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THE EFFECTS OF BACKGROUND ADAPTATION AND FOOD AVAILABILITY ON HABITAT PREFERENCE OF *CORYTHOICHTHYS FLAVOFASCIATUS*

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Abstract. Habitat preferences are intrinsically linked to factors that facilitate the survival of a species. The relationship between these factors determines how well a species does in its environment. Often habitat choice is related to the availability of food, presence of predators, and proximity to other viable habitats, amongst other variables. How these variables interact depends on fluctuations in the trophic web of which they are a part. *Corythoichthys flavofasciatus* is a species of pipefish that occurs in the fringing and back reefs of Mo'orea, French Polynesia. It feeds on zooplankton and occurs primarily on dead coral heads that are covered in algal turf. This study aimed to understand the relationship between zooplankton abundance, habitat quality, and substrate types on the habitat preferences of this pipefish. A field survey of the abundance of zooplankton in different habitats was used to determine if more food was available in habitats that were dominated by dead coral. Results suggest that more zooplankton are found above algal turf than live coral. A survey looking at pipefish abundance and amount of coral available in the habitat suggests that pipefish abundance correlates weakly to the amount of algal turf in the environment. An experiment quantifying color change in light and dark morphs of pipefish was conducted to determine if pipefish were capable of background adaptation, depending on substrate color. The results suggest that these observations were not statistically significant but warrant further research, using larger sample sizes. The findings of this study provide insight into the ecological role of pipefish in coral reef habitats.

Key words: pipefish; background adaptation; habitat preference; algal turf; zooplankton; *Corythoichthys flavofasciatus*; substrate color; Mo'orea, French Polynesia

INTRODUCTION

Coral reefs play an important role in marine ecosystems given the intricate tangle of communities they support. A diverse set of organisms interact to maintain the complex macrocosm that is a coral reef (Veron 1986). Reef health is a function of several abiotic and biotic factors that, combined, allow the proliferation of reef ecosystems (Veron 1986). Disruptions in the marine realm can heavily impact the well being of corals (Sapp 1999). There has been a worldwide decline in reefs due to a combination of anthropogenic and environmental impacts (Bellwood *et al.* 2004). Continued studies are required to fully understand the future of coral reefs and the effect on the communities that rely on them.

The coral reefs of French Polynesia are currently recovering from damage due to both a crown-of-thorns outbreak in 2003 (Adjeroud *et al.* 2005) and Cyclone Oli in

2010 (Etienne 2012). This, coupled with the increasing effects of ocean acidification (Hoffman *et al.* 2010), has drastic implications on the coral surrounding the islands. Dead coral is often covered in a layer of fine algal turf that accelerates coral smothering and may prevent coral juveniles from settling (Nugues *et al.* 2003). This algal turf is home to a plethora of microscopic invertebrates that are the basis for many marine trophic webs (Zeller 1988). A number of reef fishes are known to graze on these benthic invertebrates (Zeller 1988) as they serve an important food source in the reef community.

Pipefish (Syngnathidae) are predatory fish that are often found living in sea grass beds or coral reefs. Their prey consists primarily of copepods and amphipods, which they capture with a sucking motion, as suggested by the intact crustacean exoskeletons in their stomachs (Garcia *et al.* 2005). Pipefish in the genus *Corythoichthys* are often found in reef

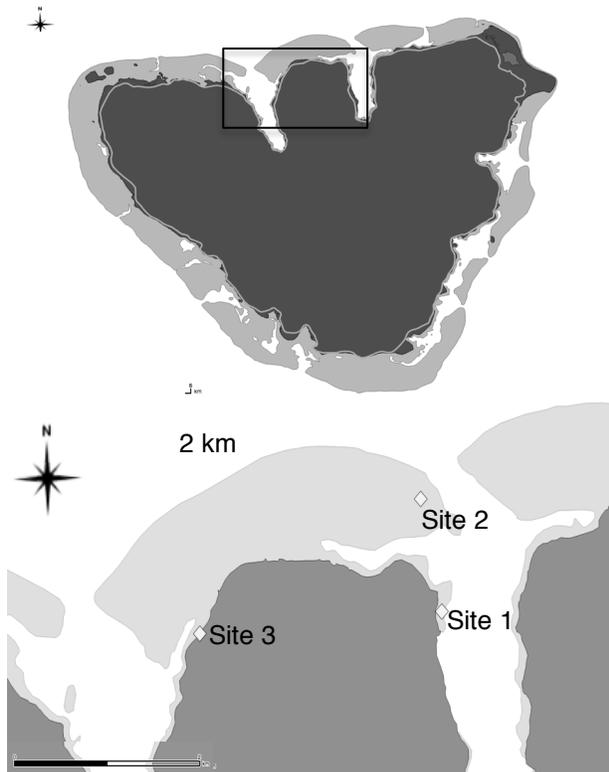


FIG. 1. Location of study sites on Mo'orea, French Polynesia.

habitats (Gronell 1984) and are common in the waters of the Indo-Pacific however they are a little-studied group (Dawson 1977).

The pipefish *Corythoichthys flavofasciatus* has been documented across the Pacific Ocean (Dawson 1977) and appears to be quite abundant in the fringing and back reefs surrounding Mo'orea, French Polynesia. Preliminary surveys suggested that it is found almost exclusively on dead corals covered by a layer of algae that the pipefish swims along. Initial observations of feeding behavior suggested that pipefish habitat preference might be linked to prey occurrence in this algal turf. The fish appear to be well camouflaged and color matched to the substrate, ranging from greenish-yellow to pale yellow. Many fish species in the class Osteichthyes are known to control and change their body color using chemical structures called chromatophores (Fujii 1993). In low contrast environments like sand, fish might appear drab and unadorned (Allen 1987).

This study aimed to elucidate the function of the specific adaptations of *C. flavofasciatus* related to its habitat choice. Investigating

this question involved understanding the underlying mechanism that controls color matching to substrate as well as assessing zooplankton abundances in algal turf. The main question was whether habitat preferences of *C. flavofasciatus* are related to background adaptation, food availability, or a combination of both. This study also provided insight into the implications of coral death on the range of viable habitat for this pipefish.

METHODS

Study site

Pipefish were observed and collected at three sites in Mo'orea, French Polynesia, from October 8 to November 16, 2012 (Fig. 1). Maps were made using QuantumGIS, Version 1.8.0 Lisboa (Quantum GIS Development Team, 2012). Sites 1 and 2 were the fringing and back reefs outside the UC Berkeley Gump Station, respectively ($17^{\circ}29'20.12''S$, $149^{\circ}49'33.04''W$ and $17^{\circ}28'48.89''S$, $149^{\circ}49'40.62''W$), and Site 3 was the fringing reef near Ta'ahiamanu Public Beach west of Cook's Bay ($17^{\circ}29'25.85''S$, $149^{\circ}50'59.88''W$). These sites were chosen due to confirmed presence and relatively high abundance of pipefish during preliminary surveys of the reef.

Study organism

The network pipefish *Corythoichthys flavofasciatus*, a member of the family Syngnathidae, has been documented across the Pacific Ocean (Dawson 1977). Pipefish are known to form monogamous bonds (Matsumoto 2001). In this family, the males are known to brood the young before they hatch out of egg sac deposited by the female (Wilson *et al.* 2007). There were no obvious differences between males and females of *C. flavofasciatus*, except for 2 blue spots on the ventral side of males, which was seen on all males regardless of breeding status. The pipefish has a highly variable pattern on its dorsal side (see Fig. 5) with dark hexagon-like shapes interlaced together on a lighter trunk. Red marks are seen on the two ridges that run along the body and yellow striations crisscross over the pale parts of the trunk. Field observations suggest that this species often occurs in pairs on the same or nearby dead coral heads covered in algal



FIG. 2. *Corythoichthys flavofasciatus* on algal turf

turf (see Fig. 2) with dominant species *Polysiphonia sertularioides* (identified using Payri *et al.* 2000). When collected, pipefish that appeared to be paired were numbered and separated in captivity. All pipefish were maintained in a tank for a maximum of four days (as per Animal Use Protocol #T042-0813). Pipefish that were originally in pairs were released together, in the general vicinity of their own coral head. When feeding, the pipefish surveyed the algal turf, their eyes constantly searching for prey. A quick pecking motion either above or in the turf was correlated with prey capture.

Zooplankton abundance and habitat type

To determine if zooplankton abundance differed on dead coral covered by algae versus live coral nearby, 20 water samples of 15 mL were taken above both these microhabitats. To simulate pipefish feeding behavior, a syringe with approximately the same diameter as a pipefish mouth was used to obtain the

samples, ensuring that zooplankton of about the right size were collected. 75% ethanol was then added to the water samples and copepods, amphipods, and isopods were counted using an MZ16 Leica® dissecting scope.

Pipefish abundance and habitat type

C. flavofasciatus were found in both the fringing and back reefs at depths of 1 to 2 meters in semi-silty to clear water. Individuals were observed while snorkeling and a distance of at least 30 cm was maintained away from the pipefish in order not to frighten them. 3 transects of 15 m were run at sites 1 and 3 and pipefish abundances were correlated with the number of live and algae-covered coral heads nearby. Transects were run 2 meters apart from each other. The transects at Site 1 were run 2 meters away from the edge of the drop-off of the reef while those at Site 3 were run 5 meters away from the start of the water line.

Color morphs and background adaptation

A field survey of the different color morphs present at the three sites was conducted to catalogue the degree of variability between pipefish. An experiment was designed to determine whether color morphs were related to substrate color and if this pipefish was capable of background adaptation. This was motivated by observations of pipefish swimming between algal turf patches, over sandy substrate. To test whether color in *C. flavofasciatus* was a phenotypically plastic trait, pipefish were collected from each of Sites 1 and 3 and



FIG. 3. Experimental Setup. Experiment 1 had a treatment group (light pipefish on dark substrate), and a control group (dark pipefish on dark substrate). Similarly, Experiment 2 had a treatment group (dark pipefish on light substrate), and a control group (light pipefish on light substrate). Dimensions of individual compartments, 8 x 19 cm².

sorted into two different categories: light and dark, distinguished from each other using the color wheel. These pipefish were then segregated into containers, with either dark green artificial turf or white sand with dark pipefish on light substrate (n=11) and light pipefish on dark substrate (n=7). The control setup put light pipefish on light substrate (n=7) and dark pipefish on dark substrate (n=8). Experiment 1 looked at the color change of dark and light pipefish on dark substrate while Experiment 2 looked at the color change of dark and light pipefish on light substrate. Pipefish were housed individually to eliminate the possibility of color change due to the presence of conspecifics. The container was a large plastic box of dimensions 65 x 38 cm². This was split into 16 compartments (each 8 x 19 cm²) using Plexiglas as separators between compartments. One side had the dark green artificial turf while the other side had white sand substrate (see Fig. 3). The walls of each compartment were covered with either dark green or sandy brown mesh. A grey mesh was placed over this setup to ensure that the pipefish stayed in their individual compartments. The pipefish were maintained in these containers, after an acclimation period of 1 hour, for a period of 24 hours, which was enough time to see change but also minimized any unwanted effects of stress on color. The setup was kept at a depth of 1 meter in a large blue tank that was in direct sunlight and had running ocean water from the Gump station flow tank system, which obtains its water from Pao Pao Bay.

The trunk of the pipefish (i.e. from head to dorsal fin) was photographed before and after, against a white background and with a color wheel of red, blue, and yellow. Photographs were taken a foot away from the subjects, using an Olympus® Tough TG-1 camera. All images were then analyzed using the program ImageJ, Version 1.46r (Abramoff, M. D. *et al.* 2012). The photographs were edited using Adobe Photoshop, Version 13.0. If the RGB values of the red, blue, and yellow on the color wheel were not consistent in the before and after shots, then they were adjusted until the RGB values were the same. Using the color picker tool, 5 random points were chosen on both the dark and light segments of the pipefish, on the same segment of each pipefish. This was done both before and after the background manipulation

Benthic Zooplankton Abundance in 2 different habitats

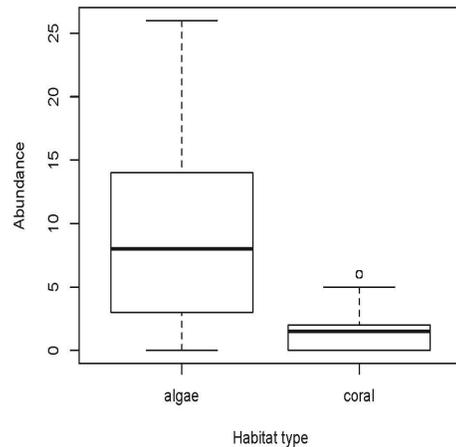


FIG. 4. Boxplots of zooplankton abundance in water samples taken above algal turf and above coral. Results are pooled for all three sites. Welch's t-test, $F_{(1,32)} = 34.7650$, $p < 0.0001$

experiment. The RGB values were recorded for each point and were averaged to obtain a mean RGB for dark and light segments, before and after. The difference between the dark and light segments of the pipefish was calculated to ensure that differences were due to actual color change and not merely an artifact of different photographing conditions. The difference between the RGB values before and after were calculated and the final results were used as the basis for the comparison of color change for each pipefish. Since high RGB values correspond to lighter colors and low RGB values correspond to darker colors, negative differences are associated with a change from dark to light while positive differences are associated with the reverse change.

Statistical methods

All data was analyzed using the statistical software package JMP, Version 10 and graphs generated using both JMP and R, Version 2.15.1. Welch's t-test was used to determine if zooplankton abundance differed on live coral versus dead coral covered in algal turf. This was done for all three sites individually, however the site had no effect on the abundance so the data were pooled when comparing zooplankton abundance to habitat type. Welch's t-test was used to determine if pipefish color change was a statistically significant phenomenon. This was done for both

experiments, with dark substrate and light pipefish. This was followed by a planned pairwise comparison between the two separate treatment groups, to look at differences between dark and light fish on oppositely colored substrates.

A multiple linear regression was used to determine the relationship between pipefish abundance and the amount of live coral and algal turf in the habitat. In other words, the effect of habitat types available on pipefish numbers.

RESULTS

Zooplankton abundance and habitat type

Welch's t-test was used to determine if zooplankton abundance differed in water samples taken above live coral versus those taken above algal turf. A two-sample t-test was not possible because of unequal variances, indicated by Levene's test ($F = 39.9002$, $p < 0.0001$), so Welch's test was used to compare means instead. There was no effect of Site on zooplankton abundance and so the data from all three sites was pooled and only the effect of habitat type was analyzed. Table 1 shows the mean zooplankton abundance for different habitats at each of the sites. The results (see Fig. 4) strongly indicate that larger numbers of zooplankton were present above dead coral covered in algal turf ($F_{(1,32)} = 34.7650$, $p < 0.0001$).

Pipefish abundance and habitat type

Analyzing the results from the multiple linear regression on the data obtained for pipefish abundance against two variables, number of live coral heads and number of dead coral heads covered in algae, suggest that pipefish abundance is in no way related to amount of live coral nearby. This was true at both Site 1 and 3. On the other hand, the amount of algal turf in the habitat was slightly predictive of the number of pipefish. However the results of this regression (RSquare = 0.172, $p < 0.0001$) are not sufficient evidence to suggest that pipefish abundance increases with amount of algal turf available. Although the low p-value suggests a significant relationship, the corresponding low RSquare value reveals merely a weak relation. In other words, only about 17% of the data can be explained by this chosen variable.

Color morphs and background adaptation

After 24 hours on either dark or light substrate, observations suggested that some dark pipefish became lighter on light substrate while some light pipefish became darker on dark substrate. These changes can be seen nicely in Figure 5. However after performing Welch's test (red: $F_{(1,6)} = 1.9510$, $p_R = 0.2033$; green: $F_{(1,6)} = 2.8455$, $p_G = 0.1272$; blue: $F_{(1,6)} = 2.1629$, $p_B = 0.1693$) on

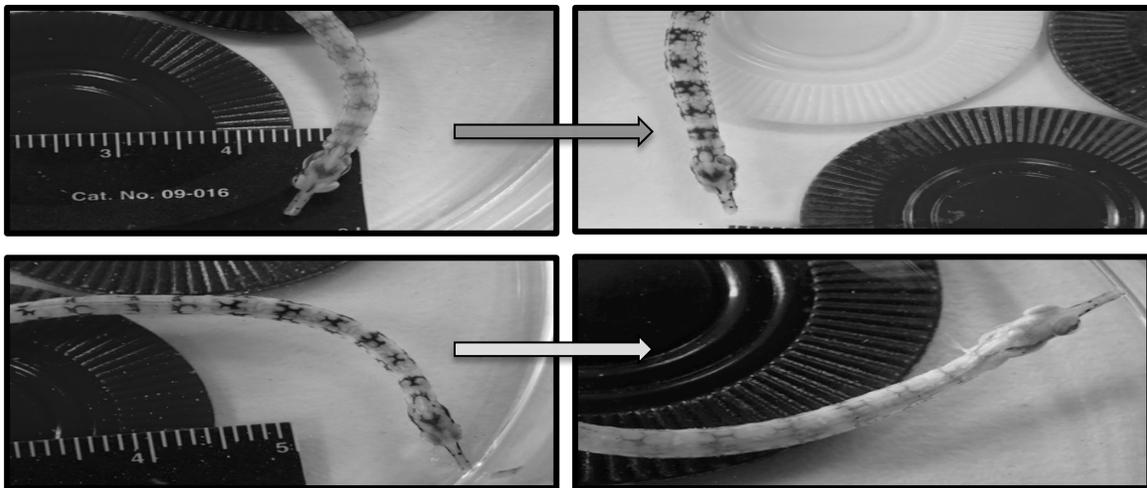


FIG. 5. Color change after 1 day in a light pipefish on dark substrate (top row), and in a dark pipefish on light substrate (bottom row). Note the contrast difference between dark and light forms.

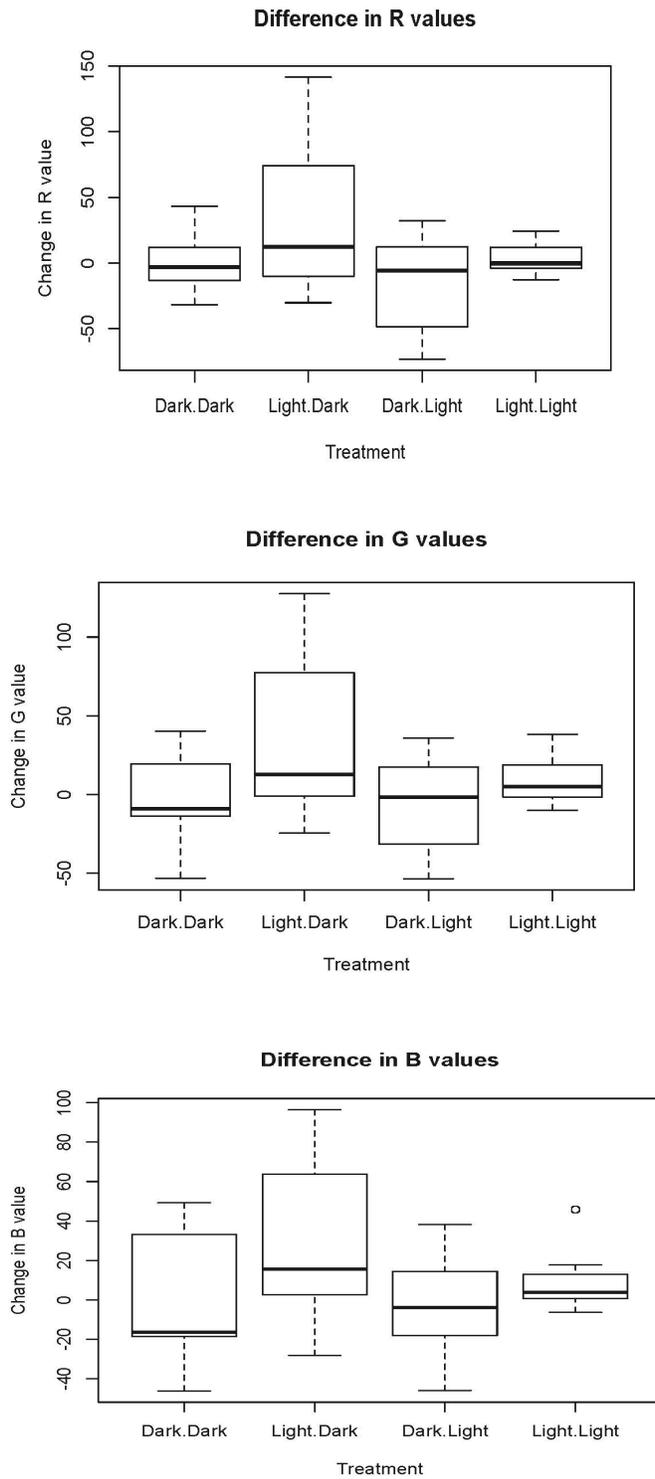


FIG. 6. Difference in R, G, B values for Experiment 1 (on left) and Experiment 2 (on right). Experiment 1 put dark and light pipefish on dark substrate and Experiment 2 put dark and light pipefish on light substrate. The difference in color between treatment and control was not significant in either experiment. Expt. 1 Welch's test, red: $F_{(1,6)} = 1.9510$, $p_R = 0.2033$; green: $F_{(1,6)} = 2.8455$, $p_G = 0.1272$; blue: $F_{(1,6)} = 2.1629$, $p_B = 0.1693$. Expt. 2 Two-sample t-test red: $F_{(1,17)} = 1.6684$, $p_R = 0.2138$; green: $F_{(1,17)} = 1.5356$, $p_G = 0.2321$; blue: $F_{(1,17)} = 1.3260$, $p_B = 0.2655$. A pairwise comparison of both treatment groups however proved significant only for R and G values, but not B values ($F_{R(1,17)} = 4.9219$, $p_R = 0.0404$; $F_{G(1,17)} = 4.8727$, $p_G = 0.0413$; $F_{B(1,17)} = 4.2544$, $p_B = 0.0548$).

Experiment 1, it was noted that these changes were not statistically significant between the treatment and control groups. Similarly, a two-sample t-test (red: $F_{(1,17)} = 1.6684$, $p_R = 0.2138$; green: $F_{(1,17)} = 1.5356$, $p_G = 0.2321$; blue: $F_{(1,17)} = 1.3260$, $p_B = 0.2655$) on Experiment 2, in which the variances across groups were equal, revealed non-significance between treatment and control (see Fig. 6). Data obtained from both Experiment 1 and 2 were normally distributed however had large variances. However, a planned pairwise comparison between the two experimental treatment groups (dark pipefish on light substrate and light pipefish on dark substrate) revealed that Differences in R and G values, but not B, were significantly distinct in both treatments ($F_{R(1,17)} = 4.9219$, $p_R = 0.0404$; $F_{G(1,17)} = 4.8727$, $p_G = 0.0413$; $F_{B(1,17)} = 4.2544$, $p_B = 0.0548$), since dark pipefish that became lighter on light substrate presented positive differences in RGB values while light pipefish that became darker on dark substrate presented negative differences (See Fig. 6).

DISCUSSION

Zooplankton abundance and habitat type

There appears to be a strong relationship between the abundance of zooplankton and the type of habitat. This was evident at all sites with higher abundances above algal turf environments. This would appear to suggest that food availability for pipefish is greater on substrate covered in algal turf. Although copepods, amphipods, and isopods were also present in water samples taken from above live coral, they are much less abundant which suggests that pipefish population dynamics might be controlled by the amount of algal turf present in the reefs. If so, it would have important implications on our understanding of reef population dynamics. Perhaps *Corythoichthys flavofasciatus* is a species of fish that might benefit from a degraded or regenerating reef ecosystem, because algae cannot grow on live coral (Diaz-Pulido *et al.* 2004).

TABLE 1. Mean zooplankton abundance for different habitat types, at each site.

Site	Gump Station Fringing Reef (Site 1)		Gump Station Back Reef (Site 2)		Public Beach Fringing Reef (Site 3)	
Habitat type	Algal turf	Live coral	Algal turf	Live coral	Algal turf	Live coral
Mean zooplankton abundance	10.00	1.80	9.20	1.50	9.00	1.30
S. Error	1.779	1.779	1.895	1.895	1.346	1.346

Pipefish abundance and habitat type

To determine if pipefish abundance is affected by habitat quality, a multiple linear regression was used to understand the relationship between amount of live coral, the amount of algal turf, and pipefish numbers. Interestingly, there was no correlation between live coral and pipefish, which was unexpected. Given the strong association between pipefish presence and algal turf environments that were revealed during field surveys, there was no inverse relationship between live coral and pipefish abundance. The amount of live coral also did not correlate with the amount of dead coral that was covered in algal turf.

This appears contradictory but might be due to a number of reasons. The simplest explanation is that the sample size was not large enough to reveal any significant trends. On the other hand, it might in fact be true that there is no relation between live coral heads and dead coral heads covered in algal turf. Algal turf is often seen as one of the first colonizers in the succession of algae on dead coral substrate (McClanahan 1997; Kattan 2005). It might not be solely the amount of algal turf that relates to quality of coral but also other species of alga that are late-successional species. These species were not counted in this study because they did not appear to affect whether pipefish were in the area or not. The fact that the amount of algal turf was not sufficiently predictive of pipefish abundance seems more likely to be due to small sample sizes, given the small R^2 and significance values obtained.

Color morphs and background adaptation

If pipefish need to disperse between patch algal turf habitats, they must inevitably cross white sand substrate. The color contrast between fish on dark versus

light substrate changes remarkably given the type of substrate they are seen on (Allen 1987). Over the course of 24 hours, not all pipefish in both experiments were seen to change color. A greater proportion of the individuals in the experimental treatments exhibited color change, in either direction, compared to each of the control setups. However, due to the small relative sample sizes, this relationship was not seen to be statistically significant. Furthermore, there may exist a great deal of variation amongst individuals in a population. Other factors, such as age, sex, and breeding status (Berglund et al. 1997), might also play a role. The best attempts were made to capture only males that were not carrying eggs however this turned out to be a very small proportion of the total number of males (i.e. most males were pregnant). As a result, pregnant males were inevitably also used in the experiment. The color change mechanism might be affected by all these factors.

Melanophores are known pigment-carrying structures in vertebrates that expand or contract, resulting in either dark or light color of the skin (Norris 2007). This mechanism might be easier one way than it is the other, which could also explain the variation seen in the results. Red and yellow on pipefish were two colors that did not change in intensity depending on substrate. It might be that these colors are genetically linked. Or they might be behaviorally modified depending on mating strategies (Berglund et al. 1997), of which little is known in *C. flavofasciatus*. Nonetheless, the fact that color change of the dark hexagons on pipefish was observed for fish put on opposing substrate colors suggests that there is an effect that could be significant given larger sample sizes. At the very least, the phenomenon is of interest biologically. A more conservative way to photograph specimens as well as a better method to

quantify color is essential to better understand this mechanism.

If habitat preference of *C. flavofasciatus* is intrinsically linked to presence of algal turf, it has interesting implications on their population dynamics. Good overall coral health has often been associated with lowered abundances of reef fishes. However, the results of this study suggest that *C. flavofasciatus* is one species that indirectly benefits from dead coral. Benthic zooplankton were more abundant above algal turf on dead coral. Pipefish are known to consume zooplankton and they were found exclusively on algal turf. It is necessary to continue monitoring pipefish abundances with time, as the coral reefs of Mo'orea recover to a healthy state. It would also be interesting to quantify pipefish abundance with their predators' abundances. This would require identifying said predators as well as determining predation rates and population dynamics of the different players in this trophic web.

A deeper understanding of predation would also give insight into the reasons why background adaptation might be an important defensive strategy in this pipefish. Further, a thorough look at the exact mechanism by which color change is possible in *C. flavofasciatus* or similar species would help clarify its importance for survival. More data on other factors that influence color (such as sex, age, genetics, mating, etc.), is also necessary. Worthy of note are the observational accounts of signs of predation on pipefish. During this study, of over 100 pipefish observed and/or caught, three pipefish were seen to possess seemingly regenerating tails. The caudal segment of the pipefish was cinched inward where it connected with a smaller than normal tail fin. Of interest is not only the regeneration aspect of the matter, but also the indirect evidence it provides towards predation on pipefish. Further research is crucial to better understand the dynamics of pipefish ecology.

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