Current Zoology, 2024, **70**, 79–86
https://doi.org/10.1093/cz/zoac099
Advance access publication 31 January 2023
Original Article

A test of the species confidence hypothesis in dusky damselfish

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Handling editor: Zhi-Yun JIA

Abstract

Visual cues are important in both interspecific and intraspecific communication. The species confidence hypothesis proposes that animals are more attracted to conspecific colors and repelled by colors, not on their bodies. Studies on terrestrial lizards and birds have tested the species confidence hypothesis and shown that conspecific colors elicit reduced antipredator behavior. To date, the species confidence hypothesis has not been tested in the marine environment, specifically on coral reefs where color communication is of vital importance. We addressed this knowledge gap by measuring flight initiation distance (the distance an individual moves away from an approaching threat) in dusky damselfish (*Stegastes nigricans*) in response to an approaching disc of 1 of 4 different color treatments: conspecific, blue, yellow, and black. If the species confidence hypothesis explained variation in damselfish flight initiation distance, then we expected individuals to tolerate closer approaches when approached by a conspecific color. In addition, we calculated the color difference between each stimulus and its corresponding background as a potential alternative explanation for flight responses. Damselfish tolerated the closest approach from the conspecific color stimulus; there were no significant differences between other colors and there was no support for the alternative color difference hypothesis. As with similar terrestrial studies, these results are relevant to ecotourists' choice of swimsuit and wetsuit colors because color choice may modify natural antipredator behavior.

Key words: antipredator behavior, flight initiation distance, risk assessment, species confidence hypothesis, *Stegastes nigricans*.

The species confidence hypothesis asserts that animals are attracted to the colors of their conspecifics and repelled by the colors of heterospecifics (Burley 1986). The initial research leading to this hypothesis discovered, by color-banding zebra finches *Poephila guttata*, that opposite-sex conspecifics perceived certain colors as more attractive than others on potential mates (Burley et al. 1982). Further research showed that the color of an approaching threat influenced risk assessment and was interpreted with respect to the species confidence hypothesis. Gutzwiller and Marcum (1993) suggested that differences in flight initiation distance (FID) in response to different colored stimuli are evidence that certain colors modify risk perception. FID is the distance at which an animal begins to flee from an approaching threat (Ydenberg and Dill 1986). Gutzwiller and Marcum (1997) showed that the color of a vest worn by a researcher who approached birds modified FID and that species tolerated a closer approach from researchers wearing a conspecific color (1997). Other studies followed. For example, Gould et al. (2004) measured variation within the FID of the spiny-cheeked honeyeater *Acanthagenys rufogularis* in response to researchers wearing colored shirts in 4 different color treatments. The results demonstrated that the spiny-cheeked honeyeater had the longest FID when approached by a researcher in a yellow shirt (a heterospecific color) and the shortest FID when approached by a researcher in a red shirt (a conspecific color), thus supporting the species confidence hypothesis. Research performed on terrestrial lizard species in both Costa Rica *Anolis aquaticus* and Southern California *Sceloporus occidentalis* further demonstrated that the perceived risk is lower when approached by conspecific colors (Putman et al. 2017; Fondren et al. 2019). However, there has been much less research analyzing risk assessment and the species confidence hypothesis in the marine environment.

Although there is limited research analyzing how the species confidence hypothesis influences risk assessment in the marine environment, previous studies have shown that conspecific coloring in fish can also trigger an aggressive response in territorial species, especially within a reproductive context. Research focusing on cichlid fish in Lake Victoria found that males acted more aggressive toward other males that shared a similar phenotype (Dijkstra et al. 2006). A neotropical cichlid species with 2 different color types also exhibited similar behavior as individuals were more aggressive toward individuals of the same color type, supporting the prediction that cichlids can differentiate between different color morphs (Lehtonen et al. 2015). In another study, 2 cichlid species were able to coexist because the body color of the guest species mimicked that of the juveniles of the host species (Ochi and Awata 2009). The host species was presented with dummies of different colors, responding the most aggressively to their conspecific color, black, and the least aggressively to the

Received 1 October 2022; accepted 8 December 2022

color of their juveniles, yellow (Ochi and Awata 2009). This research suggests that conspecific body color can serve as an important social cue, especially for territorial species, and therefore promote color-biased aggression.

Coral reefs are considered to be some of the most colorful ecosystems on the planet (Stieb et al. 2019) and coloration plays a large role in both intraspecific and interspecific interactions. Previous research on the diverse color spectrum within coral reefs has suggested that the cues used for identifying predators are primarily visual (Thresher 1976; Katzir 1981; Itzkowitz 1990). We, therefore, expected the color of visual cues to be another important factor in risk assessment considering the diversity of color within the marine environment.

Color vision requires 2 or more distinct types of photoreceptors with separate channels for signal processing (Kelber et al. 2003; Kelber 2016). Photoreceptors in vertebrates include rods and cones, and while rods are responsible for solely scotopic vision, cones are responsible for both photopic vision and color vision (Escobar-Camacho et al. 2017). The color vision systems of reef fish are considered to be highly variable (Marshall et al. 2018), with previous research suggesting that some marine animals survive as color-blind monochromats while others experience color vision that surpasses our own with trichromacy and tetrachromacy (Marshall et al. 2015). This diversity within the visual system of teleost fish is partially caused by variability in the number, type, and placement of cone visual sensitivities within each species (Carleton et al. 2020). Further variability is caused by diversity in the spectral sensitivity of fish. Unlike humans, around half of reef fish have the ability to see UV (Marshall et al. 2018) and the spectral sensitivities of teleost fish are known to range from ultraviolet to red (Carleton et al. 2020). The number of visual pigments in the eye of teleost fish can also range from 1 to 40 due to their variable ecologies, life histories, and habitats (Carleton et al. 2020). Fish have evolved these unique visual systems to survive in highly variable light habitats. Further research has even shown that vision can even vary between populations of the same species depending on their environment (Sandkam et al. 2015).

Stieb et al. (2019) suggested that damselfishes (Pomacentridae) are an ideal family to study visual discrimination in fish because they possess one of the widest ranges of spectral sensitivities of any fish family and often reside on color-diverse coral reefs. There has been previous research on color perception in multiple species of damselfish which has shown they are able to discriminate between different colors (Phillips 2008; Siebeck et al. 2008, 2014). Siebeck et al. (2014) used classical conditioning to train damselfish *Pomacentrus amboinensis* to discriminate colored patterns for a food reward, a result that was not attributed to color brightness. Additional research on color perception within damselfish has shown that the color spectrum visible to them is similar to that of terrestrial mammals and is wider than many other marine species (Hofmann et al. 2012; Emerling et al. 2015; Stieb et al. 2019). While damselfish may not perceive colors the same way as humans, research supports that they can and do discriminate between different colors, and that discrimination plays a role in decision-making.

The objective of this study was to determine if the species confidence hypothesis explained variation in risk assessment within the marine environment. We studied dusky damselfish *Stegastes nigricans* because they are territorial and are often found in large colonies (Hamb 2011). Unlike many other fish species that are free-swimming and therefore difficult to track, dusky damselfish defend individual territories (Karino and Nakazono 1993) which allowed us to avoid resampling individuals when we moved to a different location on the reef following each trial.

If the species confidence hypothesis affected FID as expected, then dusky damselfish would tolerate a closer approach when presented with a conspecific stimulus than they would with a heterospecific color stimulus. Our alternative hypothesis, which we refer to as the color difference hypothesis, was that the color difference between the stimulus and the background of the marine environment would better explain variation in FID, meaning that more detectable colors would lead to a longer FID.

Materials and Methods

Study site and species

We measured the FID of damselfish at a fringing reef site in Moorea, French Polynesia. The Public Beach Ta'ahiamanu (17°29.26S, 149°51.01W) was selected due to the abundance of dusky damselfish which allowed us to avoid pseudoreplication. Damselfish at this site routinely encounter humans. Data were collected from 15 to 25 January 2022 between 07:00 h and 16:10 h.

Color selection

To test the species confidence hypothesis, we recorded damselfish responses to the approaching conspecific color stimuli, and compared results to blue, yellow, and black stimuli. The conspecific color was taken from a photo of a dusky damselfish that resembles those found at the study site (Reef Life Survey 2015) in Adobe Photoshop 2022 (Adobe Systems Inc., San Jose, USA) using the Eyedropper tool to select the exact color for our stimulus. The same process was carried out for the other treatment colors. The blue was taken from a photo of a Pacific bullethead parrotfish *Chlorurus spilurus* (Marine Life Photography 2020) and the yellow was taken from a photo of a speckled butterflyfish *Chaetodon citrinellus* (Allen and Erdmann 2012). Both are common heterospecific, nonpredatory species found within the site and have distinct coloration. We selected black as our final color because it is a common marking color on sympatric nonpredatory fish in the region such as *Chaetodon ulietensis* and *Rhinecanthus aculeatus*. We printed these four colors onto paper with the Canon imagePRESS C700, cut the paper into 29.8-cm-diameter discs, and laminated them for underwater use. We then attached the laminated colored discs to a 29.8-cm-diameter wooden disc with Velcro™(3M), which allowed us to easily switch between treatment colors in the field. None of the wood was visible once the colored disc was attached. The interchangeable color disc was inserted into a 2-m-long pole marked in centimeter increment.

Experimental procedure

We sampled 131 dusky damselfish and our final dataset included 32 black trials, 34 blue trials, 31 yellow trials, and 28 conspecific trials. Experiments were conducted in shallow water with an average depth of 108.5 ± 22.90 cm, excluding 6 trials, by 3 observers: 1 acting as the flusher measuring FID, 1 as the scribe and photographer, and 1 measuring habitat complexity using a 1.0 m × 1.0 m quadrant. For each trial, the flusher identified an area of habitat with a group of dusky damselfish and waited 1–2 min approximately 2.0 m away from the group until an individual oriented toward the flusher. During this waiting period, the flusher quantified group size by counting the number of dusky damselfish either on the bommie (isolated patches of coral measured <1 m × 1 m across) or within the 1.0 m × 1.0 m area of contiguous habitat. The flusher chose a subject and estimated its size (±0.3 cm) based on training with 50 trials estimating objects of varying sizes in the same habitat. The flusher pushed the stimulus, at a consistent 0.25 m/s, toward the individual until it fled. Flight was defined as the moment when the subject swam away from the stimulus. The flusher then measured extension distance (ED), the distance the apparatus was extended from the flusher's body when the fish fled. Next, the flusher extended the pole until the wooden disc was at the initial position of the fish and measured the starting distance (SD)—the distance between the wooden disc and the subject at the start of the trial. FID was calculated by subtracting ED from SD. Depth was recorded using the 2.0-m pole, measuring from the surface of the water down to where the individual was when the experiment began. Water temperature was 27 °C (±1 °C) over the course of our data collection. During the flush, the second observer recorded rain (y/n) and wind level. Wind level was categorized with the Beaufort scale, which traditionally uses 12 different levels to describe weather activity (https://www.rmets.org/metmatters/beaufort-wind-scale). All trials were conducted when Beaufort ≤3, so in our experiment, 0 was no wind while 3 was an experiment conducted with a gentle breeze and breaking wavelets. After flushing, a photo was taken of the stimulus against the background from the subject's point of view by the second observer. We used a Nikon Coolpix AW130 (Nikon Corp., Tokyo, Japan) with a constant white balance setting. The distance between the camera and stimuli was ≤2 m. We did not standardize the exact distance between camera and stimuli but each photo was taken within the range of the previous experimental flush.

Once an FID trial was completed, the third observer measured and recorded the water temperature with an underwater thermometer. The third observer also measured the percent coral cover by laying a 1.0 m × 1.0 m gridded quadrant with 81 intercepts over where the subject was during the experiment. Each intercept was categorized and tallied as either "live coral cover," "dead coral cover," "macroalgae," "rubble," or "sand." "Live coral cover" was defined as living coral, and "dead coral cover" was defined as dead coral usually covered with algal turf. We defined "macroalgae" as algae with a holdfast that was not algal turf (Bruno et al. 2009), and "rubble" was considered to be broken pieces of coral or rock that were larger than sand (Rasser and Riegl 2002). Live coral cover and dead coral cover were summed to quantify the total hard substrate cover.

Color analysis

We used Adobe Photoshop 2022 to quantify the color differences between each stimulus and the background in case variation in color differences explained variation in observed FID. We imported each trial photo as a JPEG into Adobe Photoshop 2022. Because we do not know exactly how dusky damselfish perceived color, we quantified color in 2 ways: using hue, saturation, and brightness (HSB) values, and then using red, green, blue (RGB) values. We used the Eyedropper tool to select the stimulus and background colors; the Color Picker window gave us specific color information. In total, 4 sets of values were collected for each JPEG: stimulus HSB, background HSB, stimulus RGB, and background RGB. From these data, we calculated the Euclidean distance between the colors with the following equations:

$$\sqrt{(H_1 - H_2)^2 + (S_1 - S_2)^2 + (B_1 - B_2)^2} \tag{1}$$

$$\sqrt{(R_1 - R_2)^2 + (G_1 - G_2)^2 + (B_1 - B_2)^2} \tag{2}$$

Equation 1 yields the Euclidean distance between the HSB values of the stimulus and background. Equation 2 gives the Euclidean distance between the RGB values of the stimulus and the background. Subscript 1 is used for stimulus values and subscript 2 is used for background values.

Statistical analysis for species confidence hypothesis

All statistical analyses were performed using R (v. 4.1.2) statistical software (R-Core-Team 2021) with the following R packages: "emmeans" (Lenth et al. 2022), "performance" (Lüdecke et al. 2021a), "see" (Lüdecke et al. 2021b), "patchwork" (Pedersen 2020), "rsq" (Zhang 2021), "nloptr" (Ypma et al. 2018), and "ggplot2" (Wickham 2016).

Before fitting models, we plotted SD distribution by treatment and noted that three trials were outliers due to excessively short SDs (<141 cm). A single trial was conducted when Beaufort scale was 3, while all others were conducted from 0 to 2. Eliminating these 4 observations created a more homogenous dataset containing 127 total trials. We checked for multicollinearity by calculating a correlation matrix between all predictors; there was no collinearity (all r values were <0.336).

We also checked for potential confounding variables by calculating chi-square tests to test if rain or Beaufort scale differed by treatment; they did not ($P_{\text{rain}} = 0.784$, $P_{\text{Beaufort}} = 0.507$) and thus were not confounding.

For our main analysis, we fitted a general linear model to explain variation in FID with the following fixed effects: treatment color, SD, hard substrate cover, depth, fish size, and group size. Because response to a treatment could be contingent on risk, we tested the following 2-way interactions: treatment × SD, treatment × hard substrate cover, treatment × fish size, and treatment × group size. We report adjusted R^2 values as a measure of model fit. We calculated partial R^2 values using the package rsq (v. 2.2) as a measure of a variable's effect size, and calculated estimated marginal means on treatment with a Tukey adjustment for multiple comparisons using the package emmeans (v. 1.7.2). With the same package, we calculated the Cohen's d-value as a measure of treatment color effect size on FID. Throughout, we set our alpha to 0.05.

While Beaufort scale and rain could not be confounding variables, they could still obscure an effect. To examine this, we fitted the basic model sequentially containing each variable. Rain was not significant ($P = 0.434$). Beaufort was significant and we, therefore, report a final model that includes Beaufort and not rain.

Statistical analysis for color difference hypothesis

Our alternative hypothesis tests if variation in FID can be explained by the color difference between stimulus and background. To test this, we fitted several models with both HSB and RGB color difference data. The following analyses were done twice: once with HSB color difference and once with RGB color difference. We first fitted a simple linear model with color difference as the dependent variable and treatment color as the independent variable. This tested whether our treatments were significantly different from each other when deployed in the underwater environment. Then, we refitted our original linear model that tested our main hypothesis, substituting color difference for treatment color as the independent variable. By comparing adjusted R^2 values from these models to the original model, we can see whether color difference was a better explanation of our results than treatment color. Finally, we refitted our original linear model adding color difference as an additional independent variable to control for color difference while testing for treatment color.

We checked for the assumptions of the models by looking at the linearity of the plots of fitted values versus residuals, the homogeneity of the variance, and the overall distribution as well as the normality of the residuals.

Results

Our final dataset included 127 damselfish which were flushed at an average (±*SD*) depth of 108.8 ± 23.14 cm. The mean fish and group sizes were 12.2 ± 3.1 cm and 3.5 ± 2.4. The average SD was 178.4 ± 13.6 cm. The mean number of hard substrate cover the proportion in each tested area was 0.74 ± 0.2 cm.

Testing the species confidence hypothesis

After controlling for significant variation explained by hard substrate cover (estimate = 0.030, $P = 0.021$), fish size (estimate = 0.523, $P = 0.047$), and Beaufort scale (estimate = 6.553, $P = 0.010$), we found that treatment color ($P < 0.001$) significantly explained variation in FID (Table 1). Overall, the model explained 28.7% of the variation in FID. Our comparison of estimated marginal means showed that fish allowed for a closer approach with the conspecific stimulus ($P < 0.001$) compared to the rest of the treatment colors, and there was no significant difference between the remaining treatment colors in FID ($P \geq 0.615$; Figure 1). The effect sizes of conspecific versus other treatment colors were very large (all $d \geq 1.303$) while the effect sizes of the other colors were small (all $d \leq 0.329$). None of the remaining fixed effects tested in the model was significant: SD (estimate = −0.010, $P = 0.785$), depth (estimate = −0.018, $P = 0.472$), and group size (estimate = −0.783, $P = 0.228$). We checked the residuals of our main model and found that the plots of fitted values versus residuals were relatively flat, the variance was homogenous, and the distribution of the residuals was approximately normal.

Testing the color difference hypothesis

When we evaluated our alternative hypothesis and fitted the simple models with color difference as the dependent variable and treatment color as the independent variable, the color difference values of the treatment colors were significantly different whether measured in HSB ($P < 0.001$) or RGB ($P < 0.001$) color space. To homogenize variance, we $\log_{10}$ transformed the HSB color difference values. When we substituted HSB color difference for treatment color in our original model, the model was significant ($P = 0.026$) and explained 9.2% of the variation in FID. Neither the HSB color difference nor any of the interactions involving HSB color difference were significant (Table 2). When we substituted RGB color difference for treatment color in our original model, the model was not considered highly significant ($P = 0.051$) and explained 7.4% of the variation in FID. Neither RGB color difference nor any of the interactions involving RGB color difference were significant (Table 3). When we added color difference as an additional independent variable, neither HSB ($P = 0.933$) nor RGB ($P = 0.870$) had significant effects on FID (Tables 4 and 5). Thus, we found no support for our alternative color difference hypothesis. We once again checked our residuals for each model and found that the plots of fitted values versus residuals were relatively flat, the variance was homogenous, and the distribution of the residuals was approximately normal.

Table 1 Results from a linear model explaining variation in damselfish FID

Variable	F-value	P-value	Partial R^2
Treatment color	15.452	**<0.001**	0.008
Starting distance (cm)	0.075	0.785	<0.0001
Hard substrate cover	5.522	**0.021**	<0.0001
Depth (cm)	0.522	0.472	0.027
Fish size (cm)	4.052	**0.047**	<0.0001
Group size (cm)	1.470	0.228	<0.0001
Beaufort	6.967	**0.010**	0.057
Treatment color: starting distance	0.058	0.982	0.010
Treatment color: hard Substrate Cover	0.965	0.413	0.024
Treatment color: fish size	0.185	0.906	0.005
Treatment color: group size	0.223	0.880	0.007

The full model explained 28.7% of variation and was highly significant ($P < 0.001$).
Bold P -values are significant.

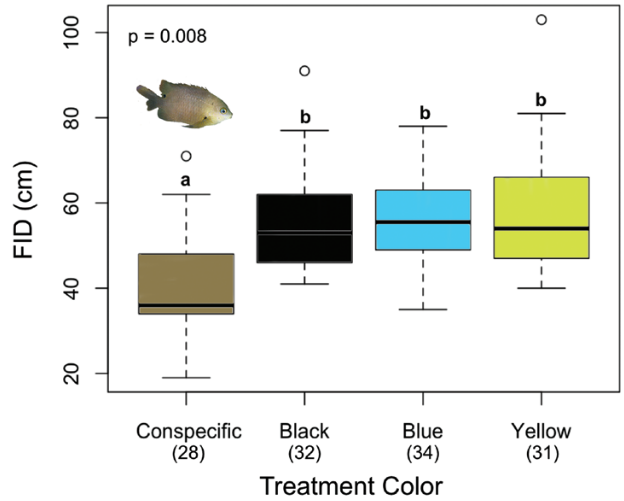

Figure 1 Boxplots of damselfish FID in response to an approaching colored disc. Letters denote significant differences in treatment color effect on FID from estimated marginal means. Sample size per treatment color in parentheses. The dotted lines represent the first and fourth quartiles. The black line in the box represents the mean. Outliers represented by circles are more than 1.5 times above or below the interquartile range denoted by the boxes. *Stegastes nigricans* photograph from Reef Life Survey (2015).

Discussion

Our results provide the first evidence for the species confidence hypothesis in the marine environment, specifically in fish. The main findings of this study were that dusky damselfish had a significantly shorter FID when approached by the conspecific

Table 2 Results from a linear model explaining variation in damselfish FID with HSB color difference substituted for treatment color

Variable	*F*-value	P-value	Partial R^2
HSB color difference	1.127	0.291	0.016
Starting distance (cm)	2.418	0.123	0.027
Hard substrate cover	1.961	0.164	0.014
Depth (cm)	1.260	0.264	0.020
Fish size (cm)	3.789	0.054	0.002
Group size (cm)	2.239	0.138	0.035
Beaufort	4.547	**0.035**	0.053
HSB color difference: starting distance	3.186	0.077	0.015
HSB color difference: hard substrate cover	0.068	0.795	0.003
HSB color difference: fish size	0.295	0.588	0.001
HSB color difference: group size	2.239	0.138	0.020

This model replaced treatment color with HSB color difference as the main independent variable. The full model explained 9.2% of variation and was highly significant (*P* = 0.026).
Bold *P* -values are significant.

Table 3 Results from a linear model explaining variation in damselfish FID with RGB color difference substituted for treatment color

Variable	*F*-value	*P*-value	Partial R^2
RGB color difference	1.553	0.215	<0.001
Starting distance (cm)	2.636	0.107	0.001
Hard substrate cover	2.058	0.154	0.019
Depth (cm)	0.664	0.417	0.014
Fish size (cm)	3.950	**0.049**	0.012
Group size (cm)	2.255	0.136	0.014
Beaufort	5.073	**0.0263**	0.043
RGB color difference: starting distance	0.064	0.800	<0.001
RGB color difference: hard substrate cover	1.232	0.269	0.012
RGB color difference: fish size	0.330	0.567	0.004
RGB color difference: group size	0.734	0.393	0.007

This model replaced treatment color with RGB color difference as the main independent variable. The full model explained 7.4% of variation and approached significance (*P* = 0.051).
Bold *P* -values are significant.

color stimulus compared to any other color stimulus. This shows that dusky damselfish tolerate a closer approach from stimuli that match their own coloration than those that do not, thus supporting the species confidence hypothesis. These findings align with previous studies that used FID in terrestrial environments as a measure of risk assessment to test the species confidence hypothesis (Gutzwiller and Marcum 1993; Gutzwiller and Marcum 1997; Gould et al. 2004; Putman et al. 2017; Fondren et al. 2019; Zhou and Liang 2020).

As an alternative hypothesis, we considered the possibility that variation in the color difference between the stimulus and background explained variation in FID. If this were the case, then fish would not respond to the color presented but rather to the magnitude of difference between the stimulus color and its corresponding background. However, our analysis of the stimuli in the underwater environment, where we quantified the color difference in both the HSB and RGB color space, provided little to no evidence in support of this alternative hypothesis. While the treatment colors were significantly different from each other in both color spaces, neither color difference model provided a comparable explanation of variance in FID to that of our original linear model. These analyses further support our hypothesis that the fish were responding to the colors of the stimuli themselves and not the color difference between the stimulus and background.

The positive estimate for hard substrate cover in our model implies that FID increased with hard substrate cover. These results differed from previous research which has suggested that the level of hard substrate cover was negatively associated with increased risk (Chan et al. 2019) but are in line with results presented in Cheh et al. (2021), which also detected a positive association between hard substrate and FID. Further previous research that differed from our results specifically tested the role of habitat structural complexity in risk assessment in a highly territorial damselfish species and found that fish in more complex habitats tolerated a closer approach (Quadros et al. 2019). One possible explanation for the difference is that the fish in areas with more hard substrate cover know that there are many hiding spots and it may be easier to hide rather than defend their territory. We did not find a significant result for SD in our model, which was similarly found in Chan et al. (2019) but differed from results in Cheh et al. (2021) where there was a significant main effect of SD in explaining FID variation. However, our experimental protocols differed slightly between this and previous studies because we waited for our subject to orient toward us and become motionless before we began our approach. This modification to prior experimental procedures used to measure FID could account for some variation in our results.

The positive estimate for fish size in our model implies that FID increased with fish size and thus larger fish took fewer risks. These results are supported by a meta-analysis on FID in fish (Samia et al. 2019), birds (Møeller 2015), and lizards (Samia et al. 2016) that all found a strong correlation between individual body size and increased FID across species. Other studies have also supported that risk taking can be size dependent, with increased risk taking in fish in a highly vulnerable size range (Biro et al. 2005). Chan et al. (2019) found that younger, smaller fish specifically took larger risks than their older counterparts, presumably to maximize growth.

The positive estimate for Beaufort scale in our model implies that FID increased with increased wind speed and sea surface variability, which typically occurred during increased storm activity. One possible explanation is that increased Beaufort led to more variation within the movements of the observer during the approach and made the stimulus more threatening. Another possible explanation is that increased Beaufort made it harder for fish to detect threats against a visually noisy background and thus led to a more cautious response. A recent study on king penguins *Aptenodytes patagonicus* found that increased wind and weather conditions were associated with an increased probability of flight combined with a decreased FID (Hammer et al. 2022). And, Blumstein and Daniel (2003) found that Bennett's wallabies *Macropus*

Table 4 Results from a linear model explaining variation in damselfish FID with HSB color difference added as an additional independent variable

Variable	*F*-value	*P*-value	Partial R^2
Treatment color	15.408	**<0.001**	0.008
Starting distance (cm)	0.104	0.748	<0.001
Hard substrate cover	5.246	0.024	<0.001
Depth (cm)	0.512	0.476	0.027
Fish size (cm)	4.515	**0.036**	<0.001
Group size (cm)	1.399	0.240	<0.001
HSB color difference	0.007	0.933	0.005
Beaufort	6.505	**0.012**	0.055
Treatment color: starting distance	0.047	0.987	0.008
Treatment color: hard substrate cover	0.973	0.409	0.026
Treatment color: fish size	0.125	0.945	0.003
Treatment color: group size	0.241	0.867	0.007

This model controlled for HSB color difference while testing for color treatment. The full model explained 28.2% of variation and was highly significant ($P < 0.001$).
Bold *P* -values are significant.

Table 5 Results from a linear model explaining variation in damselfish FID with RGB color difference added as an additional independent variable

Variable	*F*-value	*P*-value	Partial R^2
Treatment color	15.462	**< 0.001**	0.008
Starting distance (cm)	0.105	0.747	< 0.001
Hard substrate cover	5.264	0.024	< 0.001
Depth (cm)	0.513	0.475	0.028
Fish size (cm)	4.531	0.036	< 0.001
Group size (cm)	1.404	0.239	< 0.001
RGB color difference	0.027	0.870	0.008
Beaufort	6.658	0.011	0.057
Treatment color: starting distance	0.041	0.100	0.008
Treatment color: hard substrate cover	1.024	0.386	0.028
Treatment color: fish size	1.121	0.947	0.003
Treatment color: group size	0.269	0.848	0.008

This model controlled for RGB color difference while testing for color treatment. The full model explained 28.4% of variation and was highly significant ($P < 0.001$).
Bold *P*-values are significant.

rufogriseus increased vigilance under high wind conditions. Little research has addressed Beaufort as a variable impacting risk assessment in the marine environment making it a promising line of future research in the marine environment, especially since previous research has shown that turbidity and tides both create variation in irradiance (Anthony et al. 2004) and wave-induced light fluctuations can impact the appearance of an object to fish (Sabbah et al 2012).

Color cues contribute an essential role to both intraspecific and interspecific communication within the marine environment and fish specifically visually discriminate species based on shape and color (Rowland 1999). Our research indicates that fish vary their risk assessment and subsequent antipredator behavior depending on the color of the stimulus approaching them. In this specific case, conspecific coloration induced the smallest FID, and thus we infer, the lowest risk for damselfish. Dusky damselfish are a colonial species and therefore not responding fearfully to conspecific coloration might be a strategy to reduce time wasted engaged in antipredator behavior and increase time engaged in foraging and other beneficial activities such as defending their algal turf.

We considered 2 different color spaces in our analysis, and while we still lack a precise understanding of the visual system of dusky damselfish, our results suggest that the color stimuli successfully elicited different antipredator responses in our subjects. Previous research has explored aspects of the optic systems of various damselfish species (Hofmann et al. 2012; Emerling et al. 2015; Stieb et al. 2019), but there is still a lack of information concerning the visual system underwater and thus there needs to be a more developed understanding of how most fish (including dusky damselfish) perceive color underwater. More research into visual perception underwater will allow a more precise understanding of how precisely fish perceive conspecifics and heterospecifics.

However, anthropogenic impacts on the marine environment threaten to change how fish respond to visual signals underwater (Ferrari et al. 2012). In particular, increased runoff from human activity and worsening water quality affect the amount of light able to penetrate underwater and therefore color perception (Marshall et al. 2018). Previous research has considered the effects of this in the context of intraspecific communication and specifically mate selection, highlighting the detrimental effects increased turbidity can have on sexual selection in fish (Seehausen et al. 1997). Our study provides evidence that color-based risk assessment is also important to consider when examining the consequences of increased turbidity in marine environments. If water quality continues to worsen, it may become more difficult for fish to discriminate between colors and their visual range could become reduced, thus fish could potentially spend more time trying to assess threats or may have a delayed response to predators (Marshall et al. 2018). A better understanding of how anthropogenic disturbances might disrupt color signaling in the marine environment in an antipredator context is essential to inform future conservation efforts.

Clothing choice has the potential to influence the behavior of animals and thus research outcomes for biologists (Putman et al. 2017). For underwater studies specifically, deviation from traditionally all-black wetsuits has the potential to change fish behavior. In a similar respect, knowledge of these color effects can reduce the impacts of marine ecotourism in the context of tourist clothing. Putman et al. (2017) suggested that wearing certain colors when visiting natural areas may help decrease human disturbance and later proposed (Fondren et al. 2019) that making thoughtful decisions about clothing color when exploring natural environments could reduce the amount of time and energy an animal spends on antipredatory responses. We have shown that such advice may be generalizable to marine environments as well.

Acknowledgments

We thank the UC Berkeley Richard Gump South Pacific Research Station for logistical support and Connor Philson for statistical assistance and comments on an earlier version of this paper.

Funding

The UCLA Department of Ecology and Evolutionary Biology provided partial funding for this work. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

All authors designed the study which was conducted by the first three authors. DTB guided the analysis which was conducted by the first three authors. All authors interpreted the results. DTB guided the writing which was written by the first three authors. The first two authors continued revisions, while all authors edited and approve of the final paper.

Ethics Statement

Research was conducted with approval from the UCLA IUCAC (protocol # 2000-147) and the Government of French Polynesia (issued on 19 November 2021).

Data Availability

Analyses reported in this article can be reproduced using the data and code that are available on Github: https://github.com/duskydamselfish/Species_Confidence_Damselfish

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