



## Female, but not male, agonistic behaviour is associated with male reproductive success in stable bluebanded goby (*Lythrypnus dalli*) hierarchies

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### Abstract

In many social species, there are important connections between social behaviour and reproduction that provide critical insights into the evolution of sociality. In this study, we describe associations between agonistic behaviour and male reproductive success in stable social groups of bluebanded gobies (*Lythrypnus dalli*). This highly social, sex-changing species forms linear hierarchies of a dominant male and multiple subordinate females. Males reproduce with each female in the harem and care for the eggs. Since aggression tends to be associated with reduced reproduction in social hierarchies, we hypothesized that males in groups with high rates of aggression would fertilise fewer eggs. We also hypothesized that a male's agonistic behaviour would be associated with his reproductive success. Dominants often exert substantial control over their harem, including control over subordinate reproduction. To address these hypotheses, we quantified egg laying/fertilisation over 13 days and observed agonistic behaviour. We show that there was a significant, negative association between male reproductive success and the total rate agonistic interactions by a group. While no male behaviours were associated with the quantity of eggs fertilised, female agonistic behaviour may be central to male reproductive success. We identified a set of models approximating male reproductive success that included three female behaviours: aggression by the highest-ranking female and approaches by the lowest-ranking female were negatively associated with the quantity of eggs fertilised by males in their groups, but the efficiency with which the middle-ranking female displaced others was positively associated with this measure. These data provide a first step in elucidating the behavioural mechanisms that are associated with *L. dalli* reproductive success.

### Keywords

social hierarchy, agonistic behaviour, reproductive success, individual variation, agonistic efficiency, fish.

## **1. Introduction**

In social groups, the behaviour of group members has robust connections to reproduction. The complex interactions between social structure, behaviour and reproductive success frequently make it difficult to elucidate mechanistic links among these three factors, yet these mechanisms are critical to understanding the evolution of sociality. Many phylogenetically diverse social species organize in hierarchies, and there are conserved patterns of behaviour and reproduction within hierarchies (Wilson, 1980; Ellis, 1995; e.g., insects: Trunzer et al., 1999; fishes: Heg & Hamilton, 2008; birds: Nelson-Flower et al., 2013; mammals: Silk, 2007), making this social structure a useful model for understanding the links between behaviour and reproduction. Species vary in the mechanisms by which status is achieved (e.g., size, age, aggression, maternal rank), but dominance is almost always categorized as a product of agonistic interactions between group members that consistently result in the subordinate member yielding to the dominant (Wilson, 1980; Drews, 1993). Agonistic behaviour is an important subset of social behaviour that encompasses interactions related to fighting, including aggression, conciliation and retreat (Wilson, 1980), and is critical to the establishment and maintenance of the social hierarchy. Behaviours vary in a predictable way within a dominance hierarchy. Across ranks, individuals will display differences in agonistic behaviour such as aggressive and submissive behaviours (Clarke & Faulkes, 2001; Forkman & Haskell, 2004; Rodgers et al., 2007; Cleveland & Lavalli, 2010; Wong & Balshine, 2011), as well as personality traits such as boldness-shyness and activity levels (Colléter & Brown, 2011; Dahlbom et al., 2011). Although dominance is often achieved through aggression or fighting, it is not necessarily the case in stable hierarchies that dominants remain the most aggressive (Drews, 1993; e.g., Rodgers, 2007).

Hierarchical social structure also has important consequences for the ways in which individuals in the group reproduce. Dominant group members typically have higher reproductive success (Ellis, 1995; Johnstone, 2000), and the mechanisms by which that success is achieved vary across species. In extreme cases of reproductive skew, only dominants breed and subordinates are reproductively suppressed (Barrett et al., 1993; Clarke & Faulkes, 2001; White et al., 2002; Fitzpatrick et al., 2008). In other species, subordinates may be capable of reproduction but have fewer opportunities to mate, produce fewer offspring, and/or experience higher rates of abortion, egg loss, or offspring mortality (Trunzer et al., 1999; van Noordwijk & van Schaik,

1999; East & Hofer, 2001; Young et al., 2006; Robbins et al., 2007; Heg & Hamilton, 2008; Henry et al., 2013). In some of these cases, the mechanisms responsible for higher dominant reproductive success directly involve status differences in agonistic behaviour. For example, intrasexual aggression directed at subordinate Southern pied babblers (*Turdoides bicolor*) (Nelson-Flower et al., 2013), meerkats (*Suricata suricatta*) (Young et al., 2006) and golden lion tamarins (*Leontopithecus rosalia*) (Henry et al., 2013) seems to be directly related to reduced subordinate reproductive success.

In harems, social groups consisting of a dominant male and the multiple females with which he reproduces, intersexual agonistic interactions may be central to reproduction for several reasons. First, males are capable of interfering in aggressive interactions between females that might compromise his reproductive success. The male may aggressively disrupt the females and, in some cases, defend one female preferentially from the other (Schradin & Lamprecht, 2000; Kahlenberg et al., 2008). Males that are more effective at maintaining low levels of group agonism may, therefore, positively impact their reproductive success. Second, while the agonistic behaviour of each individual in a social group influences the broader pattern of group agonism (Flack et al., 2005; Pike et al., 2008; Hamilton & Ligoeki, 2012), male agonistic behaviour may be especially influential because of their top social position and, in some species, their role as a ‘conflict managers’ (Flack et al., 2005). Third, in harem species with exclusive paternal care, males aggressively defend their nests from predators including, in some species, females in the harem. For example, female gobies that gain access to the male’s nest will consume eggs, even if she is spawning (Okuda et al., 2002). Finally, parenting males may reject females attempting to enter the nest to spawn. When nest space is limited, male blennies can respond aggressively to courting females (Almada et al., 1995; Lengkeek & Didderen, 2006).

The links between agonistic behaviour and reproduction are complex and highly context-specific, so it is critical to quantify both behaviour and reproduction in order to draw valid conclusions about the associations between the two. For example, dominant banded mongooses (*Mungos mungo*) aggressively suppress subordinate reproduction; however, there is a reproductive cost of aggression for dominants (Bell et al., 2011). Subordinates may also be able to ameliorate the apparent costs of low status. Subordinate female cichlids (*Neolamprologus pulcher*) that alloparent can obtain breeding space for themselves (Heg et al., 2009), and subordinate males of many fish

species adopt alternative reproductive strategies such as sneaking (Taborsky, 1994).

The goal of this study was to investigate the associations between natural variation in agonistic behaviour, at all levels of the hierarchy, and male reproductive success, in stable social groups of bluebanded gobies (*Lythrypnus dalli*). This highly social, sex changing fish forms harems of a large, dominant male and multiple subordinate females. The females within a harem form a linear hierarchy that is influenced by size, and agonistic interactions occur frequently among group members (Reavis & Grober, 1999; Black et al., 2005; Rodgers et al., 2007). As the dominant fish in the harem, males can interfere in female–female agonistic interactions. Males reproduce with each female in the harem, resulting in reproductive success that is multiple times higher than females. Multiple females can lay eggs in the male's nest within short time intervals, and males provide sole parental care for the eggs until they hatch. Paternal care involves rubbing/fanning the eggs and aggressively defending the nest from predators, including conspecific females that try to cannibalise the eggs. Males are highly selective in allowing females into the nest (Behrents, 1983) and will refuse access even to very gravid females (Solomon-Lane & Grober, unpublished data). This is an interesting system in which to investigate links between individual behaviour and reproduction because *L. dalli* exhibits a high degree of social and sexual plasticity. Sex is socially regulated such that the dominant fish in a social group is always male (Reavis & Grober, 1999; Rodgers et al., 2007). Over a lifetime, therefore, an individual can be both female and male and may occupy multiple social statuses.

Although *L. dalli* agonistic and reproductive behaviour is well documented in a number of contexts (Behrents, 1983; Black et al., 2005; Drilling & Grober, 2005; Lorenzi et al., 2006; Rodgers et al., 2007), reproductive success has yet to be quantified in parallel with behaviour. Based on associations between agonistic behaviour and reproduction in other hierarchical and harem species, we had two *a priori* hypotheses about *L. dalli* behaviour and reproduction. First, we hypothesised that in stable *L. dalli* social groups, rates of agonistic interaction among members of the social group would be negatively associated with male reproductive success. Second, we hypothesized that male agonistic behaviour would be associated with his reproductive success. This descriptive study is a critical step to designing targeted, manipulative experiments that test the reproductive consequences of behaviour and/or the behavioural consequences of reproductive function.

## 2. Materials and methods

### 2.1. Study organism and social groups

*Lythrypnus dalli* is a small (standard length (SL) 18–50 mm), marine fish that undergoes socially regulated, bidirectional sex change (Reavis & Grober, 1999; Rodgers et al., 2007). This species lives on rocky reefs in the Pacific Ocean, from Morro Bay, CA, USA to as far south as the Galapagos Islands, Ecuador (Miller & Lea, 1976; Béarez et al., 2007). Reproduction occurs between April and September, during which time the population is female-biased (Wiley, 1976; Drilling & Grober, 2005). Under natural conditions, *L. dalli* live in mixed-sex social groups of varying sizes, from small, isolated groups (3–10 fish, similar in size to laboratory groups) to aggregations that reach densities of 120 fish/m<sup>2</sup> (Steele, 1996). Males are territorial and defend an area that encompasses his harem of females and his nest. Males reproduce with each female in the harem and provide sole parental care for the eggs (Behrents, 1983; St. Mary, 1993). Like other goby species (Tamada, 2008), multiple *L. dalli* females can lay eggs in a male's nest within short time intervals. Males, therefore, frequently care for multiple egg clutches that are at different developmental stages. High ranking females tend to be highly site attached, reproducing primarily with the resident male, while lower ranking females move more frequently among male territories (Lorenzi, 2009).

We collected *L. dalli* from reefs offshore of Santa Catalina Island, California during the reproductive season (July, California Fish and Game permit No. SC-10676). The fish were brought immediately to a holding tank (197 l, 60 × 94 × 35 cm) at the Wrigley Institute for Environmental Studies (Catalina Island, University of Southern California). The holding tank and water tables for the social groups were continuously supplied with natural seawater and exposed to a natural light cycle. We fed the fish brine shrimp twice a day. The fish used in this experiment were in the holding tank for a maximum of 3 weeks and were not released following the study. To form social groups of specific sizes and sex ratios, we briefly anesthetized the fish in tricaine methanesulfonate (MS-222; 500 mg/l salt water) before measuring SL and determining sex based on genital papilla morphology (St. Mary, 1993). We then established 10 social groups of 4 fish each: 1 large male and 3 females of varying sizes. All fish were at least 3 mm SL smaller than the next largest fish to facilitate the rapid establishment of social status (Reavis & Grober, 1999). These laboratory social groups of 1 male and 3 females are

comparable in size, sex ratio, and density to social groups naturally found on the reef (Lorenzi, 2009).

## 2.2. *Behavioural observations*

We observed agonistic behaviour 3 times during the experiment, beginning 8 days after group establishment to ensure the hierarchy was stable (Reavis & Grober, 1999). All behavioural observations were conducted in the morning and lasted for 10 min per group. The analyses presented focus on asymmetrical agonistic interactions including approaches, when one fish swims directly towards another fish within 2 body lengths, and displacements, a response to an approach in which the approached fish swims away. Displacements (i.e., the number of times a fish displaces a group member) are a measure of aggression, and being displaced is a signal of submission by subordinates (Rodgers et al., 2007). Approaches and displacements are easily quantified in lab and field *L. dalli* social groups (Black et al., 2005; Rodgers et al., 2007), have provided important insight into *L. dalli* sex, social status, and sex change in previous studies (Reavis & Grober, 1999; Lorenzi et al., 2006; Rodgers et al., 2007), and are frequently quantified in other social animals (Sommer et al., 2002; Aragón et al., 2006; Sneddon et al., 2006; Cronin & Field, 2007; Graham & Herberholz, 2008; Evers et al., 2011). In addition to these directly observed behavioural interactions, we calculated a second-order behavioural characteristic for each individual that we call agonistic efficiency, or the proportion of approaches that led to a displacement (displacements/approaches).

## 2.3. *Quantifying reproduction*

In each social group, males were given a PVC tube ‘nest’ (15.2 cm length, 1.9 cm diameter). Females lay eggs in a single layer on a sheet of acetate lining the inside of the nest tube, and males externally fertilise the eggs and then care for them until hatching. We checked the nest tube once daily, at the same time each morning, for the presence or absence of eggs. If eggs were present, we removed the acetate sheet from the nest tube and acquired a digital image, using a ruler for scale. Within 3 min, we returned the eggs to the nest, and males immediately resumed parenting.

We estimated male reproductive success as the quantity of eggs fertilised by the male. Based on the precedent established previously in gobies (Forsgren et al., 1996; Lindström, 1998; Svensson et al., 2010), we quantified

fertilised eggs as an area covered ( $\text{cm}^2$ ) using ImageJ software (Rasband, W.S., ImageJ, U.S. National Institutes of Health, Bethesda, MD, USA, available online at <http://imagej.nih.gov/ij/>) and digital images from the first day a clutch of eggs appeared in the male's nest. Images on subsequent days were used to qualitatively verify that the eggs laid in the male's nest were fertilised and went on to develop and hatch. With the exception of one clutch for one male, all clutches that were laid were fertilised and cared for until hatching.

#### 2.4. Statistics

Statistics were performed using JMP 7.0, and GraphPad Prism was used to make all figures. Results were considered significant at the  $p < 0.05$  level, and the data presented in the text are mean  $\pm$  SEM. For all behavioural analyses, rates of behaviour were averaged from the 3 observations conducted and are presented as behaviours per min. Among-status differences in approaches and displacements were analysed using a one-way analysis of variance (ANOVA, two-tailed) following a natural log transformation to normalize the data. Among-status differences in agonistic efficiency were analysed using a one-way ANOVA following a logit transformation to normalize the data. *Post hoc* analyses of significant ANOVA results were conducted using the Tukey HSD test.

We used linear regression analysis to identify associations between the quantity of eggs fertilised and agonistic behaviour in the social group, including total approaches (calculated as the sum of male, alpha, beta, and gamma approaches) and total displacements. To correct for these two linear regression analyses (total approaches, total displacements) on the same dataset (quantity of eggs fertilised), we applied a correction factor to our alpha level and considered  $p < 0.025$  significant ( $0.05/2$ ). We used multiple linear regression analysis to describe the contribution of male agonistic behaviour to the quantity of eggs he fertilised. We included male displacements, which were positively associated with male approaches, and male agonistic efficiency as predictor variables.

We used Akaike's information criterion (AICc, corrected for small sample size) to investigate associations between female agonistic behaviours and male reproductive success. This analysis identifies a set of models that approximate male reproductive success (Burnham & Anderson, 1998; Symonds & Moussalli, 2010). The best models have the lowest AICc score. We calculated the difference in AICc score between each model and the best

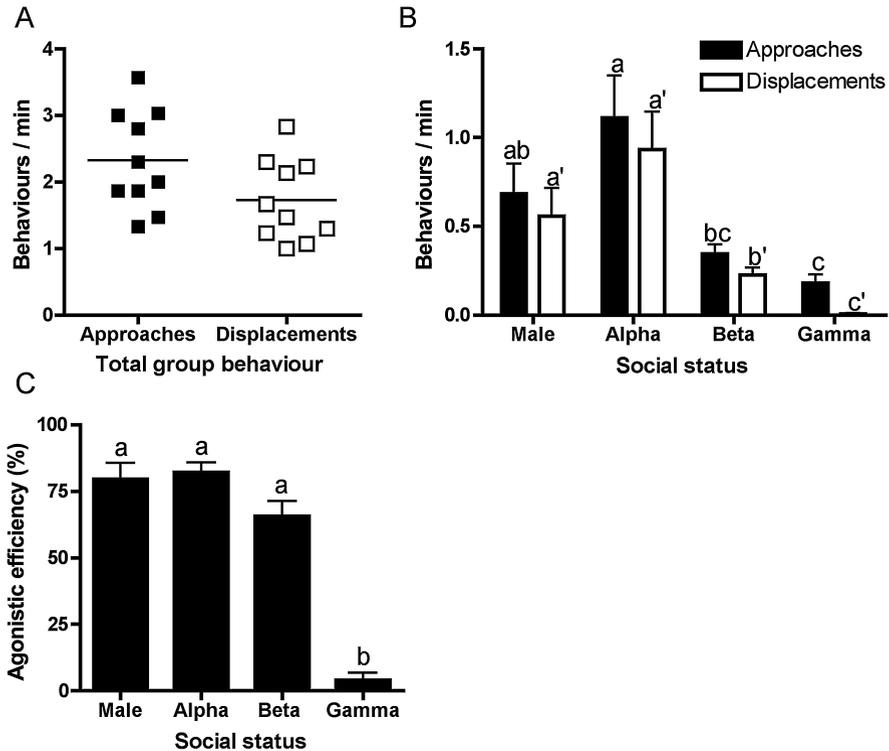
model ( $\Delta_i$ ), and then we rejected unlikely models based on the value of  $\Delta_i$ . Models with  $\Delta_i < 2$  were considered equally as good as the model with the lowest AICc score. We were less confident in models of  $\Delta_i < 6$ , but these models should still be taken into consideration. Models of  $\Delta_i > 6$  were rejected as sufficiently less plausible than the set of best models (Symonds & Moussalli, 2010). We also report the goodness of fit for each potential model ( $r^2$ ). We included alpha displacements, alpha agonistic efficiency, beta displacements, beta agonistic efficiency, and gamma approaches as predictor variables. We did not include alpha or beta approaches because both are positively associated with displacements. We did not include gamma displacements or agonistic efficiency because, as the lowest ranking group member, there were not a sufficient number of non-zero values for statistical analysis.

### 3. Results

#### 3.1. The quantity of eggs fertilised and agonistic behaviour vary among social groups

At least one clutch of eggs was laid in every male's nest, with a maximum of four clutches laid over 13 days (average  $2.73 \pm 0.36$  egg clutches per group, average clutch size  $8.00 \pm 0.72$  cm<sup>2</sup>). The quantity of eggs laid ranged from 8.65 to 33.99 cm<sup>2</sup> (average  $19.55 \pm 2.45$  cm<sup>2</sup>), and because one male did not fertilise or care for his one clutch, male reproductive success was quite variable, ranging from 0 to 33.99 cm<sup>2</sup> (average  $18.41 \pm 3.17$  cm<sup>2</sup>).

Agonistic behaviour also varied among groups (Figure 1A), with important differences among the status classes (Figure 1B, C). Rates of approaches ( $F_{3,34} = 13.00$ ,  $p < 0.0001$ ) differed significantly among males, alphas, betas, and gammas. *Post hoc* tests showed that alphas approached significantly more than betas ( $p = 0.0068$ ) and gammas ( $p < 0.0001$ ) but did not differ from males ( $p = 0.79$ ). Males approached significantly more than gammas ( $p = 0.0003$ ) but did not differ from betas ( $p = 0.082$ ). Betas and gammas did not differ in rates of approaches ( $p = 0.12$ ) (Figure 1B). Rates of displacement differed significantly across social status in a pattern similar to approaches ( $F_{3,27} = 15.93$ ,  $p < 0.0001$ ). *Post hoc* testing showed that males and alphas displaced at similar rates ( $p = 0.68$ ), and both displaced significantly more than beta (males:  $p = 0.019$ ; alphas:  $p = 0.0008$ ) and gamma (males:  $p < 0.0001$ ; alphas:  $p < 0.0001$ ). Betas also displaced significantly

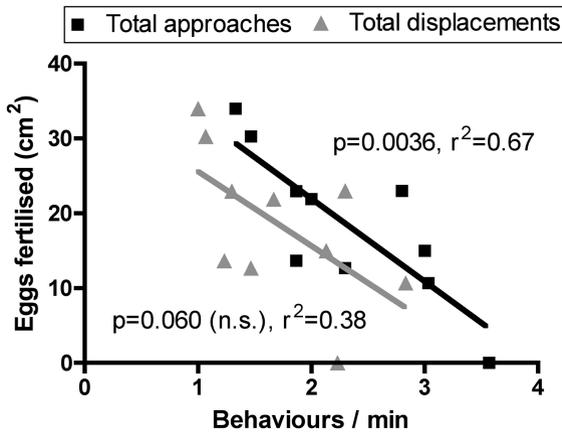


**Figure 1.** (A) Frequency of approaching and displacing in the social group ( $N = 10$ ). Total approaches and displacements refer to the sum of male, alpha, beta and gamma behaviour. The horizontal line indicates the mean. (B) Mean ( $\pm$  SEM) frequencies of approaches and displacements for each social status within a social group. (C) Mean ( $\pm$  SEM) agonistic efficiency (displacements/approaches) for each social status within a social group (male,  $N = 9$ ; alpha,  $N = 10$ ; beta,  $N = 10$ ; gamma,  $N = 9$ ). Different letters indicate significant differences.

more than gammas ( $p = 0.0096$ ) (Figure 1B). Agonistic efficiency also differed significantly among status classes ( $F_{3,25} = 7.86$ ,  $p = 0.0007$ ). *Post hoc* tests showed that males ( $p = 0.0078$ ), alphas ( $p = 0.0005$ ), and betas ( $p = 0.025$ ) all had significantly higher agonistic efficiency than gammas (Figure 1C).

### 3.2. Agonistic behaviour and male reproductive success

Total group approaches were significantly and negatively associated with the quantity of eggs fertilised by the male ( $F_{1,8} = 16.54$ ,  $r^2 = 0.67$ ,  $p = 0.0036$ ), and there was a negative trend with total displacements ( $p = 0.06$ ) (Fig-



**Figure 2.** Total approaches (sum of male, alpha, beta and gamma approaches) were significantly and negatively associated with the quantity of eggs fertilised by the male. There was a trend for a negative association between total displacements (sum of male, alpha, beta, and gamma displacements) and the quantity of eggs fertilised by the male.

ure 2). Interestingly, multiple regression analysis showed male behaviour, including displacements and agonistic efficiency, was not predictive of male reproductive success ( $F_{2,6} = 0.67$ ,  $r^2 = 0.18$ ,  $p = 0.55$ ). Using AICc, we identified a set of models of female agonistic behaviour that approximated male reproductive success (Table 1). The best models ( $\Delta_i < 2$ ) included alpha displacements, which were negatively associated with male reproductive success (Figure 3A), and beta agonistic efficiency, which was positively associated with male reproductive success (Figure 3B), independently and together. Gamma approaches, which were negatively associated with male reproductive success, may also be an important predictor (Figure 3C).

#### 4. Discussion

In this study, we show that male *L. dalli* fertilised fewer eggs in social groups with higher rates of agonistic interaction (Figure 2); however, male agonistic interactions with the females in his harem were unrelated to his reproductive success. Given that the remaining agonistic interactions in the social group were initiated by females, we conducted *post hoc* analyses of female agonistic behaviour and identified multiple associations with male reproductive success (Table 1, Figure 3). Thus, female rather than male agonistic behaviour seems to drive patterns of reproduction in *L. dalli* social groups. Nine

**Table 1.**

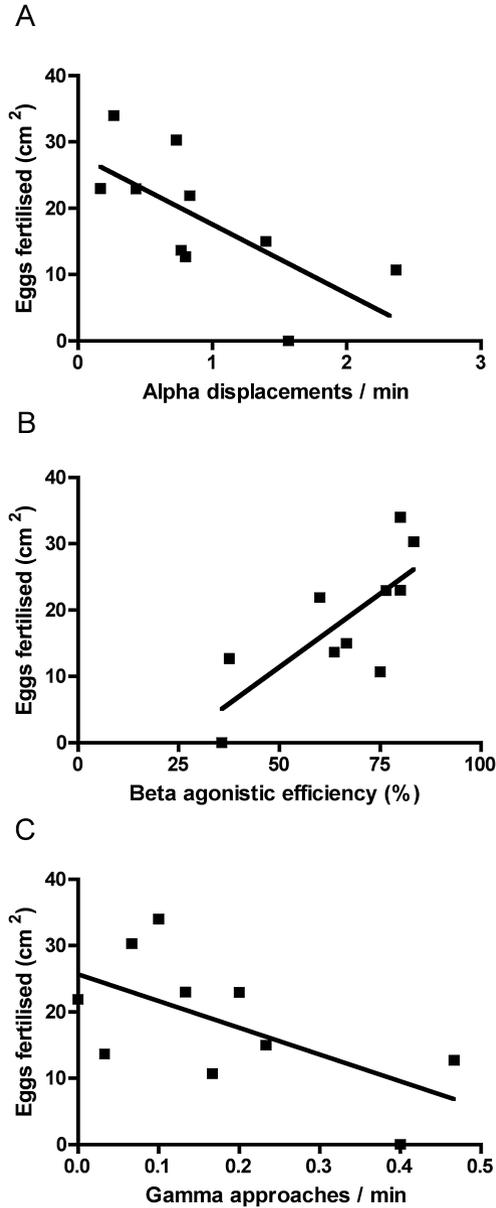
Candidate regression models ranked by Akaike's information criterion (AICc) examining the effect of female agonistic behaviour on male reproductive success.

	Candidate model	$k$	AICc	$\Delta_i$	$r^2$
1	<i><math>\alpha</math> displ; <math>\beta</math> AgEf</i>	4	71.89	0	0.76
2	<i><math>\alpha</math> displ</i>	3	73.31	1.41	0.50
3	<i><math>\beta</math> AgEf</i>	3	73.54	1.65	0.48
4	$\alpha$ displ; $\gamma$ appr	4	75.94	4.05	0.64
5	$\gamma$ appr	3	76.43	4.54	0.31

Models in italics ( $\Delta_i < 2$ ) represent the best models. Models of  $\Delta_i > 6$  were rejected.  $\alpha$ , alpha females;  $\beta$ , beta females;  $\gamma$ , gamma females; appr, approaches; displ, displacements; AgEf, agonistic efficiency.

out of 10 males in this study fertilised and cared for each of the clutches laid in their nest. This suggests that a primary source of variation among males is the quantity of eggs laid in the male's nest, although parenting behaviour and nest defence can also affect the quantity of eggs that hatch. In the cichlid *Neolamprologus pulcher* (Heg, 2008) and the angelfish *Centropyge bicolor* (Ang & Manica, 2010), dominant females lay more frequently than subordinate females, suggesting that intrasexual aggression in *L. dalli* groups might reduce the frequency of subordinate beta and/or gamma laying. Although we did not quantify female reproductive success in this study, the mechanisms underlying female reproductive success will ultimately be critical to understanding the reproductive success of male *L. dalli*. In addition to laying less frequently, agonism in the social group could affect the number of females that have opportunities to lay eggs and/or the number of eggs laid per clutch.

We hypothesized that male agonistic behaviour would be central to explaining the variation in male reproductive success. Male *L. dalli* are the dominant members of the harem and are capable of disrupting female–female aggression in the harem by displacing the involved females. Males also provide sole parental care for eggs and aggressively defend access to their nest from females attempting to spawn, cannibalise eggs, or both. However, we found that male behaviour, including displacements and agonistic efficiency, did not predict the quantity of eggs fertilised. In contrast, female behaviour accounted for a substantial portion of the variation in male reproductive success (Table 1, Figure 3). The centrality of female agonistic



**Figure 3.** (A) Alpha displacements, (B) beta agonistic efficiency (displacements/approaches) and (C) gamma approaches were identified using Akaike's information criterion as female agonistic behaviours that alone, and in combination, best approximate the quantity of eggs fertilised by the male in their social group (see Table 1 for models).

behaviour to male reproductive success is consistent with *L. dalli* natural history. When the male is in his nest parenting and/or defending his territory, he is physically separated from the females in his group, all of which occupy a shared space (Behrems, 1983). As a result, particularly for males that interact with their females less frequently and spend more time in their nest, high-ranking females may set the agonistic tone of the group (Solomon-Lane & Grober, unpublished data).

These data do not rule out a role for male behaviour in the regulation of his own reproductive success, however. The behaviour of an individual in a social group is not independent from the behaviour of other group members (Grosenick et al., 2007; Valone, 2007). Similar to the ways in which the behavioural phenotype of an individual three-spined stickleback (Pike et al., 2008), cichlid (Hamilton & Ligoeki, 2012), or pigtailed macaque (Flack et al., 2005) affects the broader social dynamics of its group, male *L. dalli* behaviour likely impacts the social network of his group, as well as the behavioural expression of females in the harem. We did not have the statistical power in this study to determine whether male agonistic behaviour was indirectly associated with reproductive success via an effect on influential female behaviours, but this is an important hypothesis to test in the future. In addition, agonistic behaviours are a subset of behaviours expressed by *L. dalli*, and male courtship, parenting, and/or nest defence could also influence male reproductive success.

Our analyses identified 5 potential models of female agonistic behaviours that approximated male reproductive success (Table 1). Three different female behaviours, alone and in combination, were associated with the variation in fertilised eggs (Table 1): alpha displacements (Figure 3A) and gamma approaches (Figure 3C) were negatively associated, and beta agonistic efficiency (Figure 3B) was positively associated, with the quantity of eggs fertilised by the male. The best model (lowest AICc) that accounted for the largest variation in male reproductive success ( $r^2$ ) included alpha displacements and beta agonistic efficiency (Table 1). Although we are cautious not to over interpret the importance of these specific patterns, these results are interesting for a number of reasons.

First, the behaviour of females in multiple status classes in the hierarchy was associated with male reproductive success. The alpha status lends itself to being an important influence on the group because she is the most dominant female in the hierarchy and the fish with the highest average rates of

aggression (Figure 1B). When the male is in his nest, alpha behaviour is left unchecked by the more dominant male. Therefore, alpha agonistic behaviour could negatively affect male reproductive success through a classic scenario of subordinate suppression (e.g., Heg, 2008), whereby aggression directed at the beta and gamma reduces their reproductive contribution.

The importance of the beta and gamma, the middle and lowest ranking females, however, suggests that the behavioural and reproductive dynamics of the social group are more complex. Analyzing the social network characteristics of *L. dalli* social groups (e.g., Dey et al., 2013; Wey et al., 2013) is a promising method to incorporate behavioural complexity into our understanding of individual reproductive success (Wey et al., 2008). In particular, this type of analysis will allow for a better understanding of the social context within which non-dominant female agonistic behaviour is expressed and, therefore, how it might affect reproduction. In addition, identifying the targets of agonistic behaviour will elucidate whether agonism within specific dyads underlies the associations we identify between female behaviour and male reproductive success. For example, high beta agonistic efficiency with alpha, a higher ranked female, likely has different social network and reproductive consequences than high agonistic efficiency with gamma, a lower ranking female. Similarly, gamma approaches and alpha displacements may have specific targets. The descriptive nature of this study made it inappropriate for hypothesis testing using network analysis techniques (see Croft et al., 2011), but these data have generated important hypotheses to be tested using these techniques in future studies.

Second, it is interesting that there were both positive and negative associations between female behavioural characteristics and reproduction (Figure 3). This highlights that agonistic interactions in the group are not inherently detrimental to male reproductive success; as a highly social and hierarchical fish, *L. dalli* interact frequently both in the lab and in the field (Black et al., 2005; Rodgers et al., 2007). Although alpha displacements and gamma approaches may be associated, directly or indirectly, with lower male (and potentially female) reproductive success, the importance of beta agonistic efficiency suggests that particular kinds of agonistic interactions — approaches that predictably lead to displacements — can be indicative of groups with a highly reproductively successful male when expressed in the right social status context. Betas achieve high agonistic efficiency by directing their approaches to fish that routinely submit to them: gamma. Therefore,

a beta with low agonistic efficiency either approached alphas and males more frequently and/or approached gammas in a manner that failed to elicit a displacement.

The positive and negative associations between behaviour and reproduction also suggest that, as in many other hierarchical species (e.g., Fedigan et al., 2008), the reproductive strategies of fish at different statuses might conflict. Male *L. dalli* maximize reproductive success by maximizing reproduction with each female in his harem, but the negative association between alpha aggression and male reproductive success suggests alphas might benefit by suppressing her subordinates. This suppression may allow alpha to monopolize reproductive opportunities and/or nest space and would indicate a direct conflict between male and alpha reproductive success. On the reef, however, males might tolerate aggressive alphas because of the potential social benefits: alpha aggression might affect cohesion among females in the harem while the male is in his nest. Alternatively, aggression might also compromise her reproductive success. In this case, alpha aggression could be adaptive if she emphasizes future rather than current reproduction (e.g., Kokko & Johnstone, 1999; Field et al., 2006). For example, aggressive alphas may have more opportunities to ascend in status and change sex.

Third, and finally, these data suggest that simple behavioural composites such as agonistic efficiency, which provide a nuanced perspective on commonly measured behaviours (approaches and displacements) (Sommer et al., 2002; Aragón et al., 2006; Sneddon et al., 2006; Cronin & Field, 2007; Graham & Herberholz, 2008; Evers et al., 2011), may be very informative in understanding behaviour and reproduction in social groups across species. For example, that the male, alpha and beta all have high agonistic efficiency (Figure 1C) demonstrates that, independent of rates of behaviour, *L. dalli* approaches are directed primarily at lower ranking fish. We might have reasonably predicted, however, that agonistic efficiency would be highest in males and then decrease with rank in the hierarchy. Therefore, although agonistic efficiency is a simple metric, it provides insights into group structure and, in this study, reproductive success, that were not gleaned from analysis of individual behaviours (e.g., beta approaches or displacements). In cichlids, a related ratio also provided insight into the action of neurochemicals (Reddon et al., 2012) and steroid receptor expression (O'Connor et al., 2013) involved in the regulation of social behaviour. In *L. dalli* social

groups, we do not anticipate that the association between beta agonistic efficiency and male reproductive success is causal. Instead, we hypothesize that beta agonistic efficiency acts as an indicator of the group's social dynamic such that reproductively successful groups allow for beta to socially navigate more successfully. Alternatively, beta's ability to navigate socially could create an environment in which reproduction is facilitated. To our knowledge, approaches and displacements have not been presented as this ratio in the literature before, although other ratios (Reddon et al., 2012; O'Connor et al., 2013) and measures of "dominance success" have been described (de Vries et al., 2006).

#### *4.1. Conclusions and future directions*

In this study, we show for the first time in *L. dalli* that variation in agonistic behaviour across stable social groups was associated with male reproductive success and that female agonistic behaviour likely plays a central role in the relationship between behaviour and reproduction. These data provide a critical foundation for testing hypotheses about (1) the direction of causation between agonistic behaviour and reproductive success (e.g., Heg & Hamilton, 2008), (2) how specific agonistic behaviours relate to each other and to the reproductive success of *L. dalli* males and females, (3) the mechanistic regulation of behaviours important to fitness and (4) selection pressures acting on behaviour and social decision-making. This study suggests that future investigations with *L. dalli*, a highly social, sex changing species in which behaviour and reproduction are simple to quantify and social context can be manipulated in ethologically relevant ways, could elucidate fundamental aspects of the fitness consequences of sociality.

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