

Protogynous hermaphroditism in *Chrysolephus laticeps* (Cuvier) and *C. cristiceps* (Cuvier) (Teleostei: Sparidae)

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A histological study of the gonads of two sparid fishes, *Chrysolephus laticeps* and *C. cristiceps* showed that these species are protogynous hermaphrodites. Both species are also monandric in which all males are derived from functional females. Intersexual individuals were restricted to a narrow size range between females and males, and no evidence for simultaneous hermaphroditism was found. Similar to other sparids, the development of the ovary and testis was delimited by a connective tissue layer.

'n Histologiese studie van die geslagskliere van twee visse van die Sparidae, *Chrysolephus laticeps* en *C. cristiceps*, het bewys dat beide spesies protoginies dubbelslagtig is. Albei is monandries, alle mannetjies was voorheen funksionele wyfies en geen blyke van gelyktydige dubbelslagtigheid is gevind nie. Individue wat besig was om geslag te verander was beperk tot 'n klein lengterêeks tussen mannetjies en wyfies. Soos in ander visse van dié familie is die ontwikkeling van die ovarium en die testis deur bindweefsel afgebaken.

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The occurrence of hermaphroditism in fishes is well documented and may have arisen independently in as many as 10 lineages (Reinboth 1970; Smith 1975; Warner 1978). New accounts are continually being reported and in certain families, for example, Sparidae, Scaridae, Scarinae and Serranidae, hermaphroditism is the rule rather than the exception.

Diverse expressions of sexuality are found in the Sparidae (sea breams or porgies), which exhibit protogyny, protandry, rudimentary hermaphroditism and true gonochorism (Atz 1964; Alexseev 1982; Buxton & Garratt 1989). Two general patterns of sexual ontogeny may be recognized in this family. The gonad may differentiate and function first as one sex and then the other (protogyny and protandry), otherwise maturation of either the testis or the ovary follows a stage of juvenile intersexuality where non-functional elements of both sexes are recognizable (rudimentary hermaphroditism). Most of the sparid species studied so far appear to be sequential hermaphrodites.

Protogynous hermaphroditism has been reported for *Chrysolephus laticeps* by Penrith (1972), and for *C. cristiceps* by Robinson (1976), both authors presenting bimodal length frequencies as evidence for sex change. The object of this study was a histological examination of the development of the reproductive organs of these fish, aimed at confirming sex change in the species.

Materials and Methods

Data were collected between June 1984 and May 1986 in the Tsitsikamma Coastal National Park, a marine sanctuary extending 5 km offshore and covering a 60 km stretch of the southern Cape coast, between Nature's Valley (34°59'S/23°34'E) and Oubosstrand (34°04'S/24°12'E).

Most specimens were obtained by handline fishing although some, particularly the smaller size classes, were

spearred. Fish were measured and sexed using macroscopic visual criteria (Table 1). Gonads were removed from a sub-sample of the catch that represented the full size range of fishes taken each month. These tissues were fixed in Bouin's solution for three days and stored in 50% propyl-alcohol. Following this they were embedded in wax, sectioned at 7 µm and stained with Harris' haematoxylin and Eosin-Y for histological examination. The total histological sample size was 109 and 104 individuals for *C. laticeps* and *C. cristiceps* respectively. Sections were taken from the anterior, mid and posterior areas of each gonad. Particular attention was paid to the posterior part of the gonad where development of the testis takes place. In addition, serial sections of immature gonads and inactive females were done to search for the first development of the testis.

Results

Length frequency and sex ratio

The length frequencies of all *C. laticeps* sampled are shown in Figure 1a. Sexes were separated on the basis of macroscopic sex determination and the ratio of male : intersex : female was 1 : 0,58 : 1,11. Fish in the process of changing sex (intersexes) were recorded between 250 and 425 mm fork length with a mean of 321 ± 29 mm.

A similar plot for *C. cristiceps* is shown in Figure 1b. The ratio of male : intersex : female was 1 : 1 : 4,22 and the mean size of intersexes was 418 ± 45 mm, ranging between 280 and 525 mm fork length.

Reproductive cycle

The development of the gonad from immaturity, through the ovarian cycle, sex change and finally to the male phase was so similar in the two species that the following description applies to both of them unless specifically stated otherwise.

Table 1 Classification of maturity stages in *Chrysoblephus laticeps* and *C. cristiceps* (modified from Buxton & Clarke 1986)

Maturity stage	Description
1. Virgin and resting	Sexual organs small. Testis thin and transparent to greyish white. Ovary long and thin, pink in colour with no visible eggs.
2. Developing	Both male and female tissues increase in size. Testis shows a lateral thickening, a marginal increase in length and is a greyish white colour. Sperm visible in the main sperm duct if cut. The ovary increases in size, particularly on the long axis, to half or more of the visceral cavity length. Colour changes from pink to reddish orange and eggs are visible to the naked eye.
3. Active	Testis greyish white with sperm in the main sperm duct and the tissue. As the breeding season progresses the testis becomes pinkish. Ovary is swollen and yellow-orange and translucent eggs are visible in the tissue and lumen. May be slightly flaccid and bloodshot if spawning has taken place.
4. Post spawning	Testis decreases marginally in size, is reddish-grey in colour with no sperm in the tissue but still present in the main sperm duct. Ovary decreases considerably in size, is flaccid and has a large empty lumen. Reddish-orange colour with few clear eggs visible.

The ovaries are paired structures, fusing posteriorly and emptying into a single short oviduct. They are suspended in the body cavity within a thin double layer of peritoneum, the mesovarium, beneath which is the ovary wall, the tunica albuginea. The composition of the tunica albuginea, which is fairly uniform in most teleosts (van der Horst 1976), consisted of smooth muscle layers, connective tissue and blood vessels. Ovigerous lamellae which arise from the internal epithelial layer, projected from the tunica albuginea and filled the ovary except for a central ovocoel which was continuous with the oviduct (Figure 2a). Although the work of D'Ancona (1949) and Alexseev (1982) suggests that a testicular primordium forms in the early stages of gonadal development in sparids, serial sections of the ovaries of young fish showed no evidence of differentiated male tissue.

During the breeding season there was a marked increase in the size of the ovary which, when ripe, occupied approximately two thirds of the body cavity. Following spawning the ovaries either regress to a resting stage or additional batches of eggs are ripened

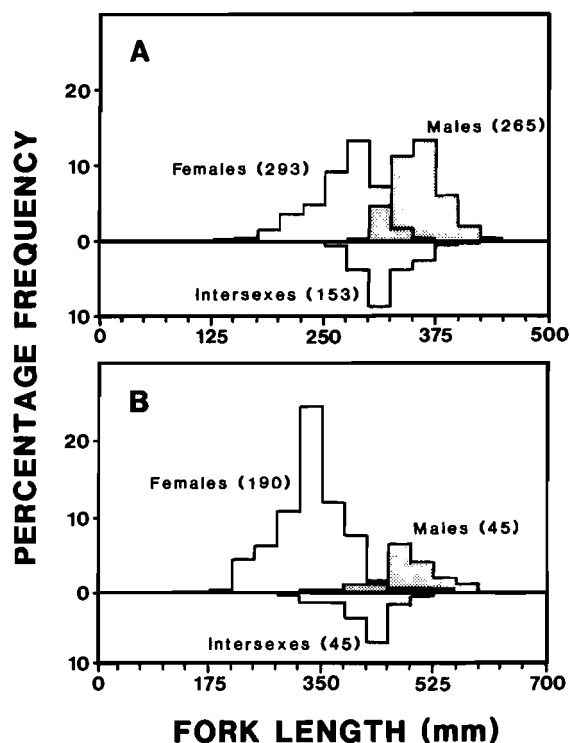


Figure 1 Sex separated length frequency analysis of *Chrysoblephus laticeps* (A) and *C. cristiceps* (B) sampled in the Tsitsikamma area between 1984 and 1986. Males are shaded and sample sizes are given in parenthesis.

and spawned. The first appearance of testicular tissue occurred at the postero-ventral part of the ovary, where it formed a thin sheet (Figure 2b). This occurred at approximately 250 mm fork length in *C. laticeps* and 280 mm fork length in *C. cristiceps*. During the initial phase of testicular development the ovary remained functional (Figure 2c), but immediately following spawning, development of the testis accelerated with a corresponding degeneration of the ovary.

Sex changing individuals were recorded throughout the year. Most of these intersexes were either functional females showing early testicular development or functional males with a degenerating ovary. Only 1.5% of intersexes had a non-functional ovo-testis with an equal development of the two sexes. This suggested that sex change was a fairly rapid process (cf. Alexseev 1982). Once sperm production began there was no evidence of continued ovarian activity and ultimately the ovary degenerated to a mass of connective tissue containing a number of 'brown bodies' (Figure 2d & e) characteristic of degenerating ovarian tissue (Smith 1965; Young & Martin 1982). Ovarian rudiments, particularly of the oviduct, were evident throughout testicular development.

The testes are paired structures, triangular in cross-section and consisted of a number of seminiferous tubules leading into secondary sperm ducts. Posteriorly these ducts join to form a common main sperm duct. Each testis is surrounded by a tunica albuginea of connective tissue, collagen and smooth muscle which appeared to be continuous with the seminiferous tubules

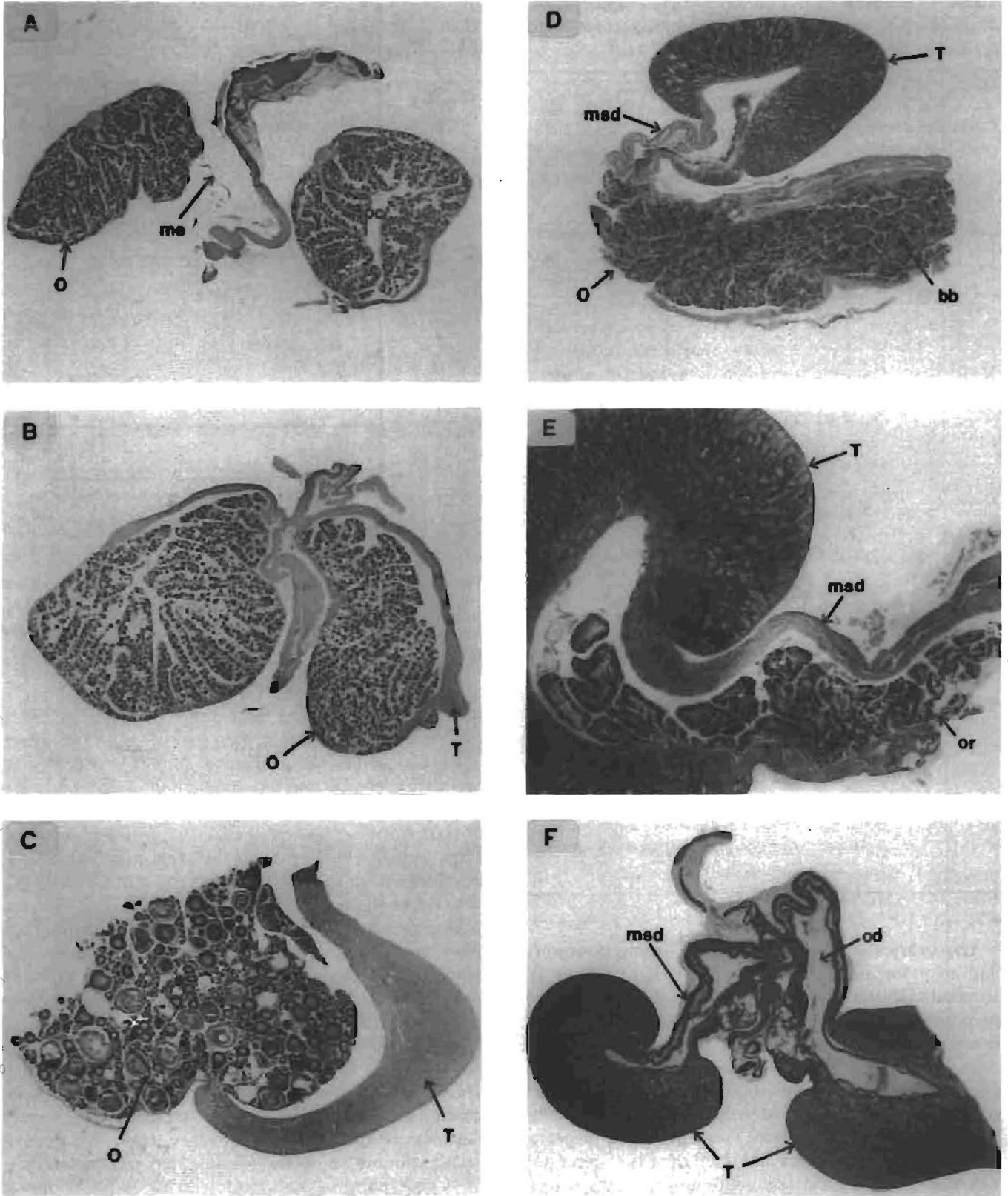


Figure 2 Development of the gonad in *Chrysoblephus laticeps* and *C. crisuiceps*. (A) Transverse section through inactive ovaries ($\times 11,0$). (B) Transverse section through the ovo-testis of an inactive functional female, showing the early development of the testis ($\times 13,0$). (C) Transverse section through the ovo-testis of a functional female showing an active ovary and an inactive testis ($\times 12,5$). (D) Transverse section through the ovo-testis of an inactive functional male showing the testis and the degenerating ovary containing numerous brown bodies ($\times 6,5$). (E) Transverse section through the ovo-testis of a functional male, showing a mature testis with sperm in the main sperm duct and a degenerate ovary ($\times 20,0$). (F) Transverse section through the testes of a functional male. Ovarian history only evident by a few brown bodies in the mesentery and by the rudimentary oviduct ($\times 10,0$). bb — brown bodies, me — mesentery, msd — main sperm duct, o — ovary, oc — ovocoel, od — rudimentary oviduct, or — ovarian remains, t — testis.

(Figure 2e & f). Evidence of a previous ovarian history was often apparent from the presence of brown bodies in the ovarian rudiments and the rudimentary oviduct (Figure 2f), although Sadovy & Shapiro (1987) caution that they may also be derived from other processes.

Discussion

Macroscopically the study species showed a bimodal length frequency distribution of smaller females and larger males, and intersexes were confined to a narrow length range between the sexes. Such a separation of the modal size of females and males has been accepted uncritically as evidence for sex change in a number of species including *C. laticeps* and *C. cristiceps* (Penrith 1972; Robinson 1976). Sadovy & Shapiro (1987) discuss this phenomenon in detail and conclude that bimodal length frequencies are a weak diagnostic criterion for sex change, and may equally result from differential growth and mortality between the sexes, spatial separation by sex and sampling error. Reinboth (1970) and Sadovy & Shapiro (1987) suggest that conclusive evidence for protogyny can only be obtained by careful histological examination of all stages in the reproductive cycle. The results presented in this study showed that in both *C. laticeps* and *C. cristiceps* all individuals functioned first as females before undergoing sex change, with no evidence of simultaneous hermaphroditism. Similar to other reports on hermaphroditic sparids (Atz 1964; Coetzee 1983; Garratt 1986), the ovary and the testis were separated by a connective tissue layer, the oviduct and main sperm duct remaining functionally separate throughout development. This differs from sex change in serranids, where the ovary is invaded by testicular elements (Smith 1965).

In many protogynous fishes two male ontogenies have been recognized and are related to the mating systems of the species studied (e.g. Choat & Robertson 1975; Robertson & Warner 1978; Warner & Robertson 1978; Robertson, Reinboth & Bruce 1982). One, monandry, prevails when all males are derived from sex-changing females. Mating usually takes place between large, territorial males and a group of females. The second type, diandry, includes primary males that develop directly from the juvenile stage, as well as secondary males that result from sex change. Again secondary males monopolize the mating by occupying preferred mating territories and mating sequentially with a number of females (Warner 1984). As with the monandric species, large size and resource defence are important determinants of mating frequency (Fischer & Petersen 1987). Primary males are aggressively excluded from most of the breeding but succeed either by interfering with the mating activities of larger males (joining a spawning couple at the moment of gamete release) or by occupying a spawning site *en masse* and group spawning with the females that appear there (Warner 1984). Primary males were not observed in *C. laticeps* and *C. cristiceps* either macroscopically or histologically and all males were considered to be derived from functional females. These observations differ from the findings of a

study on the congeneric *C. puniceus* found off Natal, South Africa. Garratt (1986) suggested that in this species small males could be regarded as primary males (i.e. direct development) and that large males were derived from females by sex reversal. No histological evidence was provided to confirm this observation. Considering the phenotypic plasticity exhibited by fish, evidenced by a change in the size at which sex change occurs in different populations (Shapiro 1981), the occurrence of primary males in protogynous members of the genus *Chrysoblephus* remains uncertain.

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