



Social Reversal of Sex-Biased Aggression and Dominance in a Biparental Cichlid Fish (*Julidochromis marlieri*)

Kelsey J. Wood*, Victoria H. Zero*, Albyn Jones† & Suzy C. P. Renn*

*Department of Biology, Reed College, Portland, OR, USA

†Department of Mathematics, Reed College, Portland, OR, USA

Correspondence

Suzy Renn, Department of Biology, Reed College, 3203 SE Woodstock Blvd, Portland OR, USA.

E-mail: renns@reed.edu

Received: December 6, 2013

Initial acceptance: January 31, 2014

Final acceptance: February 5, 2014

(W. Koenig)

doi: 10.1111/eth.12227

Keywords: parental care, plasticity, sex-role, sexual dimorphism, social behavior, territorial aggression

Abstract

In biparental species, aggression, dominance, and parental care are typically sexually dimorphic. While behavioral dimorphism is often strongly linked to gonadal sex, the environment—either social or ecological—may also influence sex-biased behavior. In the biparental cichlid fish *Julidochromis marlieri*, the typical social environment for breeding pairs consists of large females paired with smaller males. While both sexes are capable of providing territory defense and parental care, the larger female provides the majority of defense for the pair, while the smaller male remains in the nest guarding their offspring. We examine the contributions of sex and relative mate size to these sex-biased behaviors in monogamous *J. marlieri* pairs. Both female-larger and male-larger pairs were formed in the laboratory and were observed for territorial aggression (against conspecifics and heterospecifics), dominance, and parental care. In female-larger pairs, territorial aggression and intra-pair dominance were female-biased, while in male-larger pairs this bias was reversed. For both pairing types, the presence of an intruder amplified sex differences in territorial aggression, with the larger fish always attacking with greater frequency than its mate. Though less robust, there was evidence for plasticity of sex-bias for some egg care related behaviors in the inverse direction. Our study suggests that relative mate size strongly influences the sex bias of aggression and dominance in *J. marlieri* and that this aspect of the social environment can override the influence of gonadal sex on an individual's behavior. The remarkable plasticity of this species makes *Julidochromis* an exciting model that could be used to address the relationship between proximate and ultimate mechanisms of behavioral plasticity.

Introduction

In many animals, there are striking differences between the sexes in how often certain behaviors are expressed; these behaviors are said to be sexually dimorphic. Sexually dimorphic behaviors can be categorized as sex-specific (expressed by only one sex) or sex-biased (expressed in both sexes but with unequal frequencies). Aggression and parental care are two types of behavior that are often sexually dimorphic (Huntingford & Turner 1987; Clutton-Brock 1991). In biparental animals, both sexes contribute to parental

care, with males and females often emphasizing different tasks. Many biparental birds show some degree of sex bias in parental care, from incubation and feeding to active and passive defense (Owens & Hartley 1998). In biparental cichlids, males often perform territory defense, while females preferentially perform direct offspring care (see review by Keenleyside 1991). For fishes, territorial aggression in the form of egg guarding and breeding site defense can increase offspring survival by reducing egg predation (Dominy 1981), while direct offspring care, such as egg cleaning and fanning, can increase offspring survival

by removing pathogens and promoting normal development (Keenleyside 1991). Although both sexes are capable of either type of behavior, a division of labor allows one sex to specialize in a specific aspect of parental care, similar to task partitioning in eusocial insects and cooperative brooders (Ratnieks & Anderson 1999; Bruintjes & Taborsky 2011). The common pattern of male territory defense may be adaptive for males when they are the larger sex, as is the case for most cichlids (Erlandsson & Ribbink 1997), because larger fish are more effective at securing a nest site and repelling egg- and fry-eating intruders.

Sexually dimorphic behaviors often map to gonadal sex. Certain species, however, show plasticity for sexually dimorphic behaviors. For example, the sexually dimorphic courtship roles of competition and choice are conventionally associated with males and females, respectively (Bateman 1948; Trivers 1972; Emlen & Oring 1977; Clutton-Brock & Vincent 1991), yet some species such as katydids and bushcrickets, in which males provide a nutritious spermatophore to the female during mating, exhibit extraordinary plasticity in these roles. In these species, plastic courtship roles are influenced by nutritional availability, with females competing for males when food is scarce and *vice versa* when food is plentiful (Gwynne & Simmons 1990; Ritchie et al. 1998). Sex ratio and social environment are also known to influence courtship roles in other insects and fish species (Jiggins et al. 2000; Forsgren et al. 2004; Silva et al. 2010).

Plasticity of sexually dimorphic parental behavior has been observed in monogamous Central American cichlids (Itzkowitz et al. 2001; Lehtonen et al. 2011; O'Connell et al. 2012). The convict cichlid (*Amatitlania nigrofasciata*, previously *Archocentrus nigrofasciatus*) shows sex-biased parental behavior: the males, which are larger, provide the majority of territory and brood defense while females perform most egg care behaviors (Itzkowitz et al. 2005; Gagliardi-Seeley & Itzkowitz 2006; Snekser & Itzkowitz 2009). However, in experimentally size-reversed, female-larger pairs, the male decreases his aggression and increases his time with the offspring, while the female does the opposite, suggesting that convict cichlids can modify their behavior depending on the social context of relative mate size (Itzkowitz et al. 2005). While the degree of sex bias in behavior is altered in female-larger pairs, the direction of sex bias is maintained: males still show more aggression and females still spend more time with offspring relative to the other sex. That study suggests that while sex-biased territorial and

parental behaviors are influenced by social context, they are strongly influenced by sex in convict cichlids.

Among the cichlid species from Lake Tanganyika, the genus *Julidochromis* is currently the only known substrate-spawning African cichlid genus to contain some species in which larger females typically pair with smaller males (Erlandsson & Ribbink 1997; Konings 1998), as well as species in which larger males typically pair with smaller females (Erlandsson & Ribbink 1997), which is the conventional pairing type seen in other monogamous cichlid species. In naturally reversed female-larger pairs, females are more aggressive than males (Barlow 2005; Barlow & Lee 2005), are dominant to their mates (Awata & Kohda 2004), and provide the majority of territory defense (Awata & Kohda 2004). Conversely, males have smaller home ranges and spend more time at the nest, both guarding offspring and providing egg care (Yamagishi & Kohda 1996; Sunobe 2000; Awata & Kohda 2004). In some instances, the reversal in relative mate size is plastic and correlated with behavior. One field study reported 20% of the pairs to be male-larger and 80% female-larger (Awata & Kohda 2004). Regardless of sex, the larger fish was dominant over its mate, had a larger home range, and spent less time at the nest. Similarly, in the laboratory, the relative body size was found to correlate with the propensity to take a second mate (Awata et al. 2006). Those studies suggest that relative body size is an important factor in the expression of multiple behaviors in *Julidochromis* cichlids.

Here, we demonstrate plasticity of aggressive and care-related behaviors correlated with relative mate size in the naturally female-larger species *Julidochromis marlieri*. We formed both female-larger and male-larger monogamous pairs in the laboratory to measure the influence of size on typically sexually dimorphic behaviors. We predicted female-biased aggression and male-biased parental care in female-larger pairs. For male-larger pairs, for which there are no previous studies in this species, we predicted different behavioral outcomes depending on the relative importance of social environment and gonadal sex. If behaviors are determined primarily by gonadal sex, then the same sex biases seen in female-larger pairs should persist in male-larger pairs. However, if behaviors are primarily determined by relative mate size, then male-larger pairs should show the reverse pattern of sex-biased behavior. Our results suggest that in *J. marlieri* pairs, the expression of aggressive behavior is plastic and primarily depends on the social environment of relative mate size, rather than being strictly determined by gonadal sex.

Materials and Methods

Study Animals and Maintenance

Julidochromis marlieri is a member of the cichlid tribe Lamprologini, which consists exclusively of substrate or nest breeders that are endemic to Africa's Lake Tanganyika (Brichard 1989; Konings 1998). In the wild, *J. marlieri* stable pairs lay eggs in rocky crevices and they guard their eggs and territory against conspecific and heterospecific shelter competitors (Brichard 1989; Konings 1998; Sunobe 2000 Heg & Bachar 2006), as well as egg predators and piscivores (Sunobe 2000; Heg & Bachar 2006). The *J. marlieri* used in this study were obtained from commercial sources or from Dr. George Barlow (UC Berkeley) and were 2–4 yr of age during the experiments. Fish were housed in 110 liter tanks (90 × 45 × 30 cm) on a constantly circulating system at 28 ± 0.3°C, 630–650 µS/cm and pH ~ 8.3 under an 11:5:11.5 photoperiod with an additional half fade for dawn and dusk. Flake food was provided once a day in the morning.

Fish were allowed to form pairs in group tanks comprised of three to six individuals. A population of large females and small males was used to create female-larger pairing tanks, while a population of small females and large males was used to create male-larger

pairing tanks; thus, in both situations, individuals were allowed to select a mate from a size-restricted pool. In each situation, once stable (i.e., defending a territory and having laid eggs), the pair was transferred to an observation tank. Because female *J. marlieri* tend to be larger than males within an age cohort, individuals in the male-larger pairing tanks were taken from the extremes of the distribution of body size in the age-matched population (i.e., the largest males and smallest females). However, in roughly 50% of the male-larger pairs and 30% of the female-larger pairs, the smaller individuals were taken from a younger cohort to obtain appropriate size distributions in the group tanks. Standard length was measured from snout to caudal peduncle, and weight was recorded to the nearest 0.1 gram (Table 1). While age is not likely to be a confounding factor in this experimental design, growth rate is known to be dependent on social environment in some cichlid species (Fernald 2002; Hamilton & Heg 2008). Therefore, by selecting fish from the extremes of body size distribution, we may have selected for past social experiences. Without a full repeated measures design using the same individuals in each condition, it is not possible to completely eliminate the potential for such confounding factors of experience.

Table 1: Length and weight for paired males and females of both the female-larger and male-larger pairing type indicating which pairs were used in each social condition observation (C: control, R: reproductive, I: intruder). Superscripts indicate pairing type of neighbor when different than that of the focal pair (1 = ML neighbor; 2 = FL neighbor; 3 = no neighboring fish; 4 = 1 unpaired neighbor)

Female-larger pairs								Male-larger pairs										
Standard length (cm)			Weight (g)			Social Condition		Standard length (cm)			Weight (g)			Social Condition				
Pair ID	Large/Small		Female	Large/Small		C	R	I	Pair ID	Large/Small		Female	Large/Small		C	R	I	
	Female	Male		Female	Male					Female	Male							
FL1	9.0	8.1	1.1	11.3	8.6	1.3		X										
FL2	8.4	7.0	1.2	9.8	5.6	1.8	X		ML1	4.7	8.1	1.7	1.6	8.6	5.4	X ²		
FL3	7.1	5.7	1.2	7.0	2.9	2.4	X	X	ML2	4.5	6.8	1.5	1.8	5.1	2.8	X	X	
FL4	6.7	5.1	1.3	5.6	2.2	2.5	X	X ¹	ML3	4.4	6.6	1.5	1.5	5.3	3.5		X	
FL5	7.0	5.3	1.3	6.0	2.4	2.5	X	X	ML4	4.5	6.5	1.4	1.5	4.6	3.1	X ²	X	X ²
FL6	6.8	5.0	1.4	5.9	2.4	2.5	X	X	ML5	4.9	6.6	1.3	1.8	5.3	2.9	X ²		X ²
FL7	6.0	4.1	1.5	4.3	1.5	2.9		X	ML6	4.1	5.5	1.3	1.5	4.1	2.7		X	
FL8	7.0	4.7	1.5	5.9	1.8	3.3		X	ML7	3.7	4.8	1.3	1.1	2.2	2.0		X ⁴	
FL9	7.6	5.0	1.5	7.4	2.3	3.2	X	X	ML8	5.0	6.4	1.3	2.8	4.8	1.7	X	X	X
FL10	9.2	6.0	1.5	13.4	3.5	3.8	X	X	ML9	4.4	5.6	1.3	1.7	3.9	2.3		X	
FL11	6.9	4.5	1.5	5.6	2.1	2.7		X	ML10	5.0	6.3	1.3	2.2	4.6	2.1	X	X	
FL12	8.3	5.3	1.6	10.2	2.4	4.3	X	X	ML11	4.0	5.0	1.3	1.2	2.1	1.8	X		X
FL13	8.1	5.0	1.6	10.2	2.8	3.6	X		ML12	6.2	7.5	1.2	5.1	6.7	1.3			X ³
FL14	8.4	5.0	1.7	9.8	2.2	4.5		X	ML13	4.4	5.1	1.2	1.5	2.5	1.7	X		X
FL15	7.5	4.4	1.7	7.0	1.8	3.9		X	ML14	4.7	5.2	1.1	2.0	2.6	1.3	X	X	X
FL Av.	7.6	5.4	1.4	8.0	3.0	2.7			ML Av.	4.7	6.2	1.3	2.0	4.4	2.5			
FL SD	0.9	1.0	0.2	2.6	1.8	0.9			ML SD	0.6	1.0	0.1	1.0	2.0	0.2			

Observation Tanks

Each 110-liter observation tank housed two neighboring pairs separated by a transparent acrylic divider. Pairs were provided with ~2-cm gravel substrate for digging and an artificial nest crevice made of two vertical clay tiles ($15 \times 15 \times 1$ cm; entrance 8 cm) (Fig. 1) as previously demonstrated to provide a preferred site for egg-laying (Awata et al. 2006). In most instances, both pairs in the divided tank were included in the experimental dataset and were unfortunate of the same pairing type (see Table 1 for details), thus presenting a less than ideal experimental design. However, for 7 of the total 48 sets of observations, neighbor type was not confounded with the pairing type of the observed focal pairs. Therefore, the effect of neighbor type could be accounted for by inclusion in the statistical model. Average behavior measures were calculated from biweekly observations.

Behavioral Observation

Using JWatcher v1.0 (Blumstein & Daniel 2007), a freely available, Java-based event recorder (<http://www.jwatcher.ucla.edu>), behaviors were simultaneously recorded for both individuals of the pair for 10-min observations that took place 2–5 h after artificial sunrise. Reliability checks throughout the study ensured interobserver reliability. Observations for both female-larger and male-larger pairing types were conducted in three different social conditions (Table 1):

- **Conspecific control:** Pairs were observed interacting with their neighbors and with their mates four times without offspring or eggs in the nest. The four observations were conducted within a span of two weeks (female-larger pairs, $n = 9$; male-larger pairs, $n = 9$).
- **Reproductive:** Pairs were observed for egg care behaviors on the first 2 d after eggs were laid while

no other offspring were present (female-larger pairs, $n = 8$; male-larger pairs, $n = 7$).

- **Heterospecific intruder:** Pairs were observed interacting with a heterospecific intruder (the cichlid *Astatotilapia burtoni*, male, 35–49 mm standard length) that was introduced to their tank immediately prior to the observation period. No offspring or eggs were present during intruder observations (female-larger pairs, $n = 7$; male-larger pairs, $n = 9$).

The datasets for the three social conditions are not independent because some pairs were used in multiple experiments, but due to logistical constraints, only 5 pairs were used in all three conditions.

Ethogram

The ethogram included seven behaviors that could be performed by either fish (Table 2). Territory defense was measured as the number of times a focal fish approached or attacked neighbors during the control and reproductive social conditions or the number of times a focal fish approached or attacked either neighbors or the intruder during intruder condition. 'Attack mate' measured aggression within a pair. Attacks against mates are less intense than those against intruders and do not result in injury, likely functioning to maintain the dominance relationship. Care-related behaviors include both direct and indirect care behaviors. Direct egg care behavior, which occurs only when eggs are present in the reproductive social condition, was measured as 'mouthing' of eggs in addition to an 'egg care mimic'. In the absence of eggs, egg care mimic was counted as egg-care-related behavior. This behavior consists of opening and closing the mouth on the wall of the nest as if cleaning eggs (Barlow personal communication). Fanning with the pectoral fins is another care-related behavior performed to keep eggs aerated. Because the movement of the pectoral fins was difficult to discern within the nest, this behavior was not analyzed, and only the

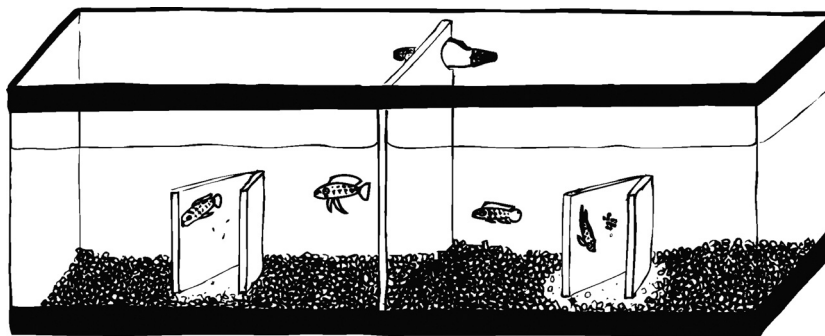


Fig. 1: Observation tank with two pairs and their nests, separated by a clear divider.

Table 2: Ethogram of behaviors recorded during observations

Measure	Behavior	Description
Aggression	Approach	Swim toward intruder/neighbor to within own body length without attacking
	Attack	Chase or bite intruder or attempt to chase, bite or jawlock neighbor across divider
	Attack mate	Chase or bite mate
Care-Related	Egg clean Mimic	Opening and closing mouth rapidly on wall of the nest while beating tail, usually in mate's presence
	Dig	Removing gravel from inside of nest with mouth
	Mouth eggs	Contacting eggs with mouth without eating them
	Fanning	Hovering ventrally over eggs accompanied by movement of the pectoral fins for more than one second
	In Nest/out of Nest	Any part of the body inside nest/No part in nest

Table 3: Variables included in the final GLMM with pair ID and tank entered as random variables. p-values based on chi-square test comparing the full model to a reduced model eliminating each variable

	Defense: Approach	Defense: Attack	Attack Mate	Egg Care	Digging	Time in Nest
Sex	<0.001	<0.001	<0.001	0.498	0.498	<0.001
Pairing type	<0.001	<0.001	<0.001	0.0992	0.099	<0.001
Social Condition	<0.001	<0.001	0.126	9.46E-06	0.290	<0.001
Neighbor type	0.027	0.130	0.808	0.3282	0.958	0.463
Three-way Interaction	<0.001	<0.001	0.358	0.011	0.011	0.001

proportion of time spent in the nest was used. Another indirect form of egg care involves 'digging', which consists of using the mouth to remove rocks from the nest area. These behaviors are similar in appearance to those described for other closely related social Lamprologine species (Taborsky 1984, 1985; Taborsky et al. 1986; Heg et al. 2005) and have been previously quantified in *Julidochromis* (Heg & Bachar 2006).

Statistical Analysis

R version 2.7.1 for Mac OS X was used for statistical analysis. Behavior was measured using occurrences for all behaviors except for time in nest (proportion of time). Using the lme4 package (Bates et al. 2011), we applied generalized linear mixed models considering the fixed effects of sex, social condition, pairing type, and neighbor pairing type, as well as the random effects of the tank and the neighbor ID. The final model included all pair-wise and three-way interactions of sex, social condition, and pairing type (BEHAVIOR ~ social condition*pairing type*sex + neighbor type + (1|pairID) + (1|tank)). While only 7 of the 48 pairs were housed with neighbors of a different pairing type, this variable was also included in the model to control for the potentially confounding factor of social environment introduced by neighbor. As determined by a chi-square test com-

paring two models, each variable was determined to have a significant effect on the model for at least one behavior analyzed (Table 3). All analyses were conducted using four control observations to obtain a solid baseline, two egg care observations to capture transient egg care behaviors soon after spawning, and a single measurement for the dramatic intruder observation.

In the *post hoc* tests, to avoid pseudo replication, analyses were conducted with the average from the four control observations, the average from the two egg care observations, and the single measurement for the intruder observation, to obtain a single value for behavior for each individual. Two-sample *t*-tests were performed on proportion of time spent in nest, as the data were normally distributed (data were treated as paired for within pairing type comparisons and as unpaired for between pairing type comparisons). Nonparametric tests were used for all other behavioral measures. Wilcoxon signed-rank tests were used for paired data, such as comparisons between paired females and males within the pair, while sum rank tests (equivalent to Mann-Whitney *U*-tests) were used for unpaired data. For comparisons between different social conditions, data were treated as unpaired because only some of the same pairs were used in both conditions (Table 1). Continuity corrections were performed, and exact p-values were not calculated in the presence of ties.

Ethical Note

All fish were housed according to animal protocol IACUC #1032007. Any fish that showed aggression leading to physical harm or excessive stress of another fish was separated from the group or pair. While allopatric to *Julidochromis*, the *A. burtoni* intruder species selected for convenience did elicit a robust aggressive response, though it should be noted that none of the fish used as intruders were physically harmed by the resident pair during intruder observations.

Results

The species-typical pairing for *J. marlieri* type is female larger. While female-larger pairs normally form under standard laboratory conditions, for this experiment, fish sizes in the group tanks were manipulated to provide only this pairing option. The male-larger *J. marlieri* pairing type has not been reported to occur in the wild, but these pairs were formed experimentally in group tanks containing only large males and small females. As observed previously in similar paradigms allowing choice (Leese et al. 2010), both pairing types spawned successfully in both the group tanks and the observation tanks. Females in female-larger pairs laid more eggs on average than those in male-larger pairs (19 ± 15 vs. 9 ± 3 eggs); however, this difference was not statistically significant ($t_{7.5} = 1.76$, $p = 0.12$). Absolute female length was significantly correlated with number of eggs (estimated slope = 4 eggs/cm, $R^2 = 0.33$, $p = 0.03$), while relative female/male length was not.

Rather than selecting a specific general linearized mixed model for each behavior, the model including sex, pairing type, social condition, and interactions thereof, as well as neighbor pairing type was used for all behavioral analyses because each variable had a significant effect on the model for one or more of the behaviors analyzed (Table 3). Importantly, the inclusion of the neighbors' pairing type significantly impacted the statistical model for only approach intruder, supporting the conclusion that the observed sex biases in behavior are primarily influenced by the social environment within the pair rather than by the relative size ratio within the neighboring pair.

Aggressive Behaviors

For all three of the aggressive behaviors analyzed (defensive approach, defensive attack, attack mate), there is strong interaction of sex and pairing type (GLMMs, approach: $z = 7.81$, $p < 0.001$; attack:

$z = 8.03$, $p < 0.001$; attack mate: $z = 9.49$, $p < 0.001$) (Table 4). This interaction results in a robust pattern of female-biased aggression in female-larger pairs that is reversed in male-larger pairs across all three social conditions. In sum, regardless of sex the larger individual in the pair assumed the aggressive role (Fig. 2a, b & c).

Under conspecific control conditions within the female-larger pairing type, territorial aggression toward neighbors, as measured by the attack and approach behaviors, was significantly higher in females than in males (Fig. 2a, b; attack: $W = 40$, $N = 9$, $p = 0.04$; approach: $W = 36$, $N = 9$, $p = 0.01$, ties = 1), whereas the opposite sex biases were significant in the male-larger pairs. Within the male-larger pairing type under control conditions, the males showed more territorial aggression than females (Fig. 2a, b; attack: $W = 0$, $N = 9$, $p = 0.02$, ties = 2; approach: $W = 0$, $N = 9$, $p = 0.009$).

The same interaction of sex and pairing type is evident in aggressive behaviors when eggs are present, during the reproductive condition. While statistically significant only for approach behavior, the females exhibit greater aggression than males in the female-larger pairing type (approach: $W = 28$, $N = 8$, $p = 0.02$, ties = 1; attack: $W = 15$, $N = 8$, $p = 0.40$, ties = 2), whereas males exhibit greater aggression than females in the male-larger pairing type, here statistically significant for attack behavior (attack: $W = 0$, $N = 7$, $p = 0.04$; approach: $W = 28$, $N = 8$, $p = 0.08$, ties = 1).

While the presence of eggs in the nest had little to no effect on the level of aggressive behaviors relative to control (GLMMs, approach: $z = -0.45$, $p = 0.65$; attack $z = -0.89$, $p = 0.37$), the introduction of a heterospecific intruder caused an increase in the overall level of aggression particularly with regard to actual attack behavior (GLMMs, approach: $z = 1.18$, $p = 0.24$; attack $z = 10.48$, $p < 0.001$). During the intruder condition, the total number of aggressive behavior events (attack and approach combined) increased for both males and females (males: $W = 47$, $N_1 = 18$, $N_2 = 18$, $p = 0.04$; females: $W = 32$, $N_1 = 18$, $N_2 = 18$, $p < 0.001$) regardless of pairing type. Again, there was a persistent interaction of sex and pairing type. As in the control and reproductive conditions, under the heterospecific intruder condition, females in the female-larger pairing type exhibited a greater number of attacks than males (attack: $W = 28$, $N = 8$, $p = 0.02$; approach: $W = 8.5$, $N = 8$, $p = 0.75$), whereas it was the males in the male-larger pairing type that exhibited a greater number of attacks than their mates (attack: $W = 1$, $N = 8$,

Table 4: Results of GLMMs for all behaviors including all fixed and interaction effects. p-values above with Z-values for Poisson distributed count data fit by the Laplace approximation or t-value for normally distributed time variable fit by REML in parentheses. Bold values indicate statistically significant effects at alpha = 0.05

	Approach	Attack	Mate	Egg Care	In Nest	Dig
Intercept: Control, FL, female	<0.001 (6.6)	0.068 (1.83)	0.035 (2.11)	0.090 (-1.7)	<0.001 (10.27)	0.001 (-3.47)
SocialCond: Intruder	0.240 (1.18)	<0.001 (10.48)	0.213 (-1.24)	0.994 (-0.01)	0.500 (0.68)	0.998 (0)
SocialCond: Reproductive	0.656 (-0.45)	0.374 (-0.89)	0.172 (-1.37)	0.285 (1.07)	0.005 (-2.88)	0.796 (-0.26)
PairingType: ML	<0.001 (-4.82)	<0.001 (-5.02)	0.009 (-2.61)	0.026 (2.23)	0.469 (0.73)	0.081 (-1.75)
Sex: male	<0.001 (-5.5)	<0.001 (-5.45)	<0.001 (-7.75)	<0.001 (4.68)	0.657 (-0.45)	0.358 (-0.92)
NeighborType: ML	0.006 (2.77)	0.016 (2.42)	0.337 (-0.96)	0.328 (0.98)	0.136 (-1.5)	0.850 (0.19)
NeighborType: none	0.0597 (1.88)	0.3134 (1.01)	0.5240 (-0.64)	0.9936 (0.01)	0.3796 (-0.88)	0.9987 (0)
NeighborType: one-fish	0.744 (0.33)	0.138 (1.48)	0.749 (-0.32)	0.548 (0.6)	0.432 (-0.79)	1.00 (0)
SocialCond: intruder; pairingType: ML	0.003 (2.94)	0.545 (0.61)	0.460 (0.74)	0.999 (0)	0.020 (-2.36)	0.999 (0)
SocialCond: reproductive; pairingType: ML	0.543 (0.61)	0.039 (2.06)	0.963 (-0.05)	0.166 (-1.38)	<0.001 (3.46)	0.998 (0)
SocialCond: intruder; sex: male	<0.001 (3.85)	0.719 (0.36)	0.835 (0.21)	0.994 (0.01)	0.561 (-0.58)	1.00 (0)
SocialCond: reproductive; sex: male	0.009 (-2.62)	<0.001 (3.78)	0.016 (2.4)	0.065 (-1.84)	0.012 (2.54)	0.860 (0.18)
PairingType: ML; sex: male	<0.001 (7.81)	<0.001 (8.03)	<0.001 (9.49)	<0.001 (-7.97)	0.032 (-2.17)	0.078 (1.76)
SocialCond: intruder; pairingType: ML; sex: male	0.001 (-3.3)	0.116 (-1.57)	0.954 (0.06)	0.991 (-0.01)	0.276 (1.09)	0.999 (0)
SocialCond: reproductive; pairingType: ML; sex: male	0.025 (2.25)	<0.001 (-4.32)	0.149 (-1.45)	0.021 (2.31)	0.002 (-3.1)	0.999 (0)

$p = 0.03$, ties = 2; approach: $W = 0$, $N = 8$, $p = 0.06$, ties = 2).

In many pair-bonding cichlid species, intrapair dominance is established by aggression directed toward the mate. As seen for aggressive behaviors directed at neighbors and intruders, sex and pairing type showed a strong interaction for mate-directed aggression (GLMM: $z = 9.49$, $p = 0.0001$), and it too followed the robust pattern of female-biased aggression in female-larger pairs that was reversed in male-larger pairs under all three social conditions (Fig. 2c). Under the control condition, in the female-larger pairing type, mate-directed aggression was higher in females than in males (Fig. 2c, $W = 36$, $N = 9$, $p = 0.01$, ties = 1), while in the male-larger pairing type males were the dominant sex, with a higher frequency of mate-directed aggression than females (Fig. 2c; $W = 3$, $N = 9$, $p = 0.04$, ties = 1). Mate-directed aggression was somewhat reduced in the male-larger pairing type (GLMM, $z = -2.61$,

$p = 0.01$). While the social condition did not have a significant effect on the overall level of mate-directed aggression (Table 4), in the presence of eggs, during the reproductive condition, there was a non-significant reduction in overall mate-directed aggression compared with the control condition (GLMM, $z = -1.37$, $p = 0.17$) that was not seen in the intruder condition.

Care-Related Behaviors

The three care-related behaviors were observed less frequently than the aggressive behaviors, thus statistical analyses were not as robust. Nonetheless, there was a discernable pattern of sex-biased behavior in several of the social conditions. Interestingly, this interaction of sex and pairing type was opposite of that seen for aggressive behaviors (Fig. 2d, e). Digging occurred very infrequently regardless of social condition (Fig. 2f).

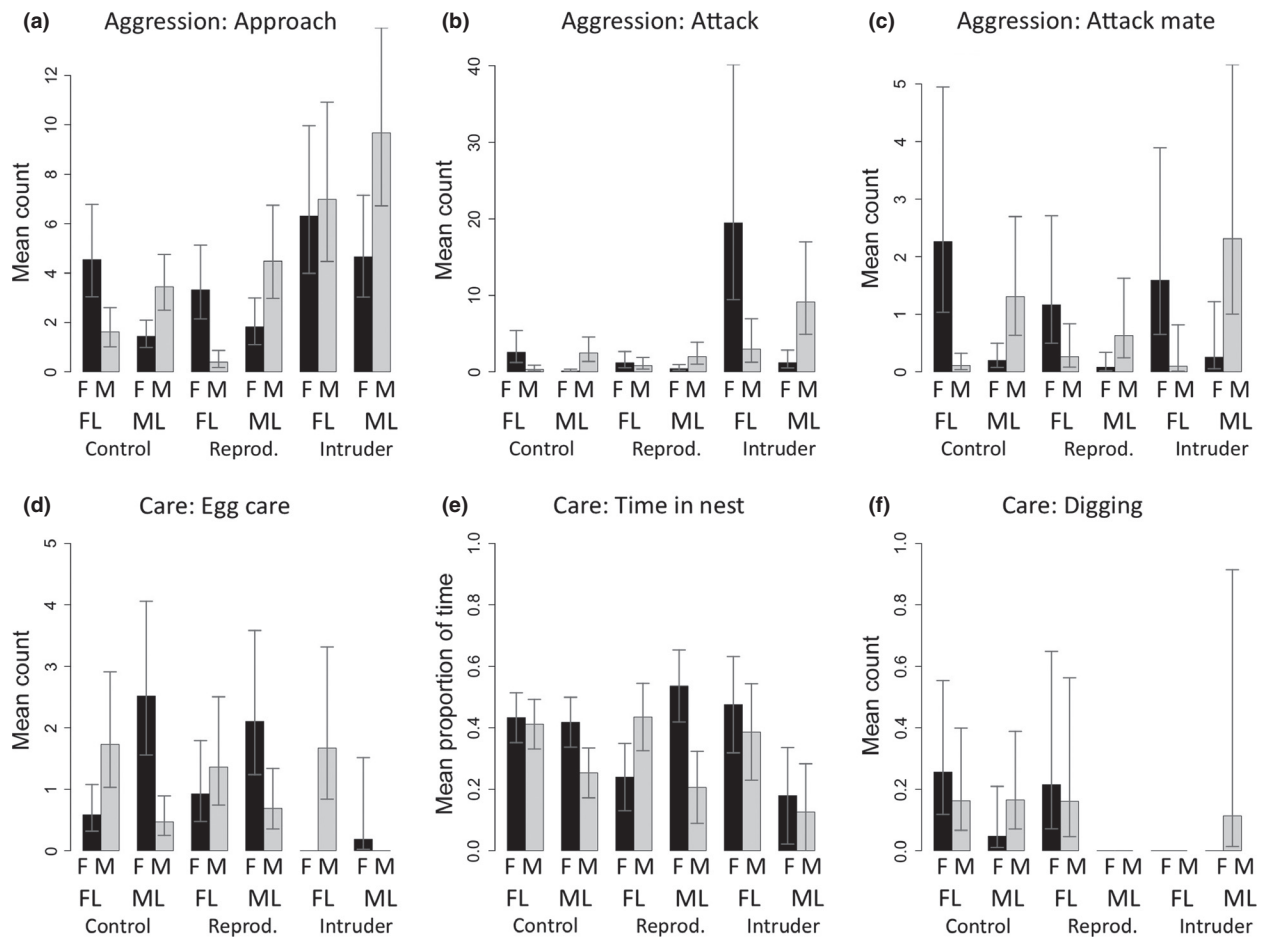


Fig. 2: Behavior data for aggressive (a–c)- and care (d–f)-related behaviors of females (black) and males (gray) in female-larger (FL) and male-larger (ML) pairing types under three social conditions. Values represent the extracted estimates of the fixed-effects parameters of the fitted model excluding the neighbor type variable. Error bars indicate 95% confidence intervals.

Among the care-related behaviors, egg care behaviors, which were scored as the egg care mimic in the control and intruder conditions and as mouthing plus egg care mimic in the reproductive condition, showed the strongest interaction of sex and pairing type (GLMM $z = -7.97$, $p < 0.001$) but were not affected by social condition (GLMMs reproductive: $z = 1.07$, $p = 0.28$; intruder: $z = -0.01$, $p = 0.99$). Under conspecific control conditions, within the female-larger pairing type, egg care was displayed more frequently by males than by females (Fig. 2d, $W = 4$, $N = 9$, $p = 0.03$), whereas the opposite sex bias was significant in the male-larger pairing type such that females displayed it more frequently than males (Fig. 2d; $W = 36$, $N = 9$, $p = 0.01$). Although the sex bias under the reproductive social condition was significant only for the male-larger pairing type, the same pattern of male-biased egg care in the female-larger pairing type ($W = 17$, $N = 7$, $p = 0.94$) and female-biased

egg care in the male-larger pairing type ($W = 26$, $N = 8$, $p = 0.05$) was observed. Similarly, under the intruder condition in the female-larger pairing type, only males performed egg care mimic, and in the male-larger pairing type, only females performed the egg care mimic.

For time in nest, the presence of eggs in the nest impacted the sex bias (GLMM $t = -2.88$, $p = 0.005$). The pattern of male bias in female-larger pairing type and female-bias in male-larger pairing type was apparent during the reproductive condition (Fig. 2e). In the female-larger pairing type males tended to spend more time in the nest ($t_7 = -1.98$, $p = 0.09$), and in the male-larger pairing type, females tended to spend more time in the nest than males ($t_6 = 1.96$, $p = 0.10$). While not statistically significant, pairwise comparisons between the social conditions present an interesting picture. As compared to the control condition, in the female-larger pairs, the male bias for

nest-time when the eggs were present was influenced by a reduction in time spent in the nest by females, rather than an increase in the amount of time spent in the nest by males (females: $t_{14.6} = 2.51$, $p = 0.02$; males: $t_{10.3} = 0.088$, $p = 0.93$). Conversely, in the male-larger pairs, the female-bias for nest-time when the eggs were present was influenced by both a reduction in the time that the male spent in the nest as well as an increase in the time that the female spent in the nest (females: $t_{10.3} = -1.04$, $p = 0.32$; males: $t_{8.23} = 0.70$, $p = 0.50$).

Discussion

Territorial aggression and dominance were found to be female-biased in *J. marlieri*, but only in the female-larger treatment that reflects the most prevalent, naturally occurring condition for this species. Reversal of size ratio resulted in a reversal in sex-biased behavior: territorial aggression and dominance became male-biased in male-larger pairs. This suggests that in *J. marlieri*, sexual dimorphism of aggressive behavior is contingent on the social environment (relative mate size) and is not determined strictly by gonadal sex. This result differs from previous reports for another species in the same genus, *Julidochromis ornatus*, in which sex bias in attacking rates were only significant when directed toward heterospecific intruders and not toward conspecifics and only in female-larger pairs but not in male-larger pairs (Awata & Kohda 2004). We found that attack rates toward both heterospecific intruders and conspecifics were biased toward the larger fish regardless of sex. The consistent pattern for attack behavior suggests that the pairing type of the focal pair is a greater determinant of behavior than the pairing type of the neighboring pair or the sex of the target of the aggression. Future experiments that explicitly manipulate the sex of the opponent could elucidate potential ultimate explanations for these sex-biased aggressive behaviors. Discrepancies between current and past results may be due to species differences or experimental setup such as laboratory vs. field or proximity of neighboring pairs. Proximity of intruders is known to alter division of labor (Richter et al. 2005) and likely explains the higher rate of attack in the current study.

The extreme plasticity demonstrated by the current study also contrasts a similar study in the convict cichlid (*A. nigrofasciata*) that measured territorial aggression against conspecifics in male-larger, same-size, and female-larger pairs (Itzkowitz et al. 2005). There, reversal of size ratio resulted in only a partial shift in aggression from males to females, but male bias in

attack rate and time spent near the intruder persisted even when the female was significantly larger (>2 cm). This suggests that sex-biased territorial behavior in the convict cichlid is determined primarily by gonadal sex although the expression of territorial behavior can be modulated by the social environment. The ultimate and proximate explanations for extreme plasticity in *J. marlieri* have yet to be investigated.

Care-related behaviors were not as consistently sex-biased for either pairing type. While under all social conditions direct egg care, in the form of mouthing and egg care mimic, did follow the pattern of sex bias opposite to that observed for aggression being higher in the smaller fish regardless of sex, time spent in the nest showed this pattern only while eggs were present. We noticed that, when eggs were present, the larger fish appeared to spend more time 'patrolling' the territory, swimming around the perimeter of the tank with fins flared in what appeared to be an aggressive display. Combined, these observations parallel field studies (Awata & Kohda 2004) and suggest that the presence of eggs or offspring will accentuate existing sex biases or initiate additional sex biases. It is possible that the egg care mimic in the absence of eggs may serve more to promote pair-bonding than to signal actual care-giving ability, which is another behavioral dimension known to show sex biases even in monogamous species (Leese 2012). The actual function of the egg care mimic may be more similar to the 'quiver' behavior seen in submissive or courting cichlids (Baerends & Baerends-Van Roon 1950).

Female fecundity was positively correlated with absolute female body size suggesting that males would have a fitness advantage if paired with larger females. This is consistent with the prevalence of female-larger pairs in the wild. Selection for increasing female body size leading to increased fecundity, a scenario broadly known as fecundity selection (Shine 1988), may have preceded the evolution of female-biased aggression and territory defense. In contrast, there was no correlation found between female body size and brood size for wild *J. ornatus*, which may explain why both male-larger and female-larger pairs are found in that species (Awata & Kohda 2004).

The degree to which the proximate environmental factors that promote plasticity from female-larger to male-larger pairing types parallel ultimate ecological pressures that favored the evolution of female-larger pairing in *J. marlieri* and male-larger pairing in *Julidochromis transcriptus* is completely unknown. Studies that show plasticity of sex-biased behavior under ecological fluctuations that cause the direction of sexual

selection to reverse support this possibility. Species such as *J. marlieri* that show reversed sex-biased behaviors provide the opportunity to examine the proximate mechanisms that underlie reversed phenotypes (Eens & Pinxten 2000), and the identification here of a genus that exhibits both evolutionary plasticity and behavioral plasticity offers the exciting possibility to address whether the mechanisms of behavioral plasticity have been co-opted during species divergence (Renn & Schumer 2013). Sex-biased neural gene expression patterns in female-larger *J. marlieri* pairs have been compared with sex-biased patterns in the naturally male-larger *J. transcriptus* pairs (Schumer et al. 2011). Those results show limited conserved sex bias for gene regulation between the two species but a significant overlap in gene expression bias associated with behavioral phenotype. In other words, the aggressive *J. marlieri* females share greater similarity of neural gene expression with aggressive *J. transcriptus* males than with care-oriented *J. transcriptus* females. Using the size ratio, manipulations to manipulate behavioral phenotype as described in the current study will provide mechanistic answers to the relationship between proximate and ultimate explanations for sex-biased phenotypes.

Conclusion

Female-biased territorial aggression and dominance were confirmed for *J. marlieri*, but only in the female-larger pairing type typical in wild populations. In pairs where males were larger than their mates, territorial aggression and dominance were male-biased. This suggests that species-typical sexual dimorphism in aggressive behavior in *J. marlieri* is a product of the species-typical sexual size dimorphism of female-larger pairs. Overall, our results suggest that sexual dimorphism in aggressive behavior is not determined by sex for *J. marlieri*, but is plastic and dependent on the social environment. These studies introduce the remarkable plasticity of *Julidochromis* as an exciting model with which to address the relationship between proximate and ultimate mechanisms of behavioral plasticity.

Acknowledgements

We thank Molly Schumer for contributions to data collection, Nadia Aubin-Horth, Julia Carleton, Cynthia O'Rourke, and Heather Machado for providing helpful comments on earlier versions of this manuscript. This work funded by NIH R15-GM080727 awarded to S.C.P.R.

Competing interests

The authors declare that they have no competing interests.

Literature Cited

- Awata, S. & Kohda, M. 2004: Parental roles and the amount of care in a bi-parental substrate brooding cichlid: the effect of size differences within pairs. *Behaviour* **141**, 1135–1149.
- Awata, S., Takeuchi, H. & Kohda, M. 2006: The effect of body size on mating system and parental roles in a biparental cichlid fish (*Julidochromis transcriptus*): a preliminary laboratory experiment. *J. Ethol.* **24**, 125–132.
- Baerends, G. & Baerends-Van Roon, J. M. 1950: An Introduction to the Study of the Ethology of Cichlid Fishes. Brill, Leiden, Netherlands.
- Barlow, G. W. 2005: How do we decide that a species is sex-role reversed? *Quart. Rev. Biol.* **80**, 28–35.
- Barlow, G. W. & Lee, J. S. F. 2005: Sex-reversed dominance and aggression in the cichlid fish *Julidochromis marlieri*. *Ann. Zool. Fenn.* **42**, 1–7.
- Bateman, A. 1948: Intra-sexual selection in *Drosophila*. *Heredity* **2**, 349–368.
- Bates, D., Maechler, M. & Bolker, B. 2011: lme4: Linear mixed-effects models using S4 classes. R package version 0.999375-42. <http://CRAN.R-project.org/package=lme4>
- Blumstein, D. T. & Daniel, J. C. 2007: Quantifying Behaviour the JWitcher Way. Sinauer Associates Inc, Sunderland, MA.
- Brichard, P. 1989: Cichlids and All the Other Fishes of Lake Tanganyika. T.F.H, Neptune City, NJ.
- Bruintjes, R. & Taborsky, M. 2011: Size-dependent task specialization in a cooperative cichlid in response to experimental variation of demand. *Anim. Behav.* **81**, 387–394.
- Clutton-Brock, T. H. 1991: The Evolution of Parental Care. Princeton Univ. Press, Princeton, NJ.
- Clutton-Brock, T. H. & Vincent, A. 1991: Sexual selection and the potential reproductive rates of males and females. *Nature* **351**, 58–60.
- Dominey, W. J. 1981: Anti-predator function of bluegill sunfish nesting colonies. *Nature* **290**, 586–588.
- Eens, M. & Pinxten, R. 2000: Sex-role reversal in vertebrates: behavioural and endocrinological accounts. *Behav. Process.* **51**, 135–147.
- Emlen, S. T. & Oring, L. W. 1977: Ecology, sexual selection, and evolution of mating systems. *Science* **197**, 215–223.
- Erlandsson, A. & Ribbink, A. 1997: Patterns of sexual size dimorphism in African cichlid fishes. *S. Afr. J. Sci.* **93**, 498–508.
- Fernald, R. D. 2002: Social regulation of the brain: Status, sex and size. In: *Hormones, Brain and Behavior* (Pfaff,

- D., Arnold, A., Etgen, A., Fahrback, S. & Rubin, R., eds). Academic Press, New York, NY, pp. 435–444.
- Forsgren, E., Amundsen, T., Borg, Å. & Bjelvenmark, J. 2004: Unusually dynamic sex roles in a fish. *Nature* **429**, 551–553.
- Gagliardi-Seeley, J. L. & Itzkowitz, M. 2006: Male size predicts the ability to defend offspring in the biparental convict cichlid *Archocentrus nigrofasciatus*. *J. Fish Biol.* **69**, 1239–1244.
- Gwynne, D. T. & Simmons, L. 1990: Experimental reversal of courtship roles in an insect. *Nature* **346**, 172–174.
- Hamilton, I. M. & Heg, D. 2008: Sex differences in the effect of social status on the growth of subordinates in a co-operatively breeding cichlid. *J. Fish Biol.* **72**, 1079–1088.
- Heg, D. & Bachar, Z. 2006: Cooperative breeding in the Lake Tanganyika Cichlid *Julidochromis ornatus*. *Environ. Biol. Fish.* **76**, 265–281.
- Heg, D., Bachar, Z. & Taborsky, M. 2005: Cooperative breeding and group structure in the Lake Tanganyika cichlid *Neolamprologus savoryi*. *Ethology* **111**, 1017–1043.
- Huntingford, F. & Turner, A. 1987: *Animal Conflict*. Chapman and Hall, New York, NY.
- 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.
- 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.
- Jiggins, F., Hurst, G. & Majerus, M. 2000: Sex-ratio-distorting *Wolbachia* causes sex-role reversal in its butterfly host. - *Proc. R. Soc. B.* **267**, 69–73.
- Keenleyside, M. H. A. 1991: *Cichlid Fishes: Behaviour, Ecology and Evolution*. Chapman and Hall, London.
- Konings, A. 1998: *Tanganyika Cichlids in their Natural Habitat*. Cichlid Press, El Paso.
- Leese, J. M. 2012: Sex differences in the function of pair bonding in the monogamous convict cichlid. *Anim. Behav.* **83**, 1187–1193.
- Leese, J., Wison, H., Ganim, A. & Itzkowitz, M. 2010: Effects of reversed size-assortative mating on spawning success in the monogamous convict cichlid, *Amantitlania nigrofasciata*. *Ethol. Ecol. & Evol.* **22**, 95–100.
- Lehtonen, T. K., Wong, B. B. M., Svensson, P. A. & Meyer, A. 2011: Adjustment of brood care behavior in the absence of a mate in two species of Nicaraguan crater lake cichlids. *Behav. Ecol. Sociobiol.* **65**, 613–619.
- O'Connell, L. A., Matthews, B. J. & Hofmann, H. A. 2012: Isotocin regulates paternal care in a monogamous cichlid fish. *Horm. Behav.* **61**, 725–733.
- Owens, I. & Hartley, I. 1998: Sexual dimorphism in birds: why are there so many different forms of dimorphism? *Proc. R. Soc. B.* **265**, 397–407.
- Ratnieks, F. & Anderson, C. 1999: Task partitioning in insect societies. *Insectes Soc.* **46**, 95–108.
- Renn, S. C. P. & Schumer, M. E. 2013: Genetic accommodation and behavioural evolution: insights from genomic studies. *Anim. Behav.* **85**, 1012–1022.
- Richter, M., Santangelo, N. & Itzkowitz, M. 2005: Biparental division of roles in the convict cichlid fish: influence of intruder numbers and locations. *Ethol. Ecol. & Evol.* **17**, 1–15.
- Ritchie, M., Sunter, D. & Hockham, L. 1998: Behavioural components of sex role reversal in the tettigoniid bush-cricket *Ephippiger ephippiger*. *J. Insect Behav.* **11**, 481–491.
- Schumer, M. E., Krishnakant, K. & Renn, S. C. P. 2011: Comparative gene expression profiles for highly similar aggressive phenotypes in male and female cichlid fishes (*Julidochromis*). *J. Exp. Biol.* **214**, 3269–3278.
- Shine, R. 1988: The evolution of large body size in females: a critique of Darwin's "fecundity advantage" model. *Am. Nat.* **131**, 124–131.
- Silva, K., Vieira, M. N., Almada, V. C. & Monteiro, N. M. 2010: Reversing sex role reversal: compete only when you must. *Anim. Behav.* **79**, 885–893.
- Snekser, J. L. & Itzkowitz, M. 2009: Sex differences in retrieval behaviour by the biparental convict cichlid. *Ethology* **115**, 457–464.
- Sunobe, T. 2000: Social structure, nest guarding and interspecific relationships of the cichlid fish (*Julidochromis marlieri*) in Lake Tanganyika. *Afr. Study Monogr.* **21**, 83–89.
- Taborsky, M. 1984: Broodcare helpers in the cichlid fish *Lamprologus brichardi*: their costs and benefits. *Anim. Behav.* **32**, 1236–1252.
- Taborsky, M. 1985: Breeder-helper conflict in a cichlid fish with broodcare helpers: an experimental analysis. *Behaviour* **95**, 45–75.
- Taborsky, M., Hert, E., von Siemens, M. & Stoerig, P. 1986: Social behaviour of Lamprologus species: functions and mechanisms. *Annales Koninklijk Museum Midden-Afrika Zoologische Wetenschappen* **251**, 7–11.
- Trivers, R. L. 1972: Parental investment and sexual selection. In: *Sexual Selection and the Descent of Man 1871–1971* (Campbell, B., ed.). Aldine, Chicago, IL, pp. 136–179.
- Yamagishi, S. & Kohda, M. 1996: Is the cichlid fish *Julidochromis marlieri* polyandrous? *Ichthyol. Res.* **43**, 469–471.