



## Original Article

# Context-dependent consequences of color biases in a social fish

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Colorful visual signals can provide receivers with valuable information about food, danger, and the quality of social partners. However, the value of the information that color provides varies depending on the situation, and color may even act as a sensory trap where signals that evolved under one context are exploited in another. Despite some elegant early work on color as a sensory trap, few empirical studies have examined how color biases may vary depending on context and under which situations biases can be overridden. Here, using *Neolamprologus pulcher*, a highly social cichlid fish from Lake Tanganyika, we conducted a series of experiments to determine color biases and investigate the effects of these biases under different contexts. We found that *N. pulcher* interacted the most with yellow items and the least with blue items. These biases were maintained during a foraging-based associative learning assay, with fish trained using yellow stimuli performing better than those trained using blue stimuli. However, these differences in learning performance did not extend to reversal learning; fish were equally capable of forming new associations regardless of the color they were initially trained on. Finally, in a social choice assay, *N. pulcher* did not display a stronger preference for conspecifics whose yellow facial markings had been artificially enhanced. Together, these findings suggest that the influence of color biases varies under different contexts and supports the situational dependency of color functions.

**Key words:** associative learning, Lake Tanganyika, *Neolamprologus pulcher*, reversal learning, social preferences, social signaling.

## INTRODUCTION

Color is an important signal that is frequently used to communicate information about danger (Noonan and Comeault 2009; Finkbeiner et al. 2014), the quality of food (Gamberale-Stille and Tullberg 2001; Schaefer et al. 2008), or social partners (Hill 1991; Stapley and Whiting 2006). Many animals exhibit color biases, yet such biases are often species specific (Burley 1986; Selz et al. 2014) and reflect the distinct evolutionary and ecological history of different species (Egger et al. 2011; Ninnis et al. 2015). One such explanation for this variation in color preferences, the sensory drive hypothesis, posits that sensory biases displayed by various species directly relate to variation in the sensory environment under which a given species has evolved (Endler 1992; Endler and Basolo 1998). However, the origins of sensory biases are often difficult to determine because while many sensory biases are thought to have evolved honestly under one context (Endler and Basolo 1998; Kokko et al. 2003)—such as foraging for colorful fruits (Rodd et al. 2002; Fernandez

and Morris 2007)—they are often exploited in other contexts (Christy 1995; Garcia and Lemus 2012). Most examples of such sensory exploitation or sensory “traps” have focused on the use of colorful signals to attract mates. As such, despite the numerous roles that color plays in the lives of animals, the majority of research on color has focused on its relationship to sexual selection via mate choice.

Many brightly colored visual signals are regulated by carotenoid intake, and these signals are frequently employed as indicators of individual quality (Hill 1991; Svensson and Wong 2011; Weaver et al. 2017). The pigments necessary to produce these colorful signals are often limited in the environment (Grether et al. 1999; Bortolotti et al. 2000) and only the highest quality individuals are capable of acquiring enough pigment to produce and maintain these signals. Consequently, animals frequently select mates that have bigger and/or brighter color patches (Hill 1991; Weaver et al. 2017). However, color also plays important roles under nonsocial contexts. The connection between the social signaling functions of color and color biases in a foraging context were elegantly demonstrated in a number of studies on Trinidadian guppies (*Poecilia reticulata*; Rodd et al. 2002; Grether et al. 2005). Female guppies

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prefer males with large orange spots and this has typically been argued to reflect the preference of females for high-quality males with superior foraging abilities (Kodric-Brown 1989). However, it has been suggested that this preference may have evolved from the exploitation of a pre-existing sensory bias toward ripe, orange-colored fruits (Rodd et al. 2002). One of the few additional studies to assess color biases under multiple contexts found that the color biases displayed by female satin bowerbirds (*Ptilonorhynchus violaceus*) while foraging (mostly on orange and red food items) were highly distinct from those displayed by males while decorating their bower (blue and violet items; Borgia and Keagy, 2006), suggesting complete context-dependent separation of color biases in this species. Because most studies assessing color biases do so using a single paradigm or context, it is difficult to determine the evolutionary origins of color biases and whether these biases are consistent across contexts. Therefore, to fully understand the integrated role that color plays, it is necessary to assess color biases under different contexts and tasks.

Cichlid fishes from the Great Lakes of Africa display phenomenal interspecific variation in body coloration (Turner et al. 2001), and female sensory drive is thought to be major contributor to this variation. As species began to occupy different habitats, their visual sensitivity shifted to better perceive food, predators, and conspecifics under the spectral distribution of their new environments (Leal and Fleishman 2004; Gray and McKinnon 2007). In particular, adjustments to the visual system of female cichlids is thought to be one of the major factors promoting interspecific color variation (Boughman 2001; Maan et al. 2006; Seehausen et al. 2008), as males of different species have developed distinct color patterns that match the visual sensitivities of conspecific (but not heterospecific) females (Christy 1995; Endler and Basolo 1998). In agreement with this hypothesis, Egger et al. (2011) observed that females of multiple species exhibited a strong bias toward carotenoid-associated colors (e.g., red, orange, and yellow) on the fin-based eggs spots of lekking male cichlids, suggesting that these colorful egg spots may have evolved to exploit the sensory bias of females toward carotenoid-associated colors. However, this bias was not unique to females, nor was it limited to species that have egg spots and mouth brood (Egger et al. 2011). As such, while sensory drive appears to be a key contributor to the dramatic speciation of African cichlids, our understanding of the origins of these color biases and their potential roles outside of mate choice is lacking.

In this study, we explored color biases in a cooperatively breeding African cichlid, *Neolamprologus pulcher*. These fish live in social groups consisting of a dominant male–female pair and 1–20 subordinate group members that help defend the territory and provide allocare toward the offspring of the dominant pair (Wong and Balshine 2011). While grouping decisions in *N. pulcher* have been shown to be influenced by complex interactions between multiple factors including group size (Reddon et al. 2011), relative individual size (Reddon et al. 2011; Wong et al. 2012), familiarity (Jordan et al. 2010), and developmental conditions (Fischer et al. 2017), it is likely that visual signals are also important. While drab in appearance compared to many other cichlid fishes, *N. pulcher* exhibit distinctive colored markings on their faces and bodies (Figure 1), and recent work has shown that the dark black facial bars (Bachmann et al. 2017; Balzarini et al. 2017; Culbert and Balshine 2019) and the ultraviolet markings along the bodies of *N. pulcher* (Sabot et al. 2017) serve important social functions. However, the potential social functions of the bright yellow markings on *N. pulcher* have not been explored. These yellow markings are most obvious on the



**Figure 1**  
Image of *Neolamprologus pulcher* displaying the prominent coloration on its face and head.

faces of *N. pulcher*—a region that plays an important role in individual recognition in this species (Kohda et al. 2015)—and the size and intensity of these yellow patches is likely related to foraging success and overall individual quality, as in other cichlids (Sefc et al. 2014). Individuals typically feed outside of their territory, consuming zooplankton located high in the water column and benthic invertebrates from the surrounding substrate (Konings 2019); both of which contain high amounts of carotenoids (O'Reilly 2001). Therefore, we hypothesized that affiliative decisions in *N. pulcher* are influenced by the size of the yellow facial patches of conspecifics and that this preference may have arisen as a consequence of a pre-existing sensory bias related to foraging for carotenoid-rich foods.

To test this hypothesis, we first assessed whether *N. pulcher* exhibited biases when interacting with colored plastic discs. We predicted that individuals would interact with yellow discs more quickly and more frequently than discs of other colors, reflecting a general bias to interact with carotenoid-related colors. We then assessed whether this bias would influence their performance on a foraging-based associative learning task, predicting that fish would make fewer mistakes when they were trained to complete the task using yellow stimuli. Additionally, we also assessed the cognitive flexibility of individuals by observing whether they could override this bias when it became disadvantageous (i.e., not rewarded) during a reversal learning assay. Such reversal learning tasks are thought to be more cognitively demanding than standard associative learning, and these increased demands can cause sensory biases to be overridden (Einhäuser et al. 2008). Thus, by measuring the performance of individuals in a reversal learning task, we could assess the relative strength of ingrained color biases in the face of conflicting information provided by other, more labile cognitive systems. We predicted that color biases would be overridden during the cognitively demanding reversal learning task and that fish would perform similarly regardless of the color of the stimuli that they were trained on. Finally, we assessed whether the predicted bias toward yellow influenced social decisions by artificially enhancing or reducing the size of the yellow patch on the face of stimuli *N. pulcher*. We tested whether enhancing or reducing yellow facial coloration influenced affiliative decisions of unmanipulated focal fish in a dyadic choice assay. We predicted that fish would prefer to affiliate with conspecifics that had more facial yellow coloration, as they would be viewed as a higher quality individual. We also predicted that this preference would be strongest when fish were presented with conspecifics of the opposite sex, as selecting a high-quality mate has direct reproductive benefits (Kokko et al. 2003).

## EXPERIMENTAL METHODS

### General housing conditions

Experiments were conducted using a colony of *Neolamprologus pulcher* housed at McMaster University in Hamilton, Ontario, Canada. The fish used in the experiment were laboratory-reared descendants of wild-caught breeders from Lake Tanganyika, Africa. All aquaria were held at 27 °C and a 13L:11D photoperiod was maintained under fluorescent (CFL) lighting (4100 K T8 Plus; Philips). Fish were fed ad libitum once a day, 6 days a week with either commercial cichlid flakes (Nutrafin Basix) or floating cichlid pellets (1 mm; Northfin). All the protocols described were approved by the Animal Research Ethics Board of McMaster University (Animal Utilization Protocol No. 18-01-02) and followed the guidelines of the Canadian Council on Animal Care regarding the treatment of animals in research and teaching.

### Experiment 1—Are there biases toward certain colors?

#### Study animals

These experiments were conducted between August and November 2016 using social groups of *N. pulcher* ( $n = 18$ ). Each group was held within 189 L aquaria and consisted of a dominant breeding male–female pair and one to four helpers (average  $\pm$  SEM of  $4.1 \pm 0.2$  group members). All social groups used in these experiments had been together for at least a month and had reared young prior to the experiment. Each aquarium contained two large sponge filters, a heater, 3 cm of off-white colored coral sand for substrate, two mirrors, and a terracotta flowerpot half.

#### Experimental protocol

Social groups ( $n = 8$ ) were presented with a combination of three-colored discs (20 mm diameter and 2 mm width) in the morning (0900–1200h), followed by three differently colored discs in the afternoon (1400–1700h). We selected colors that are representative of colors which occur in the natural environment of *N. pulcher* and would therefore be salient to the fish. These included colors that are either located on the face (black, blue, and yellow) or body of *N. pulcher* (gray and red), or is the color of their eggs (green). The specific combination of colored discs presented during each interaction period was determined using a random list generator. The same combination of colors was presented to each group. This protocol was repeated 7 days later, using different color combinations. Thus, overall, each group had two opportunities to interact with each of the six colors (black, blue, green, gray, red, and yellow) across four trials.

At the start of each trial, the discs were placed equidistantly apart on top of the coral sand substrate directly in front of the opening to the half flowerpot shelter in each tank. Fish were then given up to 15 min to begin interacting with the discs (average latency to first interaction across the two experiments was  $244 \pm 44$  s; mean  $\pm$  SEM). We recorded the latency of the fish to interact with each colored disc (adjusted to the latency of the first interaction per trial), as well as how many times the fish in each social group physically interacted with each disc (including bites, pick-ups, pushes, tosses, and touches) during the 15-min period following the first interaction. Data are reported as the average number of physical interactions with a disc across the two observation periods that each colored disc was presented.

To ensure that fish were capable of differentiating between each of the colored discs that were presented, we took 10 measurements of the spectral reflectance of each disc (as well as the coral

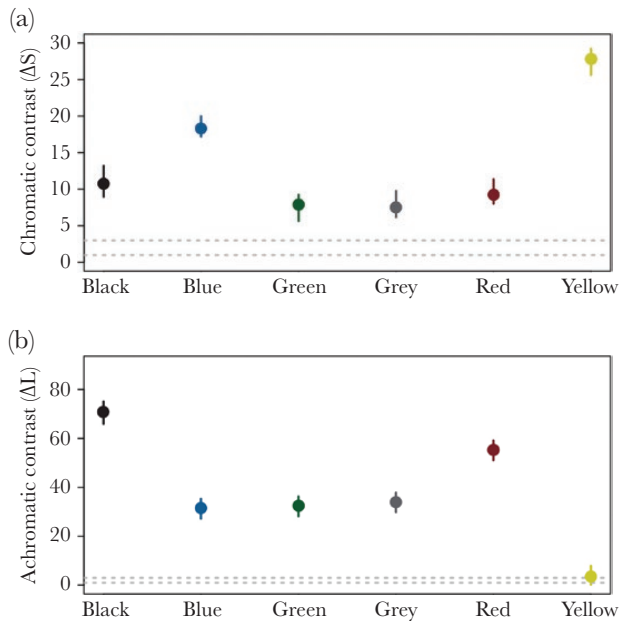
sand background) using an Ocean Optics Flame S-XR1-ES (200–1025 nm) spectrophotometer and DH-Mini Deuterium Tungsten Halogen (200–2500 nm) light source (Ocean Optics Inc.). The probe was held 3 mm away from the sample at a 45° angle, and each measurement was the mean of three (5 s) scans. The spectral reflectance outputs of each item can be found in the Supplementary Materials (Supplementary Figure 1). Chromatic ( $\Delta S$ ) and achromatic ( $\Delta L$ ) contrast between each color, and between each disc and the coral sand background, was assessed using a model of the visual system of *Neolamprologus brichardi* (a sister- or subspecies of *N. pulcher* (Duftner et al. 2007; Gante et al. 2016)), following methods outlined in Bachmann et al. (2017) using the R package “pavo” (Maia et al. 2019). Bachmann et al. (2017) used the trichromatic vision ( $\lambda_{\max}$  of 368 nm [SWS], 488 nm [MWS], and 533 nm [LWS]) of a related cichlid (*Maylandia zebra*) to represent *N. brichardi*, due to a high correlation in opsin gene expression between the two species and recorded the transmission profile of *N. brichardi* ocular media. Chromatic contrast was calculated using the SWS, MWS, and LWS cones, and achromatic contrast was calculated using the LWS cone. Our visual model used Weber fractions of 0.05 and a cone ratio (SWS:MWS:LWS) of 1:2:2 (Bachmann et al. 2017). To assess whether discs would be perceived in a similar manner under experimental and natural conditions, we repeated the model with two light environments: 1) artificial 4000K fluorescent lighting (West et al. 2011) and 2) D65 natural daylight (Maia et al. 2019). Using the bootcoldist function in the R package “pavo” (Maia et al. 2019), we generated mean chromatic ( $\Delta S$ ) and mean achromatic ( $\Delta L$ ) contrasts between each colored disc and for each disc against the coral sand background. A score of one “just noticeable difference” (JND) is commonly used as the standard minimum discrimination threshold under ideal viewing conditions, whereas a JND of three is a common conservative estimate under suboptimal lighting (Vorobyev and Osorio 1998; Bachmann et al. 2017).

#### Statistical analyses

Statistical analyses were performed using R (version 3.4.4; R Core Team, 2019) and a significance level ( $\alpha$ ) of 0.05 was used for all tests. To investigate whether the color of a disc affected latencies to interact and the number of interactions across the observation period, general linear mixed models (LMM) were fit using the lmer function in the “lme4” package (Bates et al. 2015). Models included disc color (black/blue/green/gray/red/yellow) as a fixed effect, as well as group ID as a random factor. When overall differences were detected using the Anova function in the “car” package (Fox and Weisberg 2011), Tukey’s honest significance test post hoc Tukey’s tests were performed using the emmeans function in the “emmeans” package (Lenth 2016).

#### Results

In both visual models, we found that when compared with the sand background each disc had high chromatic contrast with a mean  $\Delta S$  well above the conservative discrimination threshold (Figure 2A). Similarly, mean  $\Delta L$  between each disc and the sand was high (Figure 2B), except for the yellow disc, where mean  $\Delta L$  was below or close to the conservative discrimination threshold. Furthermore, we found that both chromatic and achromatic contrast was high between most discs (Supplementary Tables 1 and 2; Supplementary Figure 2). The exceptions were achromatic contrasts between the blue and green, and gray and green discs under CFL 4000K lighting, which were close to threshold. As all colors were well above the discrimination threshold in at least one measure of contrast, all



**Figure 2**

The chromatic (A) and luminance (B) contrasts of the colored discs used in this study compared to the coral sand substrate that the discs were presented on in Experiment 1. Values represent the mean ( $\pm$  95% confidence intervals) of 10 replicate reflectance measures from each disc. The dotted lines indicate “just noticeable differences” of 1 and 3. A JND of 1 is recognized as the visual threshold below which the difference is imperceptible under ideal conditions, whereas 3 is the visual threshold under “normal” good viewing conditions. See text for a full description of the analyses.

discs were distinct and conspicuous to the fish under the experimental conditions, with similar visual discrimination capabilities under experimental and natural lighting conditions.

The color of discs significantly affected interaction latencies (Figure 3B; LMM:  $\chi^2 = 19.26$ ,  $P = 0.002$ ), with individual fish taking longer to interact with blue discs compared to either yellow ( $z = 3.79$ ,  $P = 0.002$ ) or green discs ( $z = 3.17$ ,  $P = 0.02$ ). Individuals also physically interacted with yellow discs the most and blue discs the least, but this difference was not significant (Figure 3C; LMM:  $\chi^2 = 7.76$ ,  $P = 0.17$ ). To confirm the contrasting biases that *N. pulcher* displayed toward blue and yellow discs, we tested a set of 10 different social groups under identical conditions using only blue and yellow discs (Figure 4A). Again, we found that fish were quicker to interact with yellow discs (Figure 4B; LMM:  $\chi^2 = 30.40$ ,  $P < 0.001$ ), and interacted more with yellow discs than blue discs (Figure 4C; LMM:  $\chi^2 = 9.82$ ,  $P = 0.002$ ).

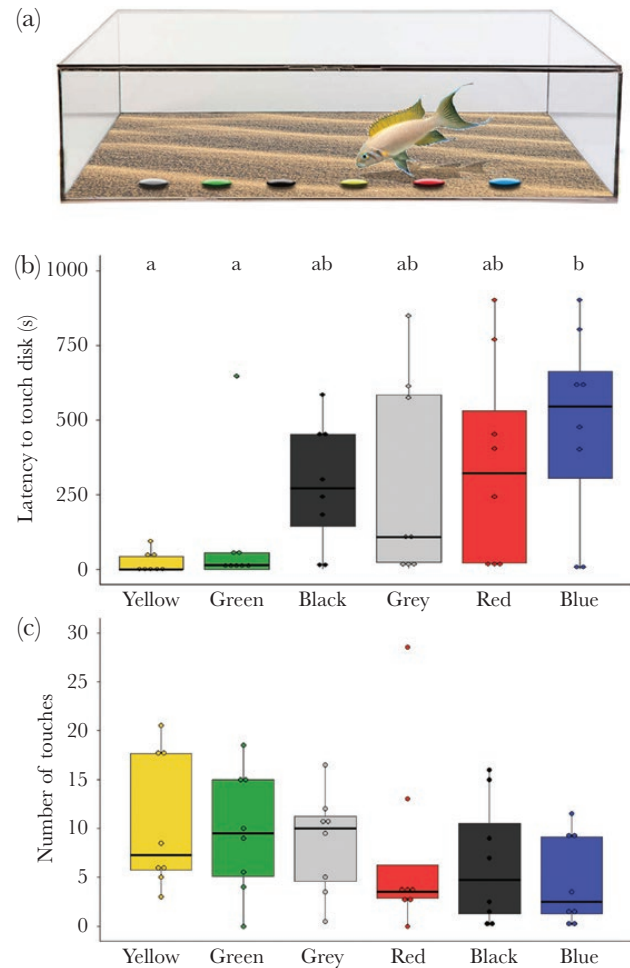
## Experiment 2—Does color influence rates of associative and reversal learning?

### Study animals

This experiment took place between November 2017 and March 2018. Fish used in this experiment ( $n = 24$ ; standard length (SL) =  $5.1 \pm 0.1$  cm) came from 527 L stock tanks containing  $\sim 75$  adults of mixed sex. Prior to the experiment, each fish was measured and sexed via visual examination of the genital papilla.

### Experimental procedures

Fish were placed individually into 38 L aquaria that contained a heater, air stone, mechanical filter, a flower pot half, 3 cm of off-white colored coral sand for substrate, and a small, white plastic



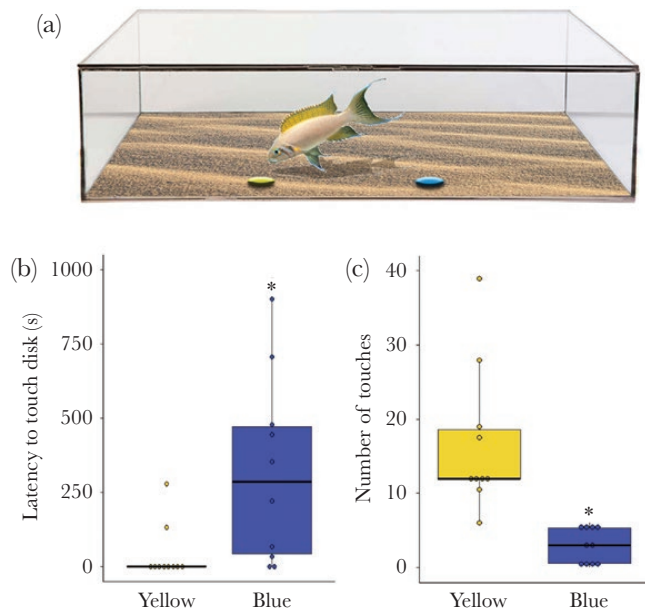
**Figure 3**

(A) Illustration of the experimental set-up used in Experiment 1. (B) The latency of *Neolamprologus pulcher* ( $n = 8$ ) to physically interact with discs of different colors when presented with multiple discs simultaneously. Each trial lasted 15 min. Boxes depict medians, first and third quartiles, and  $1.5\times$  the upper and lower interquartile ranges; points represent individual values. Colors that share a letter are not statistically different ( $P < 0.05$ ) from one another. See text for a full description of the statistical analyses.

feeding tray with 10 wells (2 rows of 5; 9 mm diameter and 7 mm depth). Each tank also contained an opaque barrier controlled by a pulley system that divided the tank in half, which allowed us to separate fish from the feeding tray.

After a 24-h acclimation period, a bloodworm was placed on top of each feeding tray. If a fish consumed the bloodworm within 15 min, another bloodworm was placed onto the tray. If the bloodworm was not consumed within 15 min, fish were given another 24 h to acclimate before another bloodworm was offered on the feeding tray. When a fish successfully consumed five bloodworms in a row, it was considered to have successfully associated the plastic tray with food.

The following day, fish were haphazardly assigned to be trained using either yellow ( $n = 12$ ; seven females and five males) or blue ( $n = 12$ ; seven females and five males) discs. The colored discs were identical to the ones used in Experiments 1 and 2 and were easy for the fish to lift and maneuver. Fish began each trial in the back compartment of the tank, separated from the feeding tray by the opaque barrier (Figure 5A). During this time, half of a bloodworm was

**Figure 4**

(A) Illustration of the experimental set-up with only blue and yellow discs. (B) The latency of *Neolamprologus pulcher* ( $n = 10$ ) to interact with discs when presented with only blue and yellow discs and (C) the number of times that blue and yellow discs were physically manipulated. Each trial lasted 15 min. Boxes depict medians, first and third quartiles, and  $1.5\times$  the upper and lower interquartile ranges; points represent individual values. An asterisk indicates a difference between blue and yellow discs ( $P < 0.05$ ). See text for a full description of the statistical analyses.

randomly placed into one of the 10 holes such that the tip of the bloodworm protruded from the hole. A disc of the assigned color was placed directly behind (but not covering) the hole containing the bloodworm. Following this, the barrier was slowly raised exposing the feeding tray and the blood worm to the focal fish. Each trial lasted 2 min, after which fish were gently guided to the back of the tank and the barrier was brought down, so it could no longer interact with the food tray. If a fish consumed the bloodworm for two trials in a row, then the disc was shifted such that it covered one-fourth of the hole containing the bloodworm. This stepwise progression continued (in one-fourth coverage increments) until the disc fully covered the hole and completely hid the bloodworm from sight at the beginning of the trial. If a fish successfully accessed the fully covered bloodworm for three trials in a row, it was considered to be trained. If at any point a fish failed to access the bloodworm for two trials in a row, then it regressed back to the previous step. Ten trials were completed per day, for a maximum of 5 days (50 trials). If a fish completed their training before the end of the 5 days, they were presented twice daily with a bloodworm in a feeding tray well that was fully covered by a disc to reinforce their training (all fish successfully accessed the bloodworm during each reinforcement trial). One fish (a female trained using yellow discs) failed to complete the training protocol and was therefore excluded from our analyses.

Following the training phase, a testing phase occurred where fish were presented with both a yellow and a blue disc. The disc that fish were trained on remained accessible, while the novel color disc was inaccessible. Discs were made inaccessible by gluing a steel nut on the bottom of the disc which fit snugly into the well and were extremely challenging for fish to lift. Only 1 of the 23 test fish managed to dislodge them and even then considerable effort was

required. The location of the two discs on the food tray (which contained 10 wells) was determined using a random order generator, and both discs had a bloodworm under them to avoid differences in olfactory cues. We recorded which disc the fish first attempted to dislodge in each test trial. Ten testing trials were completed per day for 2 days (20 trials).

Finally, we conducted a reversal phase where the colored disc that had previously been accessible became inaccessible, while the previously inaccessible colored disc became accessible (by removing or adding steel nuts). We again assessed which disc the fish first attempted to dislodge. Ten reversal trials were completed per day for a total of 6 days (60 trials).

### Statistical analyses

To investigate whether disc color influenced the number of trials necessary to train fish, a generalized linear mixed model (GLMM) with a negative binomial distribution (to account for overdispersion of count data) was fit using the `glmer.nb` function in the “lme4” package. Disc color (blue/yellow) and fish sex (male/female) were included as fixed effects. To assess whether performance (whether fish initially attempted to dislodge the accessible disc) in the associative learning and/or the reversal learning phases were influenced by the color that fish were trained on, we fitted GLMMs with a logit link function. We included fixed effects for sex (male/female), trial number (1–60), and training disc color (blue/yellow). We also included a random effect to account for individual learning curves (trial | fish ID). The summary function was used to detect statistical differences ( $P < 0.05$ ).

### Results

The number of trials required for fish to be considered “trained” on the association learning assay (average of  $14.8 \pm 1.1$  trials) was not affected by disc color (GLMM:  $z = 0.05$ ,  $P = 0.96$ ) or sex (GLMM:  $z = 1.05$ ,  $P = 0.29$ ).

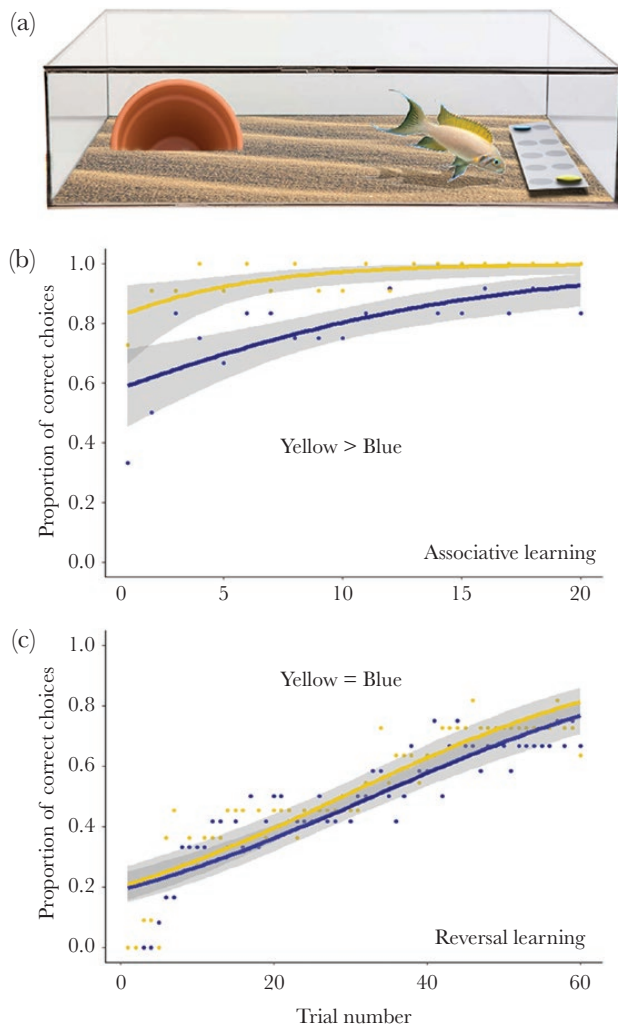
Throughout the association learning test phase (Figure 5B), fish that were trained using yellow discs had a higher success rate (correct first choice) compared to fish that were trained using blue discs (GLMM:  $z = 2.90$ ,  $P = 0.004$ ). In general, performance improved across the 20 trials (GLMM:  $z = 2.87$ ,  $P = 0.004$ ) and success rates plateaued by the final 5 trials (yellow =  $100 \pm 0\%$ ; blue:  $88 \pm 8\%$ ). Overall, females had a higher success rate than males (GLMM:  $z = 2.23$ ,  $P = 0.03$ ).

During the reversal learning phase (Figure 5C), performance increased across the 60 trials (GLMM:  $z = 5.68$ ,  $P < 0.001$ ), but performance was not influenced by the color of the disc that fish were initially trained on (GLMM:  $z = 1.02$ ,  $P = 0.31$ ). Success rates in both groups plateaued (by the final 10 trials) before either group had reached the success rates attained previously during the association learning test phase (yellow =  $73 \pm 14\%$ ; blue:  $68 \pm 13\%$ ). No differences in success rates during this reversal task were detected between sexes (GLMM:  $z = 1.34$ ,  $P = 0.18$ ).

### Experiment 3—Does conspecific yellow facial coloration influence social choices?

#### Study animals

This experiment was conducted between May and October 2017 using fish from the same stock tanks as those described in Experiment 2. Prior to this experiment, the SL of each fish was recorded, and sex was determined by examination of the genital papilla.

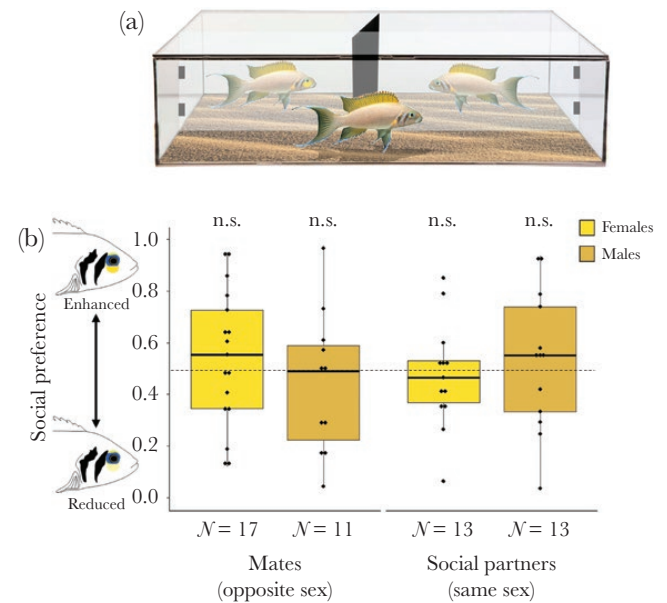


**Figure 5**

(A) Illustration of the experimental set-up used in Experiment 2. (B) The proportion of *Neolamprologus pulcher* ( $n = 23$ ) that first attempted to dislodge the colored disc ( $n = 12$  blue;  $n = 11$  yellow) that they were trained on and (C) the opposite color that they were trained on. Individual performance was assessed across 20 trials over 2 days (B) or 60 trials over 6 days (C;  $n = 10$  trials/day). Each trial lasted 2 min. Values are presented as means, and lines with 95% confidence intervals indicate learning curves for each training color. Colors represent the colored disc that fish were initially trained on. Fish trained using yellow discs chose correctly more frequently than fish trained using blue discs during the testing phase ( $P = 0.004$ ), but not the reversal phase ( $P = 0.31$ ). See text for a full description of the statistical analysis.

### Experimental procedures

We created same sex, size-matched stimuli pairs ( $N = 63$ ; 30 female pairs and 33 male pairs) with an average body size difference of  $0.14 \pm 0.1$  cm SL (mean  $\pm$  SEM) within a pair. At the beginning of each trial, the amount of yellow under the eyes of stimuli fish was manipulated. During manipulation, fish were placed on a damp towel and the area under the eyes was gently dried, and waterproof liquid eye shadow (Infallible; L'Oréal Paris) was applied directly under the eyes to either enhance (Jet Set Blond) or reduce (Cool Ivory) the amount of yellow. Thus, within each stimuli pair, the amount of yellow under the eyes of one fish was enhanced, while the amount of yellow under the eyes of the other stimuli fish was



**Figure 6**

(A) Illustration of the experimental set-up used in Experiment 3. (B) The proportion of time that individual *Neolamprologus pulcher* ( $n = 54$ ) spent associating with a conspecific with enhanced yellow facial coloration. Each trial lasted 8 min. Boxes depict medians, first and third quartiles, and  $1.5\times$  the upper and lower interquartile ranges; points represent individual values. Manipulation of yellow facial coloration did not affect social decisions. See text for a full description of the statistical analyses.

reduced. Treatment designation within each stimuli pair was determined randomly via a coin toss. After allowing the color treatment to dry for 3 s, fish were placed into one of the two back stimuli compartments within the trial tanks (side was determined via a coin toss). The entire process took less than 15 s to complete, and fish were never completely dry. Pilot tests showed that the color manipulation lasted  $\sim 20$  min, with fish resuming normal activity within a few ( $\sim 2$ – $3$ ) minutes following the color application. Accordingly, fish were given a 5-min habituation period following treatment to ensure normal activity during trials.

Sixty-three trials were performed in this experiment ( $N = 18$  females choosing males,  $N = 15$  females choosing females,  $N = 15$  males choosing males, and  $N = 15$  males choosing females). Focal fish were haphazardly selected from stock tanks and were paired with a slightly larger same or opposite sex stimuli pair (average difference in body size:  $0.34 \pm 0.16$  cm SL). Focal individuals and stimuli pairs were always taken from different stock tanks to control for familiarity. Choice trials were conducted in 38 L aquaria that had been divided into three sections. The two back compartments were separated from one another by an opaque barrier. The front compartment was separated from the back compartments by a clear barrier, as well as a removable opaque barrier that was controlled by a remote pulley system (Figure 6A). Each tank contained 3 cm of off-white coral sand as substrate, two air stones, and a heater.

At the start of each trial, all fish were separated from one another by opaque barriers. Trials began following the 5-min habituation period when the opaque barrier separating the focal fish from the two stimuli fish was raised, revealing the two stimuli fish behind the clear barrier. The two stimuli fish could not see each other or interact as they remained separated by an opaque barrier. Trials

lasted 8 min and were recorded using a video camera (VIXIA HFS100; Canon). We recorded the amount of time that focal fish spent interacting with each stimulus fish after they had visited and viewed both stimuli fish (average latency to reach this point was  $29 \pm 5$  s). In general, social decision making in *N. pulcher* occurs rapidly (within 5 min) and remains consistent across time (Reddon et al. 2011).

### Statistical analyses

To investigate whether enhanced facial yellow coloration influenced the amount of time focal fish spent associating with particular stimuli fish, one sample *t*-tests were conducted to determine whether focal fish associated with yellow enhanced stimuli more than would be expected by random chance (i.e., 50%). Trials where the focal fish did not switch sides during the trial were excluded as focal fish did not have a chance to see both stimuli fish. A total of nine trials were excluded because they did not meet this criterion, including one trial of a female choosing males, two trials of a male choosing males, two trials of a female choosing females, and four trials of a male choosing females.

### Results

Enhancement of yellow facial coloration did not affect affiliative decisions (Figure 6B). Focal males did not affiliate more than by chance with either male (One sample *t*-test:  $t = 0.45$ ,  $P = 0.66$ ) or female ( $t = 0.64$ ,  $P = 0.53$ ) conspecifics with enhanced facial coloration. Similarly, females also did not affiliate more than chance with either male ( $t = 0.73$ ,  $P = 0.48$ ) or female ( $t = 0.50$ ,  $P = 0.63$ ) conspecifics with enhanced facial coloration.

## DISCUSSION

Color biases are often studied in the context of social choices; however, few studies have assessed the context-dependent functions of color biases. We found that *N. pulcher* engaged the most with yellow plastic discs and the least with blue discs. These results are unlikely to be due to differences in detectability as the discs were highly salient and distinct both from each other and from the background. It is, however, unclear whether this bias was primarily a result of chromatic or achromatic information. In a nonsocial context, these color biases were maintained with individuals performing better on a foraging-based learning assay when they were trained using yellow stimuli compared to blue. Conversely, these biases did not influence the capability of fish to form new associations when the rewarded color was reversed. In a social context, we did not find support for the hypothesis that *N. pulcher* use yellow facial coloration as an indicator of individual quality, as individuals did not show a clear preference to affiliate with conspecifics with artificially enhanced yellow facial coloration. However, we interpret these results cautiously as it is difficult to replicate the complexity of visual signals, as well as potential interactions between visual signals and the natural sensory environment. Overall, our findings are consistent with the small number of previous studies that have identified context-specific functions or biases toward color (Gamberale-Stille and Tullberg 2001; Borgia and Keagy 2006).

As mentioned above, the social functions of colorful signals have been well studied in many species (Svensson and Wong 2011)—including several cichlids (Sefc et al. 2014). In this study, we were unable to influence the social decisions of either male or female *N. pulcher* by manipulating the size of the yellow facial markings of conspecifics. This was true whether fish were choosing

between potential social partners (same sex as focal fish) or potential mates (opposite sex as focal fish). Brightly colored signals are thought to advertise an individual's quality to others (Svensson and Wong 2011; Weaver et al. 2017), and previous studies have shown that focal individuals typically prefer to affiliate with conspecifics with larger and/or brighter signals across a wide range of species (Blount and McGraw 2008). However, making the “right” affiliative decisions can directly influence an individual's access to limited resources (Metcalfe and Thomson 1995; Senar and Camerino 1998), afford protection (Roberts 1996; Balshine et al. 2001), and affect competitive outcomes (Holekamp and Smale 1998; Pasinelli and Walters 2002), making such social decisions extremely important for group-living animals. While we assessed the potential signaling functions of the yellow facial patches of *N. pulcher* in the current study, previous work has shown that both the dark opercular bars (Bachmann et al. 2017; Balzarini et al. 2017; Culbert and Balshine 2019) and ultraviolet markings along the body (Sabol et al. 2017) serve important signaling functions in *N. pulcher*. Therefore, *N. pulcher* may rely on information acquired from each of these visual signals in combination to inform their social decisions. Additionally, signals are often multimodal (Candolin 2003), and the relative importance of visual signals compared to other sensory modalities in dictating social decisions can vary greatly between contexts and species. Olfactory signals are known to play an important communicative role in many fishes (Stacey et al. 2003; Maruska and Fernald 2012), including *N. pulcher* (Hirschenhauser et al. 2008; Bayani et al. 2017; but see O'Connor et al. 2015). Additionally, *N. pulcher* communicate using high-frequency acoustic signals (Pisanski et al. 2015; Spinks et al. 2017), which may also influence social decisions. Therefore, focal fish may have modulated their affiliative preferences had we allowed the use of additional communicative modalities.

Many colorful social signals are thought to have evolved as a consequence of pre-existing sensory biases toward these same colors in a foraging context (Christy 1995; Endler and Basolo 1998). In Lake Tanganyika, *N. pulcher* mainly feed on zooplankton in the water column, but also opportunistically feed on benthic invertebrates from the substrate of their territories. Both of these food sources are high in carotenoids (O'Reilly 2001), which may predispose *N. pulcher* to seek colors that are typically associated with carotenoids, such as yellow. We found that *N. pulcher* were indeed more successful during a foraging-based associative learning assay when individuals were trained using yellow stimuli as opposed to blue, supporting the relationship between foraging and the color biases of *N. pulcher*. However, to gain a sense of the strength of this sensory bias relative to other cognitive inputs, we also assessed how training color influenced performance during reversal learning. Reversal learning requires additional executive cognitive processes compared to traditional associative learning, as individuals must inhibit their responses toward previously rewarded stimuli, shift their attention, and then form a new association with previously unrewarded stimuli (Lai et al. 1995). The cognitive demands of complex tasks, such as reversal learning, have been suggested to have the capacity to override pre-existing sensory biases (Einhäuser et al. 2008), and our data suggest that this was likely the case in this study. We found that the influence of training color dissipated when the rewarded colors were switched during our reversal learning task, as fish performed similarly regardless of the color that they were initially trained on. Overall, our experiment emphasizes the importance of assessing sensory biases under different contexts, especially in the face of conflicting cognitive information.

Colors are expected to have variable importance in the sensory lives of different species, and multiple lines of evidence suggest that both blue and yellow are especially salient to *N. pulcher*. The visual system of *N. pulcher* is highly sensitive to both blues (~470 nm) and yellows (~580 nm); both colors penetrate to the depths (5–15 m) that *N. pulcher* are typically found in Lake Tanganyika (Bachmann et al. 2017); and both colors are located on the faces and bodies of *N. pulcher*. As such, it is likely that the blue- and yellow-colored patches on the faces of *N. pulcher* have important signaling functions. While we did not observe any differences in the affiliative choices of individuals based on the size of the yellow facial patches of stimuli fish, it is unclear how these artificial manipulations were perceived by the fish. Similar to humans, cichlids, including *N. pulcher* (Bachmann et al. 2017), have trichromatic vision (Carleton 2009). However, it is important to consider that fishes do not experience color as we do, as some fish species see UV light (Losey et al. 1999). Given that *N. pulcher* can see in the UV spectrum (Bachmann et al. 2017) and have considerable amounts of UV coloration on their faces that has been shown to be involved in regulating aggressive behaviors (Sabol et al. 2017), it is likely that there are complex interactions between these colored facial patches (blues, yellows, and UV) that collectively mediate signaling functions (Grether et al. 2004). Although lighting conditions across our experiments were constant—there was likely minimal UV irradiance in our laboratory environment, and this could have affected the perception of the manipulated markings significantly. Additionally, while we manipulated the size of the yellow patches, signal intensity may prove to be more important in influencing affiliative decisions in *N. pulcher*. Overall, it is likely that these colorful facial patches do indeed have a signaling function, and to unravel the social implications of the observed color biases, future work needs to target fish in the wild that have access to the full visual spectrum.

In many social groups, tasks are divided among individuals, and distinct specializations often occur between sexes (Cockburn 1998; Clutton-Brock et al. 2002). Such division of labor is evident in *N. pulcher* where females generally provide higher levels of parental care and hygienic duties toward young compared to males (Desjardins, Fitzpatrick, et al. 2008; Desjardins, Stiver, et al. 2008). These behavioral differences appear to reflect the distinct ecological pressures placed on each sex and may be indicative of broader differences in the cognitive specializations of females versus males. We found that females outperformed males during the testing phase of the associative learning assay. Similarly, female guppies (*Poecilia reticulata*) also outperform males in foraging-based, associative learning assays (Lucon-Xiccato and Bisazza 2014; Lucon-Xiccato et al. 2020); however, male guppies are better spatial learners (Lucon-Xiccato and Bisazza 2017; Lucon-Xiccato et al. 2020). The authors of these previous studies suggest that the enhanced spatial memory of male guppies may relate to their higher tendency to disperse (Croft et al. 2003), as well as their frequent occupancy of more spatially complex habitats compared to females (Croft et al. 2006). Similar to guppies, male *N. pulcher* are also more likely to disperse than females (Stiver et al. 2007; Hellmann et al. 2016), and many males are polygynous and hence must navigate between multiple territories (Desjardins, Fitzpatrick, et al. 2008; Wong et al. 2012), suggesting that male *N. pulcher* may too be better spatial learners than females. Indeed, male *N. pulcher* were quicker to correct a mistake compared to females during a spatial learning assay (Bannier et al. 2017). Given the apparent similarities in the ecological drivers promoting sex-specific cognitive specializations

across distantly related teleost species, this raises interesting questions regarding the breadth to which these patterns hold across other teleosts, as well as vertebrates more generally.

In conclusion, the strong bias of *N. pulcher* toward yellow items enhanced their performance in a foraging-based associative learning assay but did not influence performance when the rewarded colors were reversed. However, our results failed to offer insight into the potential social functions of the conspicuous yellow facial markings on this highly social fish, as manipulation of these markings did not influence affiliative decisions. Overall, these findings offer support for the context-dependent functions of color biases, and we encourage future studies to integrate ethology, sensory ecology, and neuroscience to further unravel the evolutionary origins and contextual perception of color as a signal.

## SUPPLEMENTAL MATERIAL

Supplementary data are available at *Behavioral Ecology* online.

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Data availability: Analyses reported in this article can be reproduced using the data provided by Culbert et al (2020).

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