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Preventing behavioural interactions with a male facilitates sex change in female bluebanded gobies, *Lythrypnus dalli*

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Abstract Sex change in marine teleost fishes is commonly regulated by social factors. In species that exhibit protogynous sex change, such as the bluebanded goby *Lythrypnus dalli*, the most dominant female typically initiates sex change when a male is removed from the social group. Females can use visual, chemical or tactile cues to assess the presence or absence of a male. The primary goal of our study was to determine whether the olfactory and visual presence of a male versus its behavioural interactions with females were important for mediating sex change. We exposed females to three different treatments: absence of a male, presence of a male that could physically interact with her and presence of a male behind a barrier that allowed visual and olfactory interactions but prohibited physical interactions. Sex change occurred in the absence of a male but not in the presence of a male that could physically interact with the female. The presence of a male behind the barrier did not prevent sex change but affected the timing of sex change. Season appeared to affect the latency to initiate male typical courtship, with a delay at the end of the reproductive season only when the male was present behind the barrier. We discuss the seasonal results in terms of *L. dalli* life history and the potential benefits and costs of changing sex late in the season in the presence or absence of aggressive reinforcement by the male. Our results identify direct behavioural interactions as an important proximate mechanism in the social regulation of sex change in *L. dalli*.

Keywords Sex reversal · Proximate mechanism · Sensory cues · Social interactions

Introduction

Social interactions can exert potent effects on reproductive behaviour, the distribution of matings within a social group and, ultimately, fitness (Ellis 1995). An extreme case of social regulation of reproduction is the inhibition of reproductive behaviour and/or function in subordinates by dominant animals, a phenomenon that has been documented in a wide range of taxa from teleost fishes (Poeciliidae, Borowsky 1987; Cichlidae, Francis et al. 1993) to mammals (dwarf mongooses, Creel et al. 1992). Subordinate individuals might perceive the dominant animal through visual, chemical or tactile cues or a combination thereof, depending on species-specific differences in sensory systems. In honeybees, for example, chemical communication plays a major role in inhibiting workers from reproducing (Keller and Nonacs 1993; Winston and Slessor 1992), while in naked mole-rats, tactile stimuli from the queen are important in preventing reproduction in subordinate females (Clarke and Faulkes 2001).

Social regulation of reproductive behaviour in sexually labile species often is manifested as control of the transition from one sex to the other by some aspect of the social environment (Fishelson 1970; Robertson 1972; Fricke and Fricke 1977; Shapiro 1979; Wright 1988; Warner and Swearer 1991; Warner et al. 1996; Baeza and Bauer 2004). Although social regulation of sex change is subtler than strict reproductive inhibition, the two phenomena are similar in that some individuals prevent others from achieving a particular reproductive phenotype, usually the phenotype with the greatest fitness potential.

In harem protogynous fishes, females change sex to male, and males typically have the greatest reproductive success (St. Mary 1994). Females do not change sex in the presence of a male, but his removal coupled with the presence of other females induces a female, usually the largest, to change sex (Fishelson 1970; Robertson 1972; Shapiro 1981a,b; Reavis and Grober 1999; for exceptions, see Muñoz and Warner 2003). Complete suppression of reproduction does not occur in these systems because both sexes are reproductively active, but some aspects of the male's

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presence inhibit females from developing male reproductive function and therefore from achieving the highest reproductive success. Although aggressive behaviour by males has been previously suggested as a possible mechanism for preventing sex change in females of some territorial species (*Labroides dimidiatus*, Robertson 1972; *Anthias squamipinnis*, Shapiro 1981a; *Centropyge potteri*, Lutnesky 1994), no studies to date have adequately partitioned the influence of male presence alone from behavioural interactions between the male and females in his harem.

We used the bluebanded goby (*Lythrypnus dalli*), a bidirectionally sex-changing benthic marine fish, to test explicitly whether preventing behavioural interactions with the male facilitates protogynous sex change in females. We use the general term behavioural interactions to account for the possibility that facilitation of sex change could be mediated by the removal of overt physical encounters or non-physical aggressive or courtship interactions. Little is known about group dynamics of *L. dalli* in the field. Behrents (1983) documented that this species lives at high densities but that individuals occupy social groups of 4–12 individuals within small territories associated with a sea urchin. It is thus possible, given close proximity, that individuals could integrate olfactory, visual or tactile (e.g. physical interaction, stimulation of lateral line system) cues to assess social group composition and individual status. Their small size allows us to establish semi-natural social groups in the laboratory, and their behavioural profile has been described in detail under both laboratory and field conditions (Reavis and Grober 1999; Black et al. 2005). Most importantly, the onset of sex change can be assessed behaviourally. Immediately after male removal, the dominant female shows a peak in her rate of aggressive displacement and a nadir in submission. One to five days later, she begins showing a distinct swimming behaviour called jerking, which is typical of male courtship (Behrents 1983; Reavis and Grober 1999) and is associated with gonadal sex change. *L. dalli* establishes a social hierarchy based mainly on body size through aggressive interactions (Behrents 1983; Reavis and Grober 1999). Thus, it is straightforward to predict which female will be the eventual sex changer and record its behaviour following male removal.

Previous studies on the inhibition or facilitation of sex change have focused primarily on disentangling the influence of olfactory and visual cues (Ross et al. 1983; Cole and Shapiro 1995) and did not employ the appropriate controls to distinguish whether sex change inhibition was due to the mere presence of, or behavioural interactions with, the male. Some of these studies implicate tactile interactions with the male as an important cue regulating inhibition of sex change in females (Ross 1981; Shapiro 1983). The primary goal of our study was to begin to assess which cues might mediate protogynous sex change by partitioning the influence of male visual or olfactory presence from behavioural interactions between the male and dominant female. We acknowledge that behavioural interactions are inherently multimodal, being composed of

any combination of tactile, visual and olfactory cues. Our aim is not to dissociate the various cues that define behavioural interactions. Rather, we explore the relative influence of multimodal behavioural interactions vs visual/olfactory cues indicative only of male presence on the probability and dynamics of sex change. We manipulated the ability of a male to interact behaviourally with the females of a social group while either maintaining or eliminating the opportunity for visual and olfactory cues to be communicated between the sexes. Assessment of dominance status appears to be critical for regulating the decision to sex change in *L. dalli* (Rodgers et al. 2005). Because dominant status is most reliably assessed through behavioural cues, whether physical or not physical, we posited that removal of cues indicative of social subordination (i.e. male behaviour) would be most salient in facilitating protogynous sex change in this species.

This experiment was conducted on fish collected during different seasons and given the potential for seasonal variation in reproductive behaviour (Reavis and Grober 1999), we accounted for season in all the analyses.

Methods

Study species

The bluebanded goby is a small benthic fish (20–45 mm adult standard length) that inhabits rocky reefs along southern California and Baja California, Mexico (Wiley 1976). The majority of individuals reproduce during a single season (Behrents 1983). Off the coast of Santa Catalina Island, California, where fish used in this study originate, *L. dalli* density ranges from 2 to 58 individuals/m² depending on season and habitat complexity, with an average of about 24 individuals/m² (Behrents 1983). The density peaks in the fall and is positively correlated with water temperature (Behrents 1983). The bluebanded goby finds shelter from predators between the spines of the sea urchin *Centrostephanus coronatus*, and 90% of the fish are found associated with an urchin (Hartney and Grover 2002) with most commonly one to four fish per urchin. *L. dalli* establishes size-based dominance hierarchies with a dominant male that defends a nest in worm tubes or empty shells and spawns with multiple females within a given season (Behrents 1983). Individuals live in harem-structured groups, the composition and location of which are stable over time (Lorenzi et al., unpublished field data). The high abundance of predators around the urchins makes movements between adjacent groups very dangerous, but no studies have examined interactions between groups in the wild. There is some evidence that individuals do emigrate from groups depending on shelter availability (Behrents 1983) and that some males can migrate when a neighbouring male is artificially removed (Black et al. 2005).

Females with developed eggs have been found in the wild from February to October, with a peak from May to August (Wiley 1976; Behrents 1983). At the functional

level, this species is a sequential hermaphrodite because individuals exhibit only one behavioural sex (St. Mary 1994; Reavis and Grober 1999). The sexes can be distinguished using genital papilla shape (Wiley 1976; St. Mary 1993). Genital papilla length-to-width ratio is a good indicator of papilla shape and therefore of sex, with female ratio being close to 1 and male ratio greater than or equal to 1.4 (Carlisle et al. 2000). Individuals with rounded, female-like papilla allocate more than 95% of their gonad to ovarian tissue, they lack accessory gonadal structures (AGS), and they do not spawn as a male. The AGS in this species consists of multiple long vesicles that produce a mucous secretion (Drilling and Grober 2005). In other goby species, the secretion is used to make sperm trails that increase sperm viability in an aqueous medium (Marconato et al. 1996; Scaggiante et al. 1999). Male *L. dalli* typically have a thin pointy papilla and always possess an AGS filled with mucus and sperm (Drilling and Grober 2005). Males also defend a nest, provide parental care to the eggs (Wiley 1976; Behrems 1983) and exhibit courtship behaviour characterized by jerk swims, which are discontinuous, choppy movements usually from the nest to the female and back to the nest (Behrems 1983; Reavis and Grober, 1999).

Experimental conditions

Fish were collected off the coast of Santa Catalina Island (CA Department of Fish and Game, permit no. 803034-01), by scuba diving in September 2003 and March 2004 with the anaesthetic quinaldine (Sigma) and hand nets. Animals were collected with the same modalities and in the same site in both seasons. Because the experiments were conducted in two different seasons and because there is some evidence that season might affect the rate of sex change (Reavis and Grober 1999), we accounted for it when comparing treatments (see “Data analysis” for details). Animals were shipped to Georgia State University, Atlanta (USA), where they initially were housed in communal tanks at a temperature of 19–20°C and a 12:12-h light/dark photoperiod. The fish were fed brine shrimp twice daily with occasional supplements of Tetra-Min (Tetra, Blacksburg, PA, USA) marine flake food. Fish were removed from the communal tank and slightly anaesthetized with tricaine methanesulfonate (MS222; Sigma). We recorded banding patterns for identification purposes, standard length (in millimetres) and wet body mass (in grams). The genital papilla was photographed, its length and width measured with callipers to calculate the papilla ratio (length/width), and its shape (rounded or pointy) was used for assignment of sex (Wiley 1976; St. Mary 1993). All fish that were smaller than 21 mm or that had ambiguous papilla shape were excluded from the study to avoid including immature individuals and fish in transitional states between the two sexes.

We established experimental groups consisting of a large male, a large female and two small females in 38-l aquaria with similar conditions as the communal tanks. Males were

at least 3 mm larger than the largest female, and the largest females were at least 4 mm longer than the small females in the same social group, which allowed us to identify unambiguously the dominant female and thus the predicted sex changer (Reavis and Grober, 1999). The presence of other females in the tank is important because sex change does not occur in social isolation (Carlisle et al. 2000). The standard length of each class of fish was similar across treatments (Table 1). A plastic screen divider (Lee’s Aquarium & Pet Products, San Marcos, CA, USA) separated the tank into one small compartment (approximately one third of the tank) and one large compartment. The divider was clear with holes of 1.5-mm diameter and allowed visual and olfactory communication but no physical contact between fish in the different compartments. A test with a coloured plume (methylene blue) showed that there was circulation of water through the divider from the side housing the male to the side containing the females; there was no significant difference in the rate of movement of the plume in a tank with or without divider (t test: $t_8=1.14$, $p=0.29$; with divider, 6.4 ± 0.40 s; without divider, 5.2 ± 0.97 s). A small PVC tube was placed in the large compartment of each tank as a nest site; a transparent acetate sheet was inserted into the tube as a lining to facilitate removal of egg clutches.

Three different treatments were established, each consisting of 10 groups: (1) social group with male present (M); (2) male behind the clear, porous barrier (MB); (3) male removed (no male present, NM). Each group (male and females) was allowed to acclimate to the new tank for 6 days and to establish a social hierarchy. On the sixth day (day –1 from male removal) and for the following 22 days, we conducted 10-min behavioural observation sessions in the morning and afternoon of each day. We observed the predicted sex changer (largest female) in the MB and NM conditions, and the male and largest female in the M condition. For these focal fish, we recorded the number of displacements (an approach within 5 cm of another fish that results in the approached fish moving away) performed towards all others occupying the same compartment, the number of displacements received from the other fish and the number of jerk swims (courtship behaviour) performed towards the nest or towards the other individuals.

On the seventh day (day 0) of the M treatment, the male was briefly netted and returned to the same side as the females so that the dominant female had visual, olfactory and physical interactions with the male. On day 0 of the MB treatment, the male was moved from the social group to the opposite side of the divider so that visual and olfactory cues indicative of male presence were still accessible to a female, but behavioural interactions with the male were prevented. On day 0 of the NM treatment, the male was removed completely from the tank so that there was no interaction at all with the dominant female. If sex changers spawned, we removed the acetate sheet and checked for fertilized eggs.

The experiment ended on day 22 from male manipulation, and predicted sex changers in all treatments were euthanized with a lethal concentration of tricaine meth-

Table 1 Standard length ranges (millimetres) and averages (mean±SE) for the animals composing the social groups

Treatment	Male	Dominant female	Larger subordinate female	Smaller subordinate female
MB	32.55–41.30 (36.58±0.92)	27.30–33.40 (30.11±0.63)	22.60–26.90 (24.77±0.46)	21.95–25.65 (23.74±0.44)
NM	33.30–44.70 (36.07±1.08)	27.30–33.70 (29.34±0.59)	22.95–26.15 (24.42±0.37)	22.40–25.50 (24.06±0.37)
M	33.00–44.20 (36.82±1.36)	26.75–32.70 (29.75±0.64)	22.55–27.20 (24.72±0.49)	21.50–27.10 (23.98±0.54)

Animals were measured to the nearest 0.05 mm.

MB Male present behind the tactile barrier, NM no male present, M male present

anesulfonate (MS222; Sigma). We again photographed their genital papillae, measured the length and width of each papilla, and extracted and photographed the gonads of the predicted sex changers to assess whether they had developed testes and AGS. We chose the day that the largest female performed the first jerk swim as an indicator of sex change initiation. We considered a dominant female to have changed sex if she exhibited male typical behaviour (jerking and defending a nest) and if she had a pointy genital papilla, typical testis morphology and an AGS at the end of the experiment. The presence of fertilized eggs has been used in the past as a criterion of sex change in *L. dalli* (Reavis and Grober 1999), but we did not use this measure because the presence of eggs (and subsequent fertilization) is highly dependent on the ovarian status of the two subordinate females.

Data analysis

We used Fisher's exact test with Tukey's post hoc test of proportions to assess treatment differences in the probability of female sex change. We used one-way analyses of variance (ANOVAs) to examine treatment differences in (1) rates of aggressive behaviour (in displacements per minute) throughout the entire 22-day period and during the first 4 days following male manipulation, (2) rates of courtship behaviour (in jerks per minute) throughout the entire 22-day period and during the 3 days prior to and on the day of the first spawn (eggs present in the tube), and (3) change of papilla ratios from the start to the end of the experiment. We used paired *t* tests to determine whether papilla ratios of the focal animal within each treatment changed during the study. Because eight groups (belonging to the MB or NM treatment) were run in the fall (September and October 2003) and the rest in the spring (April and May 2004), we accounted for season in the analyses. Two-factor ANOVAs assessed treatment (MB or NM) and season (fall and spring) effects on the latency to initiate sex change (first jerk swim) and rates of displacement and jerking behaviour; the M treatment was not included in these analyses because all trials were conducted in the spring. Planned linear contrasts examined differences among the levels of treatment and treatment × season interaction. The *p* values for all non-independent post hoc and planned comparisons were adjusted using the sequential Dunn–Sidak procedure to minimize compounding type

I error. All analyses were performed on SAS version 8.2 (SAS Institute, Inc.) using FREQ, GLM, or MIXED procedures. Data are reported as means±1 SE.

Results

Probability of sex change and associated morphological changes

None of the 10 dominant females in the M treatment changed sex. They did not exhibit male-typical courtship behaviour and they retained female-typical rounded genital papilla; average papilla ratio at the end of the experiment was not significantly different from initial values (paired *t* test: $t_9 = -1.27$, $p = 0.24$; initial, 1.01 ± 0.06 ; final, 0.92 ± 0.03). The dominant females also had distinct ovaries and did not develop an AGS.

All of the dominant females in the NM treatment changed sex; they exhibited jerking courtship behaviour consistently throughout the experiment, occupied the nest, spawned and fertilized eggs. Their genital papillae transformed into a male-typical pointy shape, and at the end of the experiment their papilla ratio was significantly larger than at the start of the experiment (paired *t* test: $t_9 = 7.38$, $p = 0.0001$; initial, 0.92 ± 0.03 ; final, 2.07 ± 0.14). All of the NM dominant females developed a testis and AGS.

Of the 10 dominant females in the MB treatment, 8 changed sex. These sex changers jerked consistently throughout the experiment, occupied the nest and developed testis and AGS. Their papillae transformed into a male-typical pointy shape, and at the end of the experiment, their papilla ratio was significantly larger than at the start of the experiment (paired *t* test: $t_7 = 8.67$, $p = 0.0001$; initial, 1.03 ± 0.06 ; final, 1.74 ± 0.04). Six of the sex changers spawned and fertilized eggs. The two fish that did not change sex jerked occasionally but did not develop AGS, and at the end of the experiment, they had ovaries full of developed eggs and female-typical papilla morphology.

There were significant differences in the frequency of sex change among treatments (Fisher's exact test: $p < 0.0001$, $N = 30$). Occurrence of sex change was significantly greater in the NM (Tukey: $q = 8.20$, $p < 0.05$) and MB (Tukey: $q = 5.98$, $p < 0.05$) treatments than the M treatment. There was no significant difference between NM and MB in the probability of sex change (Tukey: $q = 2.22$, $p > 0.05$).

Rates of displacement and courtship

We compared overall rates of aggressive behaviour (in displacements per minute over the 22-day experimental period) of sex-changed dominant females from the NM (1.43 ± 0.14 , $N=10$) and MB (1.07 ± 0.12 , $N=8$) conditions and males (1.26 ± 0.11 , $N=10$) and dominant females (0.98 ± 0.09 , $N=10$) from the M condition. There were significant overall differences in displacement rates (ANOVA: $F_{3,34}=3.17$, $p=0.04$), with NM sex changers showing greater displacement rates than females in the M treatment (Tukey's, $p<0.05$); no other comparisons were significant. Previous work on this species (Reavis and Grober 1999) indicated that sex changers exhibit robust peaks in displacement rates immediately following male removal; thus, we also examined rates of behaviour during the initial stages of sex change. There were significant differences among treatments in displacement rates performed during the first 4 days (from day 0, male removal, to day 3) of the experiment (ANOVA: $F_{3,34}=8.10$, $p=0.0003$; Fig. 1). NM dominant females had significantly higher displacement rates than all the other groups (Tukey's, $p<0.05$), but MB sex changers, males and females in M controls did not differ significantly.

The overall rate of jerking (in jerks per minute) over the 22-day experimental period did not differ among treatments (ANOVA: $F_{2,25}=0.85$, $p=0.44$). Sex changers in the NM (0.77 ± 0.17 , $N=10$) and MB (0.54 ± 0.10 , $N=8$) groups and males in the M condition (0.47 ± 0.52 , $N=10$) exhibited the same rates of courtship. Like displacements, jerking rates are not consistent over time in *L. dalli*. Peak jerking rates occur just before spawning, as the sex changer or male courts the females (Reavis and Grober 1999). There was also no significant difference among treatments in jerking rates 3 days before and on the day of first spawning (ANOVA: $F_{2,23}=1.46$, $p=0.25$; Fig. 1).

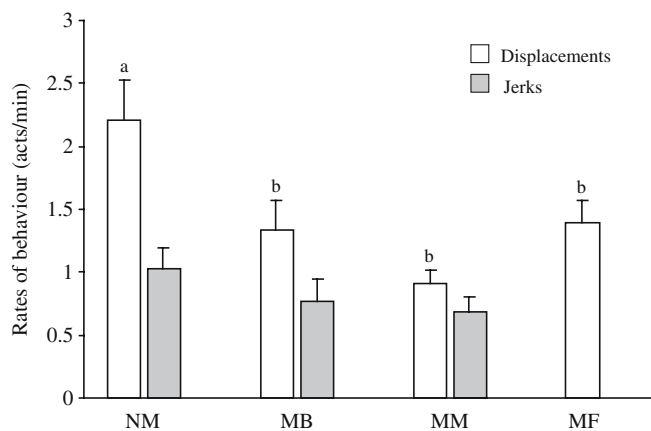


Fig. 1 Comparison of rates of behaviour across treatments: the white bars represent displacement per minute in the first 4 days from male removal; the grey bars represent jerks per minute in the day of spawning and 3 days before. The data refer to the sex changer for NM ($N=10$) and MB ($N=8$) groups and to the males (MM) and dominant females (MF) for the M group ($N=10$). Different letters represent statistical significant difference. Error bars represent the standard error of the mean

Timing of sex change

For each fish in each treatment, we calculated the difference between initial and final papilla ratio. In this analysis, we include only the eight sex changers for the MB treatment because we were interested in comparing the rate of sex change between NM and MB and the difference between sex changers and dominant females in the M treatment. We excluded the remaining two MB dominant females because, although they might have been changing sex slower, they did not exhibit male typical traits at the end of the experiment. Overall, there were significant differences among treatments in the degree to which the papilla ratio changed over the course of the experiment (ANOVA: $F_{2,25}=32.69$, $p=0.0001$). The change in papilla ratio was significantly greater in NM sex changers than in MB sex changers and dominant females in M groups (Tukey's, $p<0.05$). The change in papilla ratio was significantly greater in MB sex changers than dominant females in M groups (Tukey's, $p<0.05$).

Dominant females began jerking significantly earlier in the NM groups (day 2.6 ± 0.7 , $N=10$) than in the MB groups (day 6.2 ± 1.5 , $N=10$) (ANOVA: $F_{1,16}=4.43$, $p=0.05$), and the results were almost identical if we included only the eight sex changers for MB. There was, however, a significant treatment \times season interaction (ANOVA: $F_{1,16}=5.94$, $p=0.03$; Fig. 2). In spring (April and May), there was no significant difference between NM and MB treatments in the latency to begin jerking ($F_{1,16}=0.08$, $p=0.79$). Females in MB groups conducted during the fall (September and October), however, started jerking significantly later than females in the fall NM groups

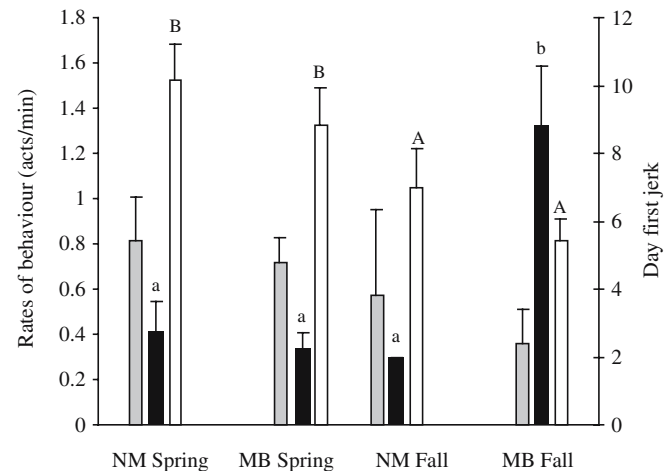


Fig. 2 Effect of treatment \times season interaction on different behaviours. The grey bars represent the sex changers' rate of jerking over the whole experimental period and the white bars the overall rate of displacement, in the absence of the male (NM: spring, $N=8$; fall, $N=2$) or with the male behind the barrier (MB: spring, $N=4$; fall, $N=4$). The black bars represent the first day after male removal that the dominant females started jerking in the absence of the male (NM: spring, $N=8$; fall, $N=2$) or with the male behind the barrier (MB: spring, $N=4$; fall, $N=6$). Different letters represent significant difference, and error bars represent the standard error of the mean

($F_{1,16}=8.06$, $p=0.01$) and females in the spring MB groups ($F_{1,16}=11.97$, $p=0.003$). In the absence of males (NM groups), there was no significant seasonal effect ($F_{1,16}=0.1$, $p=0.75$).

There was no effect of season on the rate of jerking by the sex changer in NM ($N=10$) and MB ($N=8$) groups (ANOVA: season $F_{1,14}=3.60$, $p=0.08$; treatment \times season $F_{1,14}=0.39$, $p=0.54$; Fig. 2), but rates of displacement were higher in spring for sex changers from both groups (ANOVA: season $F_{1,14}=8.34$, $p=0.01$; treatment \times season $F_{1,14}=0.07$, $p=0.80$; Fig. 2).

Discussion

The results of our experiment demonstrate that the removal of direct behavioural interactions between the male and dominant female facilitates protogynous sex change in *L. dalli*. Our results also demonstrate that, in the absence of behavioural interactions, visual and/or olfactory cues emitted by a male can modulate the timing of sex change. Furthermore, it is possible that seasonal rhythms, whether externally or internally derived, might influence the magnitude of a dominant female's response to visual and/or chemical cues indicative of male presence. We discuss the implications of this three-tiered explanation for the mediation of sex change in the context of the existing literature on the social regulation of sexual phenotypes in fishes and other vertebrate taxa.

In our study, no sex change occurred when the male was present and able to interact physically with the dominant female (M condition). Eighty per cent of the dominant females changed sex when a tactile barrier prevented direct behavioural interactions between the male and the female (MB condition). When the male was completely removed from the aquarium (NM), all dominant females changed sex. These findings are consistent with social regulation of sex change in this species (St. Mary 1994; Reavis and Grober 1999) and support our hypothesis that direct behavioural interactions exert an important influence on the final decision of a dominant female to change sex. Our results also are consistent with those of Shapiro (1983) and Ross (1981), who demonstrated that females of *A. squamipinnis* and *Thalassoma duperrey*, respectively, changed sex when they were physically separated, but still received visual or chemical cues, from the focal male. These two studies, however, either neglected to consider behavioural interactions (Ross 1981) or lacked control groups to assess whether the delay in the onset of sex change was due to the presence of the male behind a barrier (Shapiro 1983). Also, there are few species in which the mechanisms that influence sex change have been investigated despite the diversity of teleost families in which it occurs. Thus, we cannot assume that the same mechanisms apply to each species. It is interesting to note that a serranid, such as *A. squamipinnis*, might share with gobies a proximate cue involved in the inhibition of sex change.

The barrier in our experiments prevented any direct behavioural interactions between the male and the domi-

nant female. We are aware that it is difficult to distinguish strictly tactile stimuli (e.g. overt physical interactions) and general behavioural interactions (e.g. courtship and aggression without physical contact) composed of multimodal cues as proximate mechanisms mediating sex change. Without invasive manipulations, however, it is virtually impossible to partition these cues.

Behavioural interactions inform a female about her dominance status in the harem and reproductive potential as a male. If the male is unable to effectively subordinate all females due, for instance, to increased group or territory size, a female could change sex even if olfactory and visual stimuli from the male are still present (Robertson 1972; Lutnesky 1994; Perry and Grober 2003). Our results are in agreement with encounter rate models (Lutnesky 1994), but while those models could encompass any sensory modality and any level of interaction between nearby individuals, our study provides evidence that encounters involving direct behavioural interactions play an important role in inhibiting sex change. Visual and chemical cues emitted by the male were not sufficient to inhibit protogynous sex change, as demonstrated clearly by the results of the MB treatment where dominant females had visual and chemical contact with the male but exhibited a significant propensity to change sex.

The ability of females to assess their social environment using means above and beyond the mere presence of the male might be beneficial. For various reasons, the female might gain more information through direct multimodal behavioural interactions than from simply sensing male presence through visual or olfactory means. First, visual and olfactory cues might inform the female about male presence, while interactions with the male, particularly agonistic interactions, might inform her of her status in the social hierarchy. Second, during sex reversal in *L. dalli*, behaviour changes well before the gonads and secondary sexual characteristics. Behavioural interactions in the form of courtship might inform the female either that a male is present or that another female has initiated sex change in her social group. Thus, information gathered by attending to the behaviour of another individual might be more important for a female than focusing on potentially equivocal visual or hormonal messages. Contrary to other species (e.g. *Thalassoma bifasciatum*), there is no sexual colour dimorphism in *L. dalli*; thus, females cannot rely on abrupt colour changes as an indication that another individual has adopted the male phenotype.

Despite the clear importance of the removal of multimodal behavioural interactions in facilitating sex change, visual and olfactory cues indicative of male presence affected the rate of sex change. Differences in behaviour (latency to start jerking; displacements) and morphology (papilla ratio) between MB and NM treatments indicate that females perceive the male behind the partition and integrate information from visual and olfactory stimuli. *L. dalli* dominant females typically show peak displacement rates immediately following male removal (Reavis and Grober 1999), suggesting that the first few days are critical for a female to establish dominance over other females. If

visual/olfactory cues associated with male presence were not important, we would predict MB females to increase displacement rates after male removal in a similar way as NM females. Rather, the aggression rate of MB females was similar to that of dominant females in the presence of a male. Similarly, if the effects of visual/olfactory cues were negligible, we would predict MB females to initiate sex change in parallel with NM females. MB females, however, showed delayed sex change initiation (latency to first jerk swim) compared with NM females. Lastly, at the end of the experiment, the papilla ratio of MB sex changers was less male-like than NM sex changers, indicating that MB females might have initiated sex change later or that it progressed at a slower rate. Thus, although direct behavioural interactions are important in regulating final outcome (i.e. sex change), visual and olfactory cues indicative of male presence are important for mediating behavioural changes and the timing of sex change.

Experiments on the induction of sex change in isolated or all-female groups have implicated visual and chemical signals as stimulatory cues for sex change. Ross (1981) demonstrated that the visual presence of a small, female *T. duperrey* is sufficient to induce sex change in a larger female located behind a tactile barrier. Similarly, olfactory cues emitted from conspecific females increased the rate of sex change in solitary females of *Coryphopterus glaucofraenum* (Cole and Shapiro 1995). The relative importance of visual and olfactory cues requires further investigation and is likely to be a function of mating and/or social system (e.g. harem vs territorial, group stability), habitat use (pelagic vs benthic) and density. Visual and olfactory cues might also play different roles in the induction vs inhibition of sex change.

Seasonal cues also might be important in mediating how visual/olfactory information from the male is integrated. In the spring, the early reproductive season for *L. dalli* (Wiley 1976; Behrens 1983), females began jerking at the same time in all conditions without physical interaction (NM, MB). When the male is either physically or behaviourally absent early in the season, the benefits of changing sex and reproducing as a male for an entire season might significantly outweigh the costs of reallocating reproductive investment; reproductive success in *L. dalli* increases by twofold or more when assuming the male reproductive function (St. Mary 1994). In the fall, which marks the end of the breeding season, MB females delayed sex change, and two of them did not change sex. *L. dalli* is a short-lived species that rarely reproduces for more than one season (Behrens 1983) and in which females have a relatively long inter-clutch interval (up to 3 weeks; St. Mary 1994). Given these factors, allocating energy to testis development and spawning as a male for a short time in the fall might be more costly relative to spawning as a female. If after a certain period the male fails to provide behavioural reinforcement (e.g. aggression, courtship), females might opt to change sex even if late in the season. In contrast, all NM females changed sex and began jerking rapidly in both fall and spring because in the absence of all cues related to male presence, the dominant females' options are either to fore-

go spawning altogether or to allocate energy to sex change. Because our study provides only preliminary evidence for seasonal effects, we encourage future work that investigates how season and visual/olfactory cues interact to affect the timing or expression of sex change and to understand which mechanisms might be involved.

Behavioural interaction as a mechanism affecting reproductive function is widespread among social species, and the effects of behavioural interactions range from inhibition of sex change to complete reproductive suppression. Dominant female alpine marmots (Hacklander et al. 2003) and naked mole-rat queens (Clarke and Faulkes 2001) use aggression to inhibit subordinate female reproduction. Social insect workers also use aggression to suppress reproduction in other female workers (honeybees, Visscher and Dukas 1995; wasps, Platt et al. 2004). The aggressive interactions might not be as important for non-territorial species or species in which the male cannot monopolize resources (e.g. females, territories, spawning sites). Future studies are necessary to assess whether proximate behavioural mechanisms of sex change inhibition can be generalized to other sequential hermaphroditic species with different social and mating systems. Our findings provide support for behavioural interactions as an important mechanism controlling sex change inhibition (Robertson 1972; Fricke and Fricke 1977). However, the delay of sex change that we observed in the presence of a male behind a barrier shows that individuals integrate multiple levels of visual and olfactory information, which is used to guide the expression of behaviour that is most suitable to the dynamic social conditions.

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