

PROTOGYNY IN THE CARIBBEAN REEF GOBY,
CORYPHOPTERUS PERSONATUS: GONAD ONTOGENY
AND SOCIAL INFLUENCES ON SEX-CHANGE

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ABSTRACT

Coryphopterus personatus, a small Caribbean reef goby, is a protogynous hermaphrodite. Unlike the secondarily derived testes of many other hermaphroditic teleost species, those of *C. personatus* lack a residual ovarian lumen or remnant oocytes, and sperm are produced and transported through a newly formed, permanent system of anastomosing tubules that comprise the body of the gonad. Functional ovaries have no testicular tissue, but have a small, ventral outfolding situated posteriorly on each gonadal lobe. During sex-change, these outfoldings develop into apparently secretory structures associated with the newly formed testis. In the laboratory, development of spermiated testes took as little as nine days to occur. In association with gonadal transformation, the genital papilla of sex-changing fish changed in shape, and the second dorsal spine became greatly increased in length. In natural populations: (1) females outnumber males approximately four to one; (2) male and female size distributions show considerable overlap, but may differ, and; (3) there occur small males which are smaller than the smallest mature females. The testes of these small males appear to develop from an immature ovariform gonad, and are otherwise indistinguishable from those of males derived from mature females. Sex-change in laboratory groups of females was usually inhibited by the presence of a larger male, but not by the presence of either smaller males or larger sex-changing females. Multiple occurrences of sex change were frequent in small unisexual experimental groups, and sex-change proceeded most rapidly in the largest female in any group. In all experimental groups, at least one small individual remained female.

Although sex-change has been reported for numerous fish taxa, principally among marine representatives (Atz, 1964; Reinboth, 1970), hermaphroditism has only recently been described within the largest extant family of marine fishes, the Gobiidae (Lassig, 1977; Robertson and Justines, 1982; Cole, 1983). *Coryphopterus personatus* is one such hermaphrodite. It is a small (maximum 34 mm total length) protogynous gobiid (Robertson and Justines, 1982), and is found throughout the Caribbean, often in association with the edges and slopes of patch reefs from depths of 5-25 m (Böhlke and Robins, 1960). In the vicinity of the Smithsonian Tropical Research Institute's field station in the San Blas Islands, Panama (Lat. 9°34'N, Long. 78°58'W), individuals occur in aggregations of tens to several thousands of fish around topographically disjunct coral outcroppings or large sponges. Individuals hover in the water column 0.5-2 m above the substrate within a meter of coral or sponge structures, and feed on plankton.

In the laboratory, female *C. personatus* deposit eggs on the undersurface of a structure defended by a male, after which the male alone guards the eggs until hatching, usually after five or six days at water temperatures of 27-29°C (Cole, unpublished data), a pattern typical of gobiid fish reproduction (Breder and Rosen, 1966; Thresher, 1984). The sexes can be distinguished by the form of the genital papilla, which in the male is narrow, pointed, and elongate (often surpassing the first anal ray), and in the female, is short, broad, and appears ridged at the margins of the urogenital opening. Many large fish have a markedly elongate second dorsal spine in the first spinous dorsal fin.

Because little detailed information on sex-change in gobiid fishes is currently

available, we wished to examine several features associated with protogynous sex-change in *Coryphopterus personatus*, including (1) the sex-structure of natural populations; (2) gonad structure and the development of the testis from the ovary; (3) external morphological differences between the sexes and their change during sex-change, and; (4) social conditions influencing the occurrence of sex-change in the laboratory.

METHODS AND MATERIALS

Collection Method.—Five separate collections were made between August and November, 1981. Fish used for the experimental induction of sex change were collected using the anaesthetic, quinaldine, and taken in insulated containers to S.T.R.I.'s Naos Marine Laboratory in Panama City. These fish were subsequently sexed according to genital papilla structure, measured (total length, mm), and established in experimental groups within 7 h of collection. Experimental groups were maintained in 10 gallon aquaria supplied with flow-through, semi-filtered sea water, set up outdoors under roofing with natural photoperiod. All fish were fed live brine shrimp nauplii twice a day for the duration of the experiments.

Population sex-structure was assessed for two separate, nearly entire aggregations which we collected. Following collection, these individuals were sexed according to genital papilla structure, and measured (total length, mm). Further, the degree of elongation of the second spine of the first dorsal fin was assessed, based on its proportional length (0–10% longer, 10–30%, 30–50%, 50–100%, or more than 100% longer) compared to the first spine, which is 0.12 into total body length.

One additional collection was made in December 1986, and a number of females (sexed according to genital papilla structure) were immediately removed and shortly thereafter placed into separate, visually isolated 10 g tanks.

Laboratory Induction of Sex-change.—All-female groups of five females each were maintained for 2 (N = 4), 4 (N = 4), 6 (N = 4), 9 (N = 10), 12 (N = 5), 15 (N = 5), or 20 (N = 12) days to chart the progress of testicular development within the gonads, and changes in the external morphology of transforming individuals. In order to determine if sex-change is inhibited by the presence of male conspecifics, experimental groups consisting of either five females and one or more males larger than the largest female (N = 11), or five females and one or more males smaller than the largest female (N = 4) were established. To see if sex-change was restricted to a female-to-male direction, groups of six males with no females (N = 3) were established. All mixed-sex and all-male groups were maintained for 20 days. Lastly, in order to see if female conspecific presence is necessary for sex-change to occur, females from the December 1986 collection were placed either singly (N = 23) or in five-female groups (N = 7) for 7–15 days.

At the end of each experiment, all individuals were examined for papilla structure and the degree of elongation of the dorsal fin spine, then fixed for histological examination. All test females exhibited 30% or less elongation of the second dorsal spine at the outset of the experimental interval.

Histology.—Specimens were fixed in Bouin's solution, embedded in toto in paraffin blocks, and serially sectioned (7–10 μ m) in cross-section from the genital papilla anteroad. Sections were stained with Harris' Haematoxylin-Eosin. All experimental fish and small fish of undetermined sexual identity were serially sectioned through the entire length of the gonad, while all individuals from the two population collections were serially sectioned from the genital papilla through the posterior one-half to two-thirds of the gonad.

RESULTS

External Morphology.—A histological examination of 465 of 494 field-caught fish collected from two aggregations showed that in all cases but one, assignment of sex based upon genital papilla structure agreed with histological findings. One hundred percent of 379 fish with ovaries had a short, broad papilla with ridged margins surrounding the urogenital opening. Among these females, all individuals exhibited less than 100% dorsal spine elongation, with spine elongation tending to increase with fish size (Fig. 1). Eighty-four of 85 fish with testes possessed a narrow, pointed, elongate papilla. However, one individual having a fully spermiated testis with no oogenic elements had a short, broad papilla typical of fish with ovarian gonads. As with females, spine elongation increased with fish size

Frequency of Occurrence of Dorsal Spine Elongation (%DE)

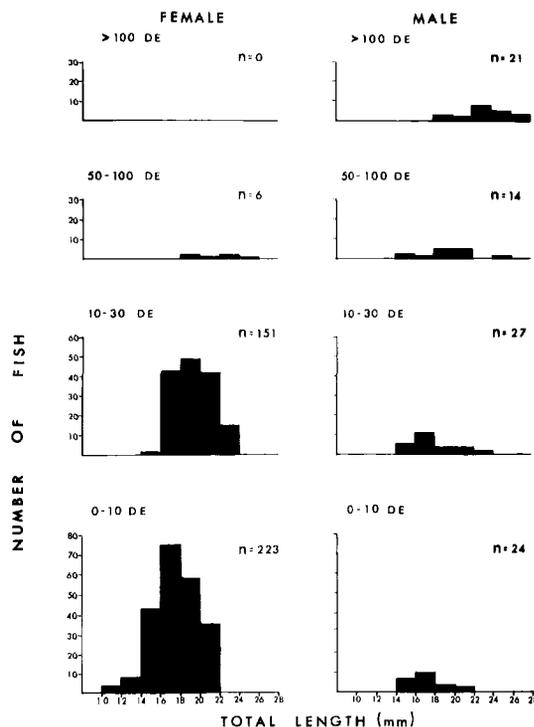


Figure 1. Degree of second dorsal spine elongation (DE) among males and females collected from two field aggregations.

among males, but pronounced elongation (more than 100%) was found only in larger-sized males among field-caught fish (Fig. 1).

Only one fish collected from field aggregations possessed an ovotestis, which was characterized by degrading ovarian tissue, incompletely formed crypts of spermatocytes, and various stages of oocytes scattered throughout the body of the gonad. This intersex had a short, pointed genital papilla intermediate between male and female shape.

Population Sex-structure.—One hundred and eighty-six (Aggregation A) and 308 (Aggregation B) fish were collected from the two aggregations (Fig. 2). In both aggregations, both males and females occurred in all size classes except the two smallest and the largest. In Aggregation B, the sex ratio was 6.0 females: 1 male, and the size-frequency distributions of males and females differed ($D = 0.265$, $P < 0.01$. Kolmogorov-Smirnov test, Siegel, 1956), with males tending to be larger than females (median sizes 21 mm and 19 mm respectively). In Aggregation A, there were 2.8 females: 1 male, but no difference was found in their size-frequency distributions (Kolmogorov-Smirnov $D = 0.113$, $P > 0.30$).

Among individuals collected for laboratory experiments over five collections, and sexed solely on the basis of genital papilla structure prior to experimental use ($N = 882$), a similar absence of males in the smallest, and females in the largest, size categories, was found (Fig. 3). The overall sex ratio was 4.3 females: 1 male,

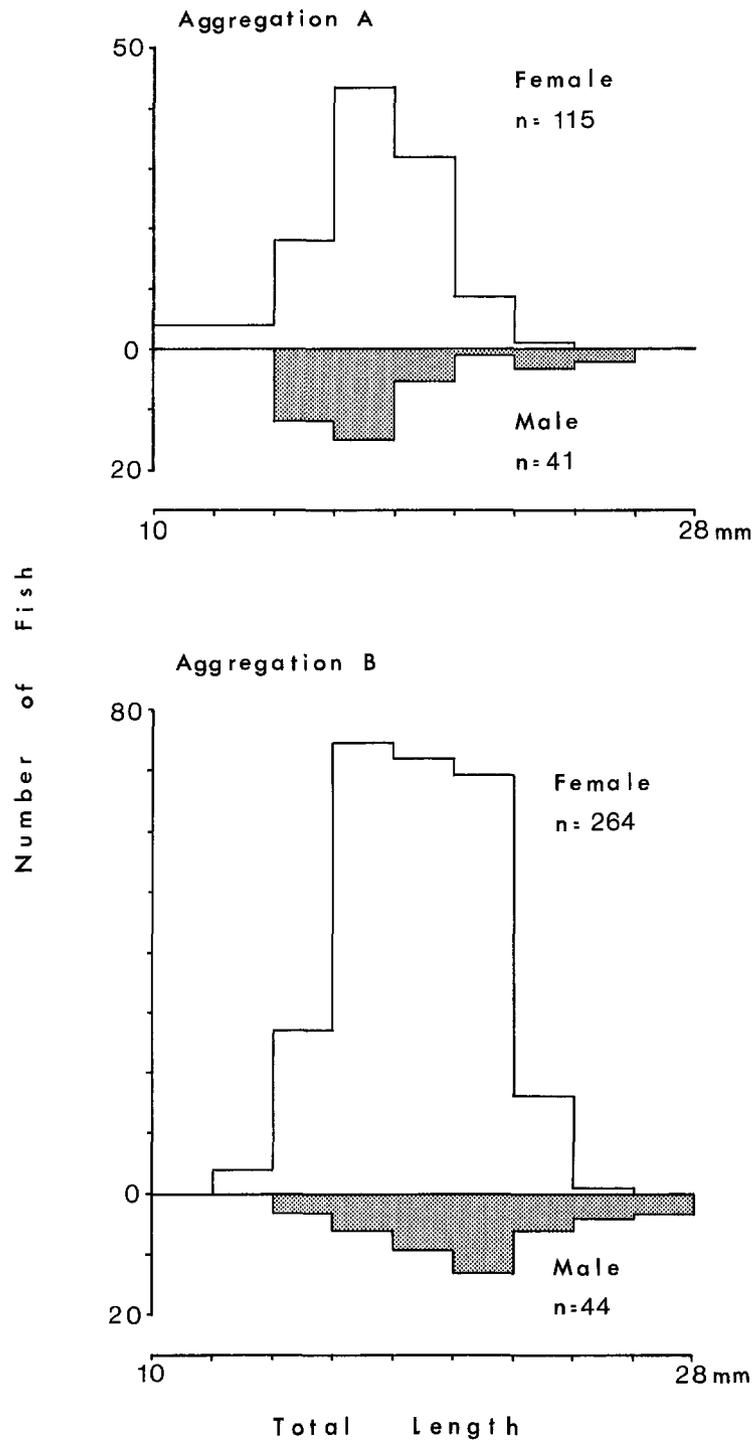


Figure 2. Number of females and males found in size categories (total length) of 2-mm increments, for Aggregations A and B. Sexual identity of individuals was confirmed histologically.

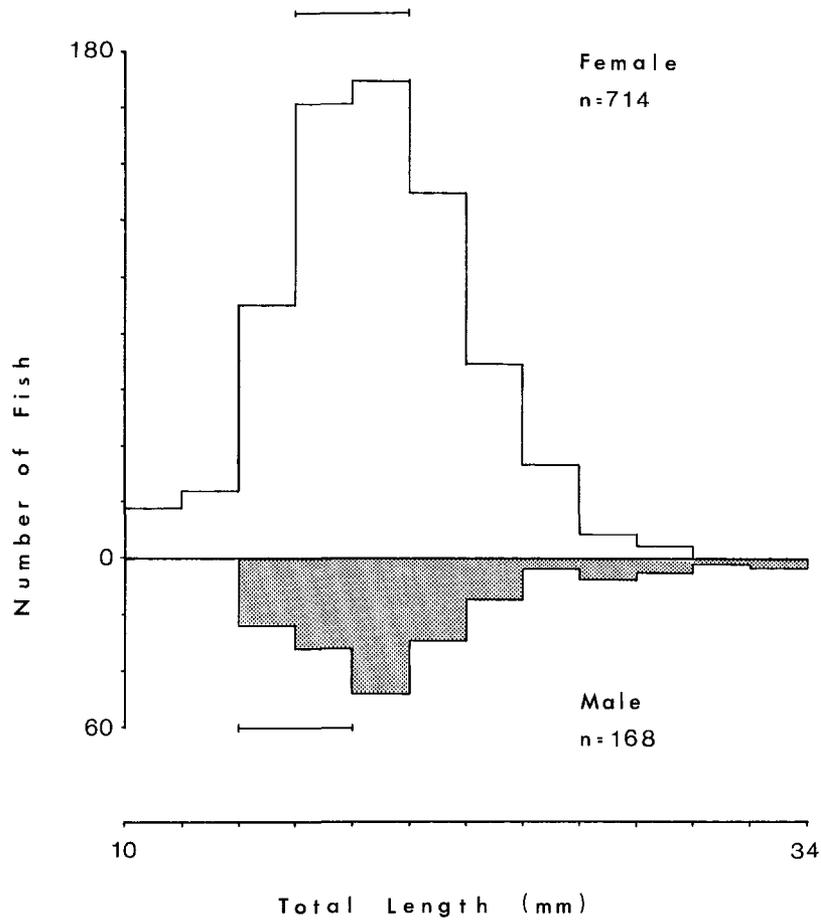


Figure 3. Number of females and males found in size categories (total length) of 2-mm increments, for experimental fish collections. Determination of sexual identities of experimental fish was based on genital papilla structure. Horizontal bars over selected size classes in female and male histograms indicate the size range for which the smallest apparently functional ovaries, and testes, respectively, were found.

and as with Aggregation A, there was no difference between male and female size distributions ($D = 0.075, P > 0.20$).

In a histological examination of 81 additional fish from experimental fish collections that had sexually undifferentiated genital papilla structure (not included in the two population samples referred to above), approximately half of the fish were less than 11 mm TL, while the remainder ranged from 11 to 19 mm TL. Among smaller fish in this group, no males were found in size classes of less than 13 mm TL, although 49 fish comprised this portion of the sample (Table 1). Thus, all individuals less than 13 mm TL had an ovariform gonad. Combining the data from individuals with indistinct papilla (sexual identity histologically confirmed) and those for which sex was determined by papilla form, in experimental collections, none of 91 fish smaller than 13 mm TL were male, while 175 of 872 individuals were male in larger size categories. Based on small individuals we

Table 1. Sexual identity, based on histological examination of individuals from experimental fish collections whose sex could not be established from the morphology of the genital papilla (not included in experimental collections in Fig. 2). Numbers in brackets indicate number of individuals found in the associated size category having either vitellogenic oocytes, or spermatozoa in the gonads

Total length (mm)	Number of fish whose gonads were	
	Ovarian	Testicular
7-9	3	0
9-11	32	0
11-13	14	0
13-15	13	3 (1)
15-17	5	2
17-19	7 (1)	2 (1)
Total	74	7

examined that had an undifferentiated genital papilla, males appear to mature at a smaller size than females (Table 1).

Both males and females exhibited varying degrees of elongation of the second dorsal spine (Fig. 1). All females and the majority of smaller males having less than 100% dorsal spine elongation showed similar size distributions (Kolmogorov-Smirnov $D = 0.116$, $P = 0.30$, females = 380, males = 65). However, males with pronounced elongation (more than 100%) were significantly larger (median total length 23 mm, $N = 21$) than their male counterparts with shorter spine lengths (median total length 17 mm, $N = 65$; $D = 0.716$, $P < 0.001$).

Gonad Structure. — **OVARY.** The immature ovary consists of small clusters of dark-staining oogonia located in two dorsally positioned gonadal lobes. The mature ovary consists of two elongate lobes that unite posteriorly to form a common genital sinus, and which anteriorly are comprised of oocyte-laden lamellae projecting into a central lumen (Fig. 4a). Of 74 females smaller than 20 mm only one had vitellogenic oocytes in her ovary (Table 1). In none of the females examined (73 immature, and 380 mature) was any testicular tissue, or clusters of small dark-staining cells typical of immature testes, evident.

TESTIS. The testes of small males (i.e., <20 mm TL, $N = 7$) were small, consisting entirely of thick layers of germinal cells organized into crypts around small-diameter lumina. Two of these individuals had spermatozoa within the lumina (Table 1). In a mature testis, the two lobes are comprised of seminiferous tubules consisting of crypts of spermatogonia or spermatocytes at various stages of development, surrounding a central, spermatozoa-filled lumen. The seminiferous tubules, numerous and narrow-diametered anteriorly, anastomose to become less numerous and larger-diametered posteriorly. Caudally, at the junction of the two lobes, these channels open into a large common genital sinus (Fig. 2f).

Two accessory gonadal structures (AGS) join the testis at the junction of the testicular lobes and common genital sinus (Fig. 4f), and extend anteriorly for approximately two-thirds of the length of the testis. Each AGS consists of channels lined by a single layer of secretory cells, and often contains large quantities of spermatozoa. At the time of release, spermatozoa are transported along a narrow channel that runs the length of the genital papilla.

Changes in Gonad Structure and External Morphology During Sex-change by Experimental Animals. — **GONAD STRUCTURE.** With the initiation of sex-change, widespread degeneration of ovarian tissue occurred throughout the length of the

gonadal lobes, and boundaries between the lamellae and the lumen rapidly disappeared. Such degeneration usually preceded the appearance of testicular tissue (three of four fish from 2–6-day groups), although small nests of spermatocytes were present in an otherwise unchanged and apparently functional ovary in the fourth individual.

Small clusters of deeply basophilic cells indicative of developing spermatogenic tissue first appeared within the field of degenerating ovarian tissue throughout the body of the gonad (Stage 1 ovotestis, Fig. 2b). With the increased presence of spermatogenic tissue, tubule formation was initiated by the formation of lumina within cell clusters (Stage 2 ovotestis, Fig. 4d). Typically, considerable amounts of ovarian material were still present in Stage 2 ovotestes, and frequently, a slender gap, located on the ventral periphery between the gonadal wall and the germinal tissue, was present (Fig. 4c, d), usually persisting for a portion of the length of the lobe.

In predominantly testicular ovotestes (Stage 3 ovotestis, Fig. 4e), each lobe consisted of a completely formed system of anastomosing tubules. The lumina of these tubules were lined with crypts of spermatogenic cells in various stages of development, but no spermatozoa were present. Thus, Stage 3 ovotestes, although sometimes testicular in appearance, were not yet functional. Occasionally, remnant pre-vitellogenic oocytes were present in the germinal tissue zone of a seminiferous tubule, and a gap, or bounded space, was usually still visible along the periphery of one or both gonadal lobes (Fig. 4e). In 35 of 43 (experimentally induced) sex-changed fish held for 9–20 days, such a gap was present to a varying extent. The remaining eight fish having no gap were all held for the maximum experimental interval, 20 days. Among 90 males obtained from various field collections that were examined for the presence of a gap, only six had a similar peripheral space in the testis. Unfortunately, no other characteristics of the gonad of these six suggested whether or not sex-change had occurred recently. Apart from the occasional presence of a gap, both field-collected ($N = 85$) and experimentally transformed ($N = 36$) males had the same testis morphology.

Accessory gonadal structures (AGS) also developed during the formation and proliferation of spermatogenic tissue within the gonadal lobes. All experimentally held ($N = 92$) and aggregation ($N = 379$) females examined histologically possessed two small outfoldings of tissue, 40–50 μm in length, on the ventral surface of, or just anterior to, the genital sinus (Fig. 4b and 4a, respectively). Examination of early-stage intersexes confirmed that AGSs arose from these presumptive (pAGS) tissue masses. Prior to or during the first appearance of spermatogenic cells in the gonad, changes in the pAGS consisted of an increased size by means of rapid cell proliferation, and the appearance of one or more lumen-like gaps (compare Fig. 4a and 4c). Subsequent tubule development within the enlarging AGS often occurred before tubule formation within the ovotestis. Unlike the developing seminiferous tubules of the gonad, however, the AGS channels consisted of a single layer of secretory cells lining the lumina (Fig. 4d). In Stage 3 ovotestes, each AGS had large lumina containing acellular, eosin-staining stroma, while in new (less than 20 days old), secondary males with spermiated testes, sperm were also present in the lumina of the AGS.

The cross-sectional area of both gonadal lobes and their associated AGS were estimated and compared for several field-caught males maintained under laboratory conditions, and for several newly transformed males. One stained section immediately anterior to the point where the two AGS joined the gonadal lobe was selected, and for each gonadal lobe and associated AGS in this section, the longest and shortest diameters were recorded. The area of an ovoid ($\pi \times r_1 \times$

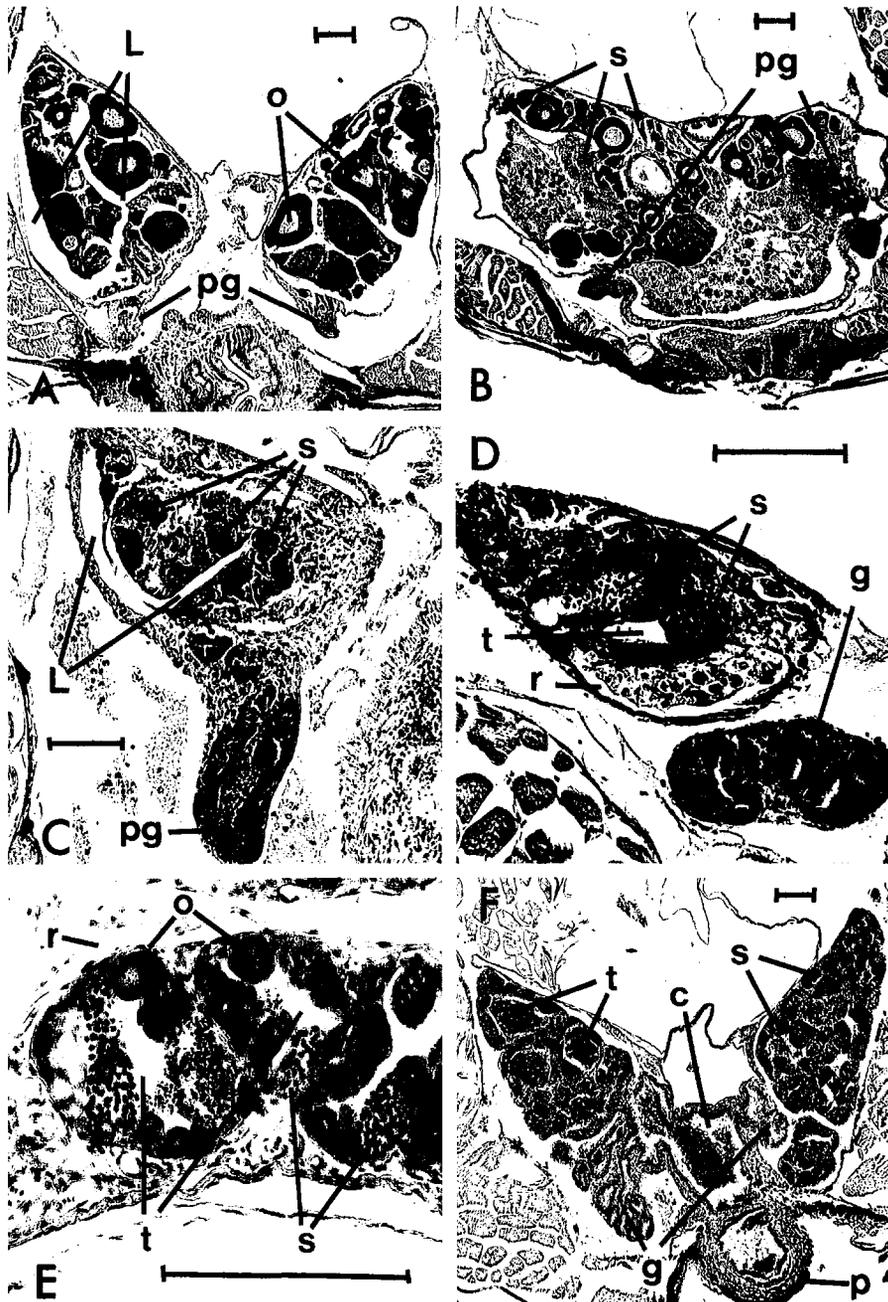


Figure 4. Functional and intersexual gonad structure: A, Functional ovary—cross-section of paired ovarian lobes just anterior to the common genital sinus showing ovarian lumen and presumptive accessory gonadal structure (pAGS) tissue; B, early Stage 1 ovotestis, cross-section through the common genital sinus, showing breakdown of ovarian material and crypts of spermatocytes; C, early Stage 1 ovotestis with developing AGS; D, Stage 2 ovotestis, with well-developed AGS tubules lined with secretory cells, crypts of spermatocytes surrounding lumina within the ovotestis proper, and with a cell-layer bound gap that may be a lumen remnant; E, Stage 3 ovotestis with fully formed spermatogenic tubules, as well as scattered remnant oocytes and a small lumen remnant; F, functional secondary

Table 2. Mean lobe area (microns²) for left and right gonadal lobes and for accessory gonadal structures (AGS) in experimentally induced (Exp.) and field-caught, laboratory-maintained (F.C.) males (see text for area calculation). Standard deviations (SD) are in brackets for each mean value. N is sample size. z and P values were calculated using combined left and right lobe areas (Mann-Whitney U test, Siegel, 1956)

	Gonadal lobe		AGS	
	Left	Right	Left	Right
Exp. N = 14	801.7 (393.0)	859.7 (389.6)	625.4 (388.0)	543.2 (231.9)
	z = 1.212 P = 0.1131		z = 3.165 P = 0.0008	
F.C. N = 21	1,457.0 (1,656.2)	1,118.1 (643.4)	287.3 (298.2)	276.0 (202.6)

r2) was then calculated to provide an estimate of the cross-sectional area. Based on these estimates, the testes in newly formed, secondary males were not significantly different in size from those males collected from the field and held under similar experimental conditions (Table 2). However, the associated AGS in new lab-induced males were approximately twice as large as those of field-caught, laboratory-maintained males (Table 2). This difference cannot be attributed to male size since there were no differences in the sizes of newly transformed males (median size 27.6 mm TL, range = 23.4–29.5, N = 14) and field-caught laboratory-maintained males (median size 27.8 mm TL, range = 20.2–33.4, N = 21; D = 0.262, P > 0.30).

CHANGES IN EXTERNAL MORPHOLOGY. Associated with completed sex-change was the elongation and narrowing of the genital papilla, with a pointed, rather than a broad, blunt terminus. In addition, the second dorsal spine in sex-changing individuals was much elongated compared to the initial spine length (less than or equal to 30% of the first dorsal spine) exhibited at the start of the experimental period. External changes in sex-changing fish were first evident in 4-day groups, where increased length of the dorsal spine (40–45%) was found in two individuals with late stage 2 ovotestes. The first indication of distal narrowing of the genital papilla was present in a late stage 2 intersex from another 4-day group. By day 6, noticeable narrowing and elongation of the genital papilla was exhibited by a late stage 3 intersex. Dorsal elongation had not yet started in this individual. Newly transformed secondary males from 9-day groups had typically male genital papillae, and dorsal elongation of up to 66%. Eighty percent dorsal elongation was exhibited by one 12-day new male, and 100% elongation was first seen in 20-day secondary males (14 of 23 transformed males).

Timing and Occurrence of Sex Change.—Because group mortality in experimental fish held for 2–6 days was high, we pooled the data. However, it was evident from even a small sample size that the progression of testicular development was rapid in experimental groups (Table 3). Ovarian degeneration started within two days, dark-staining crypts of spermatocytes were evident by 3 to 4 days, and newly formed secondary testes with spermatozoa developed in as little

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testis, cross-section through the common genital sinus showing spermatozoa-filled lumina, fully-developed AGS, but no remnant ovarian lumen. In all cases, bar = 10 μm. c, common genital sinus; g, AGS; l, ovarian lumen; o, oocyte; p, genital papilla; pg, presumptive AGS; r, possible ovarian lumen remnant; s, spermatogenic tissue; t, lumen of spermatogenic tubule.

Table 3. Number of experimental fish possessing ovarian, degenerating ovarian (D.O.), ovotesticular (Stage 1, Stage 2, Stage 3; see text for definition) or testicular gonads, after maintenance for either 2-3, 4-6, 9, 12-15 or 20 days

Experimental interval (days)	Proportion of fish in each gonadal category						Number of fish
	Ovarian	D.O.	Ovotestis stage			Testicular	
			1	2	3		
2-3	0.79	0.16	0.05	0	0	0	19
4-6	0.65	0.15	0.05	0.10	0.05	0	20
9	0.40	0.11	0.13	0.04	0.16	0.16	55
12-15	0.48	0.04	0.16	0	0.12	0.20	25
20	0.48	0.15	0	0.02	0	0.35	62

as 9 days. By 20 days, most individuals had either transformed, or remained female.

The 29 all-female groups held for 9-20 days contained either three (N = 11 groups), four (N = 8), or five (N = 10) fish at the end of the experimental interval. Mortality was generally confined to the smaller individuals. Within these 29 groups, sex-change was initiated or completed in one or more group members in all but one group. In 12 groups, a single female had an ovotestis or newly formed testis, and in all cases, this was the largest fish (mean size, TL mm, of sex-changing fish = 24.1 ± 2.3 , N = 48; mean size of fish remaining female in same groups, 21.9 ± 2.1 , N = 67). While sex-changing fish were generally larger than non-sex-changing individuals (D = 0.403, $P < 0.001$), small fish size alone did not preclude sex-change, since, in some groups the largest female was quite small (19-22 mm TL, N = 6 groups), but had developed a Stage 3 ovotestis or newly formed testis within as little as 9 days.

Multiple occurrences of sex-change within the experimental groups were more frequent than single sex-change events (Table 4). In 14 of the 16 groups having two or more sex-changing individuals, the largest individual showed an equal (N = 5) or greater (N = 9) degree of testicular development relative to other sex-changing individuals. All of the five former groups were 20-day groups, and all transformed individuals had completed sex-change and possessed spermiated testes. Of these, only 2 of 10 individuals still exhibited oogenic remnants in the transformed gonad. In no case, however, did all the individuals within an experimental group change sex. In 26 of the 28 experimental groups exhibiting sex-change, the smallest fish remained female, while in the other two groups, the smallest fish was transforming, and the second smallest remained female.

Groups that had one or more larger males present at the start of the experiment, and which we held for 20 days, showed significantly less incidence of sex-change (sex-change occurred in two of 11 groups) than did groups composed of several

Table 4. Number of experimental groups maintained for 9-20 days, that had different numbers of sex-changing individuals

Final group size	Number of groups in which different numbers of fish changed sex			Overall proportion of fish in a group that began sex-change Mean (range)
	1 fish	2 fish	3 fish	
3	5	5	0	0.50 (0.33-0.67)
4	3	3	2	0.47 (0.25-0.75)
5	4	4	2	0.36 (0.20-0.60)

females plus one or more smaller males (sex change occurred in all of four groups) ($P < 0.01$, Fisher exact probability test; Siegel, 1956). In one group with a larger male present, the largest female also had a fully developed testis after 20 days, and in another, one of the smallest females possessed a Stage 1 ovotestis. In five of six groups showing sex-change (with either larger or smaller males present) the largest female was involved. Mixed-sex groups containing larger males had females of similar sizes as those groups containing smaller males ($D = 0.208$, $P > 0.30$).

In three groups each of six males that were maintained for 20 days, all individuals had spermiated testes and no ovarian tissue at the end of the experimental interval.

The incidence of sex-change per social unit was significantly greater among groups (71% of seven groups) than solitaires. (26% of 23 individuals; $P = 0.04$, Fisher exact test; Siegel, 1956) but this was not simply a reflection of increased numbers of individuals. The incidence of sex-change among the largest individuals of each social group was greater than expected, based on the occurrence of sex-change among solitaires (71% of the seven largest individuals compared to 26% of solitaires; $P = 0.04$), while sex-change among smaller individuals was lower than that of solitaires (5% of 21 individuals comprising the three smallest individuals in each group; $P = 0.06$, Fisher exact test). Thus, the occurrence of sex-change was highly non-random among individuals within social groups; smaller individuals were apparently inhibited, and larger individuals stimulated, to change sex. This is the same pattern we found in other experimental groups previously discussed.

DISCUSSION

Coryphopterus personatus was first documented as a protogynous hermaphrodite by Robertson and Justines (1982). The data presented in that report and here show that *C. personatus* displays the female-biased sex ratio typical of many protogynous hermaphroditic species. However, our findings show that a small-scale population sampling would be unlikely to produce intersexual individuals whose presence would indicate the hermaphroditic status of this species. Moreover, the structure of the secondary testis of *C. personatus* lacks ovarian characteristics that typify the secondary testes of other species. In gonochoristic fishes, the testis is typically a solid structure (Hoar, 1969; Harder, 1975; Nagahama, 1983) with no afunctional membrane-lined spaces. Sperm transport generally occurs via an epithelially lined sperm duct that is located dorsally in each lobe, and that drains posteriorly into a common vas deferens (Harder, 1975). In contrast, the structure of the secondary testis of serranids and wrasses is usually characterized by the presence of an old ovarian lumen, which now has no gamete transport function, and a lamellar-like distribution of germinal tissue reminiscent of ovarian lamellae (Reinboth, 1970; Dipper and Pullin, 1979; Hastings, 1981; Ross, 1984; Nemtzov, 1985). To collect and drain spermatozoa from the newly formed testis, sperm sinuses are formed by the rupture of tissue layers in the old ovarian wall, resulting in peripherally located, non-epithelially lined channels that run the length of the gonadal lobe and drain into a posteriorly located vas deferens (Bruce, 1980; Hastings, 1981). In some sex-changing lethrinids, however, the sperm sinuses lie dorsally and internal to the gonad wall (Young and Martin, 1982), while in one anthiid, no duct transport system is present in secondary males other than a posteriorly located common sperm duct (Shapiro, 1981a). As with sperm duct formation, the vas deferens may arise from the rupture of col-

lagenous tissues surrounding the post-ovarian sinus, and therefore also have no discernible epithelial lining (Hastings, 1981).

In monandric protogynous hermaphrodites, all males are derived from adult females, and as a consequence, have testicular structure reflecting their former ovarian form (Reinboth, 1962). Conversely, males in most diandric fish species display one of two distinctive testis types, depending upon whether testicular development occurs directly, or follows a period of maturation and reproduction as a female. Hence, variation in testis structure in diandric species depends on whether former ovarian morphology constrains secondarily developed testis form. For this reason, the retention of the ovarian lumen is considered a reliable diagnostic trait for secondary male status among protogynous hermaphroditic fishes (Reinboth, 1967; 1980). However, in secondary males of *C. personatus*, the persistence of any space reminiscent of an ovarian lumen beyond the newly transformed male stage occurs only rarely. Sometimes a gap can be seen between the developing testicular tissue and the periphery of the lobe in the ventral interiad portion. If this gap derives from the remains of a progressively occluded ovarian lumen, its subsequent absence in well-spermiated testes of large males may be the result of complete occlusion, or breakdown of the former ovarian lamellae and lumen wall, or both. In a related protogynous hermaphroditic gobiid, *C. nicholsi*, testicular tissue arises uniformly around the periphery of the old ovarian lumen, then proliferates away from the lumen. The lumen presumably becomes compressed towards the center of the gonad and occluded as the regions of testicular tissue expand, since no trace remains in transformed males (Cole, 1983). In *C. personatus*, however, testicular tissue arises throughout the body of the gonad rather than from one location, and the transitory presence of a residual space is peripheral rather than central. For these reasons, it is not clear how structural re-organization of the gonad of *C. personatus* results in loss of the ovarian lumen. The absence of an ovarian lumen in the secondary testis has been described for *Sparisoma aurofrenatum* (Clavijo, 1982), *A. squamipinnis* (Shapiro, 1981a) and discussed in Sadovy and Shapiro (1987). In *A. squamipinnis*, instead of spermatozoa being released into lumina or ducts, partitioning between crypts uniformly and regularly breaks down, releasing sperm into a resulting sinus in the testis. This breakdown process may have a generalized effect on all membrane-bound spaces, including those which may constitute the old ovarian lumen, leading to its disappearance in some 57% (N = 44) of individuals in which the lumen remnant is absent (Shapiro, 1981a).

The distinctive sperm transport system consisting of peripheral sperm sinuses discussed above is another feature of secondary testes that is absent in *C. personatus*. In the testes of secondary male *C. personatus*, sperm is channelled through a newly formed, permanent, anastomosing network of tubules lined with spermatogenic crypts, rather than through peripheral sperm ducts. The former ovarian genital sinus and posteriorly located oviduct are retained in the male as a path of gamete egress. This testicular form is similar to that of its sex-changing congener, *C. nicholsi*, and as with *C. personatus*, typically gives no structural indication of its former ovarian history (Cole, 1983).

In addition to the testicular lobes, glandular accessory structures characteristic of gobiid fishes must develop in *C. personatus* during sex-change. These structures, when associated with well-spermiated testes, also hold moderate to large quantities of sperm, indicating that sperm storage may be one of their functions. However, the accessory gonadal structures may well have additional functions, since they are lined with secretory cells and secretions are evident within the lumina. In past

studies, these structures, (which are found predominantly, but not exclusively, in gobiids) have been called seminal vesicles (Eggert, 1931; Young and Fox, 1937). Miller (1984), suggests the term "sperm duct glands." However, since AGSs in secondary male *C. personatus* do not arise from a sperm duct structure, but rather from the walls of the ovarian lobes, or the immediately adjacent region in the common genital sinus, the term accessory gonadal structure is used here (AGS), rather than sperm duct gland.

Newly transformed male *C. personatus* have relatively large AGSs compared to individuals that have been male for a longer period of time. Both their large size and rapid development (often more rapid than the associated testis) during and shortly after sex change, suggest that the AGSs may play a role in the process of sex change. (Kiyoshi et al., 1985) showed that in the uro-haze goby, *Glossogobius olivaceus*, the glandular component of the gonad has a steroidogenic function, and suggested that the steroids produced at this site in *G. olivaceus* may play a physiological role as sexual hormones.

Although all male *C. personatus* exhibit the same testicular structure, not all males are derived from functional females. Males smaller than the size at which females first exhibit vitellogenesis were also found, indicating that some males develop directly from an immature stage. However, no males were found among the smallest-sized fish we collected. A histological examination of the gonads of small individuals revealed that all fish (N = 49) under the size of 13 mmTL had an immature ovariform gonad. As none of the small fish examined had undifferentiated gonads, it seems unlikely that males undergo differentiation at a larger size than females. Rather, all fish must first develop an ovariform gonad. Subsequently, some individuals develop into an adult female before changing sex, while others develop first into an immature, then mature, male. The development of an immature testis from an immature ovariform gonad has been reported in several gonochoristic fish species (Takahashi, 1977; Davis and Takashima, 1980; Fukayama and Takahashi, 1982; 1983; Takahashi and Shimizu, 1983; Sadovy, 1986) and in several protogynous species of parrotfish (Robertson and Warner, 1978). Robertson and Warner (1978), and Jones (1980) have referred to this phenomenon in labroid fishes as "pre-maturational" sex-change. They termed small males that develop directly from an immature female stage as "secondary gonochores," and considered them to be functional analogues of primary males (see also Robertson et al., 1982). Robertson and Warner (1978) speculate that the development of secondary gonochorism in protogynous hermaphroditic labroid species may follow the loss of primary males. A similar pattern may be present in *C. nicholsi*, where males are not found in the smallest size categories, but are present at sizes smaller than the smallest mature females found (Cole, 1983). Reinboth (1970) distinguished two developmental pathways for the ontogeny of the testis: (1) testes that are derived from a mature ovary (secondary testis), and (2) testes that never pass through an ovariform stage (primary testis). The pattern of testicular development found here, however, constitutes a type additional to those described by Reinboth (1970), indicating the existence of a third developmental pathway in some protogynous hermaphrodites.

During sex-change, external transformation processes appeared to lag behind internal changes, since the first appearance of dorsal spine lengthening and male papilla formation appeared in laboratory individuals in which testicular development had already started. However, among experimental fish, none retained external female characteristics beyond an early stage of sex-change. The exaggerated extension of the dorsal spine displayed by both experimentally induced and

large, field-collected male *C. personatus* may be analogous to terminal phase coloration in males that have been described for various labroid fishes (Robertson and Warner, 1978; Warner and Robertson, 1978). While broad overlap in size-distributions may exist between male and female *C. personatus*, the distribution of long- and short-spined individuals shows much less overlap. This situation is similar to that in some labroids in which the size-distributions of males and females also overlap extensively, but there is considerable disparity in size-distributions found between initial and terminal phase individuals (Robertson and Warner, 1978; Warner and Robertson, 1978).

The social mediation of sex-change in fishes has been proposed for numerous species (Fishelson, 1970; Robertson, 1972; Fricke and Fricke, 1977; Shapiro, 1979; 1981a; 1981b; Ross, 1984). The common feature in many cases has been the induction of sex-change among primary-sex individuals (usually female) following the removal of one or more final-sex (usually male) individuals. Because removal appears to trigger the "release" of sex change in numerous species, it has been suggested that primary-sex individuals are inhibited in their tendency to change sex by the presence of final-sex individuals (Fishelson, 1970; Robertson, 1972; Fricke and Fricke, 1977). Sex change events in *Anthias squamipinnis* appear to be controlled by a complex of female-female and male-female behavioral interactions (Shapiro, 1979), and often result in a maintenance of constant sex ratios in social groups (Shapiro and Lubbock, 1980). Ross et al. (1983) found in experimental, field-caged groups of *Thalassoma duperrey*, that both the absence of a larger conspecific and also the presence of a smaller conspecific was necessary for sex-change to occur, although the sex of larger, or smaller, conspecifics did not affect whether or not sex-change took place. Hence, sex reversal events in *T. duperrey* appear to be a function of the relative sizes of associated conspecifics, and the stimulatory presence of small conspecifics is crucial. Ross et al. (1983) suggest that the important factor in the initiation of sex-change in *T. duperrey* may be the maintenance of constant size ratios, rather than the maintenance of constant sex ratios, or the loss of dominant, terminal-sex individuals.

The wide variation in incidence of sex-change events within equal-sized social groups of laboratory-maintained *C. personatus* suggests that constancy of sex ratio may not be an important regulatory factor for sex-change in this species. However, sex-change in *C. personatus* does appear to be influenced both by the inhibitory effects of a larger male conspecific, and by the stimulatory effects of smaller female conspecifics, since the smallest female of any group almost never changed sex, and solitary females changed sex less readily than females having other conspecifics in the same tank. In *Anthias squamipinnis*, Shapiro and Boulon (1982) found that a minimum number of adult females was needed in single-male groups for male removal to induce one of them to change sex, and Robertson (1974) found that the presence of a harem was stimulatory for sex-change in *Labroides dimidiatus*. In *C. personatus*, the initiation of sex-change may be controlled by a net balance of inhibitory and stimulatory cues, rather than by the absolute presence or absence of either cue. This may also be the case in a related gobiid, *C. nicholsi*, where the incidence of sex-change in the laboratory was lower in mixed-sex groups (sex-change occurred in a mean 14% of the female population, range = 0–29%) than in all-female groups (sex-change occurred in a mean of 38% of females, range = 31–46%; Cole, 1983). In a related gobiid, *Coryphopterus glaucofraenum*, the incidence of sex-change in solitary fish held for 30, 60 or 90 days is low (21%, N = 33). However, in all-female groups of varying size held for 20 days, the proportion of groups having one or more sex-changing individuals steadily increased

(pairs: 73% of 11 groups; quartets: 82% of 11 groups; 15 females: 100% of seven groups), suggesting that an additive effect of female conspecifics occurs in progressively larger all-female groups (Cole and Shapiro, unpublished data).

Although larger, sex-changing, female *C. personatus* do not prevent sex change in some smaller females, their presence may have a retarding effect, as suggested by the less-advanced testicular elements in the ovotestes of smaller transforming females within 56% of experimental groups consisting of more than one sex-changer. Smaller females do not appear to have an inherently slower rate of sex-change than do large females, as indicated by the rapidity of sex-change events in small-female groups. The reduced testicular development among small females, in the presence of larger sex-changing females, may be the result of a slower rate of gonadal transformation, or a later initiation of the process, or both. A similar pattern of variation in the rate of gonadal development has been reported in field aggregations of *Anthias squamipinnis*, by Shapiro (1980). Robertson and Justines (1982) described multiple sex-change events within all-female experimental groups of *Gobiosoma multifasciatum*, where the degree of testicular development was greatest in the largest sex-changing individuals.

The results of this study suggest that cues for the induction of sex-change in *C. personatus* differ from those in species which conform to constant size-ratio or constant sex-ratio models. As benthic spawners with paternal egg care, gobiid fishes may face different constraints upon the timing and suitability of sex change compared to protogynous hermaphroditic species that are pelagic spawners. Alternatively, as-yet-undetermined factor(s) that are universally of primary importance among sex-changing fishes may control sex-change events.

The unusual induction processes and atypical gonadal features of sex-change in *C. personatus* are noteworthy. Whether these characteristics are prevalent throughout the genus, as might be indicated by similar traits in the protogynous hermaphroditic congener, *C. nicholsi*, is currently under investigation. At present, there is so little information available on the phenomenon of sequential hermaphroditism within the family that it remains to be determined whether sex-change is common among the Gobiidae, and, if so, whether sex-change processes we have observed in *Coryphopterus* are representative of the entire family.

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