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Simultaneous courtship and parenting in males and sex role reversal in females of the haremic bluebanded goby, *Lythrypnus dalli*

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Abstract

While males typically compete for females, species with female biased sex ratios and/or large male investment in offspring care often exhibit reversed sex roles. Here we investigated, in a haremic fish species, the bluebanded goby, *Lythrypnus dalli*, the impact of male and female courtship behaviour on male reproductive success, measured as the total number of eggs in the nest and total number of developed eggs. Reproductive success was not associated with rates of male behaviour, such as parenting, approaching and courtship, but was associated with rates of female courtship. Consistent with predictions for a role-reversed reproductive strategy, only males demonstrated nest care and females exhibited high rates of courtship and intrasexual competition, such that alpha females interrupted courtship solicitations by beta females. Overall, these data are consistent with sex role reversal in *L. dalli* and show that the expression of male courtship behaviour does not interfere with paternal care.

Keywords

mating strategy, proceptive behaviour, parenting, reproductive behaviour, sexual dimorphism.

1. Introduction

Elaborate male sexual advertisement signals, male–male competition, and female choice are the hallmarks of sexual selection (Darwin, 1871; Mac-

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Laren & Rowland, 2006; Flamarique et al., 2013). However, in at least some representatives from diverse taxa, including insects (Rutowski, 1984; Salehialavi et al., 2011), birds (Voigt & Goymann, 2007; Griggio et al., 2008), reptiles (Swierk et al., 2013), amphibians (Bush & Bell, 1997), fish (Svensson et al., 2005) and primates (Van Belle et al., 2009), the typical sex roles are reversed and females court and compete for males. Male mate choice is especially important in polygamous social systems where the operational sex ratio is skewed towards females (Trivers, 1972). In many such cases, the male invests heavily in parenting in terms of energetic expenditure, susceptibility to predators, compensation for the lack of egg care by females, and time away from courting potential mates (Westneat & Sargent, 1996). Traits that allow a male to invest heavily in parenting may be under a greater selective pressure compared to courtship, status, or territory defence, which are central to future reproductive opportunities in many species. Thus, there are important fitness trade-offs for males between current and future reproduction (Gross & Sargent, 1985; Westneat & Sargent, 1996).

Several factors influence both reproductive ability and reproductive decisions. For example, male reproductive costs can be reduced if females engage in behaviour to evoke appetitive responses (Beach, 1976; Swierk et al., 2013). Female courtship solicitation includes attractive signals (e.g., postural displays or scent marking) and proceptive behaviours (indicating sexual readiness), which are essential stimuli that initiate male interaction, influence reproductive decisions, and eventually lead to receptivity and copulation (Beach, 1976). Ability to procure mates by establishing dominance is another factor that might influence reproduction. Often, individuals living in social groups form dominance hierarchies, and mating opportunities may be restricted to more dominant individuals (Ellis, 1995). Dominants may gain higher reproductive success by suppressing subordinate reproduction (Barrett et al., 1993; Fitzpatrick et al., 2008), monopolizing mating opportunities, producing more offspring, and experiencing lower rates of abortion, egg loss, and offspring mortality (Trunzer et al., 1999; van Noordwijk and van Schaik, 1999; Young et al., 2006). Some species live in complex social hierarchies that are also very susceptible to rapid changes in social structure, and in those situations, it is difficult to tease apart factors that influence reproductive success. Hence it is important to evaluate a suite of both male and female behaviours that could impact reproductive success in stable social groups.

Bluebanded gobies, *Lythrypnus dalli*, are an ideal species in which to examine the contributions of sex-typical behaviour and social structure to reproductive success (Solomon-Lane et al., 2014). These fish are benthic dwelling marine species that inhabit the eastern Pacific from the Gulf of California, Mexico, to Morro Bay, California (Miller & Lea, 1972). *Lythrypnus dalli* live in small haremic groups consisting of several females and one male and are generally associated with the crowned sea urchin *Centrostephanus coronatus*. Social groups are found in both small isolated social groups or in large aggregations of >100 fish per m^2 (Steele, 1997). Natural nesting sites can include empty abalone shells, under surface of rocks, or small crevices in the rocks, which are difficult to observe in the field (Behrents, 1987). Agonistic interactions occur among all members of the group, to establish and maintain a linear social hierarchy in both field and laboratory conditions. Newly constructed social groups of 3–5 fish take up to 5 days to establish a stable hierarchy based on dominance, territory establishment, and size (Reavis & Grober, 1999; Black et al., 2005). Rates of female agonistic behaviour are associated with male reproductive success (Solomon-Lane et al., 2014). In the field, more dominant females generally tend to be site-attached, while more subordinate, smaller females are less so, and can move among nesting sites (Lorenzi, 2009). In stable groups in the laboratory, gonads of females from all levels of the hierarchy contain ripe eggs (D. Pradhan, pers. obs.). Removal of the male from the social groups leads to sex change in the most dominant female under both field and laboratory conditions (Reavis & Grober, 1999; Black et al., 2005). Little is known about the expression of sexually dimorphic courtship behaviour in the field, but it can be rigorously studied in a semi-natural laboratory habitat (Reavis & Grober, 1999).

Most research on *L. dalli* has been conducted when the operational sex ratio is female biased in the breeding season, which can begin as early as February and continue until September (Wiley, 1976). Little is known about the behavioural ecology and social group dynamics during the non-breeding season, when the fish enter an ambiguous reproductive phase (Wiley, 1976). In stable, reproductively active social groups, male courtship behaviour involves jerk swims towards females of his choice, whom he leads into the nest for spawning. Demersal eggs are normally attached to the roof of the nest, and in laboratory groups, eggs can be easily quantified (Solomon-Lane et al., 2014). The number of eggs in a nest is a common and effective measure of male reproductive success and, as such, is a useful proxy for male fitness (Solomon-Lane et al., 2014). Once laid, individual clutches of eggs can be tracked based on the description of the eggs through development (see methods), and more than one clutch of eggs can be laid in the same nest by multiple females (Solomon-Lane et al., 2014). Upon hatching, the larvae enter a planktonic phase where they drift in the water column for about 2 months before they settle on the reef and recruit into social groups (Behrents, 1987). As is common in many fish species, *L. dalli* males provide care to developing eggs by fanning using the pectoral and caudal fins and rubbing movements using the ventral part of their body (Zoran & Ward, 1983; Gross & Sargent, 1985; Torricelli et al., 1985). In addition to providing exclusive care for overlapping broods, the male also defends the eggs from predators, including opportunistic females who will consume eggs (Pradhan et al., 2014b). Female *L. dalli* display solicitation behaviour similar to that documented in other fish species (Rowland et al., 2002), however, its role as a reproductive signal to demonstrate readiness for spawning has not been tested previously.

In this series of studies, we evaluated a variety of basic reproductive behaviours and reproductive success in small groups of *L. dalli*. In Study 1, we examined basic questions such as the length of time it takes for egg appearance in the nest and the stages of egg development in laboratory groups of wild-caught *L. dalli*. Based on those results, we designed Study 2 to elucidate aspects of sex role reversal in this species by examining the relationship between a suite of sexually dimorphic reproductive behaviours and male reproductive success. To discern the presence of sex role reversal, we evaluated both male behaviour (nesting and courtship jerks) and female behaviour (courtship solicitations and intrasexual competition). Using groups of *L. dalli* consisting of one male and two size-mismatched females, we documented natural variation in egg numbers, including periods without eggs in the nest. Based on the variation in egg laying documented across social groups, we first asked whether egg presence affects rates of male and female reproductive behaviour. We predicted that when eggs are absent from a nest, rates of male courtship will be higher and rates of male nest care will be lower. For females, we predicted that, as in other species (Marconatto & Bisazza, 1986; Knapp & Kovach, 1991), female courtship rates will be higher in the presence of eggs. We also examined whether traits such as body size and sex specific reproductive behaviours were associated with the number of eggs in the nest. Finally, to get a deeper understanding of how female rank modulated social interactions, we analysed behavioural interactions among all fish within the group by constructing a transition matrix. Together, these data provide insights into sex role reversal and male reproductive trade-offs in *L. dalli*.

2. Methods

2.1. General procedures

All fish in the present study were collected from the reefs off the coast of Catalina Island, California by SCUBA diving and using hand nets (permit number SC-11879) in July 2011 and 2012. After capture, the fish were placed in 2-l plastic Nalgene bottles, brought to the boat, and placed in a 5 gallon bucket for transport to the laboratory in the Wrigley Institute for Environmental Sciences. Fish were housed in a $60 \times 94\ \text{cm}^2$ aquarium supplied with continuous seawater and exposed to natural ambient light cycle. The day after capture, fish were anesthetized with tricaine methanosulfate (MS-222; 0.5 mg/100 ml H_2O) and placed under a dissecting microscope for morphological measurements. Standard length (SL) was measured using a vernier calliper. The external genitalia were imaged using a camera (Motic Images) attached to an Apple MacBook, and the specific pattern of blue bands was noted. Groups were constructed based on SL of fish, such that the male was the largest and was at least 3 mm larger than the alpha female; additional females differed in SL by at least 3 mm. These differences in size ensured rapid establishment of a linear hierarchy (Reavis & Grober, 1999) and, along with the unique banding pattern, allowed for easy identification of individuals during behavioural observations. The largest female of the group (alpha female) was subordinate to the male, but dominated over the other females (beta and gamma). Prior to beginning all behavioural observations, and during the behavioural observations, we ensured that the differences in status were consistent by keeping track of agonistic efficiency of each individual (data not shown). This is a composite score of the number of approaches that lead to displacements; individuals of a higher status have a higher agonistic efficiency (Pradhan et al., 2014a; Solomon-Lane et al., 2014). Note that even though SL is one factor that contributes towards hierarchy resolution, it is not the only determinant (Rodgers et al., 2007); hence, it is important to keep track of status during the experiment. Each group was provided with a piece of PVC tube (7.62 cm in length and 3 cm in diameter), which males readily use as a nesting site. For each social group, this tube was placed in the central position within the tank, such that behaviour inside the tube could be easily recorded.

2.2. Study 1

This experiment lasted for 22 days from 5 to 27 July 2011. We evaluated the dynamics of egg laying over time after group establishment. To evaluate aspects of egg laying, we constructed groups of two different sizes; 6 groups consisted of one male and two females and 6 groups consisted of one male and three females (male, $SL = 37.28 \pm 0.82$ mm; alpha female, $SL = 31.25 \pm 0.27$ mm; beta female, $SL = 28.79 \pm 1.01$ mm; gamma female, $SL = 24.28 \pm 0.63$ mm). Fish were fed twice daily, at 8 a.m. and 4 p.m., with frozen brine shrimp (Hikari Biopure), after which the nest tubes were removed for inspection.

2.2.1. Eggs

Once the groups were constructed, nest tubes were checked twice daily for the appearance of eggs. The monolayer of eggs was visually inspected by briefly removing the tube from the aquarium. Males readily returned to their nests after this disruption. We noted whether the eggs were newly laid (orange), late embryos (‘eyed’ eggs), yolk-sac larva (transparent, with more prominent eye pigmentation) or hatched (whitish remnants of eggs without ‘eyes’) (Solomon-Lane et al., 2014). Note that we recorded the presence of clutches of eggs, but using this brief visual inspection, we could not reliably account for the disappearance (hatching) of individual clutches. The number of days for the first appearance of eggs and the number of days taken for the eggs to become eyed were calculated. Based on these data, we also determined the percentage of clutches that advanced to the eyed stage. We used unpaired t-tests to determine whether there were differences in those parameters between groups consisting of two versus three females. No extensive behavioural measurements were made in this study, except that it was noted whether the male was demonstrating nest care. The results and limitations of this study were taken in to consideration when designing Study 2.

2.3. Study 2

This experiment lasted for 26 days from July 5–31 2012. Before being placed in groups, the mass of all males was measured using an electronic balance

(Sartorium). The banding pattern and size of fish were used to construct social groups ($N = 16$), consisting of a male (SL = 42.09 ± 0.92 mm) and two size-mismatched females (alpha female, SL = 35.29 ± 0.44 mm and beta female, SL = 30.26 ± 0.36 mm). Fish were fed twice at 10 a.m. and 5 p.m. daily with brine shrimp (Hikari Biopure).

2.3.1. *Eggs*

We quantified within and between group variation in egg number by counting the total number of eggs (orange + eyed) in each group at 8 different times over the 26-day period (days 6, 8, 12, 16, 19, 21, 23 and 26). To quantify eggs, we lined each PVC nest tube with a custom-fitted sheet of acetate. To avoid disturbing the fish, we did not check the tube for eggs before the behavioural observations. Immediately after the behavioural observations, the acetate was carefully removed from the PVC tube, placed in a frame, imaged digitally (Canon SX150 IS), and replaced into the tube. ImageJ was used to count the number of eggs. On day 10, egg presence was noted, but eggs were not photographed.

2.3.2. *Behaviour*

Behaviour observations lasted for 10 min for each group and began at 9 a.m. on days 6, 8, 10, 12, 16, 19, 21, 23 and 26 after initial grouping. When one fish swam within two body lengths of another individual, we recorded that behaviour as an approach. When an approached fish swam away, we recorded that behaviour as a displacement. In the current study, we did not evaluate whether any given behaviour served an agonistic or reproductive function.

We also recorded male-specific behaviours, including courtship jerks directed towards females, non-directed courtship jerks, and the number and duration of nest care bouts. Nest care included vigorous rubbing and fanning inside the nest using fins (Pradhan et al., 2014b). Each bout of nest care lasted from one to several seconds, and each bout was separated by at least 2 s. Based on these observations, basic parameters of nest care behaviour were calculated, such as mean number of nest care bouts and mean nest care bout duration in the 10-min period. Rate of nest care/parenting was calculated as the number of fanning and rubbing bouts/total time spent fanning and rubbing (s). We observed that the male was always in the nest tube before it was removed for inspection. Upon replacement of the nest tube into the tank, the male entered it almost immediately.

Female courtship solicitation behaviour has not been previously described in *L. dalli*, hence we defined the following parameters as characterizing one

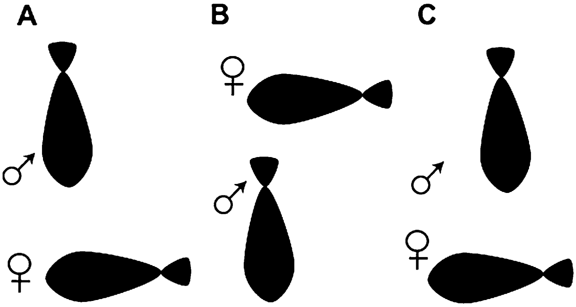

Figure 1. Scenarios and rules for scoring courtship solicitation postures by *Lythrypnus dalli* females (top views). The body of the female (♀) should be aligned perpendicular to the male (♂) so as to display the state of her distended abdomen (gravidity) to the male, the female must remain stationary and not engaged in interaction with another individual or feeding. (A) The female could be positioned anterior or (B) posterior to the male; (C) some part of the female body must be intersected by the median linear axis of the male.

form of female courtship, solicitation behaviour: the female must be oriented perpendicular to the male so as to display the state of her distended abdomen (gravidity) to the male, some part of the female body must be intersected by the median linear axis of the male, the female must remain stationary and not engaged in interaction with another individual or feeding, and the female can be positioned anterior or posterior to the male (Figure 1). Females assumed a solicitation position for two to several seconds, but we did not record the time spent per solicitation in this study. Each solicitation bout was separated by at least 2 s. Rates of all behaviours other than nest care were calculated by dividing the number of occurrences of each behaviour type by the total observation time (10 min).

In order to better understand the relationships among different behaviours, we constructed a transition matrix of behavioural events that occurred within 5 s of the preceding behaviour (see *Statistics*). This analysis allowed us to determine the common responses of the receivers of a specific behavioural display (Okanoya, 2004). As males can spend >90% of their time inside the nest tube (Pradhan et al., 2014b), it was vital to observe behaviour inside the tube. In a harem, the male and his nest is necessarily the centre of reproductive activity and behaviour, therefore, we chose to observe the most important behaviours associated with the nest as a way to narrow in on those behavioural interactions critical to reproductive success. These behaviours included interactions between females, where one female approached and displaced a second female that was in the process of soliciting (i.e., associated with the nest/male). This constituted an interruption of solicitation.

2.4. Statistics

A repeated measures ANOVA was used to analyse the number of eggs produced by 16 groups on 8 different days. Our primary goal with this analysis was to determine if there was significant within and among group variation in egg production over the 26-day period. Due to the large number of possible among group comparisons, we did not do further post hoc tests. A repeated measures ANOVA was also used to analyse the number of nest care (fanning and rubbing) bouts exhibited by males from the 16 groups on 8 different days (to match the days with egg data). Again, our goal was to determine if there was significant among group variation in nest care and due to the large number of possible among group comparisons, we did not do further post hoc tests.

To determine whether fish size has an impact on number of eggs, we used simple regression to analyse the relationship between the number of eggs and (1) SL of males and (2) total SL of both females.

To investigate the impact of egg presence on behaviour, we divided the behaviour observations of each group into two categories: no eggs and eggs present. Using paired t-tests, we determined whether there was a difference in the number of days nests were without versus with eggs. To analyse whether presence of eggs affected behaviour, we performed unpaired t-tests to compare (1) the rates of nest care and (2) male approaches towards females with and without eggs present in nest. We conducted a two-way ANOVA to investigate whether three levels of status (male, alpha, beta) and two levels of egg presence (no eggs and eggs) affected the rates of courtship behaviour (male jerks and female solicitation).

Simple regression analyses were used to determine the relationship between the total number of eggs and (1) rate of fanning and rubbing, (2) rate of males approaching females, (3) rate of male jerks and (4) rate of female solicitation behaviour. We also used simple regression analyses to determine the relationship between the number of eyed eggs and the variables mentioned above, and between rates of solicitation by alpha and by beta females.

To calculate the transition event probabilities, behaviour transcripts were converted into first order Markov chains in which rows corresponded to the preceding events and columns corresponded to the succeeding events (Klein

& de Araújo, 2010). Overall totals of all the 16 groups over 9 days of observations were calculated. Ratios between number of times each transition occurred and the total number of occurrences that followed a particular event were calculated. The ratios were converted to percentage for clarity in the resultant flow chart.

3. Results

3.1. Study 1

There were no significant differences in the timing of egg appearance and disappearance between groups consisting of two versus three females ($p >$ 0.05); therefore we have collapsed those groups. After groups were constructed, it took 5.75 ± 0.71 days for eggs to first appear. After a clutch was laid, eggs became eyed 2.42 ± 0.62 days later. Groups had 3.3 ± 0.30 clutches of eggs in 22 days ($N = 12$). Most of the clutches of eggs became eyed (77.78 ± 10.12%). In groups consisting of 3 females, there were always eggs in the nest tube; however, in groups consisting of 2 females, there were periods of time without eggs in the nest.

3.2. Study 2

3.2.1. Natural variation in number of eggs

In 93.75% of the stable social groups with two females and a male, there were periods of time when there were no eggs in the nest. Overall, there were no differences between the total numbers of days that there were no eggs in the nest (3.38 ± 0.45) versus eggs present (4.12 ± 0.48) in the nest (paired t-test, $t_{15} = 0.94$, $p = 0.36$). A repeated measures ANOVA revealed a significant variation in the number of eggs produced by groups ($F_{2,15} = 5.75$, $p < 0.0001$). Each group produced a variable number of eggs over the 26 days period (Figure 2A). For example, one group (Group 4) always had eggs in the nest while another never had eggs (Group 1).

3.2.2. Number of eggs was not associated with fish size

There was no relationship between number of eggs and SL of males ($r^2 =$ 0.03, $p = 0.54$) or total SL of females ($r^2 = 0.03$, $p = 0.54$).

3.2.3. Natural variation in nest care behaviour

A repeated-measures ANOVA revealed significant variation in the number of nest care (fanning and rubbing) bouts over the 26 days period ($F_{2,15} =$

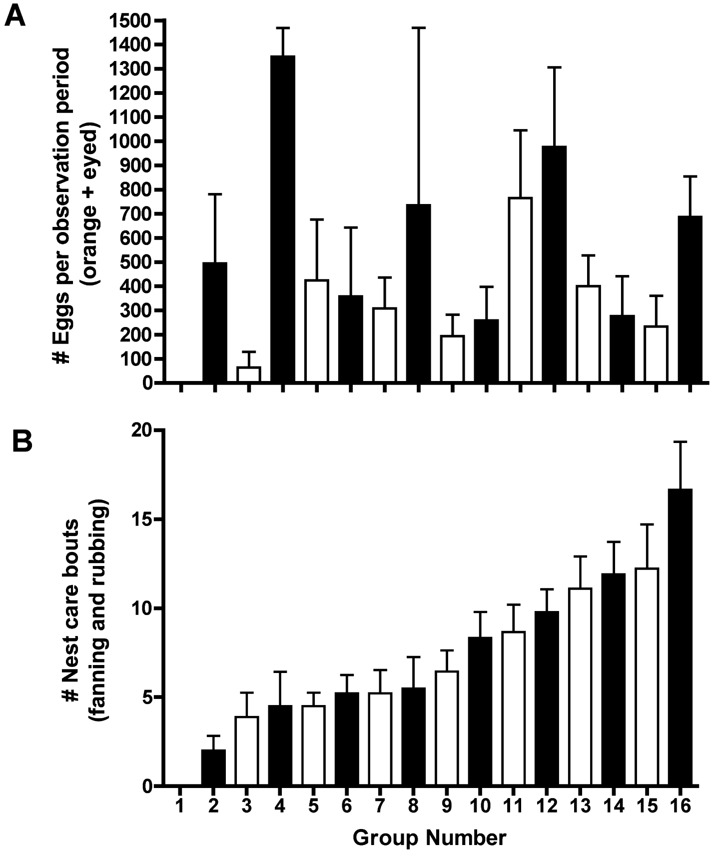

Figure 2. Social groups of *Lythrypnus dalli* show within and between group natural variation in (A) mean total number of eggs (orange + eyed) laid on 8 different days; (B) mean number of nest care (fanning and rubbing) bouts exhibited on 8 different days during 10 min observation sessions, over the course of 26 days in social groups housed in a semi-natural laboratory environment. Each social group ($N = 16$) consisted of one male and two size-mismatched females. Error bar for each group captures variation across time; Group 1 did not have any eggs during the experimental period.

5.747, $p < 0.0001$, Figure 2B). A male from one group (Group 1) did not demonstrate any nest care behaviour and the highest mean nest care rate was 16.67 ± 2.69 (Group 8). For the non-zero groups, the mean number of nest care bouts was 7.57 ± 0.53 per 10 min observation period and the mean duration of each nest care bout was 25.40 ± 3.60 s.

3.2.4. Effect of egg presence and status on rates of behaviour

There was no effect of egg presence on the rate at which males approached females (Figure 3A, $t_{29} = 0.47$, $p = 0.64$). Interestingly, males exhibited similar number of nest care bouts (Figure 3B, $t_{29} = 1.93$, $p = 0.06$) and duration of nest care bouts (Figure 3C, $t_{29} = 0.18$, $p = 0.86$) regardless of egg presence or absence. A two-way ANOVA revealed a main effect of social status on courtship behaviour ($F_{2,84} = 3.66$, $p = 0.03$; Figure 4). However, egg presence did not affect rates of courtship behaviour ($F_{1,84} = 0.26$, $p = 0.61$). There was also no interaction between social status and egg presence ($F_{2,84} = 0.85$, $p = 0.43$). Bonferroni post hoc tests revealed that when there are no eggs in the nest, beta female solicitation rates were higher than directed and non-directed male jerk rates ($t = 2.39$, $p < 0.05$). Overall, 55.86 ± 0.02% of male jerks were not directed to any particular female.

3.2.5. Effect of egg number on behaviour

The number of eggs in the nest was not associated with male nest care, male approaches, or jerks towards females ($p > 0.05$; Figure 5). However, the total number of eggs (orange + eyed) was positively associated with rate of alpha female solicitation ($r^2 = 0.31$, $p = 0.02$; Figure 6A) and with rate of alpha female + beta female solicitation ($r^2 = 0.26$, $p < 0.04$; Figure 6C). More specifically, number of eyed eggs was strongly associated with rates of alpha solicitation ($r^2 = 0.50$, $p = 0.002$; Figure 6D). Alpha females and beta females solicited males at similar rates ($t_{252} = 1.76$, $p = 0.08$; Figure 4). There was a positive correlation between the rates of alpha female and beta female solicitation ($r^2 = 0.43$, $p < 0.0001$). Beta female solicitation was not associated with total number of eggs or number of eyed eggs (Figure 6B, E).

3.2.6. Description of transitions following female solicitations

A total of 875 behavioural transitions were used to generate a transition schematic (Figure 7). The Markov chain analyses of the behavioural transitions demonstrate that following a beta female soliciting a male, there were two main outcomes: either the alpha female interfered with the beta female solicitation (39.2%) or the male approached the beta (45.5%). Following alpha female interference: (1) the beta female went back to solicit the male (34.8%), (2) the alpha female solicited the male (39.1%), or (3) the beta female approached the male (13%). In contrast, the beta female never interrupted during an alpha female solicitation of the male. Alpha female solicitation led to the males approaching the alpha female at a high frequency

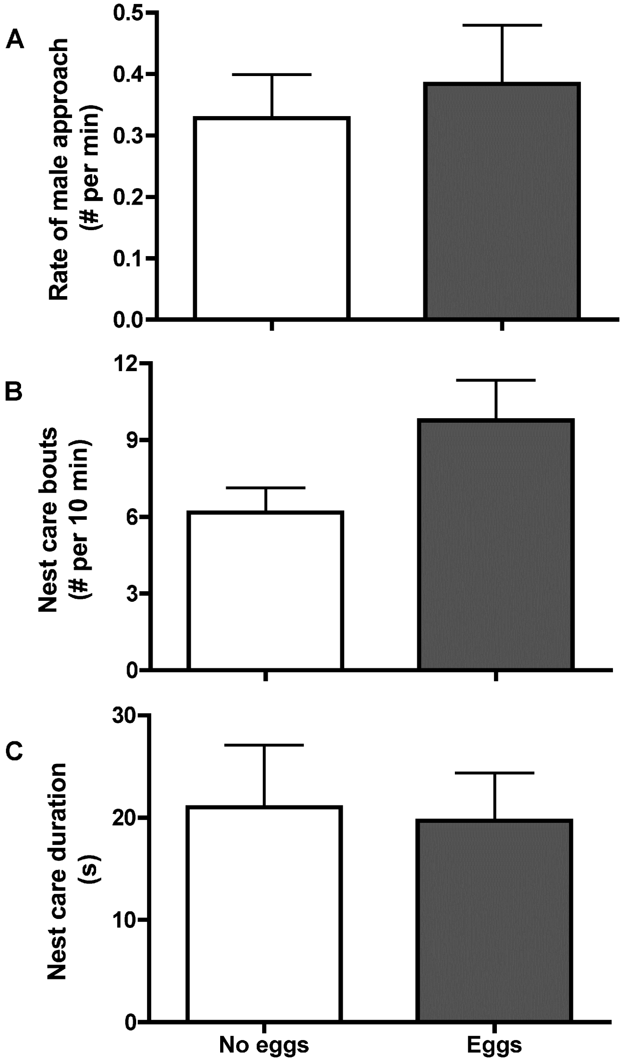

Figure 3. Effect of egg presence on behaviour exhibited by male *Lythrypnus dalli* during 10 min observation periods over a 26-day period (A) rates of male approaches towards females; (B) number male nest care bouts (fanning and rubbing); (C) nest care duration. Each group comprised of one male and two size-mismatched females. Eight behavioural observations were conducted for $N = 16$ groups. Data are means ± SEM.

(77.2%). Males displaced both females a large proportion of the times that they approached them: alpha female 99%, beta female 100%. Displacements of a female led to either the male approaching the same female again (alpha

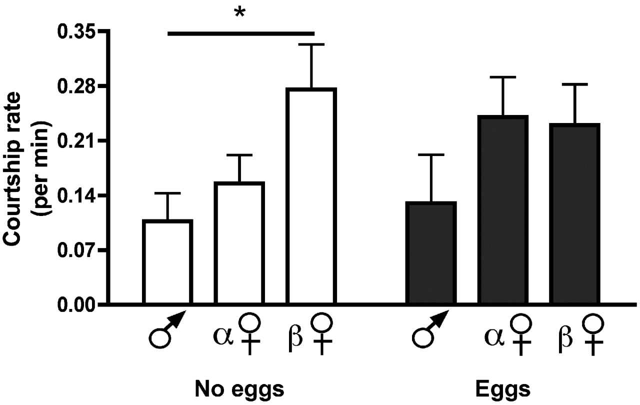

Figure 4. Effect of egg presence on rates of courtship behaviour exhibited by *Lythrypnus dalli* living in groups comprised of one male (♂) and two size-mismatched females (α♀, alpha female; β♀, beta female) during egg absence and presence over a 26-day period. Courtship behaviours quantified were jerks for males and solicitations for females. When there are no eggs in the nest, rates of beta solicitations are higher than males jerk rate. Eight behavioural observations were conducted for $N = 16$ groups. $^{*}p < 0.05$. Data are means ± SEM.

51.3%, beta 60.5%), or the male approaching the other female (alpha 21.0%, beta 25.6%).

4. Discussion

In a species where the sex ratio is skewed towards females and males demonstrate requisite parenting, we demonstrated that harems consisting of one male and two size-mismatched females varied substantially in their rates of egg production, both within groups over time and among groups. Presence of eggs in the nest had no impact on rates of male or female courtship behaviour. In addition, in the presence of eggs, males did not reduce rates of other social behaviour in lieu of nest care. Based on number of eggs produced, there appeared to be no trade-off between male nest care and other male activities, including interaction with females. As a result, males seemed to ‘multi-task’ without compromising reproductive success. Female postural displays of gravidity (courtship solicitation) were also associated with the number of eggs laid. Additionally, while alpha females did not solicit more than beta females, rates of alpha solicitation were a better indicator of male reproductive success. In this experiment, there was one male and two females, and our data showed that both females simultaneously sought access

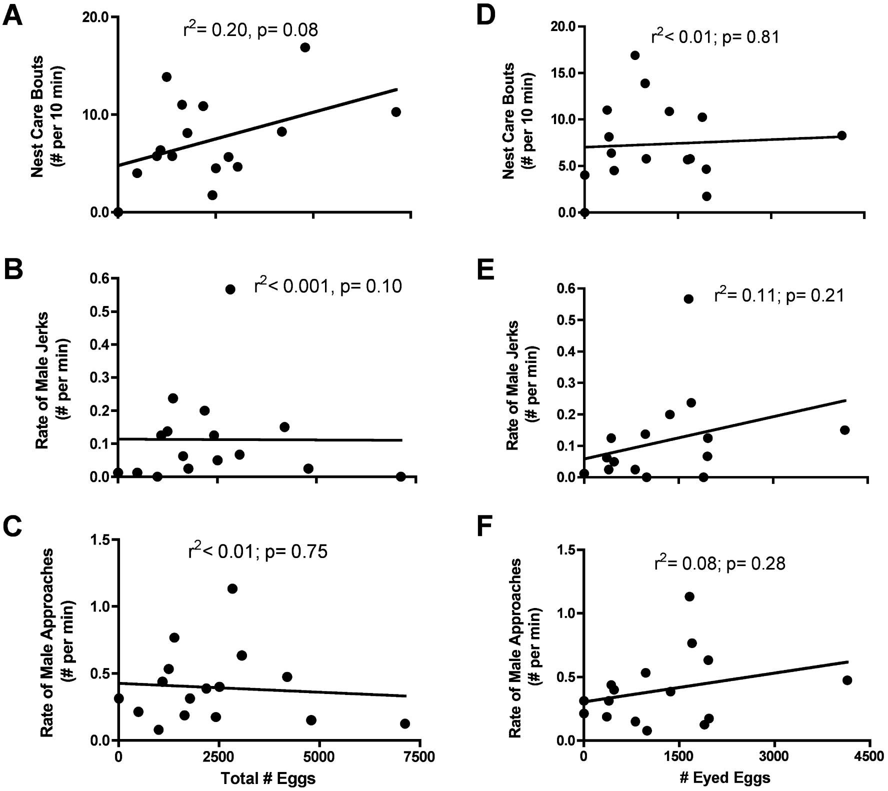

Figure 5. Relationship between number of eggs and male *Lythrypnus dalli* behaviour exhibited during 10-min observation periods over a 26-day period (A) Total number of eggs (orange + eyed) and number of nest care bouts (fanning and rubbing); (B) total number of eggs (orange + eyed) and rate of male courtship jerks; (C) total number of eggs (orange + eyed) and rate of male approaches towards females; (D) number of eyed eggs and number of nest care bouts (fanning and rubbing); (E) number of eyed eggs and rate of male courtship jerks and (F) number of eyed eggs and rate of male approaches towards females. Eight behavioural observations and 8 egg counts were conducted for $N = 16$ groups over a 26-day period.

to the male. The fact that rates of female solicitation were equal to or greater than male jerks (Figures 4–6) is evidence for the presence of sex role reversal in *L. dalli*. Moreover, transition matrix analyses revealed intra-female competition, such that alpha females were likely to interrupt beta female solicitation, and then court males. Taken together, these results support the hypothesis that *L. dalli* exhibit sex role reversal resulting in dominant females having a significant impact on male reproductive success.

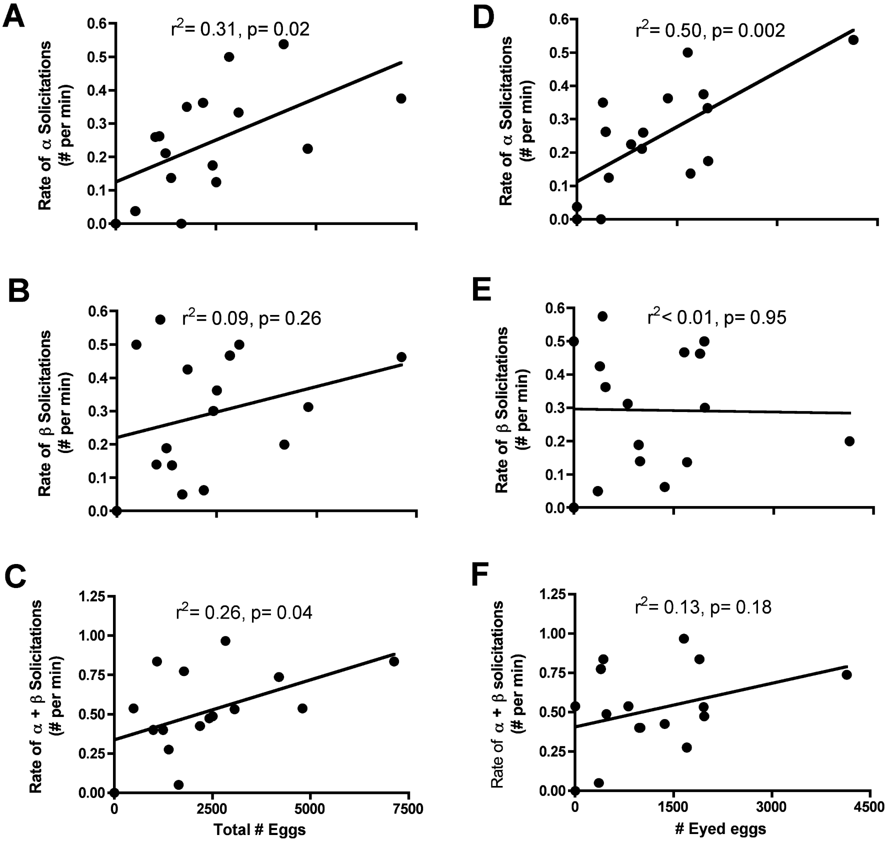

Figure 6. Relationship between number of eggs and rates of female *Lythrypnus dalli* behaviour exhibited during 10-min observation periods over a 26-day period (A) Total number of eggs (orange + eyed) and rate of alpha female solicitation; (B) total number of eggs (orange + eyed) and rate of beta female solicitation; (C) total number of eggs (orange + eyed) and rates of alpha female + beta female solicitation; (D) number of eyed eggs and rate of alpha female solicitation; (E) number of eyed eggs and rate of beta female solicitation and (F) number of eyed eggs and rates of alpha female + beta female solicitation. Eight behavioural observations were conducted for $N = 16$ groups over a 26-day period.

4.1. Egg presence had no effect on rates of courtship behaviour

Many different advertisement strategies of mate quality might affect mate choice and reproductive success (Alcock, 2001). Even though male secondary sexual characteristics are common in fishes, the presence of eggs is an important measure of male quality (Knapp & Kovach, 1991). Hence we considered the effect of egg presence on the rates of courtship behaviour

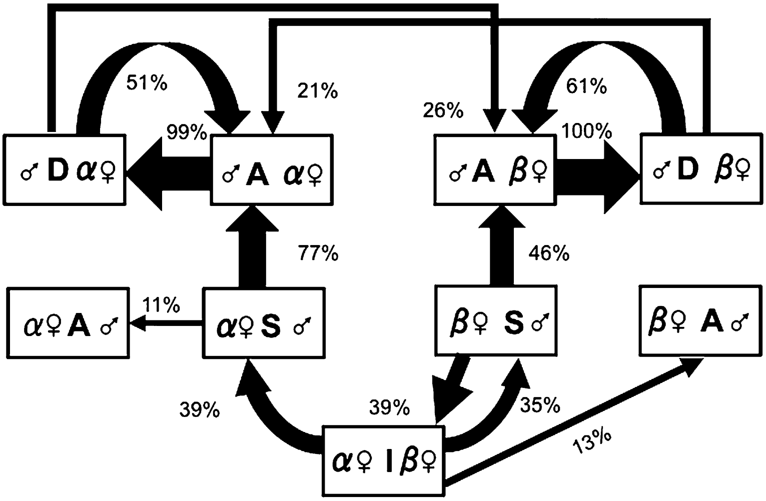

Figure 7. Simplified flow chart showing the pattern of interactions within *Lythrypnus dalli* groups consisting of one male, an alpha female, and a beta female, based on first order Markov transitions. A total of 875 transitions were used to generate the chart, and arrow thickness is proportional to the frequency (converted into percentage, and noted beside the arrow) of the transition. To reduce complexity, only frequencies > 0.1 (10%) are shown. Abbreviations: ♂, male; α♀, alpha female; β♀, beta female; A, approach; D, displacement; S, solicitation; I, interruption of solicitation.

displayed by all individuals in the social group. Importantly, we used natural changes in the cycle of egg appearance and disappearance in long-term social groups. In Study 2, we found that there was no effect of egg presence on rates of courtship behaviour; however, in the absence of eggs, beta solicitation rate was 3 fold higher than male jerk rate (Figure 4). Male courtship jerks directed toward females with or without eggs were generally low and comprised fewer than half of total jerks. It is possible that males court immediately before spawning, and we missed the display of male courtship during the observation sessions. In future studies, we can use continuous video recordings to examine the onset of male jerks shortly before spawning. Courtship can be costly, and there is inter-species variation in the timing of courtship displays. For example, in damselfish, males court the day before spawning, rather than the day of spawning (Karino, 1995). In our experimental paradigm, males may court less because the females are site attached. A consequence of our attempt to study the behaviours associated with natural variations in egg presence is that females may have laid eggs based on the visual cues associated with egg presence. The idea is that a greater number of eyed eggs indicate that the male is good at raising young (Lindström, 1992). In other species of gobies, such as sand gobies (Forsgren et al., 1996) and desert gobies (Knapp & Kovach, 1991; Symons et al., 2011), females tend to choose males with eggs and this may have an impact on egg numbers in our study. In future studies, number of eggs can be manipulated to determine effects on rates of male and female courtship behaviour.

4.2. Social interactions might limit male nest care

In Study 2 nest care rate was not associated with number of eggs in the nest. Even though it was clear that the nest care bouts were normally distributed, it did not vary predictably with the total number of eggs in a nest (Figures 2 and 5A, D). It is interesting to note that the groups where males showed highest levels of nest care were not those that had the highest number of eggs; however, when a male showed no nest care, there were no eggs in the nest.

Trivers (1972) postulated that time and energy spent on courtship and territoriality would reduce the quality of parental care (time spent tending eggs), thus reducing the chances for offspring survival. Parental investment and life history theories predict that traits that allow a male to invest heavily in nest care may be under a greater selective pressure compared to courtship (Trivers, 1972). In *L. dalli*, however, rates of male courtship were not associated with eggs in the nest (Figure 5B, E). Conflict between caring for the current brood and courting females for future reproduction is also seen in the desert goby (Symons et al., 2011) and fresh water gobies (*Rhinogobidus brunneus*) (Suk & Choe, 2002). Male desert gobies provide sole parental care and females are not site attached. In the presence of females, males significantly decrease several aspects of egg care (Symons et al., 2011). The social system of *L. dalli* is slightly different, in that they live in haremic groups and high-ranking females tend to be site-attached, while low-ranking females move among territories (Lorenzi, 2009). Due to the temporal overlap of reproductive and social behaviour and the presence of overlapping broods, during the nesting phase of *L. dalli*, males perform several overlapping activities and rapidly modulate their interaction with conspecifics. Thus time budgets and social allocation in males depend, in part, on the specific breeding system. In future studies, energetic expenditure of fanning and rubbing behaviour will lead to a better understanding of costs of parenting and the evaluation of trade-offs.

We expected that nest care would not persist without the positive sensory feedback in the form of eggs. The expectation was that the male would continue to fan only in the presence of eggs because of the sensory stimulation of egg presence. For example, in mammals, parent–offspring contact behaviour is a form of trophallactic interaction between the parent and young, in which both require sensory stimulation (Rosenblatt, 2003). In our study, however, whether or not eggs are present in the nest, male *L. dalli* constitutively exhibit nest care, including nest preparation and egg care, through fanning and rubbing behaviours. Regardless of egg presence, males also approach and jerk towards females at equal rates. Thus, males do not need to reduce rates of courtship or social behaviour with females in order to exhibit nest care. It has also been suggested that mating at the site of offspring care reduces the temporal conflict between mating and parenting (Stiver & Alonzo, 2009). Conflicts between parenting and mating are not likely in polygamous species with obligate paternal care because males usually have continuous breeding and multiple clutches of eggs at different stages of development (Kokko & Jennions, 2008). Based on the experimental paradigm we used in the present studies, trade-offs may be reduced due to both of these reasons. These data suggest that male nesting behaviour persists, regardless of the context of egg presence.

Exhibition of continuous nest care by fanning and rubbing might be adaptive when the microenvironment around the nesting site needs to be maintained consistently, and several hypotheses could explain this behaviour. First, males might be laying down sperm trails, even before spawning, to mark their territory or to prepare the nest before eggs are laid, as is the case in other goby species (Marconatto et al., 1996). Second, males might display fanning to increase aeration to keep the nesting tube clean and free of disease. In this study, as the breeding season progressed, many of the acetate sheets were coated with a thin layer of algae, and continual fanning might reduce the growth. Third, display of male nest care could induce females to breed. As mentioned earlier, in many species, females tend to lay eggs in nests where males display high rates of nest care (Forsgren et al., 1996; Karino & Arai, 2006; Forsgren, 2007); hence, males may constitutively display fanning behaviours. Fourth, fanning might aid in dispersing pheromones into the water for short-range chemical signalling towards conspecifics (Sorensen et al., 2005). Overall, we found no evidence for trade-offs in male courtship or nest care behaviour.

4.3. Female biased sex ratio permits intra-female competition and sex role reversal

The details of female mating behaviour in *L. dalli* have not been described previously. Considering that the operational sex-ratio we set up in our experiments was female biased (to mimic the natural population, and specifically, small, isolated haremic groups in the field), and brood care is only provided by males, we hypothesized that the conventional sex roles would be reversed. To evaluate this hypothesis, we evaluated both female courtship solicitations and female–female interactions when females exhibited solicitations. We found that even though alpha females did not court males more than beta females, male reproductive success was associated with the courtship behaviour of females (Figures 4 and 6). There was also a positive relationship between rates of alpha solicitation and beta solicitation, and alpha females frequently interrupted beta female solicitations (Figure 7). Thus, social rank appears to bias interactions among females, with higher-ranking individuals approaching and displacing a soliciting subordinate, but not the converse. In newly formed social groups, alpha females lay eggs more frequently than beta females, despite similar number of gravid alphas and betas (Solomon-Lane & Grober, unpublished results). In long-term stable social groups, higher-ranking females have a higher gravidity score compared to lower ranking females (Solomon-Lane et al., unpublished results), and alpha female agonistic behaviours are negatively associated with male reproductive success (Solomon-Lane et al., 2014). Here, we found that alpha female solicitation was positively associated with number of eyed eggs, and transition matrix analyses demonstrate that alpha females frequently interrupt beta female solicitation. As a result of alpha female interference, males respond at different frequencies to solicitation by alpha versus beta females. In future studies, we will keep records of gravidity scores of females because reproductive status could influence egg appearance in nests.

Preliminary studies show that *L. dalli* alpha females have priority in egg-laying compared to subordinate females (beta and gamma), but it is unknown whether they lay more eggs (Solomon-Lane & Grober, unpublished results). In future, it will be informative to track egg laying and hatching success of eggs from females of different statuses. In many species with diverse mating systems, dominant individuals have more access to copulate compared to subordinate individuals. For example, in a cooperatively breeding cichlid, *Neolamprologus pulcher*, dominant individuals reproduce and actively suppress the reproduction of subordinate individuals, who help the dominant individuals with broodcare (Balshine-Earn et al., 1998; Heg, 2008). In the angelfish, *Centropyge bicolor*, a haremic hermaphroditic species similar to *L. dalli*, higher social rank in females is associated with greater reproductive success (Ang & Manica, 2010). Further studies are required to assess the individual female contributions to the overall fitness in *L. dalli*. Finally, our analysis of egg production showed that rates of female courtship are positively associated with male reproductive success and provides robust evidence for sex role reversal in *L. dalli*. Taken together, our work on *L. dalli* demonstrates that male reproductive success could differentially affect females of different statuses, and as such, must be considered when evaluating the contribution of sexually dimorphic behavioural dynamics to fitness in this species.

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