Cichlid Fish Use Coloration as a Cue to Assess the Threat Status of Heterospecific Intruders

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Submitted February 9, 2015; Accepted May 29, 2015; Electronically published August 11, 2015

Keywords: color signal, competitor recognition, familiarity, heterospecific aggression, signal reliability, species interactions.

Abstract: The ability to assess the threat posed by competitors, and to respond appropriately, is important for reducing the costs of aggression. In this respect, aggression directed toward heterospecifics is often just as significant as aggression among conspecifics. This is especially true for cichlid fish that share breeding grounds with heterospecifics. Indeed, cichlids are known to differentiate not only between conspecifics that pose different levels of threat but also between heterospecific territorial intruders by directing more aggression toward nonbreeding individuals. To assess whether the ability to make such distinctions could be based on color cues alone, we carried out a field study in which we experimentally presented _Amphilophus sagittae_ cichlid pairs with model intruders of a sympatric congener, _Amphilophus xiloaensis_, in breeding versus nonbreeding coloration. Consistent with our prediction, we found that _A. sagittae_ exhibited more aggression toward _A. xiloaensis_ models of the latter color type. The results are, to our knowledge, the first to show that territory holders can, based on coloration alone, assess variation among individuals of a species other than their own in the threat posed to offspring survival.

Introduction

Aggressive behavior usually entails costs, such as the loss of energy (Haller 1995; Neat et al. 1998; Brandt 2003; Castro et al. 2006), time taken from other activities (Radesäter et al. 1987), and increased risk of injury (Neat et al. 1998; Lappin and Husak 2005) or predation (Marler and Moore 1988). Failing to react aggressively to a serious threat can also result in negative fitness consequences. We should therefore expect animals to have well-developed mechanisms for distinguishing among different types of opponents and adjusting their level of aggression accordingly (Grether 2011). Such biases in aggressive behavior may have significant evolutionary consequences—not only within species but also in the context of heterospecific interactions (Seehausen and Schluter 2004). Indeed, according to recent evidence, aggressive interactions between species are very common and can be just as important as aggression among conspecifics (Ord and Stamps 2009; Peiman and Robinson 2010; Ord et al. 2011; Grether et al. 2013). Nevertheless, the evolutionary consequences of heterospecific aggression are rarely addressed and, hence, remain poorly understood (Grether et al. 2009, 2013; Peiman and Robinson 2010).

In the context of the strategic allocation of aggression, patterns of phylogenetic relatedness and phenotypic similarity among competitors are likely to be important. These factors may not only affect the fitness consequences of aggressive behavior but also how easy it is to differentiate between intruders that pose different levels of threat (Grether 2011; Grether et al. 2013). In this regard, a recent meta-analysis suggests that heterospecific aggression is more pronounced among congeneric heterospecific individuals, as compared to those from more distantly related genera (Peiman and Robinson 2010). One likely contributor to such a pattern is cognitive limitations; that is, aggression may simply be biased toward those heterospecific individuals that phenotypically resemble conspecifics (Peiman and Robinson 2010), and the same cognitive and sensory systems may be used in signal recognition toward both conspecific individuals and those of closely related, phenotypically similar species (Ord et al. 2011).

Even when competing species are phenotypically similar or congeneric, aggression is not necessarily symmetrical, implying that phenotypic similarity is not the only factor governing heterospecific aggression biases. For example, when two species of grey tree frogs are calling at the same breeding pond, male _Hyla versicolor_ suffer a greater reduction in attractiveness due to call overlap than male _Hyla chrysoscelis_ (Marshall et al. 2006; Reichert and Gerhardt 2014), with male _H. versicolor_, in turn, being more likely...
to initiate physical heterospecific aggression (Reichert and Gerhardt 2014). Furthermore, there is variation among taxa in the types of cues that are most often used for distinguishing among territorial intruders at close range (Grether 2011). In fish, color is known to play an important role in both species and competitor recognition (Barlow 1974; Endler 1983; Seehausen et al. 1999). For instance, in Lake Victoria cichlid fishes of the genus Pandamila, the competitive advantage of red over blue males was diminished when encounters took place under green light conditions, which prevented the fish from seeing the color differences (Dijkstra et al. 2005). Similarly, Pseudotropheus cichlids from Lake Malawi directed more aggression in staged encounters toward members of a species with color patterns similar to their own than toward a differently colored, but more closely related, species (Pauers et al. 2008).

Neotropical cichlids are also known to exhibit biases in social interactions, including territorial aggression, depending on the color of their conspecific opponents (Barlow 1974, 1983; Lehtonen 2014). This is especially true for the Mudas cichlid species complex (Amphilophus spp.; sensu Barluenga and Meyer 2010; Elmer et al. 2010; Geiger et al. 2010), which has been one of the prime systems for evolutionary studies, especially regarding parallel and sympatric speciation (Barluenga et al. 2006; Salzburger 2009; Elmer et al. 2010). Here, we focused on aggression during the breeding season, when the breeding grounds of these fish are typically densely occupied with multiple, simultaneously breeding species of cichlids (McKay 1977; T. K. Lehtonen, personal observations). These breeding aggregations are characterized by intense competition for territory space among conspecific, congeneric, and more distantly related cichlids (McKay 1977; T. K. Lehtonen, personal observations). When ready to spawn, each cichlid pair claims a sedentary breeding territory, the defense of which (and, later, also that of juveniles) is the most notable form of aggression within and between these species in the wild (McKay 1977; Barlow 2000). Thus, territorial aggression is directed toward (i) competitors for territory space (both conspecific and heterospecific), (ii) brood predators (both conspecific and heterospecific), and (iii) conspecific sexual competitors (McKay 1977; Lehtonen et al. 2010, 2012).

In this study, we tested the hypothesis that color alone is a sufficient cue for an adjustment of territorial aggression in Amphilophus sagittae toward a sympatric congener, Amphilophus xiloaensis, displaying either breeding or nonbreeding coloration. Of note, in the majority of individuals of both species (i.e., more than 80%; Elmer et al. 2009), breeding males and females are black, often with some brighter (mostly gray) markings, while nonbreeders of both sexes have a gray base coloration with dark vertical bars (fig. 1; Barlow 1974; Lehtonen et al. 2010). Furthermore, an earlier study conducted in Lake Apoyo, Nicaragua, found that territory-holding Amphilophus cichlids directed more intense aggression toward both conspecific and heterospecific nonbreeders than toward breeders (Lehtonen et al. 2010). In the current study, we predict that coloration alone may be a sufficient cue for the adjustment of territorial aggression to the different heterospecific intruder types. We therefore expect territory holders to make a distinction between the two intruder types and adjust their behavior accordingly, even when the intruders differ only in their color markings.

**Material and Methods**

This field-based study was conducted using scuba in Lake Xiloá, Nicaragua (lat. 12°12.8'N; long. 86°19.0'W), between December 2013 and January 2014, when all cichlid species in the lake (McKay 1977) are breeding (Lehtonen et al. 2011; T. K. Lehtonen, personal observations). To investigate their aggressive responses to different types of opponents, we presented live Amphilophus sagittae breeding pairs (N = 15; at a water depth of 9.9 ± 0.4 m [mean ± SE]) with dummy stimulus models of a sympatric competitor, Amophilophus xiloaensis, which is closely related and pheno-
typically quite similar to the focal species (fig. 1) but has a deeper body and more benthic lifestyle (Elmer et al. 2009). While *A. xiloaensis* breed, on average, in deeper water than *A. sagittae* and are more often found in association with rocky rather than mixed habitat (i.e., alternating patches of rocks and finer substratum), the two species are frequent territorial neighbors (Elmer et al. 2009; Lehtonen et al. 2011; T. K. Lehtonen, personal observations).

Handmade models have been successfully used as stimuli to elicit ecologically relevant behaviors in a range of fish species (reviewed in Rowland 1999), including *A. sagittae* (Lehtonen 2014). The use of models (or dummies) have a significant advantage over the use of live stimulus animals because they allow us to explicitly control for confounding factors that might otherwise arise from differences in the behavior of the stimulus animals. However, instead of using the more traditional wax or painted shapes employed by other researchers (Barlow and Siri 1994; Rowland 1999), we chose to use more realistic-looking models based on photographs of wild-caught fish following the methods of Lehtonen (2014). Specifically, stimulus models were made by gluing waterproof, photographic color prints of the lateral side of a live or freshly euthanized specimen onto both lateral sides of a fish-shaped floating plate (thickness = 6 mm). Each model was attached to a sinker with a thin, transparent fishing line, allowing it to float in a natural position approximately 15–20 cm above the lake bottom. All models were 16 cm long, a size that was easy to handle under water and that represents an overlap in size ranges of adult males and females in the wild. Half of the models were of *A. xiloaensis* cichlids in breeding color, while the rest were of nonbreeding individuals. We tested the reactions of territory-holding *A. sagittae* toward the two model types in a habitat characterized by pebbles lying on a finer substratum of sand and organic material.

Each replicate was initiated by placing an *A. xiloaensis* model, with either breeding or nonbreeding coloration (fig. 1; see also the general description above), at a distance of approximately 40 cm from the center of the focal *A. sagittae* territory. Typical aggressive responses involved either slow movement toward the model with flared gills and fins in a threat display or a rapid advance, often followed by a bite, before retreating back to the fry. We counted the total number of such aggressive encounters by both territory owners toward the model for 5 min, giving the total aggression rate (sensu Lehtonen et al. 2012; Lehtonen 2014). After a resting period of 5 min (during which the models were removed from sight), we repeated the procedure using the alternative model type (i.e., a model with breeding coloration if the pair had initially been presented with a model with nonbreeding coloration, and vice versa).

We had in total 14 different models of *A. xiloaensis*, half of them in breeding (*N* = 7) and the other half in non-breeding (*N* = 7) coloration (with each model made using a photograph from a different fish). Following the approach of Stevens et al. (2007), to confirm that breeder and nonbreeder models were quantitatively different in their visual appearance, we assessed each model image’s sum of the red, green, and blue scores (i.e., R + G + B) in ImageJ (U.S. National Institutes of Health, Bethesda, MD) using the six landmarks (here, 50 × 50 pixels) described in Sowersby et al. (2015). We found that the nonbreeder models were, indeed, lighter colored (i.e., had a significantly higher (R + G + B)/3 score) than breeder models (twosample t-test, *t* < 3.4, *P* < .01 for all six landmarks). Hence, breeder and nonbreeder models differed from each other both in relation to their color markings and luminance. For clarity, from hereon, we refer to this visual dissimilarity between breeding and nonbreeding individuals as a difference in coloration. Because it was necessary to use individual models more than once, models of *A. xiloaensis* in breeding and nonbreeding coloration were paired haphazardly for the purpose of presentation to *A. sagittae*. As a result, one pairing of breeder and nonbreeder stimulus models ended up being used twice, whereas none of the other stimulus models were paired with the same alternate model more than once. The order of the model presentations was randomized (model with breeding coloration presented first in eight of the replicates; nonbreeding coloration presented first in the remaining seven), and a different *A. sagittae* territory was used for each replicate of the experiment.

**Statistical Analyses**

To assess the influences of sex (male vs. female) of the focal territory holders and the type of the model intruder (breeder vs. nonbreeder), we used R 3.1.0 software (R Development Core Team) to apply a generalized mixed model using the glmmPQL function of the packages nlme and MASS, with a negative binomial error distribution as appropriate for overdispersed count data (Zuur et al. 2013). To account for the nonindependence of the actions of a territory-holding male and female and the use of each model in more than one replicate, we added territory ID and model ID, respectively, as random factors (per the method described in Pinheiro and Bates 2000).

**Results**

When we applied a generalized mixed model to assess the effects of the sex of the focal territory holders and the color of the model intruder on the rate of aggression, we found no significant interaction between the two factors (*t*<sub>sex</sub> = 0.0227, *P* = .98). A model refitted without the interaction showed a significant effect of the intruder color (*t*<sub>color</sub> = 2.60,
P = .012) but not sex (t_{5} = 0.0648, P = .95): independent of their sex, *Amphilophus sagittae* territory holders were more aggressive toward nonbreeding than breeding colored *Amphilophus xiloaensis* models (fig. 1).

**Discussion**

We found that *Amphilophus sagittae* territory holders directed more aggression toward model *Amphilophus xiloaensis* intruders that had nonbreeding coloration than toward those with breeding coloration. The result is concordant with an earlier study showing that *Amphilophus zalisiosis* territory holders from Lake Apoyo were similarly more aggressive toward nonbreeding than breeding *Amphilophus astorquii* intruders (Lehtonen et al. 2010). However, in that study, behavioral responses toward heterospecifics were based on natural encounters. Therefore, it could not be established whether differences in the responses of territory holders were due to differences in the coloration of the heterospecific intruder or some other cue(s). In contrast, by using dummies to control for alternative cues such as intruder behavior or familiarity, we were able to show that *A. sagittae* adjust their aggression toward heterospecific intruders differing in breeding and nonbreeding coloration. More generally, the results of our study are also consistent with previous research in *Hetaerina* damsels. In Anderson and Grether (2010), individuals were found to direct more aggression toward heterospecific species that have colors similar to their own. However, to our knowledge, the current study is the first to show that not only is coloration (here, differences in patterns and brightness) a sufficient cue for assessment of heterospecific territorial intruders at a species level but that territory holders can use such color-related cues to distinguish between individuals that differ in their potential threat even when these individuals are heterospecific.

For *A. sagittae* to adjust their aggressive responses based solely on coloration differences among *A. xiloaensis* intruders, territory holders need to be subject to selection for differential coloration-dependent aggression (ultimate explanation), in addition to having the ability to recognize individuals with different color patterns (proximate explanation). In regard to the former, an important driver of heterospecific aggression is thought to be the extent to which heterospecifics compete over the same resources, such as food, territories, shelter, and mates (see Genner et al. 1999; Dijkstra et al. 2007). In this respect, even though neighboring breeding pairs occasionally engage in disputes over territory borders (T. K. Lehtonen, personal observations), individuals in breeding coloration are generally expected to pose a lower threat to territory owners than nonbreeders, especially in terms of offspring predation. This is because breeders already occupy a territory and are, themselves, preoccupied with caring for offspring and since they are mostly relying on previously accumulated energy reserves during the parental phase, they are less inclined to predate on the fry of other cichlids (McKaye 1977; Rogers 1988; Barlow 2000; Lehtonen et al. 2010). Nonbreeding individuals are, by contrast, more likely to predate on the eggs and juveniles of both conspecifics and heterospecifics, and pose a threat through territorial takeovers. Our results, therefore, support previous findings showing that territory holders adjust their aggression accordingly (Lehtonen et al. 2010).

At the proximate level, because heterospecific aggression may occasionally also arise from misplaced conspecific aggression (Peiman and Robinson 2010), it is possible that *A. sagittae* territory holders responded to the differences in intruder coloration because they mistook them for conspecifics. This possibility would imply a selection pressure to differentiate between conspecific, but not heterospecific, breeders and nonbreeders. Such a possibility could arise because conspecific (but not heterospecific) nonpaired individuals can pose a significant sexual threat. If the models were perceived as a sexual threat, however, we would have expected male and female responses toward them to be asymmetric, which we did not observe. It also seems likely that if territory holders are able to correctly identify the breeding status of heterospecific intruders (even if not correctly identifying their species), adjustment of responses to the intruder status should be beneficial. Furthermore, although the two species have the capacity to hybridize, pairing in the wild is species assortative (Elmer et al. 2009), suggesting that species recognition is highly tuned and mistakes, if they occur at all, are rare. In this respect, even though courtship has been observed among members of the same species, we did not see any evidence of courtship among heterospecifics—either during this or earlier studies—thus supporting our interpretation that individuals are able to properly identify and distinguish heterospecifics from conspecifics.

To conclude, our results indicate that coloration alone is a sufficient cue for intruder status identification and that consequent adjustments of aggression are relevant even among heterospecific individuals.

**Acknowledgments**

We are grateful for logistic support from M. Barluenga, L. and P. Lehtonen, K. and M. McKay, and E. van den Berghe. We also thank K. Delhey and anonymous reviewers for their helpful suggestions to improve the manuscript. Funding was provided by the Department of Biology at the University of Turku, the Hoslworth Wildlife Endowment Fund, and the Linnean Society of New South Wales.
**Literature Cited**


Above, Lake Xiloá in Nicaragua. Below left and right, cichlid fish (Amphilophus sagittae) in Lake Xiloá. Photo credit: Topi K. Lehtonen.