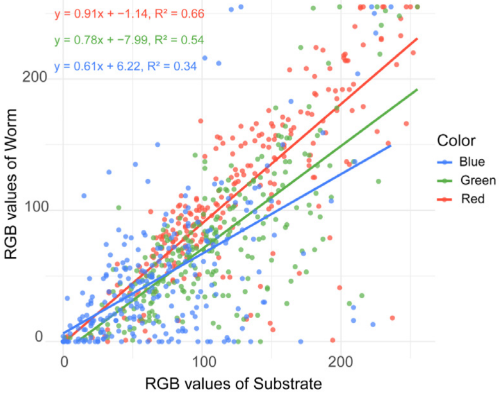

Figure 4. Correlation between the RGB values of the Christmas Tree Worms versus the coral substrates. Pearson correlation tests were significant for red, green, and blue values.

B. Most Successful Color Morph

The color morphs striped purple (Red: $R^2 = 0.69$, Green: $R^2 = 0.44$, and Blue: $R^2 = 0.66$; all $p<0.001$), purple (Red: $R^2 = 0.45$, Green: $R^2 = 0.45$, and Blue: $R^2 = 0.53$; all $p<0.001$), and striped brown (Red: $R^2 = 0.41$, Green: $R^2 = 0.41$, and Blue: $R^2 = 0.30$; all $p<0.001$) were found to have the highest correlation to the substrate as all RGB values had a statistically significant strong positive correlation (Fig. 5).

The other seven color morphs did not have a statistically significant correlation between worm RGB values and coral substrate RGB values. For CIEDE2000 values, striped brown, yellow, and white had the lowest median CIEDE2000 values, indicating the least contrast against their substrates. The colors with the highest median CIEDE2000 values were striped pink, blue, and green indicating the strongest contrast against their substrate. Further, CIEDE2000 values did differ significantly within color categories (ANOVA, F = 11, DF = 11, $p < 0.001$).

C. Predation Experiment

The relationship between the squared strike ratios and the mimic worm color was statistically insignificant (ANOVA, F = 1.902, df = 5 p = 0.111). The cryptic and non cryptic categories also had an overlapping distribution of squared strike ratios; however, the cryptic category had a slightly lower median of squared strike ratios than the non cryptic category (Fig. 6). Squared strike ratios did not differ significantly within the cryptic categories (ANOVA, F = 1.119, df = 1, p = 0.295). Worm colors were not correlated with the number of non-strikes (Kruskal-Wallis, $X^2 = 5$, p = 0.4159). A Kruskal-Wallis test was also conducted to test the effect of the cryptic category (cryptic or non cryptic) on the number of non-strikes. The results of the Kruskal-Wallis test revealed a statistically insignificant effect of the cryptic category on the number of non-strikes ($X^2 = 1.22$, p = 0.268). However, the cryptic category had a higher median number of non-strikes than the non cryptic category.

Discussion

In this study on the function of color polymorphism within the Christmas tree worm, the species exhibited crypsis and certain color morphs displayed better

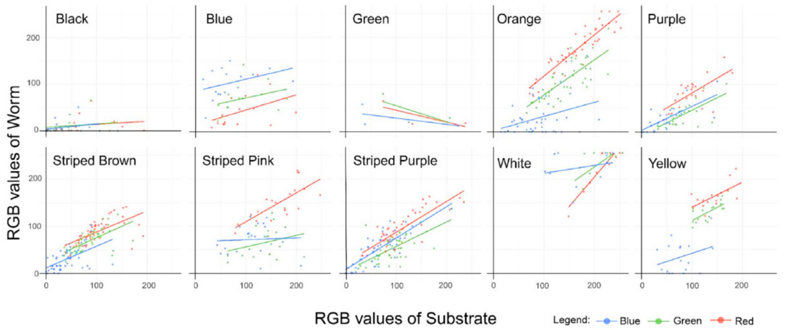

Figure 5. Correlation between the RGB values of the worm versus the RGB values of the substrate within all color.

background matching to their coral substrate than others. Although there was a small difference in predator response to cryptic worms, the color of the worm did not have a statistically significant effect on predation.

A. Background Matching as Evidence of Crypsis

Across all color morphs, the Christmas tree worms exhibited cryptic coloration in relation to their substrate (Fig. 4). The analysis of CIEDE2000 values showed a significant departure from expected proportions, as the distribution of CIEDE2000 values did not match the predicted distribution of having over 50% of the CIEDE2000 values fall below 10. This means that the majority of the Christmas tree worm's branchial crown color was distinguishable from the color of their immediate background. While the CIEDE2000 values demonstrated distinguishable difference of color between the worm and their substrate, this outcome is reasonable given that the CIEDE2000 color difference formula was made to notice extremely small differences of color, for example to match resin color to tooth color in prosthetic dentistry.[27] This means that although the majority of the worm color was not the exact color match of its immediate background, the colors were still significantly correlated to its coral substrate color. These findings are supported by existing literature in which researchers have found that Christmas tree worms have various protective adaptations, including living in a cryptic habitat, that provides the worms with physical features, such as coloration, texture, and patterns, that enable them to blend in with their surroundings.[12] This adaptation, now identified in the worm, highlights further protection they have to the variety of predators that contribute to their mortality. The analysis of the color of the worm itself in relation to its coral substrate provides further insight into the selective forces that contribute to the selection of color morphs seen within Christmas tree worms. This allows insight into the complex phenotypic plasticity of Christmas tree worms and the large variety of colors seen within the species.

B. Most Successful Color Morph

Striped purple, purple, and striped brown are all the colors that exhibited the best color matching to the coral substrate (Fig. 5). Striped purple and striped brown were also the most abundant colors within the population of 246 worms sampled. This aligns with previous research which established Porites coral as the worm's symbiont coral, which has dominant colors of purple and brown, and which matches the most common color morphs.[9,16] Previous studies indicate that carotenoids are significantly involved in the variation of crown coloration in the serpulid Polychaetes family, to which Christmas tree worms belong to.[28] However, while these pigments are derived from the diet of the worms, the study found that there was selectivity involved.[28] This means that because of the background matching exemplified in this study, cryptic colors may help to explain the maintenance and function of color polymorphism in Christmas tree worms.

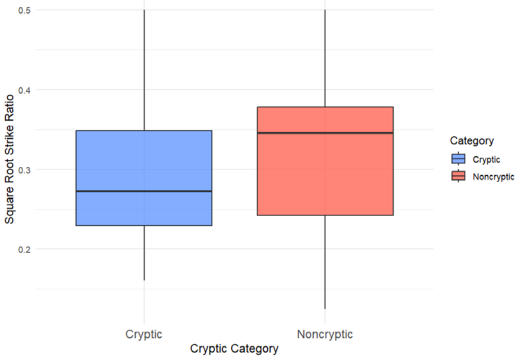

Figure 6. Relationship between cryptic category and the square root of strike ratios.

The green color morph of the Christmas tree worm possesses a slightly negative correlation to the color of its coral substrate and therefore stands out (Fig. 5). The green color morph was also the least abundant color morph. This may be explained by apostatic selection, where the green morph's rarity and distinct appearance reduces its chances of being recognized as prey by predators.[2] This suggests that polymorphism in Christmas tree worms serves two functions: promoting crypsis to blend in with the environment and providing certain morphs with a survival advantage through reduced predation. Further studies could look more into apostatic selection within the species. Together, these mechanisms could contribute to maintaining the species' diverse range of color morphs.

C. Predation Experiment

The cryptic mimic worms experienced slightly less predation, as fish bit these worms the fewest times (Fig. 6). Predation on worms of different colors was not statistically significant, conflicting with previous research that found that fish families Chaetodontidae, Acanthuridae, Balistidae, and Labridae all are predators of the worms.[29] Polychaetes and Malacostraca (the shrimp used within the experiment) have different chemical compositions due to their distinct evolutionary lineages, ecological roles, and physiological requirements.[30, 31] The differences in their biochemical makeup could have contributed to different predator-prey interactions compared to actual predator-prey interactions experienced by true Christmas tree worms. Further studies should be conducted on fish preference for different colors within the Christmas tree worm and overall, on predator-prey relationships the worms have with different species. Future studies could collect genetic data of each color morph identified within the Christmas tree worm. Intergenerational studies could also be done to study the heritability of each color morph of the species.

Conclusion

In conclusion, Christmas tree worms are cryptic and blend in well with their immediate coral background which makes up part of the composition of coral reefs of Moʻorea, French Polynesia. These findings reveal some of the functions of visible polymorphisms in Christmas tree worms and contribute to scientific knowledge of how organisms enhance their survival through crypsis. This manifests the very intricate balance between predator-prey relationships that exist in maintaining biodiversity and ecosystem stability in complex coral reef environments.

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