Copeia, 1997(1), pp. 22–31

Bisexual Juvenile Gonad and Gonochorism in the Fairy Basslet, *Gramma loreto*

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According to sex allocation theory, protogynous species ought to form social systems in which a relatively small number of males mate with a relatively large number of females. Because the fairy basslet, *Gramma loreto*, mates in this predicted manner, we examined its mode of sexuality, looking for evidence of protogyny, in 167 specimens ranging in size from small juveniles through adults. All gonads initially developed previtellogenic oocytes and a precursor of the sperm ducts. In future males, the oocytes degenerated; the ducts proliferated, anastomosed, and became available for sperm transport; and spermatogenic tissue appeared. In future females, an ovarian lumen was formed, the precursive sperm ducts degenerated, and oocytes continued to grow. No histological features indicative of protogyny were found. In an experimental attempt to induce sex reversal, males were removed from social groups transferred from natural populations into the laboratory. Five months after male removal, no female contained any histological evidence of sex change. Thus, our data strongly support the hypothesis that *G. loreto* is a gonochore. The species does not conform to the predicted relationship between protogyny and a female-biased mating system, perhaps because small, nonreproductive males evolved an alternative means of avoiding low reproductive success by growing faster than similarly sized females.

THEORY of sex allocation predicts a relationship between environmental induction of adult, female-to-male sex change (i.e., protogyny) and specific mating systems (Charnov, 1982; Shapiro, 1988; Warner, 1988a). Females ought to change sex when social conditions permit them to reproduce more successfully as a male than if they remained female. In general, such conditions are those in which a newly produced male can mate with sufficient females for a sufficiently long time for the male's reproductive success to outweigh the costs and risks of changing sex. When these conditions occur repeatedly throughout a population, natural selection is thought to favor a causative mechanism that only allows sex change to occur under these specific conditions (Shapiro, 1994). In practice, although the costs of sex change have never been fully measured, many (though not all) protogynous species mate in fixed social groups or other, more open systems in which the mating sex ratio is female-biased (Thresher, 1984; Shapiro, 1988; Warner, 1988a). These cases conform to predictions and create the general expectation that species with female-biased mating sex ratios in which males attain high mating success ought to be protogynous.

The fairy basslet, *Gramma loreto*, is a small planktivorous Caribbean fish (Böhlke and Chaplin, 1968; Randall, 1983) that lives in aggregations of up to a dozen or more individuals in close association with large coral heads, steep ledges, overhangs, and caves (Böhlke and Chaplin, 1968; Luckhurst and Luckhurst, 1978; Starck and Colin, 1978). Within these aggregations, individuals spawn in quasi-closed units in which 1–2 males each spawn with 3–9 females, with most social units consisting of 1–2 males and 4–6 females (Asoh, 1992). The overall, adult sex ratio in one Puerto Rican population was 3.17 females per male (Amador, 1982). Because both the adult sex ratio and the mating advantage of successful males fall within the range of many protogynous species and are consonant with sex allocation theory, we might expect *G. loreto* to be protogynous.

The gonads of *G. loreto* have been examined histologically twice. Among 28 specimens ranging in size from small juveniles through adults, the testes of two individuals contained degenerating oocytes, and one ovary contained testicular tissue along the periphery (Corsten-Hulsmans and Corsten, 1974). Based on this histological result and the observation that males were larger on average than females, Corsten-Hulsmans and Corsten concluded that this species is a protogynous hermaphrodite. In a second study of 58 specimens, testes contained varying densities of previtellogenic oocytes but no defined testicular and ovarian areas, no membrane-lined central cavity, and no degenerating ovarian tissue, other than early-stage oocytes and yellow-brown bodies (Amador, 1982).

The conclusion of Corsten-Hulsmans and

Corsten (1974) that *G. loreto* is protogynous has been accepted widely (Thresher, 1980, 1984; Wirtz and Nahke, 1993). However, the evidence on which their conclusion was based, namely, specific histological features and size relationships between the sexes, does not support protogyny strongly, either because the evidence itself was not convincingly presented or because it can be explained readily in alternative ways (Sadovy and Shapiro, 1987). For example, males could be larger than females simply by virtue of having a higher growth rate. Indeed, this is a common size relationship in nonhermaphroditic species (Sadovy and Shapiro, 1987). Degenerating ovarian tissue within a testis would constitute good evidence, but the photomicrograph illustrating this putative finding (Corsten-Hulsmans and Corsten, 1974) was out of focus and cannot be evaluated. Testicular tissue within an ovary could have various interpretations, e.g. might constitute a nonfunctional remnant of a bisexual juvenile gonad. Finally, previtellogenic oocytes in testes (Amador, 1982) are common in males of gonochoristic species and are thus not necessarily indicative of sex change. In brief, the sexuality of *G. loreto* remains in doubt.

The objective of this study was to determine the mode of sexuality of *G. loreto.* Because gonadal features originating during early gonadal differentiation may be misinterpreted as indications of adult sex change (Sadovy and Shapiro, 1987), we examined histologically the gonads of individuals in a wide range of body sizes, from tiny juveniles through adults, and described population characteristics that might indicate protogyny. Finally, since protogyny, in some cases, has only been discovered through observation of individuals undergoing sexual transition under experimental conditions (Sadovy and Shapiro, 1987), we attempted to induce sex change by manipulating the social system in the laboratory (Shapiro, 1990).

Materials and Methods

Gonadal differentiation and maturation.—We collected 167 specimens of *G. loreto* of various sizes from September to October 1988, from February to April 1989, and in May 1990. Collections were made at various times of the day from 0900 to 1720 h by divers using the fish anaesthetic quinaldine (1:10 quinaldine:ethanol) (Muench, 1958) and hand nets. Collection sites were forereef areas, in depths of 6–17 m, on Turrumote and Media Luna reefs located south of Magueyes Island, near La Parguera on the southwestern coast of Puerto Rico. After each collection, we transported fish to the laboratory alive, measured them to the nearest 0.1 mm standard length (SL), i.e., the distance from the most anterior part of the head to the end of the vertebral column (Hubbs and Lagler, 1949), and killed them with an overdose of 2-phenoxyethanol. We fixed each whole fish in Dietrich's fixative after slitting the abdomen to facilitate penetration of the fixative. Depending on body and gonad size, the gonad or the part of body containing the gonads was later excised. The latter was decalcified with EDTA. Tissue was dehydrated in ascending grades of ethanol, embedded in paraffin, sectioned transversely at 8 mm, and stained in Harris's haematoxylin and eosin. The gonads were either sectioned in their entirety or cut at short regular intervals throughout their length and examined under a light microscope.

Investigation of protogyny.—Of the 167 specimens examined histologically, 100 were sexually differentiated (see Results). These specimens were scrutinized for the following characteristics of protogyny: a membrane-lined central cavity, sperm sinuses within the gonadal wall, atresia of unovulated yolky oocytes, and co-occurrence of degenerating ovarian tissue and proliferating testicular tissue (Sadovy and Shapiro, 1987). In addition, each testis was scored for the presence of oocytes and each specimen was scored for the presence of yellow-brown bodies.

We attempted to induce sex reversal experimentally using 12 laboratory groups, each containing one male and five females, a common adult sex ratio in natural mating units (Asoh, 1992). Divers collected fish at the Pinnacles, a submerged reef south of La Parguera, using quinaldine and hand nets. Because no sexual dichromatism or sexual dimorphism other than size is recognized in this species (Amador, 1982; Thresher, 1984), fish were sexed underwater by the expression of ripe gonadal products. At the time of collection, each fish was placed in a separate, clear plastic bag. After most of the water from the bag was emptied, the fish's abdomen was gently squeezed to express gonadal products. The presence or absence of gametes, and their sexual identity were recorded. Milt and eggs were clearly distinguishable underwater with the naked eye. We established the reliability of this method by comparing the extruded gamete type with the histologically determined gonadal sex of 61 individuals. All fish that emitted milt were confirmed as males (n = 8), and those emitting eggs were confirmed as females (n = 30). Some juveniles (n = 7), mature males (n = 14), and females (n = 2) emit-

ted no gonadal products when squeezed. Consequently, only individuals emitting milt or eggs were sexed and used in the experiments.

After each collection, the fish were brought back to the laboratory alive, measured to the nearest 0.1 mm SL, and placed immediately in aquaria. Individuals collected from a single aggregation were placed together within an aquarium, forming a single laboratory social group, because mixing of fish from different aggregations sometimes caused high mortality. Thus, laboratory social groups consisted of one male (of 1–2 males per natural social unit) and most of the females residing within the natural aggregation from which they were removed.

The laboratory groups were numbered in order of collection and were assigned to one of two treatments using a random numbers table, either "control" or "male removal." Following a two-week acclimatization period in the aquarium, the male was netted and removed from each experimental group and was netted and immediately released in each control group. Five months later, all individuals were removed from all groups and were prepared for histological analysis. The entire gonad was sectioned at short regular intervals. The gonads of individuals initially sexed as females were examined for any indication of sex reversal, such as the presence of spermatogenic crypts, degeneration of mature ovarian tissue, and the development of sperm sinuses. The duration of this experiment exceeds the range of time (i.e., 7–103 days) needed for the completion of sex change in protogynous species whose experimental group composition and treatment were similar to those employed in this study (Sadovy and Shapiro, 1987; Asoh, 1992). Thus, the experiment could reasonably be expected to produce evidence of sex change if the species is protogynous and sex change is behaviorally controlled.

Each laboratory group was maintained in a 209-liter aquarium equipped with subgravel airlift filters and a self-contained water circulation system. Dead corals and clam shells provided shelter and nest sites. Groups were visually isolated from one another with black plastic sheets. The fish were fed frozen and live brine shrimp daily. Light through laboratory windows provided a natural photoperiod. The experiments were performed from 20 March to 26 October 1990.

Population characteristics.—The 100 sexually differentiated males and females were used in a search for population features possibly indicative of protogyny, namely, size differences between males and females, absence of males from small size classes, and sex ratios that change significantly from strongly female-biased to strongly male-biased as body size increases (Sadovy and Shapiro, 1987). A Mann-Whitney U-test (one-tailed) at a significance level of 0.05 was used to test whether male sizes (SL) exceeded female sizes. A one-tailed test was used because it was expected that males would be larger than females (Corsten-Hulsmans and Corsten, 1974; Amador, 1982). Sex ratios were calculated for each size class, and chi-square tests of equal frequencies with Yate's correction (two-tailed) at a significance level of 0.05 were performed to determine whether the observed sex ratios differed significantly from 1:1.

In addition, all individuals along a 65-m section of the base of Media Luna Reef (n = 60) were captured, sexed underwater, as described above, and measured in March 1990. The data on male-female differences in body size and on sex ratios in various body size categories were then analyzed as described above.

Results

Gonadal differentiation.—The gonad differentiated progressively as the size of the individual increased. Initially the gonads developed gonia, first-stage oocytes, and a few somatic cells concomitantly with the development of ducts in medial parts of the caudal region of both lobes. This pattern was seen in 59 of 66 specimens in the size range 10–30 mm SL (Fig. 1A–D); the remaining seven specimens contained early stage oocytes but lacked the medial duct. The medial ducts apposed the urinary bladder in nine of 41 individuals 10–25 mm SL in size (Fig. 1C) but in none of the gonads of any larger fish. Morphologically, cells in the wall of the urinary bladder were identical to ductal cells and differed from cells of the gonadal wall. The positions of the medial ducts were the same as sperm ducts in differentiated males, indicating that these early ducts were the precursors to adult sperm ducts. Thus, individuals of *G. loreto* are initially bisexual in nature (Fig. 2).

As the size of the fish increased, some gonads developed increasing degrees of empty spaces around oocytes (Fig. 1B), suggesting loss of eggs. These gonads became infiltrated with somatic cells and developed cysts of darkly staining cells, resembling spermatocytes (Fig. 1E), and enlarged medial ducts posteriorly (i.e., were becoming testes). The first fully differentiated testis was found in the 30–35 mm SL size class.

Other gonads in individuals measuring 25–35 mm SL remained packed with oocytes and gon-

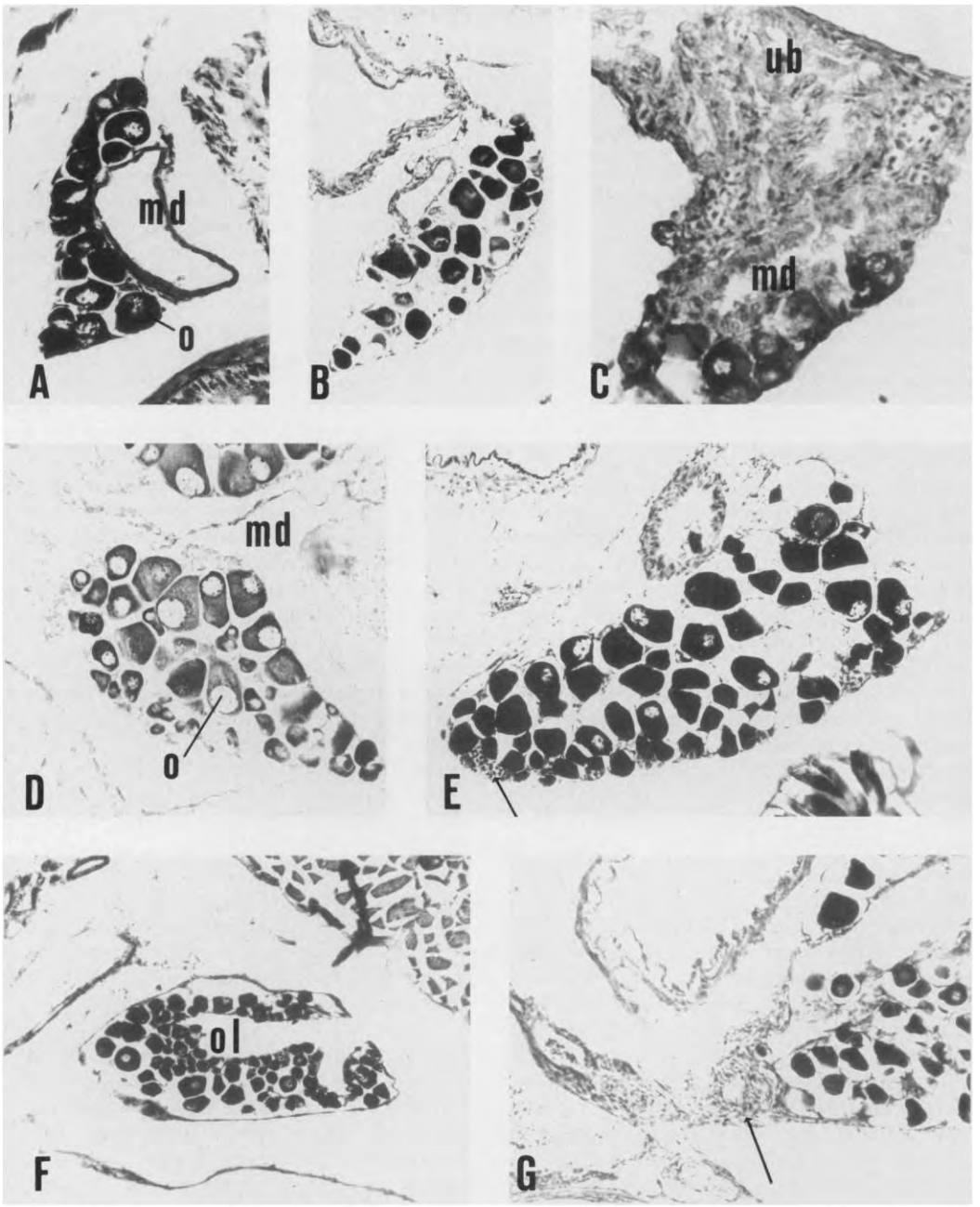

Fig. 1. Cross-sections of juvenile gonads of *Gramma loreto*. (A) a gonad from a 15.4 mm SL fish has previtellogenic oocytes (o) and medial duct (md) (×400); (B) a gonad from a 24.1 mm SL fish has empty spaces around oocytes (×400); (C) a gonad from a 16.4 mm SL fish has the medial duct (md) juxtaposing the urinary bladder (ub); (D) a gonad from a 26.2 mm fish has growing previtellogenic oocytes (o) and a dormant medial duct (md) (×200); (E) a gonad from a 26.0 mm SL fish has empty spaces around oocytes and cysts of darkly staining cells (shown by arrows) (×200); (F) a gonad from a 28.7 mm fish is forming an ovarian lumen (ol) (×100); (G) a posterior section of the same gonad as (F) showing a circular aggregation of poorly defined cells (shown by an arrow) at a medial position of the gonad (×200).

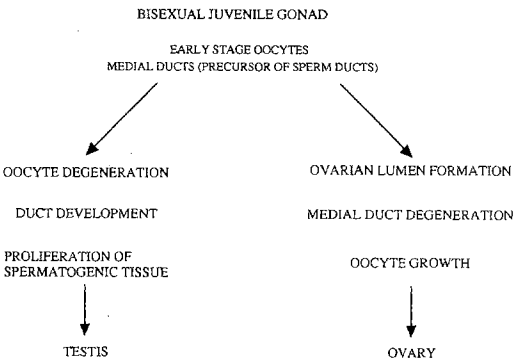

Fig. 2. Summary of process of gonadal differentiation in *Gramma loreto*.

ia, lacked empty spaces around oocytes, developed an ovarian lumen through outward folding of the gonadal lobes (Fig. 1F), and contained either comparatively undeveloped medial ducts or circular aggregations of poorly defined cells at locations occupied by ducts in other specimens (Fig. 1G). All gonads with a fully formed ovarian lumen completely lacked medial ducts.

In summary, gonads of future males differentiate from a bisexual state into testes through degeneration of oocytes, further development of the early, medial ducts, and appearance and proliferation of spermatogenic tissue (Fig. 2). Gonads of future females differentiate into ovaries through formation of an ovarian lumen, degeneration of the medial ducts, and continuous growth of oocytes.

Because the gonad of *G. loreto* initially developed both first-stage oocytes and medial ducts, a gonad was defined to have differentiated as an ovary, when it had completed the formation of an ovarian lumen, and as a testis upon the appearance of clearly recognizable spermatogenic tissue. No medial ducts were found in any ovary defined as fully differentiated by these critera. Of the 167 gonads examined histologically, 100 met the criteria for differentiation (n = 64 ovaries, n = 36 testes). Because gonads prior to differentiation contained cellular and structural elements of both sexes, they are referred to as bisexual gonads. An ovary was defined to be mature if it contained oocytes beyond the first growth stage, or showed evidence of prior spawning, such as degenerating yolky oocytes and traces of postovulatory follicles. A testis was defined to be mature if it contained mature spermatozoa or had a fully developed testis structure with occasional occurrence of residual spermatozoa. The smallest sexually differentiated male and female were 32.8 mm SL and 25.3 mm SL, respectively. Twelve of 16 fish measuring 30–35 mm SL and all fish exceeding 35 mm SL had differentiated gonads. The smallest mature male and female were 32.8 mm SL and 30.0 mm SL, respectively.

Structure of differentiated gonads.—Each lobe of the bilobed testis consisted of a medial region, containing an anastomosing network of sperm ducts lined with cuboidal cells that gradually merged into a lateral region containing germinal tissue (Fig. 3A–B). In central and posterior parts of the gonad, anastomosing ducts merged into one or several larger ducts. The large ducts from both lobes then united and, further posteriorly, coalesced to form a single common sperm duct, which descended toward the urogenital papilla behind the anus. Within the seminiferous tubules in the lateral germinal portion, germ cells occurred either singly or in cysts distributed around the central tubular lumen. All germ cells within a single cyst were approximately at the same stage of development, but development varied among cysts within the same tubule.

Both lobes of the adult ovary had a centrally located, membrane-lined cavity, the ovarian lumen (Fig. 3C–D). In cross-section, a series of folds, the ovarian lamellae, projected into each ovarian lumen. The lumen from the two lobes united posteriorly forming a short common oviduct that descended to the urogenital opening. In reproductively active ovaries, oocytes of all stages of development occurred simultaneously. Degenerating yolky oocytes of various stages were found in many ovaries, whereas 94% of differentiated ovaries contained yellow-brown bodies within ovarian lamellae, along the ovarian wall, and beside blood vessels.

Features possibly indicative of protogyny.—No differentiated testis (n = 36) had a membrane-lined cavity (i.e., a structure that could be interpreted as the remnant of an ovarian lumen). Twenty-nine of 36 testes contained a few yellow-brown bodies adjacent to blood vessels. In general, these yellow-brown bodies were smaller than those found in ovaries. All but four of the testes (n = 32 of 36) contained first-stage oocytes. The exceptional four individuals belonged to the largest two size classes and were reproductively active. When first-stage oocytes were present within a testis, they were generally located at the lateral periphery of the gonad. Oocytes beyond the first stage of development were not found in any testis. No transitional individual was found among the 100 differentiated individuals. No gonad contained degenerating ovarian tis-

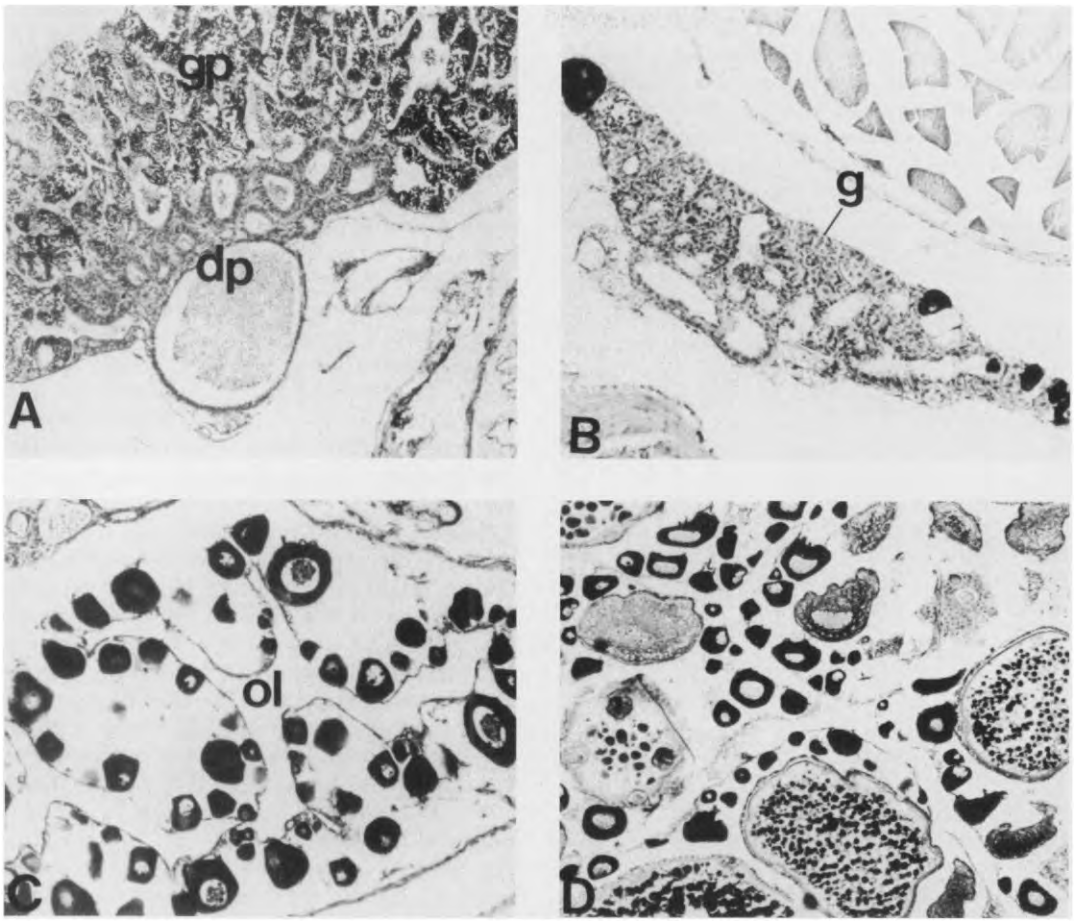

Fig. 3. Gonadal organization of adult *Gramma loreto*. (A) reproductively active testis taken from a male 54.8 mm SL consists of ductal (dp) and germinal portion (gp) (×100); (B) reproductively inactive testis taken from a male 42.6 mm SL with the germinal portion predominated by gonia (g) (×200); (C) a lobe of reproductively inactive ovary from a female 38.3 mm SL with the central ovarian lumen (ol) (×200); (D) reproductively active ovary from a female 37.5 mm SL (×100).

sue, other than first-stage oocytes and yellow-brown bodies, and proliferating testicular tissue concomitantly. Structures resembling sperm sinuses, which develop by the splitting of muscle layers of the ovarian wall and are characteristic sperm transport systems in many protogynous species (Reinboth, 1962; Choat and Robertson, 1975; Shapiro and Rasotto, 1993), were not found at any point along the testis. No ovary (n = 64) showed any sign of development of sperm sinuses in the gonadal wall.

Experimental attempt to induce protogyny.—At the beginning of the experiment, all females both in experimental groups and in control groups emitted ripe oocytes. By the end of the experiment, 154–155 days after the male had been removed from experimental groups (n = 6) or captured and released in control groups (n = 6), no female (n = 20) in the six experimental groups contained any histological sign of having changed to a male or any spermatogenic tissue in the gonad. The gonads of females in experimental groups did not differ histologically from those of females in control groups, and, by the end of the experiment (which terminated during the nonreproductive season), all females had markedly degenerated oogenic tissue, lending the appearance of an inactive ovary.

Population characteristics.—In the sample of 100 sexually differentiated specimens, male size (46.2 ± 6.4 mm SL, mean ± 1 SD) was significantly greater (Mann-Whitney U-test, $P < 0.001$, one-tailed) than female size (38.5 ± 4.8 mm SL). Sex ratios (Table 1A) were significantly female-biased in the smaller size classes and male-biased in the larger size classes. Sex ratio ap-

TABLE 1. FREQUENCY DISTRIBUTIONS AND SEX RATIOS IN VARIOUS SIZE CLASSES OF *Gramma loreto* BASED ON (A) 100 HISTOLOGICALLY DIFFERENTIATED INDIVIDUALS AND (B) 60 INDIVIDUALS SEXED BY THE EXPRESSION OF GONADAL PRODUCTS. Chi-square values and *P*-values were obtained by comparing the sex ratio in each size class to a 1:1 sex ratio.

Size class (mm)	Females N	Males N	Sex ratio M:F	Chi-Square value	*P*-value (2-tailed)
(A)					
25–30	3	0	0:1.00	1.33	$P > 0.05$
30–35	11	1	1:11.0	6.75	$P < 0.01$
35–40	24	7	1:3.43	8.26	$P < 0.01$
40–45	23	7	1:3.29	7.50	$P < 0.01$
45–50	3	9	1:0.33	2.08	$P > 0.10$
50–55	0	11	1:0.00	9.09	$P < 0.01$
55–60	0	1	1:0.00	0.00	$P > 0.90$
Totals	64	36	1:1.78	7.29	$P < 0.01$
(B)					
25–30	1	0	0:1.00	0.00	$P > 0.90$
30–35	4	0	0:1.00	2.25	$P > 0.10$
35–40	12	1	1:12.0	7.69	$P < 0.01$
40–45	16	8	1:1.63	2.04	$P > 0.10$
45–50	5	4	1:1.25	0.00	$P > 0.90$
50–55	1	4	1:0.25	0.80	$P > 0.10$
55–60	0	3	1:0.00	1.33	$P > 0.10$
60–65	0	1	1:0.00	0.00	$P > 0.90$
Totals	39	21	1:1.86	4.82	$P < 0.05$

proached 1:1 in the 40–50 mm SL size ranges. The overall male-to-female sex ratio of the sample was 1:1.78, and was significantly different from a one-to-one sex ratio ($\chi^2 = 7.29$, $P < 0.01$). Similarly, in the sample of 60 individuals collected on Media Luna Reef, male SL (mean ± SD = 48 ± 6 mm) significantly exceeded female SL (40 ± 5 mm; Mann-Whitney U-test, $P < 0.001$, one-tailed). The sex ratios (Table 1B) were female-biased in the smaller size classes, approached 1:1 in the size class 45–50 mm SL, and were male-biased in the larger size classes. However, the sex ratio did not differ significantly from 1:1 in most size classes. The overall male-to-female sex ratio of this sample was 1:1.86, and was significantly different from 1:1 ($\chi^2 = 4.82$, $P < 0.05$).

DISCUSSION

Mode of sexuality.—All available data indicate that this species is a gonochore (i.e., a species in which all individuals function exclusively as one sex throughout their lives; Atz, 1964). First, we found none of the histological features strongly indicative of protogyny (i.e., a membrane-lined central cavity in testis, degenerating yolky oocytes in testis, sperm sinuses within the gonadal wall, or transitional individuals containing proliferating testicular tissue and degenerating ovarian tissue) among 100 differentiated gonads (36 testes, 64 ovaries). The presence of previtellogenic oocytes in many testes is not a good indicator of protogyny, because such oocytes appear transiently under a variety of circumstances in testes of definitively gonochoristic fishes (Sadovy and Shapiro, 1987). Similarly, yellow-brown bodies in testes, once thought to be exclusive remnants of atretic yolky oocytes, are now known to result from a range of metabolic conditions and, consequently, are not clear indicators of protogyny (Sadovy and Shapiro, 1987). Thus, there is no histological evidence favoring a diagnosis of protogyny in *G. loreto.*

Second, experimental removal of a male from natural social units transferred intact to the laboratory failed to induce any female to change sex. Because the only known cause of adult sex change (i.e., cause for which there is positive evidence) is disruption of behavioral interactions between males and females, triggered by male removal in protogynous fishes, and because this cause operates in similar fashion in more than a dozen species (Shapiro, 1994), the failure to induce sex change in this experiment suggests that this species is not protogynous. The weight of this result is not great, however, since there could be many reasons for females in a laboratory setting to fail to change sex (e.g., inadequate diet, stress, or overly confined space for the fish), even if the species were protogynous and sex change were inducible by male removal.

Third, degeneration of the precursor of sperm ducts in differentiating ovaries of juveniles strongly suggests this species is a gonochore. We interpret our data to indicate that all juveniles develop medial ducts, which later become the sperm transport system in males. If *G. loreto* were a protogynous hermaphrodite, we would not expect females to lose the medial ducts as juveniles because they would subsequently have to reform ducts anew during adult sex change. In females of some protogynous gobies, a precursor to a testicular secretory organ develops during early gonadal differentiation (Cole, 1988, 1990; Cole and Shapiro, 1990). This precursive tissue mass remains quiescent during the female phase but grows and becomes functional during sex change. In similar fashion, if *G. loreto* were a sex-changing species, it should retain the medial ducts which develop during the juvenile phase.

Finally, although the female-biased sex ratios

among small individuals and the male-biased sex ratios among large individuals mimic sex ratios commonly observed among protogynous fishes, these results could be explained by differential male-female mortality and growth or by other factors (Sadovy and Shapiro, 1987). Indeed, small, nonreproductive males grow almost an order of magnitude faster than do females in this species. For example, during a 90-day period of the reproductive season, mean growth ± 1 SD was 0 ± 0 mm (n = 4), 4.26 ± 2.57 mm (n = 4), and 0.47 ± 0.64 mm (n = 23) for large, reproductive males, small, nonreproductive males, and females, respectively (Asoh, 1992). Furthermore, the larger size at which gonads differentiate definitively into testes than into ovaries may explain the absence of males from the smallest size classes. The difference in population sex ratios between our results and those of Amador (1982) may simply be the result of samples taken from different populations and different years. Overall, changing sex ratios with increases in body size are not sufficient to outweigh the absence of histological features of sex change, the inability to induce sex change experimentally, and the loss of medial ducts in juvenile ovaries as evidence against protogyny. We conclude, therefore, that *G. loreto* is a gonochore.

Gramma loreto was expected to be protogynous on the basis of its mating system. Individual males mate with 2–7 females, and male mating success increases with male body size, with small males not reproducing at all (Asoh, 1992). Therefore, the question arises why this species is not protogynous. Seemingly, individuals could reproduce best by being female when small and changing to males when body size or circumstance permitted increased mating success by new males (Warner, 1988b; Shapiro, 1989).

There are several possible explanations for why this species remains a gonochore. First, differences between the two sexes in size- or condition-specific reproductive success may not be large enough to overcome the cost of sex change or higher possible mortality rates for males than females (Charnov, 1986). Second, there are other theoretical options for small males to avoid low rates of mating success—to sneak into pair spawns, to overcome a large nest-guarding male by group spawning, or to grow fast to attain a size favored by females quickly. Nests of *G. loreto* are constructed inside small holes and crevices whose single openings are too small to permit sneaking or group spawning (Asoh and Yoshikawa, 1996). We hypothesize that, instead of becoming protogynous or adopting either of these spawning options, small, nonreproductive males substantially outgrow females (Asoh, 1992).

Gonadal differentiation and maturation.—During early development, the gonads of *G. loreto* had a female feature cytologically (first-stage oocytes) and a male feature anatomically (the precursor of sperm ducts). Some gonochoristic and hermaphroditic teleosts contain germ cells of both sexes in juvenile gonads (i.e., are bisexual; Yamamoto, 1969; Reinboth, 1970; Takahashi, 1977). However, the bisexuality of differentiating gonads of *G. loreto*, in which cytological features are female and anatomical features are male, is thus far unique among teleosts.

The juxtaposition of medial ducts to the urinary bladder and cytological similarity between cells of the medial ducts and cells in the urinary bladder suggests that the sperm ducts arise from the same mesonephric materials that form the bladder. Embryologically, in teleosts, the urinary bladder is formed through dilatation of the mesonephric ducts after the latter have fused at some point between the posterior end of the kidney and the urinary papilla (Hickman and Trump, 1969; Wake, 1979). In all other vertebrates, male and female ducts arise from mesonephric and coelomic materials, respectively, during a clearly defined period early in gonadal development, and later one set of ducts degenerates according to the gonadal sex (Balinsky, 1965; Hopper and Hart, 1985). To date, no vertebrate is known in which the gonad of an already sexually differentiated individual can obtain mesonephric or coelomic materials for new ducts outside of the initial, competent period (Balinsky, 1965; Hopper and Hart, 1985; M. B. Rasotto, pers. comm.). Thus, if the sperm ducts of *G. loreto* are built from mesonephric materials, then the medial ducts must be formed during a limited period early in development. It remains unclear why ovarian ducts are not also formed from mesonephric or coelomic materials at the same time.

Gramma loreto is a demersal spawner, in which males release sperm over eggs fixed to the substrate in a small, covered nest. Because water turbulence is likely to be minimal within such nests, relatively small numbers of sperm are probably adequate to fertilize all or most eggs in the clutch. Consequently, one would not expect selection to favor the development of sperm ducts that provide the male any special ability to control the number of sperm released in individual matings, beyond the simple capacity to release small numbers each spawn. In contrast, pelagic spawners release eggs and sperm directly into a turbulent, moving volume of wa-

ter, where the proportion of eggs fertilized may vary with conditions and the number of sperm released during a mating (Okubo, 1988; Denny and Shibata, 1989; Levitan et al., 1991). Under these conditions, males have been favored that allocate sperm carefully among successive spawns, e.g., that increase sperm release in accordance with female body and clutch size (Shapiro et al., 1994) and with the daily spawning rate (Warner et al., 1995). The sperm ducts of males in one such species contain special internal features and a unique muscle surrounding the sperm duct that together are likely to control sperm release. (M. B. Rasotto and D. Y. Shapiro, unpubl. data). We speculate that demersal spawners will prove to have simpler sperm ducts than will pelagic spawners and that the simple ducts of other demersal spawners will be derived from mesonephric materials early in development, as in *G. loreto*, whereas sperm ducts in pelagic spawners will be formed later in development from other embryonic sources.

Acknowledgments

We are indebted to T. Yoshikawa for field and lab assistance and to K. Cole and M. Rasotto for valuable discussion. We thank also D. Hensley, P. Yoshioka, Y. Sadovy, and G. López for encouragement and various assistance. This paper is based on a master's thesis written by KA at the University of Puerto Rico and partially supported by a graduate assistantship from the University of Puerto Rico.

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