SEEING RED: COLOR-BIASED AGGRESSION OF *RHINECANTHUS ACULEATUS*

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Abstract. This paper reviews the research done on the island of Mo'orea in French Polynesia on the color-bias aggression of Picasso triggerfish (*Rhinecanthus aculeatus*). Picasso triggerfish are infamous for being aggressively territorial. They also have impressive color vision and discrimination capabilities like many other coral reef fish. Picasso triggerfish are known to be green, magenta, and blue sensitive. However, other species of coral reef fish are known to be red and ultra violet sensitive as well. I wanted to know how color and eyesight capabilities may affect their aggression levels. Color-bias aggression has been found in other species of fish, such as damselfishes, who show more aggression towards individuals that display colors similar to their own. Using standardized colored objects, I measured their frequency of aggression as well as the distance between the individuals being tested and the observer when the individuals began to display their reaction (charging or fleeing). It was predicted that they would be less aggressive towards colors outside of wavelength range of their known eyesight capabilities as well as be less aggressive towards colors not on their own morphology. The results implied that they did not discriminate by colors found on their own morphology. Additionally, the research did find that they were most aggressive towards red, a color outside of the wavelength range of their known eyesight capabilities. Although it may not be related to their own morphology, the results imply that *R. aculeatus* is color biased in their aggressive behavior. Additionally, the results may imply that they are red-sensitive.

Key words: color bias; Rhinecanthus; *fish behavior; Moorea, French Polynesia; aggression, triggerfish*

INTRODUCTION

The depth of shallow coral reefs allows for the penetration of different colors of light. Reef fish use color and patterns for tasks such as navigation, sexual display, territorial defense and recognition of prey. Most reef fish taxa have the ability to discriminate shapes, patterns and colors (Siebeck, Litherland, and Wallis 2009, Siebeck, Wallis, Litherland, Ganeshina, and Vorobyev 2014). It has been observed that there is less aggression directed towards individuals of certain colors (Pryke 2009). Additionally, it has been found in certain damselflies that males are more aggressive towards heterospecifc males that have similar color patterns to their own in comparison to heterospecifics with a dissimilar color pattern (Tynkkynen *et al*. 2004). It's been suggested that there is an aggression bias towards individuals of the same color as the intruder may be a higher risk of resource and sexual competition (Grether *et al.* 2009). In a study done by Lehtonen (2014), found that Neotropical cichlid fish (*Amatitlania siquia*) were there was bias towards "dummies" of their own color, and even more aggressive when the dummy was of a larger size relative to the territory holder. It was also observed that there was significantly more aggression when the territory holders were defending hatched offspring. However, it is unclear if colors or shapes are used as a trigger for aggressive behaviors outside of intraspecific interactions.

Rhinecanthus aculeatus, commonly known as the Picasso triggerfish, Blackbar triggerfish, Lagoon triggerfish, and the white-banded triggerfish, is a species of triggerfish commonly found in shallow lagoons and reef flats in the Indo-Pacific (Froese and Pauly 2010). They spend a majority of their time foraging for food. Males often spend time defending their territory while females spend time defending eggs during the reproductive season. The males reach a maximum age of 13.5 years and a maximum length of 209 mm while the females live for a maximum of 9.5 years and reach 175 mm in size (Künzli and Tachihara 2012).

Picasso triggerfish are infamous for being aggressively territorial. It has been reported that territorial Picasso triggerfish adults are aggressive towards other fish species (Kuwamura 1991). Although territories have been found to be defended most aggressively during the reproductive season of July – September (Kuwamura 1997).

The reproduction cycle of the Picasso triggerfish lasts for approximately one week around a full or new moon, and mating occurs several times between 0-3 times within a cycle (Kuwamura 1997). These triggerfish continue to defend during the non-reproductive season (Ziadi-Künzli and Tachihara 2016).

The Picasso triggerfish is polygynous. It's been observed that they take part in female defense polygyny as males defend site-attached females rather than the resources the females are attracted to (Ziadi-Künzli and Tachihara 2016). The territories are defended by border fights against consexuals and encompass up to 5 females. Both males and females forage throughout the territory and mate at multiple sites (Kawase 2003). The female's highly aggressive egg defense is highly effective as the eggs are spawned at sunrise and are hatched a dawn of the same day. No predation has been observed on the egg masses. (Kawase 2003).

Picasso triggerfishes are colorful with a number of large and small stripe patterns, (Fig 1.) indicating a fair degree of visual acuity. In a behavioral test, it was observed that this species has acuities to be approximately 1.5–2 cycles · degree–1, while anatomical estimates indicate a level between 3.4 and 7.75 cycles · degree–1. Acuity, or spatial recognition, is a measurement that assesses the ability of an animal to discriminate fine detail (Champ *et al.* 2014). For comparison, humans have a visual acuity of 30 cycles · degree–1. Picasso triggerfish have color vision and are trichromatic (Pignatelli *et al.* 2010), meaning that their eyes possess one single cone (SC) and one double cone (DC). The SC's visual pigment peaks at 413 nm (violet), while one cone of the DC peaks at 480 nm (blue) and the other peaks at 530 nm (green) (Marshall *et al.* 2004). Ultra-violet-sensitive, violet-sensitive, blue-sensitive, green-sensitive, and red-sensitive have been isolated from the eyes of different coral reef fishes (Munz and McFarland 1975, Loew and Lythgoe 1978, Levine and MacNichol 1979, Lythgoe *et al.* 1994, McFarland and Loew 1994).

In the human brain, perception of color and shape can be attributed to parallel processing of visual information (Cant *et al.* 2008). Like primates, fish also generalize shape over color which may aid in the recognition of objects when color changes depending on illumination, angle, and distance (Vorobyey *et al.* 2001). When testing the ability to distinguish color and shape, it was shown that Picasso triggerfish were able to generalize shapes irrespective of color and lightness (Mitchell *et al.* 2017).

FIG. 1. Picasso triggerfish (*R. aculeatus*) displaying its variety of colors and patterns. The most vibrant colors being blue and yellow. Photo by Vivid Aquariums.

The overall goal of this study is to determine the presence of color-bias and aggression in the triggerfish species *Rhinecanthus aculeatus* conducted in the reefs of Moorea, French Polynesia. My first hypothesis is that the Picasso triggerfish will be more aggressive towards colors that are within the wavelengths of their known eyesight capabilities. My second hypothesis is that they would react more aggressively to colors that represented on their own morphology. Overall, by combining the results of my two hypotheses, I am predicting that the tested Picasso triggerfish will be most aggressive towards blue (represented on morphology and within known nm range of sight) and least aggressive towards red (unrepresented on morphology and not within known nm range of sight). I am predicting that individuals presented with colors different than their own colors or outside of their eyesight capability will swim away, or keep a distance, while individuals presented with colors similar to their own or within the wavelengths of their eyesight capability will charge at the object.

METHODS

Study site

This study was conducted on the island of Moorea in French Polynesia (17.5° S, 149.8° W), from 24 October to 14 November 2018. This study was conducted at two sites — Cook's Bay and Plage Publique (Fig. 2).

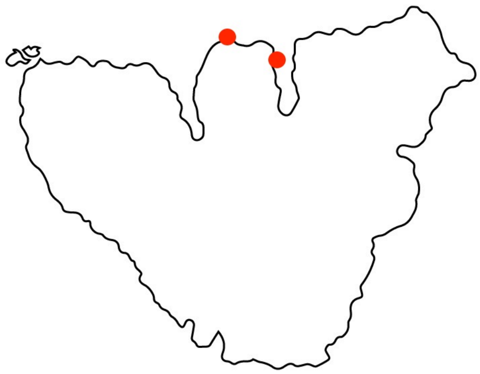

FIG. 2. Study sites on Moorea. Plage Publique (left) and Cook's Bay (right).

The majority of data were collected within Cook's Bay. The site encompasses the shallow reef outside of the Gump Station which is approximately 390 meters in length and has an average width of 110 meters across.

The Plage Publique site encompasses the shallow reef that has an approximate length of 390 meters, and an average width of 80 meters across.

Both sites contain an ecosystem of diverse coral species, both alive and dead, as well as deeper areas of flat sand. The areas that were used are approximately 1.2 meters in depth. Both sites receive regular human traffic and activity. Cook's Bay has a total area of approximately 50,200 m^2 (Google Maps). Plage Publique's reef has an approximate area of 26,000 m^2. Both locations are also surrounded by deeper sandy areas where several Picasso fish were spotted. Individuals were observed moving between the shallow reef and the deeper sandy areas.

Description of Behaviors

Prior to initiating my study, I first observed the Picasso triggerfish in nature to familiarize myself with the behaviors as described by McAlpine (2017). The behaviors are classified under two types, behaviors related to aggression and shyness (Table 1). They include posturing and charging (both related to aggression), and fleeing (a measure of shyness). Using these descriptions as a guide, I was able to determine when a fish became alert, fled, or charged.

Field Experiment

To test how aggressively Picasso triggerfish responded to different color stimuli, I conduced a field experiment. In particular, on a given survey, I swam parallel to the shore down the center of the reefs and encountered individuals opportunistically. Once spotted, the location of the fish would be noted to decrease the chances of pseudo replication. The observer would then approach the triggerfish head-on.

Four color stimuli were tested, red, blue, green, and yellow (Table 2). The colored objects are all rectangular shaped plastic lids of the same size (25.4 cm L x 16.5 cm W). I also performed a parallel set of preliminary experiments in which I used plastic lids of the same color (black) and height (10.2cm), and instead manipulated the shape. I tested

TABLE 1. Description of fish behavior and the percentage of fish tested (per color) that displayed those behaviors. There are fifteen data points per color.

Type of behavior	Behavior	Description	Red	Blue	Green	Yellow	No Color
Aggression	Posturing	Dorsal fin raised, faces observer with both eyes forward	16%	67%	0%	0%	0%
	Charging	Rapid lunge at observer, no connecting bite	50%	33%	0%	17%	17%
Shyness	Fleeing	Fish swims rapidly away from other fish	50%	67%	100%	83%	83%

TABLE 2. The four colors that were tested categorized by *R. aculeatus'* known ability to see them and their status of representation on the morphology of *R. aculeatus*.

	Represented Color	Unrepresented Color
Within capable nm range of eyesight	Blue	Green
Not within capable nm range of eyesight	Yellow	Red

multiple shapes, including a triangle, square, and circle. Regardless of whether testing the color stimuli or the shape stimuli, the field experiment proceeded in a similar manner (described next).

Colors were concealed upon approach. Once 1.5 meters (5 feet) from the individual, the observer would display a color, and note the distance (in cm) to the triggerfish when a charging or fleeing behavior was observed. Each day an individual was also approached without the observer holding a color as a control. Cook's Bay was monitored 14 times, while Temae Beach and Plage Publique were each monitored two times. All monitoring occurred at different times of day and month. No juveniles or pairs were tested.

RESULTS

Statistical Analyses

To analyze whether the distance between the observer and the location of the fish when it displayed an aggressive or shy behavior was used as the response variable in a one-way ANOVA with color (or shape) used as the explanatory factor. Using the software PAST, I first checked the assumptions of the ANOVA (normally distributed data using X test, homogeneity of variance using Y text). My data met these assumptions so I moved forward with one-way ANOVA and with alpha = 0.05 I also calculated the average distance from observer per color.

Color bias in aggressive behavior

Results for color bias in aggressive behavior in Picasso triggerfish, showed that they react fastest (at a longer distance), when presented with red (0.919 m), followed by green (0.917 m) and blue (0.90 m) (Fig. 3). There was a significant difference in the distance to observer when they were presented with yellow (0.62 m) or no color (0.67 m), as they allowed the observer to approach to a closer proximity before reacting.

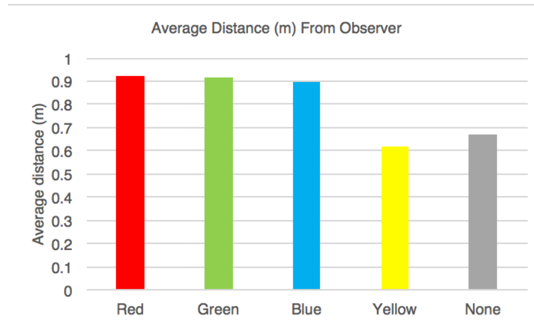

FIG. 3. Average distance between individuals tested and observer per color displayed. Fifteen points per color were

In each test, the individuals always reacted by either swimming away or charging, sometimes displaying a warning (posturing) before their reaction. To determine the average charging events per color (Fig. 4), each data point was given a value of 0 (fled) or 1 (charged). Results showed that individuals were more likely to charge red (29%) and the least likely to charge green (0%). Blue (14%), no color (14%), and yellow (7%) fell in between.

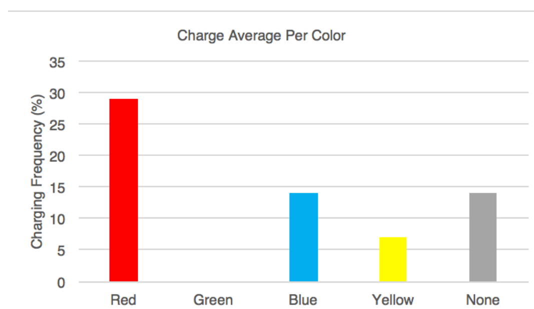

FIG. 4. Average charge frequency per color tested. Fifteen points per color were averaged.

The Shapiro-Wilks test determined that data sets for "green" (p= 0.0159), "yellow" (p=0.01993) and "none" (p=0.006707) were non-parametric, therefore I will be reporting the results for both the one-way ANOVA and the Kruskal-Wallis test.

The Kruskal-Wallis H test determined that there was a statistically significant difference in distance to observer between colors, $X^2(2) =$ 13.67 and p = 0.007097.

The Levene's test for homogeneity of variance determined that the data set was appropriate for a one-way ANOVA. According to the results of the ANOVA, there was a statistically significant difference between groups ($F_{(4,65)}$ = 3.378, p = 0.01424). Therefore, we can reject the null hypothesis.

Shape bias in aggressive behavior

Distance measurements for shape bias in aggressive behavior in Picasso triggerfish showed that there was no significant difference between shapes (square: 1.0 m; circle: 1.0 m; triangle: 0.80m) in distance to observer when they displayed a reaction (Fig. 5). None of the reported reactions were aggressive (circle: 0%; square: 0%; triangle: 0%), all individuals fled.

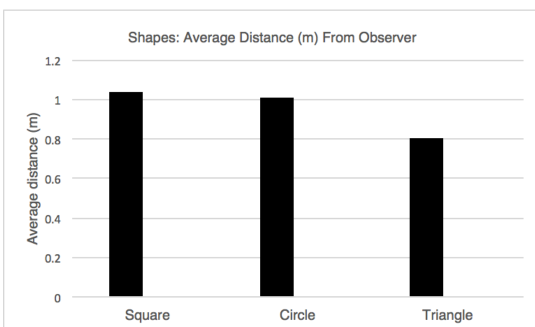

FIG. 5. Average distance between individuals tested and observer per shape displayed. Three points per shape were

A Shapiro-Wilks test showed that the data set was non-parametric, therefore a Kruskal-Wallis test was run. The test confirmed that there was no significance in the data set ($X^2(2)$ = 0.8222, p = 0.6487).

DISCUSSION

I predicted that Picasso triggerfish would show more aggression towards colors similar to their own while showing less aggression toward green and red. I also predicted that they would react quicker, or at a greater distance, when tested with colors within the wavelength range (blue and green) of their known eyesight capabilities. Additionally, I tested three shapes preliminarily.

Color Bias Aggression

Results of charging frequency per color indicated that they were most aggressive towards red and least aggressive towards green (charge frequency per color). I interpreted these results to mean that they reacted most significantly to colors unlike their own.

Looking at the average distances, red, blue, and green were all very similar in results. When presented with these colors, the fish were quicker to react as they reacted from a greater distance. When approaching with a yellow lid or with no lid, individuals were more likely to allow the observer to approach within a closer proximity.

Although the reefs at Plage Publique and Cook's Bay are similar in length, the area of Cook's Bay reef is significantly larger than Plage Publique's reef. Initial surveys of the locations showed different densities of Picasso triggerfish between Cook's Bay and Plage Publique. While swimming along a transect parallel to the shores, the length of the reefs, 11 individuals were sited in Cook's Bay while 19 individuals were sited in Plage Publique.

The majority of data were collected within Cook's Bay. However, charge averages and average distances between sites were compared. Although average distances didn't greatly differ, a significant difference in charge averages per color can be observed between sites. These results could be in response to the density differences between the sites.

Temae beach was a possible third site, however, the reef at Temae beach is approximately 1 meter deeper than the reef at Plage Publique and Cook's Bay. While testing color aggression at this site, individuals neither fled from or charged the observer. They continue to forage, seemingly unbothered

Shape Bias Aggression

Due to time restraints, I was only able to obtain 3 data points per shape during the last days of the study. With the data collected, the Kruskal-Wallis test showed no significance. A larger sample size may yield different results.

Conclusion

Research has observed that triggerfish demonstrate the most aggression during their breeding time (Ziadi-Kunzli and Tachihara 2016). This study was done outside of the breeding season which may account for the low frequency of aggressive behavior.

Triggerfish are a territorial species, though they have been known to alter their territory (Ziadi-Künzli and Tachihara 2016), this territorial lifestyle may have caused repetitive testing of the same individuals in both locations. This possibility could indicate that behaviors observed were based on an individual's normal behaviors, independent of color or shape. Future studies may encompass more study sites and a larger sample size.

Grether (2009) suggested there is an aggression bias towards individuals of the same color because they could be a bigger competitor in sexual selection. However, my results found the opposite interpretation as the most charged color (red) is not found in the morphology of Picasso triggerfish.

Future Research

Both locations were similar in amount human activity as well morphology of the reefs. I spent a day observing the Marine Protected Area on the northern side of Temae Beach. In 8 hours of observations, only one swimmer and one kayaker were spotted. Future projects could measure the difference in aggression (color bias, shape bias, or general) between sites with high rates of human activity and sites with low rates of human activity.

As mentioned earlier, a significant difference in reactions to color were measured at Temae Beach where the reef is relatively deeper. Future research may test the difference in color aggression at different depths.

Research has shown that the double cone is involved in fish color vision and color discrimination (Neumeyer 1986, McFarland 1991, Hughes *et al*. 1998). It's been reported that the cones peak at 480nm and 530nm, however they responded most aggressively to red (620 – 800nm). More colors could be tested to determine if they can discriminate between like colors, such as red and pink or green and teal.

Their defensive nature and charging behavior requires a significant amount of time and energy. Future research may look into the effects of their territorial behavior on their time and energy budget.

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