



ELSEVIER

Journal of Experimental Marine Biology and Ecology 318 (2005) 127–143

**Journal of  
EXPERIMENTAL  
MARINE BIOLOGY  
AND ECOLOGY**

www.elsevier.com/locate/jembe

## Reproduction in context: Field testing a laboratory model of socially controlled sex change in *Lythrypnus dalli* (Gilbert)

Michael P. Black<sup>a,\*</sup>, Brandon Moore<sup>b</sup>, Adelino V.M. Canario<sup>c</sup>, Denzil Ford<sup>d</sup>,  
Robert H. Reavis<sup>e</sup>, Matthew S. Grober<sup>a</sup>

<sup>a</sup>Center for Behavioral Neuroscience/Center for Brain Sciences and Health, Department of Biology, Georgia State University, PO Box 4010, 24 Peachtree Center Ave., 402, Kell Hall, Atlanta, GA 30303-4010, United States

<sup>b</sup>Department of Zoology, 223 Bartram Hall, University of Florida, Gainesville, FL 32611, United States

<sup>c</sup>Centro de Ciencias do Mar, Universidade do Algarve, Campus de Gambelas, 8000 Faro, Portugal

<sup>d</sup>Department of Life Sciences, Arizona State University, PO Box 37100, Phoenix, AZ 85069-7100, United States

<sup>e</sup>Department of Biology, Glendale Community College, 6000 West Olive Avenue, Glendale, AZ 85302, United States

Received 29 October 2002; received in revised form 20 July 2004; accepted 3 December 2004

### Abstract

Social interactions can have profound effects on reproduction and the proximate mechanisms involved are just beginning to be understood. *Lythrypnus dalli*, the bluebanded goby, is an ideal organism for analyzing the dynamics of socially controlled sex change both in the laboratory and field. As with most research species, the majority of its behavioural and physiologic study has been performed in the laboratory. The goal of our study was to induce sex change of *L. dalli* in a more natural environment and compare field dynamics with our laboratory-based model. Groups of *L. dalli*, composed of one large male and three females of varying sizes, were introduced into artificial habitats in the field. After male removal, the dominant, largest female underwent protogynous sex change in the majority of the groups. Within 15 days, 9 of 15 of the dominant females (focal fish) successfully fertilized eggs as males, compared to 13 of 17 in the laboratory. Focal fish displayed the distinctive temporal sequence of behaviour changes consisting of a dominance, quiescent, and courtship phase. In addition, focal fish had gonads, genital papillae, and accessory gonadal structures with morphology in between that of females and males. Those fish that fertilized eggs had this transitional morphology, but were functionally male. Steroids of focal fish were assayed by water sample, and morning samples of free 11-ketotestosterone (11-KT) positively correlated with the percent of male tissue in the gonad, with the size of the accessory gonadal structure but not the genitalia (genital papilla), and with aggressive displacement behaviour on the last day before the fish were sacrificed. These morphological, physiological, and behavioural patterns parallel those seen in the laboratory. Lower rates of behaviour and the dramatic

\* Corresponding author. Tel.: +1 404 463 9578; fax: +1 404 651 3929.  
E-mail address: seawater@gsu.edu (M.P. Black).

effects of ambient temperature in the field provide insights as to how the environmental context modifies the behaviour and, subsequently, the reproductive function of individuals within a social group.

© 2005 Elsevier B.V. All rights reserved.

**Keywords:** 11-Ketotestosterone; Aggression; Androgen; Genitalia; Gonad; Sex reversal

## 1. Introduction

Sex change from female to male, protogyny, is the most common form of hermaphroditism in coral reef fish (Warner, 1984). In many protogynous fish, sex change is mediated through social interactions with other fish. Removal of the dominant male and the presence of a subordinate female trigger a newly dominant female to change sex, restructuring the brain, behaviour, gonads, hormones, genitalia, and secondary sex characteristics (e.g., Robertson, 1972; Shapiro, 1981; Ross et al., 1983; Ross, 1984; Nakamura et al., 1989; Godwin et al., 2000; see also Fig. 1). *Lythrypnus dalli*, the bluebanded goby, follows this pattern, with removal of the dominant

male from a social group resulting in sex change only if a female becomes the dominant and has a female subordinate to her (Reavis and Grober, 1999; Carlisle et al., 2000).

Laboratory studies of *L. dalli* have investigated behaviour during sex change and hormonal effects on genitalia, gonads, and secondary sex characteristics, but all of these changes have not been observed in the same sex-changed fish and integrated with individual hormone levels under field conditions. As behaviour and hormones are key regulators of sex change, this study will duplicate a detailed behavioural and morphological analysis of the sex change process to determine if conclusions made about *L. dalli* from laboratory experiments are valid

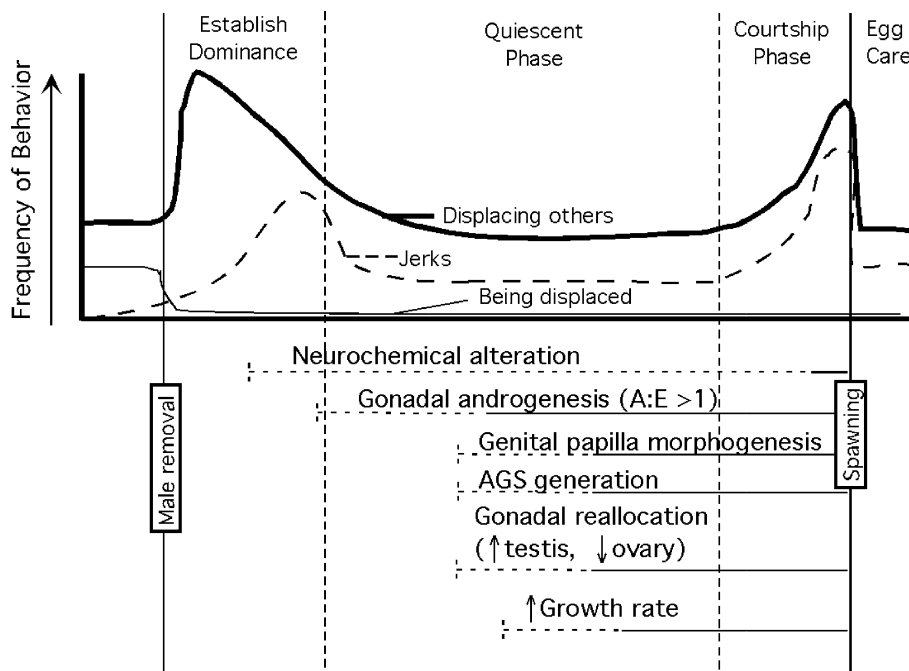


Fig. 1. Temporal sex change model for *Lythrypnus dalli* (modified from Reavis and Grober, 1999) showing frequency of displacing and jerking behavior of a focal female from before male removal until spawning as a male. Dotted lines below give putative time points for initiation of morphological and physiological changes. A:E=androgen/estrogen ratio; AGS=accessory gonadal structure.

in a more natural marine environment. In addition, both laboratory and field studies suggest that some reproductive traits (e.g., accessory gonadal structure and behaviour; Reavis and Grober, 1999; Drilling and Grober, in press) may be more insightful than others (e.g., gonad and genitalia; St. Mary, 1993, 1994a; Carlisle et al., 2000) in determining functional sex, and this study will compare those traits with regard to functional sex.

## 2. Materials and methods

### 2.1. Natural history

*L. dalli* behave primarily as sequential protogynous hermaphrodites; however they are capable of sex change in both directions (St. Mary, 1993; Reavis and Grober, 1999). These small fish (standard length 18–45 mm) commonly inhabit rocky reefs from Morro Bay, California, to the Sea of Cortez (Miller and Lea, 1976). *L. dalli* are planktivores (Hartney, 1989) and live in mixed sex groups averaging from 1.10:1 to 4:1 females to males with densities up to 100/m<sup>2</sup> (Eckert, 1974; Wiley, 1976; Behrents, 1983; St. Mary, 1994b; Steele, 1996; Drilling and Grober, in press). Spawning primarily from April to September, males externally fertilize eggs from multiple females in their group and provide all parental care to the eggs (St. Mary, 1993, 1994b).

### 2.2. Experimental paradigm

Seven artificial fish habitats were constructed, each composed of a 20.3 cm square cinder building block that had been partially filled with cement to create a cube with a single 15.2 cm square cavity on one of its faces. Each block was cemented to a 30.5 cm×30.5 cm cement paving tile with the cavity facing sideways at a slight upward angle. A 15.2 cm (length)×1.9 cm (diameter) PVC tube was attached to the top of the block and to the front of the tile base. To serve as a suitable but removable nesting site, glass test tubes were inserted into the PVC until their rims were flush with the PVC opening. *L. dalli* has been reported to lay and fertilize eggs in previous uses of this arrangement (St. Mary, 1994b; St. Mary, 1998). The habitats were placed on a reef in approximately 10 m

of seawater at Bird Rock, Santa Catalina Island, California, in March 1999. Two months later, the habitats were relocated approximately 350 m from Bird Rock to a 10-m deep, sandy bottom in Big Fisherman Cove (33°28'N, 118°29'W), near the Wrigley Institute for Environmental Studies (WIES). The habitats were placed 5 m apart in two parallel lines: one line with three habitats and the other with four. Each cavity was oriented away from other habitats and facing open sand. An appropriately sized sea urchin, *Centrostephanus coronatus*, was added into the cavity of each habitat, as *L. dalli* use urchins as a central defensive point and refuge from predators (Hartney and Grorud, 2002).

*L. dalli* were collected from Bird Rock using quinaldine sulfate (Sigma Chemicals) and dip nets (California Fish and Game Permit No. 802013-01). After collection, fish were transferred to large, flow-through seawater tables located on the WIES waterfront. For processing, fish were anesthetized using MS-222, tricaine methanesulfonate (Sigma Chemicals). Standard length of the fish was measured ( $\pm 1$  mm) and the fish were visually sexed under a dissection microscope based on genital papilla length-to-width ratio. The genital papilla is primarily sexually dimorphic, but only loosely correlates with testicular tissue allocation in the gonad. Fish with female-typical genital papillae have greater than 95% ovarian tissue (St. Mary, 1993). However, fish with male papilla morphology may have between 5% and 100% testicular tissue, so it is an imperfect diagnostic of sex (St. Mary, 1993). During protogynous sex change, the papilla is rearranged in a process of elongating and narrowing (Reavis and Grober, 1999). Fish with questionable papillae were excluded. Individuals were uniquely identified by the number of bands on each side, distinctive gaps in bands, and dorsal striping (see Reavis and Grober, 1999).

Groups were made throughout the summer starting on May 25, 1999 and consisted of a large male (>30 mm), a large female (28.29 $\pm$ 1.21 mm), a medium female (24.47 $\pm$ 1.01 mm), and a small female (22.78 $\pm$ 1.42 mm). With the exception of the small female, each fish was >3 mm smaller than the previous fish in the grouping. There were two exceptions to these criteria: in one group, there were four females instead of three; and in another, the large dominant that was removed was female, not male. In

both cases, these fish changed sex, but neither was included in the behavioural analysis. Fish with different markings were grouped for easy visual identification, sealed into ziplock bags of seawater for transport, and introduced to individual habitats.

For 4 days following introduction, the groups were allowed to acclimate and establish a social hierarchy. One day prior to male removal, groups were observed to make sure that the male was dominant in the group. Five days after introduction of the group, the male fish in each habitat was removed using dip nets and strategically placed squirts of quinaldine sulfate from a syringe. Any nesting tubes containing eggs were replaced with empty tubes.

The males were removed from all groups because sex change in artificial habitats had been shown in the past and behavioural observations of fish groups with a male remaining present had been observed in the laboratory and field without the laboratory-derived behaviour pattern or sex change occurring (St. Mary, 1994a; Black, Reavis, and Grober, unpublished). Moreover, concurrent with this experiment, daily 10-min behavioural observation following male removal from natural field groups was done off of Bird Rock. Some of these natural field groups had a male immigrate in, and the male prevented sex change of the female. These data are included for comparison. Fish introduced to the artificial habitats remained solely in and around the safety of the urchin. No evidence of emigration to or immigration from artificial habitats was observed.

### 2.3. Behavioural observations

Following male removal, two 15-min observations, starting at 0900 and 1500 h, were made daily to monitor the behaviour of individuals in each group. After the diver slowly approached the habitat, the fish were given 2 min to acclimate while the diver ascertained the identity of all of the fish in the group by size and distinctive banding/markings. Observations were consistent with Reavis and Grober (1999) for comparison to the behavioural profile observed in the laboratory (Fig. 1). Briefly, behaviour of the large female (focal fish) was categorized and recorded as approaches, being approached, displacements, being displaced, and jerks. An approach was defined as the focal fish

moving to within 5 cm of another fish, approximately two body lengths. If the approach caused the approached fish to move away, then the behaviour was termed a displacement. If another fish performed this behaviour toward the focal fish, it was recorded as approached by and displaced by, respectively. Jerks are a male-typical courtship behaviour characterized by a distinctive approach toward another fish with abrupt starts and stops and erect fins. On day 0, the male was removed in the morning, and the focal fish's behaviour was only recorded in the afternoon. All following days, the morning and afternoon behavioural rates were averaged to calculate daily rates of behaviour until the day of focal fish removal. The same procedures for observations were followed in groups on natural habitat at Bird Rock, except groups were only observed once per day for 10 min and there was an additional assessment of feeding behaviour, as determined by bites into the water column. Due to low light in the cavity of some habitats, the observer occasionally used a flashlight to illuminate the rear of the cavity in an area near the fish, casting indirect light on the observed fish.

After each observation, nesting tubes at the habitats were checked for eggs by slowly pulling the test tube from the PVC sheath, visually examining, and then reinserting it. If eggs or embryos were present, they were visually inspected for eyes to ascertain fertilization. The groups were considered complete if 15 days following male removal had passed, or if fertilization had occurred. Fifteen groups went to completion. Four of these groups had no eggs in the nest tube 15 days after male removal, 11 habitats had eggs in a nest tube, and 9 of those had egg clutches that showed developed embryos, confirming fertilization. The following analyses focus on two sets of fish: the large females from those habitats that ran to completion, called focal fish ( $n=15$ ), and a sub-set of the focal fish, sex changers ( $n=7$ ), that originally had a male and two subordinate fish and successfully fertilized eggs as a male. The groups that had three subordinate fish or had a dominant female removed were not included in this sex changer classification because of potential confounds on the behaviour and timing of sex change.

At the end of all observations and egg checks, water temperature was recorded using a Suunto Solution  $\alpha$  digital dive computer. These data were

combined with the 10-m depth daily temperatures from the nearby WIES pier to compile daily water temperature for the site over the course of the study. During the experiment, the water temperature ranged from 15.6 °C in late May to 20.1 °C in late July. Therefore, different groups of fish were exposed to increasing water temperatures as the summer progressed. Fertilized eggs were laid from 11 days after male removal in cooler temperatures to 4.5 days in warmer temperatures. The amount of time from male removal until fertilized eggs were laid negatively correlated with the average water temperature experienced by the particular habitats (ANOVA,  $r^2=0.658$ ,  $F_{1,5}=9.6$ ,  $p=0.027$ ), indicating that as temperature increased, the latency to fertilized eggs and presumably the time to change sex decreased. Therefore, to create a composite behavioural profile of sex changers, correction for temperature variation was required.

Temperature variation between the groups was controlled for by aligning their endpoints (the day each sex changer fertilized eggs as a male). Behaviour profiles were matched in length of time by elongating the shorter behavioural profiles of fish that changed sex in warm water to match the longest behavioural profiles of fish that experienced colder water (11 days). Because Reavis and Grober (1999) found that the quiescent phase was the most temperature-sensitive phase, days were added to the middle to prolong shorter time groups. Half of the data points were placed at the beginning and half of the data points were placed at the end. If there was an uneven number, the middle day was added on to the first half. For the spaces in the middle of the shorter behavioural profiles, no data were used, resulting in lower sample sizes for the middle periods. Because of this method, sample sizes were low for statistical tests, but without using this method, the variation was too high due to temperature differences.

Experiments continued from May 27 to July 29, 1999. Observations of each group of fish were terminated either when embryos with eyes were present in the nesting tube, or 15 days after male removal. If the large female disappeared or was the only fish remaining, the group was collected, identified, and examined. During the following observation, a new group of four fish including a new male was introduced into the vacant habitat and the process was repeated.

#### 2.4. Hormonal and anatomical analyses

When 15 days elapsed or embryos with eyes were found, the fish in the habitat were recaptured, remeasured, and sexed by their genital papilla. The large female was placed in a beaker containing 50 mL of seawater for 1 h to collect excreted urine. Steroids found in urine have been shown to correlate with plasma steroid levels (Scott and Liley, 1994). The water was frozen until processed for radioimmunoassay and assayed for 11-ketotestosterone (11-KT) and estradiol ( $E_2$ ) (for details, see Carlisle et al., 2000). Six fish were processed after the morning observation (AM) and nine after the afternoon observation (PM). Finally, large fish were euthanized using excess dissolved MS-222 and fixed in 4% paraformaldehyde after removal of the eyes to facilitate fixation of the brain.

The genital papilla of each preserved fish was measured by capturing a uniform  $25\times$  magnification image of the ventral region with a dissecting microscope. The image was displayed on a monitor and the dimensions were measured using a ruler, from which the length to width (L/W) ratio was calculated. The torsos containing the gonad were sunk in a 30% sucrose solution, serially sectioned ( $30\ \mu\text{m}$ ) on a cryostat from anterior to posterior, and mounted on chrom-alum coated slides. Tissues were stored at  $-20^\circ\text{C}$  until hematoxylin–eosin staining (Presnell and Schreiber, 1997).

Using NIH Image 1.62a (W. Rasband; NIH, Bethesda, MD), the  $200\times$  magnified images of a series of transverse sections of gonad for each fish were analyzed for the areas of testicular and ovarian tissues. The testicular and ovarian areas were averaged for all sections of the entire gonad for an average percent allocation of testicular and ovarian tissues. Percent testicular tissue was determined by dividing testicular tissue by total gonadal tissue. The presence of spermatozoa (tailed sperm) in the testicular regions was noted (as in St. Mary, 1993).

If an accessory gonadal structure (AGS) was present, the same imaging technique was used to calculate its area. The AGS is a pair of multi-chambered lobes containing sperm as well as mucins and/or steroid derivatives that is characteristically male (Miller, 1984; Cole and Robertson, 1988; Fishelson, 1991; Cole et al., 1994; Scaggiante et al.,

1999). The AGS structure is similar to the seminal vesicles and sperm duct glands found in other fish species (reviewed in Lahnsteiner et al., 1992) but differs in that it originates from the ovarian wall rather than the sperm ducts (Cole and Robertson, 1988). Images of the AGS and the partitions between its chambers were captured to evaluate sperm content and measure partition thickness along the central region of a bisecting midline throughout each AGS (Fig. 2). An average columnar epithelial wall thickness was calculated for the AGS of each fish for an estimate of the wall thickness between partitions. The amount of sperm seen within each AGS section was ranked as 0=no appreciable sperm visible; 1=small sperm content forming sparse, infrequent sperm aggregations; and 2=large amounts of aggregated sperm. The average amount of sperm of all AGS sections was rounded to a whole number, characterizing the AGS sperm content.

For comparison to focal fish and sex changers, five male (standard length, SL,  $34.2 \pm 1.3$  mm) and five female fish (SL  $23.4 \pm 0.9$  mm) were taken and preserved from concurrently running experiments using fish in seawater tables on the WIES waterfront that had similar group composition to those in the

field. Sex was predicted by size, observation of sex-appropriate behaviour, and papilla L/W ratio. Poor fixation and sectioning of the male fish prohibited reliable gonad analysis of the male fish, so data were collected from preserved male fish (SL  $32.6 \pm 1.9$  mm) that had been in large group tanks. These fish had exhibited male behaviour and had male morphology. After histology was performed, all of these representative male and female fish had gonads consistent with their predicted sexes.

### 2.5. Statistical analysis

Data were analyzed using SPSS 8.0 (Chicago, IL) and JMP 5.0 (Cary, NC). Simple linear regression was used to test relationships between data. Normally distributed data were analyzed using *t* test and ANOVA analysis. For data that did not distribute normally, the Mann–Whitney and Kruskal–Wallis tests were used as nonparametric alternatives. A repeated-measures ANOVA was used to compare behavioural data over time. For the jerk behaviour, the temperature-corrected data was clumped by averaging values among each of the following time points: the first day following male removal (day 0),

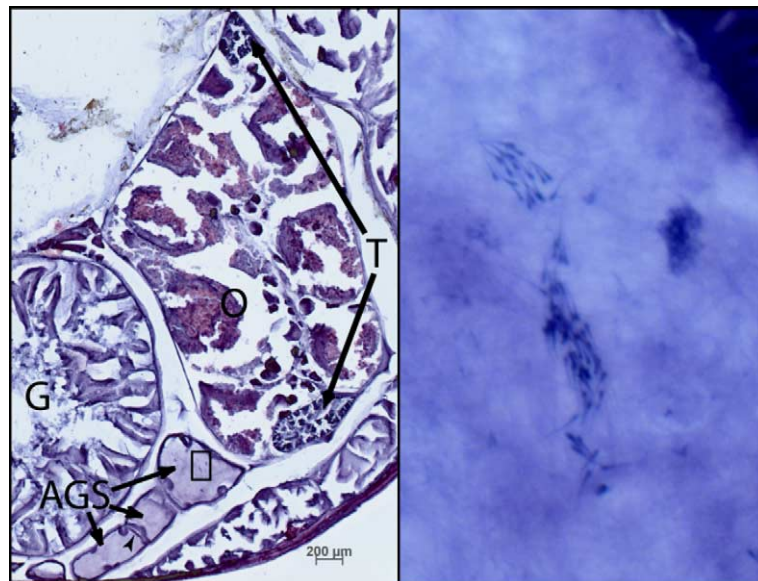


Fig. 2. Transverse section of bluebanded goby (*L. dalli*) gonad from a focal fish with 7.5% testis (T), 92.5% ovary (O) and multi-chambered accessory gonadal structure (AGS) containing sparse crypts of sperm (sperm count=1 (see text)); rectangle within AGS enlarged to the right to show sperm). Arrowhead points to AGS wall. Gut (G) labeled for reference. Scale bar length=200  $\mu$ m.

days 1–3 (dominance), days 5–8 (quiescence), and days 9–11 (courtship) were transformed by the  $2/3$  power for normality and compared using linear contrasts. Data are reported as mean  $\pm$  S.E.

### 3. Results

#### 3.1. Behaviour

When the male was removed from each field habitat, the focal fish was rarely displaced by others, and approaches by the focal fish toward smaller females resulted in their displacement over 99% of the time. Analysis of jerk and displacement rates yielded common behavioural profiles for sex changers. As

seen in sample behavioural profiles from two of the sex-changing fish (Fig. 3A and B), after male removal, the focal fish established dominance through frequent displacement of other fish. In sample profile 1 (Fig. 3A), displacements first peaked on day 2. Concomitantly, the fish began jerking behaviour that peaked on day 2. On days 5–8, both displacements and jerking subsided, followed by an increase of both on day 9. The jerking was associated with displays, leading the females toward the nesting site. On day 11, eggs were laid in the nesting tube and were subsequently fertilized. After initial spawning, this fish displayed egg care while continuing to court the other female in the group.

Sample profile 2 (Fig. 3B) shows a shorter time to change sex (only 5 days). As in profile 1, profile 2

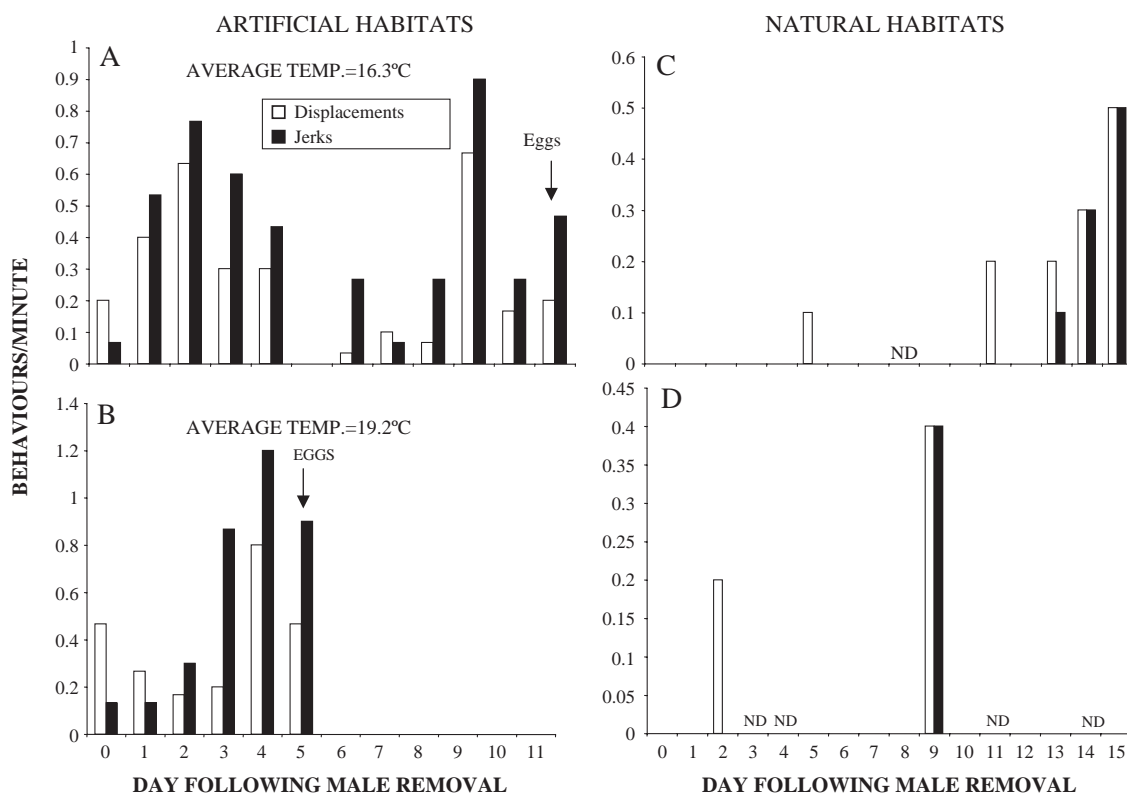


Fig. 3. Four representative behavioural profiles of *L. dalli* focal fish after the male has been removed. Displacement and jerk behavior is shown for sex changers on artificial habitats (A and B) and focal fish on natural habitats (C and D). (A) The slower rate of sex change in cooler temperatures early in the season compared to (B) the faster rate in warmer temperatures. White bars represent displacements and black bars represent jerks. Arrows indicate when eggs were laid in the nest tube by non-focal fish. Note that the y-axis scales represent much smaller rates in (C) and (D). ND=no data because fish was not seen.

shows the same high, low, then high pattern of displacement frequency. Additionally, the rate of jerks and displacements increased in association with eggs being laid.

Comparing the sample behaviour profiles, the sex-changing fish in profile 1 experienced an average temperature of 16.3 °C and took 11 days to complete functional sex change. In contrast, the sex-changing fish in profile 2 experienced 19.2 °C and only took 5 days to fertilize eggs, requiring temperature correction for comparison between profiles (see Materials and Methods).

Temperature-corrected displacements (TCD) (Fig. 4) show two peaks, one on day 1 following male removal ( $0.50 \pm 0.19$  displacements/min) and the other

on the day before fertilizing eggs as a male ( $0.36 \pm 0.12$  displacements/min). These data could not be normalized through transformation procedures and the variation between groups was too high for a repeated-measures ANOVA to reveal a difference in displacement behaviour over time.

Similarly, there were no statistical differences in displacements over time of focal females on natural habitats ( $n=6$ ), but three fish showed the same up, down, up behaviour profile (Fig. 3C and D), except at an even lower rate of displacements than those on artificial habitats. Fish in natural habitats exhibited both immigration to and emigration from the observed urchin group. This was insightful because a new male migrated in from a neighboring urchin to replace the

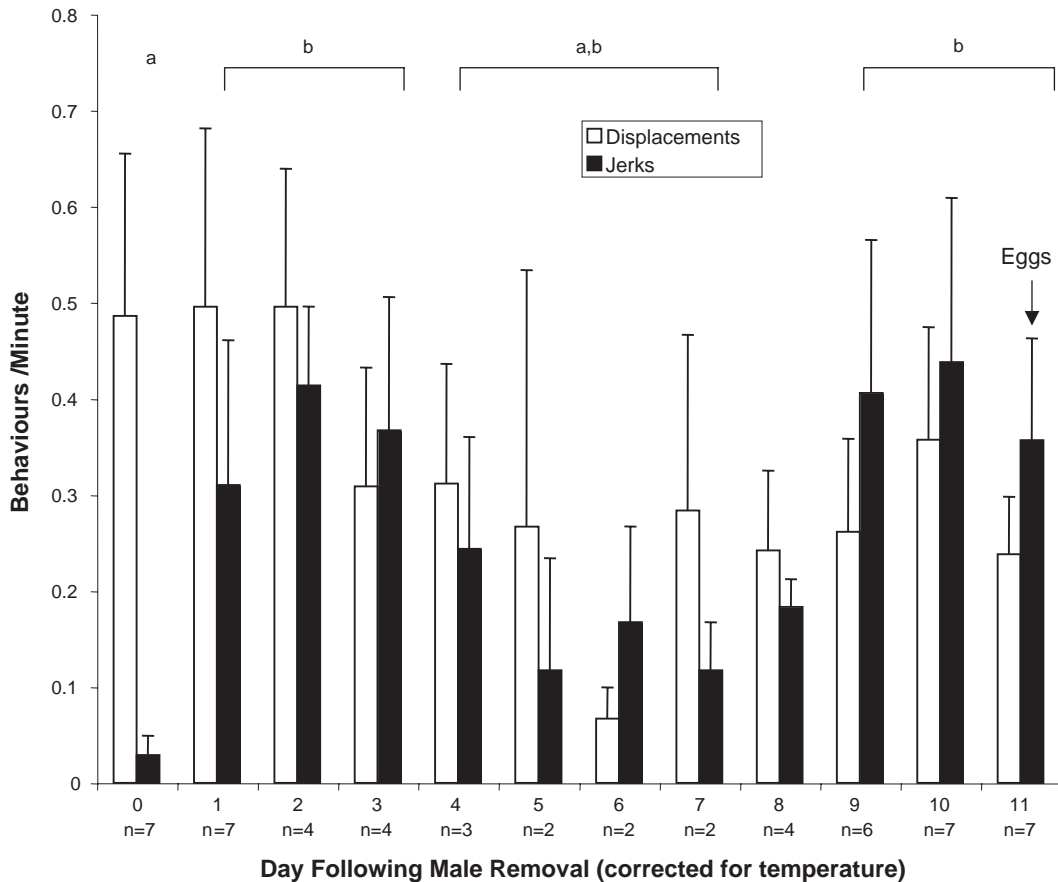


Fig. 4. Temperature-corrected displacement and jerk behaviour profiles over the course of sex change for all *L. dalli* sex changers on artificial habitats. White bars represent displacements and black bars represent jerks. Sample sizes varied across the days due to temperature correction and different letters denote differences over time in averaged jerk behaviour for clumped groups (see text for details). No significant difference was found in displacement behaviour over time.

male we had removed in the other three natural habitat groups, and instead of the behavioural profile shown by other focal fish, these females showed little to no displacements and displayed no jerking behaviour following the immigration of a new male. The most frequent behaviour in natural habitat focal fish was bites for food in the water column. Taking the average for each fish over the course of observations, 90.5% of the behaviour was bites (0.87/min), 7.2% was displacements (0.069/min), and 2.2% was jerks (0.022/min).

Females, large or small, were never observed jerking before male removal, and following male removal, only focal fish jerked. After the peak in displacements, there was a peak of jerk behaviour on day 2 after male removal ( $0.41 \pm 0.082$  jerks/min; Fig. 4). During courtship, sex changers performed jerking and leading behaviour toward the nesting tubes, and they entered the tubes repeatedly. Sex-changing fish increased jerking around the time eggs were laid, peaking the day before spawning (temperature-corrected day 10;  $0.44 \pm 0.17$  jerks/min; Fig. 4). A repeated-measures ANOVA revealed differences in jerking across time periods ( $F_{3,16}=4.05$ ,  $p=0.026$ ), with the day of male removal (day 0) being different from the average of days 1–3 ( $F_{1,16}=7.46$ ,  $p=0.015$ ) and days 9–11 ( $F_{1,16}=10.44$ ,  $p=0.005$ ), but not days 5–8 ( $F_{1,16}=2.72$ ,  $p=0.119$ ). The same pattern of jerking was seen in the focal females on natural habitats, although this was not statistically significant (Fig. 3C and D). As in the aquarium study, a leading behaviour directing females to the spawning site was observed and only males or sex changers performed egg care. Along with egg care, some sex changers continued performing courtship (jerk) behaviour toward other females in the group.

Counting only those groups maintained for more than 3 days after male removal and ignoring those fish missing due to urchin loss, 54 female fish were placed in the habitats. Fourteen fish were lost during the observation period, giving a survivorship rate of 74%. Of the missing fish, none was large, nine (64%) were medium-sized fish ( $24.6 \pm 0.51$  mm), and five (36%) were small ( $23.3 \pm 1.6$  mm). When only one fish was missing from a complete habitat (a compliment of three fish), seven out of the nine fish lost (78%) were medium-sized fish and only two (22%) were the smallest fish of the group.

### 3.2. Histology: intermediate between males and females

After the experimental period, visual examination of all fish in the group showed that only focal fish had male papilla morphology. The average length-to-width ratio of focal fish genital papillae was  $1.60 \pm 0.39$  with a range of 1.0–2.2. Focal fish papilla ratios were intermediate to and significantly different from the papilla ratios of females and males (females  $0.98 \pm 0.06$ , males  $3.48 \pm 1.07$ , Kruskal–Wallis test,  $\chi^2=16.7$ , asymp. significance  $>0.001$ ).

Focal fish gonads had a mean cross-section area of  $3.7 \times 10^5 \pm 2.6 \times 10^5 \mu\text{m}^2$  with a range of  $9.0 \times 10^4$  to  $1.0 \times 10^6 \mu\text{m}^2$ . Cross-sections of female gonads averaged  $3.5 \times 10^5 \pm 3.0 \times 10^5 \mu\text{m}^2$  and males averaged  $1.3 \times 10^5 \pm 5.8 \times 10^4 \mu\text{m}^2$ .

While possessing very little testicular tissue, females displayed a large variability in ovarian tissue size (Fig. 5). Two fish had relatively minute gonads (mean cross-section =  $6.0 \times 10^4 \pm 2.2 \times 10^4 \mu\text{m}^2$ ). Three females with larger gonads (mean cross-section =  $5.5 \times 10^5 \pm 2.0 \times 10^5 \mu\text{m}^2$ ) were visibly gravid, with swollen and pink abdomens upon visual examination with a dissecting microscope. The two females with significantly smaller gonads had sunken abdomens and presumably had recently laid clutches. Omitting the two females with small gonads, the gonad sizes were significantly different between the males, females, and focal fish (Kruskal–Wallis test,  $df=2$ ,  $\chi^2=7.97$ , asymp. significance = 0.019). Females had the largest gonads, males the smallest, and focal fish gonads were intermediate between males and females.

All focal fish gonads contained ovarian tissue and testicular tissue with spermatozoa. Percent testicular tissue in the gonad ranged widely from 0.8% to 65.4% with a mean of  $19.6 \pm 20.1\%$  for focal fish. In contrast, male fish gonads possessed a much more narrow range of  $95.4 \pm 7.5\%$  testicular tissue with spermatozoa. While the amount of testicular tissue comprising the male gonad varied, they all possessed little or no ovarian tissue (Fig. 5). Females had  $1.04 \pm 0.89\%$  testicular tissue, in which two out of five possessed spermatozoa. The amount of testicular tissue in focal fish was significantly different from and intermediate to males and females (Kruskal–Wallis,  $\chi^2=17.8$ , asymp. significance  $>0.001$ ).

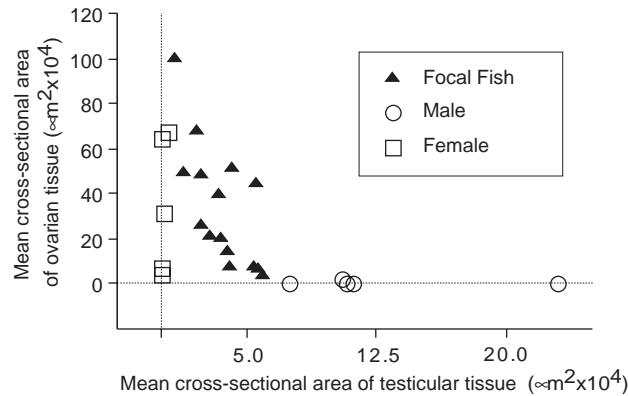


Fig. 5. Scatterplot comparing mean ovarian and testicular allocations in transverse sections of *L. dalli* gonad in focal fish (closed triangles), males (open circles), and females (open squares).

In the gonads of focal fish, the amount of ovarian tissue decreased as the amount of testicular tissue increased. Therefore, the fish with the greatest ovarian tissue had the least testicular tissue and vice versa (Fig. 5). Analysis of tissues comprising the gonad from females to focal fish to males indicates that during protogynous sex change, the rate of reduction of ovarian tissue is much less dramatic than testicular recruitment (Fig. 6). Mean ovarian cross-sections ranged from  $1.0 \times 10^6 \mu m^2$  in a focal fish having only 0.8% testicular tissue to no ovarian tissue in males with 100% testicular tissue. Conversely, testicular tissue ranged from a trace amount in females to  $2.2 \times 10^5 \mu m^2$  in male fish, which was still almost an order of magnitude smaller than the largest ovarian section of gonad.

Comparing papilla length to width ratio to percent testicular tissue in the gonad across males, females and focal fish (Fig. 7) yielded a steeply sloped sigmoid curve ( $r^2=0.68$ ,  $F_{1,23}=50.9$ ,  $p<0.001$ ). Males, with the highest percentage of testicular tissue and most elongated papilla, and females, with the least amount of testicular tissue and blunt papilla, occupy the extreme ends of the curve. The focal fish display papilla and gonadal characteristics intermediate to males and females.

All focal fish possessed an AGS, averaging  $5.8 \times 10^4 \pm 3.2 \times 10^4 \mu m^2$  in cross-sectional area (range:  $1.4 \times 10^4$  to  $1.2 \times 10^5 \mu m^2$ ). The mean AGS area of males was significantly larger than those of focal fish ( $2.7 \times 10^5 \pm 1.5 \times 10^5 \mu m^2$  vs.  $5.8 \times 10^4 \pm 3.2 \times 10^4 \mu m^2$ ,

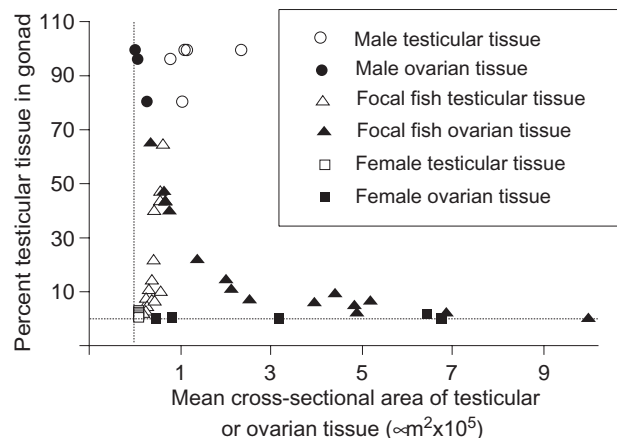


Fig. 6. Scatterplot comparing percent testis allocation in *L. dalli* gonad (testicular tissue size/gonad size  $\times 100$ ) to mean transverse cross-section size of testicular tissue (open shapes) and ovarian tissue (closed shapes) in males (circles), focal fish (triangles), and females (squares).

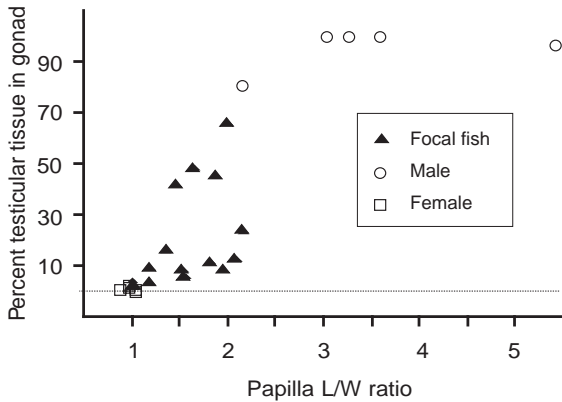


Fig. 7. Scatterplot comparing percent testicular tissue allocation in *L. dalli* gonad (testicular tissue size/gonad size $\times$ 100) to genital papilla length-to-width ratio in focal fish (closed triangles), males (open circles), and females (open squares).

Mann–Whitney  $U$  test,  $U=0$ ,  $p<0.01$ ,  $n_1=15$ ,  $n_2=5$ ). No female had an AGS.

In focal fish and males, the mean AGS area correlated with percent testicular tissue in the gonad (Fig. 8;  $r^2=0.60$ ,  $F_{1,18}=27.02$ ,  $p<0.001$ ). In fish with a small AGS, little testicular tissue, and blunt papilla, the AGS is initially restricted to the caudal portion of the gonad close to the vent. As morphology becomes increasingly male, the organ enlarges and expands anteriorly along the length of the gonad.

In focal fish and males, gonad size rapidly decreased as the percentage of testicular tissue increased (Fig. 8). Simultaneously, AGS size increased with the percentage of testicular tissue in the gonad. In fish whose gonad was composed of

more than 60% testicular tissue, the mean cross-sectional area of the AGS was larger than that of the gonad. The AGS of six focal fish had a category 0 sperm count, five had category 1, and four had category 2 sperm counts (see Materials and Methods; category 0:  $2.8\times 10^4\pm 1.4\times 10^4\ \mu\text{m}^2$ , category 1:  $7.2\times 10^4\pm 1.5\times 10^4$ , category 2:  $8.5\times 10^4\pm 3.6\times 10^4\ \mu\text{m}^2$ , Kruskal–Wallis test,  $df=2$ ,  $\chi^2=13.6$ , asymp. significance=0.001). In comparison to focal females, males had more sperm aggregations in the AGS.

Focal fish AGS had numerous, well-defined partitions between tubules spanning the width of the cross-section. In comparison, cross-sections of male AGS had far fewer interior walls. Sections of male fish AGS with mean wall thickness of less than  $10\ \mu\text{m}$  showed signs of these walls bursting (partial segments of walls not spanning the width of the organ as in focal fish). Male AGS morphology more closely resembled a single, continuous organ than the series of sub-compartments seen in the focal fish. The largest AGS of a male fish contained only one measurable internal wall. Due to insufficient sample size, the wall thickness data from this fish were not used in calculations.

Mean AGS wall thickness of focal fish ranged from 39 to  $67\ \mu\text{m}$ . Focal fish AGS walls were significantly thicker than those of males ( $51.2\pm 9.2$  vs.  $16.7\pm 7.2\ \mu\text{m}$ , Mann–Whitney  $U$  test,  $U=0$ ,  $p<0.01$ ,  $n_1=15$ ,  $n_2=5$ ). In focal fish and males, mean AGS wall thickness negatively correlated with AGS cross-sectional area and the genital papilla ratio (Fig. 9;  $r^2=0.72$  and  $r^2=0.64$ , respectively).

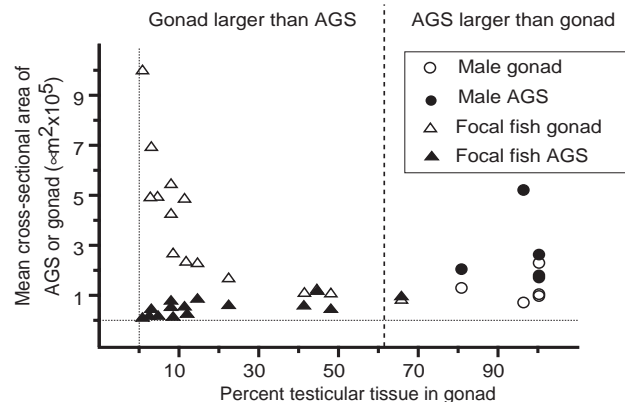


Fig. 8. Scatterplot comparing percent testicular tissue allocation in *L. dalli* gonad (testicular tissue size/gonad size $\times$ 100) to mean transverse cross-sectional area of gonad (open shapes) or AGS (closed shapes) of focal fish (triangles) and males (circles).

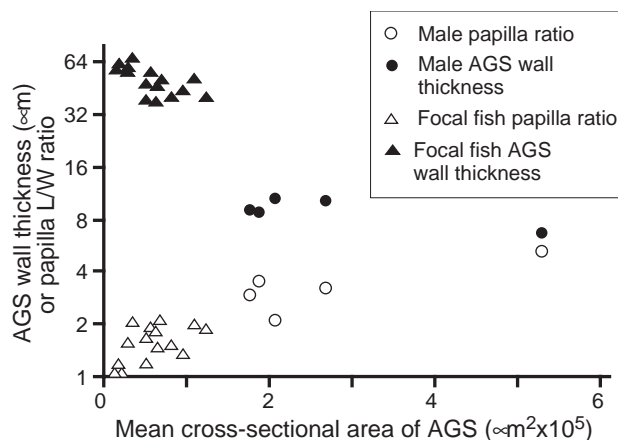


Fig. 9. Scatterplot comparing *L. dalli* AGS interior wall thickness (closed shapes) and genital papilla length-to-width ratio (open shapes) to the mean transverse cross-sectional area of AGS in focal fish (triangles) and males (circles).

Focal fish that fertilized eggs and those not fertilizing eggs showed no significant difference in papilla ratio ( $1.69 \pm 0.31$  vs.  $1.49 \pm 0.47$ , Mann–Whitney  $U$  test,  $U=20$ ,  $p=0.354$ ,  $n_1=7$ ,  $n_2=8$ ), amount of testicular tissue ( $18.5 \pm 17.4\%$  vs.  $20.8 \pm 24.3\%$ , Mann–Whitney  $U$  test,  $U=20$ ,  $p=0.354$ ,  $n_1=7$ ,  $n_2=8$ ), average AGS cross-sectional area ( $6.8 \times 10^4 \pm 2.9 \times 10^4 \mu\text{m}^2$  vs.  $4.7 \times 10^4 \pm 3.4 \times 10^4 \mu\text{m}^2$ , Mann–Whitney  $U$  test,  $U=17$ ,  $p=0.10$ ,  $n_1=7$ ,  $n_2=8$ ), or sperm count categories ( $1.13 \pm 0.83$  vs.  $0.57 \pm 0.79$ ,  $t$  test, equal variances not assumed,  $df=12.9$ ,  $t=1.3$ , two-tailed significance= $0.21$ ).

### 3.3. Steroids: linking physiology to morphology and behaviour

Free and conjugated (sulfate and glucuronide) forms of 11-KT and  $E_2$  were detected in all urine samples from focal fish. Total 11-KT (range 77.3–310.1 pg/sample, mean  $147.2 \pm 55.0$ ) was significantly greater than  $E_2$  (range 15.0–120.6 pg/sample, mean  $50.5 \pm 29.8$ , paired  $t$  test,  $p < 0.001$ ) with an 11-KT/ $E_2$  ratio of  $4.9 \pm 5.1$ . The amount of conjugated 11-KT per sample was greater than free 11-KT ( $107 \pm 51.2$  vs.  $39.7 \pm 22.5$  pg/sample, paired  $t$  test,  $p > 0.001$ ). The amount of sulfate conjugated 11-KT in each sample was greater than glucuronide conjugated 11-KT ( $70.8 \pm 28.9$  vs.  $36.8 \pm 26.7$  pg/sample, paired  $t$  test,  $p < 0.001$ ).

Total, conjugated, or free 11-KT concentrations did not significantly correlate with any histological measurements. However, teleosts have been shown to have a morning (AM) androgen peak, with basal

urinary androgens being highest in the morning and continually declining throughout the day (Oliveira et al., 2001c). Because we only took a single sample from each fish at the time of removal, a decline in androgens over the day could not be determined. However, mean 11-KT levels in samples from afternoon (PM) fish had less, although not significantly less, 11-KT than AM fish ( $45.9 \pm 9.3$  vs.  $35.6 \pm 7.6$  pg/sample; ANOVA,  $F_{1,13}=0.739$ ,  $p=0.41$ ). Controlling for time of sampling by just examining morning samples uncovered several interesting relationships between steroid levels and both behaviour and reproductive anatomy. Free 11-KT from morning samples significantly and positively correlated with percent testicular tissue ( $r^2=0.790$ , ANOVA,  $F_{1,4}=15.11$ ,  $p=0.018$ ), average size of the AGS ( $r^2=0.837$ , ANOVA,  $F_{1,4}=20.60$ ,  $p=0.011$ ), and displacement behaviour on the last day before sampling ( $r^2=0.786$ , ANOVA,  $F_{1,4}=14.68$ ,  $p=0.019$ ), but not genital papilla length-to-width ratio ( $r^2=0.022$ , ANOVA,  $F_{1,4}=0.091$ ,  $p=0.778$ ).

## 4. Discussion

### 4.1. Behaviour: lower rates, but similar patterns

Much of the behaviour that occurred in the field was similar to the description of Reavis and Grober (1999). Corresponding to aquarium-based results, sex change proceeded faster in the summer than the

spring. In May, the longest time to change sex in the field was 10.5 days, which is comparable with Reavis and Grober's finding of  $9.0 \pm 2.2$  days to change sex in the spring. In late June, the fastest sex change in the field was 5 days, again similar to the  $5.5 \pm 2.3$  days observed in aquaria. The faster sex change may be a result of physiologic changes taking place more rapidly within the sex changer, or the females in the group having a faster rate of egg laying so that the changer has eggs to fertilize more quickly. While Reavis and Grober (1999) found that the largest female fish was marginally more likely to change sex, in this study, only the largest females fertilized eggs as a male, and only one group had the second largest fish as a focal fish (dominant over the largest fish).

The sample behavioural profiles (Fig. 3A and B) show a common pattern of behaviour, despite variability in the frequency of behaviour and time needed to change sex. Because of the variability, even when behaviour profiles from all sex changers were corrected for temperature (Fig. 4), large standard errors were obtained. Despite this variance, two increases in displacements correlating with post-male removal and later courtship as a male can be seen, as in Reavis and Grober (1999). The behavioural observations from groups with males removed on natural habitats in the field show similar behaviour profiles (Fig. 3C and D), and these are distinctly different from the profiles seen in groups with a male present, suggesting that the behavioural profile described in aquaria is similar to what occurs in both artificial and natural habitats in the field.

Similar to Reavis and Grober (1999), the temporal sequence of sex change behaviour in the field consisted of an active dominance phase, a quiescent phase, and an active courtship phase (Figs. 1, 3, and 4). Although the behavioural pattern was similar between the laboratory and field, behavioural rates were lower under natural conditions. Displacements first peaked in the field at  $0.50 \pm 0.19 \text{ min}^{-1}$ , which is less than the  $2.18 \pm 1.86$  recorded in laboratory studies. As in the laboratory, the initial peak in displacements occurred in less than 3 days. In the field and laboratory, displacements peaked a second time in association with spawning as a male. During this peak, the field rate of  $0.36 \pm 0.12$  displacements/min was less than the laboratory rate of  $1.37 \pm 1.71$ .

Concomitant with the second displacement peak, sex changers displayed a peak in jerking (Fig. 3). Peak jerking rates on artificial habitats in the field,  $0.44 \pm 0.17$  jerks/min, were lower than laboratory-recorded rates,  $0.73 \pm 0.34$  jerks/min. In addition, the early peak in jerks followed the displacement peak, as in the model, and with this lag time, the jerks on the day of male removal were significantly lower than the jerk behavior during the rest of dominance (days 1–3) and courtship (days 9–11) phases, but not the quiescent phase (days 5–8) (Figs. 1 and 3). Following male removal, only focal fish performed jerks. Thus, jerking behaviour was a strong indicator of initiation of sex change and ultimately the new "maleness" of the focal fish.

Many variables could explain lower rates of behaviour in the field during sex change. Variability of individual fish, their manipulated social groups, interactions with their environments, and the experimental construct created natural and artificial stresses on the groups. However, four factors seem most influential. First, our field observations of focal fish indicate that a large portion of active *L. dalli* behaviour is foraging (90.5% for this study, with similar rates to St. Mary, 1994a; Steele, 1996, 1998). Instead of feeding to satiation on flake food twice a day as in aquarium (lab) studies, fish in the habitats fed on plankton continually throughout the day, reducing time for intraspecific interactions. Second, intruders into the habitat, most notably blackeye gobies, *Rhinogobiops (=Coryphopterus) nicholsii*, added social complexity and interspecific interactions that also reduced the time available for intraspecific behaviour. Third, some of the groups in the aquarium-based studies had one or two more females per group, so our focal fish had an average of one less fish to interact with (Reavis and Grober, 1999). Fourth, kelp bass *Paralabrax clathratus*, sand bass *Paralabrax nebulifer*, and other predators restricted the fish from interacting as often as they would in the aquaria without predators (Steele, 1996, 1998). Predation also resulted in smaller groups and fewer potential mates.

Predation had an impact on the groups that can be compared to other field studies. An overall survivorship rate of 74% was consistent with the 80% recorded in field experiments by St. Mary (1994a), and higher than the 10–48% survivorship reported by

Steele (1996, 1998) in rubble piles without urchins. However, *L. dalli* survivorship was not size-independent as previously recorded (St. Mary, 1994a). Medium-sized fish were more likely to be missing. This discrepancy may be due to differences in habitat, but it also raises the question of possible marginalization of the next smallest fish during the sex change process. If the sex changer begins inhibiting sex change in other females, the inhibition may be focused on probable competitors for dominance and sex change. The second largest fish could be marginalized from the group and exposed to higher predation as a result of this higher aggression by the sex changer. However, the data we collected do not allow us to conclusively determine this. Study of the distribution of displacements toward conspecifics of differing sizes and the possibility of increased predation is needed.

#### 4.2. Histology: transitional, but functionally male

Histology of focal fish showed a spectrum of morphologies, allowing for the analysis of the interrelationship of the gonad, the papilla, and the AGS as related to protogynous sex change. As the sex-typical behaviour of a female fish changes, histology shows that the gonad is restructured and masculinized, the papilla elongates and narrows, the AGS forms and expands, sperm production increases, and sperm aggregations are sequestered in the AGS. Our results are consistent with observations that, during protogynous sex change, the AGS develops in association with the newly formed testis (Cole and Robertson, 1988; Cole and Shapiro, 1990).

Females showed variation in gonad size, but all possessed mostly ovarian tissue and minimal amounts of testicular tissue. Gonads of males had little to no ovarian tissue and a range of testicular tissue sizes. Male gonad size is small relative to female gonad size (Fig. 8). This implies: 1) a reduction of gonad size during sex change, and 2) that small testes produce sufficient sperm for fertilization of available eggs. Only fish undergoing sex change had gonads with a relatively equal amount of testicular and ovarian tissue.

In terms of protogyny, “transitional” implies being between functioning as female and as a male, but individual *L. dalli* possessing both testicular and

ovarian tissues exclusively display only male or female behaviour and appear to reproduce only as that sex (St. Mary, 1994b; Reavis and Grober, 1999). Sex-changing fish in this study functioned as a male, and therefore should not be considered sexually transitional. Histology showed that fish with very little testicular tissue and a developing AGS were able to function as a male. Comparing the “new male” sex changers with the representative males, a difference can be observed between “functional” and “optimal” males. Male fish as defined by St. Mary (1993) can have between 5% and 100% testicular tissue, but those with large AGS, fully elongated papilla, and purely testicular gonad have optimized their male function.

Fishelson (1991), in a review of gobiid AGS morphology, found that as spermatogenic tissues proliferate, the AGS rapidly fills with mucus secretion and sperm. The initially thick cuboid cells of the internal epithelium stretch, becoming elongated and flat. The internal walls were absent in developed males. These observations are consistent with the AGS morphology of *L. dalli* and the decreased AGS wall thickness of males compared to sex-changing fish observed in this study (Fig. 9). The decreased number of chambers and the thinning of internal walls over time suggest that the AGS fills as it develops and the inner walls are stretched and burst.

The hypothesized functions of the AGS are varied (reviewed in Barni et al., 2001; Cole and Hoese, 2001), but the AGS secretions embed the sperm and can facilitate adherence to the nesting site. As the AGS incorporates sperm in the appropriate delivery medium, the elongation of the papilla may increase its effectiveness in delivering sperm trails to the nest site. If male fish use sperm trails for asynchronous fertilization, then a larger AGS could produce larger, longer-lasting sperm trails important in sperm competition (Marconato et al., 1996; Scaggiante et al., 1999). This explains why an “optimal” male has an AGS larger than its testis.

The accepted paradigm in fishes, and vertebrates more generally, is to use genitalia and/or gonadal histology to assign sex (e.g., Cole and Shapiro, 1990). However, in our study, one sex changer fertilized eggs as a male but possessed only 3% testicular tissue and had a papilla ratio of 1.2, both below the accepted norms of male morphology for *L. dalli* (greater than

5% testicular tissue and having a papilla length-to-width ratio of greater than 1.4; Carlisle et al., 2000). Compared with three other fish that had a similar testicular tissue allocation but did not fertilize eggs, this successful sex changer had a substantially larger AGS (average cross-section  $4.9 \times 10^4$  vs.  $3.3 \times 10^4 \mu\text{m}^2$ ). Another sex changer with a similar-sized AGS had 48% testicular tissue. The fertilization success of both of these individuals, despite their widely varying percentage of testicular tissue, suggests the importance of the AGS in male reproduction and that relative masculinity in *L. dalli* is not best quantified by percent testicular tissue or genital papilla, but rather by the size and functionality of the AGS. Indeed, the male genital papilla can, in some instances, be a poor indicator of gonadal function (St. Mary, 1993), and some female *L. dalli* can possess spermatozoa, but they are unlikely to function as males without effective sperm delivery through organs such as the AGS and genital papilla.

According to Sadovy and Shapiro (1987), a definitive proof of sequential hermaphroditism requires the production of sex-changing individuals experimentally, using non-hormonal techniques, in conditions that closely resemble surroundings that may occur in nature. Analysis of focal fish in this study confirmed sequential hermaphroditic sex change in our experimental groups. All focal fish were behaviourally dominant, displayed jerking behaviour, and, unlike females, possessed an AGS. However, histological analysis did not find a significant difference between fertilizers and non-fertilizers. This may be a result of our assay. In this study, we used fertilization, a definitive functional display of maleness, as a conservative measure of sex change. Within 2 weeks, focal fish fertilized eggs as a male in 9/15 (60%) groups, as compared to 13/17 (77%) in the laboratory. Some focal fish may have changed sex and possessed the ability to fertilize eggs but lacked a gravid female with which to mate. Previous studies have shown that *L. dalli* has a 3-week interclutch interval (Behrents, 1983; St. Mary, 1994b). In our experiment, only two females were available to each sex changer, and predation occasionally reduced this number to only one while some groups in the aquarium studies had more than three females in a group (Reavis and Grober, 1999).

#### 4.3. Steroids: linking physiology with behaviour and morphology

The 11-KT/E<sub>2</sub> ratio in the *L. dalli* focal fish varied widely ( $4.85 \pm 5.1$ ) but was similar to the ratio of approximately 4:1 found by Kroon and Liney (2000) for male *Rhinogobiops nicholsii*, another protogynous goby. 11-KT levels are particularly interesting because 11-KT is the most potent fish androgen (Borg, 1994). Laboratory female *L. dalli* implanted for 3–5 days with 11-KT developed an elongated male-typical papilla (Carlisle et al., 2000), enlarged testicular tissue, regressed ovarian tissue, and a male-typical sperm-sequestering AGS (Carlisle, 2001). The lack of a positive correlation between 11-KT levels and genital papilla morphology in our study is not consistent with inferences made from Carlisle et al. (2000). This may be because 11-KT promotes the lengthening of genital papillae at very high levels, such as those with the implants, but not at endogenous levels. Our results for internal morphology were in agreement with expectations from Carlisle (2001), showing that alterations in the percent testicular tissue and AGS are highly correlated with morning levels of endogenous free 11-KT and reinforcing the idea that these sex characteristics are responding to a common androgenic mechanism during sex change. Our results are also consistent with reports of 11-KT resulting in testicular tissue induction in other teleosts such as the goldfish *Carassius auratus* (Kobayashi et al., 1991), and 11-KT stimulating spermatogenesis in the Japanese eel *Anguilla japonica* (Miura et al., 1991). In our study, testicular tissue was recruited at a much faster rate than ovarian tissue was reduced (Fig. 6) as seen in aquarium studies during the course of sex change (Black, Nowak, Moore, and Grober, unpublished data). In another sex-changing species, the protogynous blue-head wrasse *Thalassoma bifasciatum*, Kramer et al. (1988) found a decrease in ovarian tissue, but no induction of testicular tissue following treatment with the 11-KT precursor, testosterone. This may be a result of a lack of conversion of testosterone to 11-KT. Elevated 11-KT is associated with protogynous sex change in *T. bifasciatum* (Grober et al., 1991), *Thalassoma duperrey* (Nakamura et al., 1989), and *R. nicholsii* (Kroon and Liney, 2000), males before protandrous sex change in the anemone fish *Amphiprion melanopus* (Godwin and Thomas, 1993), domi-

nant male fish in all male groups of *Oreochromis mossambicus* (Oliveira et al., 1996), male secondary sex characteristics in *Parablennius* (Oliveira et al., 2001a,b), and courting male morphs of species with male dimorphism (Brantley et al., 1993).

In addition to the morphological correlation, our study showed a behavioural correlation to free 11-KT. The positive correlation between morning free 11-KT and the rate of displacement just before sacrifice suggests that the behavioural interactions of the fish influence the steroid levels, as seen in the challenge hypothesis, or that higher steroid levels influenced the behaviour of the focal fish (Wingfield et al., 1990; Oliveira et al., 2001c).

In conclusion, we have established that sex change in the bluebanded goby can be induced under natural conditions by removal of the dominant male from small, isolated social groups. The dynamics of behavioural sex change in the field is quite similar to those described under laboratory conditions. The absolute amount of social behaviour is reduced in the field, and this is probably due to the increase in time spent foraging and avoiding predators. Temperature had a significant effect on the time required to change sex, with complete sex change taking almost twice as long early in the summer relative to the warmer late summer months. Histological analysis of sex-changing fish revealed gonads, genital papillae, and accessory gonadal structures with morphology that is transitional to that of representative females and males. Fish with significant testicular development, accessory gonadal structure growth, and more frequent aggressive behaviour exhibited higher levels of free 11-KT in the morning, linking endogenous levels of steroids to behavioural and morphological changes. These results demonstrate that sex change in this species can be effectively studied under field conditions and this can provide us with a unique opportunity to understand the degree to which naturally occurring variation within social groups, between species (e.g., predator/prey relationships), and in the abiotic environment can regulate sexual function in protogynous hermaphrodites.

### Acknowledgements

This paper is contribution no. 223 from the Wrigley Marine Science Center, Catalina Island. We

thank the Georgia Research Alliance and GSU-RPE program for facilities and grant support. This research was supported by NSF-IBN 9723817 to M.S.G. and the Center for Behavioral Neuroscience NSF-IBN 9876754. The authors would like to acknowledge the help of Lee Nowak, Ryan Albrecht, Syndia and Mark Marxer-Miller, Jason Miranda, Katie Marrie, Ryan Earley, Kevin Flanagan, Cyd Yonker, Grober Lab, the Wrigley Institute for Environmental Studies, and “J.P. and the Tuna.” [CS]

### References

- Barni, A., Mazzoldi, C., Rasotto, M.B., 2001. Reproductive apparatus and male accessory structures in two batrachoid species (Teleostei, Batrachoididae). *J. Fish Biol.* 58, 1557–1569.
- Behrems, K.C., 1983. The comparative ecology and interactions between two sympatric gobies (*Lythrypnus dalli* and *Lythrypnus zebra*). PhD thesis, University of Southern California, Los Angeles.
- Borg, B., 1994. Mini review: androgens in teleost fishes. *Comp. Biochem. Physiol.*, C 109, 219–245.
- Brantley, R.K., Wingfield, J.C., Bass, A.H., 1993. Sex steroid levels in *Porichthys notatus*, a fish with alternative reproductive tactics, and a review of the hormonal bases for male dimorphism among teleost fishes. *Horm. Behav.* 27, 332–347.
- Carlisle, S.L., 2001. Androgens Mediate Changes in Sexually Dimorphic Structures in the Bluebanded Goby. Master's thesis, Arizona State University, Tempe, AZ, USA.
- Carlisle, S.L., Marxer-Miller, S.K., Canario, A.V.M., Oliveira, R.F., Carneiro, L., Grober, M.S., 2000. Effects of 11-ketotestosterone on genital papilla morphology in the sex changing fish *Lythrypnus dalli*. *J. Fish Biol.* 57, 445–456.
- Cole, K.S., Hoese, D.F., 2001. Gonad morphology, colony demography and evidence for hermaphroditism in *Gobidon okinawae* (Teleostei, Gobiidae). *Environ. Biol. Fishes* 61, 161–173.
- Cole, K.S., Robertson, D.R., 1988. Protogyny in the Caribbean reef goby, *Coryphopterus personatus*: gonad ontogeny and social influences on sex change. *Bull. Mar. Sci.* 42, 317–333.
- Cole, K.S., Shapiro, D.Y., 1990. Gonad structure and hermaphroditism in the gobiid genus *Coryphopterus* (Teleostei: Gobiidae). *Copeia* 4, 996–1003.
- Cole, K.S., Robertson, D.R., Cedeno, A.A., 1994. Does gonad structure reflect sexual pattern in all gobiid fishes? *Environ. Biol. Fishes* 41, 301–309.
- Drilling, C.C., Grober, M.S., in press. An initial description of alternative male reproductive tactics in the bluebanded goby, *Lythrypnus dalli* (Teleostei, Gobiidae). *J. Fish Biol.*
- Eckert, D.B., 1974. The Systematics and Biology of the Bluebanded Goby, *Lythrypnus dalli*, in Southern California. MS thesis, Occidental College, Pasadena.
- Fishelson, L., 1991. Comparative cytological and morphology of seminal vesicles in male gobiid fishes. *Jpn. J. Ichthyol.* 38, 17–30.

- Godwin, J.R., Thomas, P., 1993. Sex change and steroid profiles in the protandrous anemonefish *Amphiprion melanopus*. Gen. Comp. Endocrinol. 91, 144–157.
- Godwin, J.R., Sawby, R., Warner, R.R., Crews, D., Grober, M.S., 2000. Hypothalamic arginine vasotocin mRNA abundance variation across sexes and with sex change in a coral reef fish. Brain Behav. Evol. 55 (2), 77–84.
- Grober, M.S., Jackson, I.M.D., Bass, A.H., 1991. Gonadal steroids affect LHRH preoptic cell number in a sex/role changing fish. J. Neurobiol. 22, 734–741.
- Hartney, K.B., 1989. The foraging ecology of two sympatric gobioid fishes: importance of behavior in prey type selection. Environ. Biol. Fishes 26, 105–118.
- Hartney, K.B., Grorud, K.A., 2002. The effect of sea urchins as biogenic structures on the local abundance of a temperate reef fish. Oecologia 131, 506–513.
- Kobayashi, M., Katsumi, A., Stacey, N.E., 1991. Induction of testis development by implantation of 11-ketotestosterone in female goldfish. Zool. Sci. 8, 389–393.
- Kramer, C.R., Koulisch, S., Bertacchi, P.L., 1988. The effects of testosterone implants on ovarian morphology in the bluehead wrasse, *Thalassoma bifasciatum* (Bloch) (Teleostei: Labridae). J. Fish Biol. 32, 397–407.
- Kroon, F.J., Liley, N.R., 2000. The role of steroid hormones in protogynous sex change in the blackeye goby, *Coryphopterus nicholsii*. Gen. Comp. Endocrinol. 118, 273–283.
- Lahnsteiner, F., Seiwald, M., Patzner, R.A., Ferrero, E.A., 1992. The seminal vesicles of the male grass goby, *Zosterisessor ophiocephalus* (Teleostei, Gobiidae), fine structure and histochemistry. Zoomorphology 111, 239–248.
- Marconato, A., Rasotto, M.B., Mazzoldi, C., 1996. On the mechanism of sperm release in three gobioid fish. Environ. Biol. Fishes 46, 1–7.
- Miller, P.J., 1984. The tokology of gobioid fishes. In: Potts, G.W., Wootton, R.J. (Eds.), Fish Reproduction: Strategies and Tactics. Academic Press, London, pp. 103–118.
- Miller, D.J., Lea, R.N., 1976. Guide to the coastal marine fishes of California. Calif. Dep. Fish Game, Fish Bull., vol. 157 (235 pp.).
- Miura, T., Yamauchi, K., Takahashi, H., Nagahama, Y., 1991. Hormonal induction of all stages of spermatogenesis in vitro in the male Japanese eel (*Anguilla japonica*). Proc. Natl. Acad. Sci. 88 (13), 5774–5778.
- Nakamura, M., Hourigan, T.F., Yamauchi, K., Nagahama, Y., Grau, E.G., 1989. Histological and ultrastructural evidence for the role of gonadal steroid hormones in sex change in the protogynous wrasse *Thalassoma duperrey*. Environ. Biol. Fishes 24, 117–136.
- Oliveira, R.F., Almada, V.C., Canario, A.V.M., 1996. Social modulation of sex steroid concentrations in the urine of male cichlid fish *Oreochromis mossambicus*. Horm. Behav. 30, 2–12.
- Oliveira, R.F., Canario, A.V.M., Grober, M.S., Santos, R.S., 2001a. Endocrine correlates of male polymorphism and alternative reproductive tactics in the azorean rock-pool blenny. Gen. Comp. Endocrinol. 121, 278–288.
- Oliveira, R.F., Carneiro, L.A., Canario, A.V.M., Grober, M.S., 2001b. Effects of androgens on social behavior and morphology of alternative reproductive males of the azorean rock-pool blenny. Horm. Behav. 39, 157–166.
- Oliveira, R.F., Lopes, M., Carneiro, L.A., Canario, A.V.M., 2001c. Watching fights raises fish hormone levels. Nature 409, 475.
- Presnell, J.K., Schreiber, M.P., 1997. Humason's Animal Techniques, 5th ed. Johns Hopkins University Press, Baltimore.
- Reavis, R.H., Grober, M.S., 1999. An integrative approach to sex change: social, behavioral and neurological changes in *Lythrypnus dalli*. Acta Ethol. 2, 51–60.
- Robertson, D.R., 1972. Social control of sex reversal in a coral-reef fish. Science 177, 1007–1009.
- Ross, R.M., 1984. Anatomical changes associated with sex reversal in the fish *Thalassoma duperrey* (Teleostei: Labridae). Copeia 1, 245–248.
- Ross, R.M., Losey, G.S., Diamond, M., 1983. The evolution of sex-change mechanisms in fishes. Science 221, 574–575.
- Sadovy, Y., Shapiro, D.Y., 1987. Criteria for the diagnosis of hermaphroditism in fishes. Copeia 1, 136–156.
- Scaggiante, M., Mazzoldi, C., Petersen, C.W., Rasotto, M.B., 1999. Sperm competition and mode of fertilization in the grass goby *Zosterisessor ophiocephalus* (Teleostei: Gobiidae). J. Exp. Zool. 283, 81–90.
- Scott, A.P., Liley, N.R., 1994. Dynamics of excretion of 17 $\alpha$ ,20 $\beta$ -dihydroxy-4-pregnen-3-one 20-sulphate, and of the glucuronides of testosterone and 17 $\beta$ -oestradiol, by urine of reproductively mature male and female rainbow trout (*Oncorhynchus mykiss*). J. Fish Biol. 44, 117–129.
- Shapiro, D.Y., 1981. Behavioural changes of protogynous sex reversal in a coral reef fish in the laboratory. Anim. Behav. 29, 1185–1198.
- Steele, M., 1996. Effects of predation on reef fishes: separating cage effect from effects of predation. J. Exp. Mar. Biol. Ecol. 198, 249–267.
- Steele, M., 1998. The relative importance of predation and competition in two reef fishes. Oecologia 115, 222–232.
- St. Mary, C.M., 1993. Novel sexual patterns in two simultaneous hermaphroditic gobies, *Lythrypnus dalli* and *Lythrypnus zebra*. Copeia 4, 1062–1072.
- St. Mary, C.M., 1994a. Sex allocation in the simultaneous hermaphrodite, the bluebanded goby (*Lythrypnus dalli*): the effects of body size and behavioral gender and the consequences for reproduction. Behav. Ecol. 5, 304–313.
- St. Mary, C.M., 1994b. The determinants of sex allocation patterns and the maintenance of simultaneous hermaphroditism in the blue-banded goby (*Lythrypnus dalli*) and the zebra goby (*Lythrypnus zebra*). PhD Dissertation, UMI Dissertation Services, Ann Arbor, Michigan.
- St. Mary, C.M., 1998. Characteristic gonad structure in the gobioid genus *Lythrypnus dalli* with comparison to other hermaphroditic gobies. Copeia 3, 720–724.
- Warner, R.R., 1984. Mating behavior and hermaphroditism in coral reef fishes. Am. Sci. 72, 128–136.
- Wiley, J.W., 1976. Life histories and systematics of the Western North American Gobies *Lythrypnus dalli* (Gilbert) and *Lythrypnus zebra* (Gilbert). Trans. S. Diego Soc. Nat. Hist. 18, 169–184.
- Wingfield, J.C., Hegner, R.E., Dufty, A.M., Ball, G.F., 1990. The “challenge hypothesis”: theoretical implications for patterns of testosterone secretion, mating systems and breeding strategies. Am. Nat. 136, 829–846.