

Colorful facial markings are associated with foraging rates and affiliative relationships in a wild group-living cichlid fish

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Abstract

Many animals use color to signal their quality and/or behavioral motivations. Colorful signals have been well studied in the contexts of competition and mate choice; however, the role of these signals in nonsexual, affiliative relationships is not as well understood. Here, we used wild social groups of the cichlid fish *Neolamprologus pulcher* to investigate whether the size of a brightly colored facial patch was related to 1) individual quality, 2) social dominance, and/or 3) affiliative relationships. Individuals with larger patches spent more time foraging and tended to perform more aggressive acts against conspecific territory intruders. We did not find any evidence that the size of these yellow patches was related to social rank or body size, but dominant males tended to have larger patches than dominant females. Additionally, patch size had a rank-specific relationship with the number of affiliative interactions that individuals engaged in. Dominant males with large patches received fewer affiliative acts from their groupmates compared to dominant males with small patches. However, subordinates with large patches tended to receive more affiliative acts from their groupmates while performing fewer affiliative acts themselves. Taken together, our results suggest that patch size reflects interindividual variation in foraging effort in this cichlid fish and offer some of the first evidence that colorful signals may shape affiliative relationships within wild social groups.

Key words: affiliation, foraging, Lake Tanganyika, *Neolamprologus pulcher*, visual signals.

Critical building blocks for the evolution of complex animal societies include the ability to differentiate between social partners and recall the nature of previous interactions with known associates (Sheehan and Tibbetts 2011; Wascher et al. 2018; Ward et al. 2020). Visual cues, markings, and/or colorful signals can aid in these efforts by providing information about the identity, quality, or behavioral motivation of a partner (Whitfield 1987; Hill 1991; O'Connor et al. 1999; Tibbetts 2002; Stapley and Whiting 2006; Kohda et al. 2015). However, while the success of animal societies hinges on several different types of exchanges between social partners (e.g., affiliative, agonistic, cooperative, and sexual), previous studies investigating the role of colorful signals during social interactions have overwhelmingly focused on agonistic and sexual behaviors (Blount and McGraw 2008; Svensson and Wong 2011; Weaver et al. 2017). The role(s) that colorful visual signals may play in the selection and maintenance of affiliative and/or cooperative relationships remains unclear.

The African cichlid fish *Neolamprologus pulcher* lives in permanent social groups consisting of a dominant male–female

breeding pair and 1–20 mixed-sex subordinates that help to maintain and defend the territory (Balshine et al. 2001; Heg et al. 2004). In the wild, *N. pulcher* routinely interact and maintain relationships with dozens of conspecifics including both groupmates and neighbors (Balshine et al. 2001; Bergmüller et al. 2005; Heg et al. 2005). Visual signals likely aid in the maintenance of these relationships by providing information regarding the identity, quality, and/or behavioral state of conspecifics. These fish have complex, multicomponent color patterns with high contrast patterns that include black, blue, ultraviolet, and yellow markings, which are particularly concentrated on the head and around the eyes (Figure 1). Previous studies have shown that during agonistic interactions, *N. pulcher* are attentive to both the melanic (Bachmann et al. 2017; Balzarini et al. 2017; Culbert and Balshine 2019) and the ultraviolet markings around the head (Sabol et al. 2017) on conspecifics, but the role(s) of the conspicuous yellow coloration located on the face and body remains elusive. Bright colorful signals (such as these yellow patches) are often related to the amount of high-quality, carotenoid-rich food that

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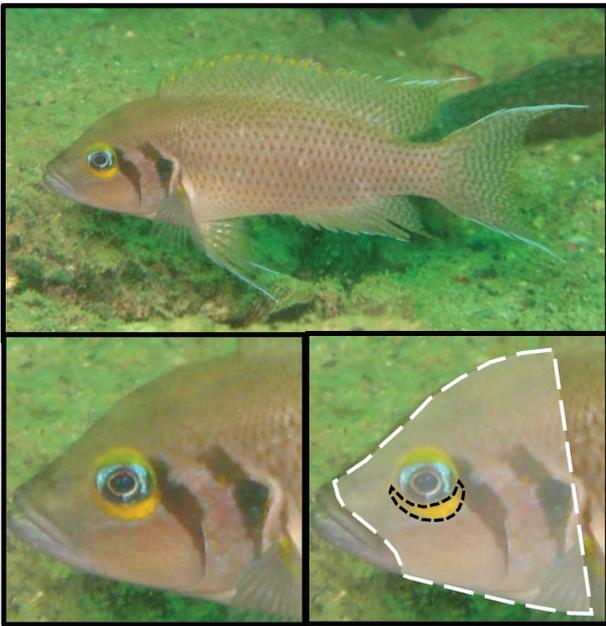


Figure 1 Image of *Neolamprologus pulcher* displaying the conspicuous yellow coloration on its face, as well as a diagram depicting how the size of these color patches was determined.

individuals consume (Blount and McGraw 2008). Colorful signals are, therefore, generally larger and/or brighter on individuals that acquire lots of high-quality food either because they manage to spend more time foraging or forage more effectively and/or selectively than others (Grether et al. 1999; Senar and Escobar 2002; Walker et al. 2014). Several studies have reported that *N. pulcher* exhibit a preference for yellow items in nonsocial contexts (Culbert et al. 2020; Fischer et al. 2021; Reyes-Contreras and Taborsky 2022); however, the potential relevance of this color preference under social contexts is less clear. Jungwirth et al. (2019) found that fish which received artificial yellow markings along their body were less likely to be accepted into a new social group compared to fish with no markings or markings of other colors, and Culbert et al. (2020) reported that fish with experimentally enlarged yellow facial patches were not preferred as social partners. But both experiments were conducted using laboratory-reared fish that experienced simplified social settings and were regularly provided high-quality food. It is likely that the heightened complexity observed in the natural environment of *N. pulcher*—where food acquisition (mostly zooplankton; Gashagaza and Nagoshi 1986) is more challenging, group membership is more dynamic (Dierkes et al. 2005; Stiver et al. 2006; Fitzpatrick et al. 2008; Jordan et al. 2010) and individuals routinely interact with groupmates, neighbors, and heterospecifics (Bergmüller et al. 2005; Desjardins et al. 2008b; Jungwirth et al. 2015a, 2015b)—forces individuals to pay greater attention to all forms of social signals, including color-based signals.

In the present study, we evaluated 3 nonexclusive hypotheses regarding the potential social function(s) of conspicuous yellow facial patches in wild *N. pulcher*, calling these hypotheses 1) the *individual assessment hypothesis*, 2) the *social dominance hypothesis*, and 3) the *affiliation hypothesis*. To test these 3 hypotheses, we recorded the social behavior of wild *N. pulcher* social groups in Lake Tanganyika

using SCUBA and then measured the size of the yellow facial patches on dominant males, dominant females, and mixed-sex subordinates.

We first tested the *individual assessment hypothesis*, which posits that facial patch size functions as an honest indicator and allows conspecifics to quickly assess various metrics of an individual's quality (Zahavi 1974, 1977; Penn and Számadó 2020). We predicted that larger patches would be found on individuals that were in better physical condition, consumed more food, and/or were more active contributors to the group (i.e., performed more territory defense and brood care). Additionally, because dominant males often “own” more than 1 territory but dominant females never do (Desjardins et al. 2008a; Wong et al. 2012; Jungwirth et al. 2016), males are likely to experience more reproductive competition compared to females and thus may be under stronger selection to advertise their individual quality (as has been observed in many species [Dixon et al. 2005; Dunn et al. 2015; Grueter et al. 2015; McQueen et al. 2019]). We, therefore, predicted that male *N. pulcher* would have larger color patches than females.

Our second hypothesis, the *social dominance hypothesis*, was that patch size is related to how dominant an individual is (reviewed by Senar 2006; Svensson and Wong 2011). We predicted that dominant individuals would have larger patches than subordinates (potentially owing to differences in food intake; see *individual assessment hypothesis*) and that individuals who performed the highest levels of dominance behaviors would have the largest patches.

Our final hypothesis was the *affiliation hypothesis*. If patch size is indeed related to individual quality, then individuals with larger patches might receive more affiliative acts from their groupmates (see Zahavi 1995; Henrich and Gil-White 2001). Group members might gain benefits from having strong bonds with high-quality groupmates and affiliative acts could be used to strengthen these bonds since affiliation is thought to promote prosociality and cohesion in *N. pulcher* (Schürch et al. 2010; Brintjes et al. 2016; Anderson et al. 2020; Culbert et al. 2021a; Stettler et al. 2021). Alternatively, strong affiliative relationships with groupmates might be required for individuals to be able to spend the extra time/energy required to attain the resources necessary to develop and/or maintain large patches. Subordinates, in particular, benefit from having strong affiliative relationships with their groupmates because a well-connected subordinate is less likely to be in conflict with other group members and will have a relatively low risk of being exiled and losing the protection of the social group (Taborsky 1984, 2016). Moreover, subordinates that are well connected can benefit further because dominants may be more likely to allow them to reproduce (Heg and Hamilton 2008; Heg et al. 2009; Brintjes et al. 2011) and may also be more likely to eventually assume the dominant position in their group (Dierkes et al. 2005; Stiver et al. 2006; Fitzpatrick et al. 2008). We, therefore, predicted that individuals with larger patches would receive more affiliative acts from their groupmates, and more specifically, that this relationship would be strongest among subordinates.

Materials and Methods

Field site and animals

This study was conducted off the shore of Mutondwe Island, Lake Tanganyika, Zambia (8°42'45”S, 31°7'27”E) in

December of 2019. *Neolamprologus pulcher* are abundant in this region and are listed as “Least Concern” on the IUCN Red List of Threatened Species. Turbidity is generally low and visibility is high (>5 m) in this region of the lake (Langenberg et al. 2003; Plisnier et al. 2018) providing the opportunity for the color signals of *N. pulcher* to be highly salient and used effectively as a form of communication between conspecifics. Using SCUBA, 28 social groups were located between 6 and 8 m depth for this study (see Freudiger et al. 2021, for a detailed description of the study site). Groups contained an average (mean \pm SEM) of 7.6 ± 0.5 individuals. We performed a series of behavioral observations and then collected focal individuals for morphological measurements and assessment of color patch size. To minimize our impact on individual groups, we collected dominant females from half of the social groups ($N = 14$), and dominant males and subordinate helpers from the other half of groups ($N = 14$). Additionally, all fish used in this study were collected as part of several other studies evaluating the physiological consequences of group living and the neuroendocrine regulation of social rank (Culbert et al. 2021b, 2022; Balshine et al., unpublished data).

Behavioral observations

The behavior of 14 dominant males, 14 dominant females, and 14 large subordinate helpers was observed over two 10-min observation periods conducted on separate days (mean of 31 h between observation periods). Before recording behaviors, we allowed a 2-min period for fish to acclimate to the presence of the underwater observer, and then we scored all aggressive (chases, bites, rams, opercular flares, head-down postures, and lateral displays), submissive (submissive postures, tail quivers, j-hooks, and flees), and affiliative behaviors (follows, parallel swims, and soft touches) for 10 min (see Sopinka et al. 2009, for further species-specific behavioral details). Additionally, we counted the number of times that each fish visited the brood chamber (as a proxy for brood care effort) and how much each fish contributed to territory defense (the number of aggressive acts performed toward intruding conspecifics [nongroup members] and heterospecifics).

We calculated a dominance index for each focal fish by subtracting the combined number of aggressive acts received and submissive acts given from the total number of aggressive acts given and submissive acts received (Dom Index = $[\text{Agg}_{\text{Given}} + \text{Sub}_{\text{Rec}}] - [\text{Agg}_{\text{Rec}} + \text{Sub}_{\text{Given}}]$; Aubin-Horth et al. 2007; Fitzpatrick et al. 2008). We also measured the amount of time that focal fish spent foraging in the water column. We controlled for the variable amount of time that individuals spent on versus off their group’s territory by scaling. For example, if a fish was on its territory for 5 min of the 10-min focal watch and performed 2 aggressive acts, we then scaled the aggressive acts to 4 acts over 10 min.

Fish collection and measurement

Within 72 h of the second observation period, all focal fish ($N = 42$) were captured using fence nets and hand nets. Fish were identified based on individual differences in body size, unique markings, behavior, and discrete home ranges within each group’s territory (Werner et al. 2003). Once caught, fish were brought to the water surface using a custom floatation apparatus that allowed fish to ascend at a controlled and steady speed. We subsequently returned to each territory to

confirm both that the correct individual had been captured and that all other group members remained on the territory. On the surface, fish were immediately euthanized via terminal anesthesia (0.5 g L^{-1} ethyl-p-aminobenzoate; Sigma-Aldrich) to facilitate other studies investigating physiological/neuroendocrine endpoints (see Culbert et al. 2021b, 2022; Balshine et al., unpublished data). Following euthanasia, we measured each fish’s standard length to the nearest millimeter using Vernier calipers (dominant males: 71 ± 1 mm, dominant females: 63 ± 1 mm, subordinates: 54 ± 1 mm), weighed them to the nearest milligram using an electronic balance (OHAUS Scout Pro), and identified their sex via visual examination of their genital papilla. As a general indicator of an individual’s health/physiological condition, we calculated condition factor (Fulton’s K ; Ricker 1975) as ($K = W/L^3$), where W is the weight in grams and L is the length in centimeters.

Analysis of facial patch size

To minimize reflection and help maintain consistent environmental conditions across images, fish were gently patted with a damp microfiber cloth to remove water droplets from their body surface prior to imaging. Pictures were taken at a constant distance (~20 cm above each fish) under diffuse lighting conditions using an Olympus TG-820 digital camera (manual setting; ISO = high; white balance = cloudy; color = normal; exposure correction = -1.3). Using ImageJ v1.53e (Schneider et al. 2012), we measured the area of the yellow patch below the eye in each photo (see Figure 1). We divided the area of the yellow patches by the total area of each fish’s head to control for individual differences in body size (head area was strongly correlated with body length; linear model [LM]: $R^2 = 0.79$, $F_{1,38} = 143.11$, $P < 0.001$). To define the area of the head, we drew a line from the beginning of the first spine of the dorsal fin down to the attachment site of the pectoral fin which served as the posterior margins of the head (see Figure 1). The upper and lower jaws were not included in our measurements of head size to avoid biases resulting from differences in the position of the mouth at the time of imaging. We focused on the size of these yellow patches because conditions in the field precluded our ability to reliably assess other qualities of these colored patches (e.g., intensity). After controlling for individual differences in head size, we found no evidence that larger fish had larger patches (LM: $R^2 = 0.02$, $F_{1,38} = 0.85$, $P = 0.36$). Due to technical issues during imaging, we were unable to evaluate patch size for 2 dominant males. Thus, we were left with a final sample size of 12 dominant males, 14 dominant females, and 14 large subordinate helpers (11 females and 3 males) for our measurements of patch size.

Statistical analyses

Statistical analyses were conducted using R (version 4.2.0; R Core Team 2022) and a significance level (α) of 0.05 was used for all tests. We performed a series of LMs to evaluate the relationship between patch size and our measures of behavior and morphology. All models were fit using the lm function and model assumptions were evaluated visually using the “performance” package (Lüdtke et al. 2021). Preliminary analyses that included Group ID as a random effect did not improve the fit of the overall models and caused singularity issues. Therefore, we did not include Group ID as a random effect in our final models to avoid issues with overparameterization (see Bolker et al.

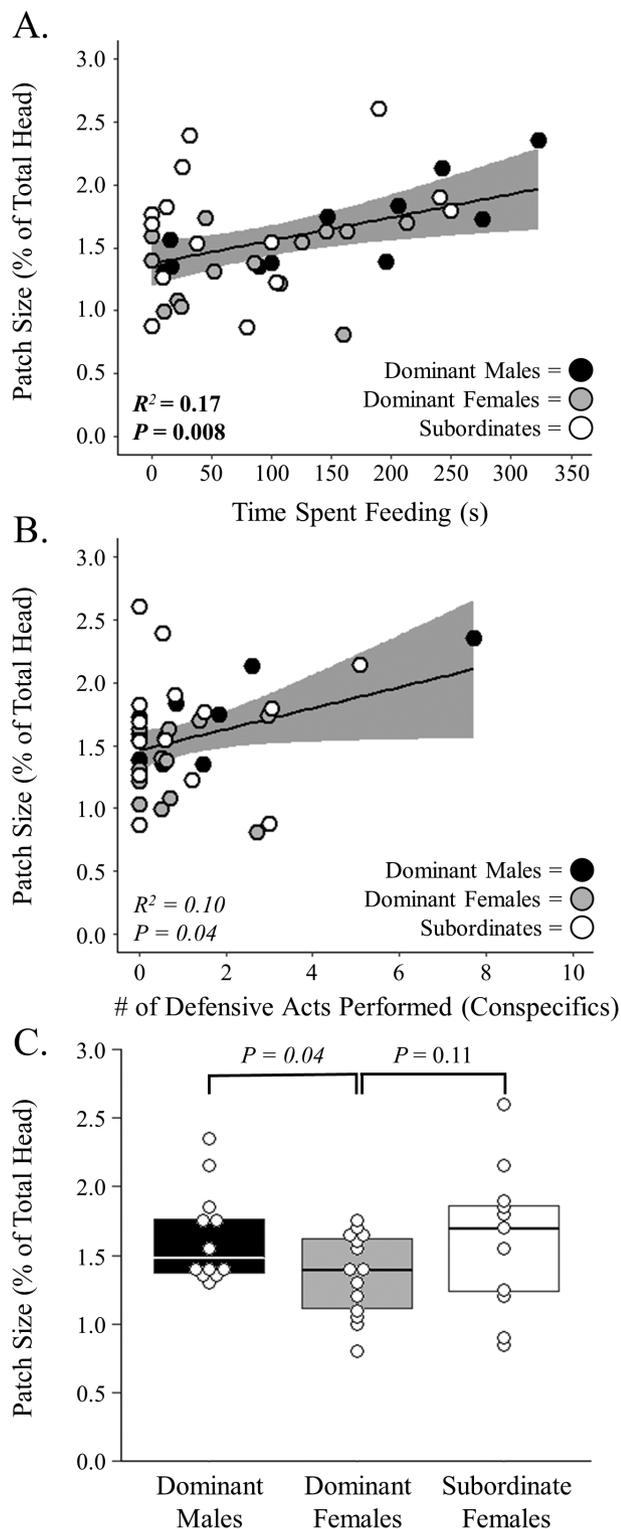


Figure 2 Relative patch size increased as individuals spent more time feeding (A) and fish that contributed more toward territory defense against intruding conspecifics tended to have larger patches (B). Additionally, relative patch size was ~20% greater on dominant males than dominant females but did not differ between dominant females and subordinate females (C). A linear regression was fitted in Panels A and B, with the shaded areas showing the 95% confidence interval around the regression. Values in Panel C are presented as boxplots where the horizontal lines through the boxes represent the median and the limits of the box represent the 1st and 3rd quartiles. Filled and open circles represent individual values. **Bolded** P -values indicate significance following false discovery corrections and *italicized* P -values indicate differences that were only significant prior to false discovery corrections (see text for detailed statistical results).

2009). For all behavioral analyses, we initially included the interaction term between the behavior and social rank to determine whether the relationship between patch size and behavior differed depending on social rank. If this interaction term was significant (see [Supplementary Table 1](#)), analyses were then conducted separately for each social rank to further tease apart patterns. If the interaction term was not significant, then it was dropped from the model and the analyses included fish of all social ranks in a single model. Overall differences were evaluated using the Anova function in the “car” package (Fox and Weisberg 2011) and effect sizes were estimated by calculating R^2 values using the “performance” package (Lüdecke et al. 2021). To reduce our false discovery rate (FDR), Benjamini–Hochberg corrections (Benjamini and Hochberg 1995) were applied to control for multiple tests within each hypothesis.

We first evaluated the evidence for the *individual assessment hypothesis* using LMs to assess the relationship between the size of yellow facial patches and measures of physical quality (foraging time or condition factor) or behavioral workload contributions (number of brood chamber visits or acts of territory defense performed). As well, we examined whether facial patch size differed between males and females to assess whether patches might be under sexual selection in males of this polygynous fish. Note that this analysis of patch size between sexes was restricted to dominant fish as very few subordinate males ($N = 3$) were captured. To evaluate the *social dominance hypothesis*, we used LMs to assess whether the size of yellow facial patches was related to social rank (dominant or subordinate; note that this analysis was restricted to females because only $N = 3$ subordinate males were captured) or dominance index scores. Finally, we evaluated the *affiliation hypothesis* using LMs to explore the relationship between yellow facial patch size and affiliative behaviors (number of affiliative acts performed or received).

Results

Individual assessment hypothesis: Patch size increased with foraging durations

Fish that spent more time foraging in the water column had larger patches (Figure 2A; Table 1). Fish with larger patches also tended to perform greater amounts of territory defense against intruding conspecifics (Figure 2B; Table 1); however, this result was not significant following FDR corrections. Similarly, dominant males had 20% larger color patches than dominant females (Figure 2C; Table 1), but this result was also not significant following FDR correction. Patch size was not related to individual body condition, nor was it related to how much defense that fish performed toward intruding heterospecifics or the amount of brood care that fish provided (Table 1).

Social dominance hypothesis: Patch size was *not* related to dominance

Patch size did not differ between dominant and subordinate females (Figure 2C; Table 1). Additionally, while a significant relationship between patch size and the interaction term of dominance index scores and social rank was detected (Supplementary Table 1), subsequent analyses did not reveal any significant relationships between patch size and dominance index scores within any individual social rank (Table 1).

Table 1 Statistical results used to evaluate the 3 proposed hypotheses for facial patch size in *Neolamprologus pulcher*

	Variable	Group analyzed	R ²	F _(DF)	P
Individual assessment hypothesis	Feeding (s)	All individuals	0.17	7.84 _(1,38)	< 0.01
	<i>Acts of territory defense (conspecifics)</i>	<i>All individuals</i>	<i>0.10</i>	<i>4.42</i> _(1,38)	<i>0.04</i>
	<i>Sex</i>	<i>Dominants only</i>	<i>0.16</i>	<i>4.58</i> _(1,24)	<i>0.04</i>
	Condition factor (K)	All individuals	0.01	0.05 _(1,38)	0.83
	Acts of territory defense (heterospecifics)	All individuals	0.01	0.22 _(1,38)	0.64
	Brood care visits	All individuals	0.01	0.31 _(1,38)	0.58
Dominance hypothesis	Social rank	Females only	0.10	2.83 _(1,24)	0.11
	Dominance index score	Dominant males	0.07	0.79 _(1,10)	0.40
		Dominant females	0.14	2.03 _(1,12)	0.18
		Subordinates	0.19	3.08 _(1,12)	0.11
Social integration hypothesis	Affiliative acts received	Dominant males	0.40	7.36 _(1,10)	0.02
		Dominant females	0.12	1.83 _(1,12)	0.20
		<i>Subordinates</i>	<i>0.32</i>	<i>6.12</i> _(1,12)	<i>0.03</i>
	Affiliative acts performed	Dominant males	0.03	0.29 _(1,10)	0.60
		Dominant females	0.05	0.72 _(1,12)	0.41
		Subordinates	0.37	7.55 _(1,12)	0.02

Relationships that remained significant following false discovery corrections are indicated in **bold** and differences that were only significant prior to false discovery corrections are indicated in *italics* (see main text for details of analyses).

Affiliation hypothesis: Affiliative relationships varied with patch size in a *rank-specific* manner

Patch size was related to affiliative behavior in a rank-specific manner (Supplementary Table 1). Dominant males with larger patches received *fewer* affiliative acts from their groupmates compared to dominant males with smaller patches (Figure 3A; Table 1). In contrast, subordinates with larger patches tended to receive *more* affiliative acts from their groupmates compared to subordinates with small patches (Figure 3B; Table 1); however, this result was not significant following FDR corrections. Additionally, subordinates with larger patches performed *fewer* affiliative acts toward their groupmates (Figure 3C; Table 1). Patch size was not related to the number of affiliative acts performed by dominant males or dominant females, or how many affiliative acts dominant females received (Table 1).

Discussion

We found that the conspicuous yellow patches located on the face of *N. pulcher* serve as a cue related to foraging efforts in the wild. Specifically, patch size was positively related to how much time an individual spent feeding in the water column indicating that developing/maintaining large patches might at least partly be related to the amount of food that an individual consumes. In addition, the size of an individual's patch was related to affiliative relationships with groupmates in a rank-specific way. Dominant males, but not dominant females, with large patches received fewer affiliative acts from other group members. In contrast, subordinates with larger patches performed fewer affiliative acts while tending to receive a greater number of affiliative acts from their groupmates. In combination, these data suggest that patch size may have important signaling functions that are associated with social relationships within wild groups of this highly social cichlid fish.

Individuals that consume large amounts of high-quality food are expected to have larger and brighter color patches (Blount and McGraw 2008; Svensson and Wong 2011; Sefc et al. 2014). However, while many laboratory-based studies have shown that diets that are rich in certain nutrients (e.g., carotenoids) can increase the size and/or brightness of colorful patches in birds, fishes, amphibians, and reptiles (reviewed by Blount and McGraw 2008; Svensson and Wong 2011), far fewer studies have reported such a relationship between feeding and color patches in the wild (Grether et al. 1999; Hill et al. 2002; Walker et al. 2014). Here, we found that *N. pulcher* which spent a greater amount of time foraging in the water column had larger facial patches. These fish predominantly feed on zooplankton located in the water column (Taborsky and Limberger 1981; Gashagaza and Nagoshi 1986; Konings 2019), which can contain high amounts of carotenoids and other nutrients (Kurki et al. 1999; O'Reilly 2001). Even though fish with larger patches spent more time feeding in the water column, fish with larger patches did not have a higher condition factor—a measure that is often used as a proxy for the size of energy reserves in fishes (Ricker 1975; Herlinger and Friars 1991; Chellappa et al. 1995). Instead, it is possible that individuals with large patches were using the energy acquired from increased feeding to fuel energetically costly activities because fish with larger patches tended to contribute more toward aggressively defending the group's territory from intruding conspecifics. Agonistic interactions (such as territory defense) are energetically costly (Grantner and Taborsky 1998; Taborsky and Grantner 1998; Ros et al. 2006) and greatly increase an individual's risk of injury or death (Peake and McGregor 2004). Therefore, patch size may not signal individual quality in terms of energy reserves but instead might reflect how much an individual can afford to contribute toward energetically costly acts that benefit the group.

Affiliative acts are thought to promote social cohesion and maintain prosocial relationships between group members

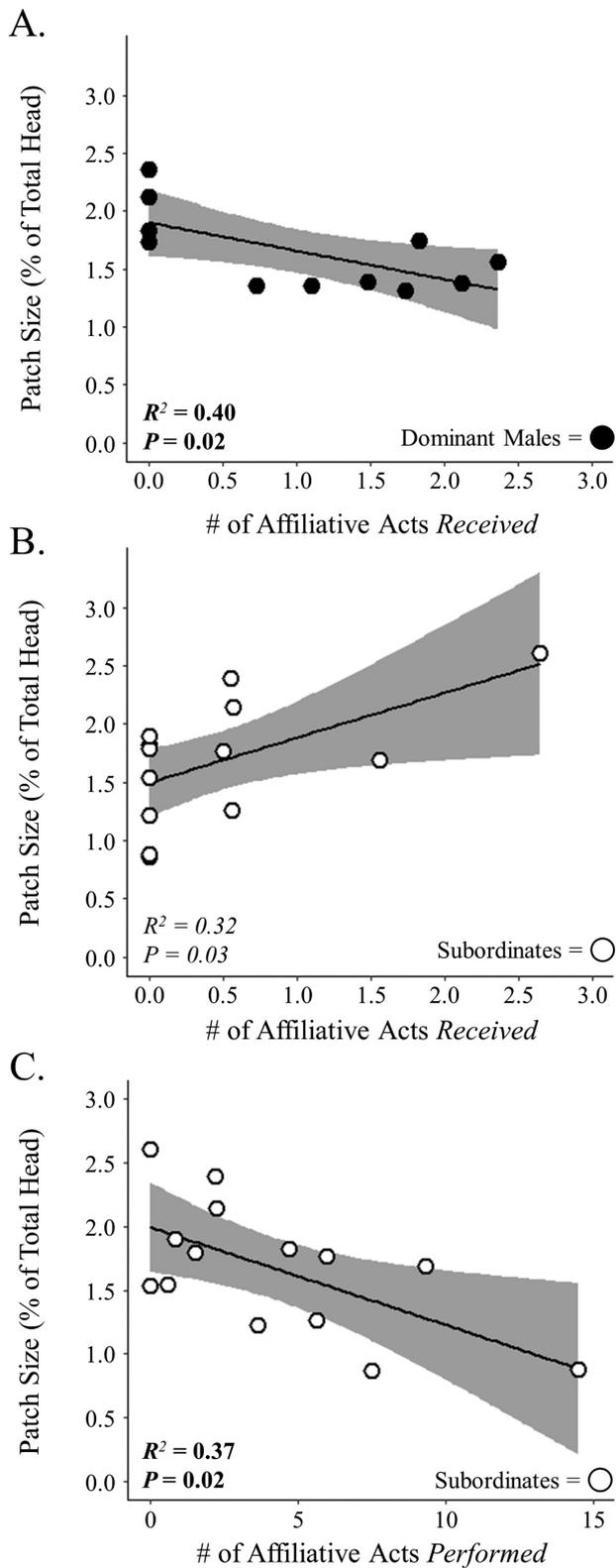


Figure 3 Dominant males with larger facial patches received fewer affiliative acts from their groupmates (A), while subordinates (both sexes combined) with larger facial patches tended to receive more affiliative acts from their groupmates (B). Subordinates with larger patches also performed fewer affiliative acts toward their groupmates (C). Linear regressions were fitted in all panels, and the shaded area shows the 95% confidence interval of the regression line. **Bolded** P -values indicate significance following false discovery corrections and *italicized* P -values indicate differences that were only significant prior to false discovery corrections (see text for detailed statistical results).

across many species (Fraser et al. 2008; Radford 2008; Burkett et al. 2016), including in *N. pulcher* (Bruintjes et al. 2016; Anderson et al. 2020; Culbert et al. 2021a). We found that subordinates (both sexes combined) with large patches tended to receive more affiliative acts, even though these same individuals performed fewer affiliative acts themselves. Since individuals with large patches tend to contribute more toward territory defense, it is likely that groupmates of individuals with large patches have lightened workloads (e.g., they are not required to contribute as much towards territory defense). Because of the energy savings associated with load lightening (Grantner and Taborsky 1998; Taborsky and Grantner 1998), it is likely beneficial for group members to maintain strong affiliative relationships with individuals that have larger patches. Curiously, dominant males with large patches received fewer affiliative acts compared to dominant males with small patches. This difference between subordinate and dominant males might reflect a shift in social strategies that group members use to maintain relationships with dominant males versus subordinates. Dominant males typically display high levels of aggression toward other group members and group members rely on submissive displays to help appease dominant males to reduce the amount of aggression that they receive (Ruberto et al. 2020; Reddon et al. 2019, 2021). Therefore, it is possible that submissive acts are more important than affiliative acts for maintaining prosocial relationships with dominant males; although, we did not find any relationship between patch size and dominance index scores (or any individual component of these scores) for any social rank. Schürch et al. (2010) reported that the amount of prosocial behaviors (affiliation and submission combined) received by dominants was more affected by their body size and personality compared to subordinates. While we did not have the statistical power to control for these traits in our analyses, it is possible that these factors might help to explain the rank-based differences that we observed. Regardless, further experiments are clearly required to evaluate the underlying cause(s) of the observed relationships between patch size and affiliative behavior across social ranks, including assessing how manipulations of patch size affect individual social relationships and overall group dynamics in the field.

Colorful signals are often favored/driven by sexual selection (Grether et al. 2004; Schaefer and Ruxton 2015). Dominant male *N. pulcher* often have multiple breeding partners (Desjardins et al. 2008a; Wong et al. 2012), and it is possible that male *N. pulcher* use their colorful facial patches to advertise their high quality to potential breeding partners. Indeed, dominant males tended to have larger patches than dominant females in the current study, raising the possibility that sexual selection may influence the size of color patches in this otherwise relatively drab, sexually monomorphic cichlid. However, the difference in patch size between dominant males and dominant females was rather small (~20%). Additionally, we were unable to conduct a full factorial analysis of patch sizes across sexes and social ranks because so few subordinate males were caught; and therefore, it is difficult to fully interpret this result. Consequently, future experiments should include dominant and subordinate individuals of both sexes, as well as different size classes of subordinates since subordinate *N. pulcher* exhibit size-dependent task specialization (which could influence relationships between patch size and behavior [Bruintjes and Taborsky 2011; Groenewoud et al. 2016]). Finally, since all but one dominant male that was

included in our study were polygynous, future studies should also assess whether patch size differs between monogamous and polygynous males.

Overall, our findings suggest that the size of bright colorful facial patches is primarily driven by foraging rates in this model cichlid species from Lake Tanganyika. Additionally, these data offer some of the first evidence that colorful visual signals are associated with affiliative relationships between nonsexual partners in wild social groups. Collectively, our results indicate that colorful signals might have an important modulatory role on the development and/or maintenance of affiliative/cooperative relationships in complex animal societies.

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Conflict of interests

The authors declare no competing interests.

Ethics statement

All protocols were approved by the Animal Research Ethics Board of McMaster University (Animal Utilization Protocol No. 18-04-16) and the Zambian Department of Fisheries. In addition, all protocols were designed following the guidelines of both the Canadian Council on Animal Care and [ASAB/ABS \(2022\)](#) regarding the treatment of animals in research and teaching.

Author contributions

BMC, IYL, MGS, MYLW, and SB conducted the behavioral observations and collected the fish. BMC analyzed the data and wrote the first draft of the manuscript. All authors contributed to the design of the experiment, discussed results, provided feedback on the manuscript, and approved the final draft.

Data availability

Supporting data can be found in the attached supplemental file.

Supplementary Material

Supplementary material can be found at <https://academic.oup.com/cz>.

References

- Anderson HM, Little AG, Fisher DN, McEwen BL, Culbert BM et al., 2020. Behavioural and physiological evidence that increasing group size ameliorates the impacts of social disturbance. *J Exp Biol* 223:jeb217075.
- ASAB/ABS. 2022. Guidelines for the treatment of animals in behavioural research and teaching. *Anim Behav* 183:I–XI.
- Aubin-Horth N, Desjardins JK, Martei YM, Balshine S, Hofmann HA, 2007. Masculinized dominant females in a cooperatively breeding species. *Mol Ecol* 16:1349–1358.
- Bachmann JC, Cortesi F, Hall MD, Marshall NJ, Salzburger W et al., 2017. Real-time social selection maintains honesty of a dynamic visual signal in cooperative fish. *Evol Lett* 1:269–278.
- Balshine S, Leach B, Neat FC, Reid H, Taborsky M et al., 2001. Correlates of group size in a cooperatively breeding cichlid fish *Neolamprologus pulcher*. *Behav Ecol Sociobiol* 50:134–140.
- Balzarini V, Taborsky M, Villa F, Frommen JG, 2017. Computer animations of color markings reveal the function of visual threat signals in *Neolamprologus pulcher*. *Curr Zool* 63:45–54.
- Benjamini Y, Hochberg Y, 1995. Controlling the false discovery rate: A practical and powerful approach to multiple testing. *J R Stat Soc* 57:289–300.
- Bergmüller R, Heg D, Peer K, Taborsky M, 2005. Extended safe havens and between-group dispersal of helpers in a cooperatively breeding cichlid. *Behaviour* 142:1643–1667.
- Blount JD, McGraw KJ, 2008. Signal functions of carotenoid colouration. *Carotenoids* 4:213–236.
- Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR et al., 2009. Generalized linear mixed models: A practical guide for ecology and evolution. *Trends Ecol Evol* 24:127–135.
- Bruintjes R, Bonfils D, Heg D, Taborsky M, 2011. Paternity of subordinates raises cooperative effort in cichlids. *PLoS ONE* 6:1–7.
- Bruintjes R, Lynton-Jenkins J, Jones JW, Radford AN, 2016. Out-group threat promotes within-group affiliation in a cooperative fish. *Am Nat* 187:274–282.
- Bruintjes R, Taborsky M, 2011. Size-dependent task specialization in a cooperative cichlid in response to experimental variation of demand. *Anim Behav* 81:387–394.
- Burkett JP, Andari E, Johnson ZV, Curry DC, de Waal FBM et al., 2016. Oxytocin-dependent consolation behavior in rodents. *Science* 351:375–378.
- Chellappa S, Huntingford FA, Strang RHC, Thomson RY, 1995. Condition factor and hepatosomatic index as estimates of energy status in male three-spined stickleback. *J Fish Biol* 47:775–787.
- Culbert BM, Balshine S, 2019. Visual threat signals influence social interactions in a cooperatively breeding fish. *Anim Behav* 151:177–184.
- Culbert BM, Ligocki IY, Salena MG, Wong MYL, Bernier NJ et al., 2021a. Glucocorticoids do not promote prosociality in a wild group-living fish. *Horm Behav* 127:104879.
- Culbert BM, Ligocki IY, Salena MG, Wong MYL, Hamilton IM et al., 2021b. Rank- and sex-specific differences in the neuroendocrine regulation of glucocorticoids in a wild group-living fish. *Horm Behav* 136:105079.
- Culbert BM, Ligocki IY, Salena MG, Wong MYL, Hamilton IM et al., 2022. Galanin expression varies with parental care and social status in a wild cooperatively breeding fish. *Horm Behav* 146:105275.
- Culbert BM, Talagala S, Barnett JB, Stanbrook E, Smale P et al., 2020. Context-dependent consequences of color biases in a social fish. *Behav Ecol* 31: 1410–1419.
- Desjardins JK, Fitzpatrick JL, Stiver KA, Van der Kraak GJ, Balshine S, 2008a. Costs and benefits of polygyny in the cichlid *Neolamprologus pulcher*. *Anim Behav* 75:1771–1779.
- Desjardins JK, Stiver KA, Fitzpatrick JL, Balshine S, 2008b. Differential responses to territory intrusions in cooperatively breeding fish. *Anim Behav* 75:595–604.
- Dierkes P, Heg D, Taborsky M, Skubic E, Achmann R, 2005. Genetic relatedness in groups is sex-specific and declines with age of helpers in a cooperatively breeding cichlid. *Ecol Lett* 8:968–975.

- Dixson A, Dixon B, Anderson M, 2005. Sexual selection and the evolution of visually conspicuous sexually dimorphic traits in male monkeys, apes, and human beings. *Annu Rev Sex Res* 16:1–19.
- Dunn PO, Armenta JK, Whittingham LA, 2015. Natural and sexual selection act on different axes of variation in avian plumage color. *Sci Adv* 1:e1400155.
- Fischer S, Balshine S, Hadolt MC, Schaedelin FC, 2021. Siblings matter: Family heterogeneity improves associative learning later in life. *Ethology* 127:897–907.
- Fitzpatrick JL, Desjardins JK, Milligan N, Stiver KA, Montgomerie R et al., 2008. Female-mediated causes and consequences of status change in a social fish. *Proc R Soc B* 275:929–936.
- Fox J, Weisberg S, 2011. *An R Companion to Applied Regression*. 2nd edn. Thousand Oaks: Sage Publications.
- Fraser ON, Stahl D, Aureli F, 2008. Stress reduction through consolation in chimpanzees. *Proc Natl Acad Sci USA* 105:8557–8562.
- Freudiger A, Josi D, Thünken T, Herder F, Flury JM et al., 2021. Ecological variation drives morphological differentiation in a highly social vertebrate. *Funct Ecol* 35: 2266–2281.
- Gashagaza MM, Nagoshi M, 1986. Comparative study in the food habits of six species of *Lamprologus* (Osteichthyes: Cichlidae). *Afr Study Monogr* 6:37–44.
- Grantner A, Taborsky M, 1998. The metabolic rates associated with resting, and with the performance of agonistic, submissive and digging behaviours in the cichlid fish *Neolamprologus pulcher* (Pisces: Cichlidae). *J Comp Physiol B* 168:427–433.
- Grether GF, Hudon J, Millie DF, 1999. Carotenoid limitation of sexual coloration along an environmental gradient in guppies. *Proc R Soc B* 266:1317–1322.
- Grether GF, Kolluru GR, Nersissian K, 2004. Individual colour patches as multicomponent signals. *Biol Rev* 79:583–610.
- Groenewoud F, Frommen JG, Josi D, Tanaka H, Jungwirth A et al., 2016. Predation risk drives social complexity in cooperative breeders. *Proc Natl Acad Sci USA* 113: 4104–4109.
- Grueter CC, Isler K, Dixon BJ, 2015. Are badges of status adaptive in large complex primate groups? *Evol Hum Behav* 36:398–406.
- Heg D, Bachar Z, Brouwer L, Taborsky M, 2004. Predation risk is an ecological constraint for helper dispersal in a cooperatively breeding cichlid. *Proc R Soc B* 271:2367–2374.
- Heg D, Brouwer L, Bachar Z, Taborsky M, 2005. Large group size yields group stability in the cooperatively breeding cichlid *Neolamprologus pulcher*. *Behaviour* 142:1615–1641.
- Heg D, Hamilton IM, 2008. Tug-of-war over reproduction in a cooperatively breeding cichlid. *Behav Ecol Sociobiol* 62:1249–1257.
- Heg D, Jutzeler E, Mitchell JS, Hamilton IM, 2009. Helpful female subordinate cichlids are more likely to reproduce. *PLoS ONE* 4:e5458.
- Henrich J, Gil-White FJ, 2001. The evolution of prestige: Freely conferred deference as a mechanism for enhancing the benefits of cultural transmission. *Evol Hum Behav* 22:165–196.
- Herbinger CM, Friars GW, 1991. Correlation between condition factor and total lipid content in Atlantic salmon *Salmo salar* L., parr. *Aquac Res* 22:527–529.
- Hill GE, 1991. Plumage coloration is a sexually selected indicator of male quality. *Nature* 350:337–339.
- Hill GE, Inouye CY, Montgomerie R, 2002. Dietary carotenoids predict plumage coloration in wild house finches. *Proc R Soc B* 269:1119–1124.
- Jordan LA, Wong MYL, Balshine S, 2010. The effects of familiarity and social hierarchy on group membership decisions in a social fish. *Biol Lett* 6:301–303.
- Jungwirth A, Balzarini V, Zöttl M, Salzmann A, Taborsky M et al., 2019. Long-term individual marking of small freshwater fish: the utility of visual implant elastomer tags. *Behav Ecol Sociobiol* 73:49.
- Jungwirth A, Brena PF, Keller I, Taborsky M, 2016. Polygyny affects paternal care, but not survival, pair stability, and group tenure in a cooperative cichlid. *Behav Ecol* 27:592–600.
- Jungwirth A, Josi D, Walker J, Taborsky M, 2015a. Benefits of coloniality: Communal defence saves anti-predator effort in cooperative breeders. *Funct Ecol* 29:1218–1224.
- Jungwirth A, Walker J, Taborsky M, 2015b. Prospecting precedes dispersal and increases survival chances in cooperatively breeding cichlids. *Anim Behav* 106:107–114.
- Kohda M, Jordan LA, Hotta T, Kosaka N, Karino K et al., 2015. Facial recognition in a group-living cichlid fish. *PLoS ONE* 10:e0142552.
- Konings A, 2019. *Tanganyika Cichlids in Their Natural Habitat*. 4th edn. El Paso: Cichlid Press.
- Kurki H, Vuorinen I, Bosma E, Bwebwa D, 1999. Spatial and temporal changes in copepod zooplankton communities of Lake Tanganyika. *Hydrobiologia* 407:105–114.
- Langenberg VT, Nyamushahu S, Roijackers R, Koelmans AA, 2003. External nutrient sources for Lake Tanganyika. *J Great Lakes Res* 29:169–180.
- Lüdtke D, Ben-Shachar M, Patil I, Waggoner P, Makowski D, 2021. Performance: An R package for assessment, comparison and testing of statistical models. *J Open Source Softw* 6:3139.
- McQueen A, Kempnaers B, Dale J, Valcu M, Emery ZT et al., 2019. Evolutionary drivers of seasonal plumage colours: Colour change by moult correlates with sexual selection, predation risk and seasonality across passerines. *Ecol Lett* 22: 1838–1849.
- O'Connor KI, Metcalfe NB, Taylor AC, 1999. Does darkening signal submission in territorial contests between juvenile Atlantic salmon *Salmo salar*? *Anim Behav* 58:1269–1276.
- O'Reilly CM, 2001. *The effects of land use change on littoral zone dynamics of Lake Tanganyika, East Africa* [PhD thesis]. Tuscon (AZ): Univiversity of Arizona.
- Peake TM, McGregor PK, 2004. Information and aggression in fishes. *Anim Learn Behav* 32:114–121.
- Penn DJ, Számadó S, 2020. The Handicap Principle: How an erroneous hypothesis became a scientific principle. *Biol Rev* 95:267–290.
- Plisnier PD, Nshombo M, Mgana H, Ntakimazi G, 2018. Monitoring climate change and anthropogenic pressure at Lake Tanganyika. *J Great Lakes Res* 44:1194–1208.
- R Core Team, 2022. *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing. Available from: <http://www.R-project.org/>.
- Radford AN, 2008. Type of threat influences postconflict allopreening in a social bird. *Curr Biol* 18:R114–R115.
- Reddon AR, Dey CJ, Balshine S, 2019. Submissive behaviour is mediated by sex, social status, relative body size and shelter availability in a social fish. *Anim Behav* 155:131–139.
- Reddon AR, Ruberto T, Reader SM, 2021. Submission signals in animal groups. *Behaviour* 159:1–20.
- Reyes-Contreras M, Taborsky B, 2022. Stress axis programming generates long-term effects on cognitive abilities in a cooperative breeder. *Proc R Soc B* 289:20220117.
- Ricker WE, 1975. Computation and interpretation of biological statistics of fish populations. *Bull Fish Res Board Can* 191:1–382.
- Ros AF, Becker K, Oliveira RF, 2006. Aggressive behaviour and energy metabolism in a cichlid fish *Oreochromis mossambicus*. *Physiol Behav* 89:164–170.
- Ruberto T, Talbot JL, Reddon AR, 2020. Head up displays are a submission signal in the group-living daffodil cichlid. *Behav Processes* 181:104271.
- Sabol AC, Hellmann JK, Gray SM, Hamilton IM, 2017. The role of ultraviolet coloration in intrasexual interactions in a colonial fish. *Anim Behav* 131:99–106.
- Schaefer HM, Ruxton GD, 2015. Signal diversity, sexual selection, and speciation. *Annu Rev Ecol Evol Syst* 46:573–592.
- Schneider CA, Rasband WS, Eliceiri KW, 2012. NIH Image to ImageJ: 25 years of image analysis. *Nat Methods* 9:671–675.
- Schürch R, Rothenberger S, Heg D, 2010. The building-up of social relationships: Behavioural types, social networks and cooperative breeding in a cichlid. *Philos Trans R Soc B* 365:4089–4098.
- Sefc KM, Brown AC, Clotfelter ED, 2014. Carotenoid-based coloration in cichlid fishes. *Comp Biochem Physiol Part A* 173:42–51.
- Senar JC, 2006. Color displays as intrasexual signals of aggression and dominance. In: Hill GE, McGraw KJ, editors. *Bird Coloration: Function and Evolution*. Cambridge: Harvard University Press.

- Senar JC, Escobar D, 2002. Carotenoid derived plumage coloration in the siskin *Carduelis spinus* is related to foraging ability. *Avian Sci* 2:19–24.
- Sheehan MJ, Tibbetts EA, 2011. Specialized face learning is associated with individual recognition in paper wasps. *Science* 334:1272–1275.
- Sopinka NM, Fitzpatrick JL, Desjardins JK, Stiver KA, Marsh-Rollo SE et al., 2009. Liver size reveals social status in the African cichlid *Neolamprologus pulcher*. *J Fish Biol* 75:1–16.
- Stapley J, Whiting MJ, 2006. Ultraviolet signals fighting ability in a lizard. *Biol Lett* 2:169–172.
- Stettler PR, Antunes FD, Taborsky B, 2021. The serotonin 1A receptor modulates the social behaviour within groups of a cooperatively-breeding cichlid. *Horm Behav* 129:104918.
- Stiver KA, Fitzpatrick JL, Desjardins JK, Balshine S, 2006. Sex differences in rates of territory joining and inheritance in a cooperatively breeding cichlid fish. *Anim Behav* 71:449–456.
- Svensson PA, Wong BBM, 2011. Carotenoid-based signals in behavioural ecology: A review. *Behaviour* 148:131–189.
- Taborsky M, 2016. Cichlid fishes: A model for the integrative study of social behavior. In: Koenig WD, Dickinson JL, editors. *Cooperative Breeding in Vertebrates: Studies of Ecology, Evolution, and Behavior*. Cambridge: Cambridge University Press, 272–293.
- Taborsky M, 1984. Broodcare helpers in the cichlid fish *Lamprologus brichardi*: Their costs and benefits. *Anim Behav* 32:1236–1252.
- Taborsky M, Grantner A, 1998. Behavioural time-energy budgets of cooperatively breeding *Neolamprologus pulcher* (Pisces: Cichlidae). *Anim Behav* 56:1375–1382.
- Taborsky M, Limberger D, 1981. Helpers in fish. *Behav Ecol Sociobiol* 8:143–145.
- Tibbetts EA, 2002. Visual signals of individual identity in the wasp *Polistes fuscatus*. *Proc R Soc B* 269:1423–1428.
- Walker LK, Thorogood R, Karadas F, Raubenheimer D, Kilner RM et al. 2014. Foraging for carotenoids: Do colorful male hihi target carotenoid-rich foods in the wild? *Behav Ecol* 25: 1048–1057.
- Ward AJW, Kent MIA, Webster MM, 2020. Social recognition and social attraction in group-living fishes. *Front Ecol Evol* 8:1–16.
- Wascher CAF, Kulahci IG, Langley EJJ, Shaw RC, 2018. How does cognition shape social relationships? *Philos Trans R Soc B* 373:15–18.
- Weaver RJ, Koch RE, Hill GE, 2017. What maintains signal honesty in animal colour displays used in mate choice? *Philos Trans R Soc B* 372:20160343.
- Werner NY, Balshine S, Leach B, Lotem A, 2003. Helping opportunities and space segregation in cooperatively breeding cichlids. *Behav Ecol* 14:749–756.
- Whitfield DP, 1987. Plumage variability, status signalling and individual recognition in avian flocks. *Trends Ecol Evol* 2:13–18.
- Wong MYL, Jordan LA, Marsh-Rollo S, St-Cyr S, Reynolds JO et al., 2012. Mating systems in cooperative breeders: The roles of resource dispersion and conflict mitigation. *Behav Ecol* 23:521–530.
- Zahavi A, 1995. Altruism as a handicap: the limitations of kin selection and reciprocity. *J Avian Biol* 26:1–3.
- Zahavi A, 1977. The cost of honesty (further remarks on the handicap principle). *J Theor Biol* 67:603–605.
- Zahavi A, 1974. Communal nesting by the Arabian babbler. A case of individual selection. *Ibis* 116:84–87.