Environmental Biology of Fishes **41**: 301–309, 1994.

Does gonad structure reflect sexual pattern in all gobiid fishes?

Kathleen S. Cole[1], D. Ross Robertson[2] & Alcibiades A. Cedeno[2]
[1] *Department of Biology, Bishop's University, Lennoxville, Quebec J1M 1Z7, Canada*
[2] *Smithsonian Tropical Research Institution (Panama), Unit 0948, APO AA 34002-0948, U.S.A.*

Received 9.2.1993 Accepted 1.3.1994

Key words: Ontogeny, Protogyny, *Gobiosoma*, Gobiidae

Synopsis

In immature and adult females of protogynous gobies, small distinctive masses of cells associated with the ovarian wall develop into testis-associated glandular structures during sex change. These precursive accessory gonadal structures, or pAGS, have been found in females of known protogynous goby species, but not among gonochoric goby species, suggesting that their presence can be used as a species-specific indicator of protogyny within the family. However, a detailed examination of a developmental series of ovaries in two gonochoric species, *Gobiosoma illecebrosum* and *G. saucrum*, revealed the presence of a gonadal feature previously thought to be restricted to protogynous gobies. Among immature females of both species, pAGS-like structures having a similar appearance and placement as functional pAGS of protogynous gobies were found. In female *G. illecebrosum*, the size of these structures among immatures progressively decreased with maturation and were absent in all but the smallest adult females. A similar pattern was evident in a small sample of *G. saucrum*. Population demography based on field collections showed that *G. illecebrosum* exhibits sex ratios and male and female size-frequency distributions typical of gonochores and laboratory experiments indicated that final sexual identity was unaffected by social environment during the juvenile period. Thus, the presence of pAGS in juvenile female *G. illecebrosum* is not related to an ability to change sex at that ontogenic interval. Whether the transient pAGS observed here are vestiges of an ancestral protogynous condition is unknown. Based on their presence among immatures in two gonochore gobies, however, only the presence of pAGS in adult females should be used to predict protogyny among gobies.

Introduction

As far as is known, males of all fishes in the suborder Gobioidei share unique glandular structures typically associated with the sperm duct of the testis. These have variously been referred to as seminal vesicles (Egami 1960, Arai 1964), sperm-duct glands (Miller 1984) and, in some protogynous gobies, where they derive from cell masses located in the ovarian wall, accessory gonadal structures (AGS – Cole & Robertson 1988). The precursive cell masses (pAGS; illustrated in Fig. 1a) which develop into AGS during sex change have been found in all females examined (both immature and mature) among 11 experimentally confirmed protogynous goby species within five genera (Cole 1983, 1988, 1990, Cole & Robertson 1988, Cole & Shapiro 1990, Cole unpublished data) but not among a small number of similarly examined females of four gonochore goby species (Cole 1988). Consequently, Cole (1988) postulated that among gobies the presence of pAGS associated with the ovary should be considered a reliable indicator of protogyny.

Hermaphroditism has now been reported for 13

gobiid genera, including *Gobiodon* and *Paragobiodon* (Lassig 1977), *Coryphopterus* and *Gobiosoma* (Robertson & Justines 1982), *Lythrypnus* (Cole 1988), *Pleurosicya, Bryaninops, Luposicya* (Fishelson 1989) and *Lophogobius, Fusigobius, Eviota, Trimma* and *Priolepis* (Cole 1990). In seven genera in which more than one species has been examined (*Gobiodon, Paragobiodon, Lophogobius, Lythrypnus, Fusigobius, Trimma, Priolepis* and *Coryphopterus*), hermaphroditism has been found in all species (n = 33) examined to date (Cole unpublished data, Cole & Hoese unpublished data). This includes *Coryphopterus,* in which hermaphroditism has been found in all 10 of the 11 described species that have been examined (one rare species remains unexamined). Universal protogyny among examined congeners within the above genera suggests that hermaphroditism in gobiids may be a shared trait among closely related species and, possibly, an ancestral condition (Cole & Shapiro 1990).

One gobiid genus, however, does not follow this pattern. In *Gobiosoma,* protogyny has been reported for one species, *G. multifasciatum* (Robertson & Justines 1982) but is absent in three others, *G. saucrum, G. illecebrosum* (Robertson & Justines 1982) and *G. evelynae* (Cole 1988). If hermaphroditism is ancestral in *Gobiosoma*, species such as *G. saucrum, illecebrosum* and *evelynae* which demonstrate no functional hermaphroditism may nevertheless reveal some features of gonad structure typically restricted to protogynous species. Given that the only consistent gonadal feature of protogyny in gobiids is the presence of pAGS associated with the ovary of females prior to sex change (Cole & Robertson 1988, Cole & Shapiro 1990), it seems likely that any structural anomalies in gonochore species that are closely related to hermaphroditic species will be found associated with the ovary.

Previous work (Robertson & Justines 1982) indicated that *G. illecebrosum* is a gonochore with a 1:1 sex ratio and no sex-change potential among adult females. However, with the occurrence of protogyny in a closely-related species and the proposition that hermaphroditism may be an ancestral condition in *Gobiosoma*, the gonochore designation for *G. illecebrosum* deserves closer examination. Previous experiments that tested for sex change ability in gonochoric species of *Gobiosoma* were performed only with adult females. Since in some other protogynous fishes (i.e. Scaridae – Robertson & Warner 1978) females appear capable of transforming the ovary to a testis prior to maturity, it is possible that immature females of some 'gonochore' gobies could have similar precocious sexual lability. If so, one might expect such lability in apparently gonochoric species that have protogynous congeners.

This paper describes an investigation of several aspects of ovarian morphology and sexual development in *G. illecebrosum* and, to a lesser extent, *G. saucrum* and *G. multifasciatum.* The first part examines the histostructure of ovaries of immature and mature females of these three species for the possible presence in *G. illecebrosum* and *G. saucrum* of structural features typically associated with ovaries of protogynous goby species, including *G. multifasciatum.* The second part presents information on population sexual demography based on field collections and results of a series of laboratory experiments with *G. illecebrosum* that were designed to test for: (a) sex change potential in adult and subadult females and adult males, and (b) lability in the development of sexual identity by undifferentiated immature fish. We predicted that if *G. illecebrosum* is a strict gonochore, both immatures and adults from field collections and laboratory experiments would exhibit a 1:1 primary sex ratio, irrespective of the natural or experimental social environment.

Methods and materials

Field collections

A large field collection of *G. illecebrosum* was carried out in August 1993 to obtain baseline data on sex-ratios and size-frequency distributions. Individual *G. illecebrosum* were collected from small patch reefs in the vicinity of the Smithsonian Tropical Research Institute's (S.T.R.I.) field station in the San Blas Islands, Panama (Lat. 9 34′ N, Long. 78 58″W) using a fish anesthetic quinaldine sulfate and a dip net. *Gobiosoma illecebrosum* live in small groups, or aggregations, on isolated coral heads. As the so-

cial structure and mating systems of these groups and whether, in fact, they comprise cohesive social groups, is unknown, all individuals collected from a single coral head henceforth will simply be referred to as an aggregation. Collected individuals were killed by over-anesthetization immediately after collection, preserved in Dietrich's fixative, then examined with a dissecting microscope to establish sex based on genital papilla structure. In this species, the male genital papilla is elongate with a pointed terminus, has a small genital pore at the apex, and often has melanocytes scattered along the length; the female genital papilla is shorter, square to rectangular in outline with a blunt terminus, has a broad genital pore at the apex and exhibits few, or no, melanocytes (Cole & Robertson unpublished data). Individuals were classified as male or female accordingly, then measured (mm, standard length). The resulting data were then combined with information taken from similarly collected individuals previously collected from the same locale in 1980 (Robertson & Justines 1982) to provide information on sex ratio and size-frequency distributions for males and females.

Histological examination

In separate collections from those described above, individual *G. illecebrosum* were collected from small patch reefs in the vicinity of the S.T.R.I. San Blas field station with quinaldine sulfate. In the first collection, made in October 1988, all fish (n = 93) were killed immediately after collection, preserved in Dietrich's fixative, decalcified in Fisher's Cal-Ex solution and embedded in toto in paraplast. The posterior portion of the body was then serially sectioned at 10 μm and all sections were mounted, stained with Harris' haematoxylin and eosin and viewed with a light microscope. This series was used to examine gonad structure in sexually undifferentiated fish, immatures, adult females and adult males. Some additional immature fish (n = 28) obtained from subsequent collections were treated similarly and added to the histological sample for a total of 121 histologically examined specimens.

Gobiosoma multifasciatum and *G. saucrum* of a range of sizes including both immatures and adults (see Results) were collected at the same site and treated in the same manner as the *G. illecebrosum* prepared for histological examination.

Rearing experiments

In separate collections from the above, additional *G. illecebrosum,* including adults, sexually distinct immatures and smaller, sexually indistinct immatures, were collected between October 1988 and June 1989, transported in insulated containers to S.T.R.I.'s Naos Marine Laboratory in Panama City and within 24 h of initial capture were placed in aquaria for the different experimental treatments.

In each experimental replicate one or more fish, depending on the treatment (see below) were maintained in a visually isolated 50 l aquarium provided with rocks and coral skeletons for shelter, flow-through sea water, aeration and a natural photoperiod. A superabundance of freshly hatched brine shrimp nauplii was added to each aquarium daily as food for the test fish. Each experiment ran between 1–2 months until all immatures had surpassed the minimum size at which maturity occurs in the wild (21 mm SL, see Results).

To establish experimental groups, individuals were first divided into juveniles and adults according to size (based on size of first gamete production according to histological information described above and presented in Results). The sex of individuals having sexually differentiated papillae were further identified as male or female, as described above. Most individuals up to 12 mm standard length (SL) had sexually undifferentiated papillae and could not be sexed externally.

Five series of experiments were run:

1. *Groups of adult or subadult females:* To substantiate previous reports of an absence of protogyny in this species, we established three experimental groups each comprising three adult-sized females and six groups each comprising three immature-sized fish that had female-shaped genital papillae, for 3 weeks.

2. *Group of immature males:* Up to now, protogyny is the only known pattern of sequential sex

change among gobies but, as a preliminary test of sexual lability among adult fish with male papillae, we kept one group of five immature males together for two months.

3. *Small immatures in varying social environments:* In these experiments, test fish were all less than 10 mm standard length (i.e. individuals of a size that typically do not have sexually distinct germ cells within the gonad, as described in Results) and had undifferentiated genital papillae.

(a) *Solitary immatures:* 27 randomly chosen fish were reared singly.

(b) *Pairs of immatures:* 44 randomly constructed pairs were reared to maturity.

(c) *Single immature and single adult:* single, randomly chosen immatures were reared either with an adult male (n = 16) or an adult female (n = 18).

Each of these experiments ran for 5–6 weeks.

Results

Population demography of G. illecebrosum

Thirteen aggregations collected in 1980 by Robertson & Justines (1982) and an additional 21 aggregations collected in August 1993 were examined for adult (i.e. ≥ 21 mm SL) sex ratios. The sex ratios for 1980 (18 female, 20 male) did not differ significantly from that of 1993 (41 female, 34 male) (Yates adjusted $X^2 = 0.29$, $p = 0.59$, Sokal & Rohlf 1981). In the combined sample of 113 adults, the adult sex ratio (59 female, 54 male) did not differ significantly from 1:1 ($G = 0.105$, $p = 0.75$, G-test for goodness of fit, Sokal & Rohlf 1981), and the two sexes did not differ significantly from one another in terms of size-frequency distributions (Kolmogorov-Smirnov two sample test, $p = 0.77$).

Similarly, female:male sex ratios among juveniles measuring 17–20 mm SL collected in 1993 (35 female, 25 male) did not differ significantly from those collected in the 1980 sample (17 female, 12 male; $X^2 = 0.15$, $p = 0.70$) and combined, did not differ significantly from a 1:1 sex ratio ($G = 1.67$, $p = 0.20$).

In terms of sexual composition of aggregations, seven aggregations collected in August 1993 and seven more collected in 1980 (Robertson & Justines 1982) included four or more adults (i.e. individuals ≥ 21 mm SL). In the former, 22 were male and 22 were female while in the latter 16 were male and 22 were female.

Gonad structure of field-collected immatures and adults of both sexes of G. illecebrosum

Of 93 fish collected in October 1988, 28 exhibited a short, blunt papilla typical of female gobies. Thirty-three other fish had an elongate, pointed papilla characteristic of males in other goby species (Miller 1984). In all cases, histological examination of the gonad verified these sexual designations. This sex ratio was not significantly different from 1:1 (Yates corrected $X^2 = 0.072$, $p = 0.79$). The remaining 32 fish had a small, immature papilla having no sexually distinct features.

Of these 32 immature fish, plus 28 other individuals with undifferentiated papillae obtained from subsequent collections which were all examined histologically, eight ranging in size from 9–11 mm SL had clearly differentiated gonadal lobes but germ cell identity was indistinct and sex could not be assigned. Ten individuals ranging from 13–18 mm SL were immature males. Their gonads consisted of two small testicular lobes having seminiferous tubules lined with crypts of spermatogonia and spermatocytes, but no spermatozoa. Associated with each testicular lobe was a smaller body having anastomosing, cell-lined lumina but little other structural differentiation. These bodies joined with their respective testicular lobes posteriorly at the point of union of the two testicular lobes and were identical in appearance to typical early-stage accessory gonadal structures (i.e. sperm duct glands sensu Miller 1984) found in immature males of other gonochoric goby species (Cole unpublished data). No oocytes or other ovarian features were evident in any of the immature testes observed in this sample.

Twenty-one individuals ranging from 10–22 mm SL were immature females and their bilobed ovaries contained previtellogenic oocytes in various stages of development, as well as localized protruberances of the ventral ovarian wall in the region of

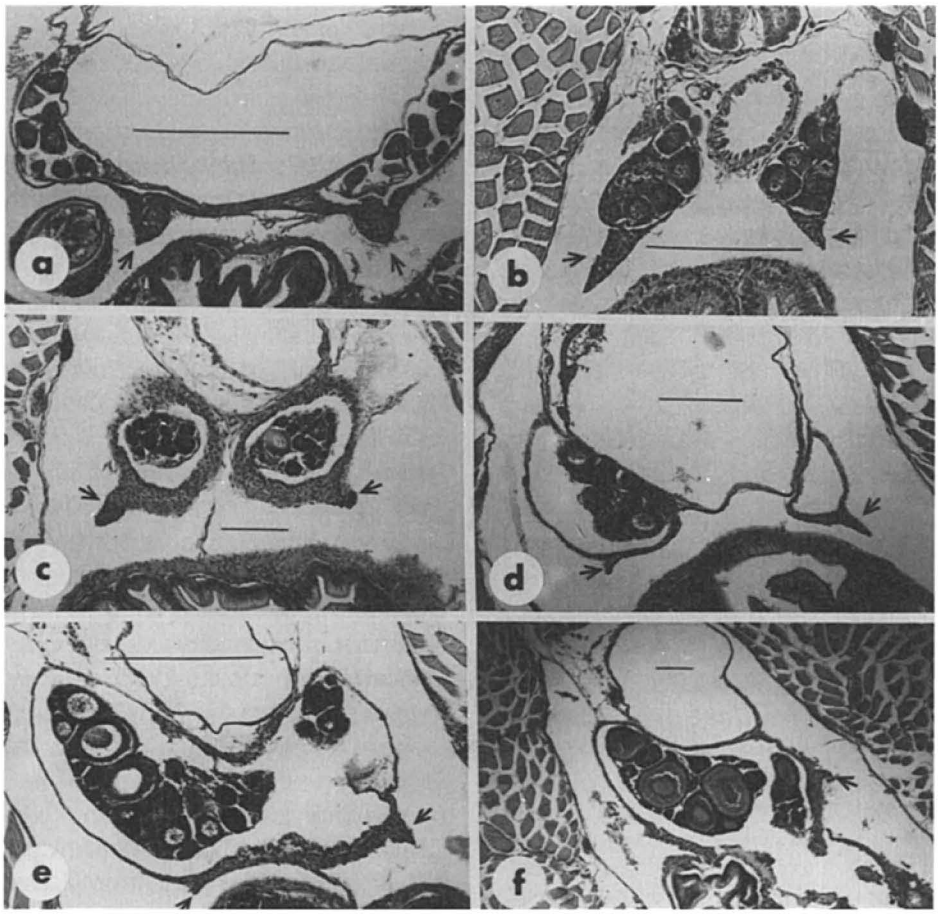

Fig. 1. Ovarian structure in a protogynous goby and in immature and early adult *Gobiosoma illecebrosum*. a) – cross-sectional view of the posterior portion of the ovary, including associated pAGS (indicated by arrows), in the protogynous goby, *Coryphopterus hyalinus*. b–f: *G. illecebrosum*. b) – ovary of immature female (11.8 mm SL) showing (arrow) large pAGS-like structures associated with the ventral wall in the posterior region close to the point of union of the two lobes; c) – pAGS-like structures of immature female, 19.1 mm SL; d) – reduced pAGS-like structures of slightly larger immature female, 20.4 mm SL; e) – remnant pAGS-like structures of small adult female, 23.6 mm SL; f) – remnant pAGS-like structures of slightly larger adult female, 24.2 mm SL. Bar is 100 µm.

the union of the two ovarian lobes (Fig. 1b–d). These structures, which were quite large in the smallest immature females (Fig. 1b–c), were similar in both appearance and location to precursive cell masses found in females of protogynous goby species (Fig. 1a) and described elsewhere (Cole 1988, 1990) which, upon sex change, develop into accessory gonadal structures.

The remaining fish (17 females and 4 males) were all adult. While localized enlargements of the ventral ovarian wall were large in small immature females (Fig. 1b), these cell masses became both relatively and absolutely smaller (Fig. 1c,d) as females approached the size of maturity. Based on the presence of vitellogenic oocytes in the ovary of the smallest adult female present in this sample, we estimated the size for first maturity for females to be 21 mm SL. Remnants of these cell masses were still visible in three adult females (21, 23 and 24 mm SL, Fig. 1e, f) but were absent in all of the remaining 14 adult females whose size ranged from 21–32 mm SL (Fig. 2). Neither immature nor adult females exhibited any testicular tissue, either in the form of seminiferous tubules or unorganized crypts of spermatocytes, within the body of the ovary, or accessory gonadal structures.

No oocytes were evident within the testes proper

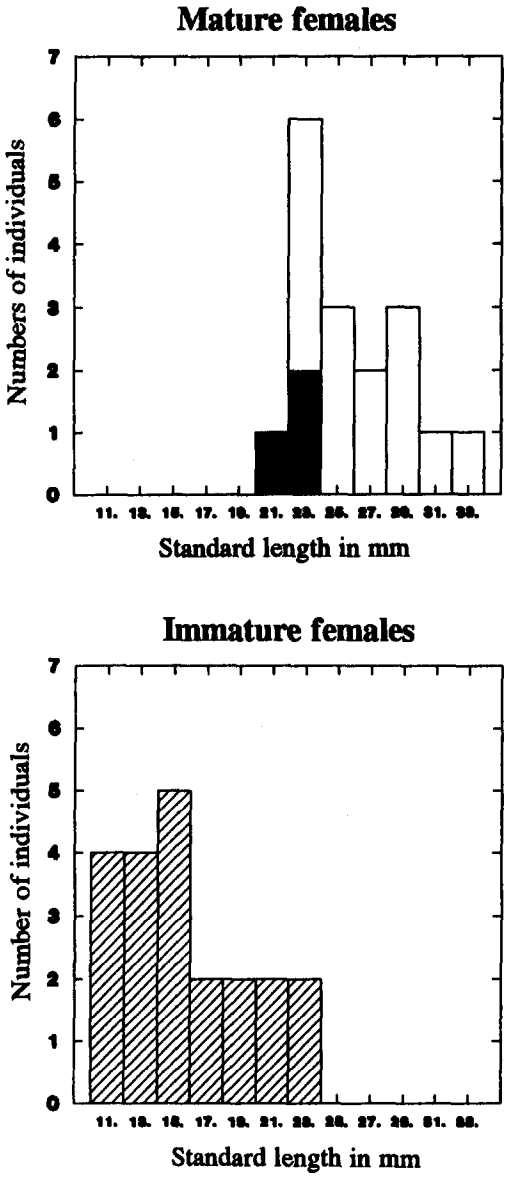

Fig. 2. Bar histogram illustrating the occurrence of pAGS-like structures among (i) immature (n = 21); and (ii) adult (n = 17), female *G. illecebrosum*. Horizontal scale represents increasing size (standard length) in 2 mm increments. Open bar, females with no pAGS-like structures; black bar, females with vestigal pAGS-like structures; hatched bar, females with pAGS-like structures.

or the associated AGS of the four adult males (18–23 mm SL) examined in this sample.

Gonad structure of G. saucrum *and* G. multifasciatum

Of the nine histologically examined adult female *G. saucrum* (13–17.5 mm SL), none had pAGS associated with the ovary. Six of 10 immature females (9–10 mm SL) had distinct pAGS and the remaining four had smaller pAGS-like structures associated with the caudo-ventral ovarian wall.

Three adult male *G. saucrum* (15–19 mm SL) and four immature males (9–11 mm SL) all exhibited fully differentiated AGS associated with the testis. Among all of the mature males and one immature male (11 mm SL) the lumina of the AGS were filled with an acellular, acidophillic (i.e. eosin-staining) secretion. The AGS of the remaining three immatures were fully formed but inactive. No ovarian tissue was visible in the testes or AGS of any of the seven males examined.

Among three immature female *G. multifasciatum* of 12–14 mm SL, all had pAGS. No adult females were present in this sample, although pAGS have been reported elsewhere in adult females of *G. multifasciatum* (Cole 1988). All three males of 19–21 mm SL were adult with large, well-developed AGS, the lumina of which were filled with spermatozoa. No ovarian tissue was visible.

Experimental rearings of G. illecebrosum

Robertson & Justines (1982) previously ran experiments in which groups of 4–6 adult females were maintained together for 3–5 weeks. Since the number of replicates of those experiments was small (n = 4) and they found one male among the females at the end of the experiments, we set up some additional all-female groups to verify gonochorism. Upon histological examination of the six groups each consisting of three immature females, and the three groups each made up of three adult females, no individuals showed any indication of any partial or complete sex change, ovarian degeneration or early-stage testicular tissue. In the single group of five immature males, no individual showed any signs of testicular degeneration or the appearance of early-stage ovarian tissue in their gonads.

Of 27 immatures reared in isolation, 12 were female and 15 male. This ratio is not significantly different from 1:1 (Yates corrected $X^2 = 0.143$, $p = 0.71$). Among the 44 pairs of immatures reared to maturity, 20% (n = 9) were made up of two females, 18% (n = 8) of two males and 62% (n = 27) of a female and male, for a total of 45 females and 43 males. This distribution was not significantly different from that expected by chance (1F:2MF:1M) from random assemblages of pairs from a population with a 1:1 sex ratio ($G = 1.19$, $p = 0.60$). Of 18 immatures reared with an adult female, 33% were female and 67% were male; of 16 immatures reared with an adult male, 31% were female and 69% were male. Neither of these sex ratios was significantly different from 1:1 (with adult female, $G = 2.039$, $p = 0.15$; with adult male, $G = 2.306$, $p = 0.13$) and did not differ from each other (G test for independence, $G = 0.017$, $p = 1.0$, Sokal & Rohlf 1981).

Discussion

In various species of sequentially hermaphroditic fishes, including gobies, sex-change can be induced in some adult individuals by a change in the social environment (Fishelson 1970, 1975, Robertson 1972, Fricke & Fricke 1977, Warner 1978, 1988, Shapiro 1979, 1981, 1987, Shapiro & Lubbock 1980, Robertson & Justines 1982, Fricke 1983, Ross 1983, Cole & Robertson 1988). By the same token, one might expect that variation in social conditions during development from an undifferentiated juvenile state to adulthood should expose lability in gonadal development that may be present in a species, particularly one with close relatives that do show adult sexual lability. In our experiments we varied the social conditions under which differentiated adult *G. illecebrosum* were maintained and under which both differentiated and undifferentiated juveniles developed to adulthood. We predicted that if *G. illecebrosum* is a strict gonochore with a 1:1 primary sex ratio that lacks lability in sexual potential, then no sex-change should occur among either groups of mature females, immature females or mature males; and that the adult sex-ratio of fish that developed from an undifferentiated state to maturity should not differ from 1:1 regardless of the social situation in which they were reared.

The results of the present study support and extend those of Robertson & Justines (1982) which indicated a lack of sexual lability in adult *G. illecebrosum*. Comparisons of adult sex-ratios and size-frequency distributions from field collections presented here demonstrate that *G. illecebrosum* exhibits male and female size-frequency distributions and a 1:1 sex-ratio typical of gonochore fish species. In addition, the failure of sexually undifferentiated juveniles that were reared to maturity under similar, and varying, social conditions, and of experimental groups of adults, to show signs of any developmental plasticity indicates that *G. illecebrosum* is an illabile gonochore with a 1:1 sex ratio.

Since sample sizes in each of the experiments we ran were not large, the power of each statistical test to detect a deviation from a 1:1 sex ratio is not high. In particular, it should be noted that the sex ratio of single juveniles reared with a female was (non-significantly) male-biased. Considering the experimental social situation, this bias is in the direction one would expect if sexual differentiation was plastic. However, we do not give much weight to this result since the experiment in which juveniles were reared with a male also produced a (non-significant) male bias. Further, it is possible to combine the results of a series of statistical tests that aim to test the same hypothesis. We used this 'combination of probabilities' (Sokal & Rohlf 1981) technique to test whether the four experiments in aggregate produced sex ratios different from 1:1 and obtained a probability value of 0.75, indicating that the non-significant sex ratio biases in each individual experiment do not indicate an overall sex ratio different from 1:1.

Immature and mature females of all protogynous gobies so far studied bear distinctive cell masses associated with the ovary, termed precursive accessory gonadal structures (pAGS) which, during sex-change, develop into accessory glandular structures similar to sperm duct glands (sensu Miller 1984) described for gonochoric gobies (Cole & Robertson 1988, Cole 1988, 1990, Cole & Shapiro 1990, Cole unpublished data). As we expected, pAGS were present in the ovaries of immature, protogynous *G.*

multifasciatum and no such structures were present among adult females of the two examined gonochore species, *G. illecebrosum* and *G. saucrum*. However, among immature females of these latter two species, ovary-associated structures that seem to be similar, both in appearance and location on the ovary, to pAGS, were found. This indicates that both *G. illecebrosum* and *G. saucrum*, while functional gonochores, possess some hermaphroditic features in the immature ovary.

The presence of pAGS-like structures among immature females of *G. illecebrosum* evidently is not related to an ability to change sex at that stage, since our experiments do not indicate that there is any lability in the sexual development of *G. illecebrosum*. This suggests that the pAGS-like structures found in immature *G. illecebrosum* and *G. saucrum* are either unresponsive, or not competent to respond, to changes that activate pAGS in sex-changing gobies. The progressive diminution of the pAGS-like structures as females of *G. illecebrosum* approach the size of maturity, and their absence in all but the smallest mature females, is striking. Were these structures merely extensions of the ovarian wall, maturation should have had a neutral or positive effect on their development. Their gradual reduction in absolute size with approaching maturity and ultimate disappearance among adults suggests that endogenous changes associated with maturation in females are antagonistic to their retention. This would be consistent with the fate of testis-associated tissue which presumably requires a certain level of circulating androgens for continued maintenance or further development.

According to Cole (1988), the presence of pAGS-like structures associated with the ovary in gobiid species is a reliable indicator for protogyny. However, based on our findings here, it is evident that this postulation requires modification. Clearly, gonad structure, particularly among immatures, does not necessarily reflect functional sexual pattern, at least in some *Gobiosoma* and possibly in other gobies. Consequently, only the presence of pAGS in *adult* females may reliably predict protogyny among gobies.

The development and subsequent disappearance of pAGS-like structures in two gonochore *Gobiosoma* species is intriguing. It is tempting to speculate that these species, like present-day *G. multifasciatum*, were once protogynous and that this sexual pattern has been secondarily lost. If so, the transient pAGS observed here are simply ontogenetic remnants of a former labile sexual pattern and, as such, their ephemeral presence during development, if observed in other gonochore goby species, may be useful indicators for ancestral protogyny. However, inferring ancestral states and directionality for evolutionary transitions on the basis of a single morphological feature is risky at best, particularly in this case since little is known about gonad development in fishes in general, and gobies in particular.

In order to make educated guesses regarding ancestral sexual patterns in *G. illecebrosum* and *G. saucrum*, we first need to establish generalities for patterns of gonad development in *Gobiosoma,* including examinations of gonad development at the cellular level for both gonochoric and protogynous species. Comparative work on other gonochoric and protogynous gobies, and other teleosts (see also Fishelson 1992), is also essential. As a family, gobies exhibit a variety of sexual patterns including gonochorism, simultaneous hermaphroditism (St. Mary 1994) and protogyny (i.e. Cole 1990). In order to understand the evolution of this diversity, the existence of patterns in the distribution of gonochorism and protogyny among gobiids, and the role of phylogeny in that distribution, need to be determined. Unfortunately, phylogenetic relationships within this most speciose family of marine fishes (Nelson 1984) are still poorly understood (Hoese 1984, Birdsong et al. 1988). Finally, details of the life histories and mating systems of gonochoric and protogynous gobies will need to be elucidated in order to understand the role of selective forces that have favored the development of protogyny in other fishes (e.g. Charnov 1982, Warner 1978, 1988), in producing this diversity of sexual patterns among the gobiids.

Acknowledgements

We thank the Kuna General Congress and the Government of the Republic of Panama for permission

to carry out this study in the San Blas Islands, Marvalee Wake for helpful conversations regarding the difficulties in determining the evolutionary status of morphological features in vertebrates and two anonymous reviewers. Photographic assistance was generously provided by C. Coleby, Royal Ontario Museum and the Photographic Department of the Smithsonian Tropical Research Institute. J. Porter and R. van Hulst provided assistance with manuscript preparation, and statistical analyses and graphics, respectively. This research was supported by a Smithsonian Short-term Fellowship, a Bishop's University Senate Research Grant awarded to KSC and general STRI research funds. Portions of this manuscript were written while the senior author was supported by a National Science and Engineering Research Council of Canada Operating Grant.

References cited

Arai, R. 1964. Sex characters of Japanese gobioid fishes (I). Bull. Nat. Sci. Mus. Tokyo 7: 295–306.

Birdsong, R.S., E.O. Murdy & F. Pezold. 1988. A study of vertebral column and median fin osteology in gobioid fishes with comments on gobioid relationships. Bull. Mar. Sci. 42: 174–214.

Charnov, E. 1982. Alternative life-histories in protogynous fishes: a general evolutionary theory. Mar. Ecol. Prog. Ser. 9: 305–309.

Cole, K.S. 1983. Protogynous hermaphroditism in a temperate territorial marine goby, *Coryphopterus nicholsi.* Copeia 1983: 809–812.

Cole, K.S. 1988. Predicting the potential for sex-change on the basis of ovarian structure, in gobiid fishes. Copeia 1988: 1082–1086.

Cole, K.S. 1990. Patterns of gonad structure in hermaphroditic gobies (Teleostei: Gobiidae). Env. Biol. Fish. 28: 125–142.

Cole, K.S. & D.R. Robertson. 1988. Protogyny in a Caribbean reef goby, *Coryphopterus personatus:* gonad ontogeny and social influences on sex change. Bull. Mar. Sci. 42: 317–333.

Cole, K.S. & D.Y. Shapiro. 1990. Gonad structure and hermaphroditism in the gobiid genus *Coryphopterus* (Teleostei: Gobiidae). Copeia 1990: 966–973.

Egami, N. 1960. Comparative morphology of the sex characters in several species of Japanese gobies, with reference to the effects of sex steroids on the characters. J. Fac. Sci. Univ. Tokyo, Ser. IV 9: 67–100.

Fishelson, L. 1970. Protogynous sex reversal in the fish *Anthias squamipinnis* (Teleostei, Anthiidae) regulated by the presence or absence of a male fish. Nature 227: 90–91.

Fishelson, L. 1975. Ecology and physiology of sex reversal in *Anthias squamipinnis* (Peters), (Teleostei, Anthiidae). pp. 284–294. *In:* R. Reinboth (ed.) Intersexuality in the Animal Kingdom, Springer Verlag, New York.

Fishelson, L. 1989. Bisexuality and pedogenesis in gobies (Gobiidae: Teleostei) and other fish, or why so many little fish in tropical seas? Senckenbergia marit. 20: 147–169.

Fishelson, L. 1992. Comparative gonad morphology and sexuality of the Muraenidae (Pisces, Teleostei). Copeia 1992: 197–209.

Fricke, H.W. 1983. Social control of sex: field experiments with the anemonefish *Amphiprion bicinctus.* Z. Tierpsychol. 61: 71–77.

Fricke, H.W. & S. Fricke. 1977. Monogamy and sex change by aggressive dominance in a coral reef fish. Nature 266: 830–832.

Hoese, D.F. 1984. Gobioidei: relationships. pp. 588–591. *In:* H.G. Moser et al. (ed.) Ontogeny and Systematics of Fishes, Spec. Pub. No. 1, Am. Soc. of Ichthy. and Herpet., Allen Press, Lawrence.

Lassig, B.R. 1977. Socioecological strategies adopted by obligate coral-dwelling fishes. pp. 565–570. *In:* D.L. Taylor (ed.) Proc. Third Intern. Coral Reef Symp. I, Biology, Miami.

Miller, P.J. 1984. The tokology of gobioid fishes. pp. 119–153. *In:* G.W. Potts & R.J. Wootton (ed.) Fish Reproduction: Strategies and Tactics, Academic Press, New York.

Nelson, J.S. 1984. Fishes of the world, 2nd ed. John Wiley and Sons, New York. 416 pp.

Robertson, D.R. 1972. Social control of sex reversal in a coral reef fish. Science 177: 1007–1009.

Robertson, D.R. & G. Justines. 1982. Protogynous hermaphroditism and gonochorism in four Caribbean reef gobies. Env. Biol. Fish. 7: 137–142.

Robertson, D.R. & R.R. Warner. 1978. Sexual patterns in the labroid fishes of the western Caribbean, II. The parrot fishes (Scaridae). Smithsonian Contributions to Zoology 255: 1–26.

Ross, R.M., G.S. Losey & M. Diamond. 1983. Sex change in a coral reef fish: dependence of stimulation and inhibition on relative size. Science 221: 574–575.

St. Mary, C.M. 1994. Novel sexual patterns in two simultaneously hermaphroditic gobies, *Lythrypnus dalli* and *Lythrypnus zebra.* Copeia (in press).

Shapiro, D.Y. 1979. Social behavior, group structure and the control of sex reversal in hermaphroditic fish. Advances in the Study of Behavior 10: 43–102.

Shapiro, D.Y. 1981. Size, maturation and the social control of sex reversal in the coral reef fish *Anthias squamipinnis* (Peters). J. Zool. (Lond.) 193: 105–128.

Shapiro, D.Y. 1987. Differentiation and evolution of sex change in fishes. BioScience 37: 490–497.

Shapiro, D.Y. & R. Lubbock. 1980. Group sex ratio and sex reversal. J. Theor. Biol. 82: 411–426.

Sokal, R.R. & F.J. Rohlf. 1981. Biometry. The principles and practice of statistics in biological research, 2nd ed. W.H. Freeman and Company, San Francisco. 859 pp.

Warner, R.R. 1978. The evolution of hermaphroditism and unisexuality in aquatic and terrestrial animals. pp. 78–101. *In:* E.S. Reese & F.J. Lighter (ed.) Contrasts in Behavior, John Wiley & Sons, New York.

Warner, R.R. 1988. Sex change in fishes: hypotheses, evidence and objectives. Env. Biol. Fish. 22: 81–90.

Kassi Cole and her typical smile. Photograph by M. Keenleyside.