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Agonistic reciprocity is associated with reduced male reproductive success within harem social networks

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While individual variation in social behaviour is ubiquitous and causes social groups to differ in structure, how these structural differences affect fitness remains largely unknown. We used social network analysis of replicate blue-banded goby (*Lythrypnus dalli*) harems to identify the reproductive correlates of social network structure. In stable groups, we quantified agonistic behaviour, reproduction and steroid hormones, which can both affect and respond to social/reproductive cues. We identified distinct, optimal social structures associated with different reproductive measures. Male hatching success (HS) was negatively associated with agonistic reciprocity, a network structure that describes whether subordinates 'reciprocated' agonism received from dominants. Egg laying was associated with the individual network positions of the male and dominant female. Thus, males face a trade-off between promoting structures that facilitate egg laying versus HS. Whether this reproductive conflict is avoidable remains to be determined. We also identified different social and/or reproductive roles for 11-ketotestosterone, 17 β -oestradiol and cortisol, suggesting that specific neuroendocrine mechanisms may underlie connections between network structure and fitness. This is one of the first investigations of the reproductive and neuroendocrine correlates of social behaviour and network structure in replicate, naturalistic social groups and supports network structure as an important target for natural selection.

1. Introduction

A central approach to understanding the evolution of social behaviour involves directly measuring the fitness consequences of natural variation in behaviour. Individual variation in social behaviour is ubiquitous [1,2], even among individuals of similar demographics (e.g. sex, size and status) within similar social and environmental contexts (e.g. [3–5]). It is well-established that individual behaviour can affect fitness [2]; however, the social group within which behaviour occurs can modulate the behaviour–fitness association. Social group structure emerges from the local interactions of individuals, which are non-random for social species [3,6,7]. For example, shoaling guppies preferentially associate by size [8], while house finch attractiveness influences association patterns [9]. Sex, age, status and personality are also influential [4,5,10–13], and these non-random interactions can affect future reproductive state, success and fitness [4,5,13–15].

The structure of a social group can also impact the fitness of individuals within the group, suggesting that selection could act directly on group structure (e.g. [4]). For example, hierarchical social structure is central to the reproductive benefits experienced by dominants [16]. Hierarchies are defined by asymmetrical agonism, directed from dominants to subordinates [17]. Reproductive skew is remarkably common across hierarchical species, and the mechanisms by which dominants enforce their reproductive advantage (e.g. physiological

suppression, limiting mating and inducing abortion) are facilitated by the hierarchical social structure [16].

More nuanced aspects of social structure are also related to fitness, and the application of social network analysis to animal social groups is a valuable tool for elucidating these connections [6,18–20]. Social networks are made up of the individuals (nodes) in a group or population and the connections (edges) between individuals that interact. Quantitative measures of social network structure, while based on dyads, incorporate the social context within which interactions occur. Thus, this type of analysis can place a numerical value on social properties important for fitness, such as conflict and stability [6,21,22]. For example, in social networks of degus, a pluralistic breeder, fewer pups are produced per female when individuals associate more with some members of the group than others [11]. Highly variable patterns of association among dyads are a proposed indicator of conflict [11]. A number of social networking studies include direct fitness measures and identify important connections with various social network parameters [5,9,11,14,23–25]. Few of these studies, however, use replicate networks to understand inter-network variation (e.g. [26,27]). Valuable knowledge about sociality has come from investigations of single groups or populations, but the conclusions are difficult to generalize because of the lack of statistical power [5]. Studying the natural variation in social networks in conjunction with fitness is critical because, if network structure has fitness consequences, selection may act on this variation. The inclusion of replicate social networks has increased recently in studies of fish [8,12,28,29], birds [25,27,30] and mammals [31,32], but fitness was rarely measured in those studies [25,27].

Our goal was to identify the reproductive correlates of social network variation in replicate harems of bluebanded gobies (*Lythrypnus dalli*). This highly social, sex-changing fish forms linear social hierarchies of a dominant male and multiple subordinate females. The male can reproduce with each female in the harem. After fertilization, the male cares for the eggs alone until they hatch. *Lythrypnus dalli* is a useful research model for a number of reasons. First, agonistic and reproductive behaviours have been well characterized in replicate laboratory and field social groups [33–38]. Second, reproductive success (RS) can be measured directly: females routinely lay eggs and males readily parent in the laboratory. Distinct social network parameters may be associated with different aspects of reproduction; therefore, it is important that egg laying can be quantified separately from the number that hatch, our best measure of male RS. Third, agonistic behaviour is associated with male RS. Fewer eggs are fertilized in groups with higher rates of agonistic interaction, and female-initiated agonism appears responsible. This individual behavioural variation within and across social statuses [36] should drive differences in social networks that are relevant to reproduction.

This study expands on previous social networking studies, and *L. dalli* research, in several important ways. Drawing from natural *L. dalli* populations during the reproductive season, we formed a relatively large number of replicate social groups ($n = 34$) in the laboratory, of identical size and sex ratio. By observing agonistic interactions three times in the stable groups, we can identify which individuals interacted and the nature of the interaction, including who initiated and the behavioural response. Based on these data, we formed social networks with weighted and directional ties to indicate

the frequency (weight) and direction of interaction. We also analysed two kinds of networks relevant to hierarchies. Approach networks describe patterns of association, while displacement networks describe dominance interactions in which aggression results in submission [29]. Finally, we quantified systemic steroid hormones, including 11-ketotestosterone (KT), a potent fish androgen, 17 β -oestradiol (E2) and cortisol, the primary teleost glucocorticoid, in a subset of males and females. In vertebrates, hormones are reciprocally related to both social behaviour and reproduction [39,40]. To our knowledge, only one other study has incorporated endocrine measures with social network analysis [10], yet neuroendocrine mechanisms are critical to generating adaptive, context-specific behaviour [40]. Understanding endocrine covariation with social network structure and reproduction may provide insight into whether and which hormones affect RS directly and/or indirectly via the social network.

Here, we first tested the hypothesis that social network density, a measure of interaction frequency, is negatively associated with male RS. This test confirms a previously demonstrated association that did not use social network measures [36] and supports the utility of network metrics. Second, we investigated the importance of network structure and hypothesized that agonistic reciprocity predicts male RS. Reciprocity refers to the proportion of interactions between individuals that are reciprocated, i.e. both individuals initiate interaction with each other, and is well suited to describe hierarchies. Because social hierarchies are characterized by asymmetrical agonism [17], reciprocity should be low, by definition [21,41]. Third, previous data identified a central role for female behaviour in male RS [36]; therefore, we hypothesized that female social network position is central to male success. Lastly, we investigated potential roles for KT, E2 and cortisol in the reciprocal connections with social network measures and reproduction. We took a general approach to these analyses and hypothesized that each hormone would be implicated differentially in social and/or reproductive processes. Our extensive work on *L. dalli* neuroendocrine regulation [33,42,43] provides a solid foundation for these analyses.

2. Material and methods

(a) *Lythrypnus dalli* social groups

In nature, the population sex ratio is female-biased [44], and social groups are mixed-sex and vary in size. Small, isolated groups (3–10 fish) include the territory of a single nesting male and a harem of females. Large aggregations (up to 120 fish m⁻²) include the territories of multiple nesting males and many females. Within aggregations, high-ranking females associate closely with the territory of one male, while lower ranking females move among territories [45]. The number of nesting males in the population is determined by socially regulated sex change. Following the removal of the male from the social group, the dominant female will change sex [34,38]. The most commonly observed social behaviours are agonistic interactions within harems, and male–male aggression across territories is rarely observed.

We collected *L. dalli* from reefs offshore of Catalina Island, California, during the reproductive season (July, California Fish and Game permit SC-11879) using hand nets while SCUBA diving. Males were identified, in part, by their territory holding behaviours, indicating their ability to defend a territory in nature. Fish were first housed in a holding tank (60 × 94 × 35 cm) at the Wrigley Institute for Environmental Studies (Catalina Island,

University of Southern California) and then in groups in divided seawater tables (20 × 31 × 15 cm). Tanks were continuously supplied with natural seawater and exposed to a natural light cycle. Fish were fed brine shrimp twice a day. We formed 34 social groups of one large male and three females of varying sizes. Fish from the holding tank were briefly anaesthetized in tricaine methanesulfonate (MS-222; 500 mg l⁻¹ salt water) to measure standard length (SL; 24–47 mm) and determine sex based on genital papilla morphology [44]. All fish were at least 3 mm SL smaller than the next largest fish to facilitate status establishment [34]. We refer to females based on their decreasing status: alpha, beta and gamma. Social groups of this size, sex ratio and density are observed in nature, and laboratory social groups of wild-caught fish exhibit similar social and reproductive behaviours as natural and semi-natural groups [37,38]. All research was conducted in accordance with animal care guidelines.

(b) Quantifying reproduction and female reproductive state

Males were provided with a PVC nest tube (7.62 cm long × 3 cm diameter) lined with acetate. Females lay adhesive eggs on the acetate, which the male externally fertilizes and cares for alone until hatching (approx. 6 days). We checked for eggs once each morning. If present, we removed the acetate, took a digital image and returned the eggs to the nest (less than 3 min). Males immediately resumed parenting. We used IMAGEJ software [46] to count the number of eggs laid ('new eggs') and the number of eggs hatched ('hatched eggs') over 14 days from sequential images. New eggs were counted the first day they were laid. We then tracked the disappearance of eggs daily from the digital images. Because the planktonic larvae cannot be counted directly, we quantified hatched eggs from the final image before a clutch disappeared, usually on day 6. Eggs from the same clutch typically hatched together and left behind a visible residue. Eggs that disappeared before day 5 and without developed eye spots and tails were considered eaten. Hatching success (HS) was calculated by dividing hatched eggs by new eggs. We also visually estimated female reproductive state. The ovaries of gravid females cause the abdomen to obviously distend. Each morning before feeding, we scored females categorically from 0 (not at all gravid) to 3 (very gravid). We were not able to determine which female laid a particular clutch of eggs.

(c) Agonistic behavioural observations

Agonistic behaviour is a subset of social behaviour that encompasses fighting, aggression, conciliation and retreat [2]. During the 14-day experiment, we observed behaviour in the stable groups (more than 5 days after group formation [34]) on 3 different days for 10 min each, between 09.00 and 12.00 (as in [36]). Social networks were based on approaches, when one fish swam directly towards another fish, within two body lengths, and displacements, a response to an approach in which the approached fish swam away. Displacements are a common measure of aggression, and being displaced is a signal of submission [35].

(d) Social network analysis

Social networks represent the interactions between each dyad in a social group. For each social group, we constructed two weighted, directional networks: one network using approaches per minute and one using displacements per minute. Rates of behaviour were averaged from the three behavioural observations. Using UCINET software [47], we calculated five social network measures. Density and reciprocity were calculated for both approach and displacement networks and describe the network as a whole. Density is the sum of all edges (edge value is higher

between individuals that interact frequently) divided by the number of possible edges, or a network's average edge strength. Reciprocity is the ratio of dyads with reciprocated edges (e.g. fish that approach/displace each other; unweighted edges) relative to the number of dyads that have any edge (reciprocated or not) connecting them. We also calculated measures of individual position (i.e. centrality or connectedness) within the approach networks. Out-degree is the sum of edges (number and weight) originating at a node (e.g. male) and directed to other group members (e.g. alpha, beta gamma female), while in-degree is the sum of edges directed at a single node. Power increases if an individual is well connected to group members that are also well connected (attenuation = 0.5) [21,41].

(e) Quantifying waterborne hormones

Collecting waterborne hormones is a non-invasive method of measuring systemic hormone levels [42] that does not impact group stability (e.g. [43]). Waterborne hormone levels correlate with circulating concentrations in plasma [48,49]. We collected hormones two times from a subset of fish on days that behaviour was also observed. The social groups from which fish were sampled were selected randomly, independent of social or reproductive factors. From each sampled group, we measured hormones for either the male and alpha female or the alpha and beta females (males: collections 1 and 2: $n = 10$; alpha females: collection 1: $n = 19$, collection 2: $n = 20$; beta females: collection 1: $n = 9$, collection 2: $n = 10$). We used hand nets to gently remove all of the fish from the group and placed them individually in 200 ml beakers containing 100 ml of fresh salt water for 1 h (14.30–15.30). Fish were then returned to their tanks. We used established protocols for hormone extraction (using 3 cc Sep-Pak Vac C18 columns, Water Associates), and samples were analysed using specific KT, E2 and cortisol enzyme immunoassays (Cayman Chemical) (standard curves: $r^2 \geq 0.96$). Data were analysed as pg sample⁻¹ h⁻¹ [42].

(f) Data analysis

Statistics were performed with JMP v. 11. Values are presented as averages ± s.e.m., and α was set at 0.05. We used linear regression analysis to test for associations between reproductive measures (new eggs, HS and hatched eggs); between social network measures/agonistic behaviour and reproductive measures; and between hormones and social network measures. One-way ANOVA was used to test for status differences in gravidity, with Tukey's HSD test for post hoc analysis. Because centrality measures were correlated within a network, we used a factor analysis with a varimax (orthogonal) rotation to reduce the number of variables (approach network male, alpha, beta and gamma female in-degree, out-degree and power) to the underlying latent factors. To correct for multiple linear regressions with reproduction (new eggs: 7; HS: 13; hatched eggs: 8), we applied a Benjamini Hochberg correction to control for false discovery rate [50]. Adjusted p -values are reported in the results and figures. One outlier each was excluded from analyses of new eggs (0 eggs) and HS (0%), and both were excluded from hatched eggs analyses.

We used regression analyses to determine whether hormones were differentially implicated in social and/or reproductive processes. After identifying relevant ($p < 0.1$) associations between hormones (KT, E2 and cortisol) and (i) social network measures (displacement network reciprocity, approach network density, in-degree, out-degree and power for male, alpha female, beta female and gamma female; 126 regressions); (ii) gravidity (alpha, beta and gamma females; 27 regressions); and (iii) reproduction (new eggs, HS, hatched eggs, average clutch size, number of clutches laid; 45 regressions), we calculated the proportion of relevant associations that included cortisol versus

KT versus E2 for each category and average r^2 values. All combinations of male, alpha female and beta female hormones with social and reproductive measures were analysed. We included associations between an individual's hormone levels and their own social network position/gravidity, as well as that of group members, because hormones can respond to social and reproductive context (e.g. [51]).

3. Results

(a) Reproduction and female reproductive state

Over 14 days, 106 443 eggs were laid in 33 of 34 social groups. A maximum of six clutches was laid in a group (3.68 ± 0.23), and clutch size ranged from 319 to 2333 eggs (892.5 ± 62.8). There was a significant, negative association between the number of clutches laid and average clutch size ($r^2 = 0.21$, $p = 0.0079$). New eggs were not associated with HS ($r^2 = 0.02$, $p = 0.43$), but by definition, new eggs ($r^2 = 0.67$, $p < 0.0001$) and HS ($r^2 = 0.48$, $p < 0.0001$) were positively associated with hatched eggs. HS ranged from 0 to 100% ($66.1 \pm 3.7\%$), and hatched eggs ranged from 0 to 4995 (2106 ± 189.8).

Average female gravidity differed significantly across social statuses (one-way ANOVA: $F_{2,99} = 22.75$, $p < 0.0001$). Post hoc analysis revealed that alpha females ($p < 0.0001$) and beta females ($p < 0.0001$) were significantly more likely to appear gravid than gamma females but did not differ from each other ($p = 0.84$). Beta female gravidity was significantly and positively associated with new eggs ($r^2 = 0.16$, $p = 0.018$), but there was no association for alpha ($r^2 = 0.0093$, $p = 0.59$) or gamma female gravidity ($r^2 = 0.016$, $p = 0.48$).

(b) Hypothesis 1: network density was negatively associated with male reproductive success

There were significant, negative associations between hatched eggs and network density for approach ($r^2 = 0.20$, $p = 0.023$) and displacement networks ($r^2 = 0.28$, $p = 0.015$) (figure 1a).

(c) Hypothesis 2: agonistic reciprocity was negatively associated with male reproductive success

Hatched eggs were negatively associated with displacement network reciprocity ($r^2 = 0.16$, $p = 0.04$), but not approach network reciprocity ($r^2 = 0.041$, $p = 0.31$) (figure 1b). Specifically, displacement network reciprocity was negatively associated with HS ($r^2 = 0.23$, $p = 0.013$), not new eggs ($r^2 = 0.025$, $p = 0.71$) (figure 1c).

(d) Hypothesis 3: female social network position was central to male reproductive success

Factor analysis of male, alpha, beta and gamma female in-degree, out-degree and power (approach networks) revealed four underlying latent factors that together accounted for 80.65% of the variance (table 1). Factor 1 ($r^2 = 0.16$, $p = 0.046$) and factor 3 ($r^2 = 0.22$, $p = 0.030$) were significantly and negatively associated with hatched eggs, but there were no associations with factor 2 ($r^2 = 0.015$, $p = 0.50$) or factor 4 ($r^2 = 0.07$, $p = 0.19$). For the components of male RS, there was a significant, negative association between factor 1 and new eggs ($r^2 = 0.22$, $p = 0.039$) (figure 2a) but not HS ($r^2 = 0.00017$, $p = 0.94$). There was also a significant, negative

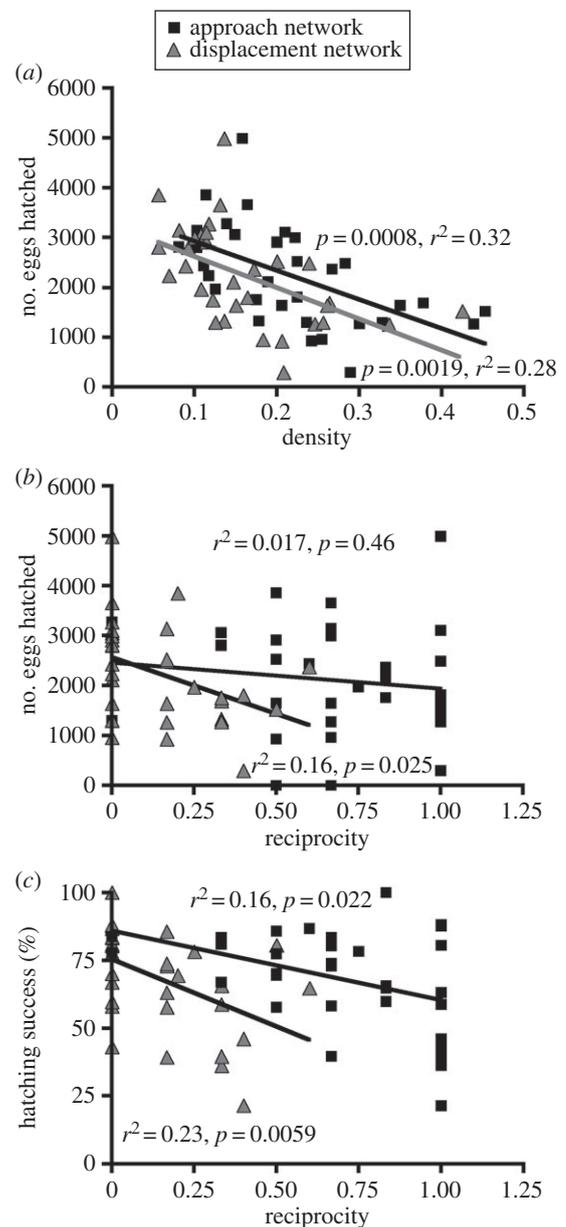


Figure 1. Associations between (a) approach and displacement network density and the number of eggs that hatched, (b) approach and displacement network reciprocity and the number of eggs that hatched, and (c) approach and displacement network reciprocity and HS.

association between factor 3 and HS ($r^2 = 0.25$, $p = 0.016$; figure 2b) but not new eggs ($r^2 = 0.06$, $p = 0.24$).

Only male and alpha female social network positions loaded on factor 1, including male and alpha female out-degree; male and alpha female power; and alpha female in-degree (table 1). Dyadic interactions between male and alpha female were not sufficient to explain the association with new eggs. Following Benjamini Hochberg correction, there were no significant associations between new eggs and male approaches to alpha female ($r^2 = 0.089$, $p = 0.32$) or alpha female approaches to male ($r^2 = 0.086$, $p = 0.23$). Factor 3 included alpha and gamma female out-degree and male, beta female and gamma female in-degree (table 1). Dyadic interactions provided insight into the directed agonism important for HS. There were significant, negative associations between alpha female approaches ($r^2 = 0.24$, $p = 0.014$) and displacements ($r^2 = 0.23$, $p = 0.014$) to beta females and HS (figure 3a). Gamma female approaches ($r^2 = 0.27$, $p = 0.014$)

Table 1. Rotated factor loading for factor analysis of social group agonism. Social network analysis of approach networks was used to calculate male, alpha female, beta female and gamma female out-degree, in-degree and power. The agonistic measures that contribute significantly to each factor are italicized. Factors in italic are significantly associated with reproduction in the social group.

agonistic measure	factor 1	factor 2	factor 3	factor 4
male out-degree	<i>0.9357</i>	-0.2268	0.0299	-0.1877
male power	<i>0.8317</i>	-0.3112	-0.1861	-0.1760
alpha female in-degree	<i>0.6453</i>	<i>0.4782</i>	0.1663	-0.0923
beta female out-degree	0.0338	<i>0.9814</i>	0.1883	0.0030
beta female power	-0.1617	<i>0.8372</i>	-0.1038	0.1577
alpha female out-degree	-0.4520	0.0098	<i>0.8910</i>	0.0408
gamma female in-degree	-0.0821	<i>0.5743</i>	<i>0.7126</i>	0.0601
beta female in-degree	0.1792	-0.0555	<i>0.6849</i>	0.1671
male in-degree	-0.2459	0.1152	<i>0.3712</i>	0.2177
alpha female power	-0.8792	-0.1489	0.2764	0.0179
gamma female power	-0.1801	0.0358	0.0269	<i>0.9337</i>
gamma female out-degree	-0.1023	0.1204	<i>0.4682</i>	<i>0.8694</i>

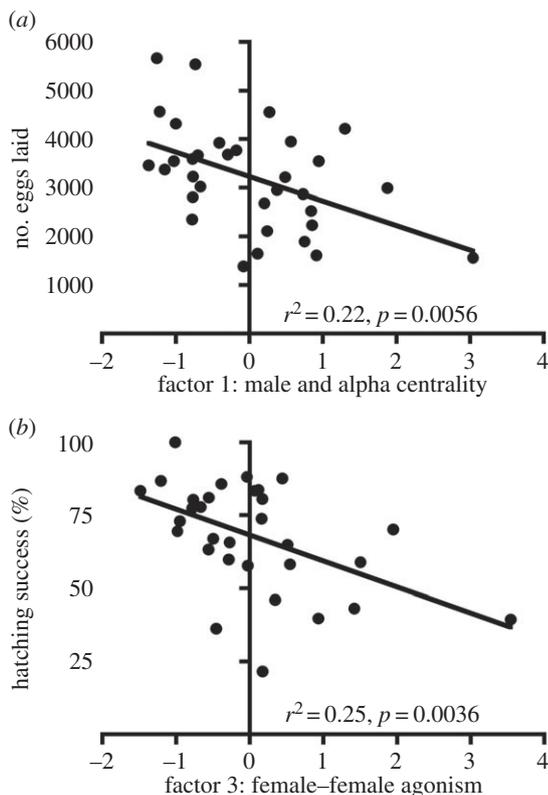


Figure 2. Factor analysis was used to identify four latent factors underlying male, alpha female, beta female and gamma female in-degree, out-degree and power. (a) Associations between factor 1 and the number of eggs laid in a social group and (b) between factor 3 and HS (number of eggs laid/number of eggs hatched; %). Factor 1 ('male and alpha centrality') included measures of male and alpha female social network positions. Factor 3 ('female-female agonism') primarily included measures of female-initiated, female received agonism.

and displacements to beta females ($r^2 = 0.46$, $p = 0.0013$) were also negatively associated with HS (figure 3b). There were negative trends between alpha female approaches ($r^2 = 0.13$, $p = 0.076$) and displacements ($r^2 = 0.13$, $p = 0.076$) to gamma females and HS (figure 3c), but not for gamma female

approaches ($r^2 = 0.074$, $p = 0.19$) and displacements to alpha female ($r^2 = 0.0053$, $p = 0.75$). Neither alpha female ($r^2 = 0.009$, $p = 0.71$) nor gamma female approaches to the male ($r^2 = 0.018$, $p = 0.60$) were associated with HS.

(e) Hypothesis 4: hormones were differentially implicated in social networks, gravidity and reproduction

Overall, 12.6% of hormone associations were relevant ($p < 0.1$), with an average r^2 value of 0.39 ± 0.025 . These associations and average r^2 were relatively evenly distributed across social network measures (11.9%; 0.40 ± 0.029), gravidity (14.8%; 0.31 ± 0.065) and reproduction (13.3%; 0.42 ± 0.066); however, cortisol, KT and E2 were not equally represented across categories (figure 4). For example, cortisol was overrepresented for social network measures. Most associations involved male (40%) or beta female (47%) cortisol, and 80% involved individual cortisol and social network structure or the network position of a group member. For gravidity, KT and E2 were both implicated, but cortisol associations were absent. All hormones were equally represented for reproduction. Beta female hormone levels accounted for 50% of the relevant associations, and 83% of all associations with reproduction were negative. See electronic supplementary material for additional details.

4. Discussion

We identified strong connections between *L. dalli* social network structure and multiple aspects of reproduction, including male RS. Overall, fewer eggs hatched in high-density social networks (figure 1a), similar to our previous study [36]. We demonstrated that a specific pattern of agonistic interaction was responsible: males were more successful in social networks with low agonistic reciprocity (figure 1b,c). Across species, hierarchies are defined by asymmetrical agonism, directed from dominant to subordinate [17]. Although *L. dalli* hierarchies were linear, the variation we observed in reciprocity indicates that subordinates can and

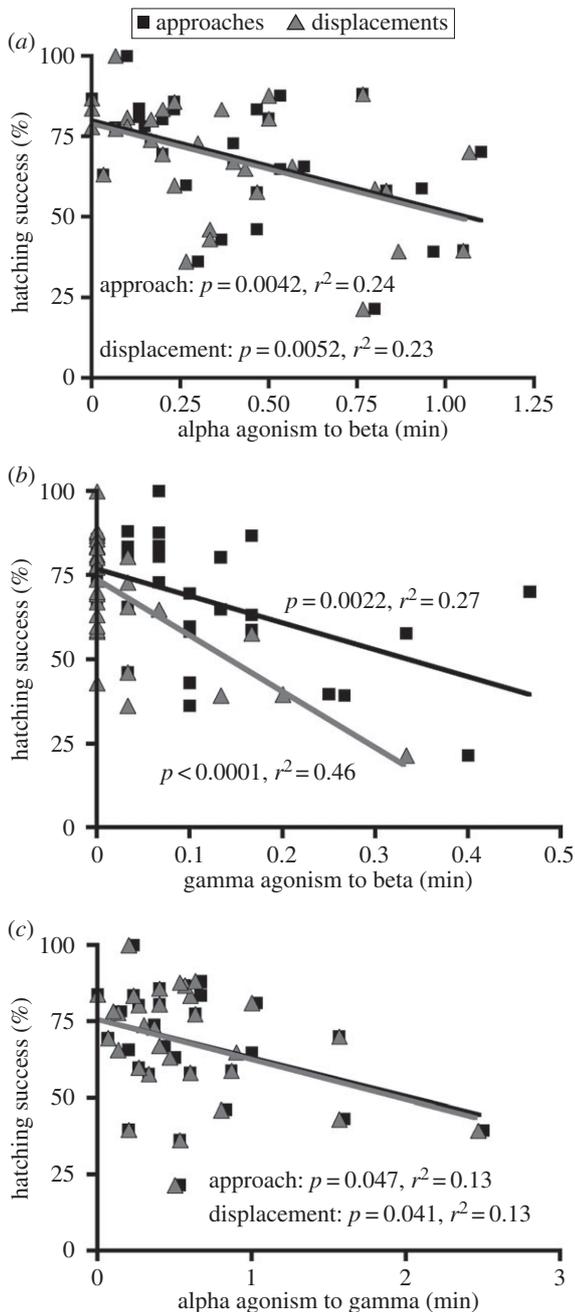


Figure 3. Associations between HS (%) and dyadic agonistic interactions: (a) alpha female approaches and displacements to beta female, (b) gamma female approaches and displacements to beta females, and (c) alpha female approaches and displacements to gamma females.

do displace dominants, an action that reduced, or was reduced by, fitness. Reciprocity was negatively related to HS (figure 1c), specifically, and factor analysis identified a central role for agonism directed at beta females by alpha and gamma females (figure 3a,b). Using different analytical methods, we previously identified a role for these females, suggesting consistency in the interplay of social and reproductive dynamics [36]. Gamma female displacements of beta females, in particular, explained nearly half of the variation in HS, and, as a subordinate displacing a dominant, contributed to increased reciprocity (figure 3b). The concept of reciprocity has provided important insights into the evolution of behaviour, including for networks based on cooperation [52], grooming [53] and agonistic intervention [54]. This is the first study, to our knowledge, to report on agonistic reciprocity or its implications for fitness.

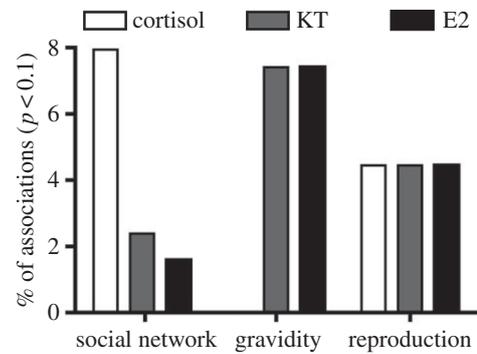


Figure 4. Proportion of relevant associations ($p < 0.1$) between hormones (cortisol, 11-ketotestosterone (KT) and 17 β -oestradiol (E2)) and social network measures (density, reciprocity, in-degree, out-degree and power); gravidity (alpha, beta and gamma females); and reproduction (new eggs, HS, hatched eggs, average clutch size and number of clutches laid).

The links among HS, reciprocity and intrasexual female agonism contradicts our previous assumption that, after females lay eggs, the male is solely responsible for egg fate. This insight came from providing a reproductive context for the variation in social network structure that we observed across replicate groups. While it is clear that male parenting and nest defence are critical to male goby RS [33,37,55], *L. dalli* females influence post-spawning success. We cannot yet determine how reciprocity is controlled or identify the mechanisms linking high reciprocity to substantial egg loss. One hypothesis is that males that fail to control their harem, leading to high reciprocity, are also bad parents. Individuals of any status can affect network structure [3], however, and alpha females are ideally positioned to be influential. Alpha females are the most aggressive [36] and the most dominant while the male is away in his nest [37]. Alpha female-, but not male-, initiated agonism (out-degree) also contributed to factor 3/HS (table 1). Low HS could be caused by females or males eating eggs and/or poor egg quality. Females, and alphas specifically (see below), may time their egg laying so that other eggs were already in the nest [56], providing the opportunity to consume eggs while spawning [33,57]. Low beta female HS could also drive total HS down. Alpha and gamma female-initiated agonism towards beta females was associated with HS and could affect her physiology and, subsequently, her egg quality. Social stress is a potent activator of the neuroendocrine stress axis, and stress axis activation can negatively impact reproduction [58], including HS (e.g. [59]).

The success of low reciprocity *L. dalli* networks suggests that there could be fitness benefits, across species, to meeting idealized expectations of social structure. For example, degus are pluralistic breeders that communally care for offspring. Strong associations among females do not benefit RS; however, when interactions are equally distributed among group members, pup production is higher [11]. Similarly for rock hyrax, another pluralistic breeder, variation in individual centrality is detrimental to longevity [23]. For both pluralistic species, individuals benefit when social network position is more homogeneous across the group. Our data suggest that adhering strictly to asymmetrical agonism could benefit fitness for hierarchical species. Thus, whether hetero- or homogeneity in individual social network position benefits/detracts from fitness depends on species-specific social organization.

There were different optimal social network structures associated with egg laying versus HS. Both egg laying and

HS are critical to maximize RS, yet in *L. dalli* groups, the social correlates of these reproductive phases could conflict. Factor analysis revealed that only measures of male and alpha female network position were associated with new eggs (table 1 and figure 2*a*). Simple, dyadic interactions between the dominant male and female were not associated with new eggs, demonstrating that unlike factor 3 with HS, for which dyadic agonism was illuminating, social network analysis provided unique insight [24]. Although we could not quantify individual female RS, these data suggest alpha females contributed the most reproductively, as expected for her status and size [16,60]. Alpha females were often gravid, and the absence of an association with new eggs suggests her egg laying could be dissociated, in part, from reproductive state. Dominant fish often lay more frequently [60], and alpha females might time their spawning to maximize their own success, for example, when the male's nest already contained eggs [56].

Although male RS increases when the reproductive contribution of all females is maximized, our data indicate that alpha, beta and gamma females served different reproductive roles, which is common in hierarchies [16]. Beta females appeared gravid as often as alpha females, but unlike alphas, there was a positive association with new eggs. If beta females laid less frequently, the betas that contributed reproductively may have appeared more gravid for longer as they waited for a spawning opportunity. Despite the importance of agonism targeting beta for male RS, the association was with HS not egg laying, suggesting this agonism may not reduce her spawning opportunities. By contrast, gamma females seem to experience partial reproductive suppression [16,60]. The insights gained from these social network analyses support its use for understanding reproductive dynamics and conflict. Quantifying individual female RS will be critical to future investigations.

Steroid hormones are optimally positioned to affect and respond to social network structure and reproduction but are rarely included in networking studies [10]. A relatively small proportion of hormone associations were deemed relevant ($p < 0.1$) in this study, which is consistent with data from stable groups of *L. dalli* and other species. Hormones are more likely to be associated with behaviour during social transitions [43,61], although the corollary for transitioning social networks (e.g. [30]) remains to be tested. Here, the r^2 values suggest the associations we did observe were robust. Cortisol, KT and E2 could each affect fitness directly via RS or indirectly via the social network. It was our goal to narrow this scope by identifying social and/or reproductive roles for each hormone

that can be tested directly in manipulative studies (e.g. [33]). For example, cortisol was implicated for social network associations (figure 4). The neuroendocrine stress axis can regulate agonistic behaviour and respond to social stress and status [62]. Because the majority of associations involved male and beta female cortisol with the network position of another group member, we hypothesize that network structure causes status-specific changes in stress axis activation. By contrast, only sex steroids were important for gravidity (figure 4). Oestrogens and androgens fluctuate during the female reproductive cycle and in response to group member reproductive state [51,63,64].

Overall, we show that stable *L. dalli* social networks vary in structure, and this variation was associated with distinct aspects of reproduction. Male *L. dalli* face a trade-off, therefore, between promoting networks that facilitate egg laying versus HS. It remains to be determined whether it is feasible to maximize both or whether reproductive conflict is inherent. The extent to which any individual can influence network structure will also be critical to understanding this potential tug-of-war over RS, as socially influential individuals [7] may shape networks to their benefit. Alternatively, selection could act on the ability to adjust reproductive strategy to the social context. Our work suggests that future social network studies that manipulate social behaviour, social and reproductive context (e.g. status, sex and gravidity), and neuroendocrine state will provide important insight into the evolution of social behaviour and network structure in social species.

Data accessibility. Behaviour, social network and hormone data: Dryad <http://dx.doi.org/10.5061/dryad.n0h37>.

Authors' contributions. T.K.S.L. contributed to the study design, data acquisition, conducted the statistical analyses and drafted the article. D.S.P. and M.C.W. contributed to data acquisition and revised the article for intellectual content. M.S.G. contributed to the study design and the drafting and revision of the article. All authors gave final approval for publication.

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References

1. Darwin C. 1859 *On the origin of the species by means of natural selection*. London, UK: John Murray.
2. Wilson E. 1980 *Sociobiology: the abridged edition*. Cambridge, UK: The Belknap Press of Harvard University Press.
3. Pike T, Samanta M, Lindström J, Royle N. 2008 Behavioural phenotype affects social interactions in an animal network. *Proc. R. Soc. B* **275**, 2515–2520. (doi:10.1098/rspb.2008.0744)
4. Pruitt JN, Goodnight CJ. 2015 Site-specific group selection drives locally adapted group compositions. *Nature* **514**, 359–362. (doi:10.1038/nature13811)
5. Silk JB, Beehner JC, Bergman TJ, Crockford C, Engh AL, Moscovice LR, Wittig RM, Seyfarth RM, Cheney DL. 2009 The benefits of social capital: close social bonds among female baboons enhance offspring survival. *Proc. R. Soc. B* **276**, 3099–3104. (doi:10.1098/rspb.2009.0681)
6. Krause J, Croft DP, James R. 2007 Social network theory in the behavioural sciences: potential applications. *Behav. Ecol. Sociobiol.* **62**, 15–27. (doi:10.1007/s00265-007-0445-8)
7. Flack JC, Krakauer DC, de Waal FBM. 2005 Robustness mechanisms in primate societies: a perturbation study. *Proc. R. Soc. B* **272**, 1091–1099. (doi:10.1098/rspb.2004.3019)
8. Croft DP, James R, Ward AJ, Botham MS, Mawdsley D, Krause J. 2005 Assortative interactions and social networks in fish. *Oecologia* **143**, 211–219. (doi:10.1007/s00442-004-1796-8)
9. Oh KP, Badyaev AV. 2010 Structure of social networks in a passerine bird: consequences for sexual selection and the evolution of mating

- strategies. *Am. Nat.* **176**, E80–E89. (doi:10.1086/655216)
10. Hansen H, McDonald DB, Groves P, Maier JAK, Ben-David M. 2009 Social networks and the formation and maintenance of river otter groups. *Ethology* **115**, 384–396. (doi:10.1111/j.1439-0310.2009.01624.x)
 11. Wey TW, Burger JR, Ebensperger LA, Hayes LD. 2013 Reproductive correlates of social network variation in plurally breeding degus (*Octodon degus*). *Anim. Behav.* **85**, 1407–1414. (doi:10.1016/j.anbehav.2013.03.035)
 12. Schürch R, Rothenberger S, Heg D. 2010 The building-up of social relationships: behavioural types, social networks and cooperative breeding in a cichlid. *Phil. Trans. R. Soc. B* **365**, 4089–4098. (doi:10.1098/rstb.2010.0177)
 13. Sih A, Chang AT, Wey TW. 2014 Effects of behavioural type, social skill and the social environment on male mating success in water striders. *Anim. Behav.* **94**, 9–17. (doi:10.1016/j.anbehav.2014.05.010)
 14. McDonald DB. 2007 Predicting fate from early connectivity in a social network. *Proc. Natl Acad. Sci. USA* **104**, 10 910–10 914. (doi:10.1073/pnas.0701159104)
 15. White DJ, Gersick AS, Snyder-Mackler N. 2012 Social networks and the development of social skills in cowbirds. *Phil. Trans. R. Soc. B* **367**, 1892–1900. (doi:10.1098/rstb.2011.0223)
 16. Ellis L. 1995 Dominance and reproductive success among nonhuman animals: a cross-species comparison. *Ethol. Sociobiol.* **16**, 257–333. (doi:10.1016/0162-3095(95)00050-U)
 17. Drews C. 1993 The concept and definition of dominance in animal behaviour. *Behaviour* **125**, 283–313. (doi:10.1163/156853993X00290)
 18. Croft DP, Madden JR, Franks DW, James R. 2011 Hypothesis testing in animal social networks. *Trends Ecol. Evol.* **26**, 502–507. (doi:10.1016/j.tree.2011.05.012)
 19. Pinter-Wollman N *et al.* 2013 The dynamics of animal social networks: analytical, conceptual, and theoretical advances. *Behav. Ecol.* **25**, 242–255. (doi:10.1093/beheco/art047)
 20. Sih A, Hanser SF, McHugh KA. 2009 Social network theory: new insights and issues for behavioral ecologists. *Behav. Ecol. Sociobiol.* **63**, 975–988. (doi:10.1007/s00265-009-0725-6)
 21. Croft DP, James R, Krause J. 2008 *Exploring animal social networks*. Princeton, NJ: Princeton University Press.
 22. Wey T, Blumstein DT, Shen W, Jordán F. 2008 Social network analysis of animal behaviour: a promising tool for the study of sociality. *Anim. Behav.* **75**, 333–344. (doi:10.1016/j.anbehav.2007.06.020)
 23. Barocas A, Ilany A, Koren L, Kam M, Geffen E. 2011 Variance in centrality within rock hyrax social networks predicts adult longevity. *PLoS ONE* **6**, e22375. (doi:10.1371/journal.pone.0022375)
 24. Formica VA, Wood CW, Larsen WB, Butterfield RE, Augat ME, Hougen HY, Brodie 3rd ED. 2012 Fitness consequences of social network position in a wild population of forked fungus beetles (*Bolitotherus cornutus*). *J. Evol. Biol.* **25**, 130–137. (doi:10.1111/j.1420-9101.2011.02411.x)
 25. Ryder TB, McDonald DB, Blake JG, Parker PG, Loisele BA. 2008 Social networks in the lek-mating wire-tailed manakin (*Pipra filicauda*). *Proc. R. Soc. B* **275**, 1367–1374. (doi:10.1098/rspb.2008.0205)
 26. Ryder TB, Parker PG, Blake JG, Loisele BA. 2009 It takes two to tango: reproductive skew and social correlates of male mating success in a lek-breeding bird. *Proc. R. Soc. B* **276**, 2377–2384. (doi:10.1098/rspb.2009.0208)
 27. Royle NJ, Pike TW, Heeb P, Richner H, Kolliker M. 2012 Offspring social network structure predicts fitness in families. *Proc. R. Soc. B* **279**, 4914–4922. (doi:10.1098/rspb.2012.1701)
 28. Thomas POR *et al.* 2008 Does defection during predator inspection affect social structure in wild shoals of guppies? *Anim. Behav.* **75**, 43–53. (doi:10.1016/j.anbehav.2007.06.004)
 29. Dey CJ, Reddon AR, O'Connor CM, Balshine S. 2013 Network structure is related to social conflict in a cooperatively breeding fish. *Anim. Behav.* **85**, 395–402. (doi:10.1016/j.anbehav.2012.11.012)
 30. Hobson E, Avery M, Wright T. 2013 An analytical framework for quantifying and testing patterns of temporal dynamics in social networks. *Anim. Behav.* **85**, 83–96. (doi:10.1016/j.anbehav.2012.10.010)
 31. Madden JR, Drewe JA, Pearce GP, Clutton-Brock TH. 2009 The social network structure of a wild meerkat population: 2. Intragroup interactions. *Behav. Ecol. Sociobiol.* **64**, 81–95. (doi:10.1007/s00265-009-0820-8)
 32. McCowan B, Anderson K, Heagarty A, Cameron A. 2008 Utility of social network analysis for primate behavioral management and well-being. *Appl. Anim. Behav. Sci.* **109**, 396–405. (doi:10.1016/j.applanim.2007.02.009)
 33. Pradhan DS, Solomon-Lane TK, Willis MC, Grober MS. 2014 A mechanism for rapid neurosteroidal regulation of parenting behaviour. *Proc. R. Soc. B* **281**, 20140239. (doi:10.1098/rspb.2014.0239)
 34. Reavis R, Grober M. 1999 An integrative approach to sex change: social, behavioural and neurochemical changes in *Lythrypnus dalli* (Pisces). *Acta Ethol.* **2**, 51–60. (doi:10.1007/PL00012232)
 35. Rodgers EW, Earley RL, Grober MS. 2007 Social status determines sexual phenotype in the bi-directional sex changing bluebanded goby *Lythrypnus dalli*. *J. Fish Biol.* **70**, 1660–1668. (doi:10.1111/j.1095-8649.2007.01427.x)
 36. Solomon-Lane T, Willis MC, Pradhan DS, Grober MS. 2014 Female, but not male, agonistic behaviour is associated with male reproductive success in stable bluebanded goby (*Lythrypnus dalli*) hierarchies. *Behaviour* **151**, 1367–1387. (doi:10.1163/1568539x-00003188)
 37. Behrems K. 1983 *The comparative ecology and interactions between two sympatric gobies* (*Lythrypnus dalli* and *Lythrypnus zebra*). Los Angeles, CA: University of Southern California.
 38. Black M, Moore B, Canario A, Ford D, Reavis R, Grober M. 2005 Reproduction in context: field testing a laboratory model of socially controlled sex change in *Lythrypnus dalli* (Gilbert). *J. Exp. Mar. Bio. Ecol.* **318**, 127–143. (doi:10.1016/j.jembe.2004.12.015)
 39. Adkins-Regan E. 2009 Neuroendocrinology of social behavior. *ILAR J.* **50**, 5–14. (doi:10.1093/ilar.50.1.5)
 40. Oliveira RF. 2009 Social behavior in context: hormonal modulation of behavioral plasticity and social competence. *Integr. Comp. Biol.* **49**, 423–440. (doi:10.1093/icb/icip055)
 41. Hanneman R, Riddle M. 2005 *Introduction to social network methods*. Riverside, CA: University of California.
 42. Lorenzi V, Earley R, Rodgers E, Pepper D, Grober M. 2008 Diurnal patterns and sex differences in cortisol, 11-ketotestosterone, testosterone, and 17 β -estradiol in the bluebanded goby (*Lythrypnus dalli*). *Gen. Comp. Endocrinol.* **155**, 438–446. (doi:10.1016/j.ygcen.2007.07.010)
 43. Lorenzi V, Earley RL, Grober MS. 2012 Differential responses of brain, gonad and muscle steroid levels to changes in social status and sex in a sequential and bidirectional hermaphroditic fish. *PLoS ONE* **7**, e51158. (doi:10.1371/journal.pone.0051158.t003)
 44. St. Mary CM. 1993 Novel sexual patterns in two simultaneously hermaphroditic gobies, *Lythrypnus dalli* and *Lythrypnus zebra*. *Copeia* **4**, 1062–1072. (doi:10.2307/1447085)
 45. Lorenzi V. 2009 *The behavioral neuroendocrinology of fish sex change: the role of steroids and monoamines*. Atlanta, GA: Georgia State University.
 46. Rasband WS. 1997–2014 *ImageJ*. Bethesda, MD: U. S. National Institutes of Health. See <http://imagej.nih.gov/ij/>.
 47. Borgatti SP, Everett MG, Freeman LC. 2002 *Ucinet for windows: software for social network analysis*. Harvard, MA: Massachusetts Analytic Technologies.
 48. Gabor CR, Contreras A. 2012 Measuring water-borne cortisol in *Poecilia latipinna*: is the process stressful, can stress be minimized and is cortisol correlated with sex steroid release rates? *J. Fish Biol.* **81**, 1327–1339. (doi:10.1111/j.1095-8649.2012.03411.x)
 49. Kidd CE, Kidd MR, Hofmann HA. 2010 Measuring multiple hormones from a single water sample using enzyme immunoassays. *Gen. Comp. Endocrinol.* **165**, 277–285. (doi:10.1016/j.ygcen.2009.07.008)
 50. Benjamini Y, Hochberg Y. 1995 Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Soc. Ser. B* **57**, 289–300.
 51. Kidd MR, O'Connell LA, Kidd CE, Chen CW, Fontenot MR, Williams SJ, Hofmann HA. 2013 Female preference for males depends on reproductive physiology in the African cichlid fish *Astatotilapia burtoni*. *Gen. Comp. Endocrinol.* **180**, 56–63. (doi:10.1016/j.ygcen.2012.10.014)
 52. van Doorn GS, Taborsky M. 2012 The evolution of generalized reciprocity on social interaction networks. *Evolution* **66**, 651–664. (doi:10.1111/j.1558-5646.2011.01479.x)
 53. Silk JB, Seyfarth RM, Cheney DL. 1999 The structure of social relationships among female savanna

- baboons in Moremi Reserve, Botswana. *Behaviour* **136**, 679–703. (doi:10.1163/156853999501522)
54. de Waal F, Luttrell LM. 1988 Mechanisms of social reciprocity in three primate species: symmetrical relationship characteristics or cognition? *Ethol. Sociobiol.* **9**, 101–118. (doi:10.1016/0162-3095(88)90016-7)
 55. Karino K, Arai R. 2006 Effect of clutch size on male egg-fanning behavior and hatching success in the goby, *Eviota prasina* (Klunzinger). *J. Exp. Mar. Bio. Ecol.* **334**, 43–50. (doi:10.1016/j.jembe.2006.01.018)
 56. Forsgren E, Karlsson A, Kvarnemo C. 1996 Female sand gobies gain direct benefits by choosing males with eggs in their nests. *Behav. Ecol. Sociobiol.* **39**, 91–96. (doi:10.1007/s002650050270)
 57. Okuda N, Ito S, Iwao H. 2002 Female spawning strategy in *Rhinogobius* sp. OR: how do females deposit their eggs in the nest? *Ichthyol. Res.* **49**, 371–379. (doi:10.1007/s102280200056)
 58. Breuner CW, Patterson SH, Hahn TP. 2008 In search of relationships between the acute adrenocortical response and fitness. *Gen. Comp. Endocrinol.* **157**, 288–295. (doi:10.1016/j.ygcen.2008.05.017)
 59. Mingist M, Kitani T, Koide N, Ueda H. 2007 Relationship between eyed-egg percentage and levels of cortisol and thyroid hormone in masu salmon *Oncorhynchus masou*. *J. Fish Biol.* **70**, 1045–1056. (doi:10.1111/j.1095-8649.2007.01362.x)
 60. Heg D. 2008 Reproductive suppression in female cooperatively breeding cichlids. *Biol. Lett.* **4**, 606–609. (doi:10.1126/science.230.4729.1037)
 61. Almeida O, Gonçalves-de-Freitas E, Lopes JS, Oliveira RF. 2014 Social instability promotes hormone–behavior associated patterns in a cichlid fish. *Horm. Behav.* **66**, 369–382. (doi:10.1016/j.yhbeh.2014.05.007)
 62. Solomon-Lane TK, Crespi EJ, Grober MS. 2013 Stress and serial adult metamorphosis: multiple roles for the stress axis in socially regulated sex change. *Front. Neurosci.* **7**, 210. (doi:10.3389/fnins.2013.00210)
 63. Brooks S, Tyler CR, Sumpter JP. 1997 Egg quality in fish: what makes a good egg? *Rev. Fish Biol. Fish.* **7**, 387–416. (doi:10.1023/A:1018400130692)
 64. Ramsey ME, Wong RY, Cummings ME. 2011 Estradiol, reproductive cycle and preference behavior in a northern swordtail. *Gen. Comp. Endocrinol.* **170**, 381–390. (doi:10.1016/j.ygcen.2010.10.012)