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Perplexing problems of sexual patterns in the fish genus *Paralabrax* (Serranidae, Serraninae)

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Abstract

For 40 years there have been problems in diagnosing sexual patterns in the serranid genus *Paralabrax*. Difficulties stem from a combination of factors including terminology, histological and morphological characteristics, and the complex inter-relationships in the Serranidae between gonad morphology, phylogenetic position and gonad function. A detailed study of four species of *Paralabrax*: *P. maculatofasciatus, P. nebulifer, P. auroguttatus* and *P. clathratus,* clarifies the problems and improves the diagnosis of sexual pattern in the Serranidae, and for similar families that do not have distinct morphologies for primary- and secondarily-derived males. An hypothesis was developed to account for the multiple appearance of gonochorism in the Serranidae, better known for its widespread hermaphroditism.

Key words: Serranidae, sexual pattern, hermaphroditism, *Paralabrax*

INTRODUCTION

The family Serranidae is well-known for its diverse expressions of sexual pattern in the wild, which range from gonochorism (separate sexes) to simultaneous hermaphroditism and various forms of protogyny (female to male sex change). Only protandry (adult male to female sex change) has never been described, although it does occur in captivity. Sexual pattern is defined as the typical expression of sexuality exhibited by individuals of a population or species. We use the term in a strictly functional sense to reflect the sum of reproductive function(s) of individual fish expressed within their lifetime.

Protogyny can be divided into two functional types, monandry and diandry. Monandry denotes a single male pathway of development, whereby all males develop from functional females by sex change (secondary males). Diandry signifies two possible male developmental pathways, one via adult sex change (secondary males), as in monandry, the other by direct male development from the juvenile phase with no adult sex change (primary males). The terms monandry (literally single male) and diandry (double male) were originally coined by Reinboth (1962, 1967) to distinguish different forms, or pathways, of male development within certain protogynous species. Referring specifically to the labrids, Reinboth (1970) notes '*Fortunately*, in these fishes the structure of the testis reflects clearly whether a male originated by sex-inversion (secondary male) or has been born as a male (primary male)' (our italics). Indeed, in many families and species, such as certain labrids, scarids, several synbranchids and in *Rivulus marmoratus* (Harrington, 1967, 1971; Liem, 1968), primary and secondary males can be readily distinguished histologically because of distinct morphological differences between the two testis types. However, this morphological distinction may not be so clear in all labrids (Rasotto & Shapiro, 1992; Shapiro & Rasotto, 1993), while in other families, such as the Serranidae, Pomacentridae, Scaridae and Mugiloididae, a morphological distinction among males, reflecting different developmental pathways, is not apparent, making primary and secondary males impossible to distinguish by morphology alone. Hence sexual pattern, in the functional sense in which we use it, is particularly difficult to determine using testis morphology alone. We distinguish between function and morphology throughout.

Interest in the different sexual patterns and gonadal morphologies within the Serranidae initially focused on their potential usefulness as a phylogenetic character, but subsequent work caused workers to question this application. Smith (1965) proposed that serranids have 'structural and functional patterns of sexuality that can be utilized in phylogenetic studies' and noted that such characters could possibly be used to determine the limits of the family, as well as arrangements within it. He recognized differences in gonad structure and sexual patterns that appeared to follow subfamily lines. Smith & Atz (1969) used gonad morphology to hypothesize evolutionary relationships among certain serranid genera.

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Thought to be conservative, sexual patterns and gonad morphology were considered applicable as a taxonomic character in the study of family limits, and Gosline (1966) used gonad structure (from Smith, 1965) and osteological data to remove the gonochoristic Percichthyidae from the hermaphroditic Serranidae. Bortone used patterns of testicular tissue to determine relationships within the genus *Diplectrum* and proposed that differences in gonad morphology between *Serranus* and *Diplectrum* may be important for understanding systematic relationships within the two genera (Bortone, 1977). He none the less recognized the need for more data to realize the potential and limits of morphology and sexual patterns in systematics. More recent studies have shown that not only are functional sexual patterns considerably more variable within the Serranidae than previously realized, but that the arrangement of female and male tissue, relative to each other, may not as clearly follow taxonomic lines as originally proposed: the character was not used by Baldwin & Johnson (1993) in their revision of the family. None the less, it appears to be a useful phylogenetic characteristic in the Gobiidae (Cole, 1990) and Platycephalidae (Fuji, 1974) and merits a closer look in the Serranidae (Hastings & Petersen, 1986; Nakazono & Kuwamura, 1987).

As more workers examined sexual patterns in fishes, not only from a phylogenetic perspective, but increasingly in relation to reproductive biology, ecology, sex allocation theory and responses to fishing, difficulties emerged in reaching definitive diagnoses of sexual pattern in certain families. The core of the problem was twofold; sexual development appears to be particularly plastic in some teleost families or species, while others may not exhibit morphological differences in the gonads that reflect different *functional* male developmental pathways and hence the sexual pattern (in this case the type of protogyny) is difficult to detect. Perhaps inevitably, the confusion brought about by mismatches in form and function led to a proliferation of terms that have fuelled rather than resolved the confusion. This is because it is often unclear whether such terms are intended to refer to gonad function or gonad ontogeny/morphology, and, moreover, whether they are intended to have phylogenetic implications. Hence, the literature abounds with such classifications as pre-maturational and post-maturational sex change, non-functional protogyny, partial protogyny, secondary gonochorism, etc.

The problem reflected in this unwieldy terminology is not usually one of disagreement, but rather of our continuing poor understanding of early sexual development, differentiation and developmental plasticity in many fishes. For example, in functionally diandric species, do both primary and secondary testes pass through fundamentally different developmental sequences, as might be suggested in labrids but not in other families such as pomacentrids or serranids? Or, do all individuals undergo an initial female-like or female phase whereby the difference between primary and secondary males is simply one of timing, relative to sexual maturation, at which the ovarian-like gonad develops into a testis (Rasotto & Shapiro, 1992; Shapiro & Rasotto, 1993)? In families that express various forms of hermaphroditism, is hermphroditism the ancestral form (with gonochorism thus a derived condition) from which other sexual patterns develop, or is it derived from a gonochoristic ancestor? Are such families particularly plastic in their expression of sexual patterns?

Recognizing some of the earlier problems of diagnosing sexual patterns, Sadovy & Shapiro (1987) attempted to establish rigorous criteria for determining (functional) sexual pattern based on morphological characteristics. However, they did not address in depth the problem of distinguishing different male developmental pathways in protogynous species in families for which testicular morphology provided no clue to testicular ontogeny. The Serranidae is particularly challenging in this respect because functional sexual pattern and gonad developmental pathways do not always match. Only when sexual patterns are properly diagnosed can we begin to explore questions of their adaptive significance expressed under natural conditions, their phylogenetic incidence and significance, and understand the responses to exploitation of sexually labile species.

Criteria used to determine sexual pattern involve the detection of several characteristics, some being more reliable indicators than others. For example, transitional fish changing from functional female to functional male show degenerating vitellogenic, or later, stage oocytes (or other sign of prior mature female function), an ovarian lumen and sperm sinuses surrounding the testis suggesting previous female function in some, but not all, species (Sadovy & Shapiro, 1987). A testis containing a lumen with surrounding sinuses is not, for example, sufficient to detect functional female to male sex change in families like the Pomacentridae and Serranidae since all males have a secondary testis structure, irrespective of sexual pattern and male developmental pathway (Reinboth, 1970; Sadovy & Colin, 1995; Asoh, Yoshikawa & Kasuya, 2001; Adams, 2003; Liu & Sadovy, 2004). Induction of sex change in captivity may reflect the functional sexual pattern of the species in the field, although sex change can be induced in captivity in non sex-changing species. Also, male to female sex change has been noted in the serranids *Epinephelus akaara* and *Cephalopholis boenak* in captivity although it is not clear whether this occurs naturally (Tanaka *et al*., 1990; Liu & Sadovy, 2004). Other characteristics, such as significantly larger or older males among mature adults, and the absence of smaller males may also suggest sex change but, again, are not definitive since other biological attributes can produce such effects. In other words, it is typically a combination of characters that must be used to diagnose sexual pattern (Sadovy & Shapiro, 1987). Particularly in families, like the Serranidae, that do not exhibit distinctive morphological differences between primary and secondary male developmental pathways, multiple indicators are needed to determine functional sexual pattern. The genus *Paralabrax* exemplifies many of the difficulties of diagnosing sexual pattern in the Serranidae, in particular, and in fishes in general.

The genus *Paralabrax* (10 species) is particularly interesting both for its phylogenetic position as a basal serranine (Meisler, 1987), itself considered the basal subfamily in the Serranidae on both morphological and genetic grounds (Smith, 1959; Gosline, 1966; Lauder & Liem, 1983; Baldwin & Johnson, 1993; Pondella, Craig & Franck, 2003), as well as for the continuing difficulties encountered by workers over four decades in determining functional sexual patterns within the genus. *Paralabrax maculatofasciatus*, for example, has variously been diagnosed as protogynous with pre-maturational sex change (Hastings, 1989; Oda, Lavenberg & Rounds, 1993), and as having 'diandric origins' with different populations ranging from gonochorism to protogyny (Hovey & Allen, 2000). *Paralabrax clathratus* was diagnosed as a secondary gonochore (Smith & Young, 1966). The extent to which these apparent differences stem from methodological problems of diagnosing sexual pattern in this genus, differing terminology, or reflect plasticity in functional sexual pattern in the species in nature calls for further examination. Only when diagnoses of sexual patterns are accurate can a meaningful discussion of the adaptive significance of (functional) sexual pattern, on the one hand, and its utility as a phylogenetic (functional and/or morphological) character on the other, progress, and apparent inconsistencies be resolved.

We examined in detail four of five species of the North American clade of *Paralabrax*: *P. nebulifer, P. maculatofasciatus, P. auroguttatus* and *P. clathratus*. Our objectives were to: (1) establish the functional sexual pattern for each species; (2) develop improved criteria for diagnosing sexual patterns in the Serranidae; (3) re-examine gonad morphology and functional sexual patterns in the Serranidae in relation to their proposed phylogeny.

MATERIALS AND METHODS

Paralabrax specimens were collected using hook and line with live, dead and artificial bait, the exception being a few *P. nebulifer* collected by fish trap. Sampling was concentrated during the months the fish were reproductively active, but fish were also taken during non-spawning times of the year, except for *P. auroguttatus*. Five hundred and twenty-one *P. clathratus* were collected, between 1993 and 1995, from Los Angeles to San Diego, California, and near Punta Eugenia and surrounding islands, Baja California, Mexico. All *P. clathratus* samples were taken between March and August of each year in water depths of 6–30 m. Two hundred and fifty-five *P. maculatofaciatus* specimens were collected, from depths of 1–10 m, in San Diego Bay between May and July of 1999. Three hundred and sixty-four *P. nebulifer* were collected, between 1993 and 1995, throughout southern California from depths of 3–50 m. All *P. nebulifer* specimens were collected between the months of February and November. Two hundred and forty-seven *P. auroguttatus* were collected during a single sampling trip to the Gulf of California in May of 1998, in depths of 40–100 m (Table 1).

Each specimen was measured to the nearest mm and weighed to the nearest g before gonads were removed for histological preparation. Gonads were weighed to the nearest g before fixation in Davidson's solution. In most cases the entire gonad was preserved, but for large fish only 1 lobe of the gonad was fixed.

Gonads were prepared using standard paraffin embedding techniques and stained in haematoxylin and eosin. Serial sections of 6–8 μm each were taken extensively from anterior, middle and posterior segments. Germ cell stages are described and stage of sexual maturation determined based on the most advanced stages of cell type present, indications of previous spawning or other structural details (see below). Gonads of *P. nebulifer, P. clathratus* and *P. maculatofasciatus* were sectioned transversely at 3 different levels along the lengths of the gonads. Additional sectioning was conducted in all cases where testicular, or presumptive testicular, islets were found to determine the extent of their distribution, or for large gonads that could not be sectioned transversely in their entirety. Since testicular or presumptive testicular islets were always found towards the middle part of the gonad, testes and ovaries of *P. auroguttatus* were only sectioned in their central portion. For *P. clathratus* and *P. nebulifer*, not all gonads sampled were prepared histologically; all those in smaller size classes were selected for histological preparation as well as a random subset of larger ones from all available size classes.

Stages of germ cell development

Stages of oogenesis

Stages 1 & 2: oocyte stages 1 and 2 are part of the primary growth phase and their development is independent of pituitary control (Wallace & Selman, 1981). These oocytes are previtellogenic with a low cytoplasm to nucleus ratio, and perinucleoli visible at later stages.

Stage 3: stage 3 is gonadotropin-dependent and is the cortical alveolus stage (a precursor to the yolk vesicle phase). This is also a previtellogenic phase of early oogenesis. The cytoplasm expands in area relative to the nucleus, stains lighter in H&E than earlier phase oocytes and the pale-coloured cytoplasmic inclusions of cortical alveoli increase in number as this phase progresses.

Stage 4: this is the vitellogenic phase in which yolk is transferred into the oocyte from the liver. Yolk globules accumulate and produce a massive expansion of oocyte volume. The zona radiata is thick and the oocytes appear increasingly circular with growth. The nucleus is not apparent in the later stages.

Stage 5: hydrated oocytes or partially hydrated stage 4 oocytes. Hydration typically occurs shortly before spawning and is an indication that spawning is imminent. The yolk globules progressively coalesce until the oocyte is smooth and greatly expanded and

the zona radiata is thin. The fully hydrated stage may be within the follicle or within the ovarian lumen and does not retain its shape after histological processing, becoming highly convoluted.

Stages of spermatogenesis

Spermatocyte stage 1: cysts of deep-staining cells embedded in lobule walls.

Spermatocyte stage 2: as above but each cyst contains greater numbers of smaller, darker staining cells than in previous stage.

Sperm (i.e. spermatids/spermatozoa): as above but cysts are large and sperm extremely numerous. Cells are smaller and more deeply stained than above. Spermiogenesis probably occurs while spermatids are still within cysts (from tailed sperm), and as cyst walls break down spermatozoa enter the lobule lumen. The presence or absence of sperm sinuses, with or without sperm, was noted.

Stages of maturation and reproductive function

Ovary

F1: immature, or inactive (i.e. developing or recovering from spawning), females with oocytes of stages 1, 2 and sometimes 3 are evident; no indication of previous spawning but it is possible that spawning has occurred and that the gonad is between spawning seasons and regressed. Some of the oocytes may be atretic. Females could be sexually immature (Fig. 1a, b).

F2: mature, ripe or recently post-spawned females. Oocytes of stages 1, 2, 3 and 4 present, possibly with the occasional hydrated, stage 5 oocyte; post-ovulatory follicles would indicate recent spawning. Gonad is large and gonad wall is thin. Includes mature post-spawning females in which spawning has finished, as indicated by all or some of the following features: post-ovulatory follicles, significant atresia of vitellogenic oocytes, few healthy vitellogenic oocytes and generally disrupted appearance. For recently post-spawned females, ovarian wall may be thick as it contracts post-spawning (Fig. 1c).

Testis

M1: inactive male. Testis dominated by early stages of spermatogenesis. Gonia prominent at the borders of the lamellae. Sperm may or may not be present but, if present, are few. Sperm sinus is present which may have a few sperm (Fig. 1d).

M2: mature, ripe or recently post-spawned male. Testis has all stages of spermatogenesis and is capable of spawning or has recently finished spawning. Sperm sinuses are always present. In post-spawning males, there are collapsed and partially empty seminiferous lobules and stages of early spermatogenesis and gonia are particularly evident; some sperm may remain in sinuses and the testis wall may be thick. Spermatozoa tails within individual cysts all oriented in a similar direction (Fig. 1e, f).

Bisexual stages of gonad development

Individuals with gonads containing both ovarian and testicular tissues at different stages of development (both immature and mature), or presumptive testicular tissue, were all defined as bisexuals. The term bisexual refers to gonad morphology and not to gonad function. The functional sex of each bisexual stage is indicated based on the most advanced stage(s) of germ cell development present. In bisexual females the testicular portion either consists of 1–2 small islets of spermatogenic tissue located at the alamellar margin of the gonad periphery and ventrally in the gonad, or presumptive testicular islets containing tissue that occurs in the same location as testicular islet proliferation and distinctive clusters of gonia found nowhere else within the gonad. Not included in the definition of bisexual is the occasional occurrence of previtellogenic oocytes scattered within mature testicular tissue because these isolated cells are assumed to be of little functional significance and indicate nothing about past or future reproductive function (Sadovy & Shapiro, 1987).

BI(a and b): immature or inactive bisexual – gonad includes previtellogenic oocytes of stages 1, 2 and sometimes 3 that may or may not be degenerating. Also present are presumptive testicular islets (BIa) (Fig. 2a, b) or testicular islets (BIb) (Fig. 2c, d) with early spermatogenic tissue (i.e. mainly stages 1 and 2 spermatocytes and few, if any, spermatids or spermatozoa). BI fish are reproductively inactive.

BF: mature female bisexual – contains primary growth, cortical alveolus and vitellogenic stage oocytes that may be degenerating following spawning, and cysts of early stages (1 and 2) spermatogenesis in testicular islets or presumptive testicular islets. The reproductive function of this stage is female (Fig. 2e, f).

BM: mature male bisexual – contains cortical alveolus and/or primary growth stage oocytes that may or may not be degenerating, and all, or mostly later, stages of spermatogenesis, and sperm sinuses with sperm. The reproductive function of this stage is male (Fig. 3a, b).

BT: transitional fish, changing from an adult female to an adult male were defined as individuals with degenerating vitellogenic, or later stage, oocytes (indicating prior female function) and spermatogenic tissue of different stages of spermatogenesis.

RESULTS

The gonad morphology was similar in all species examined and is described in detail along with a

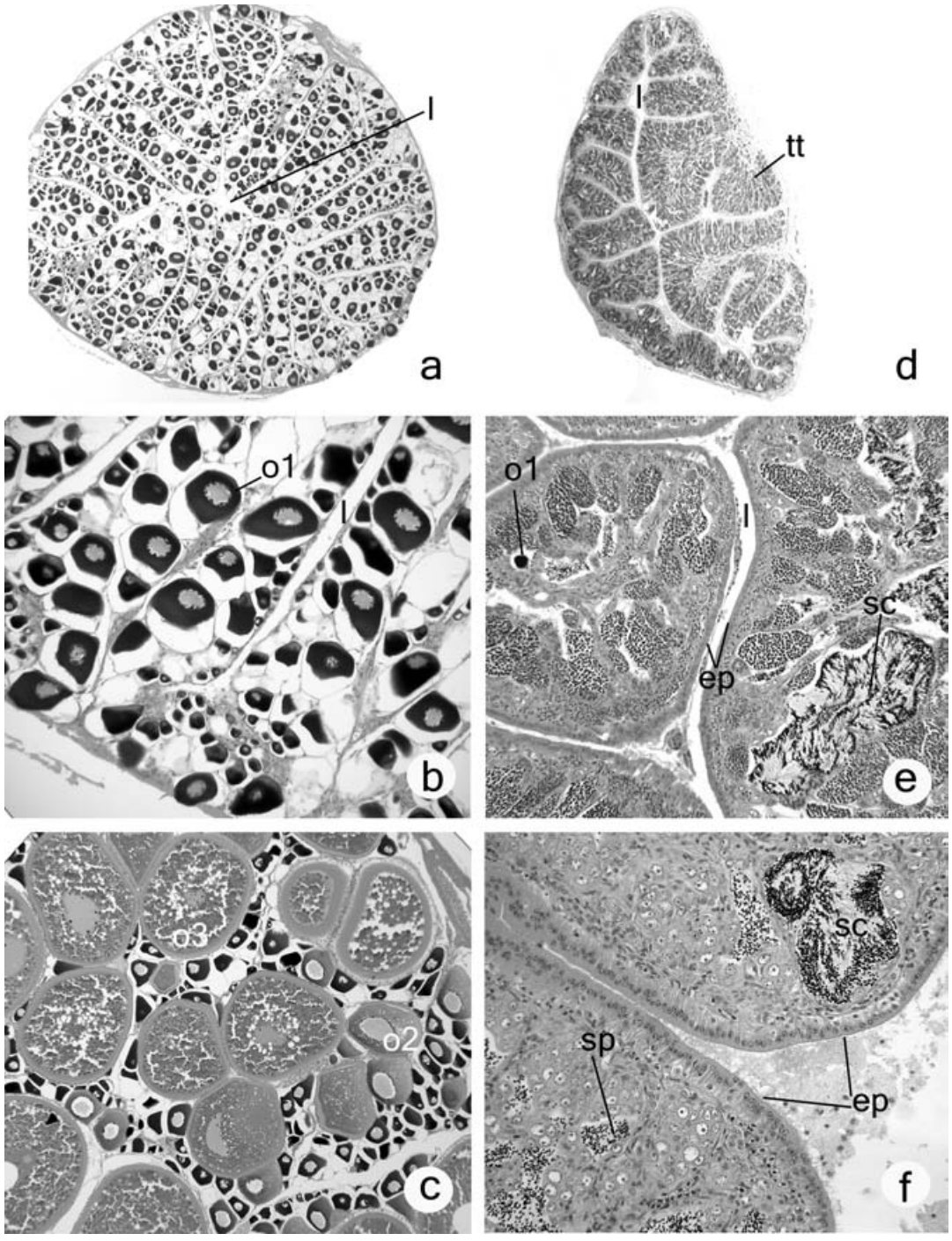

Fig. 1. Transverse sections of gonads. (a) Immature/inactive (F1) female *Paralabrax auroguttatus* (mag. × 40), SL = 187 mm; (b) immature/inactive (F1) female *P. auroguttatus* (mag. × 200) – detail of (a); (c) mature active (F2) female *P. auroguttatus* (mag. × 100), SL = 270 mm; (d) inactive male (M1) *P. maculatofasciatus* (mag. × 40), SL = 181 mm; (e) mature, active (M2) male *P. auroguttatus* (mag. × 200), SL = 300 mm; (f) mature, active (M2) male *P. auroguttatus* (mag. × 400), SL = 301 mm. ep, Epithelium; l, ovarian lumen; o1, primary stage oocyte; o2, cortical alveolus stage oocyte; o3, vitellogenic oocyte; sc, sperm in cyst (sperm tails are the pale grey area); sp, spermatogenic tissue; tt, testicular tissue.

hypothesized sequence of gonadal changes and sexual development for each species. Because we had very few individuals at or below the size of sexual maturation for either *P. clathratus* or *P. auroguttatus*, we were unable to fully evaluate sexually immature fish of these two species. However, similarities to the other two *Paralabrax* species examined suggest like gonad development in all species.

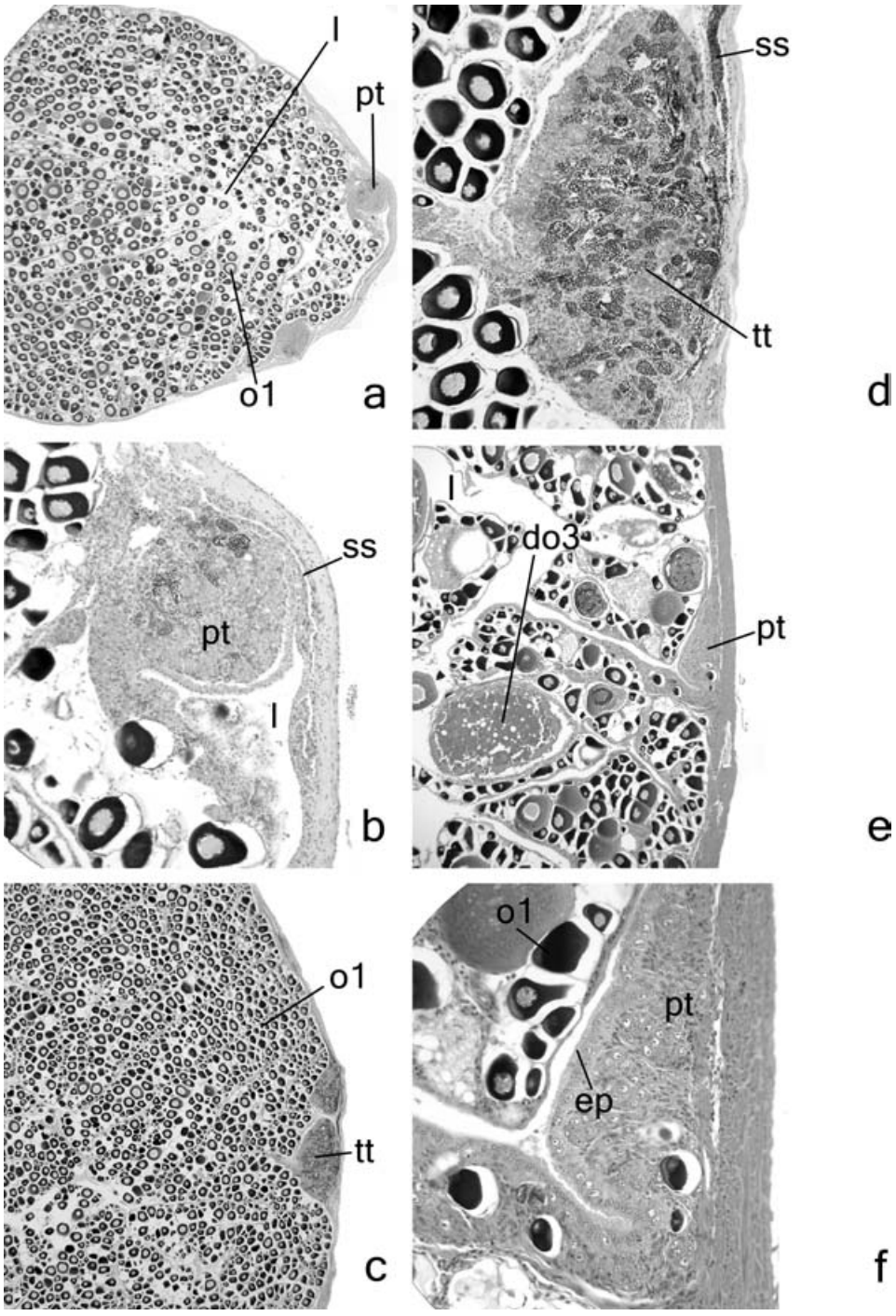

Fig. 2. Transverse sections of gonads. (a) Bisexual juvenile with presumptive testicular tissue (BIa) *Paralabrax maculatofasciatus* (mag. × 40), SL = 186 mm; (b) bisexual juvenile with presumptive testicular tissue (BIa) *P. maculatofasciatus* (mag. × 200) – detail of (a); (c) bisexual juvenile with testicular tissue (BIb) *P. maculatofasciatus* (mag. × 40), SL = 178 mm; (d) bisexual juvenile with testicular tissue (BIb) *P. maculatofasciatus* (mag. × 200) – detail of (c); (e) mature bisexual female (BF) with presumptive testicular tissue *P. auroguttatus* (mag. × 100), SL = 192 mm; (f) mature bisexual female (BF) with presumptive testicular tissue *P. auroguttatus* (mag. × 400) – detail of (e). Do3, Degenerating vitellogenic stage oocyte; ep, epithelium; l, ovarian lumen; o1, previtellogenic stage oocyte; pt, presumptive testicular tissue; ss, sperm sinus; tt, testicular tissue.

Gonad morphology – all species

The ovary is similar to that of most serranids, with two lobes fused posteriorly (Smith, 1965). The lamellae contain oocytes and project into the ovarian lumen and all gonads have a distinct alamellar section roughly opposite the dorsal blood vessel. In many individuals of various sizes and states of sexual maturation, presumptive

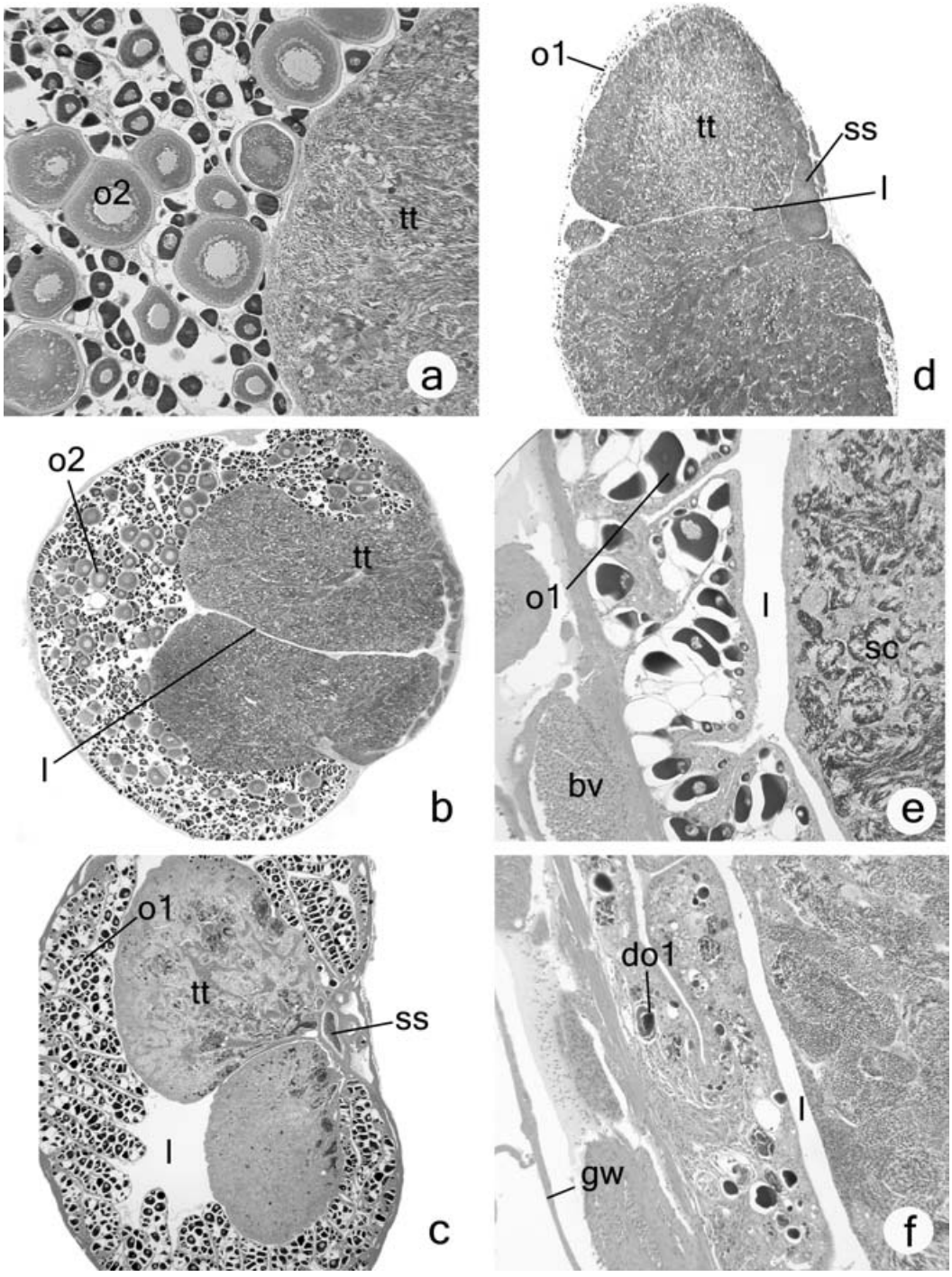

Fig. 3. Transverse sections of gonads. (a) Mature bisexual male (BM) *Paralabrax maculatofasciatus* (mag. × 100) – detail of (b); (b) mature bisexual male (BM) *P. maculatofasciatus* (mag. × 20), SL = 177 mm; (c) mature bisexual male (BM) *P. auroguttatus* (mag. × 40), SL = 256 mm; (d) mature bisexual male (BM) *P. maculatofasciatus* showing band of oocytes at gonad margin (mag. × 20), SL = 183 mm; (e) mature bisexual male (BM) *P. auroguttatus* showing band of oocytes (mag. × 200), SL = 238; (f) mature male (M2) *P. auroguttatus* showing a few remnant degenerating oocytes (mag. × 200), SL = 259 mm. bv, Blood vessel; do1, degenerating previtellogenic stage oocyte; gw, gonad wall; l, ovarian lumen; o1, primary oocyte; o2, cortical alveolus stage oocyte; sc, sperm in cyst; ss, sperm sinus; tt, testicular tissue.

testicular tissue, in the form of a distinctive group of cells, and sometimes with gonia, arise ventrally within the gonad at the two points of junction between the alamellar gonad wall and the lamellae (Fig. 2a, b), and run as bands laterally and medially along the gonad. Evidence that this band of tissue is presumptive testicular tissue (BIa) comes from the following characteristics: it occurs in the same location as developing testicular tissue in other fish (Fig. 2c, d)

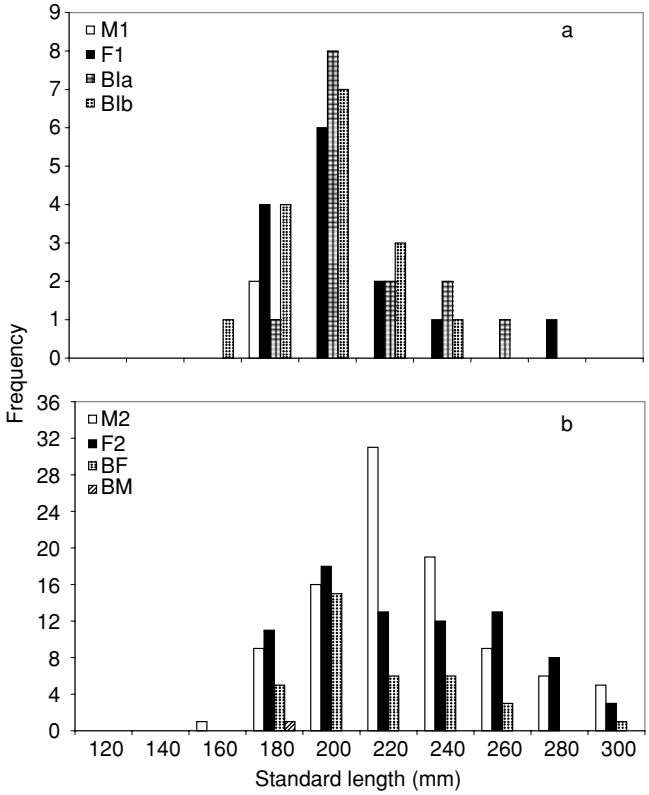

Fig. 4. Size frequency distributions of all stages determined histologically for *Paralabrax maculatofasciatus*. Details of sample sizes are given in Table 1. M1, inactive male; M2, mature, active or recently post-spawned male; F1, immature or inactive female; F2, mature, active or recently post-spawned female; BIa, immature/inactive bisexual fish with presumptive testicular islets; BIb, immature/inactive bisexual fish with testicular islets; BF, mature female bisexual with mature ovarian tissue and testicular islets or presumptive testicular islets; BM, mature male bisexual with previtellogenic oocytes and spermatogenic tissue and sperm sinuses.

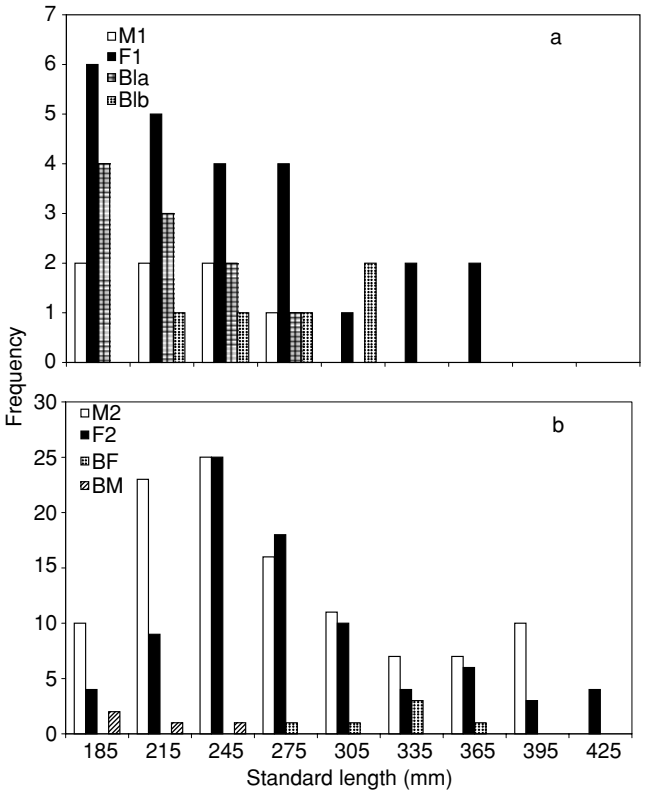

Fig. 5. Size frequency distributions of all stages determined histologically for *Paralabrax nebulifer*. For abbreviations see Fig. 4. Details of sample sizes are given in Table 1.

and nowhere else, it is sometimes interspersed with recognizable testicular tissue, and the size range of fish in which it occurs overlapped with that of bisexual fish which had clearly recognizable testicular islets (BIb) (Figs. 3, 4a and 5a). In gonads that were predominantly ovarian (i.e. bisexual but functionally female – BF), presumptive or early testicular tissue tended to be limited to the mid section of the gonad (Fig. 2e, f) and, therefore, not visible in all sections, as determined from serially sectioned gonads. Amongst both immature and mature females of all sizes, spermatogenic, or presumptive testicular, tissue was visible in the majority of gonads; it is likely that sectioning simply missed the testicular tissue in larger, especially ripe, gonads and that most, if not all, ovaries retain some testicular component from the bisexual juvenile phase throughout much of their adult life. All males had a distinctive central cavity (Fig. 1d, e), a lamellar structure and multi-chambered sperm sinuses within the gonadal wall and surrounding much of the gonad. Spermatozoa in all species were arranged in cysts with their heads oriented distinctly in a single direction, toward the periphery of the cyst; this feature has not been reported from any other serranid genus (Fig. 1e, f). The layer of epithelial cells lining the central cavity was initially associated only with testicular islets or presumptive testicular islets but expanded throughout the gonad, together with expanding testicular tissue, until it lined the gonadal lamellae that had filled with testicular tissue (Fig. 1f, 2f). In larger males, the ovarian lumen was visible but very little ovarian tissue was retained, other than the occasional, small, scattered previtellogenic oocyte (Fig. 1e). No individuals in sexual transition were confirmed in any species and the separation between male and female tissue was generally clear.

Hypothesized sequence of gonadal development and sexual maturation

Based on gonadal morphology in association with size and sex in all species examined, a sequence of gonad development is proposed for the four species of *Paralabrax* sampled by us. All species in this study had similar gonad development patterns, differing only in sizes of sexual maturation and at which different stages were apparent (Table 1). All four study species, *P. maculatofasciatus* (Fig. 4a, b), *P. nebulifer* (Fig. 5a, b), *P. clathratus* (Fig. 6a, b)

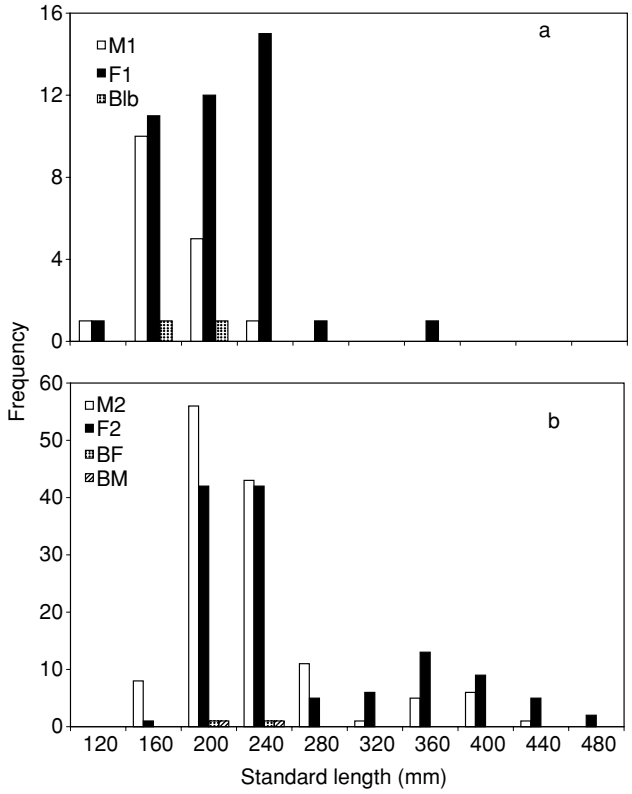

Fig. 6. Size frequency distributions of all stages determined histologically for *Paralabrax clathratus*. For abbreviations see Fig. 4. Details of sample sizes are given in Table 1.

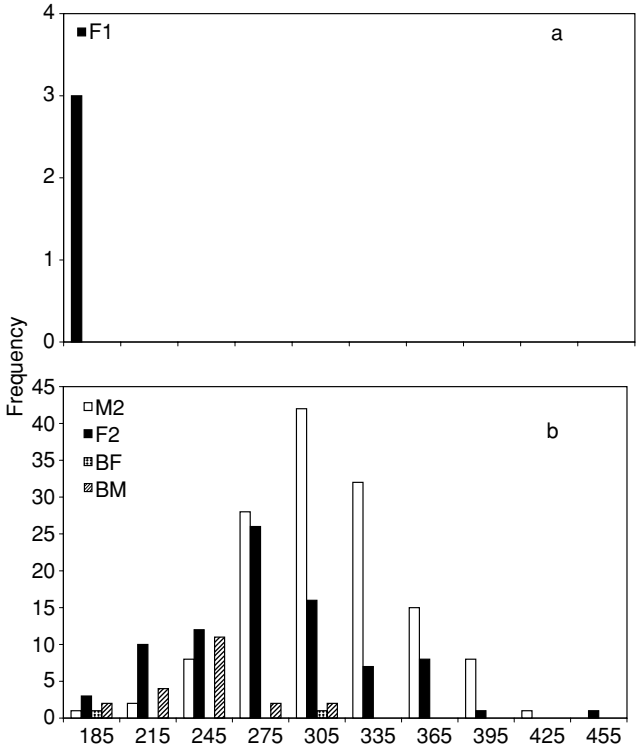

Fig. 7. Size frequency distributions of all stages determined histologically for *Paralabrax auroguttatus*. For abbreviations see Fig. 4. Details of sample sizes are given in Table 1.

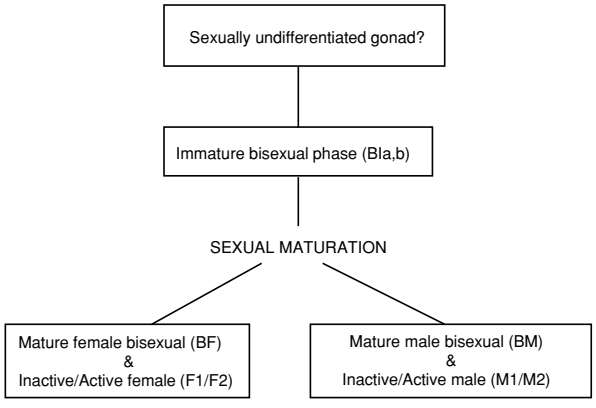

Fig. 8. Hypothesized schemata of gonad development in *Paralabrax* spp. based the present study. Further histological work is needed on sexually immature fish to understand the earliest stages of gonadal development.

and *P. auroguttatus* (Fig. 7a, b) were determined to be functionally gonochoristic. The likely developmental pathway (Fig. 8), based on this study, and assuming that *P. clathratus* and *P. auroguttatus* development is similar prior to sexual maturation, suggests that fish pass through a juvenile (non-functional) morphologically bisexual phase (BI) in which male and female tissue occur separately in distinct areas of the gonad (Fig. 2a, b). Bisexual juveniles then develop in either a male (Fig. 3c, d) or female (Fig. 2e, f) direction and elements of the early bisexual phase may be retained into adulthood (Figs. 2e, f & 3d, e). Tissue of the non-functional sex diminishes with increase in body size (Figs. 3f, 4b, 5b, 6b & 8b). This suggests the possibility that a potential for functional sex change is retained, although no transitionals (BT) were noted. All individuals of both sexes retain an ovarian lumen derived from the initial bisexual phase, although in mature males this may sometimes become occluded as the lamellae press closely together. In such cases, careful examination will reveal the distinctive epithelial layer separating the testicular tissue from the former cavity. In functional males, the testicular section of the bisexual juvenile phase proliferates from the testicular islets, ovarian tissue regresses and sperm sinuses develop in the gonad wall (Figs. 2d & 3d). In functional females, the oocytes mature and expand, while the presumptive testicular tissue or islets of early spermatogenic tissue remain small.

DISCUSSION

We must begin the discussion with a clarification on terminology. The term ‘secondary gonochore’ has been used in the literature to describe an evolutionarily derived gonad form, and gonochoristic reproductive function. It is necessary to separate clearly function from inferred phylogeny. With respect to reproductive *function*, we use the term ‘secondary’ for males developed from functional females, with ‘primary’ males developing directly from

Table 1. Summary of samples taken and samples prepared histologically for four species of *Paralabrax*, showing size ranges, maximum known size for the species, size of sexual maturation determined from the specimens, incidence of bisexual fish and adult sex ratios. [a] Histologically prepared samples only. TL, total length; SL, standard length; F, female; M, male; BI, immature or inactive bisexual; BF, mature female bisexual; BM, mature male bisexual

Species (common name)	Total *n* sampled	Maximum known size (mm TL)	Size range (histologically prepared samples only)	Minimum size of maturation (mm SL)		Bisexuals			Adult sex ratio[a] (F:M)
				F	M	BI	BF	BM	
P. auroguttatus (goldspotted sand bass)	247	720	178–452 (F) 176–419 (M) $n = 247$	178	176	0	2	21	0.61
P. clathratus (kelp bass)	521	720	121–486 (F) 114–426 (M) $n = 321$	174	142	2	2	2	0.95
P. maculatofasciatus (sand bass)	255	600	170–297 (F) 158–296 (M) $n = 255$	170	158	30	36	1	0.81
P. nebulifer (barred sand bass)	364	670	163–422 (F) 176–409 (M) $n = 248$	181	177	15	6	4	0.76

the juvenile form. It is important to note that the testes of these two male 'types' in the serranids appear to be similar in morphology. With respect to phylogeny, we use the traditional evolutionary terms 'ancestral' and 'derived' as descriptors of the inferred evolutionary derivation.

All four species of *Paralabrax* were determined to be functionally gonochoristic, with a non-functional bisexual juvenile phase of gonad development and clear separation of male and female tissues. Are these ancestral or derived gonochores from a phylogenetic perspective? If *Paralabrax* has a functionally hermaphroditic ancestor (Smith, 1965), then the four *Paralabrax* species are derived gonochores, as proposed for *P. clathratus* (Smith & Young, 1966) If, however, the ancestral *Paralabrax* gonad was not functionally hermaphroditic, but rather a pre-hermaphroditic state with both male and female tissue present in early development and only one or the other tissue type functional after sexual maturation, then it might be more appropriate to consider these *Paralabrax* species to be ancestral gonochores. Proposing, thus, two possible routes to gonochorism in the serranids would distinguish gonochorism and differences in gonad morphology and sexual pattern in *Paralabrax* spp. from those of the gonochorism found in the more derived epinephelines (such as *Epinephelus striatus;* Sadovy & Colin, 1995). Under such a scenario, gonochorism would be considered to be derived only in more advanced groups, such as the epinephelinae, and ancestral in the basal genus *Paralabrax*. These two proposed pathways to gonochorism have not been developed with any reference to other phylogenetic characteristics, but the hypothesis could explain observed differences in gonad morphology among phylogenetically distinct gonochoristic serranids, and is consistent with serranid phylogeny as we currently understand it.

The simultaneous hermaphroditism of the serranines, under this scenario, could have developed from a prehermaphroditic condition through physiological developments that allowed the sexual maturation of tissues of both sexes simultaneously in the same gonad, while maintaining the physical separation of male and female tissue found in all studied serranines (e.g. *Serranus, Diplectrum* and *Hypoplectrus* species, etc.). Hints of the sequential hermaphroditism (protogyny) that seems to typify derived serranids, appear in several species of *Serranus* that occasionally exhibit a male phase following an earlier simultaneous phase (Bruslé, 1983; Hastings & Petersen, 1986; Petersen & Fischer, 1996). This sequential sex change tends to be associated with behavioural mediation, which may itself be a derived condition, of sex change. Most studied anthiines and epinephelines exhibit protogyny, while we see a trend from the physical separation of ovarian and testicular tissue in the serranines, including in *Paralabrax*, to various mixtures of separation and integration of the sex tissues in the anthiines on the one hand, and their complete integration in the epinephelines, on the other (Smith, 1965; Hastings, 1981).

Sexual pattern is particularly difficult to diagnose in the Serranidae for three reasons. First, all individuals appear to go through a developmental phase that includes oocytes and an ovarian-like lumen, making primary and secondary males impossible to distinguish by testicular morphology alone. Second, among the serranines and some anthiines, testicular tissue arises in discrete islets, only visible in some areas of the gonad and thus difficult to detect if the gonad is not carefully examined throughout its length. Third, there appears to be considerable phenotypic plasticity of sexual expression in this family, with functional sexual patterns differing over time (e.g. some *Serranus* spp. change from simultaneous to sequential hermaphrodites with increase in body size (Hastings & Petersen, 1986)), or across space, as in *Plectropomus leopardus* (Adams *et al*., 2000). Male

to female sex change has been recorded in captivity in *Epinephelus akaara* (Tanaka *et al*., 1990) and in *Cephalopholis boenak* (Liu & Sadovy, 2004); whether this occurs in the field is not known. Moreover, given the apparent plasticity of sexual expression indicated in some species, experimental induction of female to male sex change under laboratory conditions cannot be used as sole determinant of the typical sexual pattern expressed in the wild, nor the assumption be made that all populations of a species exhibit the same sexual pattern. Finally, gonochorism can evidently derive in more than one way within a given phylogeny, with different gonad configuration of male and female tissue and suggesting that functional sexual pattern is not a reliable taxonomic character.

The diagnosis of sexual pattern in several *Paralabrax* spp. has posed problems for 40 years, largely because of differing interpretations of histological criteria and because of the other difficulties already discussed. Importantly, and of broader relevance to the study of hermaphroditism in fishes, these problems also illustrate lessons learned since criteria to determine sex change in fishes were published in 1987 (Sadovy & Shapiro, 1987). Clearly it is now time to refine some of these criteria as they apply to families like the Serranidae. *Paralabrax maculatofasciatus* is the most extensively studied *Paralabrax* species and provides an excellent example of some of the challenges of diagnosing sexual patterns in the Serranidae. Although fish undergoing transition were reported by both Hastings (1989; 33 fish prepared histologically, three cited as transitional) and by Hovey & Allen (2000; 1155 fish prepared histologically; five cited as transitional), the photos in both publications illustrating this phase do not show sexually transitional fish. Fish undergoing transition from adult female to adult male need to show clear evidence of former female function (e.g. degenerating vitellogenic oocytes or other signs of former female function) to indicate that adult transition is occurring. The concomitant occurrence of male and inactive female tissue depicted in the plates probably reflect, instead, the bisexual juvenile phase. Moreover, in the latter study, the use of yellow-brown pigment bodies as indications of sex change is not valid, as argued in Sadovy & Shapiro (1987: 146); such bodies may derive in many organs and are accumulations of pigment from a range of processes, only one of which involves vitellogenic oocyte degeneration. On the basis of evidence provided, therefore, and bearing in mind what we have come to learn of early bisexual development in the genus, and the presence of an ovarian lumen in all male serranids, we consider that female to male adult sex change was not demonstrated in either case by the histological features presented.

In the Hovey & Allen (2000) study, *P. maculatofasciatus* was diagnosed as having diandric origins with gonochorism and protogyny variously expressed in six different populations (two protogynous; four gonochoristic); we believe that insufficient evidence was provided for diandry on two counts. The first is that the definition of lumen applied excluded any cavity not lined by squamous epithelial cells, and any testis without a lined cavity was therefore considered to be a primary male. This resulted in the apparent absence of a true ovarian lumen in a large proportion of males (up to 99% in some populations). This is not only inconsistent with Reinboth (1970) but also with the studies of Hastings (1989) and ourselves in this species, both also noting that all males had an ovarian-like lumen. The testicular area in several *Paralabrax* species is closely associated with a distinctive epithelial layer, not found in association with ovarian tissue and we believe that this possibly led to an erroneous distinction of primary and secondary males by Hovey & Allen (2000) using the epithelial characteristic (Fig. 1f). The second problem was that sperm sinuses were reported in up to 63% of the mature females sampled (different populations showing different percentages); our histological analyses showed that functional females never had a sperm sinus and we suggest that these are only found in functional males, and in bisexual juveniles or mature bisexual males. In conclusion, we suggest that there is no published evidence showing adult sex change in *P. maculatofasciatus*; our own specimens of this species were taken from San Diego Bay, one of the two protogynous populations identified in the Hovey & Allen (2000) study.

We suggest that there is no published evidence to date showing adult sex change in any *Paralabrax* species. This is not to say that sex change never occurs, for our own study also fell short of what we would consider to be an ideal sampling protocol (see below). However, based on histological grounds and size-frequency data, we suggest that protogyny does not occur, or is likely to be uncommon, naturally. Problems we encountered in our study were an inability to sample fish well below the size of sexual maturation, as well as the lack of larger fish and those outside of the reproductive seasons for several study species. Since fish undergoing sexual transition in many species tend to be found between reproductive seasons, this could have reduced our chances of encountering transitional individuals. None the less, the sizes of mature fish we sampled are not dissimilar from those in other studies on this genus and gonochorism has also been diagnosed in *P. clathratus* (Smith & Young, 1966) and *P. auroguttatus* (Pondella *et al*., 2001). *Paralabrax humeralis* was also considered to be gonochoristic, with males developing via a juvenile bisexual phase (Borquez, Olivares & Tapia, 1988). Absence of very small fish in all of these studies as well as our own mean that it is not possible to know what stage may precede the bisexual juvenile phase. The significance of the distinct epithelial border associated with testicular tissue and the distinctive orientation of the sperm in this genus has not been reported in other serranid genera and suggests a close evolutionary relationship among *Paralabrax* spp. investigated (Hastings, 1989).

From the histological evidence of the present and earlier studies, and an overview of sexual patterns and gonad morphology in the family Serranidae, we conclude that *certain* aspects of gonad morphology, such as relative position and degree of intermixing, of male and female tissues are of value for phylogenetic studies in the family. However, the plasticity of sexual patterns *per se*, even

at the level of the genus, over time and space, limit their value for such studies and also argue strongly for particular care in diagnosing sexual patterns in the Serranidae. An understanding of the adaptive significance of sexual patterns requires the application of clear criteria and careful study. In 1987, a set of criteria was developed to diagnose sexual patterns in fishes (Sadovy & Shapiro, 1987). The need to apply several (rather than just a single) characteristics, and the importance of histology, were emphasized and finds further strong support in the history of work on *Paralabrax* spp.

Two characters, in particular, need careful consideration in diagnosing functional sexual pattern and are reiterated here. Adult fish undergoing female to male transition are typically recognizable by the presence of unovulated atretic oocytes at stages that clearly reflect their prior vitellogenesis (yolky state), together with spermatogenic tissue (Sadovy & Shapiro, 1987: 146). For identifying adult sex change, maturation of the first sex must be demonstrated – the atresia of non-yolky ova cannot be used to identify a transitional fish since these are known to appear in gonochore males as well as immature females (Liu & Sadovy, 2004). The second character is the presence or absence of a lumen in a testis often used to distinguish developmentally primary from secondary males. In many families this does indicate a former ovarian state; however, it is not an infallible indicator of adult female to male sex change (Sadovy & Shapiro, 1987: 143; Fennessy & Sadovy, 2002; Adams, 2003; Liu & Sadovy, 2004), since in some families, such as the Serranidae, all testes bear a lumen, irrespective of former sexual function and testis structure, and thus, gives no indication of male developmental pathway. Males with solid testes and no ovarian lumen – the primary males of labrids and scarids – are not known in the Serranidae.

Finally, given the marvellous diversity of sexual patterns and developmental pathways found in fishes, it is of utmost importance to apply descriptive terminology that is neutral and does not inadvertently infer a particular developmental pathway or sexual pattern that has not been demonstrated. As just one of several possible examples, the term 'pre-maturational sex change', to us at least, infers that individuals destined for one sex have changed to the other before sexual maturation. The term has been applied to account for small males (similar to or below the size of female sexual maturation) in which the testes bear a lumen. In such cases, sex change before sexual maturation has not been demonstrated and an equally possible explanation is that a lumen arises as a natural part of early gonad development and has nothing to do with sex change. The problem is that the term influences the way in which we think about this particular phenomenon and in this it is a distraction. In determining sexual pattern in serranids, therefore, it is essential to apply several types of diagnostic criteria, select a large sample of fish of a broad enough size range, ideally including juveniles and taken throughout the year, to conduct a thorough histological examination of sections along the lengths of the gonads, and to apply suitably neutral descriptive terminology. Only thus can we prepare ourselves for the surprises with which fishes will no doubt continue to delight us, as far as their sex lives are concerned.

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