

MICROHABITAT SELECTION AND FUNCTIONAL MORPHOLOGY IN THE HETEROGENEOUS ENVIRONMENT OF THE CUSHION STARFISH *CULCITA NOVAEGUINEAE* ON MO'OREA

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Abstract. The colonization and dispersal of organisms on volcanic islands is reliant on various traits driven by natural selection. Phenotypic plasticity is a survival mechanism known to aid organisms in colonizing novel habitats, especially when these habitats are highly heterogeneous and organisms might benefit from occupying a distinct ecological niche. Microhabitat is one of the theoretical drivers behind organismal plasticity, particularly in a reef ecosystem with a variety of available substrates. *Culcita novaeguineae* is a common tropical reef starfish widespread in the Pacific that exhibits a range of phenotypic variation in its colors and patterning. Factors related to the morphological variation in this species have not been examined, and little is known about the ecology and life history of this species in general. The color diversity of thirty-one *C. novaeguineae* individuals was quantified using digital photography and an HSB color space, and these values were assessed for correlation to reef substrates that stars selected. Substrate preference by individuals was investigated and found to be non-random among substrate types present on the fringing reef. Additional analysis showed that the coloration of a star can serve as a predictor of which substrate it may select. The findings of this study reveal a relationship between color variation and the microhabitat selection of *C. novaeguineae*. These results further the understanding of phenotypic plasticity as a mechanism for survival and increased ecological fitness, shedding light on island ecology and species colonization and dispersal, as well as the natural history of this understudied species.

Key words: starfish; *Culcita*; phenotypic plasticity; camouflage; color; variation; French Polynesia

INTRODUCTION

Phenotypic plasticity and morphometric variation are characteristics of natural selection that have long been enigmatic. Different environments can result in phenotypic variation in populations of the same species, but the precise mechanisms and costs behind this variation are not well

understood (Pigliucci 2005). Islands provide unique environments helpful for studying phenotypic plasticity; when a population arrives in a new environment, plasticity can be a key mechanism that aids in colonization and survival (Price 2006, Whitely et al. 2009). This can precede or be independent of genetic change. In novel, challenging habitats plasticity can lead to the ecological success of

a species (Agrawal 2001, Ghalambor et al. 2007). Plasticity in morphological traits can thus lead to the presence of different morphotypes in a new environment originally colonized by a single species or population. Islands have a variety of microhabitats, that facilitate specialization into different ecological niches (Gillespie 2016). Variation of relatively plastic traits such as patterning and color by microhabitat has been shown to exist within heterogeneous environments, and to have an impact on increasing species' fitness within them (Schlichting and Pigliucci 1998, Merilaita et al. 1999, Sultan and Spencer 2002).

Geographic color variation is often driven by factors such as sexual selection, signaling behavior, diet and habitat (Krohn and Rosenblum 2016). Color also has an advantage in escaping predation. Camouflage and high levels of color variance in a species may both make it difficult for visually oriented predators to find and develop a search image of their prey (Merilaita et al. 1999). Previous studies that have investigated color as an adapted trait in ectotherm species often describe a tradeoff between camouflage or background matching and thermoregulatory ability (Stuart-Fox and Moussalli 2009, Langkilde and Boronow 2012, Smith et al. 2016). Darker colored ectotherms are generally able to conduct more heat with less energy expenditure but face greater risks of overheating when temperatures are higher and solar radiation is more direct (Norris 1967), such as in the tropics.

In Mo'orea, French Polynesia, the cushion star *Culcita novaeguineae* Müller and Troschel (1842) is a common corallivore found on the shallow fringing and patch reefs. *C. novaeguineae* is widespread throughout the tropical Pacific (Glynn and Krupp 1986), yet little is known about this species. The morphology of *C. novaeguineae* includes a wide variety of colors and mottling patterns, all of which are believed to comprise a single

species (Mah 2018). Color variation in the cushion star is unlikely caused by sexual selection or signaling, as they are broadcast spawners that can disperse across reefs. Diet is also an unlikely factor in color variation, as *C. novaeguineae* have been shown to be generalist feeders on algae and sponges in addition to multiple species of coral. Its feeding preferences are likely driven by the local abundance of each food type within its foraging range and the energetic challenges of reaching its food source (Glynn and Krupp 1986, Hawkins 2006). The color and patterning variations seen in *C. novaeguineae* do vary geographically across and within its distribution range (Mah 2014). This suggests that local habitat and microhabitat may be influencing the diversity of colors and patterns exhibited by this species. Therefore, both habitat selection and thermoregulation within microhabitats are factors that may possibly be related to phenotypic plasticity in the cushion star, neither of which have been investigated for this species. Behavioral thermoregulation has been observed in other groups of tropical invertebrates and it has been shown that some have the ability to select thermally favorable microhabitats in the wild (Chaparron and Seuront 2011). Correspondingly, in increased temperatures *C. novaeguineae* moves into cooler, deeper waters to avoid overheating, and temperature was shown to be a controlling factor in cushion star distribution on the fringing reef of Mo'orea (Yokley 2016). Thus, if the microhabitats that *C. novaeguineae* selects, for either thermoregulatory or camouflage purposes, are related to the color diversity it exhibits was the focus of this study.

The research objectives of this study were to determine which cushion star color morphs are found on the fringing reef of Mo'orea, in which microhabitats they are found and if a correlation exists between microhabitat selection and color variation. I hypothesized

that microhabitats selected by *C. novaeguineae* on the fringing reef were correlated with color differences amongst morphs. To test this hypothesis, whether cushion stars non-randomly select microhabitat substrates from their available environment was determined, and substrate alignment tendencies of different color morphs was investigated in both the field and the lab. Tradeoffs between camouflage and thermoregulation in light and dark color morphs of *C. novaeguineae* were also observed.

METHODS

Field surveys

This study was conducted on the island of Mo'orea, French Polynesia. Five field sampling sites were visited on the northern coast of the island between October 9-30, 2018 from 15:00 to 18:00 h local time. Field sites were on the fringing reef encompassing the northern coast of the island (Fig. 1). The sites were: (1) Temae Beach, (2) Cook's Bay, north of the UC Berkeley Richard B. Gump Research Station, (3) Pih'aena MPA, (4) Public Beach, Opunohu Bay, (5) Snack Mahana Beach, Papeto'ai. An area of 3,000 – 6,000 meters squared (average 4,817 m sq.) of the patch reef was searched by snorkeling for 3 hours in total. Collection time, handling and photography were an estimated 10 minutes per animal, so subtracting one hour from search time, search effort across sites averaged 65 seconds per every meter squared. Differences in the sampled area between sites were due to the limited area of cushion star habitat at some fringing reef flats and sloping reef across the island, and so the sampled area was adjusted to feasibly accommodate the standard search time for each survey. The length of shoreline searched at each site ranged from 62 – 86 meters (average 69 m).

Search area was plotted using GPS points taken in the field and mapped as a polygon on Google Earth Pro (Fig. 1).

Cushion stars were sampled opportunistically within the survey area. Two snorkelers swam the width of each search polygon, searching in parallel lanes perpendicular to the shore until the entire area was covered. *C. novaeguineae* have been shown to be most active in movement and foraging for coral and algae at night, beginning around 18:00 h (Hawkins, 2006), so sampling occurred during late afternoon and early evening to increase likelihood of encountering enough organisms. *C. novaeguineae* can also be found at different depths on the reef at different times of the day, to depths of 20 meters in Opunohu Bay (Parmentier and Das 2004), so much of its distributional range during early and midday is beyond snorkeling range.



FIG 1. Mo'orea, French Polynesia, with survey sites indicated with white pins. Experimental site at Cook's Bay (2) is also noted. Inset showing the polygon search area at Temae Beach (1) with horizontal substrate transects, an example of the typical sampling setup used at each site.

Each time a cushion star was found during our survey, substrate and microhabitat

features were recorded and photographed in the field. Type categories for substrate were determined based on the predominant substrate directly underneath the cushion star when encountered. Categories were algae on coral (ALGC), algae on coral rubble (ALGCR), coral rubble (CR), sand with coral rubble (SCR), rock (R) and live coral (C). At the same time, we noted whether shelter for the cushion stars was present or absent. We also determined whether or not the individual was eating when encountered by checking if the stomach was extroverted when picked up. Lastly, aboral and oral diameters of each cushion star were measured, along with ambulacral groove length.

To determine the overall substrate composition and available habitat at each field survey site, three transects were drawn parallel to shore within the original search area polygons on Google Earth Pro. Each of these was 50 x 2 meters. The length of each site's polygon search area was divided into three portions, near, intermediate, and distant from shore. Within these three portions, a random number generator was used to determine the exact meter at which the transect would be placed. Each of the 50 m three transect lines was then searched by snorkeling, categorizing the area 1 m to the left and 1 m to the right of the transect. The predominate substrate encountered every 5 meters was recorded according to the same substrate categories used for cushion star sampling. Three transects were done at each of the five sites to generate data on habitat availability and heterogeneity of the reef within search areas.

Collection and photography analysis

In order to determine the extent of variety and morphological differences between cushion star color morphs found on different substrates, digital photographs were taken for

later analysis (McKay 2013). When an organism was encountered, it was collected and brought to shore in a round 4.5-liter plastic tub. Each individual was photographed on the iPhone 6 8-megapixel, 1.5 μ m pixel size camera (Apple Inc.) in HDR mode, inside a flat white bin filled with shallow water. Photos were taken from directly overhead and lighting was controlled by fully shading the bin to prevent glare. A color standard showing true white, grey, RGB and CMY values was present in each photograph (Whiteley et al. 2009, O'Hanlon et al. 2017). Photographs were taken of the aboral and oral sides of each animal.

Each photo was standardized relative to the true white and grey values of the color standard using the Curves function in Adobe Photoshop CC 2018 (Adobe Systems Inc., San Jose, CA) as in Whiteley et al. (2009) and Krohn and Rosenblum (2017). Values were adjusted to achieve identical values for the RGB channels, with white balance scores set to 255 for R, G and B (O'Hanlon et al. 2017). A rectangular 1500 X 60 pixel transect was drawn through the middle of each cushion star (see Appendix A) and was analyzed for RGB values using the plugin RGB Measure in ImageJ (ImageJ 1.x, National Institutes of Health, Bethesda, MD). The size and shape of this transect allowed for more accurate average RGB values to be taken for each cushion star, as their mottling patterns result in the presence of different color patches between the center and outer ring of the aboral surface.

Hue, Saturation and Brightness (HSB) values were measured by converting each photo to a HSB stack and simultaneously analyzing the same 1500 X 60 pixel transect for each image in ImageJ (McKay 2013). This method allowed these values to be extracted repeatedly from the same location on each individual. Coral reef-dwelling starfish have eyes that form low-resolution images and are

mostly spectral sensitive, allowing them to distinguish differences between light and dark, but not between colors (Garm and Nilsson 2014). Their experiments indicated that reef starfish eyes are well-adapted for navigating back towards the reef, which they perceive as 'dark,' and this prevents them from venturing out into the open ocean, which they perceive as 'light.' Thus, RGB values are not meaningful to the asteroid visual system and introduce a human bias of color perception. Therefore, RGB values were not used in quantifying the role of color on habitat selection of *C. novaeguineae*.

Here, HSB values were used to quantify cushion star color. Hue, Saturation and Brightness were used cumulatively to create a comprehensive color space that was compared to substrate selection preferences. Average Brightness values were used as a measurement of dark versus light cushion star color, to determine if darkness and lightness values of stars independently influenced microhabitat.

Laboratory experiments

To determine if darker cushion stars have a higher tendency to select darker substrates, i.e. sheltered substrates, and if light cushion tend to remain exposed on light substrates, individuals were brought to the laboratory to conduct a habitat alignment experiment. All laboratory animals were collected from the reef flat and reef slope north of and in front of the UC Berkeley Richard B. Gump Station in Cook's Bay, Mo'orea, outside Piha'ena MPA range. Cushion stars were housed in the outdoor wet lab at the Gump Station in circular blue tanks supplied with a constant flow of seawater. Cushion stars were collected opportunistically as they were encountered along areas of the reef. All individuals were housed in an acclimation tank for at least 24 hours before conducting the experiment in

order to minimize effects of the stress of collection and transportation (Yokley 2016).

A treatment tank was set up to determine if microhabitat selection is correlated to cushion star coloration. Up to three 'light' morphs and three 'dark' morphs (see Appendix B), based purely on the appearance of their visible colors, were held in the lab at a time. Half of the tank was shaded with a tarp to provide options for shelter throughout the day. Cushion stars in holding were fed with heads of live *Pocillopora* and *Acropora* coral found broken off naturally by wave action from the Snack Mahana Beach site in Papeto'ai. No individual was held for longer than six days. Light-colored sand was collected north of the Gump station to evenly cover the entire bottom of the treatment tank, to prevent differences in texture from influencing the microhabitat selection of the cushion stars. The temperature of the tank was maintained at an average of 29-30° Celsius to prevent high variances in temperature from influencing cushion star behavior. Two hiding areas were constructed of cement bricks on one side of the tank, with sufficient space for both cushions star to burrow underneath for shade or shelter. These shelters were designed so that two cushions could hide within them without interaction, as to prevent spatial competition from influencing their behavior. No food was present in this tank as *C. novaeguineae* has been shown to move persistently towards sources of food (Hawkins 2006), and this would have affected its microhabitat selection. For this reason, bricks were used to provide shelter instead of rocks from the ocean.

Two cushion stars, one light morph and one dark morph, were allowed to roam free in the treatment tank for 24 hours. Each two-star trial was observed three times in 24 h, once in the afternoon (between 16:00-17:00 h), once at night (between 20:00-21:00 h) and once in the morning (between 7:00-8:00 h). The morning

and afternoon observations were intended to account for changes in weather and direct sunlight exposure throughout the day, and the nighttime observation was to account for a treatment in an absence of sunlight. At each observation, cushion stars were recorded as either (1) blending in or standing out from the substrate and (2) hiding or not hiding in the cement structure. All cushion stars used in experimental trials were marked with a superficial X pattern using a razorblade on the aboral surface (Olliff, 2011) to prevent utilizing the same individual twice. Following the experiment, all individuals were released alive back to the site of initial capture (Gump Station reef).

Statistical Analyses

To determine if geographic habitat variation was tied to cushion star coloration, habitat selection by cushion stars within their overall environments was first analyzed. To test if cushion stars non-randomly select microhabitats from the substrates available to them, observed proportion of cushion star-inhabited substrate was compared to proportions of available substrate types. A Chi-squared test was used to analyze the proportional percentage differences in substrate used by cushion stars in the field and the frequencies of substrates that were available to them.

Next, the color of all cushion stars sampled in the field was quantified using a HSB color space, for which a Principle Component Analysis (PCA) was performed on Hue, Saturation and Brightness values extracted from ImageJ. To determine clustering patterns associated with the HSB values of 'dark' and 'light' cushion stars, to see if their color differences were in fact variable, this data was visualized using 3 axes to represent Hue, Saturation and Brightness in the PCA. Color values obtained for each

cushion star were plotted alongside substrate selected by that star to determine whether clustering of color values in relation to certain substrate types was apparent. All three axes in the color space, Hue, Saturation and Brightness, contributed to PC1, as in Krohn and Rosenblum (2016).

PC1 represented 82% of variation in the data, and thus was a reasonable metric of cushion star coloration. To examine correlation between color and substrate selection, logistic regression was used to determine correlation between a cushion star's HSB color and the substrate it selected (Smith et al. 2016). PC1 scores were the explanatory variable in each regression, and microhabitat substrate type was the binary response variable. Logistic regressions for each substrate type were tested for goodness of fit using a Chi-squared goodness of fit test with residual and null deviances.

As Sand was distinct from other substrates, being the lightest substrate available on the reef and the substrate on which stars were found to be most visible and exposed, this substrate in particular was explored for further relationship to cushion star color. The lightness and darkness of stars, quantified using average Brightness values, was tested for significant differences using a two-sided two-sample t-test (Sowersby et al. 2014). Brightness values were tested for normality using a Shapiro-Wilks normality test and homogeneity of variance using Levene's Test. Data were found to be normally distributed and display equal variance. Average Brightness of stars sampled on Sand was compared to the average Brightness of individuals found on all other substrate types. To assess if any relationship existed between Brightness of stars and their selection of shelter in the field, Brightness of individuals found exposed (no shelter) was compared to the Brightness of individuals found under shelter.

To further investigate the hypothesized substrate alignment differences between 'light' and 'dark' cushion stars, dark and light individuals were brought into the lab to determine if this pattern persisted in a binary habitat choice test. Dark stars were expected to align to dark substrates and light stars to light substrates. For these experiments, a Chi-squared test was used to determine if substrate alignment was significantly different for dark and light color morphs in comparison to an expected equal alignment between morphs. All statistical analyses were conducted in R using the packages *ggplot2*, *ggfortify*, *jtools* and *car* (v1.1.419; R Development Core Team 2015) with $\alpha = 0.05$.

RESULTS

Microhabitat selection in the field

All field sites surveyed were found to be heterogeneous in habitat type, with at least two different substrate types found per site. Substrate types available on the reef were compared to substrate types on which cushion stars were found (Fig. 2). These data were lumped across field sites, which served as replicates. As no site had equally available substrate types, count values were converted to proportional percentages divided by the total number of observations for Available and Used surveys ($n = 150$, $n = 33$).

Data was found to be normally distributed by Shapiro Wilks. There were significant differences between percentages of Available versus Used substrates across the 6 substrate categories (Chi-sq. = 20.78, $df = 5$, $p < 0.01$).

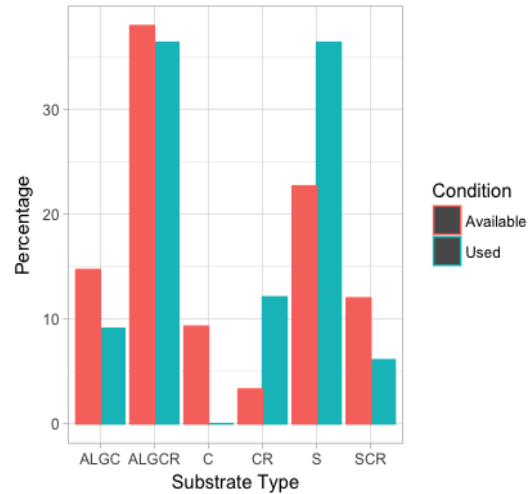


FIG 2. Relative percentages of the 6 substrate categories found at field survey sites. Categories were ALGC = algae on coral, ALGCR = algae on coral rubble, C = live coral, CR = coral rubble, S = sand and SCR = sand with coral rubble. Available (red) represents percentage of times that substrate category was seen in the field, with used (blue) representing the percentage of times a cushion star was observed on that substrate type.

Photography analysis: HSB clustering

Visibly darker morphs had lower Hue, Saturation and Brightness values altogether (80-140), while visibly lighter morphs had higher HSB values (140-200). Distribution of points within the color space indicates that color variance exists between individuals ($n = 31$) (Fig. 3). To determine color variability between morphs, HSB values were clustered in a scaled Principle Component Analysis (Fig. 3). 82% of variation was explained by PC1, and all three vectors contributed to overall variation in cushion star color.

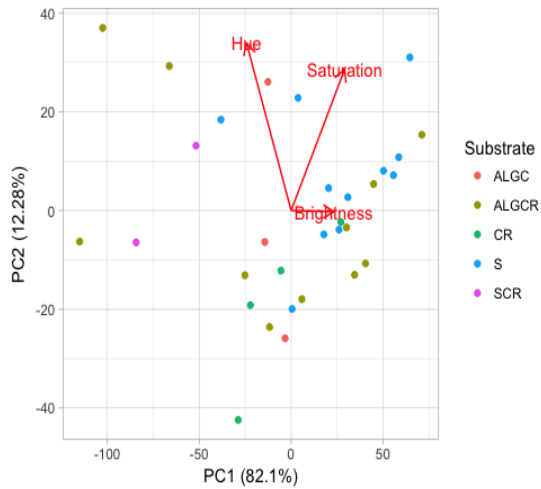


FIG 3. PCA showing clustering of Hue, Saturation and Brightness values with percent variation within the data explained by PC1 and PC2. Vectors showing correlation between the three color parameters. Substrates that each sampled cushion star was found on are shown in different color points.

Photography analysis: Color correlation to substrate selection

A logistic regression was used to determine correlation between PC1 values and individual substrates (Fig. 4). A single logistic regression was run separately for each substrate and PC1. Of the five substrates, only Sand (S) was found to be statistically explained by cushion star coloration using a logistic regression (Wald test, $p = 0.04$). Goodness of fit for logistic regression was tested against a null model using the difference between deviance of residuals and the null deviance. The logistic model was found to fit the data for Sand only significantly better than an empty model (Chi-sq. goodness of fit, $df = 1$, $p = 0.01$).

The average Brightness (darkness or lightness of color only) of individuals on Sand was found not to be significantly different from Brightness of individuals on all other

substrates ($t = -1.51$, $df = 29$, $p = 0.14$). Average Brightness of individuals found exposed versus under shelter in the field was also found not to be significant with $\alpha = 0.05$ ($t = -0.22$, $df = 29$, $p = 0.83$).

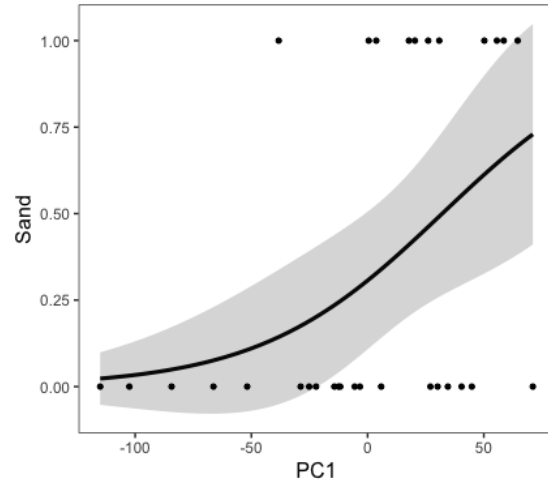


FIG 4. Logistic regression showing correlation between PC1 as a representation of cushion star coloration and the probability of a star being found on sand. PC1 as an explanatory variable significantly predicts the probability of a cushion star selecting sand (Wald test, $p = 0.03$). Area in grey indicates the 95% CI of the model.

Laboratory microhabitat selection

In the 10 trials completed using 20 stars for a total of 30 observations, dark color morphs selected dark substrates more frequently than light substrates, aligning color to substrate 63% of the time. Light morphs selected light substrates more often than dark substrates, aligning 73% of the time. Light stars were slightly more likely to match their substrate than dark stars were (Table 1). However, these trends were not significant (Chi-sq. = 4.54, $df = 3$, $p = 0.21$).

TABLE 1. Results from microhabitat selection trials, lumped across observations times. Dark substrates indicate sheltering beneath bricks, light substrates indicate exposure on sand or side of the tank.

Substrate	Light star	Dark star
Light	22	11
Dark	8	19

DISCUSSION

The variation in color found in sea stars, as within many invertebrates, can be a challenging evolutionary trend to unravel. In this study, habitats on the fringing reef of Mo'orea were determined to be heterogeneous and cushion stars were found to non-randomly select substrates within their environment, indicating microhabitat preference. Color was found to be significantly correlated with the selection of certain substrates. The findings of this research illuminate potential drivers for phenotypic plasticity in a species that exists within microhabitat niches in coral reef environments.

In accordance with these results, geographic patterns in morphological color variation have been shown to exist in the heterogeneous habitats of other sea stars (Harley et al. 2006), and habitat differences have been shown to play a discernible role in the coloration of both terrestrial and marine ectotherms (Stevenson 1985, Rosenblum 2005, Duarte et al. 2016). Distinct substrates could be related to background matching or camouflage tendencies (Stevens and Merilaita 2008), as well as thermoregulation, which is an important life history trait of ectotherms that can be closely linked with microhabitat (Stuart-Fox et al. 2017).

In the HSB color space created to describe cushion star coloration, clustering patterns revealed that sampled stars have different

values of Hue, Saturation and Brightness. This showed that individuals of *C. novaeguineae* have colors that are highly variable within the same population. While phenotypic plasticity of color in this species has many possible evolutionary mechanisms, significant correlation found between PC1 and substrate selection for Sand shows that cushion star coloration does play a role in substrate alignment. The significance of sand initially suggested that the lightness or darkness of a star may be the most important factor in an organism's overall color when it comes to microhabitat selection (Krohn and Rosenblum 2016). This was further supported by a trend towards higher Brightness values in stars that were found on sand versus on other substrates. Small sample size (n=11) likely contributed to this trend not reaching statistical significance.

Contrastingly, both the shortness of the Brightness vector in the PCA and the lack of statistical significance in the t-tests using Brightness values indicate that Brightness may not be an appropriate measurement of cushion star coloration. This suggests that overall PC1 was a more comprehensive and accurate color metric. Based on this data, darkness versus lightness of a cushion star cannot be concluded to be a sufficient representation of its coloration, or a feasible predictor of microhabitat selection.

Although reef starfish are thus far believed to be colorblind (Garm and Nilsson 2014), their photoreceptive ability to distinguish between darkness and lightness was observed in the lab experiment: cushion stars were able to seek out an opening to the cement structure and shelter beneath it repeatedly. *C. novaeguineae* may strategically seek out light or dark substrates at different times of day, as this behavior was observed within each 24-hour trial (stars being consistently more exposed at night and sheltered during the morning), but this did

not appear to be statistically significant in relation to color differences. However, darker stars did select to shelter themselves in the lab more than twice as often as light stars (ratio of 19:8), which suggests that 'dark' stars might experience stronger pressure for both camouflage and thermoregulation than light-colored stars. These pressures have been shown to be a tradeoff that many ectotherms face (Stuart-Fox and Moussalli 2009). As sand and algae on coral rubble were the two most commonly encountered substrates in shallow waters across the fringing reef, light-colored stars may possess both a camouflage and thermoregulatory advantage when exposed on these reef substrates (Stevens et al. 2013).

Ultimately, it is possible that shelter is less important altogether than the actual darkness, lightness or color of the substrate a cushion star selects. Both the lab trials comparing stars on sheltered (dark substrates) versus on exposed (light substrates) and Brightness values for exposed versus sheltered stars on sand in the field showed no statistically significant differences. With combined evidence from the laboratory and field trials that were based on the use of shelter, it is difficult to conclude whether shelter is as important to the microhabitat selection of a dark starfish than a truly dark substrate would be. It is known that in organisms which exhibit background matching, substrate color often dictates color variation among morphs (Sowersby et al. 2014). Extracting numeric color values for each microhabitat substrate type encountered in the field, to determine if PC1 or Brightness values for sampled stars are correlated to these values, would be further informative.

In the half-shaded acclimation tank that was set up as a control for observing daily movements of the stars, no distinct patterns were observed in certain color morphs moving to the shaded side of the tank more frequently than others. Under increased

thermal stress—such as more direct exposure to sunlight—*C. novaeguineae* does have the ability to move into deeper, cooler waters (Yokley 2016). Individuals are able to augment this by burrowing and shading themselves beneath rocks and dead coral (Stevenson 1985). Dark morphs, as well as morphs with extensive black mottling (see Appendix B), were observed to hide or burrow more frequently under objects available to them in the acclimation tank. These observations align with the expected reduced ability of darker colored ectotherms to withstand higher temperatures without overheating (Trullas et al. 2007). Experiments isolating thermoregulation as an explanatory variable for habitat selection would be warranted to draw conclusions on this trend's possible link to color or mottling in the cushion star.

Microhabitat selection of *C. novaeguineae* was found to be nonrandom within a heterogeneous environment. This preferential habitat selection in an island ecosystem supports prior evolutionary theories that plasticity both arises in heterogeneous environments and can promote fitness of colonizing organisms in these environments (Sultan and Spencer 2002, Rosenblum et al. 2007, Hendry 2015). The cushion star has escaped predation in its habitat on the fringing reef of Mo'orea, indicating that it is ecologically successful in its niche. To the best of my knowledge, *C. novaeguineae* has no known natural predators in the entirety of its South Pacific range. This suggests that camouflage may be less of a selection pressure to this species relative to thermoregulation, but further studies regarding *C. novaeguineae*'s ecology are required to validate this notion.

This study was designed under the standards of well-developed methodology for effectively and repeatedly quantifying the color of animals through digital photography (Stevens et al. 2007, Whiteley et al. 2009,

McKay 2013, O'Hanlon et al. 2017). However, image analyses were subject to the innate biases of the human eye, although this was substantially decreased by selecting an HSB color space over an RGB model or CIE LAB color space (Alexander Krohn, pers. comm. 2018). Additional technology and methodology for removing the bias of human color vision from describing the morphology of non-vertebrates is needed to provide neutral interpretations of animal coloration (McKay 2013, Kelley and Kelley 2014).

Ultimately, the research objective of this study was to determine how color morphology impacts the microhabitat selection of *C. novaeguineae*. Through an increased understanding of what may be an evolutionary pressure of phenotypic plasticity in this starfish, the role of plastic traits in species' survival and dispersal patterns on islands can be better understood. However, the geographic range sampled in this study does not encompass the greater geographic diversity of this species, which is widespread across much of the Pacific (Mah 2018). Future work to explore the color variation in *C. novaeguineae* populations on islands throughout the Indo-Pacific region would be beneficial to determine if genotypic differences exist between separated populations of this species. Such genetic differences have been found between color morphs of the crown-of-thorns starfish *Acanthaster planci* across their similarly large distribution range (Benzie 2017). Spatial genetic differences in different color morphs have also been recorded in *Linckia laevigata* (Williams and Benzie 2017), another common tropical starfish, suggesting that color variation in tropical reef starfish with wide distribution ranges may in fact be related to genetic divergences. Williams and Benzie (2017) connects the recency of these genetic divergences to changes in modern ocean conditions. Rising sea level and global

warming are increasing the capacity for tropical species to disperse, through the phenomenon known as "tropicalization," by which the distribution ranges of many tropical species are now moving into higher latitudes (Zarco-Perello et al. 2017). This allows populations of tropical phyla to colonize past their current distribution range, which may increase plasticity and lead to more spatial genetic divergences between populations. More information on the juvenile stage of the cushion starfish is needed to understand how its larvae travel and react to settlement cues, and thus how its distribution range and phenotypic variation may expand across the Pacific with ocean warming.

Alternatively, it is also possible that plasticity is working independently of genetic change in *C. novaeguineae*, as other molecular work on sea stars has revealed that phenotypically variant color morphs do not show large genetic variances (Harley et al. 2006). It remains unknown whether color variation in the cushion star is a trait independent of genetic change within populations, or if it is one mechanism of natural selection. As organisms attempt to survive the constantly changing, heterogeneous environments of coral reefs, especially in the era of climate change, population dynamics and plasticity are both important fields of study that can provide insight on the fitness abilities and survival strategies of tropical reef species.

ACKNOWLEDGMENTS

I would like to thank Professors Cindy Looy, Ivo Duijnste and Vince Resh for building a strong foundation for the beginning of this class, and Noah Whiteman and Seth Finnegan for fostering a lovely and welcoming learning environment during their brief time with us. I would like to thank Professor Stephanie Carlson for her invaluable expertise,

insight and patience in the final weeks of data collection and analysis. A huge, huge thanks to Jim LaChance, Clay Noss and Renske Kirchholtes, who were both a pleasure and a privilege to work and live with on an island, and who all went above and beyond expectations to help the students of this course. The utmost thanks to my friends, classmates and steadfast field buddies who were there with me throughout the thick and thin moments in the lengths of this study, and whom I was very lucky to learn alongside: Veronica Ellis, Daisy Stock, Jacques Jouglu, Emma Reich, Jasper Chao, Ben Malit and most especially Madeline Moore, without whom none of this would have been possible. Lastly, Mauruuru roa to the island of Mo'orea, a place special beyond words.

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APPENDIX A

Pixel transects were drawn in ImageJ for measuring the HSB values of each individual image. Transects were drawn on the original RGB image and were automatically copied by ImageJ onto each image generated in the image's conversion to an HSB stack. Transecting was standardized according to the methodology shown below. These methods were created due to a lack of prior literature on transecting the bodies of radially symmetric animals, and the need for repeatability for all images used this study. A straight, horizontal line was drawn using the Arrow tool and the Draw function from the bottom left eyespot to the bottom right eyespot, the points where a modified tube foot with a simple eye extends out to sense the animal's surroundings. The tube foot itself was usually not visible in the images taken, but the "corner" point where it would extend from was distinguishable after developing familiarity with the animals. The middle of this line was shown automatically in ImageJ and was marked with a permanent crosshair point using the ROI Manager, which records the x and y coordinates of each marked point (Fig. A1). From the middle point of the horizontal line, another straight, vertical line was drawn using the Arrow tool and the Draw function, through the middle of the animal from bottom to top (Fig. A1). The center of this vertical line was also shown automatically and was marked with a second crosshair point in the ROI Manager. The x and y coordinates of the middle point on the vertical line became the middle of the rectangular box used to create the pixel transect (Fig. A2). The top of the rectangle was centered at the same ROI coordinate points of the point marked to be the middle of the vertical line. The rectangle was extended out until it reached a height of 60 and a width of 1,500 (Fig. 3A). The area within the rectangular box was selected and analyzed using the Measure function separately for each image, Hue, Saturation and Brightness respectively (Fig. A4).

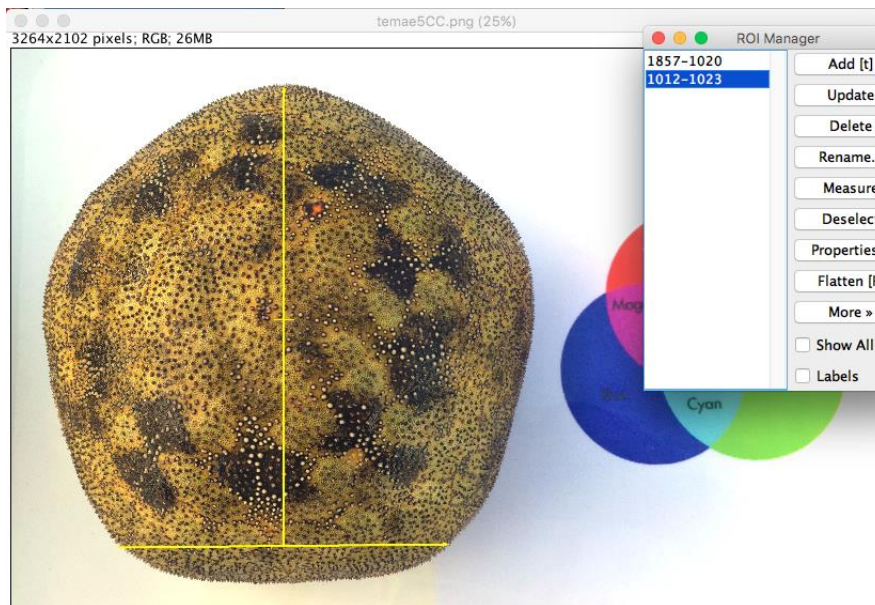


FIG 1A. Horizontal and vertical lines drawn using the Arrow tool from eyespot to eyespot and through midline areas of each specimen. Coordinate points at the middle of each line marked using the ROI Manager, shown at right.

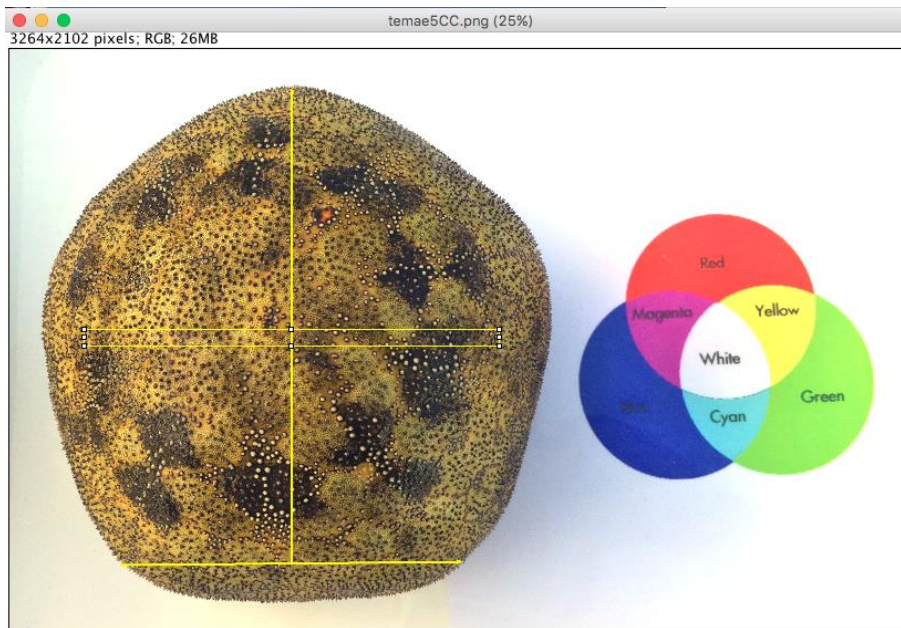


FIG 2A. 1500 x 60 pixel transect selected using the Rectangle tool, standardized to location by aligning the top center of the rectangle to the middle point of the vertical reference line.

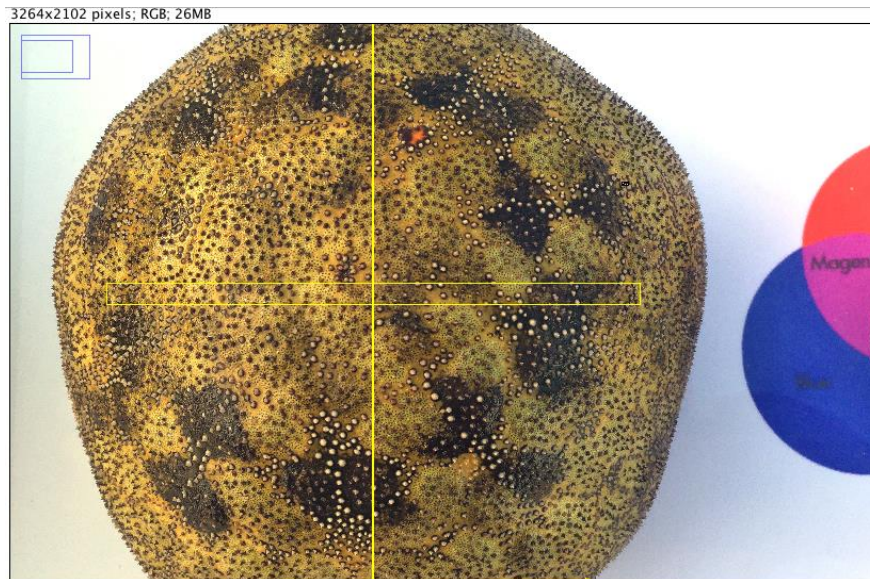


FIG 3A. Rectangular transect set in place using the Draw tool, to be copied over to the HSB stack.

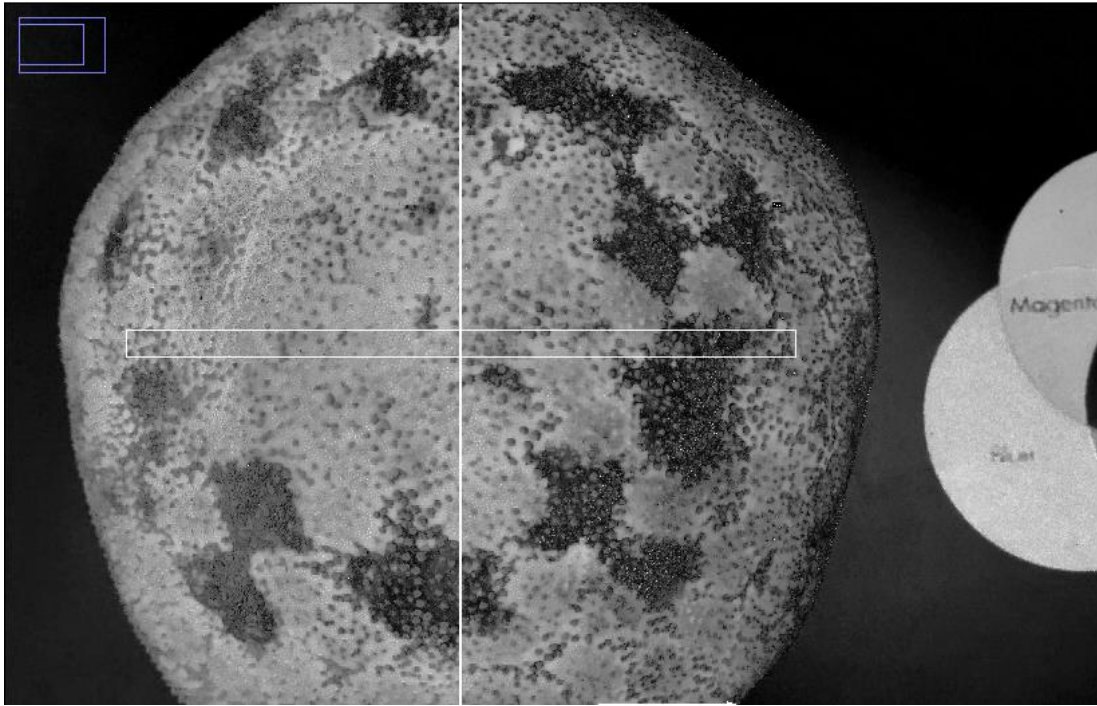


FIG 4A. Brightness image from RGB image conversion to HSB stack. Each of the three images in the stack were analyzed using the same rectangular transect to obtain numeric HSB values.

APPENDIX B

Photos of example 'light and 'dark' morphs used in laboratory experiments. (A) Only clearly distinguishable 'light and 'dark' morphs were collected and used in lab trials. Only easily categorized 'light' and 'dark' morphs such as the individuals shown below were used in substrate alignment laboratory trials. Ambiguous morphs were found during collection, but were excluded from these trials, as this would have added uncertainty to the binary color categorization used for this part of the study. Ambiguous 'dark' and 'light' color morphs (B) included any light morphs with excessive black mottling or significant dark-colored or black patches and light stars with dark spines that contrasted the aboral color. For dark morphs, only stars with dark coloration and matching dark spines were used, as lightness of spines also added ambiguity to their color assignment.



FIG. 1B. Example of clearly distinguishable dark and light morphs used in laboratory trials.



FIG. 2B. Example of two ambiguous color morphs not used in laboratory trials.