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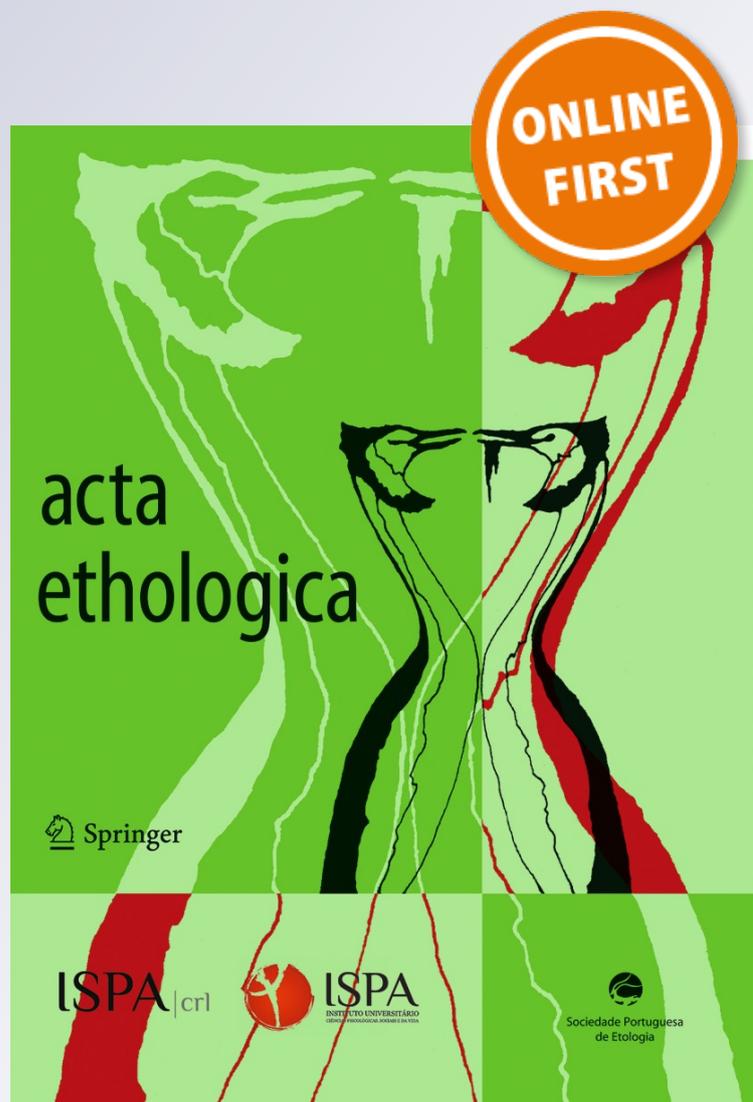
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# The effects of previous experience and current condition on status contests in the bluebanded goby (*Lythrypnus dalli*)

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**Abstract** Social status can change over a lifetime and affect fitness. Status transitions are often influenced by previous experience such that previous winners tend to dominate in future interactions, while losers tend to remain subordinate. We investigated the role of social status experience on future status contests in the bluebanded goby (*Lythrypnus dalli*), a highly social, sex-changing fish. Female *L. dalli* were given long-term experience as alpha (dominant) or beta (subordinate) in a stable social group of three fish (two females, one dominant male). We hypothesized that females with previous dominance experience would be more likely to become dominant in a novel context and change sex, a status-dependent transition. We found that in size-matched pairs, half of the previous alphas and half of the previous betas established and maintained dominance in the novel pair. Previous betas were more likely to become dominant in pairs with small relative differences in physical condition. Betas that initiated more interactions with the male in the stable group were also more likely to become dominant. Experience only had a transient effect on agonistic behavior during status establishment. In pairs in which the previous beta established dominance, individual agonistic efficiency, or the proportion of their approaches that led to a displacement, was lower. There was no long-term effect of experience on the rate of sex change. These data

suggest that long-term tenure as a subordinate does not, itself, prevent status ascension and that physical condition and individual behavior are relevant factors that influence *L. dalli* contests.

**Keywords** Agonistic efficiency · Condition index · Sex change · Social experience · Social status · Winner and loser effects

## Introduction

In many social species, individuals ascend and descend in social status over their lifetime. Understanding the intrinsic and extrinsic factors influencing transitions between social statuses is critical because status is closely tied to fitness (Ellis 1995; Wilson 1980). In general, status transitions are expected if status is determined by traits like body size, weapon size, fighting ability, age, or behavior (Colléter and Brown 2011; Drews 1993; Emlen 2008; Wilson 1980). These traits can change over time and/or the benefits afforded can change relative to other group members. Within social groups, there are often extended periods of stability punctuated by transient instability resulting from life history transitions (e.g., immigration/emigration, death) and/or changes in the environment (e.g., resource availability, season) (Wilson 1980). In comparison, hierarchies determined by stable traits, such as maternal rank, are relatively constant over a lifetime (Silk et al. 2009).

Multiple factors influence the outcome of status contests, including behavior, the physical condition of each competitor, the context within which the contest occurs, and previous social experience, the focus of this study. Evidence from species as diverse as insects and mammals suggests that after winning an agonistic interaction (i.e., establishing dominance), winners are more likely to dominate in future agonistic interactions.

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The losers are more likely to be subordinate in future interactions (Hsu et al. 2006). Social experience is typically divided into two types: the experience of establishing status vs. establishing and maintaining status (i.e., achieving social stability, Franck and Ribowski 1987). Most studies focus on the former, where winner and loser effects are very common (reviewed in Hsu et al. 2006). In social species, the experience of establishing and maintaining social status (e.g., Drummond and Osorno 1992; Franck and Ribowski 1989; Whiteman and Côté 2004) may be more ethologically relevant, however, because status transitions often follow periods of stability (Wilson 1980). In species with robust winner and/or loser effects and established fitness benefits of dominance, previous social experience could be an important predictor of future social status(es) and, therefore, a useful estimate of fitness.

Although winners can gain the fitness benefits associated with high status, contests cost time and energy and risk injury. Therefore, individuals should only engage in and/or escalate a contest when the benefits outweigh the costs (Hsu et al. 2006). In addition to, or in the absence of, winner/loser effects, current environmental conditions and internal states are also influential. Physical size and condition are among the most common proxies for fighting ability, and physical assessments of oneself and/or one's competitor should be central to agonistic behavior, fight duration and intensity, and status outcome. In many species, but not all (e.g., Reddon and Hurd 2009), size and condition are associated with social status, which can itself constrain growth rates and impact body condition (Hofmann et al. 1999; Huang et al. 2011; Jonart et al. 2007). Larger, high condition individuals are also more likely to win contests (Cloutier and Newberry 2000; Earley and Dugatkin 2006; Jonart et al. 2007; López and Martín 2001).

In this study, we investigated the effects of previous stable social status experience and physical condition on future status outcome in the bluebanded goby (*Lythrypnus dalli*), a highly social, sex-changing fish. This is a useful model system for a number of reasons. First, *L. dalli* social status naturally changes over time. Adults form linear social hierarchies composed of a large, dominant male and multiple, subordinate females. Status contests occur following the emigration or death of a group member, when an immigrant joins an existing group, and between novel individuals when establishing a group. Second, *L. dalli* is capable of bidirectional sex change, which is regulated by social status: the dominant fish is male, subordinates are female. Therefore, contests between same-sex competitors can result in a female becoming male or vice versa (Reavis and Grober 1999; Rodgers et al. 2007). Third, there is empirical evidence that *L. dalli* social status and sex affect fitness. Males, which are always dominant, have substantially higher reproductive success than females (St Mary 1996), and high status females take

priority when laying eggs in the male's nest (Solomon-Lane and Grober, unpublished data).

Winning and losing has important consequences for *L. dalli* social experience. Status classes differ in how frequently they initiate agonistic interactions (Solomon-Lane et al. 2014), and by definition, status dictates the outcome of this agonism (i.e., subordinates submit to dominants but not vice versa) (Drews 1993). In a stable laboratory social group, individual *L. dalli* are involved in over a thousand agonistic interactions per day, on average (Solomon-Lane and Grober, unpublished data), leading to dramatically different histories of winning and losing depending on social status (Solomon-Lane et al. 2014). These highly stereotyped agonistic interactions in stable groups are critical for *L. dalli* status maintenance. For example, if a male is prevented from interacting with the females in his harem, the dominant female will change sex to become the male of the group (Lorenzi et al. 2006). Based on the importance of status in determining *L. dalli* agonistic experience, we hypothesized that females with experience establishing and maintaining dominance would be more likely to establish dominance, and subsequently change sex, in a future contest. Alternatively, cues assessed during the contest, such as physical condition, may be a stronger influence on contest outcome. We tested these hypotheses by pairing novel, size-matched females, one with stable dominance experience and one with stable subordinate experience. This work can elucidate factors that influence life history transitions in *L. dalli* and other social species.

## Methods

### Study species

*L. dalli* is a small (18–45 mm adult standard length; SL) marine fish that undergoes socially regulated, bidirectional sex change (Reavis and Grober 1999; Rodgers et al. 2007). This species lives in mixed-sex social groups on rocky reefs in the Pacific Ocean, from Morro Bay, California as far south as the Galapagos Islands, Ecuador (Béarez et al. 2007; Miller and Lea 1972). Under natural conditions, groups range from small (3–10 fish) isolated harems to large aggregations (up to 120 fish/m<sup>2</sup>) (Steele 1996). Within small aggregations, high ranking females tend to remain in the same area, while lower ranking females move among social groups (Lorenzi 2009). Patterns of agonistic interaction in laboratory social groups (Reavis and Grober 1999; Rodgers et al. 2007) parallel those observed in natural social groups (Black et al. 2005). There are differences across statuses in agonistic behavior, including aggression, submission, and agonistic efficiency (displacements/approaches) (Rodgers et al. 2007; Solomon-Lane et al. 2014), body size (Behrens 1983), neuroendocrine

function (Lorenzi et al. 2012; Solomon-Lane et al. 2013), and reproductive success (Behrens 1983).

We collected *L. dalli* on reefs offshore of Catalina Island, California using hand nets while SCUBA diving (California Fish and Game permit no. SC-10676) and transported them to our fish facility at Georgia State University (Atlanta, GA). The fish facility was maintained on a 12:12 light/dark cycle at 18–20 °C, and fish were fed brine shrimp once daily until satiation. In the 8 months prior to the start of this experiment, we housed fish in social groups of one large male and three to six females of varying sizes in 38-l aquaria. Similar studies have included periods of isolation before their social manipulation to limit the effects of pre-experiment housing conditions (e.g., Dugatkin and Druen 2004); however, *L. dalli* do not respond well to isolation. To minimize the effect of pre-experiment experience, we imposed either dominant or subordinate stable social status experience by “rigging” contests between a larger dominant and smaller subordinate female (Beaugrand and Goulet 2000). Sufficient size differences bias *L. dalli* dominance to the larger fish (Rodgers et al. 2007).

### Stable social status experience

Females were selected to be either the alpha (dominant) or beta (subordinate) female in a social group with a larger, dominant male. To form groups of specific sizes and sex ratios, we briefly anesthetized fish in tricaine methanesulfonate (MS-222; 500 mg/L salt water) to measure SL and mass (females 26.6–39 mm SL, 0.29–0.91 g) and determined sex based on genital papilla morphology (St. Mary 1993). We took a digital image of the genital papilla to assess sex-typical morphology, and for females, we calculated the length-to-width ratio using ImageJ software (Rasband, W.S., ImageJ, U.S. National Institutes of Health, Bethesda, MD, USA, <http://imagej.nih.gov/ij/>, 1997–2012). Males have long, pointed papillae with a length-to-width ratio greater than 1.4. Females have short, rounded papillae with ratios less than 1.4 (St. Mary 1993) (average female ratio  $1.23 \pm 0.025$ ). Males were at least 3 mm SL longer than the alpha female, and alphas were at least 3 mm SL longer than their beta, in order to rapidly induce the desired social status. The male was dominant over both females, and the alpha female was dominant over the beta female. As a result, the alpha gained dominance experience while the beta female did not. The fish remained in these groups for 7 weeks (April 15–June 6), during which time no changes in status occurred. Two, 10-min behavioral observations (described below) were conducted approximately 6.5 weeks after group formation (June 1 and June 3) to confirm female social status. These observations were also used to determine whether agonistic behavior in the stable social groups was predictive of social status outcome in the novel pair.

### Social status contest in a novel pair

After 7 weeks in the stable social groups, we removed males and re-paired females such that an alpha was paired with a novel beta. We removed fish from their aquaria using hand nets and isolated them for approximately 1 h, a logistical necessity to guarantee that each novel pair was placed into a novel aquarium. Isolated females were then gently transferred into a novel aquarium at the same time as their contest opponent.

Females were paired in two different size conditions. To determine whether previous stable social status experience affected social status outcome in this novel context, we formed size-matched pairs ( $n=10$ ) of one previous alpha and one previous beta. Standard length measurements taken 1 week prior to re-pairing were used for size matching. We did not measure fish closer to the re-pairing to avoid an effect of handling. We also re-measured female SL and mass 24 h after females were placed in their novel pair. Based on this measurement 24 h post-pairing, size-matched females differed in SL (average  $\pm$  standard error of the mean, s.e.m.) by  $0.23 \pm 0.05$  mm and in mass by  $0.04 \pm 0.007$  g. Size differences this small do not bias status outcome (Rodgers et al. 2005). We also formed size-mismatched pairs ( $n=12$ ) in which the previous alpha was at least 3 mm SL longer than the previous beta (average differences: SL  $6.03 \pm 0.34$  mm, mass  $0.29 \pm 0.03$  g). In this control condition, the larger previous alpha should easily establish and maintain dominance over the smaller previous beta. Females remained in these pairs for 10 days.

One minute after female pairs were transferred into their novel aquarium, we began three rolling 10-min behavioral observations. We observed up to 30 min or until dominance criteria were met by one of the females. Dominance establishment was defined as one female displacing the other five times without being displaced herself (similar to Beaugrand et al. 1991; Reddon and Hurd 2009). Agonistic interactions were initiated with an approach, when one fish swam directly towards any part of another fish's body within two body lengths. Approaches led to a displacement if the approached fish swam away within 5 s. From these data, we calculated individual agonistic efficiency, the proportion of approaches that led to a displacement (displacements/approaches). To confirm that status was maintained, we conducted additional behavioral observations 8, 9, and 10 days after females were paired. Dominance was determined by patterns of submission and occupying the nest territory.

Pairing two female *L. dalli* in the absence of a male is permissive to sex change: the female that establishes and maintains dominance changes sex (Rodgers et al. 2005). We evaluated whether sex change had been initiated based on digital images of the sexually dimorphic genital papilla 10 days after pairing (Reavis and Grober 1999; St. Mary 1993).

## Body size and physical condition

We calculated relative differences in size between paired females as follows:  $(\text{dominant} - \text{subordinate mass}) / (\text{average dominant, subordinate mass})$ . We estimated physical condition based on condition index, actual mass/expected mass (%), a value used across species to evaluate general health, stress, and energy stores (Stevenson and Woods 2006). Using an established variation of Fulton's condition factor,  $K$ , which estimates expected mass as  $SL^3$ , we calculated expected mass empirically from a regression of  $SL$  by mass for 396 wild-caught *L. dalli*:  $\text{mass} = 0.000019055 \times SL^{2.94}$  (Stevenson and Woods 2006). We calculated the relative difference in condition index between a pair as follows:  $(\text{dominant} - \text{subordinate condition}) / (\text{average dominant, subordinate condition})$ .

## Data analysis

Statistics were performed using JMP 11.0, and the data presented in the text are means  $\pm$  s.e.m. For clarity, we will explicitly refer to the different social contexts as "stable social groups" or "pairs." We will refer to females in the stable social groups as "alpha" and "beta" (the dominant fish was male) and fish in the pairs as "dominant" and "subordinate." In the pairs, either the previous alpha or previous beta could establish dominance. In the size-mismatched (control) pairs, all of the larger alphas established and maintained dominance; therefore, we will continue to refer to these pairs as "size-mismatched." We will refer to size-matched pairs in which the previous alpha became dominant as size-matched "SAME status pairs" (i.e., alphas remained dominant and betas remained subordinate: individual status was the *same* in the stable social group and in the pair). Size-matched pairs in which the previous beta became dominant will be referred to as "SWITCHED status pairs" (i.e., alphas became subordinate and betas became dominant: individual status *switched* between the stable social group and the pair) (Fig. 1, summary of the social contexts included in this study: stable social groups, novel pairs, and stable pairs). For statistical comparisons, "treatment groups" refer to comparisons among size-mismatched, size-matched SAME status, and size-matched SWITCHED status pairs. A significant effect of treatment group indicates an effect of experience and size difference. If size-mismatched and size-matched SAME status pairs differ from SWITCHED status pairs, this indicates an individual-specific effect of previous agonistic experience in the stable group. If size-mismatched differ from the size-matched pairs, this indicates an effect of size difference in the pair.

Pairwise comparisons of alpha and beta behavior and condition in the stable groups were compared using paired  $t$  tests or Wilcoxon signed rank tests, for data that did not meet the assumptions of parametric statistics. Independent  $t$  tests were used to compare stable group behavior for fish that became

dominant vs. subordinate. Logistic regressions were used to test the effect of behavior and condition on status outcome in the pairs. Alpha levels were adjusted for multiple pairwise comparisons or logistic regressions using sequential Dunn-Sidak corrections. Exact  $p$  values are provided in the text. We used mixed factorial analysis of variance (ANOVA) to determine whether behavior, condition, or genital papilla ratio differed among treatment groups (size-mismatched, size-matched SAME status, size-matched SWITCHED status; between-subject factor), between status outcomes (dominant, subordinate; within-subject factor), or if there was an interaction. Status outcome is the "within-subject" factor because individual status is not independent of the other member of the pair (Briffa and Elwood 2010). Behavior during status establishment was summed from the rolling observations and analyzed as behaviors per minute. Two size-matched pairs were excluded from the behavior analyses on the day of pairing because the social status of the females reversed between that day and day 8. One-way ANOVA was used to analyze treatment differences in the time required to establish dominance. Post hoc analyses of significant ANOVA results were conducted using Tukey HSD tests. Linear regression analysis was used to identify associations among agonistic behavior, physical condition, time to establish dominance, and change in genital papilla ratio.

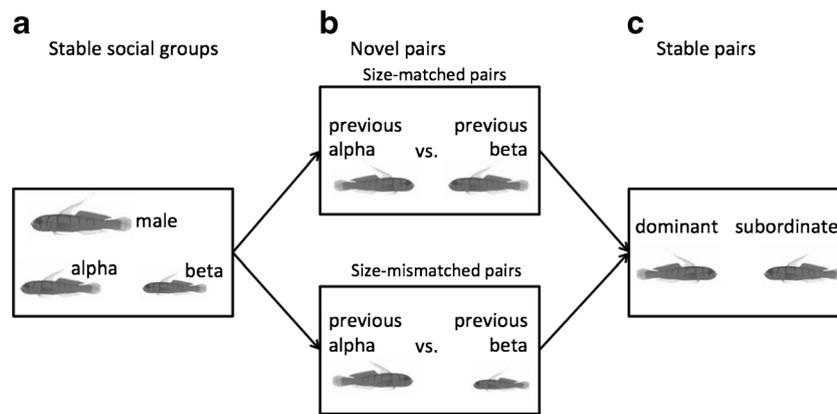
## Results

### Agonistic behavior, but not physical condition, was status-dependent in stable social groups

In the stable social groups, agonistic behavior differed between females of different statuses. Alpha females approached (paired  $t$  test, natural log transformation:  $t = -4.38$ ,  $d.f. = 19$ ,  $p = 0.0003$ ,  $n = 20$ ) and displaced (Wilcoxon signed rank test:  $S = -114.50$ ,  $p < 0.0001$ ,  $n = 21$ ) significantly more than betas, and alphas had significantly higher agonistic efficiency than betas (Wilcoxon signed rank test:  $S = -105.0$ ,  $p < 0.0001$ ,  $n = 20$ ) (Fig. 2a). There were no significant differences between alpha and beta condition index (paired  $t$  test:  $t = -0.30$ ,  $d.f. = 20$ ,  $p = 0.77$ ,  $n = 21$ ) (Fig. 2b).

### Social status outcome in the novel pairs

In the size-mismatched control pairs, all of the previous alphas ( $n = 12$ ) established dominance within 30 min according to our criteria (five consecutive displacements without being displaced) and maintained dominance for the following 10 days. Eight out of ten size-matched pairs established dominance within 30 min. In six of those eight pairs, the previous alpha established dominance. On days 8–10, all pairs had a clear dominant and subordinate. In the size-matched pairs,



**Fig. 1** Overview of the three social contexts, and associated nomenclature, for fish in this study: **a** stable social groups, **b** novel pairs (in which status has yet to be established), and **c** stable pairs. In **b** and **c**, we will refer to size-matched pairs in which the previous alpha (in

**a**) established dominance as size-matched SAME status pairs. Pairs in which the previous beta (in **a**) established dominance are size-matched SWITCHED status pairs

dominance reversed in two size-matched pairs before day 8, resulting in five previous alphas (SAME status pairs) and five previous betas (SWITCHED status pairs) establishing and maintaining dominance.

**Physical condition, status contest duration, and status outcome in the novel pairs**

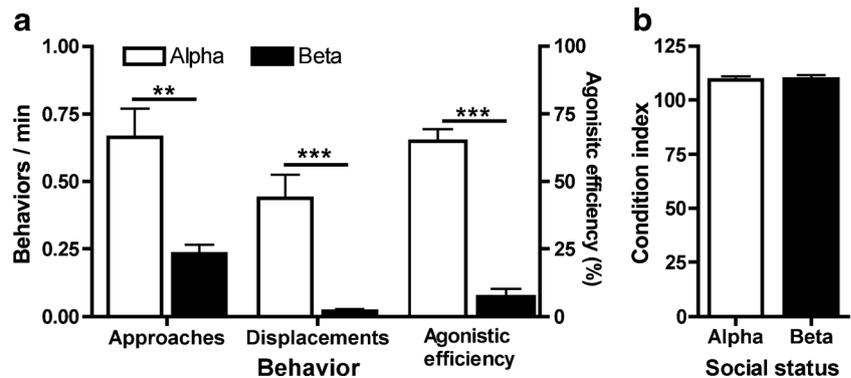
In size-matched pairs, pairs with a larger relative difference in condition between dominants and subordinates were significantly more likely to have a SAME status outcome than a SWITCHED status outcome (logistic regression:  $\chi^2=6.16$ ,  $d.f.=1$ ,  $p=0.013$ ,  $n=10$ ). There was a trend for pairs with a larger relative difference in mass to have a SAME status outcome rather than a SWITCHED status outcome (logistic regression:  $\chi^2=3.58$ ,  $d.f.=1$ ,  $p=0.058$ ,  $n=10$ ). In the size-matched pairs, condition index did not differ between dominants and subordinates (mixed factorial ANOVA:  $F_{1,8}=2.47$ ,  $p=0.15$ ) or between individuals in SAME and SWITCHED status pairs ( $F_{1,8}=1.48$ ,  $p=0.26$ ). The interaction between status and SAME vs. SWITCHED outcome was significant ( $F_{1,8}=7.74$ ,  $p=0.024$ ). Post hoc analysis showed a trend for

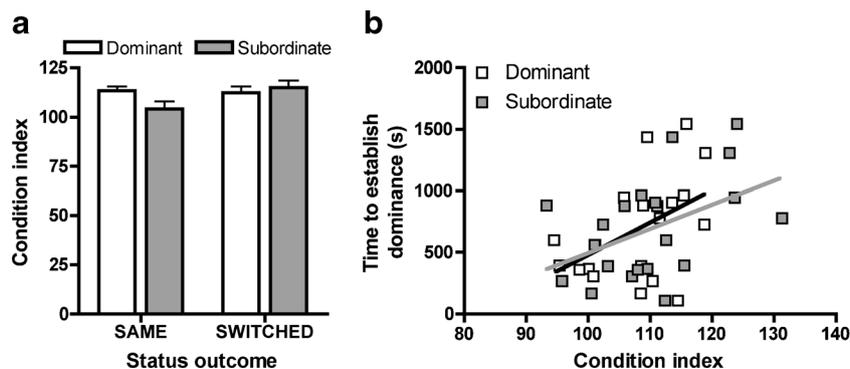
SAME status dominants to have higher condition than SAME status subordinates ( $p=0.06$ ), and no other pairwise differences were significant ( $p>0.13$ ) (Fig. 3a). A regression model including both dominant and subordinate condition explained a significant amount of the variation in the time required to establish dominance ( $r^2=0.32$ ,  $F_{2,17}=5.52$ ,  $p=0.014$ ). Both parameters contributed significantly to the model: dominant condition ( $\beta=0.42$ ,  $p=0.039$ ) and subordinate condition ( $\beta=0.42$ ,  $p=0.043$ ) (Fig. 3b). For pairs that established dominance within 30 min, there were no significant differences in the time to establish dominance among size-mismatched, size-matched SAME status, and size-matched SWITCHED status pairs (one-way ANOVA:  $F_{2,15}=1.77$ ,  $p=0.20$ ).

**Behavior in the stable social groups was associated with status outcome in the size-matched pairs**

There were no differences in alpha approaches ( $t$  test, natural log transformation:  $t=-0.21$ ,  $d.f.=6.87$ ,  $p=0.84$ ) (Fig. 4) in the stable social groups between alphas that went on to be dominant in the size-matched pairs (SAME status pairs,  $n=5$ ) and those that became subordinate (SWITCHED status pairs,  $n=5$ ).

**Fig. 2** Behavior and physical condition in stable social groups. **a** Mean ( $\pm$ s.e.m.) alpha and beta approaches ( $n=20$ ), displacements ( $n=21$ ), and agonistic efficiency ( $n=20$ ) in the stable social groups. **\*\*** $p<0.001$ , **\*\*\*** $p<0.0001$ . **b** Mean ( $\pm$ s.e.m.) alpha and beta condition index ( $n=21$ )

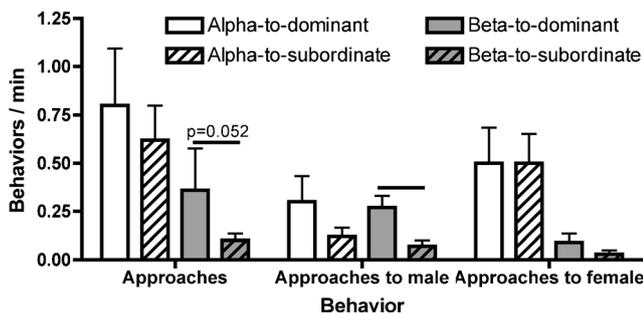




**Fig. 3** Physical condition in stable social groups and contest duration in novel pairs. **a** Mean ( $\pm$ s.e.m.) dominant ( $n=10$ ) and subordinate ( $n=10$ ) condition index (actual mass/expected mass) in size-matched SAME ( $n=$

5) and SWITCHED status pairs ( $n=5$ ). **b** Associations between dominant ( $n=20$ ) and subordinate ( $n=20$ ) condition index (all treatment groups) with the time required to establish dominance in the novel pairs

There was a trend for betas that established and maintained dominance in the pairs (SWITCHED status pairs,  $n=5$ ) to approach more in the stable social groups than betas that became subordinate (SAME status pairs,  $n=5$ ) ( $t$  test:  $t=-2.53$ ,  $d.f.=5.05$ ,  $p=0.052$ ) (Fig. 4). To better understand the importance of beta approaches for status outcome in the pairs, we analyzed approaches to the male and approaches to the female separately. Betas that approached the male more frequently were significantly more likely to have a SWITCHED status outcome (logistic regression:  $\chi^2=7.75$ ,  $d.f.=1$ ,  $p=0.0054$ ,  $n=10$ ). Beta approaches to the alpha did not predict status outcome in the pairs (logistic regression:  $\chi^2=1.64$ ,  $d.f.=1$ ,  $p=0.20$ ,  $n=10$ ). Alpha approaches to the male did not predict status outcome in the pairs (logistic regression:  $\chi^2=2.11$ ,  $d.f.=1$ ,  $p=0.14$ ,  $n=10$ ) (Fig. 4). For all females, rate of approaching in the stable group was not associated with their condition (linear regression:  $r^2=0.014$ ,  $F_{1, 42}=0.61$ ,  $p=0.44$ ,  $n=44$ ).



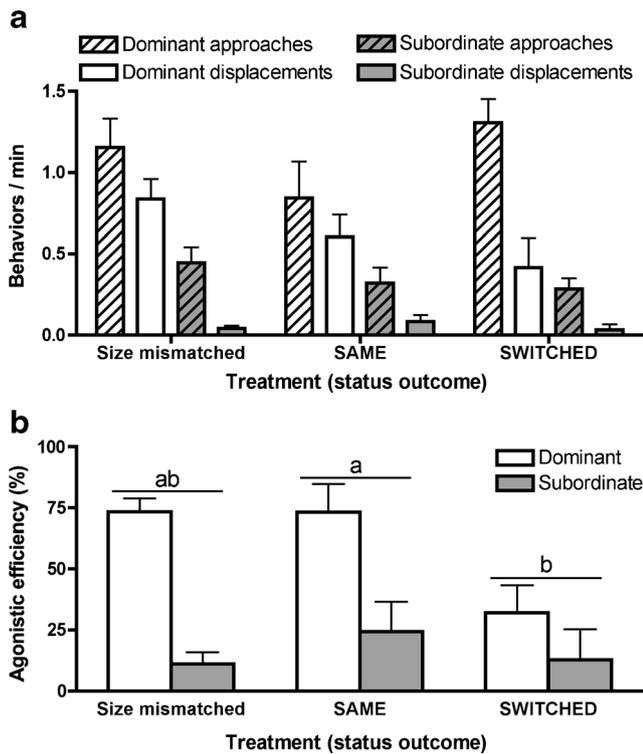
**Fig. 4** Behavior from stable social groups and status outcome in novel pairs. Mean ( $\pm$ s.e.m.) alpha and beta approaches in the stable social groups. Approaches refer to the total number of approaches, including approaches to the male and approaches to other female. Alphas that went on to become dominant in the pairs (alpha-to-dominant; size-matched SAME status pairs;  $n=5$ ) are compared to alphas that became subordinate in the pairs (alpha-to-subordinate; size-matched SWITCHED status pairs;  $n=5$ ). Betas that went on to become dominant in the pairs (beta-to-dominant; size-matched SWITCHED status pairs;  $n=5$ ) are compared to betas that became subordinate in the pairs (beta-to-subordinate; size-matched SAME status pairs;  $n=5$ ). Betas that approached the male more were more likely to have a SWITCHED status outcome (logistic regression:  $p=0.0054$ , black bar)

### Agonistic behavior during status establishment

We used mixed factorial ANOVAs to determine whether there were differences in agonistic behavior across status outcomes (dominant or subordinate) or treatment groups or whether there was an interaction. Approach rate differed significantly across statuses ( $F_{1, 17}=42.13$ ,  $p<0.0001$ ), but there were no differences across treatment groups ( $F_{2, 17}=0.61$ ,  $p=0.56$ ), and there was no interaction ( $F_{2, 17}=1.19$ ,  $p=0.33$ ). Post hoc analysis showed that dominants approached significantly more than subordinates ( $p<0.0001$ ) (Fig. 5a). Displacement rates also differed significantly across statuses ( $F_{1, 17}=33.20$ ,  $p<0.0001$ ). There was no difference in displacement rate across treatment groups ( $F_{2, 17}=1.43$ ,  $p=0.27$ ) and no interaction ( $F_{2, 17}=1.94$ ,  $p=0.17$ ). Post hoc analysis showed that dominants displaced significantly more than subordinates ( $p<0.0001$ ) (Fig. 5a). Agonistic efficiency differed significantly across statuses ( $F_{1, 16.2}=27.1$ ,  $p<0.0001$ ) and treatment groups ( $F_{2, 15.7}=4.13$ ,  $p=0.036$ ), but there was no interaction ( $F_{2, 16.2}=2.21$ ,  $p=0.14$ ). Post hoc analysis showed that dominants had significantly higher agonistic efficiency than subordinates ( $p<0.0001$ ). Post hoc tests also showed that individuals in SAME status pairs had significantly higher agonistic efficiency than in SWITCHED status pairs ( $p=0.036$ ). There was a trend for individuals in size-mismatched to have higher agonistic efficiency than in SWITCHED status pairs ( $p=0.064$ ), but agonistic efficiency in size-mismatched and SAME status pairs did not differ ( $p=0.68$ ) (Fig. 5b).

### Status and sex change in the stable pairs

The same individuals were dominant and subordinate during the behavior observations 8, 9, and 10 days after pairing, indicating that status was stable. There was a trend for dominant fish to have more male-typical genital papilla ratios (mixed factorial ANOVA:  $F_{1, 19}=4.22$ ,  $p=0.054$ ). There were no differences in papilla ratio among treatment groups (mixed factorial ANOVA:  $F_{2, 19}=1.01$ ,  $p=0.38$ ) and there was no

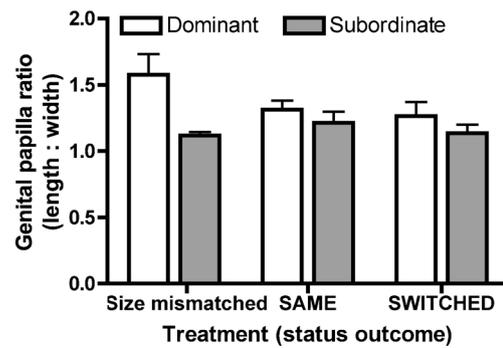


**Fig. 5** Behavior and status outcome in novel pairs. **a** Mean ( $\pm$ s.e.m.) dominant and subordinate approaches and displacements expressed during status establishment in size-mismatched ( $n=12$ ), size-matched SAME status ( $n=5$ ), and size-matched SWITCHED status ( $n=3$ ) pairs. **b** Mean ( $\pm$ s.e.m.) dominant and subordinate agonistic efficiency during status establishment in size-mismatched ( $n=12$ ), size-matched SAME status ( $n=5$ ), and size-matched SWITCHED status pairs ( $n=3$ ). Different letters indicate significant differences ( $p<0.05$ )

interaction (mixed factorial ANOVA:  $F_{2, 19}=1.39$ ,  $p=0.27$ ). Dominant fish in size-mismatched pairs were the only group with male-typical papilla ratios (size-mismatched,  $1.58\pm 0.15$ ; SAME status,  $1.31\pm 0.068$ ; SWITCHED status,  $1.27\pm 0.11$ ) (Fig. 6). There was a significant, positive association between the rate of dominant displacements in the stable pairs and the change in genital papilla ratio (day 10–day 1 genital papilla ratio) ( $r^2=0.41$ ,  $p=0.0014$ ,  $n=22$ ).

## Discussion

Quantifying individual agonistic behavior and physical condition before, during, and after a status contest provided insight into factors relevant to the establishment and maintenance of dominance in *L. dalli*, as well as sex change, a status-dependent transformation (Rodgers et al. 2007). We effectively gave alpha and beta females in the stable social groups dramatically different agonistic experiences (Fig. 1 and 2a). Pairing females that had previously been alpha with a size-matched or smaller previous beta identified important roles for physical condition and previous agonistic experience in the resolution of *L. dalli* contests. We cannot yet draw



**Fig. 6** Genital papilla morphology in stable pairs. Mean ( $\pm$ s.e.m.) genital papilla length-to-width ratios in size-mismatched ( $n=12$ ), size-matched SAME status ( $n=5$ ), and size-matched SWITCHED status ( $n=5$ ) pairs 10 days after pairs were formed (male-typical  $>1.4$ ; female-typical  $<1.4$ )

strong conclusions about the role of previous stable status experience because of sample size limitations; however, in this study, half of the dominants in size-matched pairs were previously alpha and half were previously beta. Testing the effects of previous status experience in a stable *L. dalli* social group is especially interesting because, across species, the effects of establishing and maintaining dominance are much more variable than following a paradigm that only includes dominance establishment (Hsu et al. 2006). While crickets (Simmons 1986), green sunfish (McDonald et al. 1968), and blue-footed boobies (Drummond and Canales 1998; Drummond and Osorno 1992) all show long-term effects of previous stable status experience, green swordtails (Franck and Ribowski 1989), hens (Cloutier and Newberry 2000), common marmosets (Saltzman et al. 1996), and cleaning gobies (Whiteman and Côté 2004) do not.

Condition index can be affected by resource availability, allocation to reproduction and/or growth, and stress (Stevenson and Woods 2006). In this study, contests involving females in better condition took longer to resolve (Fig. 3b), but there were no differences among size-mismatched and the size-matched groups in time to establish dominance. These data suggest that contest resolution is affected by a female's own condition and persistence rather than the difficulty of the contest (Gammell and Hardy 2003; Taylor and Elwood 2003), which should be lower in size-mismatched pairs that were "rigged" for previous alphas to win. Both dominant and subordinate condition independently explained a significant amount variation in contest duration (Fig. 3b). Models of this scenario have demonstrated that a significant association for subordinate condition can drive a parallel association between dominant condition and contest duration (Taylor and Elwood 2003). It remains to be tested whether subordinate condition alone determines contest duration in *L. dalli* pairs. While longer contests can require a greater energetic investment, there is unlikely to be a substantially increased risk of injury because *L. dalli* agonistic interactions rarely escalate.

In size-matched pairs, individuals competed for dominance on a more level playing field, providing an opportunity to identify factors other than size that may have a more nuanced effect on status outcome. Although these data must be interpreted with caution because only ten size-matched pairs were included in this study, we identified two significant predictors of SAME vs. SWITCHED status outcome. First, the relative difference in condition between size-matched dominants and subordinates predicted status outcome. Relatively large condition differences were more likely to have a SAME status outcome. This suggests that when paired previous alphas and previous betas were in similar condition, any potential advantage of previous dominance experience decreased. In other species, superior physical condition can, itself, be an advantage in a status contest (Jonart et al. 2007; López and Martín 2001). Condition can also influence decisions to engage or escalate a contest or serve as a signal of fighting ability or status (Hsu et al. 2006; Jonart et al. 2007; López and Martín 2001; Schuett 1997). To identify a causal role for *L. dalli* condition, it will be necessary to pair fish of specific conditions and relative condition differences. Second, in the stable groups, betas that approached the male more frequently were more likely to have a SWITCHED status outcome. Behavior and social experience within a status class can vary for *L. dalli* (Solomon-Lane et al. 2014) and other social species (e.g., Nelson-Flower et al. 2013; Wey et al. 2013; Young et al. 2006). Our data suggest that within-status variation in agonistic experience, in addition to categorical experiences as a particular social status, may be relevant for understanding contest outcomes.

Our data suggest there may be a specific and limited effect of status experience on agonistic behavior during status establishment. Agonistic efficiency was significantly lower in size-matched SWITCHED status pairs than SAME status pairs (Fig. 5b). There were no treatment differences in approaches or displacements (Fig. 5a). During status establishment in this species, agonistic efficiency starts low for all group members. As dominance relationships form, agonistic efficiency increases dramatically in all individuals except the lowest ranking (Solomon-Lane et al. 2014). In SWITCHED status pairs, dominants failed to achieve an agonistic efficiency higher than their subordinates before meeting dominance criteria. This could be caused by the inability of previous betas (now dominants) to execute an approach that reliably elicits a displacement or by previous alphas (now subordinates) being reluctant to submit (i.e., be displaced). Subordinates may be more likely to drive down dominant agonistic efficiency because, although dominants can initiate an approach, subordinates dictate whether they submit, ignore, or escalate (e.g., Schuett 1997). To test this hypothesis directly, females with the same stable status experience should be paired so that only one individual per pair experiences a change in status (as in Franck and Ribowski 1989).

For *L. dalli*, establishing and maintaining dominance in a sex change permissive context can lead to dramatic increases in reproductive success. One of the most important reasons for understanding the mechanisms of status contests and transitions is the strong connection to fitness (Ellis 1995; Wilson 1980). In this study, we detected no differences in the rate of sex change, as indicated by the genital papilla ratio, between dominants and subordinates or across treatment groups (Fig. 6). Interestingly, dominants in size-mismatched pairs were the only group with obviously male-typical papilla (ratios >1.4). These data are consistent with previous studies showing that the social context in which *L. dalli* dominance is established is central to the rate of sex change. In groups of unfamiliar or similarly sized fish, sex change is delayed because dominance establishment takes longer (Reavis and Grober 1999). Although we predicted that dominant papilla ratios would be most similar in size-mismatched and size-matched SAME status pairs because of their behavioral similarities during status establishment (Fig. 5), our data suggest that the size difference and behavioral factors, such as the rate of dominant aggression in the stable pair, may impact the social context within which sex change occurs more than the previous experience of the dominant (or subordinate) fish or transient differences in agonistic efficiency.

### Limitations and future directions

This study was designed to test the effect of previous stable social status experience on status outcome in a future contest. Although there was an even split between the number of previous alphas and previous betas that became dominant in size-matched pairs, too few pairs were included to draw strong conclusions. In addition, including pairs that were “rigged” for a larger previous beta to establish and maintain dominance over a previous alpha would have allowed us to discriminate between the role of previous experience and size differences on contests and sex change. This will be an important experimental group to include in future studies, along with pairs matched or mismatched for experience and condition. Overall, we think that future investigations with *L. dalli*, a highly social, sex-changing fish in which many factors relevant to contests can be manipulated in ethologically relevant ways, will further elucidate the regulation of social status and status transitions across social species.

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**Compliance with ethical standards** All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed were in accordance with the ethical standards of Georgia State University, at which the studies were conducted (IACUC protocol #A13023).

**Conflict of interest** The authors declare that they have no competing interests.

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