

1 Social reversal of sex-biased aggression and dominance in a biparental cichlid fish
2 (*Julidochromis marlieri*)

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10 11 12 **ABSTRACT**

13
14 In biparental species, aggression, dominance and parental care are typically sexually dimorphic.
15 While behavioural dimorphism is often strongly linked to biological sex, the environment, either
16 social or ecological, may influence sex-biased behaviour in some species. In the biparental
17 cichlid fish *Julidochromis marlieri*, large females paired with smaller males characterizes the
18 typical social environment. While both sexes are capable of providing territory defence and
19 parental care, the larger female provides the majority of defence, while the smaller male
20 remains in the nest guarding their offspring. We examine the contributions of sex and relative
21 mate size in these sex-biased behaviours in monogamous *J. marlieri* pairs. Both female-larger
22 and male-larger pairs were formed in the lab and were observed for territorial aggression
23 (against both conspecifics and heterospecifics), dominance, and parental care. In female-larger
24 pairs, territorial aggression and intra-pair dominance were female-biased, while in male-larger
25 pairs this bias was reversed. For both pairing types, the presence of an intruder amplified sex
26 differences in territorial aggression, with the larger fish always attacking with greater frequency
27 than its mate. There was no evidence for differences in time spent in the nest or for direct egg
28 care behaviours for either pairing type. Our study suggests that relative mate size strongly
29 influences the sex-bias of aggression and dominance in *J. marlieri*, and that this aspect of the
30 social environment can override the influence of biological sex on an individual's behaviour.

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32 **Keywords:** social behaviour; plasticity; sexual dimorphism; sex-role; parental care; territorial
33 aggression

INTRODUCTION

In many animals there is a striking inequality between the sexes with regard to how often certain behaviours are expressed; these behaviours are said to be sexually dimorphic. Specifically, sexually dimorphic behaviours are considered to be either sex-specific (expressed by only one sex) or sex-biased (expressed in both sexes but with unequal frequencies). Aggression and parental care are two aspects of animal behaviour 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 defence (reviewed in Owens & Hartley 1998). In biparental cichlids, territory defence is often performed by the male, while direct offspring care is performed by the female (e.g. *Aequidens vittatus*, Keenleyside & Bietz 1981; *Cichlasoma cyanoguttatum*, Itzkowitz 1984; *Lamprologus toae*, Nakano & Nagoshi 1990; also see review by Keenleyside 1991). For fish, territorial aggression in the form of egg guarding and breeding site defence can increase offspring survival by reducing egg predation (Dominey 1981) while direct offspring care, such as egg cleaning and fanning, can increase offspring survival by removing pathogens and promoting normal development (Keenleyside 1991). While either sex is capable of either type of behaviour, the division of parental roles may allow one sex to specialize in a specific aspect of parental care, similar to task partitioning as seen in eusocial insects (Ratnieks & Anderson 1999). The pattern of male territory defence may be adaptive since males are the larger sex in most cichlids (Erlandsson & Ribbink 1997), making them more effective at securing a nest site and repelling egg- and fry-eating intruders.

Sexually dimorphic behaviours usually have their basis in biological sex. However, certain species show plasticity in the expression of sexually dimorphic behaviours in response to the environment. For example, mating behaviour often consists of sexually dimorphic courtship roles: one sex courts and competes (usually males) while the other sex chooses (usually females) (Bateman 1948; Emlen & Oring 1977; Trivers 1972, Clutton-Brock & Vincent, 1991), but a few species appear to have extraordinary plasticity in regards to which sex fills which role. This is particularly true in insect species, such as katydids and bushcrickets, in which males provide a nutritious spermatophore to the female during mating. In these species, courtship roles are plastic and determined by nutritional availability, with females competing for males when food is scarce and vice versa when food is plenty (Gwynne & Simmons 1990; Ritchie et al. 1998). Similarly, butterflies, gobies, and pipefish have been shown to reverse the sex-bias of courtship roles depending on the operational sex ratio, with the more abundant sex competing more intensely for access to the rarer sex (Jiggins et al. 2000; Forsgren et al. 2004; Silva et al. 2010).

Plasticity of sexually dimorphic behaviour has also been observed in the monogamous South American convict cichlid in regards to parental behaviour (*Amatitlania nigrofasciatum*, previously *Archocentrus*) (Itzkowitz et al. 2005). The convict cichlid shows sex-biased parental behaviour: the males, which are larger, provide the majority of territory and brood defence while females provide the majority of egg care behaviours (Itzkowitz et al. 2002, 2003, 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 behaviour depending on the social context of relative mate size (Itzkowitz et al. 2005). While the degree of sex-bias in behaviour was altered in female-larger pairs, the direction of sex-bias was maintained: males still showed more aggression and females still spent more time with offspring relative to the other sex. This study suggests that while sex-biased territorial and parental behaviours are influenced by social context, they are determined primarily by sex in convict cichlids.

In *Julidochromis* cichlids, relative mate size may be more important than biological sex in the expression of sexually dimorphic aggressive and parental behaviours (Awata & Kohda 2004). The five species in the genus *Julidochromis* are primarily monogamous, biparental, substrate-brooding cichlids from Lake Tanganyika (Konings 1998). *Julidochromis* is currently the only known non-mouthbrooding African cichlid genus to contain species where females are larger than males (*J. ornatus*: Erlandsson & Ribbink 1997; *J. regani* and *J. marlieri*: Konings 1998). Therefore, monogamous pairs are generally female-larger. Interestingly, in female-larger pairs, males and females show a reversal of the sex-biased parental roles that are seen in other monogamous cichlids. Females are more aggressive than males (*J. marlieri*: Barlow 2005, Barlow & Lee 2005), are dominant to their mates (*J. ornatus*: Awata & Kohda 2004), and provide the majority of territory defence (*J. ornatus*: Awata & Kohda 2004). Males, on the other hand, have smaller home ranges and spend more time in and around the nest, presumably guarding offspring and providing egg care (*J. marlieri*: Sunobe 2000; Yamagishi & Kohda 1996; *J. ornatus*: Awata & Kohda 2004). In these studies, *Julidochromis* female-larger pairs show a complete reversal not only in relative size, but also in the typical pattern of sex-biased behaviour seen in other monogamous cichlids. The reversal in relative mate size may actually cause the reversal in behaviour, as suggested by a field study of *J. ornatus* (Awata & Kohda 2004). Out of 55 *J. ornatus* pairs in that study, 43 were female-larger and 12 were male-larger. Regardless of sex, the larger fish was dominant to its mate (as measured by number of mate-directed attacks), and the smaller fish had a smaller home range and spent more time in and around the nest. In a separate laboratory study, using a typically male-larger species, *J. transcriptus*, relative body size was found to influence both parental behaviour and the propensity to take a second mate (Awata et al. 2006). These studies show that relative body size is an important factor in the expression of multiple behaviours in *Julidochromis* cichlids. The remarkable plasticity of behaviour that *Julidochromis* species show in response to the social environment makes them an apt model system in which to study the effects and interactions of biological sex and environment on the expression of sexually dimorphic behaviour.

Here we use *Julidochromis marlieri* to determine the extent to which the expression of aggressive and parental behaviour is influenced by sex and the social environment of relative mate size. We were able to form both female-larger and male-larger monogamous pairs in the laboratory. Both types of pairs were observed for measures of territorial aggression (both against conspecifics and heterospecifics), dominance, and parental care. Based on previous research with *J. marlieri* (Barlow & Lee 2005; Yamagishi & Kohda 1996) and other *Julidochromis* species (Awata & Kohda 2004), we expected to confirm female-biased territorial aggression and dominance, and we predicted male-biased parental care. There is no previous research on male-larger *J. marlieri* pairs. We predicted different outcomes depending on the relative importance of sex and of the social environment of relative mate size in determining behaviour. If territorial and parental behaviours are determined only by biological sex in *J. marlieri*, then the same sex-biases seen in female-larger pairs would be expected to be maintained in male-larger pairs. However, if territorial and parental behaviours are determined by relative mate size, then we expected male-larger pairs to show the reverse pattern of sex-biased behaviour. Our results were consistent with behaviour being determined by the social environment of relative mate size. We found that territory defence behaviours were consistently biased towards the larger fish of the pair regardless of sex, as was intra-pair dominance. The expression of egg care behaviours did not appear to be determined by size or sex. Our results suggest that, in *J. marlieri* pairs, the expression of aggressive behaviour is plastic depending on the social environment, and is not determined by biological sex alone.

METHODS

Study animals and maintenance

Julidochromis marlieri belongs to 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* lay their eggs on the ceiling of rocky caves or crevices, and a pair will guard their eggs, cave, and territory against intruders (Brichard 1989; Konings 1998). They tend to spawn in small broods every 2-4 weeks, which results in a constant presence of eggs, wrigglers, or fry to be protected (Brichard 1989). Pairs must defend their nesting cave against shelter competitors, which include congeners as well as other cave brooders such as *Neolamprologus* species (Brichard 1989; Sunobe 2000; Heg & Bachar 2006). When offspring are present, the parents defend them from egg predators and piscivores, including *Lamprologus* and *Lepidolamprologus* species (Sunobe 2000; Heg & Bachar 2006). The pair bond may not be life-long, but is stable across multiple spawning events (Brichard 1989).

All fish were housed in tanks measuring 90 x 45 x 30 cm (110 L). Water temperature was maintained at $28 \pm 0.3^{\circ}\text{C}$ by ambient room heat. Salinity was kept between 630 – 650 $\mu\text{S/cm}$ and pH 8.3. The photoperiod was 11.5 h:11.5 h light:dark, with an additional half hour of graded light changes in order to mimic dawn and dusk. Flake food was provided once a day in the morning. *Julidochromis marlieri* were obtained either from commercial sources or from the lab of Dr. George Barlow (UC Berkeley) and were approximately 2-4 years of age during the experiments.

Fish were allowed to form pairs in group tanks comprised of three to six individuals. Once a stable pair could be identified they were removed from the pairing tank and transferred to an observation tank (see below). Fish were considered to have formed a pair if they defended a territory and laid eggs, however some pairs that were observed to defend a territory together and occupy the same nest were moved to observation tank before eggs were laid. Large females and small males were used to create female-larger pairing tanks, while small females and large males were used to create male-larger pairing tanks. Due to the fact that female *J. marlieri* are 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 population (i.e. the largest males and smallest females) or taken from stocks of different age. Fish were measured from the tip of the snout to the start of the tail (standard length) to the nearest millimetre using a ruler and were weighed to the nearest 0.1 gram on a digital scale. Tables 1 and 2 show female and male size differences and the ratio of the larger fish to the smaller fish for each pair in the experiment.

Table 1. Individual size differences for females (F) and males (M) in female-larger pairs

Table 2. Individual size differences for females (F) and males (M) in male-larger pairs

Female-larger pairs								Male-larger pairs								
Pair ID	Standard length (cm)				Weight (g)				Pair ID	Standard length (cm)				Weight (g)		
	Female	Male	Large	Female	Male	Large		Female		Male	(Large)	Female	Male	(Large)		
			Small			Small	(Small)				(Small)					
FL1	9.0	8.1	1.1	11.3	8.6	1.3			ML1	4.7	8.1	1.7		1.6	8.6	5.4
FL2	8.4	7.0	1.2	9.8	5.6	1.8			ML2	4.5	6.8	1.5		1.8	5.1	2.8
FL3	7.1	5.7	1.2	7.0	2.9	2.4			ML3	4.4	6.6	1.5		1.5	5.3	3.5
FL4	6.7	5.1	1.3	5.6	2.2	2.5			ML4	4.5	6.5	1.4		1.5	4.6	3.1
FL5	7.0	5.3	1.3	6.0	2.4	2.5			ML5	4.9	6.6	1.3		1.8	5.3	2.9
FL6	6.8	5.0	1.4	5.9	2.4	2.5			ML6	4.1	5.5	1.3		1.5	4.1	2.7
FL7	6.0	4.1	1.5	4.3	1.5	2.9			ML7	3.7	4.8	1.3		1.1	2.2	2.0
FL8	7.0	4.7	1.5	5.9	1.8	3.3			ML8	5.0	6.4	1.3		2.8	4.8	1.7
FL9	7.6	5.0	1.5	7.4	2.3	3.2			ML9	4.4	5.6	1.3		1.7	3.9	2.3
FL10	9.2	6.0	1.5	13.4	3.5	3.8			ML10	5.0	6.3	1.3		2.2	4.6	2.1
FL11	6.9	4.5	1.5	5.6	2.1	2.7			ML11	4.0	5.0	1.3		1.2	2.1	1.8
FL12	8.3	5.3	1.6	10.2	2.4	4.3			ML12	6.2	7.5	1.2		5.1	6.7	1.3
FL13	8.1	5.0	1.6	10.2	2.8	3.6			ML13	4.4	5.1	1.2		1.5	2.5	1.7
FL14	8.4	5.0	1.7	9.8	2.2	4.5			ML14	4.7	5.2	1.1		2.0	2.6	1.3
FL15	7.5	4.4	1.7	7.0	1.8	3.9										
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 observation tank housed two neighbouring pairs of the same pairing type separated by a transparent acrylic divider. Each pair was provided with ~2 cm gravel substrate for digging, and an artificial nest crevice. The nests were made of two clay tiles (15 x 15 x 1 cm) with an entrance width of 8 cm, designed to mimic the caves and crevices that are used as nests in the wild (Fig. 1). Previous studies of *J. transcriptus* (Awata et al. 2006) showed that females preferentially laid eggs on the inner sides of such artificial nests, making it easy to see when eggs have been laid and to directly observe parental behaviours such as egg cleaning.

Behavioural Observation

Behaviours were recorded live and continuously on a Macintosh laptop using the program JWatcher v1.0 (Blumstein & Daniel 2007), a freely available, Java-based event recorder (<http://www.jwatcher.ucla.edu>). Reliability checks were made throughout the study to ensure inter-observer reliability between three trained observers. An observer, using separate keys for the male and the female, recorded behaviours of both fish in a pair simultaneously. Five minutes before the observation, an overhead incandescent light was turned on to facilitate viewing of in-nest behaviours, and the observer sat quietly in front of the observation tank to allow the fish to acclimate to the presence of the observer. Observations were 10 minutes in duration and took place between two to five hours after artificial sunrise. There were three types of observations:

Control: Pairs were observed interacting with their neighbours and with their mates four times without offspring in the nest. The four observations were conducted within a span of two weeks. The sample size for control observations was nine female-larger and nine male-larger pairs.

Egg care: Pairs were observed for egg care behaviours the first two days after eggs were laid. The sample size for egg care observations was eight female-larger and seven male-larger pairs.

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 were present during intruder observations. The sample size for intruder observations was eight female-larger and eight male-larger pairs.

For statistical analysis, tests were conducted on the averages from four control observations, the averages from the two egg care observations, and the single measurement for the intruder observation.

Ethogram

The ethogram included eight behaviours that could be performed by either fish (Table 3). Territory defence was measured as the number of times a focal fish attacked or approached their neighbours or the intruder during the control and intruder trials, respectively. "Attack mate" was used as a measure of dominance within a pair. Here, the fish that performed more attacks against its mate is assumed to be "dominant". Attacks against mates were less intense than those against intruders, and did not result in injury. Thus, these attacks likely function to maintain the dominance relationship rather than cause actual harm. The "egg clean mimic", which consists of opening and closing of the mouth on the wall of nest as if cleaning eggs, is not a known cichlid behaviour, but was frequently observed during initial observations in this study. Because the egg clean mimic almost always occurred in the presence of the mate, we consider it a mate interaction behaviour. The actual function of the egg clean mimic behaviour is unclear, although it may play a role similar to the "quiver" behaviour seen in submissive or courting cichlids (Baerends & Baerends-Van Roon 1950). *Julidochromis marlieri* pairs remove rocks from their nest area ("digging"), even though they lay their eggs on the walls of the nest, perhaps to

protect larval fry from becoming trapped in the substrate. Egg care was measured by egg “mouthing” and “fanning”. Mouthing cleans the eggs and fanning by the pectoral fins aerates them. As the movement of the pectoral fins was difficult to discern, hovering over the eggs was used as a proxy for fanning.

Table 3: Ethogram of behaviours recorded during observations.

Measure	Behaviour	Description
Territory Defence	Approach	Swim towards intruder/neighbour to within own body length without attacking
	Attack	Chase or bite intruder or attempt to chase, bite or jawlock neighbour across divider
Mate interaction	Attack mate	Chase or bite mate
	Egg Clean Mimic	Opening and closing mouth rapidly on wall of the nest while beating tail, usually in mate’s presence
Nest Maintenance	Dig	Removing gravel from inside of nest with mouth
Egg Care	Mouth Eggs	Contacting eggs with mouth without eating them
	Fanning	Hovering ventrally over eggs for more than one second
Time in nest	In Nest/Out of Nest	Any part of the body inside nest/No part in nest

Statistical Analysis

R version 2.7.1 for Mac OS X was used for statistical analysis. Behaviour was measured using occurrences for all behaviours except for time in nest (proportion of time) and fanning (seconds). Two sample t-tests were performed on proportion of time spent in nest, as the data were normally distributed. Nonparametric tests were used for all other behavioural measures. Wilcoxon signed rank tests were used for paired data, such as comparisons between paired females and males, while sum rank tests (equivalent to Mann-Whitney tests) were used for unpaired data. For comparisons between observation types, data were treated as unpaired because only some of the same pairs were used in both types of observation. The **R** command “wilcox.test” was used and the test statistic “W” is reported here. 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. None of the fish used as intruders were physically harmed by the resident pair during intruder observations.

RESULTS

Female-larger pairs

The species-typical pairing type is female-larger for *J. marlieri*. For the female-larger pairs in this study, territorial aggression toward conspecifics, as measured by the behaviours attack and approach, was significantly higher in females (Fig. 2A&B; attack: $W = 40$, $N = 9$ $P = 0.044$; approach: $W = 36$, $N = 9$, $P = 0.014$, ties = 1). Territorial aggression toward heterospecific intruders was also higher in females, though both males and females approached

the intruder (without attacking) with similar frequencies (Fig. 4C; attack: $W = 28$, $N = 8$, $P = 0.016$; approach: $W = 8.5$, $N = 8$, $P = 0.75$). Mate-directed aggression was higher in females, (Fig. 2C, $W = 36$, $N = 9$, $P = 0.014$, ties = 1) suggesting that females were the dominant sex in these female-larger pairs. The egg clean mimic, which may play a role in submission, courtship, or both, was displayed more frequently by males (Fig. 2D, $W = 4$, $N = 9$, $P = 0.032$). There were no differences between males and females in the amount of time spent in the nest during control and intruder observations (Fig. 5A&C; control: $t_8 = 0.40$, $P = 0.70$; intruder: $t_6 = 0.68$, $P = 0.47$). Although not significant, when eggs were present, males spent more time in the nest than females (Fig. 3C; $t_7 = -1.98$, $P = 0.087$). Interestingly, comparison of behaviour when eggs are present to behaviour during control observations shows that the male-bias for nest-time with eggs present was due to a significant reduction of time spent in the nest by females, rather than an increase in the amount of time spent in the nest by males (Fig. 5A&B; females: $t_{14.6} = 2.51$, $P = 0.024$; males: $t_{10.3} = 0.088$, $P = 0.93$). There were no significant differences in the amount of egg care behaviours displayed by males and females (Fig. 3A&B; mouthing: $W = 19.5$, $N = 8$, $P = 0.39$, $n = 8$, ties = 1; fanning: $W = 5$, $N = 7$, $P = 1.0$).

Male-larger pairs

Male-larger *J. marlieri* pairs have not been reported in the wild, but were formed experimentally in group tanks containing only large males and small females. These male-larger pairs showed a reversal in the direction of sex-bias for certain behaviours. In these pairs, males showed more territorial aggression than females toward conspecific neighbours (Fig. 2A&B; attack: $W = 0$, $N = 9$, $P = 0.022$ ties = 2; approach: $W = 0$, $N = 9$, $P = 0.009$) as well as toward the heterospecific intruder (Fig. 4C; attack: $W = 1$, $N = 8$, $P = 0.034$, ties = 2; approach: $W = 0$, $N = 8$, $P = 0.059$, ties = 2). Males were the dominant sex, with a higher frequency of mate-directed aggression than females (Fig. 2C; $W = 3$, $N = 9$, $P = 0.042$, ties = 1). The egg clean mimic was displayed more frequently by females than males during control observations (Fig. 4C; $W = 36$, $N = 9$, $P = 0.014$, $n = 9$). For both control and egg care observations, females spent more time in the nest than males, although the difference is only marginally significant (control: Fig. 2F, $t_8 = 2.08$, $P = 0.07$; eggs: Fig. 5C, $t_6 = 1.96$, $P = 0.10$). Comparison of behaviour when eggs are present to behaviour during control observations shows that the female-bias for nest-time when eggs were present was due to 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 (Fig. 5A&C), however the differences between control observations and reproductive observations for time in nest were not significant for either sex (females: $t_{10.3} = -1.04$, $P = 0.32$; males: $t_{8.23} = 0.70$, $P = 0.50$). There were no significant differences in the amount of egg care behaviours displayed by males and females (Fig. 3A&B; mouthing: $W = 22$, $N = 9$, $P = 0.20$; fanning: $W = 3$, $N = 9$, $P = 0.58$, ties = 2).

Interactions between relative mate size and sex

There was no interaction between relative mate size and sex on territorial aggression, with both larger males and larger females attacking both conspecifics and heterospecifics at similar frequencies. However, for mate-directed attacks there was a non-significant trend for larger females to attack their mates more than the larger males did in the male-larger pairs ($W = 62$, $N = 9$, $P = 0.057$). Time in nest also showed an interaction between relative mate size and sex. During control observations, both larger and smaller females spent similar amounts of time in the nest, whereas males spent significantly less time in the nest when they were the larger of the pair (Fig. 5A, $t_{15.9} = 3.13$, $P = 0.006$). When there was an intruder present, there was not a significant sex difference in time spent in the nest for male-larger pairs (Fig 5C, $t_6 = 0.68$, $P = 0.52$), however both sexes in the male larger-pairs spent significantly less time in the nest than did their counterparts in the female-larger pairs during intruder observations (males: $t_{8.27} = 2.81$, $P = 0.021$; females: $t_{9.34} = 2.27$, $P = 0.048$).

Reproduction and parental behaviour

Behaviour measures for egg care observations were averaged between the first and second day after laying eggs and statistics were performed on these averages. The only significant difference found between the first and second day after laying eggs was that females in female-larger pairs approached their neighbours more on the first day ($W = 28$, $N = 8$, $P = 0.02$).

Aside from the onset of egg care behaviours, there were few differences between observations with eggs and without eggs. There was a non-significant trend toward an increased total number of attacks (male and female) between the control and the egg care observations (Fig. 4A&B; $W = 82$, $N_1 = 18$, $N_2 = 15$, $P = 0.057$). In addition, the larger individual of the pair spent more time outside of the nest when eggs were present than during the control trials (Fig. 5A&B, $t_{28.1} = 2.17$, $P = 0.038$). As might be expected, the egg clean mimic was observed less frequently while eggs were actually present ($W = 814.5$, $N_1 = 18$, $N_2 = 15$, $P = 0.0002$).

Differences in fecundity were tested between female-larger and male-larger pairs. Females in female-larger pairs laid more eggs on average than those in male-larger pairs (19 ± 15 vs. 9 ± 3 eggs), although this difference was not significant in this sample ($t_{7.5} = 1.76$, $P = 0.12$). 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. Surprisingly, amount of time spent in the nest was not correlated with amount of egg care behaviours (mouthing or fanning).

DISCUSSION

Territorial aggression and dominance were found to be female-biased in *J. marlieri*, but only for female-larger pairs. Reversal of size-ratio resulted in a reversal in the sex-bias of behaviour: territorial aggression and dominance became male-biased in male-larger pairs. This suggests that, in this species, sexual dimorphism of aggressive behaviour is contingent on the social environment (relative mate size) and is not determined strictly by gonadal sex. It remains to be tested whether this holds for other members of the genus *Julidochromis*, or for other monogamous cichlids. Awata and Kohda (2004) did not detect significant sex-bias in attacking rates towards conspecifics in female-larger and male-larger *J. ornatus* pairs in the field, although they did find a marginally significant female-bias in attacking rate, towards heterospecifics in female-larger pairs only. In our study, we found that attacking rates towards both heterospecific intruders and conspecifics was biased towards the larger fish regardless of sex. Discrepancies between our results and those of Awata and Kohda (2004) may be due to differences between *Julidochromis* species or due to different conditions between the lab and the field. For example, in our experimental set-up, a pair was in constant proximity to conspecifics, whereas in the field, pairs are not generally found in such close proximity to each other. This would explain why the *J. marlieri* in this study had a much higher attacking rate than the *J. ornatus* in the field (Awata & Kodha 2004).

A similar study by Itzkowitz et al. (2005) measured territorial aggression against conspecifics in male-larger, same-size, and female-larger pairs of brooding convict cichlids (*A. nigrofasciatum*). Reversal of size ratio resulted in a partial shift in aggression from males to females, males still spent more time near the intruder and had a higher attack rate than females even when the female was significantly larger (>2 cm). This suggests that sex-biased territorial behaviour in the convict cichlid is determined primarily by sex, and that although the expression of territorial behaviour can be modulated by the social environment, the degree of plasticity is less than is observed in *J. marlieri*.

Egg care behaviours were not consistently sex-biased for either pairing type, though time spent in the nest while eggs were present showed a trend of being biased towards the

smaller fish. However, this was due primarily to the larger fish spending more time outside the nest when eggs are present, rather than the smaller fish increasing its time spent in the nest. 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 a defensive display. This casual observation, in addition to the fact that time spent in the nest is not correlated with amount of egg care behaviour, suggests that time spent in the nest may not fully capture the details of parental care in this species or under this experimental paradigm.

In their field study on *J. ornatus*, Awata & Kohda (2004) found that parental care, as measured by time spent in the nest, was biased toward the smaller fish of the pair. Mouthing and fanning were not measured due to difficulty of observation in the field. In a laboratory study on *J. transcriptus*, Awata et al. (2006) were able to measure mouthing and fanning due to the use of artificial nests (as used here). Similar to the present study, they did not find a significant sex-bias in mouthing or fanning in monogamous pairs, but did find that these behaviours were highly female-biased or male-biased in polygynous and polyandrous trios, respectively.

Female fecundity was found to be positively correlated with absolute female body size. In this study, males were of similar absolute size in both female-larger and male-larger pairs. We did not give males the option of pairing with either a large female or a small female, but the positive correlation between fecundity and female body size suggests that males would have a fitness advantage if they paired with larger females. This is consistent with the prevalence of female-larger pairs in the wild. 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 were found in this species (Awata & Kohda 2004).

CONCLUSION

Female-biased territorial aggression and dominance was confirmed for *J. marlieri*, but only in female-larger pairs. In pairs where the males were larger than their mates, territorial aggression and dominance were male-biased. This suggests that species-typical sexual dimorphism in aggressive behaviour in *J. marlieri* is likely a product of the species-typical sexual size dimorphism and preference for forming female-larger pairs. Overall, our results suggest that sexual dimorphism in aggressive behaviour is not determined by sex for *J. marlieri*, but is plastic and dependent on the social environment.

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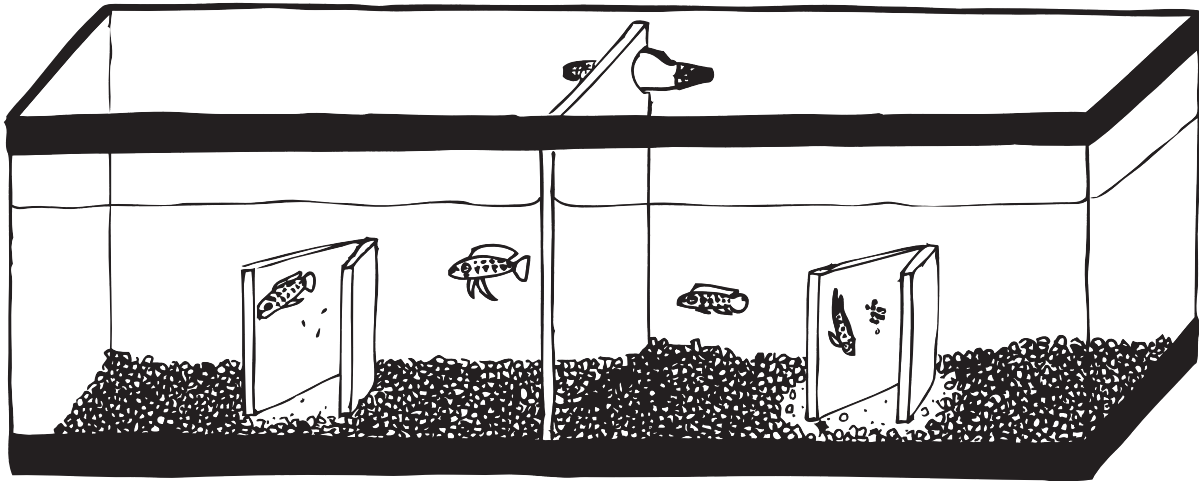


Figure 1. Observation tank with two pairs and their nests, separated by a clear divider.

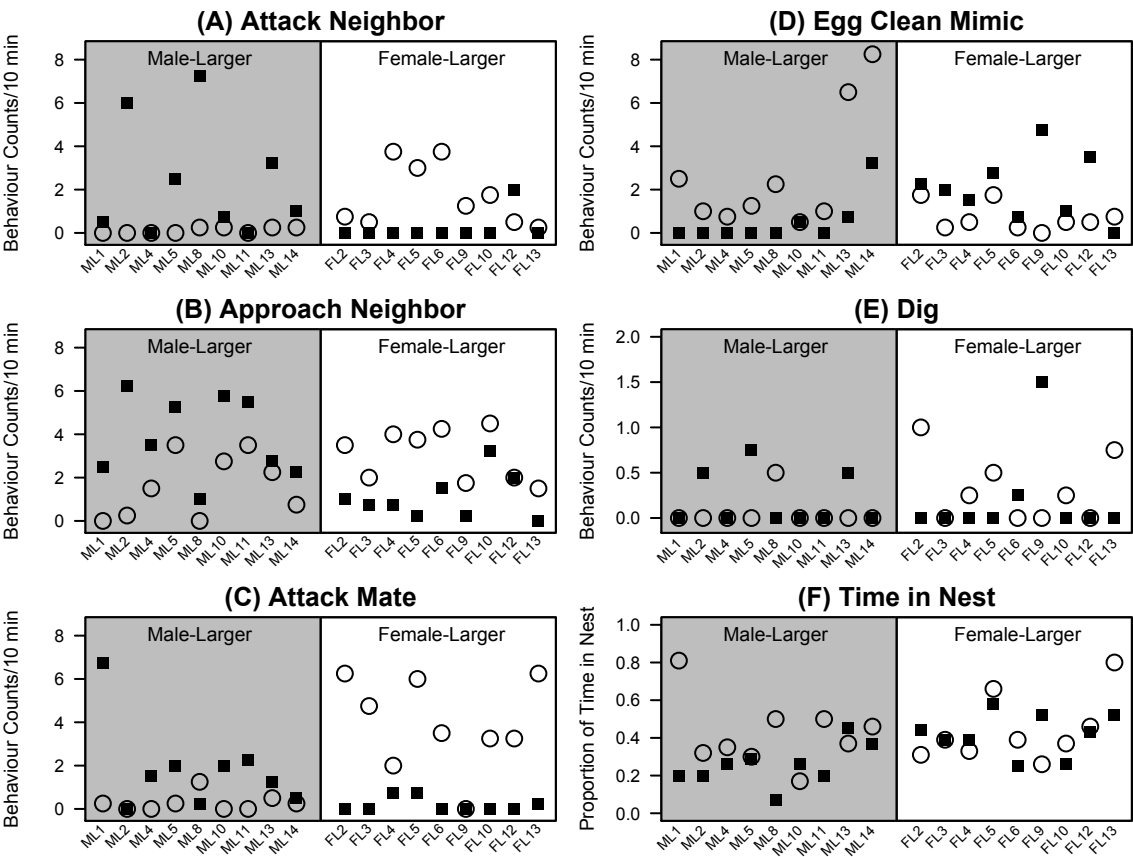


Figure 2. Behaviour data for territory defence (A&B), mate interaction (C&D), nest maintenance (E) and time in nest (F) from males (■) and females (○) during control observations. X-axis labels indicate pair ID (see Tables 1&2). Y-axis values indicate behaviour averages from four 10-minute observations. Pairs are ordered according to male to female size ratio (Tables 1&2).

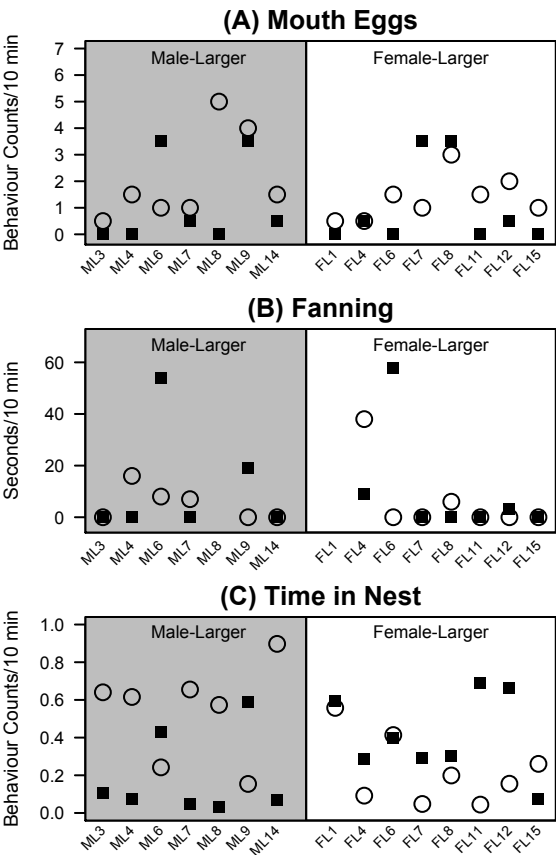


Figure 3. Behaviour data for egg care (A&B) and time in nest (C) from males (■) and females (○) during egg care observations. X-axis labels indicate pair ID (see Tables 1&2). Y-axis values indicate behaviour averages from the first two days after spawning. Fanning not recorded for ML8 or FL1. Pairs are ordered according to male to female size ratio (Tables 1&2).

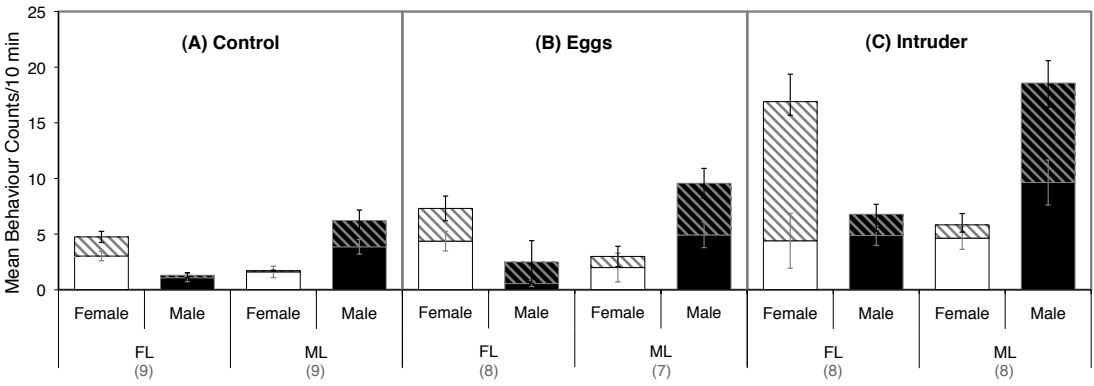
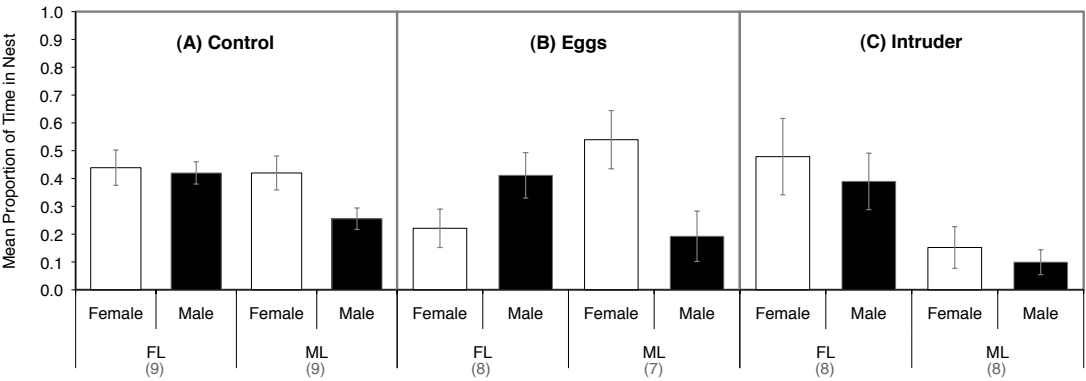


Figure 4. Comparison of territorial aggression between control (A), egg care (B), and intruder (C) observations for female-larger (FL) and male-larger (ML) *J. marlieri* pairs. Attacks (against neighbours or intruder) are represented by diagonal patterned bars, approaches towards neighbours or intruder (without attacking) are represented by unpatterned bars. Error bars show standard error. Numbers in parentheses indicate sample size.

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Figure 5. Comparison of time in nest between control (A), egg care (B), and intruder (C) observations for female-larger (FL) and male-larger (ML) *J. marlieri* pairs. Error bars show standard error. Numbers in parentheses indicate sample size.