

An initial description of alternative male reproductive phenotypes in the bluebanded goby, *Lythrypnus dalli* (Teleostei, Gobiidae)

Cathleen C. Drilling & Matthew S. Grober

Department of Biology, Georgia State University, MSC 8L0389, 33 Gilmer St SE Unit 8, Atlanta, GA 30303-3088 U.S.A. (e-mail: mgrober@gsu.edu)

Received 1 December 2003 Accepted 14 July 2004

Key words: accessory gonadal structure, parasite, sneaker, density, sperm competition

Synopsis

Contrary to the generally accepted life history theory regarding sequential hermaphroditism ('size-advantage model'), we have recently identified 'mini males' in *Lythrypnus dalli*, the bluebanded goby. These are small (female-sized) fish that appear male based on their external genitalia. We investigated the reproductive anatomy and demography of these mini males. Based on their small size and the sexual plasticity of this species, we expected that mini males are not actually reproductively functioning males. However, when we examined their gonad anatomy, with particular attention to the male-typical accessory gonadal structure (AGS), we found that mini males have gonads that are comparable to those of nesting males in relative size and the percentage of tissue that is 'male' (testicular and AGS). Although this is contradictory to theories of sperm competition in alternative male phenotypes, reproductive strategies of these two types of males were clearly distinguishable based on their AGS content – as described in other examinations of differing ejaculate quality in alternative male phenotypes in the Gobiidae. Regular sampling of the *L. dalli* population showed that mini males make up a small fraction of the small size classes and that as the breeding season progressed, mini male frequency decreased and standard length increased significantly – thus allowing us to discuss their role within the social system. Based on these findings as well as comparisons with other species with multiple male phenotypes, we can begin to categorize *L. dalli* males as either bourgeois or parasitic, primarily based on their secondary sex characteristics and AGS contents.

Introduction

Lythrypnus dalli, the bluebanded goby, is a small (adult standard length 18–45 mm; St. Mary 1993) hermaphroditic fish (Fishelson 1989) with individuals that allocate reproductive tissues (RTs) to male, female or both sexes simultaneously (St. Mary 1993). This species can be found from the Gulf of California, Mexico, to Morro Bay, California, at depths ranging from intertidal to 64 m (Miller & Lea 1972). Living in densities that can range from 30 to 120 individuals m⁻² (Wiley 1972, Behrens 1983, Steele 1996), these benthic fish are typically clustered around the long-spined sea

urchin *Centrostephanus coronatus* (Hartney & Grorud 2002) or found on rocky vertical faces (Wiley 1976). Within their clusters, they generally exhibit a harem social structure, in which small groups consist of one large male (median standard length 29.5 mm; St. Mary 1993) that aggressively dominates a group of females of varying sizes (median standard length 23.2 mm; St. Mary 1993). The removal of the male and his aggressive behavior triggers sex change within a large female – as long as there are other females with which to spawn (Carlisle et al. 2000).

In the case of studying intact *L. dalli*, sexual phenotypes are usually defined by external genital

morphology, slight differences in fin (dorsal, anal) morphometrics and behavioral characteristics (Wiley 1976, Behrents 1983, Reavis & Grober 1999). In general, females have short, blunt genital papillae ('female-typical') and typically deposit ripe eggs from April to September (although they begin developing the eggs as early as February; Wiley 1976, Behrents 1983). During this time, males – which are characterized by a long, pointed genital papilla ('male typical') and longer fins – court gravid females, maintain nesting sites and presumably lay trails of sperm before and after the courted female releases her eggs (as in other gobies, see Scaggiante et al. 1999; Reavis & Grober, unpublished data). The males then proceed to defend the nests of demersal eggs (Behrents 1983). After hatching, the planktonic larvae drift in the water column for 2–3 months before settling at a standard length of 9–11 mm (typically May to January; Behrents 1983). These 'recruits' grow rapidly, reaching maturation within 1 month of settlement, and living for approximately 2 years with the potential to breed in both (Behrents 1983).

On a broad, ecological scale, sex change in *L. dalli* has been examined in terms of the size-advantage model presented by Warner (1975) (St. Mary 1993, 1994; but see also St. Mary 1997). This model predicts that in a protogynous hermaphroditic system, smaller individuals will have greater reproductive success as females, while large individuals benefit more from reproducing as males. In describing the population structure of *L. dalli*, however, researchers have come across anomalous fish that do not fit this model (St. Mary 1993). Now termed 'mini males,' this small proportion of the population consists of fish that are approximately 17–25 mm in standard length (a female-typical size range) but have male-typical genitalia. Neither external genital morphology nor behavioral characteristics are absolutely indicative of sexual function in this species (St. Mary 1993, Reavis & Grober 1999) and up to 10% of *L. dalli* with pointed genitalia may allocate as much as 95% to female gonadal tissue (St. Mary 1993, 1994). Thus the male-typical genitalia that define mini males as 'male' are not necessarily an indication of their capacity to function as a male.

Since external morphology is not definitive of sexual function, examination of internal (RTs) is a

more explicit way to identify an individual's reproductive function (St. Mary 1993). One essential male characteristic in gobies is the accessory gonadal structure (AGS) (Cole & Robertson 1988), a pair of multi-chambered lobes containing sperm as well as mucins and/or steroid derivatives (Miller 1984, Fishelson 1991, Scaggiante et al. 1999). This structure is similar to seminal vesicles and sperm duct glands found in other fish species (reviewed in Lahnsteiner et al. 1992). Therefore, in this anatomically based study, the presence or absence, size and contents of the AGS, as well as the histological characteristics of the entire of the gonad, are used as indications of sexual function in males.

We hypothesize that mini males do not have the capacity to reproduce as males. Carlisle et al. (2000) found that male size and AGS area increased concomitantly – therefore, we predict that small mini males lack AGS. Additionally, previous demonstrations of the flexibility of genital papilla morphology (especially male-typical papillae) indicated that the most apparent 'male' characteristic is not necessarily a reliable indicator of sexual function (St. Mary 1993, Carlisle et al. 2000). The first specific aim of this study is to properly characterize and compare mini male gonadal morphology to that of the other *L. dalli* phenotypes: recruits, small ambiguously sexed fish (meaning that their genital morphology is neither distinctly male nor female), females and large dominant males.

The second aim of this study is to integrate mini males into our understanding of the *L. dalli* social system. To address this issue, we regularly sampled *L. dalli* populations throughout the breeding season. Overall, these population data, in combination with the gonadal analyses, will provide a more comprehensive investigation of the role of mini males in the *L. dalli* social system.

Materials and methods

Gonad analyses

We collected the fish used for gonad analysis ($n = 59$, see below) in June and July 2001 near the Wrigley Institute of Environmental Studies (University of Southern California), Santa

Catalina Island, California (CA Department of Fish and Game Permit #: 803034-01). We made all collections by scuba diving and using the anesthetic quinaldine (Sigma Chemical; solution diluted one part quinaldine to nine parts acetone) and hand nets. We identified clusters of urchins with large breeding groups of fish and attempted to collect all fish within the groups. We measured (standard length to nearest 0.5 mm), weighed (to nearest 0.001 g) and classified each fish as male, female or ambiguous based on inspection of its genital papilla under a dissecting microscope. We then divided each individual into three parts: head, midsection (from just behind pectoral fins to just beyond anus – containing the gonads) and tail. We stored the midsections in Bouin's fixative (picric acid, formaldehyde and acetic acid) at 4°C until we processed them for histology.

In the laboratory at Georgia State University (Atlanta, Georgia), we dehydrated and infiltrated with paraffin (using the ThermoShandon Citadel 1000) the midsections of 18 mini males (ranging in length from 17 to 24 mm), 14 females (19–29 mm), five small ambiguously sexed individuals (19–21 mm), 12 nesting males (31–38 mm) and 10 recruits (9–12 mm). We then embedded the tissues in paraffin blocks and sliced them into 15 μm sections. We deparaffinized, rehydrated and stained these sections with Ehrlich's hematoxylin and eosin.

Initially, we analyzed the entire gonads from a number of individuals. We then systematically decreased the number of sections that we analyzed until we reached the minimum number of sections that accurately characterized the gonad. Therefore, we evaluated approximately 10 transverse sections from each fish – spaced throughout the gonad – using the AxioVision image analysis software (Zeiss, Inc.). We analyzed each section in terms of: (1) the area (μm^2) of all RTs, as well as the portions that were ovarian, testicular and AGS; (2) the presence or absence of tailed sperm and hydrated oocytes (indicators of active gonads, St. Mary 1993); and (3) AGS content. AGS content was rated on a discrete scale of one to three based on the relative proportion of mucus to sperm: one indicated high sperm densities with very little mucus while a three indicated only a few pockets of sperm in a mucus-filled AGS.

The classical measure of gonadal investment is the gonadosomatic index (GSI). While GSI is

typically found by dividing the weight of the gonad by the body weight of the fish, we were unable to dissect out and weigh the gonads of these fish because such a procedure may destroy the tissues and it was critical that we analyze the contents of the AGS. In order to evaluate GSI while also leaving the fish intact, we therefore planned to use the cross sectional area of the gonad, AGS and entire body to calculate the relationship. However, while assessing these structures under the microscope, the entire cross section of many fish was too large to be measured at low magnification on a compound microscope. Lacking analysis software on a dissecting microscope, we measured the length (L) and width (W) of two random cross sections from each fish using dial calipers. We used these values to calculate the body area (area of an ellipse = $\pi \times (L/2) \times (W/2)$) and averaged the two calculated cross sections for each fish. These data were used as the body area values to calculate GSI. In order to check the appropriateness of our cross section estimations, we regressed digitally imaged microscopic measurements (from those fish that could be measured) of cross sectional area with our caliper-based estimations from the same fish. There was a significant linear relationship ($R^2=0.86$, $F=113.34$, $p < 0.0001$), thus we felt justified in using the caliper-based estimations for the larger fish that were included in the GSI analyses.

Normally distributed data (i.e., there was homogeneity of variances) were examined using ANOVA and Tukey post hoc analyses. GSI data that were non-normally distributed were analyzed using Mann-Whitney (for two samples) or Kruskal-Wallis (for greater than two samples) nonparametric analyses. The effects of body size on female sexual characteristics were examined using standard linear regression analysis. When appropriate, data are presented as the mean \pm standard error.

Population composition

We collected the fish used in the population composition analyses from May to August 2002 using the same methods in the same location as the previous collections (CA Department of Fish and Game Permit #: 803028-05). As in the previous catches, we measured, weighed and classified each collected fish on the basis of its genital papilla. The

standard length, sex and frequency of individuals gathered from these collections allowed for an examination of temporal changes in population size and composition (i.e., sex and size) throughout the sampling period. Most of these fish were then either used in other studies; shipped to Atlanta, Georgia, for laboratory studies; or released back into the wild. When appropriate, pooled data were examined using ANOVA or log-linear analysis.

Results

Gonad analyses

Microscopic analysis of the gonads revealed that nearly all males and females did contain both ovarian and testicular tissues. The gonads of the females, although not entirely packed with gametes, consisted primarily of vitellogenic (developing) and secondary (undeveloped) oocytes (Figure 1). They also had small pockets of tailed sperm (typically less than 5% of the total gonad)

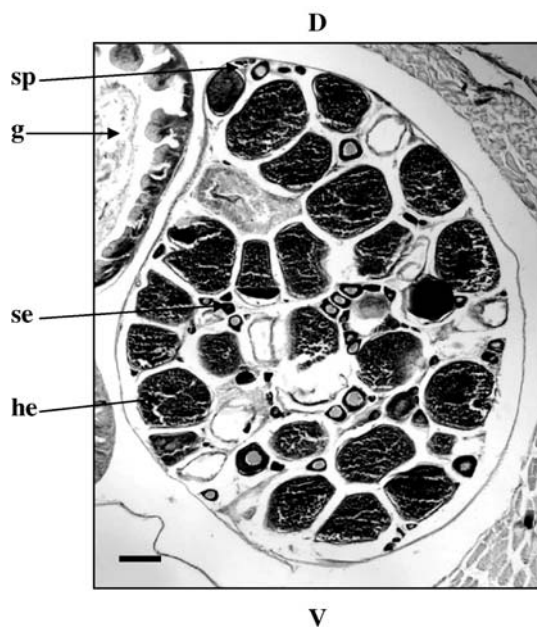


Figure 1. Image of one *L. dalli* female ovary. Female gonads are primarily filled with oocytes, although there are typically small pockets of spermatic tissue at the periphery. Abbreviations: he, hydrated egg; se, secondary egg; sp, spermatic tissue; g, gut; D, dorsal; V, ventral. Scale bar represents 200 μm .

usually located at the dorsal and ventral poles of the gonad. Females also lacked an AGS. There were, however, two extraneous females with more than 5% spermatic tissue and an AGS. These individuals may have been misclassified as female or were in the process of sex change.

In general, there was no effect of body size on sexual characteristics of females. The percent of the gonad that was ovarian tissue did not increase with body size ($R^2 = 0.19$, $F = 2.28$, $p = 0.16$). Although there was a tendency for gonad area to increase with body size, this relationship also was not significant ($R^2 = 0.029$, $F = 4.17$, $p = 0.068$).

The small individuals with ambiguous genital papillae were highly variable. Some possessed gonads that could be considered male (an allocation of more than 95% to testicular and AGS tissue), others could be considered female (an allocation of more than 95% to ovarian tissue) and still others were a mix of ovarian and testicular tissue.

None of the newly recruited individuals possessed male-typical papilla, nor did they possess any testicular tissue or AGS. They all had small ovaries consisting of undeveloped oocytes (not shown).

Both mini males and nesting males had testes that were solidly packed with tailed and developing sperm (Figure 2a, b). A sparse number (< 5% of gonadal tissue) of secondary oocytes were also found in the testes, typically located along the edges of the gonad. In addition to the testis, all male subjects (mini and nesting) had a multi-chambered AGS. The mean percent of male tissue (testicular and AGS) differed significantly amongst the groups of individuals sampled (Figure 3). The percent male tissue of mini males was significantly greater than that of females and ambiguously sexed fish. However, mini males did not differ significantly from nesting males in their percent testicular and AGS tissue.

Mini males and nesting males also did not differ significantly in the percent of the total cross sectional area composed of testis, AGS or total RTs (testis plus AGS) (Table 1). However, they did differ in terms of the contents of their AGS (Figure 2), which was rated one to three based on the relative proportion of mucus to sperm. Analysis of the characterization data revealed that mini males had significantly less mucus (and therefore more sperm) in their AGS than nesting males (Figure 4).

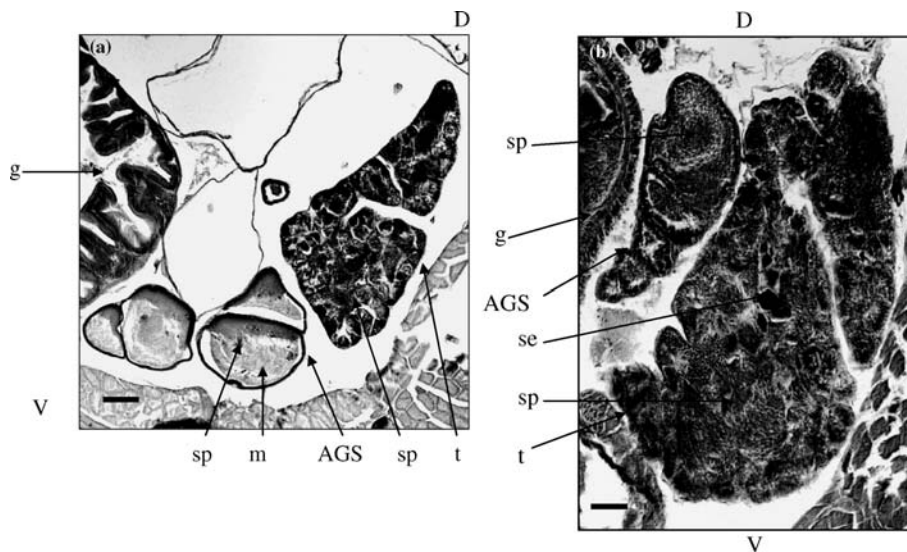


Figure 2. Images of one *L. dalli* (a) nesting male gonad and (b) mini male gonad. Male gonads are comprised of a testis (packed with sperm) as well as an AGS. The contents of the AGS appear to differ by male phenotype: mini males' are filled mostly with sperm, while nesting males' mostly contain mucus with small pockets of sperm. Abbreviations: AGS, accessory gonadal structure; *t*, testis; sp, sperm; se, secondary egg; m, mucus; g, gut; D, dorsal; V, ventral. Scale bars represent 200 μm and 400 μm in nesting males and mini males respectively.

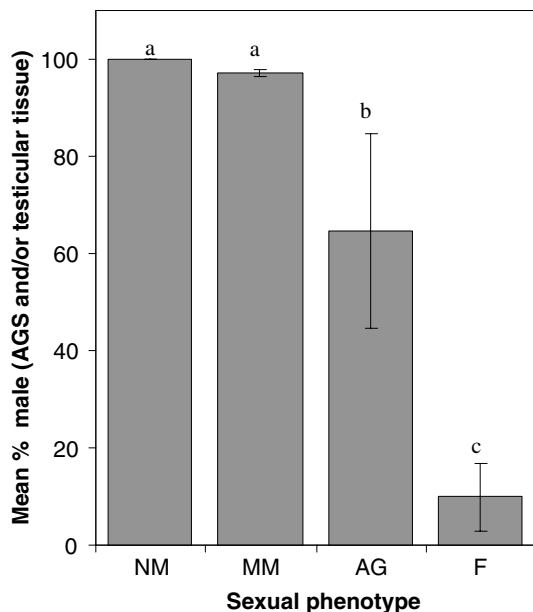


Figure 3. The mean percent of male tissue (spermatic and AGS) differed amongst the categories of *L. dalli* individuals sampled (ANOVA; $F_{3,48} = 65.76$, $p < 0.001$). Groups with the same letter above the error bar were statistically similar, while groups with differed letters differed (Tukey post-hoc test, $p < 0.05$). NM, nesting males; MM, mini males; AG, ambiguous genitalia; F, females.

Therefore, mini males did not differ from nesting males in terms of the relative size of their RTs, but rather in terms of the contents of these structures.

As a result of the gonad measurements in both sexes, we also had an opportunity to examine whether male and female *L. dalli* invested differently in their RTs. The mean total GSI of nesting males, mini males and females differed significantly (Kruskal–Wallis; $\chi^2 = 6.24$, $df = 2$, $p = 0.044$; nesting males = 6.30 ± 0.56 ; mini males = 7.93 ± 1.11 ; females = 11.01 ± 1.35). Mini males did not have a GSI that was significantly greater than nesting males (multiple comparison test for the Kruskal–Wallis analysis: Zar 1996; $Q = 0.80$, $p > 0.05$) or significantly less than females ($Q = 1.81$, $p > 0.05$). However, females had a significantly greater GSI than nesting males ($Q = 2.40$, $p > 0.05$).

Population composition

The population data demonstrated a female to male ratio of approximately 4:1. Mini males (17–25 mm; male genital papilla) made up a relatively small percentage of the *L. dalli* population, found at a maximum of 4.47% early in the breeding season (May 2002). From the bimonthly large

Table 1. A comparison of *L. dalli* mini males (MM) and nesting males (NM) in terms of the percent testis, AGS, or testis and AGS in their total cross section.

	Testis/ Cross	AGS/ Cross	Testis + AGS/Cross
MM	5.83 ± 1.06	2.08 ± .31	7.91 ± 1.12
NM	3.55 ± .34	2.74 ± .32	6.29 ± .56
Significance (<i>p</i>)	0.1	0.16	0.27
$F_{1,28}$	2.91	2.07	1.25

The two male phenotypes did not differ significantly in any of the measurements.

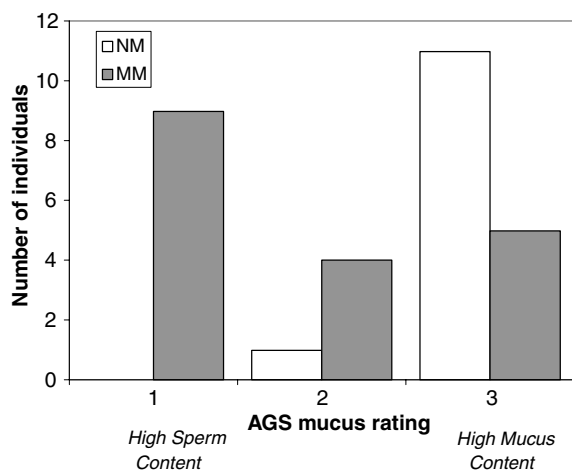


Figure 4. The number of mini (MM) and nesting (NM) *L. dalli* males in each AGS mucus category. AGS content was rated one to three based on the relative proportion of mucus to sperm (one indicated high sperm densities with very little mucus while a three indicated only a few pockets of sperm in a mucus-filled AGS). As a group, mini males had significantly more sperm and less mucus in their AGS than nesting males (Mann-Whitney, $U = 34.50$, $n_{MM} = 18$, $n_{NM} = 12$, $p < 0.001$).

catches, we observed a significant decline over the course of our sampling period in the frequency of these individuals (log-linear analysis, $\chi^2 = 12.33$, $p = 0.031$, $df = 5$; Table 2). In addition, the standard length of mini males and the standard length of the smallest mini male from each sampling period increased significantly (Figure 5). This suggests that for a given mini male cohort, individuals do 'grow up' out of their size class. It is also reasonable to suggest that recruitment of the next cohort (which begins in late August and early September and was after our sampling period for this study) would replenish the mini male population. It is also interesting to note that females

Table 2. A compilation of descriptive data from *L. dalli* bimonthly large catches as well as the total from all catches throughout the breeding season.

Date	MM avg SL (mm)	total avg SL (mm)	% MM	% F	F : M	Total # fish
19.5.03	21.8	27.7	4.47	11.73	4.00	179
2.6.03	22.8	28	3.64	8.61	3.63	302
16.6.03	22.9	27.5	3.03	13.6	4.27	132
30.6.03	23.3	28.1	3.23	3.23	4.63	62
14.7.03	23.5	29.4	0.72	7.19	3.97	278
28.7.03	24.5	30.3	0.62	0.62	4.52	161
Total	22.7	28.6	2.54	7.63	3.97	1180

Mini male (MM) average standard length (SL) increased throughout the season, as did the total average standard length of all fish caught. Among the small fish (mini males and females less than 25 mm) sampled, females (F) typically represented a much larger percent of the population. The female to male ratio is consistently female-biased throughout the breeding season.

made up a larger percentage of the small size range (Table 2).

Discussion

Contrary to our expectations, the gonad analyses clearly indicate that *L. dalli* mini males have the

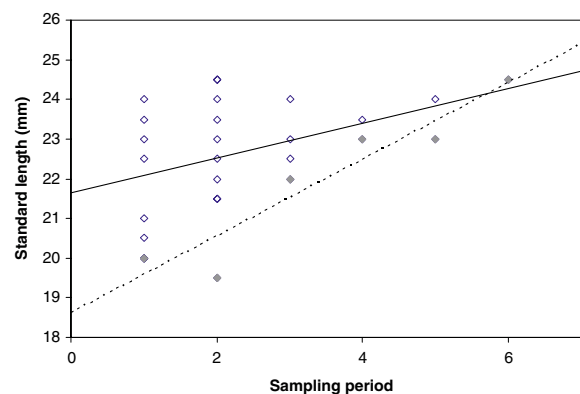


Figure 5. *L. dalli* mini male standard length increased significantly (simple regression, solid line and hollow diamonds; ANOVA, $F_{1,26} = 5.211$, $R^2 = 0.167$, $p = 0.031$) throughout the sampling period. The standard length of the smallest mini male from each sampling period also significantly increased over time (simple regression, dashed line and solid diamonds; ANOVA, $F_{1,4} = 33.266$, $R^2 = 0.893$, $p = 0.004$), suggesting that the mini male population is not replenished throughout the breeding season and that mini males do 'grow up' out of their size class.

gonadal physiology necessary to act as functional males. Based on the differences in reproductive morphology between mini and nesting males, it also appears that this species has multiple male phenotypes, thus refuting previous assumptions of monomorphism in *L. dalli* males (e.g., Grober 1998). This finding may also clarify previous speculations in St. Mary (1993), in which the author both declared the absence of small males within the population and used GSI data to suggest the presence of small male streak spawners. Our study provides one additional clarification with respect to the reproductive biology of *L. dalli*. Individuals that were previously thought to be immature juveniles (Wiley 1976: 13–22 mm; Behrents 1983: less than 24 mm; Hartney & Grorud 2002: less than 20 mm) now should be considered functional and be included in examinations of reproductive function. This includes small-sized females, which did not differ in reproductive capacity from larger females in this study. Specifically, *L. dalli* juveniles should be defined as individuals 9 mm (the smallest recruit collected) up to 17 mm (the smallest mini male or female). This is supported by Fishelson's (1989) study of 150 species of fish, in which he found that all gobies 'start to reproduce when they are small (12–25 mm total length), possibly a very short time after metamorphosis and settlement from the planktonic stage.'

To better understand the role of mini males in the *L. dalli* system, it is important to consider the major concepts of alternative male reproduction. Taborsky (1994, 2001) has reviewed much of the literature and classified males into four major categories: (1) males that participate in mass spawnings are forced to invest heavily in spermatogenesis in order to overcome intense sperm competition, (2) dominant ('bourgeois') males that are able to monopolize resources, mates or territories and typically invest in reproduction behaviorally, morphologically and physiologically, (3) sneaker parasitic males that utilize decreased conspicuousness and increased gonadal investment to allow them to evade the bourgeois monopoly, (4) satellite males, which also parasitize the bourgeois effort, that gain some access to fertilizations through 'cooperating' in nest defense, nest building and mate attraction with the larger males.

To understand how mini males might fit into the study of alternative male reproduction and what

further work is necessary, we compared mini males in *L. dalli* to a select group of species with extensively studied alternate male phenotypes (Table 3). An important factor in classifying alternate males is whether or not they have life histories that are fixed (due to genetics or an ontogenetic switch that irreversibly commits them to one strategy) or flexible (based on environmental or social conditions, body size, etc.; reviewed in Taborsky 2001). This characteristic appears to be variable. A few of the parasitic males (*Porichthys notatus*, *Lepomis macrochirus*, *Xiphophorus nigrensis*, *Salmo salar*) are fixed in their strategies, while most are plastic, changing to bourgeois males throughout their life span in response to social cues (*Z. ophiocephalus*, *S. pavo*, *T. bifasciatum*, *Symphodus ocellatus*). We do not know if mini males change to nesting males throughout their lifetime (see below). This must be further examined by means of experimental social manipulations.

In general, all parasitic males have a smaller body size than bourgeois males, a relationship also observed in *L. dalli* males (Table 3). Additionally, bourgeois males invest behaviorally (e.g., territory defense and courtship) and morphologically (e.g., body size and coloration) while parasitic males use sneaking, streaking or cooperative behaviors – often in conjunction with female mimicry (e.g., body size and coloration) – to steal fertilizations (as in Taborsky 1994, 2001). While mini males do resemble female *L. dalli* (small body size, lack of enlarged dorsal fin), their behavioral repertoire is unknown. Future behavior analyses are critical in understanding their reproductive strategy: are they sneakers or satellite males?

While examining the role of GSI in the classification of sexual phenotypes, it is interesting to note that GSIs in *L. dalli* males (nesting male = 6.3 and mini male = 7.93) and females (11.01) are not as different as those found in other species. For example, in *Porichthys notatus* females have a GSI of 15, type II (parasitic) males 9, type I (bourgeois) males 1 (Bass & Marchaterre 1989); *Anthias squamipinnis* females at two sites averaged GSIs of 25.96 and 18.72 while males averaged 4.46 and 3.20 (Shapiro 1981), and in a review of 30 gobiid species most female GSIs ranged 12–25, males 0.3–3.6 (Miller 1984). Thus, *L. dalli* does follow the general trend of the larger female GSI.

Table 3. A comparative look at alternative male reproduce strategies

Species	RS ^a	ALH ^b	Body size	Behavior		Secondary characteristics		GSI ^c		Ejaculate characteristics	
				B	P	B	P	B	P	B	P
Bluebanded goby ¹	H	?	B > P	territorial, guard eggs, court females	?	enlarged dorsal fin	similar to females	B = P	mucus in AGS for laying sperm trails	sperm-packed AGS	
Grass goby ²	G	PI	B > P	territorial, dig nests, guard eggs	sneak spawns	same	same	B < P	mucins help sperm trails last longer and release sperm more constantly	seminal final vesicles mucin deficient; sperm release shorter and less constant	
Peacock blenny ³	G	PI	B > P	territorial, nest holder	non-functional males or sneak spawners	head crest, anal gland	similar to females in morphology	B < P			
Bluehead wrasse ⁴	H	PI	B > P	territorial, court females	group, sneak or spawn	blue coloration	similar to females	B < P	not enough sperm in ejaculate to fertilize entire clutch	greater quantity of sperm	
Ocellated wrasse ⁵	G	PI	B > P	territorial, build nests, court females, care for eggs	parasitize spawning by sneaking or associating with nesting males	colorful	similar to females	B < P			
Bluegill sunfish ⁶	G	F	B > I > P	territorial, build nests, court females, guard eggs	parasitize spawning by sneaking or associating with nesting males	light in body color with dark yellow-orange breasts	sneaker; light color satellite; dark body with dark bars-	B < I < P	ejaculate volume and total number sperm greater	ejaculate concentration greater	
Swordtail ⁷	G	F	B > I > P	gain access to females via courtship behaviors	chase females and force copulation	large dorsal fins and swords; blue color	lacking elaborate fins; drab color	B < I < P*			
Plainfin midshipman ⁸	G	F	B > P	territorial, guard nests, produce hum to attract females, guard eggs	parasitic spawning	well developed swim bladder muscles	lacking swim bladder muscles	B < P			
Atlantic salmon ⁹	G	F	B > > P	dominates spawning beds	sneak spawns	silver color for ocean camouflage	dark backs and bars for river camouflage	B < P	more actual sperm	more relative sperm and more active for longer	

Note. B, bourgeois males; P, parasitic males; I, intermediate males.

^aRS, reproductive strategy; H, hermaphroditic; G, gonochoristic.

^bALH, alternative life history strategy; PI, plastic; F, fixed.

^cGSI, gonadosomatic index; GSI data do not include accessory gonadal structures.

¹*Lythrypnus dalli*; Wiley 1976; Behrens 1983; St. Mary 1993, 1994.

²*Zosterisessor ophiocephalus*; Scaggitante et al. 1999; Mazzoldi et al. 2000.

³*Salarias pavo*; Ruchon et al. 1995; Oliveira et al. 2001.

⁴*Thalassoma bifasciatum*; Warner & Robertson 1978; Warner & Swearer 1991; Warner et al. 1995.

⁵*Symphodus ocellatus*; Sojian 1930; Taborsky et al. 1987; Alonzo et al. 2000; Alonzo & Warner 2000.

⁶*Lepomis macrochirus*; Dominey 1980; Gross & Charnov 1980; Gross 1991; Leach & Montgomerie 2000.

⁷*Xiphophorus nigrensis*; Ryan et al. 1990; Ryan et al. 1992; Ryan & Rosenthal 2001; * this data applies to a related species, *X. maculatus*, Schreibman & Kallman 1977.

⁸*Porichthys notatus*; Bass & Marchaller 1989; Bass 1992; Barni et al. 2001.

⁹*Salmo salar*; Gafte et al. 1995.

In bourgeois and parasitic males, comparisons of relative GSI estimates are often explained using sperm competition theory (Parker 1970, 1982, 1990) to make a smoother transition. This usually results in a higher relative GSI in parasitic males relative to the larger, behaviorally invested males (Table 3). In *L. dalli*, however, the relative size of the RTs is statistically similar in mini and nesting males. While this does not conform to classic sperm competition theory, these two male phenotypes may use their structures differently, thus providing a different form of reproductive differentiation. The higher mucus content in nesting male AGS is probably important for laying sperm trails in their nests (Fishelson 1991, Scaggiante et al. 1999), however, mini males (which most likely have to sneak fertilizations) will not have the opportunity to lay sperm trails and thus do not need a mucus-filled AGS. Therefore, mini males may not need a relatively large testis (contrary to sperm competition theory) because they have an AGS that is packed with sperm, releasing the testes from this constraint. In sum, *L. dalli* males do not have the GSI differences that are predicted by sperm competition theory (reviewed in Stockley et al. 1997), but instead can be differentiated by means of the contents of their AGS.

Comparisons of ejaculate characteristics also have been useful in distinguishing male phenotypes, especially within the family Gobiidae (Table 3). Parasitic males consistently release a larger quantity of sperm (relative to their size) than their bourgeois counterparts. Therefore, ejaculate quality and quantity in *L. dalli* males may be the defining characteristic of their reproductive strategy and requires further investigation. This supports the suggestion by Scaggiante et al. (1999) that the actual function of accessory structures must be considered when studying sperm competition in diandric (two male phenotypes) systems.

Therefore, although mini males in *L. dalli* do not have GSIs that are significantly higher than those of nesting males, our comparative analysis indicates that they do possess characteristics that are consistent with sneaker males in the most closely related representatives in our analysis (Table 3). Given the similarity between *L. dalli* mini males and sneaker males from closely related gobies, we suggest that there is an alternative male phenotype in *L. dalli*. Based on comparisons with other species with

multiple male phenotypes, we can begin to categorize *L. dalli* males as either bourgeois or parasitic, primarily based on their relative body size, secondary sex characteristics and AGS contents. Specifically, differences in AGS contents suggest a novel means of discerning sperm competition when relative GSIs are not significantly different.

The data presented here do not allow us to assess the factors that influence sexual plasticity and the generation of alternate male phenotypes in *L. dalli*. However, we are interested in how these concepts may apply to this system. For example, if *L. dalli* males are genetically determined, it appears that the AGS – which may be coded for in the male genotype – is utilized for a different purpose in mini males (e.g., not primarily a mucus-producing organ).

It seems more likely, though, that environmentally determined sexual differentiation ‘is more the rule than the exception, and [that] it occurs in a great variety of organisms’ (Warner 1988). For instance, if (1) the absence of a dominating male and (2) a female-biased sex ratio are necessary to induce female sex change, then theoretically, a high density of females in a social group may provide the opportunity for sex change (see Shapiro 1984). This may be especially true for individuals that are small enough to use habitat complexity for shelter from aggressive conspecific males (Warner 1984, Behrems 1987). Additionally, high densities of females may saturate the nests of dominant males (as in the peacock blenny, *Salarias pavo*; Almada et al. 1994) and lead to selective mate choice by males (if given the choice, males choose to mate with larger females in both *S. pavo*, Almada et al. 1995, and *T. bifasicatum*, van den Berghe et al. 1989), thus providing conditions that would allow small individuals to have a higher reproductive fitness as male than as female. Ghislen (1969) also predicted sex change in these smaller (and therefore younger) individuals. He hypothesized that birth date could act as an environmental determinant of sex: ‘an individual delayed in finding suitable locality [for settlement] could still reproduce when small, but could do so better if male than female.’ To combine these two theories of environmental sex determination and apply them to *L. dalli*, mini males may be late recruits (settling in January, rather than early in the previous May; thus their small size relative to

the other individuals in the population) that have settled upon habitats with a high density of females (adults and all earlier recruits). This would provide them the social conditions to 'choose' the alternative male strategy (perceptual and motor abilities of fish larvae: Avise & Shapiro 1986; effects of conspecific densities: Behrens 1987, Steele 1997, Forrester 1999; settlement cues reviewed in Wilson & Osenberg 2002).

If mini males are plastic in their sexual differentiation, they could continue to respond to social conditions and change sex when the conditions warrant it. However, it is unknown if *L. dalli* males can change to females in nature and precisely what conditions would drive the process. St. Mary (1994) was able to change one in a group of five males (in four out of six laboratory groups) in 2 weeks time, although the initial male behavioral and functional status of those sex-changers was unknown. Reavis & Grober (1999) were also able to change males in the laboratory, although the rate was much slower (in three groups of males, eggs appeared 32, 46, and 53 days after group construction), which may be because those males were known spawners and/or had exhibited parental care (meaning that they had previously functioned as males). Female to male sex change usually occurs within 14 days (Reavis & Grober 1999). The slow rate of change from male to female (especially when considered in the context of a 4 month breeding season and two year lifespan) may indicate the difficulty of male-to-female sex change. Additionally, the 4:1 female-biased sex ratio that we found in our population studies predicts unidirectional hermaphroditism in *L. dalli*, rather than bi-directional sex change (which is typically associated with a 1:1 in sex ratio) (Sadovy & Shapiro 1987, Cole & Hoese 2001). Therefore, based on the slow rate and low probability of male to female sex change as well as the population sex ratio, we suggest that this species is primarily a unidirectional protogynous sex changer with a sneaker male alternative phenotype.

Acknowledgements

First we thank Michael Black for his assistance in formulating and carrying out the field experiments as well as his thorough reading of this manuscript.

We also thank Ryan Earley, Ed Rodgers and anonymous referees for their comments on this manuscript. We are grateful to Elizabeth Stokes, Brandon Moore and the WMSC staff for their assistance in the field. Additionally, we thank our colleagues at Georgia State University for their generosity with laboratory equipment. This study was supported in part by the Center for Behavioral Neuroscience, an STC Program of the National Science Foundation under Agreement No. IBN-9876754 and NSF IBN-9723817 to MSG. Additional support was provided by the Georgia Research Alliance and GSU-RPE program. This is contribution #229 from the University of Southern California's Wrigley Marine Science Center on Santa Catalina Island. The research was performed in compliance with all current laws of the USA.

References

- Almada, V.C., E.J. Gonçalves, A.J. Santos & C. Baptista. 1994. Breeding ecology and nest aggregations in a population of *Salaria pavo* (Pisces: Blenniidae) in an area where nest sites are very scarce. *J. Fish Biol.* 45: 819–830.
- Almada, V.C., E.J. Gonçalves, R.F. Oliveira & A.J. Santos. 1995. Courting females: ecological constraints affect sex roles in a natural population of the blennioid fish *Salaria pavo*. *Anim. Behav.* 49: 1125–1127.
- Alonzo, S.H., M. Taborsky & P. Wirtz. 2000. Male alternative reproductive behaviours in a Mediterranean wrasse, *Symphodus ocellatus*: evidence from otoliths for multiple life-history pathways. *Evol. Ecol. Res.* 2: 997–1007.
- Alonzo, S.H. & R.R. Warner. 2000. Dynamic games and field experiments examining intra- and intersexual conflict: explaining counterintuitive mating behavior in a Mediterranean wrasse, *Symphodus ocellatus*. *Behav. Ecol.* 11: 56–70.
- Avise, J.C. & D.Y. Shapiro. 1986. Evaluating kinship of newly settled juveniles within social groups of the coral reef fish *Anthias squamipinnis*. *Evolution* 40: 1051–1059.
- Bass, A.H. 1992. Dimorphic male brains and alternative reproductive tactics in a vocalizing fish. *Trends Neurosci.* 15: 139–145.
- Bass, A.H. & M.A. Marchaterre. 1989. Sound-generating (sonic) motor system in a teleost fish (*Porichthys notatus*): sexual polymorphism in the ultrastructure of myofibrils. *J. Compar. Neurol.* 286: 141–153.
- Barni, A., C. Mazzoldi & M.B. Rasotto. 2001. Reproductive apparatus and male accessory structures in two batrachoid species (Teleostei, Batrachoididae). *J. Fish Biol.* 58: 1557–1569.
- Behrens, K.C. 1983. The comparative ecology and interactions between two sympatric gobies (*Lythrypnus dalli* and *Lythrypnus zebra*). Ph.D. dissertation. Los Angeles: University of Southern California.

- Behrents, K.C. 1987. The influence of shelter availability on recruitment and early juvenile survivorship of *Lythrypnus dalli* Gilbert (Pisces: Gobiidae). *J. Exp. Mar. Biol. Ecol.* 107: 45–59.
- Carlisle, S.L., S.K. Marxer-Miller, A.V.M. Canario, R.F. Oliveira, L. Carneiro & M.S. Grober. 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. & D.R. Robertson. 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. & D.F. Hoese. 2001. Gonad morphology, colony demography and evidence for hermaphroditism in *Gobidon okinawae* (Teleostei, Gobiidae). *Environ. Biol. Fish.* 61: 161–173.
- Dominey, W.J. 1980. Female mimicry in male bluegill sunfish – a genetic polymorphism? *Nature* 284: 546–548.
- Fishelson, L. 1989. Bisexuality and pedogenesis in gobies (Gobiidae: Teleostei) and other fish, or, Why so many little fish in the tropical seas? *Senckenbergiana maritima* 20: 147–169.
- Fishelson, L. 1991. Comparative cytology and morphology of seminal vesicles in male gobiid fishes. *Jpn. J. Ichthyol.* 38:17–30.
- Forrester, G.E. 1999. The influence of adult density on larval settlement in a coral reef fish, *Coryphopterus glaucofraenum*. *Coral Reefs* 18: 85–89.
- Gage, M.J., P. Stockley & G.A. Parker. 1995. Effects of alternative male mating strategies on characteristics of sperm production in the Atlantic salmon (*Salmo salar*): theoretical and empirical investigations. *Philos.Trans. Roy. Soc. Lond. B* 350: 391–399.
- Ghiselin, M.T. 1969. The evolution of hermaphroditism among animals. *Quart. Rev. Biol.* 44: 189–208.
- Grober, M.S. 1998. Socially controlled sex change: integrating ultimate and proximate levels of analysis. *Acta Ethol.* 1–2: 3–17.
- Gross, M.R. 1991. Evolution of alternative reproductive strategies: frequency-dependent sexual selection in male bluegill. *Philos. Trans. Roy. Soc. Lond. B* 332: 59–66.
- Gross, M.R. & E.L. Charnov. 1980. Alternative male life histories in bluegill sunfish. *Procs. Nat. Acad. Sci. USA* 77: 6937–6940.
- Hartney, K.B. & K.A. Grorud. 2002. The effect of sea urchins as biogenic structures on the local abundance of a temperate reef fish. *Oecologia* 131: 506–513.
- Lahnsteiner, F., M. Seiwald, R.A. Patzner & E.A. Ferrero. 1992. The seminal vesicles of the male grass goby, *Zosterisessor ophiocephalus* (Teleostei, Gobiidae), fine structure and histochemistry. *Zoomorphology* 111: 239–248.
- Leach, B. & R. Montgomerie. 2000. Sperm characteristics associated with different male reproductive tactics in bluegills (*Lepomis macrochirus*). *Behav. Ecol. Sociobiol.* 49: 31–37.
- Mazzoldi, C., M. Scaggiante, E. Ambrosin & M.B. Rasotto. 2000. Mating system and alternative male mating tactics in the grass goby *Zosterisessor ophiocephalus* (Teleostei: Gobiidae). *Mar. Biol.* 137: 1041–1048.
- Miller, P.J. 1984. The tokology of gobioid fishes. pp. 119–153. *In: G.W. Potts & R. J. Wootton (eds.), Fish Reproduction: Strategies and Tactics.* Academic Press, London.
- Miller, D.J. & R.N. Lea. 1972. Guide to the Coastal Marine Fishes of California. Fish Bulletin No. 157. California Department of Fish and Game, Sacramento, California. 187 pp.
- Oliveira, R.F., L.A. Carneiro, D.M. Gonçalves, A.V.M. Canario & M.S. Grober. 2001. 11-ketotestosterone inhibits the alternative mating tactic in sneaker males of the peacock blenny, *Salaria pavo*. *Brain, Behav. Ecol.* 58: 28–37.
- Parker, G.A. 1970. Sperm competition and its evolutionary consequences in the insects. *Biol. Rev.* 45: 525–567.
- Parker, G.A. 1982. Why are there so many tiny sperm? Sperm competition and the maintenance of two sexes. *J. Theoret. Biol.* 96: 281–294.
- Parker, G. A. 1990. Sperm competition games: sneaks and extra-pair copulations. *Proc. Roy. Soc. Lond. B* 242: 127–133.
- Reavis, R.H. & M.S. Grober. 1999. An integrative approach to sex change: social, behavioural and neurochemical changes in *Lythrypnus dalli* (Pisces). *Acta Ethol.* 2: 51–60.
- Ruchon, F., T. Laugier & J.P. Quignard. 1995. Alternative male reproductive strategies in the peacock blenny. *J. Fish Biol.* 47: 826–840.
- Ryan, M. J., D. K. Hews & W. E. Wagner Jr. 1990. Sexual selection on alleles that determine body size in the swordtail *Xiphophorus nigrensis*. *Behav. Ecol. Sociobiol.* 26: 231–237.
- Ryan, M.J., C.M. Pease & M.R. Morris. 1992. A genetic polymorphism in the swordtail *Xiphophorus nigrensis*: testing the prediction of equal fitness. *Am. Nat.* 139: 21–32.
- Ryan, M.J. & G.G. Rosenthal. 2001. Variation and selection in swordtails. pp. 133–148. *In: L.A. Dugatkin (ed.), Fish, Amphibian, & Reptile Model Systems,* Princeton University Press, New Jersey.
- Sadovy, Y. & D.Y. Shapiro. 1987. Criteria for the diagnosis of hermaphroditism in fishes. *Copeia* 1987: 136–156.
- Scaggiante, M., C. Mazzoldi, C.W. Petersen & M. Rasotto. 1999. Sperm competition and mode of fertilization in the grass goby *Zosterisessor ophiocephalus* (Teleostei: Gobiidae). *J. Exp. Zool.* 283: 81–90.
- Schreibman, M.P. & K.D. Kallman. 1977. The genetic control of the pituitary-gonadal axis in the platyfish, *Xiphophorus maculatus*. *J. Exp. Zool.* 200: 277–294.
- Shapiro, D.Y. 1981. Size, maturation and the social control of sex reversal in the coral reef fish *Anthias squamipinnis*. *J. Zool. Lond.* 193: 105–128.
- Shapiro, D. Y. 1984. Sex reversal and sociodemographic processes in coral reef fishes. pp. 103–118. *In: G.W. Potts & R.J. Wootton (eds.), Fish Reproduction: Strategies and Tactics,* Academic Press, London.
- Soljan, T. 1930. Nestbau eines adriatischen Lippfisches. *Zeitschrift für Morphologie Ökologie der Tiere* 17: 145–153.
- Steele, M.A. 1996. Effects of predators on reef fishes: separating cage artifacts from effects of predation. *J. Exp. Mar. Biol. Ecol.* 198: 249–267.
- Steele, M.A. 1997. The relative importance of processes affecting recruitment of two temperate reef fishes. *Ecology* 78: 129–145.
- St. Mary, C.M. 1993. Novel sexual patterns in two simultaneously hermaphroditic gobies, *Lythrypnus dalli* and *Lythrypnus zebra*. *Copeia* 4: 1062–1072.
- St. Mary, C.M. 1994. Sex allocation in a simultaneous hermaphrodite, the blue-banded goby (*Lythrypnus dalli*): the

- effects of body size and behavior gender and the consequences for reproduction. *Behav. Ecol.* 5: 304–313.
- St. Mary, C.M. 1997. Sequential patterns of sex allocation in simultaneous hermaphrodites: do we need models that specifically incorporate this complexity? *Am. Nat.* 150: 73–97.
- Stockley, P., M.J. Gage, G.A. Parker & A.P. Møller. 1997. Sperm competition in fishes: the evolution of testis size and ejaculate characteristics. *Am. Nat.* 149: 933–954.
- Taborsky, M. 1994. Sneakers, satellites, and helpers: parasitic and cooperative behavior in fish reproduction. *Advan. Study Behav.* 23: 1–100.
- Taborsky, M. 2001. The evolution of bourgeois, parasitic, and cooperative reproductive behaviors in fishes. *J. Heredity* 92: 100–110.
- Taborsky, M., B. Hudde, & P. Wirtz. 1987. Reproductive behaviour and ecology of *Symphodus crenilabrus ocellatus*, a European wrasse with four types of male behaviour. *Behaviour* 102: 82–118.
- van den Berghe, E., F. Wernerus & R. R. Warner. 1989. Female choice and the mating cost of peripheral males. *Ani. Behav.* 38: 875–884.
- Warner, R.R. 1975. The adaptive significance of sequential hermaphroditism in animals. *Am. Nat.* 109: 61–82.
- Warner, R.R. 1984. Mating behavior and hermaphroditism in coral reef fishes. *Am. Scientist* 72: 128–136.
- Warner, R.R. 1988. Sex change and the size-advantage model. *Trends Ecol. Evol.* 3: 133–136.
- Warner, R.R. & D.R. Robertson. 1978. Sexual patterns in the labroid fishes of the western Caribbean, I: the wrasses (Labridae). *Smithsonian Contribut. Zool.* 255: 1–27.
- Warner, R.R. & S.E. Swearer. 1991. Social control of sex change in the bluehead wrasse, *Thalassoma bifasciatum* (Pisces: Labridae). *Biol. Bull.* 181: 199–204.
- Warner, R.R., D.Y. Shapiro, A. Marcanato & C.W. Petersen. 1995. Sexual conflict: males with highest mating success convey the lowest fertilization benefits to females. *Proc. Roy Soc. Lond. B* 262: 135–139.
- Wiley, J.W. 1976. Life histories and systematics of the western North American gobies *Lythrypnus dalli* (Gilbert) and *Lythrypnus zebra* (Gilbert). *San Diego Soc. Nat. Hist.: Trans. Mem.* 18: 169–184.
- Wilson, J. & C.W. Osenberg. 2002. Experimental and observational patterns of density-dependent settlement and survival in the marine fish *Gobiosoma*. *Oecologia* 130: 205–215.
- Zar, J. H. 1996. *Biostatistical Analysis*, 3rd edn. . Prentice Hall, Inc., New Jersey. 226 pp.