



Original Article

Threat sensitive adjustment of aggression by males and females in a biparental cichlid

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Defending offspring provides fitness benefits to parents, but is costly. To moderate costs, parents should adjust aggressive responses to the threat posed by different species entering their territory. However, few studies have experimentally tested behavioral adjustments in response to the threat posed by different types of intruders, particularly in the field, and in environments with an array of heterospecific intruders. Here, using a biparental cichlid, the poor man's tropheus (*Hypsophrys nematopus*), we investigated whether males and females in the wild invest equally into offspring defense and what impact the absence of a partner might have on the quality of offspring defense provided by a solitary parent. In a separate experiment, we assessed responses of breeding pairs to 3 common heterospecific intruders that pose different levels of threat to the breeding pair and their brood. We found that both paired and unpaired females invested significantly more into territorial aggression than males. However, unpaired females were unable to fully compensate for the absence of their partner, with intruders left to venture closer to their offspring. Furthermore, we show that breeding pairs can readily discriminate between heterospecific intruders, with pairs responding quicker and more aggressively towards species that posed a greater potential threat. Our results demonstrate that biparental species can have extensive behavioral flexibility in their aggressive responses towards intruders, even in environments with a high frequency of territory incursion. The quality of territorial defense can nevertheless be compromised if one parent is left to defend the brood alone.

Key words: aggression, cichlid fish, crater lake, intruder recognition, mate desertion, parental care, sexual conflict, species interaction.

INTRODUCTION

In many taxa, defending offspring from predators plays a key role in reproductive success (Ridley 1978; Perrone and Zaret 1979; Clutton-Brock 1991; Alonso-Alvarez 2012), with broods that are more vigorously defended often experiencing reduced predation (Greig-Smith 1980; Tryjanowski and Goławski 2004) and higher rates of offspring recruitment (Kontiainen et al. 2009). For example, when White's skinks (*Egernia whitii*) aggressively defend their territories from intruders, offspring survival is higher (Sinn et al. 2008). Likewise, more aggressive red-backed shrikes (*Lanius collurio*) have greater reproductive success than less aggressive individuals (Tryjanowski and Goławski 2004), highlighting the important role that territorial aggression can have in offspring survival and parental fitness. However, territorial aggression can also incur costs to parents, such as energy loss and an increased risk of injury or mortality (Marler and Moore 1988; Haller 1996; Lappin and Husak

2005; Requena et al. 2012). An important strategy for reducing such costs is to readily identify the risks, and react accordingly to the danger posed by different types of intruders (Nakano and Nagoshi 1990; Ochi and Yanagisawa 1998; Swaisgood et al. 2004; Grether 2011; Lehtonen et al. 2015). For instance, female ground squirrels (*Spermophilus beecheyi*) assess the body temperature and size of Pacific rattlesnakes (*Crotalus viridis oreganus*) to evaluate the danger individual snakes pose to their offspring (Swaisgood et al. 2004). Nonetheless, despite such findings, few studies have experimentally investigated whether individuals can readily recognize and discriminate between an array of heterospecific intruders (for an exception, see Matsumoto and Kohda 2004; Fischer et al. 2014). This is surprising given that animals within many ecological communities can encounter, and potentially interact with, a myriad of different species that vary in the level of threat they pose to offspring.

In some species, both sexes play a role in parental duties (i.e. biparental care; Clutton-Brock 1991). However, the sexes may have evolved to take care of different parental tasks (Barta et al. 2014), with the unequal division of labor (Trivers 1972; Wynne-Edwards 1995) being driven, for example, by differences in potential reproductive rates (Baylis 1981; Reynolds 1996, also see Kokko and

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Jennions 2008), or a lack of assurance over paternity (Trivers 1972; Keenleyside 1991; Neff 2003). Studies investigating the relative parental investment of the sexes in biparental species have tended to focus on offspring provisioning rather than offspring defense (Johnstone and Hinde 2006; Hinde and Kilner 2007; Harrison et al. 2009). For instance, Sanz et al. (2000) demonstrated that male, but not female, great tits (*Parus major*) reduce their offspring provisioning rate when their wings have been experimentally clipped to make flying more difficult. In great tits, males and females also differed in terms of how much they compensated their feeding rate to that of their handicapped partner (Sanz et al. 2000). Yet, only a few studies have explicitly investigated the interplay between the sexes in aggression towards intruders, even though this can be an important component of parental care (Harrison et al. 2009; Trnka and Grim 2012).

In biparental species, it is not uncommon for one partner to desert its mate and offspring (Keenleyside 1983; Keenleyside 1991; Székely and Cuthill 1999; Amat et al. 2000). Of the 2 sexes, males are generally more likely to abandon their mates than females (McNamara et al. 2002), with the latter often continuing alone to provide care for the offspring (Trivers 1972; Keenleyside 1991; McNamara et al. 2002; Lehtonen et al. 2011a). Caring for offspring, however, can present a cost for the abandoned parent, as shown in burying beetles (*Nicrophorus pustulatus*), in which single parents suffer more injuries when protecting offspring without help from their mate (Trumbo 2007). Nevertheless, it remains unclear, especially in nonavian species, to what extent abandoned females attempt to compensate for the loss of their partner and how successful they might be at doing so, particularly under ecologically relevant (i.e. field-based) conditions (Marques 2004; Houston et al. 2005; Harrison et al. 2009; but see Lehtonen et al. 2011b).

Previous studies that have investigated compensatory behaviors by solitary parents have typically focused on quantitative changes in parental care, without accounting for the quality of care provided (Harrison et al. 2009). For example, although many studies (mostly in birds) have measured changes in provisioning rates, the quality of the food brought to the offspring has rarely been considered (Harrison et al. 2009). Furthermore, studies that have focused on taxa whose primary form of parental care is brood defense have generally limited their investigations to quantitative changes in defense behaviors, such as the total number of aggressive behaviors directed towards a predator, rather than investigating qualitative aspects of aggression, such as the type or duration of aggressive responses (Lehtonen et al. 2011b).

Here, we conducted a field-based study to investigate patterns of sex-specific investment in aggressive responses toward intruders by using a territorial and biparental cichlid fish, the poor man's tropheus (*Hypsophrys nematopus*), as our model. We began by firstly observing the behaviors of breeding pairs in their natural habitat to assess sex differences in their ability to adjust aggressive responses toward intruders. This also allowed us to compare the responses of breeding pairs with naturally occurring solitary parents (i.e. individuals who are looking after the brood on their own) to determine whether the latter can compensate for the loss of their mate, with regard to the quantity and quality of the care they provide. In a separate field experiment, we then assessed whether breeding pairs can discriminate between different, commonly encountered species of territorial intruders. We were particularly interested in testing whether pairs responded more quickly to the presence of species that pose a higher level of threat to their offspring or territory, as

well as assessing their subsequent rate of aggression towards the different levels of threat.

MATERIALS AND METHODS

Study species and site

The poor man's tropheus is a small monogamous substratum-spawning cichlid that is native to Central America, including Lake Xiloá, Nicaragua (McKaye 1977, 1986; McKaye et al. 2010). We conducted this study in Lake Xiloá during the first 3 weeks of January 2014. The poor man's tropheus is an ideal candidate for in-situ behavioral observations and experimental manipulations because of its abundance in this clear-watered volcanic Crater Lake, its prolonged breeding season, and stationary breeding territories that are typically located in rocky habitat (McKaye 1986). Reproductively active poor man's tropheus are recognizable by their distinct breeding coloration (vertical white stripe on dark body, see Figure 1) and their territorial behavior, making them distinct from non-breeding individuals, which are lighter in body color and do not maintain stable territories (McKaye et al. 2010; personal observations). Poor man's tropheus fry rely on their parents to diligently protect them from brood predators, until the juveniles become independent several weeks after hatching (McKaye 1977, 1986). Nevertheless, the rates of brood failure are high due to intense predation pressure, with only a small percentage of young surviving to independence and even fewer eventually reaching sexual maturity (McKaye et al. 2010).

Territory defense in breeding pairs and unpaired females

We observed poor man's tropheus pairs to investigate how parental investment, in regard to aggressive response to territory intruders, is shared between the sexes in breeding pairs. Similarly, we observed solitary parents, to determine whether they can compensate for the loss of their mate, both in terms of the quantity and quality of care they provide. To do this, we directly observed poor man's tropheus territories that were occupied by either a breeding pair ($n = 36$) or a solitary parent ($n = 8$). In all cases, the latter was an unpaired female, and we never encountered broods that were guarded by a



Figure 1
Hypsophrys nematopus breeding pair with fry in Lake Xiloá. The larger fish on the left is the male.

solitary male. We assume that the unpaired females that continued to raise the brood alone had either been abandoned by their partners or their partner had died (e.g. due to infection or predation). To control for any sex differences in behavior that may occur at different stages of the brood cycle (see Nakano and Nagoshi 1990; Sowersby et al. 2017), we standardized our observations of poor man's tropheus, so that all broods consisted of free-swimming fry. Territories were observed by standing at a distant vantage point or, if required, with the aid of a mask and snorkel. After a 5-min acclimation period, we observed and recorded, for 10 min, aggressive behaviors of a pair or unpaired female toward naturally occurring territory intruders. Firstly, we counted the total number of aggressive responses during the observation period (for $n = 80$ territory owners comprising 36 paired males, 36 paired females, and 8 unpaired females). Secondly, to gain a qualitative measure of each aggressive response, we estimated the reaction distance, i.e. the distance between the intruder and the center of the brood when a parent initiated an aggressive response ($n = 70$ territory owners, as 10 individuals did not exhibit any aggressive responses). Lastly, we classified each individual aggressive response according to one of 2 categories: "attacks" (rapid movement towards the intruder until the intruder fled) and "chase" (an attack that was continued even after the intruder had started to flee; *sensu* Lehtonen et al. 2010). If a territory owner chased any of the intruders ($n = 49$ territory owners), we measured the chase distance, i.e. the distance between where the chase was initiated by the parent to where the parent stopped chasing the intruder. We estimated distances with the aid of a measuring tape (50 cm) that was placed on the substrate outside of the breeding territory before the 5-min acclimation period. All territory intruders were identified to species level or, if this was not possible, the lowest possible taxonomic level (i.e. family or genus). In addition, after each observation period, we estimated the total lengths of the parental fish (to the nearest 1 mm with the measuring tape as a scale), the number of fry they were guarding, and the median total length of the fry (as per Lehtonen et al. 2010, 2011b). We then numbered a nearby rock with a permanent marker to prevent assessing a territory (pair or unpaired female) more than once.

We used R 3.2.2 software (R Development Core Team) for the statistical analyses. To assess the frequency of aggressive responses (i.e. their count per observation period), we ran a generalized mixed model ("glmmADMB" package in R) with a negative binomial error distribution and "log" link function (as appropriate for overdispersed count data). For the remaining 2 response variables, i.e. the distance between the intruder and the center of the brood when a parent initiated an aggressive response ("reaction distance," averaged for each territory owner), and the distance the parent chased the intruder ("chase distance," averaged for each territory owner), we applied linear mixed models ("nlme" package, "lme" function), after log transforming the data, as this improved the normality of model residuals (assessed by scatterplots of residuals and predicted values). In all 3 models, we investigated the effects of the sex/status of the parent (i.e. paired male, paired female, unpaired female), and the number of fry within the territory. Each of the initial, full models also included the interaction between these 2 effects. Moreover, in each model, the effect of parent length (mm) was accounted for by adding it as a covariate, and territory ID was added as a random effect, to account for non-independence of the actions of a male and female defending a territory (in the case of pairs). We note that our estimations of fry length and fry number had a strong negative correlation (Pearson's correlation, $r = -0.34$, $df = 78$, $P = 0.002$), and we therefore included only the latter in

the models. The statistical significance of each of these effects was assessed by stepwise model simplification, with each step being assessed using log-likelihood tests. We used removal criterion of $P > 0.10$. Where applicable, we then performed separate post hoc contrast analyses to directly compare the aggressive behaviors of the different categories of sex/status of the parent (i.e. paired male, paired female or unpaired female).

Experimental exposure to regular territory intruders

In a separate field-based experiment, we tested the aggressive response of poor man's tropheus pairs towards 3 species of fish that regularly encroached on their territories: bigmouth sleepers (*Gobiomorus dormitor*), convict cichlids (*Amatitlania siquia*), and a species of molly (*Poecilia* sp.). Not only were these 3 species found to be common heterospecific intruders in our first field assessment, but they also represented distinct levels of threat to poor man's tropheus offspring and territories. Specifically, the bigmouth sleeper is an ambush predator and a specialized predator of cichlid fry and small juveniles (Alonzo et al. 2001; Bedarf et al. 2001), the convict cichlid is a breeding site competitor and an opportunistic fry predator (authors' personal observations), whereas the molly likely poses little or no risk to poor man's tropheus territories and offspring as it is a nonterritorial shoaling fish that feeds on small invertebrates, plant matter and detritus.

Here, we were interested in testing whether poor man's tropheus breeding pairs discriminate between different territory intruder species by responding quicker to the presence of species that pose a higher level of threat to their offspring or territory, and adjust the rate of their aggressive responses according to the different levels of threat. In addition, we investigated whether the number of fry influences patterns of parental aggression.

For these purposes, we haphazardly collected representatives of our intruder stimulus species from the shallows of Lake Xiloá and presented them to poor man's tropheus breeding pairs, one at a time, in a cylindrical glass container (17-cm high, 7.5 cm circumference; *sensu* van Breukelen 2015). To control for any potential biases in aggression by poor man's tropheus towards different sized intruders, we purposely selected individuals that did not significantly differ in body size (convict cichlid total length [mean \pm SD]: 5.5 cm \pm 0.55, bigmouth sleeper: 5.6 cm \pm 0.64, molly: 5.3 cm \pm 0.50, Anova: $F_{2,71} = 1.62$, $P = 0.20$, $n = 24$ for each intruder species). Each poor man's tropheus pair ($n = 24$) was exposed to all three intruder species and a control (the empty glass container), in a randomized presentation order. Each intruder was placed within the reaction distance of the territory-guarding parents (30 cm from the brood center). Pairs were exposed to each intruder for 2 min. During that time, we first recorded the latency to attack and then the total number of aggressive responses directed towards the intruder by both the male and female in each pair. We note that the 2 variables provide very similar information, as they are highly correlated (regarding aggressive reactions, when present, over the three intruder categories: Spearman rank correlation, $\rho = -0.39$, $n = 55$ observations in which pairs reacted to a stimulus, $P = 0.004$). Therefore, we expect them to reveal the same behavioral patterns (either significant or nonsignificant). Nonetheless, both are provided here to allow direct comparisons with previously published studies on aggressive behaviors (e.g. Nakano and Nagoshi 1990; Lehtonen et al. 2011b). We provided pairs with a resting period of 5 min between subsequent intruder presentations. After a pair

had been presented with all 4 treatments (3 intruders and the control), we estimated the total lengths of the male and female and the median length and total number of fry in the brood. For one pair, we were not able to reliably estimate the number of fry, and therefore only the remaining 23 pairs were used for the analyses that required complete observations. Furthermore, none of the pairs responded to the control stimulus and this stimulus type was therefore excluded in the subsequent analyses.

We analyzed the latency of poor man's trophus pairs to attack the different types of stimulus intruders (bigmouth sleeper, convict cichlid, molly) as a time-to-event analysis (i.e. time passed until the first attack by one of the parents), using a Cox proportional hazards model. This was done to accommodate the cases in which the pair did not perform any attacks within the 2-min observation period. We used a cluster distribution to accommodate the "repeated" design of the data (i.e. each pair being exposed to all stimulus classes). To account for fry number, as well as male and female body size (total length), these variables were added as covariates (i.e. the full model did not include any interactions). We refitted the model, using $P > 0.10$ removal criterion, until a minimal adequate model was obtained.

The frequency of aggressive responses by the focal pair (i.e. count of responses per observation period) was analyzed using a generalized mixed model (GLMM) with a negative binomial error distribution and "log" link function (as appropriate for overdispersed count data), intruder category and fry number as fixed factors, male and female lengths as covariates, and replicate ID as a random effect to account for each pair being exposed to all 3 stimulus classes. Hence, besides the random effect, the full model contained 2 fixed effects, 1 interaction (between the 2 fixed effects), and 2 covariates. The statistical significance of these effects was assessed by stepwise model simplification and assessing each step using log-likelihood tests. We used removal criterion of $P > 0.10$ until a minimal adequate model was obtained. To assess significant multilevel effects in more detail, we then performed a post hoc contrast analysis.

RESULTS

Territory defense in breeding pairs and unpaired females

The simplification process of the model for the number of aggressive behaviors showed that the interaction between parent sex/status and fry number, as well as the effects of fry number and parent length were all nonsignificant ($P > 0.10$; Table 1). In contrast, parent sex/status had a significant effect (Table 1). A post hoc contrast analysis revealed that both paired (response ratio \pm SE = 3.219 ± 0.649 , z -ratio = 5.796, $P < 0.001$) and unpaired (response ratio \pm SE = 4.896 ± 1.920 , z -ratio = 4.050, $P < 0.001$) females exhibited a higher number of aggressive responses toward

Table 1

Results of hierarchical model simplification process of a generalized mixed model assessing the number of aggressive responses towards naturally occurring intruders

Effect	Order	χ^2	df	P
Sex/status: Fry number	1	1.364	2	0.51
Parent length	2	1.358	1	0.24
Fry number	3	2.462	1	0.12
Sex/status	4	30.52	2	<0.001

The table shows the removed effect terms, order of their removal and statistics related to each step.

intruders than did paired males, while there was no significant difference between paired and unpaired females (response ratio \pm SE = 0.6575 ± 0.2089 , z -ratio = -1.320 , $P = 0.38$; Figure 2).

Regarding parents' reaction distance to intruders, we found that the larger the number of juveniles, the longer the reaction distance (Table 2). In particular, the model simplification process revealed that fry number had a significant effect, whereas the interaction term, parent sex/status and parent length (covariate) were nonsignificant (Table 2).

Lastly, while other factors were nonsignificant (Table 2), parent sex/status had a significant effect on the distance parents chased intruders from their brood (Table 2; Figure 3). In particular, a post hoc contrast analysis revealed that paired females chased intruders significantly farther away from their broods in comparison to unpaired females (estimate \pm SE = 0.8901 ± 0.3366 , t -ratio = 2.645, $df = 36$, $P = 0.032$), and similarly, paired males had a marginally nonsignificant tendency to chase intruders farther than unpaired females (estimate \pm SE = -0.9295 ± 0.3642 , t -ratio = -2.552 , $df = 10$, $P = 0.068$) (Figure 3). There was no significant difference in the distance intruders were chased by paired males as compared to paired females (estimate \pm SE = -0.0393 ± 0.2031 , t -ratio = -0.193 , $df = 10$, $P = 0.97$).

Experimental exposure to regular territory intruders

The Cox proportional hazard model showed that the effects of male ($z = 0.346$, $P = 0.73$) and female ($z = 0.013$, $P = 0.99$) body lengths were not significant on the latency to attack intruders. We refitted the model without these two terms and found that fry number had a significant effect ($z = 2.198$, $P = 0.028$): pairs reacted quicker when their brood was larger. In addition, a local test over the different intruder categories suggested a highly significant overall intruder effect ($P < 0.001$). We therefore proceeded with a post hoc contrast analysis, which showed that pairs reacted quicker to both convict cichlids (hazard ratio \pm SE = 4.191 ± 1.478 , z -ratio = 4.063, $P < 0.001$) and bigmouth sleepers (hazard ratio \pm SE = 2.018 ± 0.603 , z -ratio = 2.348, $P = 0.049$) than mollies (Figure 4). Pairs also had a marginally nonsignificant tendency to react quicker to convict cichlids than bigmouth sleepers (hazard ratio \pm SE = 2.077 ± 0.667 , z -ratio = 2.275, $P = 0.059$; Figure 4).

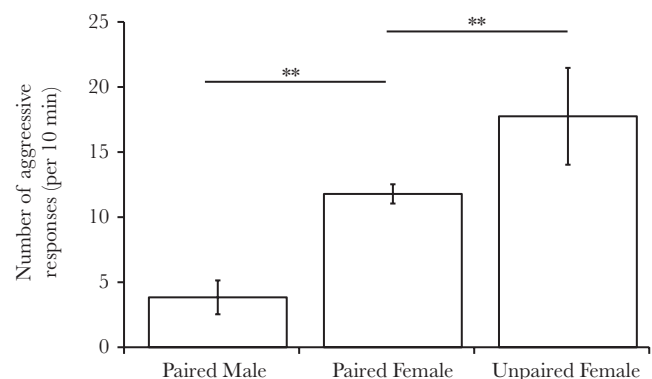


Figure 2

The mean number of aggressive responses (attacks and chases) directed towards territory intruders by paired males ($n = 36$), paired females ($n = 36$) and unpaired females ($n = 8$). Error bars indicate standard errors (SE).

Table 2
Hierarchical model simplification of 2 linear mixed models, showing the response variables, removed effect terms, order of the model simplification steps, and statistics related to each step

Response variable	Reaction distance				Chase distance				
	Effect	Order	χ^2	df	P	Order	χ^2	df	P
Sex/status: Fry number		1	1.112	2	0.57	1	0.587	2	0.75
Parent length		2	0.315	1	0.52	2	0.016	1	0.90
Sex/status		3	3.064	2	0.22	4	7.177	2	0.028
Fry number		4	4.487	1	0.034	3	0.561	1	0.45

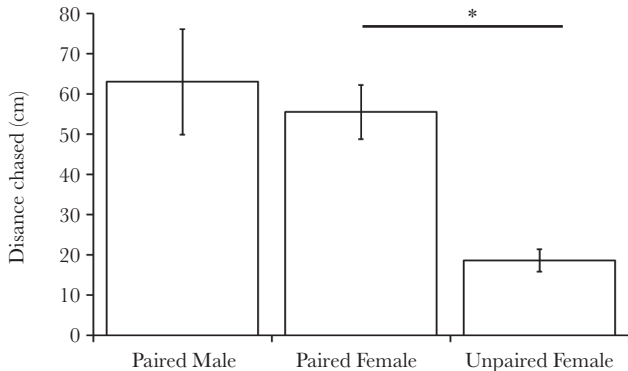


Figure 3

The mean distance paired males ($n = 13$), paired females ($n = 29$) and unpaired females ($n = 7$) chased territory intruders. Error bars indicate SE.

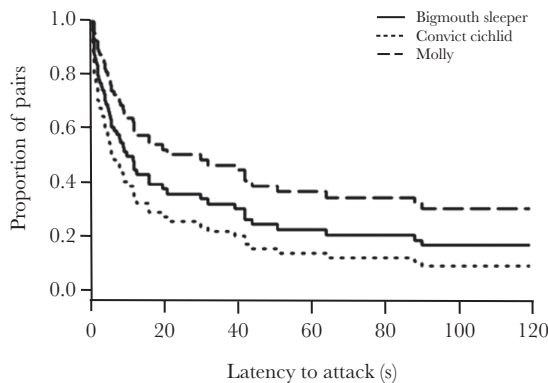


Figure 4

Survival curves showing latency to attack in poor man tropheus pairs ($n = 23$) directed towards three categories of territory intruders. No attacks were directed towards the empty jar control.

Lastly, regarding the total number of aggressive responses by pairs directed towards the intruders presented to them, we found that the effects of the interaction term (intruder \times fry number), male length, and female length were nonsignificant (Table 3). In contrast, pairs responded aggressively more often when they had a larger brood (Table 3), and the intruder category also had a significant effect (Table 3). Specifically, both convict cichlids (Post hoc contrast analysis, response ratio \pm SE = 10.83 ± 3.668 , z -ratio = 7.033, $P < 0.001$) and bigmouth sleepers (response ratio \pm SE = 4.535 ± 2.161 , z -ratio = 3.173, $P = 0.004$) provoked significantly more aggressive responses than mollies (Figure 5). Furthermore, convict cichlids provoked more aggressive responses than bigmouth sleepers (response ratio \pm SE = 2.388 ± 0.735 , z -ratio = 2.828, $P = 0.013$) (Figure 5).

Table 3

Hierarchical model simplification of a generalized mixed model of number of aggressive responses towards experimentally presented intruders

Effect	Order	χ^2	df	p
Intruder category: Fry number	1	2.366	2	0.31
Female length	2	0	1	1.0
Male length	3	0.026	1	0.87
Fry number	4	5.676	1	0.017
Intruder category	4	31.12	2	< 0.001

The table shows the removed effect terms, order of the model simplification steps, and statistics related to each step.

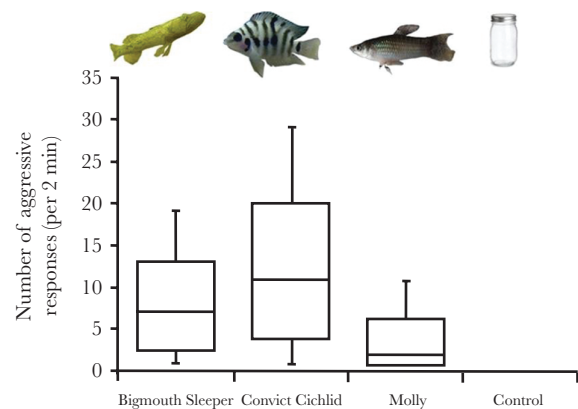


Figure 5

The number of aggressive responses poor man tropheus pairs ($n = 23$) directed towards three categories of territory intruders.

DISCUSSION

In the wild, paired poor man's tropheus females contributed significantly more aggressive responses than their male partners. Furthermore, unpaired females were not fully able to compensate for the absence of their partner. Specifically, the quality of aggressive responses was reduced, as unpaired females did not chase intruders as far away from their broods as did paired females. When we presented man's tropheus breeding pairs with commonly encountered intruder species, we found them to be flexible in their aggressive responses. In particular, pairs appeared to readily discriminate between intruder species, which posed different levels of threat to their offspring or territory. Additionally, offspring number played an important role in shaping patterns of parental aggression, with larger broods being defended more vigorously.

We found that female poor man's tropheus contributed more aggressive behaviors than their male partners during the parental care period. By contrast, previous studies in several other biparental

cichlid species have shown the opposite pattern (e.g. *Lamprologus toae*: Nakano and Nagoshi 1990; *Julidochromis marlieri*: Yamagishi and Kohda 1996; *Amatitlania siquia*: Lehtonen 2008, Lehtonen and Lindström 2008; *Hyposphyris nicaraguensis*: Lehtonen et al. 2015; *Neolamprologus multifasciatus*: Suriyampola and Eason 2015), no significant sex differences in aggression towards territorial intruders (e.g. *Herichthys cyanoguttatus*: Itzkowitz 1984), or flexible relative aggression levels between the sexes, depending on which of the sexes is the smallest in a breeding pair (e.g. *Julidochromis ornatus*: Awata and Kohda 2004). There are several possible reasons for the differences among studies, other than there simply being interspecific differences in the contribution of males and females towards offspring defense. First, evidence shows that sex-specific patterns in territory defense can shift over time (e.g. Sowersby et al. 2017). Hence, differences in the timing of the brood cycle could, at least partly, contribute to sex differences in the patterns observed. Second, males and females could respond differently towards intruders depending on whether they are conspecific or heterospecific. For example, McKaye and Murry (2008) found that male *Amphilophus xiloensis* cichlids were more aggressive towards intruders of their own species, whereas females were more aggressive towards other species. This would be consistent with the findings of the current study in which pairs of poor man's tropheus were largely defending their broods from heterospecific intruders. Third, the size of intruders may also be influential (Lehtonen 2014). Although we deliberately controlled for intruder size in our manipulative experiment, future work may wish to examine whether interspecific differences in the patterns of parental behavior could also be dependent on the size of intruders. Nevertheless, concordant with theory, any increase in female parental investment could enable males to decrease their parental effort (and vice versa), thereby contributing to the patterns seen in our study (*sensu* Hammerstein and Parker 1987).

While female parents in many birds adjust their workload with regard to decreased male participation, they are often unable to completely compensate for the absence of a partner (Marques 2004; Houston et al. 2005; Harrison et al. 2009). In our study, the overall quality of territory defense provided by unpaired females appeared to be lower than that provided by pairs. Specifically, unpaired females did not (or could not) chase territory intruders as long distance as paired individuals. Consequently, territory intruders were probably able to remain closer to the broods of unpaired females, potentially leaving the fry more vulnerable to predation and reducing average offspring survival. Therefore, our findings underscore the importance of considering not only the quantity, but also the quality of parental behaviors, when assessing the impact of mate loss in biparental species (see also Lehtonen et al. 2011b).

We found that breeding poor man's tropheus pairs can readily discriminate between different intruders placed into their territories. Specifically, pairs reacted with a higher rate of aggression to convict cichlids compared to the other intruder species. Such flexibility in aggressive behavior is likely to be an important component of reproductive success, particularly in environments such as Lake Xiloá, where incidences of territory incursion by both innocuous and threatening heterospecifics are high. Regarding the latter, competition for breeding sites and predation pressure on offspring are intense in the lake (McKaye 1977). In this respect, the heightened rate of aggression directed towards convict cichlids may be due to the 2 species having similar breeding patterns, reproductive behaviors, and peak breeding periods (McKaye 1977, 1986; McKaye et al. 2010). Both species are highly

limited by suitable breeding sites (McKaye 1977; Lehtonen and Lindström 2008), which may lead to heightened interspecific competition for breeding territories. Furthermore, convict cichlids are opportunistic predators of fish eggs and fry and therefore poses a direct threat to the offspring of poor man's tropheus (Mackereth and Keenleyside 1993). Interestingly, the rate of aggression was even higher towards convict cichlids than bigmouth sleepers, which are specialized ambush predators of juvenile fish (Alonzo et al. 2001; Bedarf et al. 2001). We also found that the molly elicited the slowest and least aggressive response by poor man's tropheus breeding pairs. This is consistent with a previous observational study, which noted that poeciliid fish species are often tolerated closer to the broods of breeding cichlids than other species (Wisenden et al. 2015). Importantly, by actively discriminating between heterospecifics entering their breeding territories, poor man's tropheus can avoid performing unwarranted, costly aggressive behaviors, while still efficiently protecting their offspring from potential predators.

Finally, we found that offspring number was positively associated with the reaction distance towards naturally occurring intruders, as well as the intensity (both latency and frequency) of aggression in the experimental assay. In environments with high rates of predation and brood failure, parents should adjust their level of aggressive responses according to the value of the brood at stake (Trivers 1972; Clutton-Brock 1991). Moreover, previous studies have predicted that larger broods are more valuable to parents and should be defended more vigorously than smaller broods (Andersson et al. 1980; Greig-Smith 1980; Montgomerie and Weatherhead 1988; Redondo 1989). For example, both the imperial shag bird (*Phalacrocorax atriceps*) and the redwing blackbird (*Agelaius phoeniceus*) invest more into nest defense as brood size increases (Robertson and Biermann 1979; Svagelj et al. 2012). Similarly, female *Aequidens coeruleopunctatus* cichlids adjust their parental behaviors in response to manipulated changes in brood size, i.e. females with experimentally reduced broods are easier to scare away when threatened and stay away for longer than females with experimentally augmented broods (Carlisle 1985). Together with the results of the current study, such findings show that offspring number plays an important role in shaping patterns of parental aggression towards territory intruders and potential brood predators.

In conclusion, we found that, in breeding pairs of poor man's tropheus, females display higher rates of aggression towards intruders than males. However, the quality of aggressive responses provided by unpaired females is lower than that of breeding pairs, as shown by territory intruders being chased significantly shorter distances away from the broods of unpaired females than paired individuals, which conceivably affects offspring survival of the former negatively. Our results therefore demonstrate the importance of assessing not only the quantity, but also the quality of parental behaviors, when evaluating the costs of mate loss in biparental species. Additionally, we show that biparental species, such as poor man's tropheus, can readily discriminate between different heterospecific intruders and react accordingly to the threat they pose to the brood or territory. This ability allows poor man's tropheus to flexibly adjust their behavioral responses depending on the threat posed by different intruders, which is likely to be beneficial for offspring survival and also in moderating costs of aggressive behaviors, particularly in ecological communities with high intruder pressure.

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