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Seeing orange: breeding convict cichlids exhibit heightened aggression against more colorful intruders

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Abstract

Female convict cichlids (*Amatitlania siquia*) exhibit bright orange ventral coloration that males lack. The behavioral implications of this color are poorly understood, particularly in naturally occurring populations where female coloration could play a role in the expression of territorial nest-guarding behaviors. In this field experiment, monogamous breeding pairs of convict cichlids were presented with 3D printed model conspecific intruders of three body sizes (small, medium, and large) exhibiting three orange patch sizes (large, small, or none) to observe how territorial aggression varied as a function of intruder size and female coloration. Individuals occupying breeding pairs that were defending hatched offspring were significantly more aggressive toward intruders with small and large amounts of orange than toward models lacking orange, indicating that color is an important context-dependent elicitor of aggression in this species. Males were significantly more aggressive toward the intruder than females, and male aggression was strongly influenced by their size relative to the intruder. When males were smaller than the intruder, they performed significantly more aggressive acts than when they were the same size or larger than the intruder; this trend persisted across three putative populations in Lake Xiloa, Nicaragua. A potential explanation for these findings is that the orange color functions as a signal of individual quality or breeding readiness and that breeding pairs increase aggression to repel intruders that pose the greatest threat to pair bond and nest maintenance.

Significance statement

One or both sexes of many animal species possess brightly colored features that might communicate information about overall heath or reproductive status. In convict cichlid fish, males and females establish pair bonds and jointly defend their nest and offspring. Single females exhibit striking orange coloration that males and breeding females lack. Our field-based experiment provided evidence that more colorful females, which likely pose a threat to pair bond stability and nest maintenance, incite more aggression from breeding pairs than drab females. Our study suggests that color provides salient information about, perhaps, female quality or readiness to breed in natural populations, and adds to a growing body of research that seeks to understand the varied roles that colorful ornaments play in animal communication.

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Introduction

Condition-dependent signals are an important component of animal communication because they may provide honest

information to receivers about the signaler's quality. For example, the intensity of a visual signal such as a colorful ornament can advertise an individual's health to potential mates (e.g., Hamilton and Zuk 1982; Mougeot et al. 2006). However, the honesty of a signal may be compromised if individuals can cheat by misrepresenting their quality via exaggerated signal intensity. Thus, honest signals of quality may be characterized by constraints on cheating such as a cost that is more difficult for individuals of lower quality to afford (Emlen et al. 2012).

Color signals produced from carotenoid pigments are hypothesized to be relatively honest because carotenoids cannot typically be synthesized de novo by animals and must be derived from their diet (Olson and Owens 1998; Sefc et al. 2014). A low-quality diet that lacks carotenoids might prevent an animal from expressing intense carotenoid-based coloration, thereby maintaining the honesty of the color signal as an indicator of individual quality. Furthermore, carotenoids affect numerous physiological processes other than coloration that could result in a trade-off occurring under circumstances of carotenoid limitation, whereby carotenoid allocation toward physiological functions that would promote survival reduces the pigment available to support ornamental features. Carotenoids may contribute to aspects of reproduction such as egg production and hatching rates (Güroy et al. 2012) and embryonic development (Levi et al. 2011). Carotenoid supplementation increases overall growth rates in adult fish (Güroy et al. 2012; Sheikhzadeh 2013; Teimouri et al. 2013), and can affect the growth and survival of offspring via maternal effects (Brown et al. 2014). Multiple aspects of immune function also respond to dietary carotenoids. For example, dietary β-carotene supplementation enhanced lymphocyte proliferation in larval parrotfish (Tachibana et al. 1997), and a synthetic astaxanthin supplement significantly reduced pathogen-induced mortality in rainbow trout (Amar et al. 2012). Studies of fish immune response to environmental pollutants have indicated that carotenoids may reduce oxidative stress (Elseady and Zahran 2013) and thus protect against oxidative damage (Pereira et al. 2011). Carotenoid supplementation studies also have demonstrated that multiple indices of oxidative stress are inversely correlated with dietary carotenoid levels (Wang et al. 2006; Pan et al. 2011; but see Sullivan et al. 2014).

The convict cichlid *Amatitlania siquia* is an excellent model system for studying the implications of carotenoid-based condition-dependent signaling in the context of a reversed sexual dimorphism (Brown et al. 2013). One of the diagnostic characteristics of convict cichlids is orange carotenoid-based ventral coloration that is expressed by sexually mature females but not by males. Like many Neotropical cichlid species, convict cichlids are biparental substrate brooders that form monogamous breeding pairs, protect their offspring until they reach the juvenile developmental stage, and then seek new mates (but see Keenleyside 1985 for a discussion of bigamous behavior in male convict cichlids). Convict cichlids

often exhibit size-assortative mating in nature (Wisenden 1994a), and although brood desertion is uncommon, males are more likely to abandon their partners in locations with low brood predation risk (Wisenden 1994b). Thus, the nest site fidelity and territorial behaviors of breeding pairs make them a practical choice for field-based behavior experiments.

While a convict cichlid breeding pair defends their offspring, the fish are exposed to numerous stressors such as predation and territorial competition (Itzkowitz et al. 2005; Snekser et al. 2011), which might be sufficient to stimulate mobilization of integument carotenoids to prioritize other physiological demands such as immune function (Svensson and Wong 2011). Field observations of convict cichlid breeding pairs have indicated that breeding status is a reliable predictor of female coloration; females defending older offspring are less likely to have orange coloration than those with offspring at earlier developmental stages (Anderson et al. 2015) and the vast majority of non-breeding females exhibit an orange patch (CA, RLE personal observation). While the mechanisms controlling the seasonal dynamics of female coloration are largely unknown, it is possible that non-breeding females exhibit significantly more orange color than breeding females because they do not have to partition carotenoid pigments between the integument and physiological functions related to survival. Thus, an orange female intruding into a breeding territory could be a higher-quality individual or it could be prospecting for its own breeding territory, either of which might elicit a strong aggressive response from an established breeding pair.

The signaling potential of female convict cichlid coloration is not fully understood. One hypothesis concerning female coloration is that it is used by male convict cichlids to select a mate, a reversal of the typical role that sexual dichromatism plays in mate choice patterns, but this hypothesis has not received strong experimental support (Beeching et al. 1998). However, there is evidence that intensity of female intrasexual aggression is positively correlated with the amount of orange coloration that an opponent exhibits (Beeching et al. 1998). Although colorful female ornaments have been observed and studied in other cichlid species (e.g., Tobler 2007; Baldauf et al. 2011), the diversity of cichlid breeding strategies and types of coloration impedes any general inferences about the function of reversed sexual dichromatism. Thus, the behavioral implications of female color in convict cichlids requires further study in order to determine its value as a signal and the contexts in which it operates.

The primary aims of this study were to evaluate how convict cichlid breeding pairs respond to variation in the orange coloration of intruding female conspecifics under field conditions and to determine whether that response varies across putative populations within Lake Xiloa, a volcanic crater lake in western Nicaragua. We focused our study on breeding pairs of convict cichlids because they exhibit territorial aggression and strong nest site fidelity, whereas non-breeding individuals

exhibit relatively little aggression, move freely among shallow vegetation, and cannot be tracked easily. Lake Xiloa is an excellent location for conducting field studies of convict cichlids because they breed throughout the year, with increased breeding pair densities in the middle of the dry season (February–April) and the middle of the rainy season (August–September) (McKaye 1977; RLE personal observation). Three locations within Lake Xiloa were chosen for a population comparison based on habitat discontinuity and geographic separation from one another (Fig. 1a). One site (Site 1) was more intensively studied because it was more easily accessible to divers and had the highest density of breeding pairs. The other two sites were only accessible by boat, so an abbreviated version of the experiment was used for purposes of population comparison. It was hypothesized that varying amounts of orange color on an intruding convict cichlid would elicit different aggressive responses from conspecific breeding pairs. Although color signals are complex and have myriad quantifiable characteristics such as hue, brightness, and intensity (Kemp et al. 2015), orange patch size was chosen for the purposes of this study because the amount of coloration is known to be an important elicitor of behavior in convict cichlids (Beeching et al. 1998). If female orange color is an honest signal of individual quality, it was predicted that intruders with larger orange patches would elicit more aggression. In addition, it was hypothesized that breeding pairs from the three sites in Lake Xiloa would display significantly different responses to the same model intruders. Variation in environmental characteristics can induce variation in color signals and conspecific responses to them (e.g., Fuller 2002; Zoppoth et al. 2013), so convict cichlids at different sites might also exhibit varying responses to female orange color.

Materials and methods

3D model fabrication

The convict cichlid models (hereafter, "intruders") used in this experiment were generated using Google Sketchup 8 and printed with Catalyst EX software on a Dimension SST 1200es 3D Printer with ABS ivory polymer. This technique allowed for the generation of models with different lengths but identical proportions. Three intruder sizes (small, SL 35 mm; medium, SL 39 mm; and large, SL 48 mm; Fig. 1b) were chosen based on the variation in size of wild female *A. siquia* as well as the manufacturing limitations of the 3D printer. Nontoxic acrylic craft paint was used to color the intruders. An Ocean Optics Jaz spectrometer was used to test the reflectance spectra of several brands of orange paint, and Martha Stewart Satin Carrot acrylic paint was found to resemble the spectral qualities of the female orange patch most closely (Supplementary Fig. 1), so it was used for all orange color on the intruders. The intruders were painted using photographs of female *A. siquia* from Lake Xiloa as references. Three color treatments were chosen to approximate the natural variation in female orange color: no orange patch, small orange patch (2–3 % of the area of one side of the intruder), and large patch (8–12 % of the area of one side of the intruder). A total of nine model treatments were used in this experiment, consisting of all possible combinations of intruder body sizes and patch sizes (Fig. 1b). Nine 3D models were fabricated, one for each treatment.

The intruders were mounted with monofilament fishing line on rigs constructed from metal forestry flag stakes and black plastic cable ties (Fig. 1c). A horizontal stake suspended the intruders several centimeters above the substrate and two

Fig. 1 **a** Map of Lake Xiloa with locations of study sites 1, 2, and 3; **b** diagram of nine intruder treatments; **c** example of intruder presentation rig
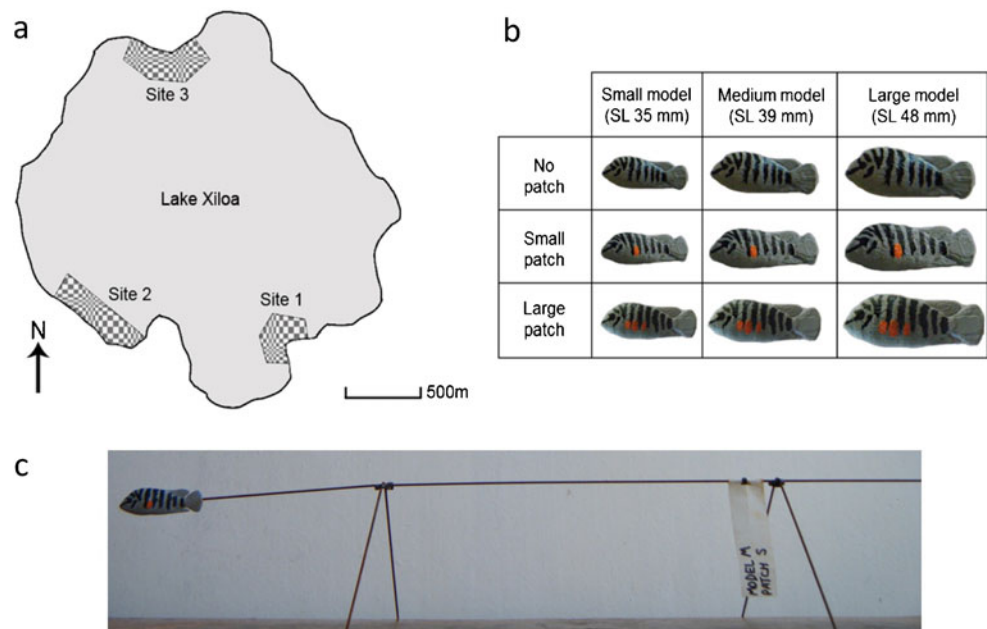
additional stakes were used to form four stabilizing legs. Pilot tests of the intruder presentation rig indicated that the metal components of the rig did not influence the behavior of convict cichlid breeding pairs. Sixteen non-experimental breeding pairs were presented, simultaneously, with a rig containing an intruder and a rig containing a small piece of black duct tape (control) approximating the size of an intruder. The amount of aggressive behavior directed by males and females toward the control was negligible and significantly lower than the amount of aggressive behavior directed toward the intruder (female toward control [mean $\pm$ SEM: 0.125 ± 0.08] vs. female toward intruder [2.81 ± 0.98]: paired t test, $t_{15} = 2.88$, $P = 0.006$; male toward control [0.125 ± 0.13] vs. male toward intruder [4.38 ± 1.62]: paired t test, $t_{15} = 2.61$, $P = 0.01$).

Field behavioral observations

This experiment was conducted in Lake Xiloa in February 2013. All presentation dives lasted 60 to 90 min and were conducted between 0900 and 1700 CST. Site 1 is characterized by gently sloping sandy substrate, shallow weed beds occupied by non-breeding convict cichlids, and a deeper rocky zone where the majority of breeding pairs were located. Presentations at Site 1 were conducted in the rocky zone at depths of 7.5–12.5 m ($N = 171$ pairs, 19 pairs per treatment [Fig. 1b]). For 1 min immediately prior to an intruder presentation, the observer would kneel on the substrate approximately 1 m from the focal nest and note the time, depth, presence or absence of female orange ventral coloration, relative size of the male and female of the breeding pair compared to the intruder (larger, equal, or smaller), developmental stage of any offspring present, and intruder treatment to be used. This pre-presentation time also served to minimize the effects of observer disturbance on breeding pair behavior. Coloration was treated as a binary variable (present vs. absent) due to the limitations of observing free-swimming fish in poor visibility conditions. Offspring were categorized as "none/eggs" or "wrigglers/fry." Direct confirmation of egg presence, which requires excavating the nest, likely would have induced nest desertion by the focal pair and disrupted the behavior of adjacent breeding pairs, so the only viable indicator of egg presence was the display of guarding behaviors generally associated with eggs. "Wrigglers" were defined as recently hatched larval offspring that were not yet free-swimming and moved closely to the substrate, whereas "fry" were defined as free-swimming larval offspring. After noting relevant characteristics of the focal pair and nest, the observer presented the intruder by moving the rig slowly toward the nest from one side. The rig was then placed on the substrate so that the lateral side of the intruder was facing the nest and within 15 cm of the nest. Aggressive behaviors delivered by

the male and female convict cichlids (pair-mates) toward the intruder were counted for 5 min immediately following presentation of the intruder. Aggressive behaviors included any rapid action directed toward the intruder such as approaching, biting, lateral displaying (the fish extending its branchiostegal rays and unpaired fins and presenting its side to the intruder), frontal displaying (the fish extending its branchiostegal rays and unpaired fins and flaring its opercular flaps while facing the intruder), and tandem attacking (the male and female performing a simultaneous rapid approach while remaining within one body length of each other); behavior descriptions were partially adapted from Itzkowitz et al. (2001).

To compare breeding pair responses to an intruder across multiple sites within Lake Xiloa, two additional locations were selected based on their relative distance from the primary study site and *A. siquia* densities. Site 2 was characterized by a steeper incline than Site 1 and patchy rocks and vegetation on a sandy substrate, while Site 3 had the shallowest incline of the three sites and rocky reefs and large logs on a sandy substrate with significantly more light penetration. These sites could only be accessed by boat, unlike the primary site, which was close to an access point. Due to the reduced efficiency of conducting intruder presentations at the additional two sites, only the medium intruder size was used in order to obtain an acceptable number of replicates for the three color patch treatments. The medium-sized intruder was chosen because it most closely resembled the size of a mature female *A. siquia* in Lake Xiloa. Breeding pair density at the additional sites was lower than at the main site, so presentations were conducted at a wider range of depths. At Site 2, presentations were conducted from near the surface to a depth of 12 m ($N = 55$ pairs, 18–19 per treatment), while presentations were conducted from near the surface to a depth of 9 m at Site 3 ($N = 50$ pairs, 16–17 per treatment). Because the experiment was conducted using focal animals in the field and because behavioral data were collected at the time of observation, it was not possible for the observer to be blind to the experimental treatment.

Data analysis

This study examined numerous factors that potentially are relevant to convict cichlid aggression: intruder body size (large, medium, small), patch type (amount of orange possessed by an intruder: small, large, no orange patch), sex, breeding status (no eggs/eggs only or wrigglers/fry), relative size compared to the intruder (smaller, equal to, larger), time of day, and depth. Data were analyzed using a mixed model within-subjects approach with sex as the within-subjects factor and pair as a random effect to account for non-independence of the male and female of a given pair (Briffa and Elwood 2010). All of the aforementioned factors (as fixed

effects) and their interactions resulted in a highly complex model, so PROC GLMSELECT and the ALLMIXED2 macro (Fernandez 2007) were used in SAS 9.1 to perform a model-fitting procedure that identified a simplified set of best-fit models using a corrected Akaike's Information Criterion (AICc)-based approach. The reference model was the simplest, containing only the fixed effects associated with the hypotheses that we wished to test—intruder size, intruder orange, sex of the focal animal, breeding status, and size of the focal animal relative to the intruder. Additional models were built to include two-way interactions between the fixed effects (e.g., sex × size relative to intruder; intruder orange × breeding status), the covariates (water depth and time of day), and interactions between the fixed effects and the covariates. Models representing all possible combinations of the fixed effects (reference), covariates, and two-way interactions were subjected to the model selection process performed by the ALLMIXED2 macro (Fernandez 2007). Best-fit models were identified as those with ΔAICc < 2.0; top models receive a ΔAICc = 0 and additional models within two ΔAICc units of the top model are considered to be candidate best-fit models. Thus, the results from all models with ΔAICc < 2.0 are reported.

The first analysis was performed on behavior data from Site 1 to determine which treatment factors (intruder size and patch size) and breeding pair characteristics (sex, breeding status, size relative to model, depth, and time) predicted aggression toward the model. The color status of the focal female was not included in the models because the frequency of females possessing orange color was low (12 of 171 females at Site 1 [all treatments]) and because the vast majority (83 %) of females possessing orange also were nesting without hatched offspring. Aggression data were natural-log transformed to achieve normality. Because a within-subjects design was employed to account for non-independence of behavior in the male and female of a given pair, the most appropriate covariance structure (i.e., behavioral covariance between the male and female) was determined using ALLMIXED2. The unstructured and Huynh-Feldt covariance structures were the best choices for a model of the Site 1 data (AICc = 0.00 for both); an unstructured structure was chosen because it makes no assumptions about covariance matrix structure.

Next, data from all three sites were analyzed using the ALLMIXED2 macro to evaluate differences among sites in the aggressive response of the breeding pair toward the intruder. The aggression data were again natural-log transformed to achieve normality. Statistical models were constructed, and model-fitting was conducted, in the same fashion as those for the analysis of Site 1 data except that site was included as a fixed effect and intruder size was eliminated because only medium-sized intruders were used. As with the Site 1 analysis, the color status of the focal female was not included in the models because the frequency of females possessing orange

color was low at most sites (1 of 57 pairs at Site 1 [medium-sized intruder only]; 15 of 55 at Site 2; but 27 of 50 at Site 3) and because the majority (100 % at Site 1; 87 % at Site 2; and 67 % at Site 3) of orange females also were nesting without hatched offspring. ALLMIXED2 found that unstructured and Huynh-Feldt covariance structures were the best choices for a model of the multi-population data (AICc = 0.00 for both), and an unstructured structure was used as in the Site 1 analysis.

Results

Behavior at site 1

Five statistical models met the threshold criterion (ΔAICc < 2.0) to be considered candidate best-fit models in the Site 1 analysis (Table 1). Breeding status, sex, and size of the focal fish relative to the intruder were significant predictors of aggression exhibited toward the intruder in all statistical models (Table 1). Focal fish defending hatched offspring (wrigglers/fry: $N = 134$ pairs) were significantly more aggressive toward the intruder than focal fish without hatched offspring (no eggs/only eggs: $N = 36$ pairs) (mean ± SEM aggressive acts per 5 min observation period; wrigglers/fry: 11.69 ± 0.88; no eggs/only eggs: 4.97 ± 1.07). Males of a pair were significantly more aggressive toward the intruder than females (mean ± SEM; males: 13.66 ± 1.28; females: 6.83 ± 0.53). Overall, individuals responded more aggressively when they were larger than the intruder compared to when they were smaller or equal in size to the intruder (mean ± SEM; larger: 11.28 ± 1.75, smaller: 10.42 ± 0.98, equal: 8.63 ± 1.43).

There was a significant "sex × size relative to intruder" interaction in four of the top statistical models, and linear contrasts were used to resolve differences among levels of this effect; data are reported for top-ranking Model #1 but all candidate best-fit models including this term showed the same results. Males were significantly more aggressive toward the intruder than females when the two sexes were smaller than the intruder ($F_{1,165} = 50.8$, $P < 0.0001$) or the same size as the intruder ($F_{1,165} = 6.9$, $P = 0.0095$), but not when the two sexes were larger than the intruder ($F_{1,165} = 0.5$, $P = 0.47$) (Fig. 2). Females were significantly more aggressive when they were larger than the intruder compared to when they were the same size as the intruder ($F_{1,165} = 5.4$, $P = 0.02$); comparisons between females that were larger vs. smaller than the intruder ($F_{1,165} = 3.2$, $P = 0.08$) and smaller vs. same size as intruder ($F_{1,165} = 1.9$, $P = 0.17$) were not significant (Fig. 2). On the contrary, males were significantly more aggressive when they were smaller than the intruder compared to when they were the same size as the intruder ($F_{1,165} = 9.2$, $P = 0.003$) or larger than the intruder ($F_{1,165} = 6.1$, $P = 0.015$); there was no

Table 1 Best statistical model candidates and selection criteria for Site 1 model presentations and the results of mixed model within-subjects analyses for each of the top models with ΔAICc < 2.0

	Fixed effects and covariates	AICc	ΔAICc	AICc weights	AICc weight ratio
Model #1	Reference + (sex × size relative to intruder)	2669.14	0.000	0.303	1.000
Model #2	Reference + (sex × size relative to intruder) + time of day	2669.28	0.140	0.283	0.932
Model #3	Reference + (sex × size relative to intruder) + (intruder orange × breeding status) + time of day	2670.50	1.358	0.154	0.507
Model #4	Reference + time of day	2670.65	1.515	0.142	0.469
Model #5	Reference + (sex × size relative to intruder) + (intruder orange × breeding status)	2671.03	1.894	0.118	0.388

Model	F value$_{df}$	P value	Model	F value$_{df}$	P value
Reference			Model #1		
Intruder size	$0.02_{2,165}$	0.980	Intruder size	$0.06_{2,165}$	0.946
Intruder orange	$1.76_{2,165}$	0.175	Intruder orange	$2.29_{2,165}$	0.104
Sex	$49.2_{1,165}$	*<0.0001*	Sex	$6.15_{1,165}$	*0.014*
Breeding status	$31.3_{1,165}$	*<0.0001*	Breeding status	$31.57_{1,165}$	*<0.0001*
Size relative to intruder	$4.19_{2,165}$	*0.017*	Size relative to intruder	$4.75_{2,165}$	*0.0099*
			Sex × size relative to intruder	$3.61_{2,165}$	*0.029*
Model #2			Model #3		
Intruder size	$0.03_{2,165}$	0.968	Intruder size	$0.02_{2,163}$	0.983
Intruder orange	$2.12_{2,165}$	0.123	Intruder orange	$0.10_{2,163}$	0.905
Sex	$6.49_{1,165}$	*0.012*	Sex	$6.71_{1,163}$	*0.011*
Breeding status	$31.96_{1,165}$	*<0.0001*	Breeding status	$39.23_{1,163}$	*<0.0001*
Size relative to intruder	$4.86_{2,165}$	*0.009*	Size relative to intruder	$4.78_{2,163}$	*0.0096*
Sex × size relative to intruder	$3.50_{2,165}$	*0.033*	Sex × size relative to intruder	$3.08_{2,163}$	*0.049*
Time of day	$1.18_{1,165}$	0.279	Intruder orange × breeding status	$5.22_{2,163}$	*0.006*
			Time of day	$1.95_{1,163}$	0.165
Model #4			Model #5		
Intruder size	$0.00_{2,165}$	0.997	Intruder size	$0.04_{2,163}$	0.963
Intruder orange	$1.62_{2,165}$	0.201	Intruder orange	$0.07_{2,163}$	0.931
Sex	$49.23_{1,165}$	*<0.0001*	Sex	$6.28_{1,163}$	*0.013*
Breeding status	$31.72_{1,165}$	*<0.0001*	Breeding status	$38.1_{1,163}$	*<0.0001*
Size relative to intruder	$4.21_{2,165}$	*0.016*	Size relative to intruder	$4.64_{2,163}$	*0.011*
Time of day	$1.41_{1,165}$	0.237	Sex × size relative to intruder	$3.22_{2,163}$	*0.043*
			Intruder Orange × breeding status	$4.83_{2,163}$	*0.009*

Significant effects ($P < 0.05$) are shown in italics

difference between males that were the same size vs. larger than the intruder ($F_{1,165} = 0.3$, $P = 0.61$) (Fig. 2).

Two of the top-ranking models included a significant 'intruder orange × breeding status' interaction, and linear contrasts were used to resolve differences among levels of this effect; data are reported for top-ranking Model #3 but the two candidate best-fit models containing this interaction showed the same results. Individuals occupying pairs that were defending hatched offspring (wrigglers/fry) were significantly more aggressive toward intruders with orange patches than individuals occupying pairs without hatched offspring (i.e., no eggs/eggs only) (large orange patch: $F_{1,163} = 20.2$, $P < 0.0001$; small orange patch: $F_{1,163} = 21.8$, $P < 0.0001$; Fig. 3). Individual responses to intruders without an orange patch, however, did not depend on breeding status ($F_{1,163} = 1.8$, $P = 0.18$; Fig. 3).
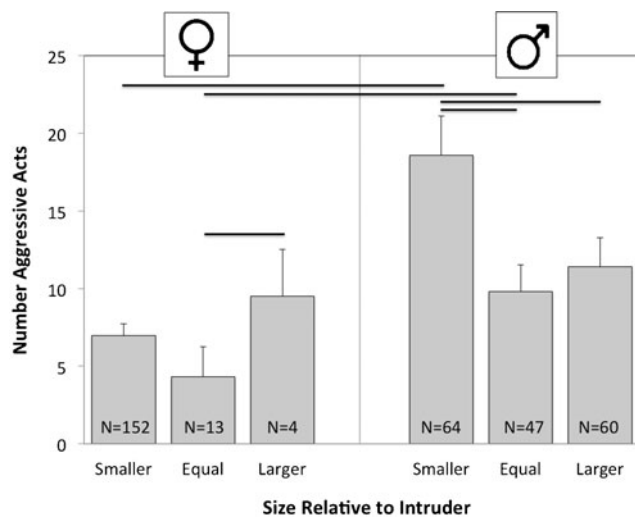
Fig. 2 Variation in aggressive behavior (mean ± SEM, untransformed) exhibited toward the intruder based on sex and individual size relative to model intruder at Site 1. Samples sizes for each category are shown within the histogram bars. The size of two females relative to the intruder could not be determined because the females did not exit the nest; hence, $N = 169$ for females and $N = 171$ for males. Lines above the bars indicate significant differences between categories as determined by linear contrasts

Individuals occupying pairs without hatched offspring did not show differential responses to intruders based on the size of the orange patch (no patch vs. small patch: $F_{1,163} = 2.9$, $P = 0.09$; no patch vs. large patch: $F_{1,163} = 1.54$, $P = 0.22$; small patch vs. large patch: $F_{1,163} = 0.3$, $P = 0.56$; Fig. 3). However, individuals occupying pairs that were defending hatched offspring were significantly more aggressive toward intruders possessing an orange patch compared to intruders
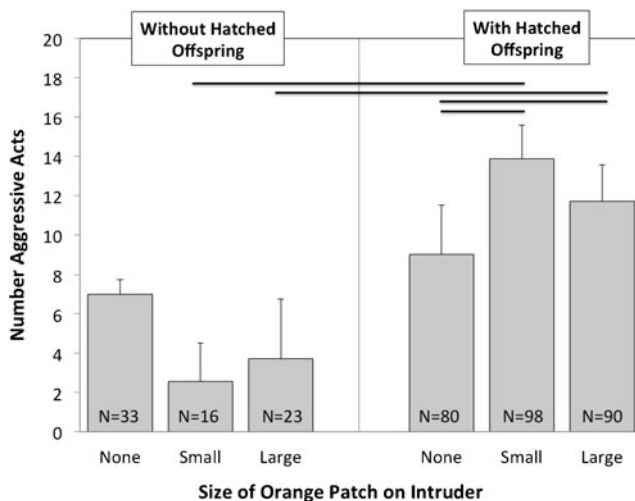

Fig. 3 Variation in aggressive behavior (mean ± SEM, untransformed) exhibited toward the intruder based on breeding status (with or without free-swimming offspring [wrigglers/fry]) and the size of the intruder's orange patch. Samples sizes for each category are shown within the histogram bars. Lines above the bars indicate significant differences between categories as determined by linear contrasts

without the orange patch (no patch vs. small patch: $F_{1,163} = 9.5$, $P = 0.002$; no patch vs. large patch: $F_{1,163} = 7.8$, $P = 0.006$; Fig. 3); the animals responded similarly to intruders with small and large patches ($F_{1,163} = 0.06$, $P = 0.81$; Fig. 3).

Variation in behavior among sites

Two statistical models met the threshold criterion ($\Delta AICc < 2.0$) to be considered candidate best-fit models in the among-site analyses (Table 2). Breeding status and sex were significant predictors of aggression exhibited toward the intruder in all statistical models (Table 2), and the interpretations were the same as the Site 1 analyses – males were significantly more aggressive toward intruders than females and focal fish that were defending hatched offspring (wrigglers/fry) were significantly more aggressive toward the intruders than focal fish without hatched offspring (no eggs/eggs only).

There was a significant "sex × size relative to intruder" interaction in the two non-reference statistical models, and linear contrasts were used to resolve differences among levels of this effect; data are reported for top-ranking Model #1 but the two candidate best-fit models containing this interaction showed the same results. Across all three sites, males were significantly more aggressive toward the intruder than females when the two sexes were smaller than the intruder ($F_{1,156} = 11.3$, $P = 0.001$) but not when the two sexes were the same size as the intruder ($F_{1,156} = 0.1$, $P = 0.72$) or larger than the intruder ($F_{1,156} = 2.2$, $P = 0.14$). Females were significantly more aggressive when they were larger than the intruder compared to when they were smaller than the intruder ($F_{1,156} = 4.6$, $P = 0.03$); comparisons between females that were larger vs. same size as the intruder ($F_{1,156} = 2.2$, $P = 0.14$) and smaller vs. same size as intruder ($F_{1,156} = 1.0$, $P = 0.31$) were not significant. Similar to the Site 1 analysis, males that were smaller than the intruder were significantly more aggressive than males that were the larger than intruder ($F_{1,156} = 3.8$, $P = 0.05$) and tended to be more aggressive than males that were the same size as the intruder ($F_{1,156} = 3.1$, $P = 0.08$); there was no difference between males that were the same size vs. larger than the intruder ($F_{1,156} = 0.03$, $P = 0.86$).

There was some evidence of variation in aggression among populations in all of the statistical models (Table 2). Cichlids from Site 2 were less aggressive (mean ± SEM aggressive acts per 5-min observation period: 5.37 ± 0.65, $N = 109$) than those from Site 1 (10.49 ± 1.49, $N = 114$) and from Site 3 (8.56 ± 1.34, $N = 96$); cichlids from Sites 1 and 3 did not differ in their aggression levels toward the intruder. These results were significant prior to Tukey-Kramer adjustments (Site 1 vs. 2: $t_{156} = 2.0$, $P = 0.047$; Site 3 vs. 2: $t_{156} = 2.13$, $P = 0.035$), after which statistical significance was lost (Site 1 vs. 2: $P_{adjusted} = 0.116$; Site 3 vs. 2: $P_{adjusted} = 0.087$).

Table 2 Best model candidates and selection criteria for model presentations at all three sites and the results of mixed model within-subjects analyses for each of the top models with $\Delta AICc < 2.0$

	Fixed effects and covariates		AICc	ΔAICc	AICc weights	AICc weight ratio
Model #1	Reference + (sex × size relative to model)		2451.15	0.000	0.595	1.000
Model #2	Reference + (sex × size relative to model) + time of day		2451.92	0.772	0.405	0.680

Model	F value$_{df}$	P value	Model		F value$_{df}$	P value
Reference			Model #1			
Population	$3.00_{2,156}$	0.053	Population		$2.50_{2,156}$	0.085
Intruder orange	$2.04_{2,156}$	0.134	Intruder orange		$2.08_{2,156}$	0.129
Sex	$3.90_{1,156}$	*0.050*	Sex		$0.24_{1,156}$	0.625
Breeding status	$45.7_{1,156}$	*<0.0001*	Breeding status		$45.3_{1,156}$	*<0.0001*
Size relative to model	$4.19_{2,156}$	0.894	Size relative to model		$0.88_{2,156}$	0.416
			Sex × size relative to model		$4.91_{2,156}$	*0.009*
Model #2						
Population	$2.07_{2,156}$	0.129				
Intruder orange	$2.08_{2,156}$	0.129				
Sex	$0.19_{1,156}$	0.665				
Breeding status	$44.0_{1,156}$	*<0.0001*				
Size relative to model	$0.92_{2,156}$	0.402				
Sex × size relative to model	$4.88_{2,156}$	*0.009*				
Time of day	$0.30_{1,156}$	0.587				

Significant effects ($P < 0.05$) are shown in italics

Discussion

The results from our primary study site (Site 1) partially support the prediction that aggression exhibited by breeding convict cichlids would increase in response to intruders with larger orange patch sizes. Breeding pairs with hatched offspring exhibited differential aggression based on the presence or absence of orange color, and intruders with orange received significantly more aggression than those lacking orange (Fig. 2). Previous observations of breeding pairs in Lake Xiloa (Alonzo et al. 2001; Anderson et al. 2015) and a Costa Rican population (Snekser et al. 2011) also demonstrated that convict cichlids exhibit increased territorial aggression as the brood ages. Thus, the egg hatching process may stimulate differentially greater aggression toward colorful females in addition to a general increase in aggressive behavior. Female cichlids did not display strong differences in aggression based on their size relative to the intruder, whereas male aggression was significantly higher when the males were smaller than the intruder than when they were of equal size to or larger than the intruder (Fig. 2). Higher male aggression may be a consequence of parental role specialization in which the male prioritizes repelling threats (Itzkowitz et al. 2001). Elevated male aggression in the presence of a relatively larger intruder may

be a mechanism by which the breeding male attempts to compensate for the size disparity (but see Itzkowitz et al. 2005).

Differential aggression based on the presence or absence of a color signal has been demonstrated in numerous species. For example, in cichlid species that exhibit color polymorphisms, males will preferentially attack male conspecifics of the same color morph (e.g., Dijkstra et al. 2009; Tyers and Turner 2013). In a study of *Amphilophus sagittae*, another cichlid species found in Lake Xiloa, territorial males and females both were found to preferentially attack individuals of the same color morph in addition to exhibiting assortative mating based on color morph (Lehtonen 2014). Red-headed females were found to dominate females of other color morphs in Gouldian finches (Pryke 2007), and a population-dependent association between aggression in dyadic contests and throat coloration was demonstrated in mesquite lizards (Bastiaans et al. 2013). Thus, convict cichlids may employ a similarly coarse assessment of female conspecific coloration and increase their aggression toward any individual possessing orange color.

A potential explanation for the observed differential aggression at Site 1 concerns the relationship between female breeding status and color. A previous field study of convict

cichlid coloration indicated that paired breeding females are less likely to express orange color than unpaired non-breeding females (Anderson et al. 2015). In addition, there is notable breeding status-based segregation of convict cichlids at Site 1, with most unpaired non-breeding fish located in shallow weed beds and breeding pairs primarily located at greater depths in a rocky area that is preferable for nesting. Increased resource overlap is generally associated with increased aggression, particularly toward conspecifics (Peiman and Robinson 2010), so the presence of orange color on an intruding female may indicate to a breeding pair that the intruder is attempting to establish a nesting territory or obtain a mate, two potentially major threats to nest and pair bond maintenance, and thus should be repelled aggressively. It might also be the case that female orange coloration indicates a relatively high-quality individual that is capable of superior nest defense (Svensson and Wong 2011), as the female orange patch may serve as a reservoir of excess dietary carotenoids that currently are not needed to support immunity (e.g., Tachibana et al. 1997; Amar et al. 2012) and oxidant/antioxidant balance (e.g., Pereira et al. 2011; Elseady and Zahran 2013). In the context of a territorial intrusion, if orange color indicates an intruder of superior quality, it may be more difficult for a breeding pair to repel an orange female from the nest area, so increased territorial aggression might be a mechanism through which the pair would more successfully deter a threat to their pair bond or nest maintenance.

Nest defense at Site 1 can be very costly because multiple cichlid species including convict cichlids nest and defend their broods from one another in that area; in response, selection should favor strategies that minimize these costs. Perhaps it is favorable to treat nearby conspecific or heterospecific breeding pairs as "dear enemies" (Leiser and Itzkowitz 1999) but to pay the energetic cost to repel unpaired non-breeding females that pose the greatest threat to the nest or pair bond. Non-breeding females also may present an opportunity for polygyny (Keenleyside 1985), which could reduce a male's nest defense efforts, prompting increased aggression by the breeding female toward intruders possessing orange coloration as a preventative measure. However, males also increased aggression toward colorful intruders, possibly in an attempt to preserve the pair bond with his current mate by deterring prospective mates; female convict cichlids are attentive to male behavior in other contexts and will abandon the pair bond under certain circumstances (van Breukelen and Draud 2005).

The hypothesis that the three convict cichlid populations would differ significantly in their aggressive response to a model intruder was not well supported by our results. Habitat discontinuity has not been studied for the entirety of Lake Xiloa, so there may be sufficient gene flow to impede

behavioral differentiation among populations. Alternatively, the environmental characteristics of the three study sites may not differ enough to drive strong selection for divergent behavioral responses to orange color. Mechanisms driving population differences in breeding pair aggression could include environmental differences (e.g., light penetration and food availability), variation in color perception, and variation in signal value of the orange coloration. The significant population differences prior to Tukey-Kramer adjustment and the significant population effect in the reference model indicate that further study of these convict cichlid populations is necessary to establish the presence or absence of such differences. In particular, it would be interesting to determine whether the differences, if they persist with larger samples sizes, might be attributed to behaviorally plastic responses to habitat variation or a combination of restricted gene flow and variable selection pressures among sites.

An important limitation to this study is that one representative model was generated for each of the nine treatments. This was done to standardize the stimulus to which the focal breeding pairs in each treatment were exposed (e.g., Locatello et al. 2015) and because there was restricted availability of the 3D models. However, this approach also presents an issue with pseudoreplication (Kroodsma et al. 2001) because there were not replicate models in each treatment, leading to the possibility that subtle differences in model fabrication could have influenced our interpretation of among-treatment differences. Although various perspectives have been forwarded regarding limitations to ideal experimental designs in the field and the issue of pseudoreplication (e.g., Wiley 2003; Davies and Gray 2015), the experimental approach used in this study would have been sounder had replicate models been used for each treatment (e.g., Lehtonen 2014). Because the models that were employed had nearly continuous variation for the two traits of interest (size and orange), the interpretations forwarded above would likely be robust to slight among-treatment variation in model design and the lack of within-treatment replication. This, however, should be confirmed with future studies that prioritize avoiding pseudoreplication vs. standardizing the model type.

The findings of this study indicate that female orange coloration elicits significantly greater amounts of aggression from breeding conspecifics, suggesting that orange females are perceived as a greater threat to a nest, brood, or pair bond than females lacking orange. In addition, breeding males appear to alter their aggression based on their size relative to an intruder, but females demonstrate no such sensitivity to intruder size. These results contribute to an understanding of the breeding dynamics of convict cichlids and the behavioral implications of a reversed sexual dimorphic color signal, but further laboratory and field work is necessary to determine precisely what information is conveyed via carotenoid-based coloration in female convict cichlids.

References

Alonzo JJ, McKaye KR, van den Berghe EP (2001) Parental defense of young by the convict cichlid, *Archocentrus nigrofasciatus*, in Lake Xiloá, Nicaragua. J Aquaricult Aquat Sci 9:208–228

Amar EC, Viswanath K, Akutsu T, Satoh S, Watanabe T (2012) Resistance of rainbow trout *Oncorhynchus mykiss* to infectious hematopoietic necrosis virus (IHNV) experimental infection following ingestion of natural and synthetic carotenoids. Aquaculture 330–333:148–155

Anderson C, Wong SC, Fuller A, Zigelsky K, Earley RL (2015) Carotenoid-based coloration is associated with predation risk, competition, and breeding status in female convict cichlid fish (*Amatitlania siquia*) under field conditions. Environ Biol Fish 98:1005–1013

Baldauf SA, Kullmann H, Bakker TCM, Thünken T (2011) Female nuptial coloration and its adaptive significance in a mutual mate choice system. Behav Ecol 22:478–485

Bastiaans E, Morinaga G, Gaytán JGC, Marshall JC, Sinervo B (2013) Male aggression varies with throat color in 2 distinct populations of the mesquite lizard. Behav Ecol 24:968–981

Beeching SC, Gross SH, Bretz HS, Hariatis E (1998) Sexual dichromatism in convict cichlids: the ethological significance of female ventral coloration. Anim Behav 56:1021–1026

Briffa M, Elwood RW (2010) Repeated measures analysis of contests and other dyadic interactions: problems of semantics, not statistical validity. Anim Behav 80:583–588

Brown AC, Leonard HM, McGraw KJ, Clotfelter ED (2014) Maternal effects of carotenoid supplementation in an ornamented cichlid fish. Funct Ecol 28:612–620

Brown AC, McGraw KJ, Clotfelter ED (2013) Dietary carotenoids increase yellow nonpigment coloration of female convict cichlids (*Amantitlania nigrofasciata*). Physiol Biochem Zool 86:312–322

Davies GM, Gray A (2015) Don't let spurious accusations of pseudoreplication limit our ability to learn from natural experiments (and other messy kinds of ecological monitoring). Ecol Evol 5:5295–5304

Dijkstra PD, Hemelrijk C, Seehausen O, Groothuis TGG (2009) Color polymorphism and intrasexual competition in assemblages of cichlid fish. Behav Ecol 20:138–144

Elseady Y, Zahran E (2013) Ameliorating effect of β-carotene on antioxidant response and hematological parameters of mercuric chloride toxicity in Nile tilapia (*Oreochromis niloticus*). Fish Physiol Biochem 39:1031–1041

Emlen DJ, Warren IA, Johns A, Dworkin I, Lavine LC (2012) A mechanism of extreme growth and reliable signaling in sexually selected ornaments and weapons. Science 337:860–864

Fernandez GC (2007) Model selection in PROC MIXED—a user-friendly SAS® macro application. SAS Global Forum 2007 Orlando, FL

Fuller R (2002) Lighting environment predicts the relative abundance of male colour morphs in bluefin killifish (*Lucania goodei*) populations. Proc R Soc Lond B 269:1457–1465

Güroy B, Şahin İ, Mantoğlu S, Kayalı S (2012) *Spirulina* as a natural carotenoid source on growth, pigmentation and reproductive performance of yellow tail cichlid *Pseudotropheus acei*. Aquacult Int 20:869–878

Hamilton WD, Zuk M (1982) Heritable true fitness and bright birds: a role for parasites? Science 218:384–387

Itzkowitz M, Santangelo N, Cleveland A, Bockelman A, Richter M (2005) Is the selection of sex-typical parental roles based on an assessment process? A test in the monogamous convict cichlid fish. Anim Behav 69:95–105

Itzkowitz M, Santangelo N, Richter M (2001) Parental division of labour and the shift from minimal to maximal role specializations: an examination using a biparental fish. Anim Behav 61:1237–1245

Keenleyside MHA (1985) Bigamy and mate choice in the biparental cichlid fish *Cichlasoma nigrofasciatum*. Behav Ecol Sociobiol 17:285–290

Kemp DJ, Herberstein ME, Fleishman LJ, Endler JA, Bennett ATD, Dyer AG, Hart NS, Marshall J, Whiting MJ (2015) An integrative framework for the appraisal of coloration in nature. Am Nat 185:705–724

Kroodsma DE, Byers BE, Goodale E, Johnson S, Liu WC (2001) Pseudoreplication in playback experiments, revisited a decade later. Anim Behav 61:1029–1033

Lehtonen TK (2014) Colour biases in territorial aggression in a Neotropical cichlid fish. Oecologia 175:85–93

Leiser JK, Itzkowitz M (1999) The benefits of dear enemy recognition in three-contender convict cichlid (*Cichlasoma nigrofasciatum*) contests. Behaviour 136:983–1003

Levi L, Ziv V, Admon A, Levavi-Sivan B, Lubzens E (2011) Insight into molecular pathways of retinal metabolism, associated with vitellogenesis in zebrafish. Am J Physiol Endocrinol Metab 302:E626–E644

Locatello L, Poli F, Rasotto MB (2015) Context-dependent evaluation of prospective mates in a fish. Behav Ecol Sociobiol 69:1119–1126

McKaye KR (1977) Competition for breeding sites between the cichlid fishes of Lake Jiloá, Nicaragua. Ecology 58:291–302

Mougeot F, Redpath SM, Piertney SB (2006) Elevated spring testosterone increases parasite intensity in male red grouse. Behav Ecol 17:117–125

Olson VA, Owens IPF (1998) Costly sexual signals: are carotenoids rare, risky or required? Trends Ecol Evol 13:510–514

Pan CH, Chien YH, Wang YJ (2011) Antioxidant defense to ammonia stress of characins (*Hyphessobrycon eques* Steindachner) fed diets supplemented with carotenoids. Aquac Nutr 17:258–266

Peiman KS, Robinson BW (2010) Ecology and evolution of resource-related heterospecific aggression. Q Rev Biol 8:133–159

Pereira AA, van Hattum B, Brouwer A (2011) Hepatic retinoid levels in seven fish species (teleosts) from a tropical coastal lagoon receiving effluents from iron-ore mining and processing. Environ Toxicol Chem 31:408–416

Pryke SR (2007) Fiery red heads: female dominance among head color morphs in the Gouldian finch. Behav Ecol 18:621–627

Sefc KM, Brown AC, Clotfelter ED (2014) Carotenoid-based coloration in cichlid fishes. Comp Biochem Physiol A 173:42–51

Sheikhzadeh N (2013) Influence of Dietary Vegetable Crops on Rainbow Trout (*Oncorhynchus mykiss*) Immune System and Growth Performance. Acta Sci Vet 41:1109

Snekser JL, Santangelo N, Nyby J, Itzkowitz M (2011) Sex differences in biparental care as offspring develop: a field study of convict cichlids (*Amatitlania siquia*). Environ Biol Fish 91:15–25

Sullivan M, Brown AC, Clotfelter ED (2014) Dietary carotenoids do not improve motility or antioxidant capacity in cichlid fish sperm. Fish Physiol Biochem 40:1399–1405

Svensson PA, Wong BBM (2011) Carotenoid-based signals in behavioural ecology: a review. Behaviour 148:131–18

Tachibana K, Yagi M, Hara K, Mishima T, Tsuchimoto M (1997) Effects of feeding of β-carotene-supplemented rotifers on survival and lymphocyte proliferation reaction of fish larvae (Japanese parrotfish (*Oplegnathus fasciatus*) and Spotted parrotfish (*Oplegnathus punctatus*)): preliminary trials. Hydrobiologia 358:313–316

Teimouri M, Amirkolaie AK, Yeganeh S (2013) The effects of *Spirulina platensis* meal as a feed supplement on growth performance and pigmentation of rainbow trout (*Oncorhynchus mykiss*). Aquaculture 396–399:14–19

Tobler M (2007) Reversed sexual dimorphism and female courtship in the Topaz cichlid, *Archocentrus myrnae* (Cichlidae, Teleostei), from Costa Rica. Southwest Nat 52:371–377

Tyers AM, Turner GF (2013) Signal and preference divergence among populations of the non-endemic basal Lake Malawi cichlid fish *Astatotilapia calliptera* (Perciformes: Cichlidae). Biol J Linn Soc 110:180–188

van Breukelen NA, Draud M (2005) The roles of male size and female eavesdropping in divorce in the monogamous convict cichlid (*Archocentrus nigrofasciatus*, Cichlidae). Behaviour 142:1029–1041

Wang Y, Chien Y, Pan C (2006) Effects of dietary supplementation of carotenoids on survival, growth, pigmentation, and antioxidant capacity of characins, *Hyphessobrycon callistus*. Aquaculture 261:641–648

Wiley RH (2003) Is there an ideal behavioural experiment? Anim Behav 66:585–588

Wisenden BD (1994a) Factors affecting mate desertion by males in free-ranging convict cichlids (*Cichlasoma nigrofasciatum*). Behav Ecol 5:439–447

Wisenden BD (1994b) Reproductive behaviour of free-ranging convict cichlids, *Cichlasoma nigrofasciatum*. Environ Biol Fish 43:121–134

Zoppoth P, Koblmüller S, Sefc KM (2013) Male courtship preferences demonstrate discrimination against allopatric colour morphs in a cichlid fish. J Evol Biol 26:577–586