COMPARATIVE ASPECTS OF THE REPRODUCTIVE BIOLOGY OF SEABREAMS (PISCES: SPARIDAE)

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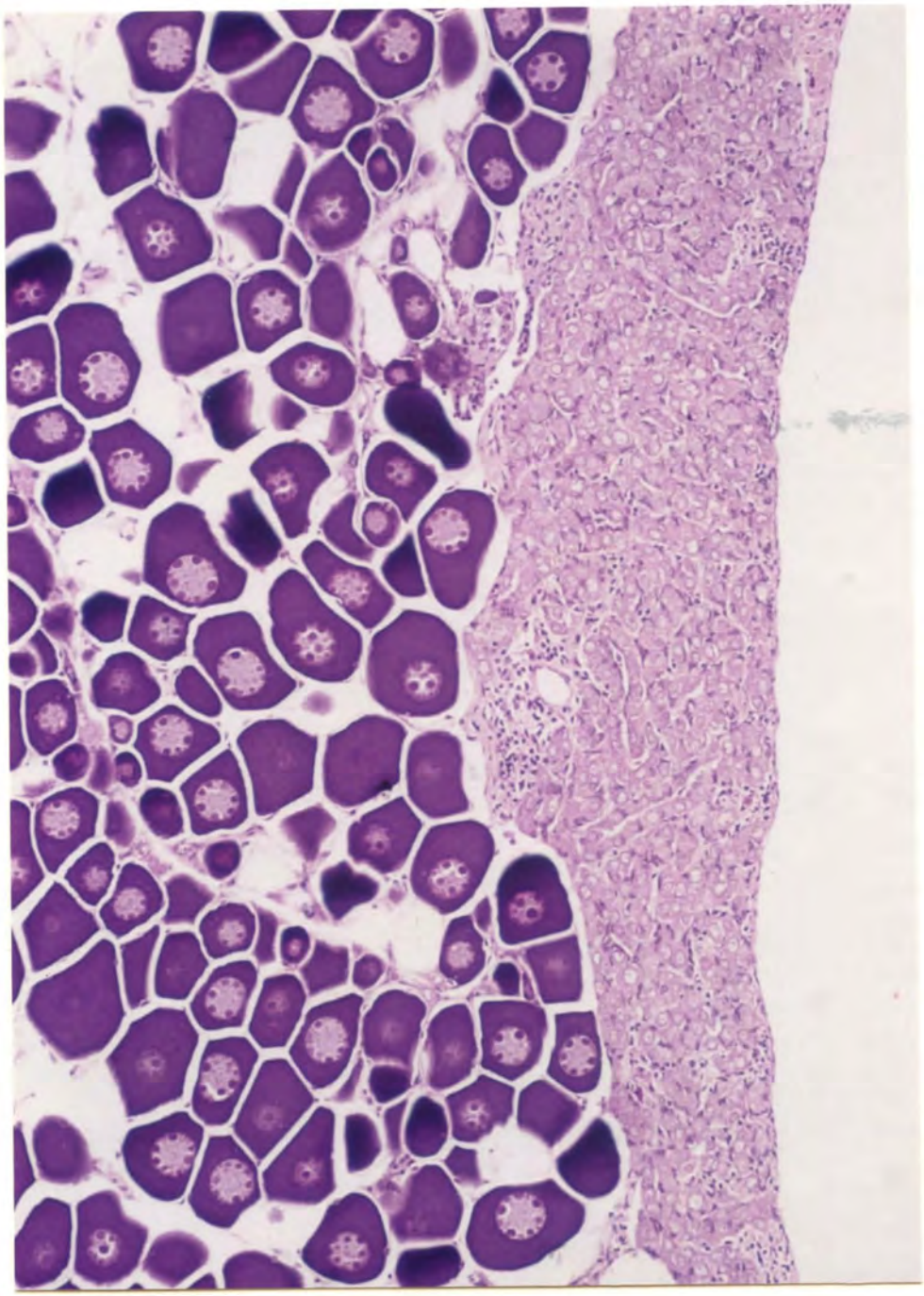

"Hermaphroditism finds its most complex expression among the sparid fishes".

James W. Atz, 1964.

Frontispiece: Gonial cells proliferating in the male element (right) of a predominantly female Cheimerius nufar gonad.

DEDICATED TO MY PARENTS

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ABSTRACT

Sexuality in seabreams (Sparidae) is considered to be more complex than in any other family of fishes. Early work indicated five reproductive styles within the family: protandry, protogyny, simultaneous hermaphroditism, rudimentary hermaphroditism and gonochorism. More recently two reproductive styles have been suggested: sex change (protandry and protogyny) and secondary gonochorism (rudimentary hermaphrodites). The need for detailed descriptions of sex differentiation, gonad development and spawning behaviour in this family has been identified by a number of workers in this field.

The aims of the present study were: i) to provide accurate, detailed descriptions and comparisons of gonadal development in representatives of each reproductive style, ii) to investigate their spawning strategies, and iii) to relate these findings to current theories on hermaphroditism and sex change in fishes. Four species were investigated. Slinger, Chrysoblephus puniceus, the only known protogynous hermaphrodite in Natal. Santer, Cheimerius nufar, described in the literature as a rudimentary hermaphrodite. Riverbream, Acanthopagrus berda, suspected to be a protandrous hermaphrodite. Natal stumpnose, Rhabdosargus sarba, reported elsewhere as a protandrous hermaphrodite.

Detailed histological analysis showed that morphological and cytological development of all gonads proceeded initially in a female direction, irrespective of reproductive style, but that differentiating gonads of protandrous and protogynous hermaphrodites could easily be distinguished from one another. Early gonadal development was similar in R. sarba and A. berda with gonadal primordia differentiating into distinctly bisexual organs. In C. puniceus and C. nufar gonadal primordia differentiated into ovaries with reduced, inert male elements in the tunica albuginea. Sex differentiation occurred relatively late (100-150mm fork length) in all the species investigated.

Few cells conforming to primordial germ cells (PGC's) described in other teleosts were identified. These cells only became evident after the appearance of gonial cells and their identity is questioned. Gonial cells appeared to develop within less-electron-dense cysts of cells. Gonial cells in presumptive male and female elements could not be distinguished from one another morphologically, suggesting the bipotentiality of these cells.

All R. sarba and A. berda gonads pass through a predominantly male phase and all fish function first as males, indicating protandrous sex change in both species. All C. puniceus and C. nufar gonads develop initially into ovaries. Sex change thus occurs in both species and protogyny in C. puniceus is confirmed. In C. nufar, sex change may occur before or after sexual maturity and its reproductive style remains uncertain.

Investigations into the spawning habits of A. berda have shown that this species spawns inside the Kosi estuary at night. Eggs are released during peak ebb tides. Spawning occurs in large aggregations and several to many males compete to spawn with individual females. This spawning strategy does not conform to predictions made from the size advantage model for protandrous species.

Chrysoblephus puniceus appears to have preferential spawning sites on down-current outer reef margins. Spawning was not observed in this species, but changes in behaviour, social structure and colour during the spawning season suggest that it may have a mating system similar to several protogynous labrids and scarids, in which territories are temporary. Cheimerius nufar has a similar mating system. Temporary territories are established by large males during the spawning season, which extends from August to November. Mating is by pair-spawning and dominant territorial males obtain a disproportionate number of matings. 'Streaking' appears to represent an alternative mating strategy for males until they attain a sufficient size to establish and defend territories. The mating pattern of C. nufar suggests that it is either a gonochorist which does not conform to current theoretical predictions; or that it is a protogynous hermaphrodite incorrectly diagnosed as a rudimentary hermaphrodite; or that protogyny in the Sparidae is an ancestral condition and C. nufar is in the process of evolutionary change from a protogynous to a gonochoristic form (or visa versa).

CHAPTER 1 - GENERAL INTRODUCTION

Seabreams (family: Sparidae) are found in virtually all the oceans of the world, but nowhere are they as diverse as in the waters around southern Africa. Of the 41 species which occur in these waters, 21 are endemic to the region (Smith & Heemstra 1986) and 29 form important components of various linefisheries and net fisheries (van der Elst & Adkin 1991). Because of their importance to these fisheries, considerable research effort has been directed at the family in recent years (Hecht & Baird 1977, Coetzee & Baird 1981a,b, Joubert 1981a,b, Coetzee 1983, 1986, Buxton 1984a,b, 1989, 1990, 1992, 1993, Buxton & Clarke 1986, 1989, 1991, 1992, Buxton & Smale 1989, Buxton & Garratt 1990, Garratt 1985a,b, 1986a,b,c, 1988, 1991, Smale 1988, Garratt et al. 1989, Smale & Punt 1991, Mann 1992, Garratt et al. 1993, Punt et al. 1993).

A striking fact that has arisen from this work is that many members of the family are relatively slow growing and a number of species have complex reproductive styles. Several commercially exploited seabreams are reported to change sex during their lives (Hecht & Baird 1977, Garratt 1986b, Buxton 1989) and there is evidence of significant changes in sex ratios brought about by selective exploitation (Garratt 1985a,b, Buxton 1993). This has resulted in considerable debate into the possibility that fishes with such reproductive styles require special manangement and, recently, some valuable research has been undertaken in this direction (Buxton 1992, 1993, Punt et al. 1993).

Hermaphroditism is widespread amongst teleosts (for reviews see Atz 1964, Reinboth 1970, Smith 1975, Policansky 1982, Buxton & Garratt 1990) and, as more data accumulate, it is becoming increasingly evident that within several families of teleosts sex change is the rule rather than the exception (Warner 1984). The range of sexuality in fishes is unparalleled among the vertebrates (Gold 1979) and the physiological and genetic basis for this sexuality is not well understood (Price 1984, Reinboth 1988). Fish chromosomes tend to be small and numerous (Atz 1964) and sex chromosomes appear to be in a primitive state of evolutionary development (Ohno 1967, Mittwoch 1975). In most fishes sex chromosomes are morphologically indistinguishable from the autosomes and it has been postulated that, in some fishes, sex determination is determined by a number of minor genes distributed throughout the chromosome compliment i.e. there are no sex chromosomes as such (Price 1984).

Control of sex must be primarily genetic, but even though the primary determinants of sex reside in the genome of the individual, the transformation from genotypic sex to phenotypic sex appears to be accomplished by biochemical processes which are susceptible to environmental influences i.e. sex control in fishes is governed by both genetic (intrinsic) and environmental (extrinsic) factors (Chan & Yeung 1983). "Insofar as sex determination in mammals is concerned, a single Y-chromosome is sufficient to determine maleness. Among the fishes, no such generalizations can be made" (Gold 1979, p. 364).

Atz (1964), in his review of hermaphroditism in teleosts, concluded that sexuality in the family Sparidae was more complex than in any other family. Atz did not research sexuality in fishes personally, but he collated all the available information at that time. Further collation of results by Garratt (1986b) suggested that there were five reproductive styles within the family: protandry (sex change from functional male to functional female), protogyny (sex change from functional female to functional male), simultaneous hermaphroditism (both sexes functional simultaneously), rudimentary hermaphroditism (immature fish possess bisexual gonads but mature either as males or females) and gonochorism (separate sexes). More recent work has, however, suggested that there are only two reproductive styles within the family: sex change (protogyny and protandry) and secondary gonochorism (rudimentary hermaphrodites) (Buxton & Garratt 1990). Initially protandry was considered the most common reproductive style found within the Sparidae (Reinboth 1970), but presently there are more reported protogynous hermaphrodites.

Since the early work of d'Ancona (1949a), which described gonadal development in several Mediterranean seabreams from sex differentiation through sexual maturity to sex change, various pathways describing the sexuality of sparids have been postulated (Michèle 1977, Zohar et al. 1978, 1984, Alekseev 1982, Coetzee 1983, Pollock 1985, Yeung & Chan 1987, Matsuyama et al. 1988, Buxton & Garratt 1990, Shapiro 1992, Mann 1992). Whereas both histological and behavioural studies on many other protogynous species are accumulating evidence of alternative pathways of sexual development ((primary and secondary males (diandry) and females which do not change sex)) (Robertson & Choat 1973, Choat & Robertson 1975, Robertson & Warner 1978, Warner & Robertson 1978, Warner & Hoffman 1980, Charnov 1982, Robertson et al. 1982, Warner 1984, Shapiro 1987a,b, 1989, Warner 1988a,b) there is, as yet, no evidence of such alternative pathways in protogynous sparids (Buxton 1989). There is, however, accumulating evidence which suggests that sexuality is fairly plastic in protandrous sparids. Partial protandry, in which some individuals within the population change sex whilst others do not, has been suggested for a

number of species (Michèle 1977, Yeung and Chan 1987, Coetzee 1986, Zohar et al. 1978, 1984, Shapiro 1992, Mann 1992). Work on captive fishes has been particularly convincing in this regard (Zohar et al. 1978, 1984). A remarkable sequence of sexuality has been obtained from captive shoals of the protandrous hermaphrodite, Chrysophrys auratus (Shapiro 1992), but it is not known if a similar pattern exists in wild populations.

Despite the above work, confusion and uncertainty about the various reproductive styles in this family still exists and the need for detailed descriptions of both gonad development (especially early development) and spawning behaviour has been identified by a number of workers in this field (Bannerot et al. 1987, Sadovy & Shapiro 1987, Matsuyama et. al. 1988, Buxton & Garratt 1990, Chang & Yueh 1990). Virtually no detailed work has been conducted on early gonadal development or spawning behaviour in the Sparidae and, for most species, these aspects of their reproductive styles remain unknown.

The structural morphology of sparid gonads makes it difficult to determine sex change in fishes sampled from wild populations, yet it is for wild populations that there is a growing need for more information. The main reason for this is that male and female elements are separated by connective tissue and sex change is manifested simply in the development of one tissue and regression of the other. Determination of protandrous sex change is especially difficult, as degeneration of male tissue does not necessarily indicate sex change. The presence of non-ripe testicular tissue at the time that the ovary becomes functional does not constitute protandry (Buxton & Garratt 1990). In protogynous species in which male and female elements are mixed (eg. Serranidae, Labridae, Scaridae), remnants of the opposite sex, such as an ovarian lumen, are considered fairly conclusive evidence of sex change (Sadovy & Shapiro 1987). This evidence remains throughout the life of the individual, the morphology of the functional testis remaining ovarian in nature. In the Sparidae the remnants of the ovarian lumen, which is separate from the testis, may be evident for only a short period and may later disappear altogether.

Other than results obtained from captive fish (which may reveal patterns of sexuality which differ from wild populations) there has been no direct demonstrative evidence of sex change in any of the wild populations of Sparidae. Sex change in these populations has been based largely on indirect evidence such as a separation in the size of the sexes combined with histological evidence of degenerating tissue within the non-functional element of the gonad. Whereas this evidence has been fairly conclusive in many species, in most instances it has not been possible to adequately fulfill the criteria suggested by Sadovy

and Shapiro (1987). A means by which this problem can be overcome, which seems to have been seldom used by workers in this field, is the use of tag and recapture. Fish tagged and sexed during their peak spawning season could produce conclusive evidence of sex change if they were recaptured as the opposite (and functioning) sex during the following spawning seasons. This avenue of investigation was incorporated into part of the present study.

A growing awareness of functional hermaphroditism in exploited species has led to the need to incorporate this phenomenon into fisheries modelling (Shapiro 1987a). Only a few such studies have been made on teleosts (Bannerot et al. 1987, Buxton 1992, Punt et al. 1993), the latter producing some valuable insights into the sustainability of slinger Chrysoblephus puniceus, a protogynous hermaphrodite exloited in Natal and southern Mozambique. Even though the above studies have given some insight into the possible effects of exploitation on the population structures and reproductive output of such species, they rely heavily on assumptions regarding reproductive styles, for which there is no information. It is considered that the models will remain of limited value until more is known about reproductive behaviour; the responses of spawning shoals to the disruptive effects of fishing; and the consequence of possible sperm limitation (shortage of males in heavily fished populations of protogynous hermaphrodites) (Bannerot et al. 1987). Recent work by Peterson (1987) confirms this need.

The aims of this present study were thus threefold. The first was to provide accurate, detailed descriptions and comparisons of gonadal development in representatives of each reproductive style, from gonad formation and sex differentiation through to sexual maturity and sex change, where this occurs. These descriptions would confirm or reject earlier postulations regarding the sexuality of these species and also provide information which is needed to clarify pathways of sexuality in the family. The second was to investigate the spawning strategies of these fish and the third was to relate these findings to current theories on the evolution of hermaphroditism and sex change in fishes.

Four species were investigated: the Natal stumpnose Rhabdosargus sarba, the riverbream Acanthopagrus berda, the slinger Chrysoblephus puniceus and the santer Cheimerius nufar. Rhabdosargus sarba, found in inshore waters (<20m) has been reported elsewhere as a protandrous hermaphrodite (Yeung & Chan 1987). Acanthopagrus berda is a euryhaline estuary-dependent species (Begg 1978, van der Elst 1988) suspected to be a protandrous hermaphrodite. This is based on macroscopic evaluation of the gonads and the fact that all other members of the genus are reported protandrous hermaphrodites

(Kinoshita 1939, Abu-Hakima 1984, Pollock 1985, Chang & Yueh 1990). Chrysoblephus puniceus occurs offshore between 10-100m and is, at present, the only known protogynous hermaphrodite in Natal (Garratt 1985a,b, 1986b). Known locally as the soldier, Cheimerius nufar is found offshore to depths of 200m and has been described as a rudimentary hermaphrodite (Coetzee 1983, Garratt 1985a,b).

Rhabdosargus sarba was included in the study for the following reasons: firstly, there was some doubt that Acanthopagrus berda was a protandrous hermaphrodite. Much of the earlier work on protandrous species, including work on several members of the genus Acanthopagrus, has recently been questioned (Buxton & Garratt 1990) on the grounds that few reports of protandry have demonstrated that females are derived from reproductively active males: rather they have shown that males mature at a smaller size than do females. Secondly, it afforded an opportunity to investigate early gonadal development in this species (for which there is no information) and to compare this, and later gonadal development, with that of A. berda.

CHAPTER 2 - SEX DIFFERENTIATION AND GONADAL DEVELOPMENT

2.1 INTRODUCTION

Gonadal development and gametogenesis in teleosts have received a great deal of attention and investigations into the development of sparid gonads were recorded soon after the turn of the century (Williamson 1911). The early works of Williamson (op. cit.), Le Gall (1929) and Kinoshita (1936, 1939) described hermaphroditism in several sparids and were followed by a number of papers which appeared in the forties, fifties and early sixties (Pasquali 1941, D'Ancona 1941, 1945, 1946, 1949a,b, Zei 1950, Coupé 1952, Larranéta 1953, Aoyama 1955, Salekhova 1961, Zei & Zupanovio 1961, Reinboth 1962). D'Ancona is considered to be the pioneer of research into hermaphroditism, intersexuality and sex change in the Sparidae.

It was not until the review of Atz (1964), however, that the complexity of sexuality in this family was fully appreciated. Subsequent reviews by Garratt (1986b) and Buxton & Garratt (1990) have highlighted this complexity, but they have also indicated that some of it may be due to a lack of clarity in terminology and, in some instances, superficial observation.

Early gonadal development in sparids, from the formation of the gonadal primordia (anlagen) through sex differentiation to sexual maturity, has received little attention. There are, to my knowledge, no studies which have followed the entire gonadal ontogeny from the formation of the gonads through sexual maturity to sex change (where this occurs) within the various reproductive styles . D'Ancona (1949a) presented a schematic representation of early development in sparid gonads which, he suggested, was representative of both gonochorists and functional hermaphrodites. His study was, however, restricted to light microscopy and, apart from a recent study of the protandrous sparid Lithognathus mormyrus (Besseau 1991), there are no ultrastructural studies which present details of the colonization of sparid gonads by primordial germ cells (PGC's), the differentiation of germ cells into either spermatogonia or oogonia, or the morphological structure and development of the early gonads.

According to Persov (1972), the principal steps in the phenotypic differentiation of sex can be separated into two phases. The first is the undifferentiated, pre-gonadic stage in which

segregation, migration and concentration of primordial germ cells (PGC's) in the region of the future gonads occurs. The second phase consists of the formation of the gonads and gonadal differentiation. The present study investigates gonad development and gametogenesis from the beginning of the second phase (formation of the gonads and sex differentiation) through sexual maturity to sex change. Four sparids, representing three reproductive styles within the sparid family, were studied in order to present descriptions of this nature for the group. In doing so, the following key questions were asked:

Sex differentiation:

i) Does early morphological development of sparid gonads follow the same pattern, irrespective of reproductive style?

Since the discriptions of D'Ancona (1949a) virtually no work has been carried out on early gonad development in sparids. D'Ancona presented a schematic representation of early gonad development, suggesting that this pattern applied to all sparids. This may have been more misleading than helpful, especially if there are differences early in life which distinguish one reproductive style from another. The inclusion of early gonadal development in a study of sparid sexuality may allow for a clear distinction to be made between protandrous, protogynous and rudimentary hermaphrodites.

ii) Are primordial germ cells (PGC's) in sparids similar to those described for other teleosts? Do they conform to the general vertebrate pattern, being relatively large (in relation to surrounding somatic cells) undifferentiated cells which colonise the gonad early during the first year of life?

Apart from a recent doctoral thesis on early gonadal development in the sparid Lithognathus mormyrus (Besseau 1991), no work has been conducted on sparids at the ultrastructural level. This study will add to our knowledge of early gonadal development in the family and critically appraise earlier descriptive studies on teleosts.

iii) Do PGC's give rise to differentiated gonial cells i.e. in sparid gonads, can spermatogonia and oogonia be distinguished from one another?

It is not known if gonial cells become differentiated in the early stages of gonadal development, or if they remain undifferentiated until later in life when they are

masculinized or feminized (Reinboth 1982, Nakamura et al. 1989). Bruslé & Bruslé (1978) have suggested that in the mullet (Mugil auratus) spermatogonia and oogonia can be distinguished from one another morphologically. In the sparids, the early separation of gonia by connective tissue allows for measurements to be made on germ cells in presumptive male and female tissues and this study will test whether such a distinction can be made in sparids. Differences in morphology and cytology may indicate that gonia differentiate when they first appear in the gonad.

iv) Is the presumptive female area colonized by germ cells before the male area in all sparids, including protandrous species?

Recently it has been suggested that gonadal ontogeny in all fishes, including protandrous species, may follow the mammalian paradigm of primacy of female development i.e. all individuals develop in a female direction unless redirected on a male pathway by a masculinizing mechanism (Shapiro 1992). This study will investigate if ovarian development is the primary developmental scheme in sparids.

Gonadal development after differentiation:

v) Is sex change in protogynous and protandrous sparids characterised by the simultaneous degeneration of tissue of the one sex and development of the other? If so, there should be sound histological criteria to determine sex change in sparids.

In their paper entitled: "Criteria for the diagnosis of hermaphroditism in fishes" Sadovy & Shapiro (1987) suggest that definitive histological evidence of sex change in fishes consists of transforming gonads which contain degenerating tissue of one sex and proliferating tissue of the other. These authors maintain that most studies of protogynous species report the simultaneous presence of both tissues in transitional individuals, but that they do not indicate if ovarian tissue is degenerating. They suggest that the paucity of reports providing descriptions of simultaneous degeneration and development of male and female tissue raises doubts as to whether transitional gonads do, in fact, display such a histological picture.

vi) Is testicular tissue evident in the gonads throughout the life of protogynous sparids?

Sadovy & Shapiro (1987, p.142) state that "gonads of the initial sex in most protogynous species are either purely ovarian, with no trace of testicular tissue, or undelimited in type". In sparid gonads, male and female elements are separate (delimited type) and it is unlikely that there are no traces of testicular tissue in the gonads of protogynous species. Detailed study may show that this hypothesis does not apply to sparids and that testicular tissue is evident in all gonads during the initial female stage.

vii) Is A. berda a protandrous hermaphrodite, with all individuals functioning first as males and later as females during their normal ontogeny?

All members of the genus Acanthopagrus so far studied are reported to be protandrous hermaphrodites. It has been assumed that A. berda is also protandrous, but its sexuality remains unknown. Detailed descriptions of gonad development in this species, with comparisons to a known protandrous species (R. sarba), will be used to determine the sexuality of this species.

viii) Is C. puniceus a diandric protogynous hermaphrodite, some males developing directly from immature fish (primary males) and others from females through sex change (secondary males)?

In an earlier study, Garratt (1986b) produced size-separation and histological evidence of protogynous sex change in C. puniceus. Histological evidence consisted of degenerating pre-vitellogenic oocytes and extensive brown bodies in the female element of bisexual gonads. Sadovy & Shapiro (1987) consider size-separation to be a weak indicator of sex change and Bruslé-Sicard et al. (1992) have recently suggested that the histological evidence for sex change in C. puniceus (viz. brown bodies and degenerating pre-vitellogenic oocytes) is not very convincing. Garratt (1986b) also suggested that C. puniceus may be diandric, a small number of small males in the Natal population representing primary males which develop directly from immature fish. However, no histological evidence was presented to confirm this observation and primary males have not been observed in the congenerics C. laticeps and C. cristiceps (Buxton 1989). Further data were collected to confirm protogyny in C. puniceus and to investigate the possibility of diandry in this species.

ix) Is C. nufar a rudimentary hermaphrodite in which all young fish possess an immature bisexual gonad and mature either as male or female fish with no evidence of sex change?

Coetzee (1983) presented evidence of rudimentary hermaphroditism in C. nufar. Based on the small number of hermaphrodites reported in both studies, and a preponderance of females in the smaller size classes in the Natal population, Garratt (1985a) suggested that hermaphrodites in this species represented abnormal fish which were not representative of a transitional stage. A shortcoming of both studies was that they were based on catches made by sport and commercial fisheries in which there were relatively few immature fish. Garratt (1985a) suggested that further work should be undertaken to confirm the sexuality of this species.

2.2 MATERIALS AND METHODS

Sampling strategy:

This study involved the microscopic and macroscopic investigation of gonad morphology and development in wild populations. Macroscopic assessment of gonads was used to determine the relative abundance of gonad types in wild populations. The aim of the microscopic study was to produce detailed information on developmental stages which could be used, together with the macroscopic assessment, to compare gonadal development in each reproductive style. Material for this purpose was obtained by subsampling.

All samples were collected from wild populations, apart from gonads which were removed from captive C. puniceus to investigate gonad development in relation to territorial behaviour and gonads removed from two juvenile C. nufar raised in captivity. Recently, studies which have investigated hermaphroditism and sex change using samples collected from fishermen's catches have been criticised on the grounds that these catches can be taken from several, or many, communities (Sadovy & Shapiro 1987). As such they may not accurately reflect population structure (size and sex ratios) within separate communities. Also, fisheries generally do not include immature fish and they can bias size frequency distributions through selective capture. Accordingly, where possible, attempts were made to collect samples from single communities, or at least from the same system with regard to estuarine species. In this respect, sampling was most successful regarding A. berda and C. puniceus (see below).

Samples of R. sarba were collected during the period October 1988-March 1992 along the length of the Natal coast (Fig. 1). Adults, ranging in size from 200-580mm (FL), were collected from personal and anglers' catches made in the surf zone. Nowhere are R. sarba caught in great numbers and samples were collected on an opportunistic basis. Juveniles (20-200mm) were collected during the same period by net and line in the Kosi, St Lucia and Richards Bay estuaries and Durban harbour.

Samples of A. berda were collected during the period June 1987-June 1993 from the Richards Bay estuary, the Kosi estuary, the Umgeni river and Durban harbour (Fig. 1). Adults, ranging in length from 150-348mm (FL), were caught by line, spear and net throughout these estuaries. Juveniles (12-150mm) were caught by net in areas which were suitable for netting. In the Richards Bay sanctuary, netting was limited to the lower reaches of the estuary.

Samples of adult C. puniceus, ranging in size from 250-436mm (FL), were collected by line and spear during the period April 1986 - November 1992 from shoals which appear to be resident on the Leadsman Shoal (Garratt, unpublished data), which is situated in the sanctuary area of the St Lucia Marine Reserve (Fig. 1). Juvenile C. puniceus (70-250mm) were collected, over the same period, on shallow inshore reefs (<20m) off Tongaat, as there are no known nursery areas in the St Lucia Marine Reserve.

It was not possible to obtain samples of adult C. nufar from a specific area. Adults of this species occur at depths of 50-100m along the Natal coast (Garratt 1985b) and, for much of the year, they are dispersed over wide areas (Garratt pers. obs.). Samples of adults ranging in length from 250-630mm (FL) were collected for histological purposes in the vicinity of Durban between June 1989 - June 1992. In order to produce sufficient samples to assess the proportions of gonad types in the wild population, fish ranging in length from 130-650mm (FL) were collected along the entire Natal coast. Juvenile C. nufar, in their first year of life (55-110mm FL), have been found inshore in depths of 3-33m (Buxton et al. 1984) and samples of fish in this size range were obtained from shallow reefs in the vicinity of Durban for histological investigation. The gonads of two C. nufar (37 & 44mm FL) which had been successfully raised from the eggs of captive adults (Garratt et. al. 1989) were also included in the study.

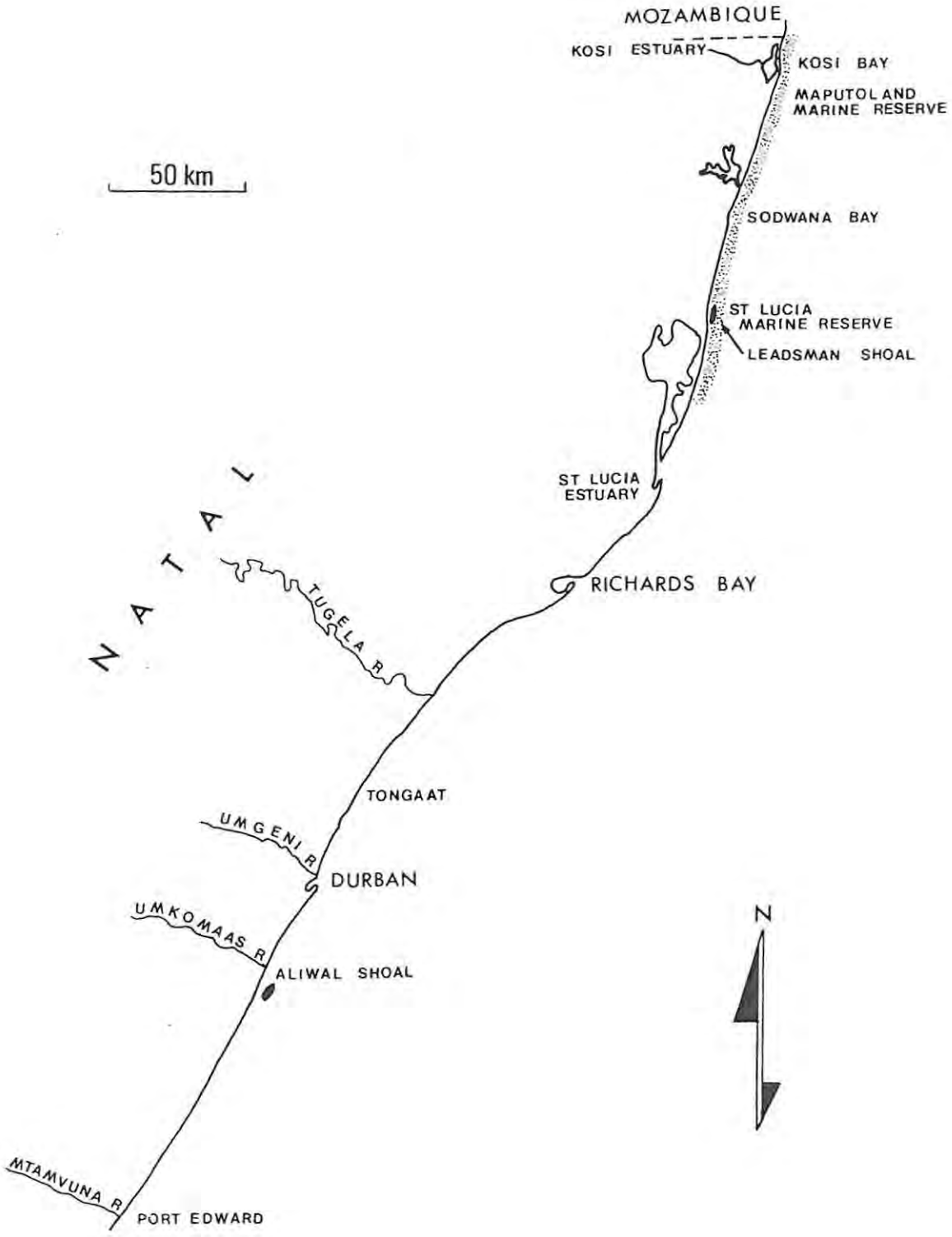

Figure 1. Map of the study area showing places mentioned in the text.

Gonads for histological investigation were removed from fish soon after capture and fixed in buffered 10% formalin. For electron microscopy, fish were anaesthetised with MS-222 and the dissected gonads fixed in a 2.5% glutaraldehyde solution buffered to pH 7.2 with 0.1% sodium cacodylate buffer. They were stored at $4^{o}C$ until further processing.

Laboratory techniques:

i) Electron Microscopy

After several buffer rinses, the material was post-fixed in a 0.5% osmium tetroxide (OsO_4) solution for one hour, dehydrated in a graded acetone series, and embedded in low viscosity epoxy resin (Spurr 1969). Sectioning was carried out using a Reichert Ultracut E microtome. Semi-thin sections of 3-7μm were stained with toluedine blue and viewed and photographed with a Nikon Biophot light microscope to investigate and record early morphological development of the gonads. Ultrathin sections (60-100nm) were stained with a saturated aqueous uranyl acetate solution (15 min) followed by lead citrate (Reynolds 1963) for 40 min. Images were recorded with a Jeol 100C transmission electron microscope.

ii) Histology

Fixed tissues were processed using a Biorad H2500 microwave processor and embedded in paraffin wax. The tissue was sectioned at 3-7μm and stained with Erlich's haematoxylin and eosin. Photographs of sections were taken using a Nikon Biophot light microscope.

Assessment of gonad differentiation and development:

In order to describe gonad differentiation and development in relation to growth, all species were divided into 50mm length classes. These classes were somewhat arbitrary, but they were selected to encompass major events in the development of the species under investigation (such as sexual differentiation, maturity and sex change), while accommodating individual variability in development.

Macroscopic gonad staging was used to establish general patterns of gonad development in large populations of the sparids studied. Gonads removed from subsamples were used for detailed descriptions in each length class and to confirm the accuracy of macroscopic gonad staging.

Anterior, medial and posterior sections were taken from the gonads of 3-8 fish in each length class in order to establish patterns of development throughout the gonads. These sections were also used to ascertain if there was one region which would adequately describe changes in gonad development with growth in the majority of fish sampled. It was ascertained that the area slightly posterior of the mid-region was representative of the functional state of the gonad, and the majority of sections were taken from this area. In some instances, however, the number of sections was increased to five, thereby yielding a better picture of development throughout the gonads.

Statistical procedures:

To quantify volumes of tissue types within gonads, the stereological techniques of Freere & Wiebel (1967) were used. Volumes were estimated from the proportion of points lying on the type of tissue when a point grid was superimposed on a micrograph.

To investigate if spermatogonia and oogonia could be distinguished from one another morphologically, the relative sizes of gonial cells and their nuclei, cell/nucleus ratios, and the relative positions of nuclei in the gonia were determined for gonia in presumptive male and female areas. Analysis of variance was used to test if there were significant morphological differences between spermatogonia and oogonia.

2.3 RESULTS

2.3.1 Classification of gonad types - R. sarba and A. berda

In all the species investigated, the gonads were paired structures suspended from the dorsal abdominal wall by a thin mesovarium. In R. sarba and A. berda male and female elements were evident in most gonads. In gonads in which male and female elements were conspicuous, the ovarian element was dorsal to the ventro-lateral male element. Male and female elements were separated from one another by collagenous connective tissue.

Preliminary histological investigation of the gonads of R. sarba and A. berda showed that gonads could be classified as follows: I - immature, II - male, III - predominantly male, IV - predominantly female, V - female. Each of these gonad types could be identified in the field macroscopically and macroscopic staging was thus used to determine their percent occurrence in wild populations in each of the 50mm length classes.

Definitions of the types are: I - IMMATURE, macroscopically the sex is indeterminate (Fig. 2). Microscopically, these gonads ranged from gonadal primordia in which no sex cells were evident, through the colonization and proliferation of germ cells in the gonads, to gonads in which male and female elements were clearly separated by connective tissue (see Figs 3,8,11). Spermatogenesis and oogenesis commenced soon after proliferation of gonia. Oocytes were arrested in the previtellogenic stage but spermatogenesis included all stages through to the production of sperm in isolated lobules. The sperm duct was not yet fully formed; II - MALE, macroscopically the gonad appeared to be composed entirely of male tissue. No oocytes were visible, but a hollow, flattened sac (ovarian remnant) was evident in the dorsal region between the lobes of each gonad. Microscopically, a thin band of previtellogenic oocytes (mostly one layer) lined one side of the ovarian remnant (see Fig. 44); III - PREDOMINANTLY MALE, macroscopically both elements were conspicuous but the male element composed more than half the volume of the gonad. Microscopically the male element was functional. The female element consisted of an ovary with a large lumen and lamellae containing numerous previtellogenic oocytes (see Figs 20,21); IV - PREDOMINANTLY FEMALE, macroscopically both elements were conspicuous but the female element composed more than half the volume of the gonad. Microscopically the male element was either active or appeared to be degenerating, suggesting earlier activity (see Figs 22,24). V - FEMALE, macroscopically the gonad appeared to be entirely female. Microscopically, the gonad consisted of a round to oval female element with ovigorous lamellae protruding into a central cavity. Part of the tunica albuginia consisted of a thickened sterile zone from which no lamellae protruded. The male element consisted either of an inactive remnant or merely a thickening of the ovarian wall (sterile zone) in the ventro-lateral region (see Figs 21e,26)

2.3.2 Gonadal development in R. sarba

Early morphological development and sex differentiation:

Length class: 0-49mm

Anterior, medial and posterior sections were cut from gonadal primordia of seven fish in the length class 0-49mm. Serial sections from three of these fish were prepared for electron microscopy. The smallest specimen measured 32mm and the largest 49mm. At 32mm the formation of the gonads had begun. Each primordium consisted of a single elongate, membrane-lined organ attached to the dorsal abdominal wall by a thin mesovarium (Fig. 2).

In specimens of 39-49mm a second element was developing on each primordium which extended from the mesovarium down alongside the first (Fig. 3). A caudo-cranial gradient of somatic growth was evident. The least developed anterior sections consisted of two small elements. One contained the major blood vessel, fibrillar tissue and dense clusters of granulocytes (Fig. 4a) and the other was composed of somatic cells, granulocytes and loose fibrillar tissue (Fig. 4b,c). Medial and posterior sections showed further development, with both elements of each primordium extending further into the abdominal cavity. The major blood vessel was situated in the larger element which was composed largely of somatic cells and granulocytes. The smaller element was composed of these cells and also a large amount of fibrillar and loose connective tissue. At this stage there was no evidence of colonization of the gonadal primordia by primordial germ cells (PGC's), or the development of gonial cells, but occasional clusters of less-electron-dense (LED) cells were evident in the stroma (Fig. 4d).

Length class: 50-99mm

A central cavity was formed in the length class 50-99mm through the extension of the second element in each gonadal primordium and fusion of the two elements ventrally. The formation of the cavity was completed first in the anterior region and progressively towards the medial and posterior regions (Figs 5,6). In smaller fishes within this length class (55-79mm) there was no evidence of germ cells, but there were distinct cellular regions within each primordium. Large clusters of granulocytes were evident in the dorsal region immediately below the mesovarium (Fig. 7a). The medial elements of each primordium, which contained the major blood vessels, and a distinct ventral apex, were composed largely of somatic cells and scattered granulocytes (Fig. 7b,c). The lateral elements were composed mainly of fibrillar and loose connective tissue interspersed with somatic cells and occasional granulocytes. The margins of the central cavity, lined with epithelial cells, became convoluted (Fig. 7d).

In larger fishes within this length class the central cavity was fully formed and nests of LED cells and gonial cells had appeared along the inner margins of the cavity (Fig. 8a-c). The ventral apex developed into the presumptive male element through a differential growth of somatic cells. These developments signified the transition from primordia to gonads.

The appearance of gonial cells was preceded by the development of nests of LED cells along the inner margins of the central cavity (Fig. 9a). Gonial cells appeared to develop

within these nests (Fig. 9b) and, as they developed, the associated less-electron-dense cells became flattened and apppeared to develop into prefollicular cells which surrounded the gonial cells (Fig. 9c). Gonial cells had the same general characteristics of those reported for other teleosts (Bruslé & Bruslé 1978a,b, Bruslé 1989, Bruslé-Sicard et al. 1992). They had a mean length of 15.94μm (SD=1.71) and a mean width of 12.33μm (SD=2.43) and had a high nucleus to cell ratio (0.31, SD=0.07) and a low electron density (n=37). Chromatin in the nucleus was granular and dispersed. Mitochondria, which were round in shape, had few cristae and were thus rather electron opaque. Small irregular masses of electron dense substance ('nuage' sensu Clérot 1968, 1976), were located outside the nuclear membrane (Fig. 9c). Independant masses ('ciment' sensu Clérot 1968, 1976) were associated with clusters of mitochondria in the cytoplasm.

In this length class, gonial cells were restricted to the lining of the central cavity. There was no development of germinal tissue in presumptive male element. Between the gonial cells lining the central cavity and the outer membrane of the gonads the tissue was composed largely of somatic cells, granulocytes and vacuolated cells (Fig. 9d).

The development of the left and right gonads was asynchronous both morphologically and cytologically in all specimens. In each case the larger gonad was the most well developed, containing a larger number of somatic nests and gonia in the female element. It could not be established if the larger gonad was consistently on the left or right side. Macroscopic inspection of the gonads of larger fishes suggested that the larger of the two gonads could be on either side. Asynchronous development of the gonads continued through to sexual maturity and beyond, but became less evident with increasing size.

Length class: 100-149mm

Differentiation of male and female tissue occurred in the length class 100-149mm with the development of germ cells in both male and female elements of all specimens examined (n=16). All gonads were bisexual at this stage of development. Presumptive oogonia proliferated along the margins of the central cavity while presumptive spermatogonia, which were not evident in the previous length class, developed simultaneously within the dense stroma of the ventral regions (Figs 10,11).

Gonial cells first appeared in the presumptive female area (along the margins of the central cavity) of the anterior region of both gonads. In the middle and posterior regions of each gonad pair, an equal or greater number of gonial cells developed in the presumptive

male region shortly thereafter (Fig. 10a-c). In larger specimens, oogonia and spermatogonia proliferated simultaneously (Fig. 11). In the female element the development of pre-perinucleolar oocytes had also commenced, but development was more advanced in male elements with all stages of spermatogenesis evident. The male element thus could be regarded as the dominant sex in this length class.

The female element dominated the anterior region of all gonads in this length class (Fig. 11a), the proliferation of oogonia in nests resulting in the enlargement of lamellae which began to protrude into the central cavity in larger fish (Fig. 11b,e). In medial sections both elements were of similar size (Fig. 11b) due to the simultaneous development of spermatogonia and oogonia. In the posterior region, the male element constituted the greater proportion of the gonad (Fig. 11c).

The male element formed into a lobular type of structural organization (Fig. 11d) commonly found in teleosts (Grier 1981) and there was the simultaneous formation of sperm ducts. Sperm ducts formed in the sterile zone of each gonad as a result of extensive atresia of somatic and connective tissue in that zone (Fig. 12a). Numerous granulocytes in phagocytic activity were evident at this time (Fig. 12b).

It is not known if the gonial cells which first colonize early gonads are undifferentiated, bipotential cells which develop according to the masculinizing or feminizing effects of surrounding tissue within male and female elements, or if they have already differentiated into spermatogonia and oogonia from bipotential primordial germ cells (PGC's). Accordingly, an investigation was carried out to determine if gonial cells in presumptive male and female elements of R. sarba (Fig. 13) could be distinguished from one another not only topographically, but also morphologically, as they have been for the golden grey mullet (Mugil auratus) (Bruslé & Bruslé 1978a).

Analyses of variance (one-way ANOVA) on measurements of gonial cells in male (n=23) and female (n=19) elements indicated that there was no significant difference ($p > 0,05$) between gonial cells in these elements with respect to size, shape, nucleus to cell ratio, or the relative position of the nucleus within the cells (Table 1).

An ultrastructural investigation of cellular organization within male and female elements of gonads from fish ranging in size from 120-135mm (n=5) showed that in female elements development of germ cells occurred within lamellae which extended progressively into the central cavity. Between these lamellae and the tunica albuginia, there were somatic cells,

TABLE 1. Test of significant difference (ANOVA) in morphological characteristics between gonial cells in presumptive male and female elements of undifferentiated R. sarba gonads (p= 0.05).

Source of variation	F-ratio	Sig. level
Cell area	1.844	.1821
Nucleus area	0.890	.3611
Nucleus/cell ratio	0.792	.3882
Centrality of nucleus		
smallest distance *	1.497	.2283
greatest distance *	1.803	.1869
ratio of distances	.295	.5957

* Distances between nucleus and cell membrane.

dense clusters of granulocytes and vacuolated cells (Fig. 14a,b). In the anterior regions of all gonads lamellae containing oogonia formed around the entire circumference of the central cavity. In medial and posterior regions a sterile zone, which consisted mainly of loose connective tissue and fibrillar tissue, was evident adjacent to the newly forming sperm duct. No female germinal tissue formed along this sterile zone.

Within nests of germ cells in the lamellae, gonia were proliferating and the production of previtellogenic oocytes had commenced. Early previtellogenic oocytes (Fig. 14c) were characterised by a similar size to gonial cells (mean length= 16.63μm, SD=1.40, mean width= 10.83μm, SD=2.68), an almost regular outline and a voluminous nucleus (N/C ratio from 0.3-0.35) with an undulating envelope and one large nucleolus or several smaller nucleoli. The cytoplasm was more electron dense than the nucleus due to a substantial increase in the number of ribosomes in the cytoplasm. Early oocytes were surrounded by pre-follicle cells characterized by irregular outlines (Fig. 14c). As previtellogenic oocytes developed further, both cell and nucleus became more spherical with smooth outlines and each oocyte was surrounded by flattened follicle cells (Fig. 14d).

Cells conforming to the diagnostic features of primordial germ cells (PGC's) described for other teleosts (Bruslé & Bruslé 1978a,b, Bruslé 1986, Bruslé-Sicard et al. 1992), first became evident in fishes measuring 120-135mm. They were more abundant in the female element where they were located in, and amongst, nests of oogonia (Fig. 15a,b). In the male element they were discernible amongst spermatogonia and between lobules of spermatocytes and spermatozoa (Fig. 15c,d). These cells were slightly smaller than surrounding gonial cells (mean length = 15.63μm, SD = 3.34, mean width = 9.32μm, SD = 3.11) and were characterized by the following features: irregular outlines, high electron density of nucleus and cytoplasm (related to a finely granular chromatin and many free ribosomes), a voluminous eccentrically located nucleus with prominent nucleolus having a small fibrillar centre and granular cortex, and aggregates of an electron dense substance in the cytoplasm associated either with the nuclear membrane ('nuage') or with groups of mitochondria ('ciment'). Nowhere in the gonads of R. sarba were PGC's numerous and they could not be distinguished at the light microscope level.

Spermatogonia proliferated in the male element at the same time that oogonia were proliferating in the female element and numerous spermatocytes in meiotic prophase were evident (Fig. 16a). Throughout the male element nests of spermatogonia, in which each spermatogonium was surrounded by Sertoli cells, were transforming into spermatogonial cysts delimited by a single layer of Sertoli cells (Fig. 16b). Leydig cells, which had not been observed in the gonads of younger fish, also became evident in the male element at this time. Clusters of these prominent cells occurred between spermatogonial cysts (Fig. 16c,d). Leydig cells occur only in male tissue and are the main source of testicular androgens (Christensen 1975). These cells are characterized by a large nucleus, abundant smooth endoplasmic reticulum and prominent rod shaped mitochondria with densely packed tubular cristae (Fig. 16c). The appearance of Leydig cells in the interstitium of the male element coincided with spermatogenesis and spermiogenesis.

The formation of spermatogonial cysts resulted in a lobular organization within the male element and, within a number of these lobules synchronous spermatogenesis was evident. Lobules of primary and secondary spermatocytes (Fig. 17) were more numerous towards the centre of the male element, with lobules of spermatids and spermatozoa (Fig. 18) coalescing to form larger sinuses.

Gonadal development after differentiation:

Descriptions of gonadal development in R. sarba after differentiation were based on random samples taken from the Natal coast (see explanation in methods). Samples were collected during spring and summer months (October-March) and included the peak-spawning and post-spawning period of R. sarba in this region. Sampling was restricted to these months because it was difficult to determine the dominant sex (or the sex which would function during the following spawning season) during the inactive phase, and also because sex change in a number of sparids was known to occur during the post-spawning period.

Macroscopically, all specimens smaller than 150mm were classed as immature (Fig. 19). Histological analysis (above) of gonads in these length classes confirmed that this assessment had been accurate. Even though spermatogenesis and spermiogenesis had commenced in the gonads of larger fish within the length class 100-149mm, the production of sperm was limited, few lobules had ruptured forming larger sinuses and the sperm duct was not yet fully formed (see Fig. 11).

Length class: 150-199mm

Sexual maturity was attained in this length class, with the majority (54%) of the fish having a predominantly male gonad (TYPE III) (Figs 20,21a-d). Male gonads (TYPE II) were uncommon, constituting only 4% of the fish sampled. Females were almost equally represented by predominantly (13%) and totally female (17%) ovaries (TYPES IV & V respectively). The overall sex ratio was 1.59 : 1.00.

Histological analysis was conducted on the gonads of fifteen specimens in this length class, including five from which anterior, medial and posterior (AMP) sections were taken. AMP sections revealed that mid-sections were best suited to illustrate the dominant sex (Fig. 20), and all subsequent sections were taken from this region.

Ten of the fish sampled for histological purposes were TYPE III males, three were TYPE IV females and two were TYPE V females. Structurally, the gonads of TYPE III males represented the 'basic type' which was common to all fishes in the previous length class (Figs 20,21a-d). Developmental changes which had occurred in these gonads included the dorsal extension of the sperm duct into the sterile zone and the partial enclosure of the female element by male tissue.

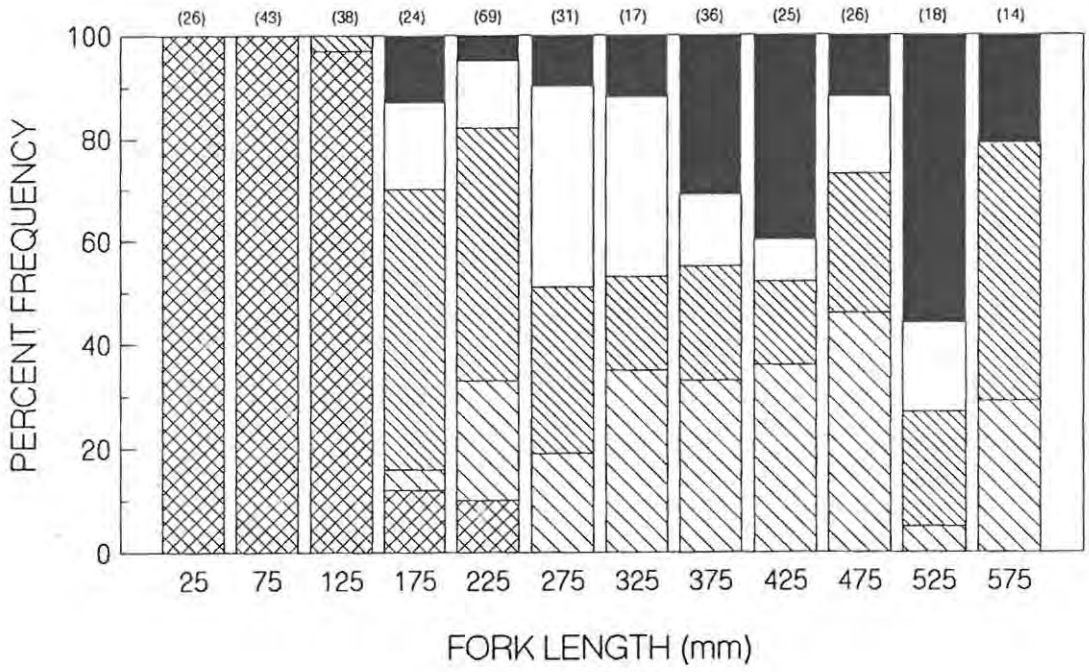

Figure 19. Percentage composition, in 50mm length classes, of gonad types in Rhabdosargus sarba sampled along the Natal coast. ▨ Immature (I), ▧ male (II), ▧ predominantly male (III), □ predominantly female (IV), ■ female (V). Sample sizes in parenthesis.

All males had well developed testicular zones in which spermatogenesis was taking place. Lobules towards the centre of each gonad had commenced rupturing, resulting in large sinuses of spermatozoa in the central regions. The caudo-cranial gradient of development noted in the previous length class persisted in these fishes with relatively little spermatogenic activity evident in the anterior regions of the gonads and most activity occurring in the posterior regions (Fig. 20d,e).

Development had also continued in the female elements of TYPE III gonads, but this was limited to the extension of lamellae into the central cavity as a result of continued proliferation of oogonia and the production of oocytes which were arrested in the perinucleolar stage (Fig. 21e).

The three TYPE IV gonads were found to be functional males. Even though the female element in each gonad was substantially larger than the male element (Fig. 21f,g), all stages of spermatogenesis were evident in the male tissue of two of these fish (Fig. 21h), including spermatozoa in the sperm ducts. The third specimen had functioned as a male, as there was still sperm in the posterior section of the sperm duct, but the entire male element was undergoing extensive atresion (Fig. 22). In the latter, the blood vascular system had increased substantially and there was extensive phagocytosis of gonial cells. Such extensive degeneration of gonial cells is not a general feature of fishes in post-spawning condition (see Fig. 24e) and it was concluded that this fish was in the process of changing sex.

TYPE V gonads were functional females. A male remnant, consisting of gonial cells which were located in the walls of both ovaries immediately below the thickened sterile zone, was evident in each gonad studied.

Length class: 200-249mm

Ninety percent of the fish in this length class were mature. The majority were males (72%) with TYPE III gonads predominating (49%) (see Fig. 19). Gonads of twenty-six fish were sectioned for analysis, including eight from which AMP sections were cut. Neither TYPE II nor TYPE III (Fig. 23) gonads differed morphologically from those described in the previous length class. Cytologically, there was more extensive degeneration of tissue in the male elements of some TYPE III post-spawning gonads than in others (Fig. 23d,e), but there were no other features to suggest that any of these fish may have been in the process of changing sex.

The TYPE IV gonads in this size class exhibited structural changes not evident in the smaller size classes. The male element of these gonads (all in post-spawning condition) was reduced in size and convoluted (Fig. 24). In two of the gonads studied, the male element consisted of a remnant with two flattened lobes and a sperm duct which had closed (Fig. 25). Numerous spermatogonia evident throughout the lobes, and lacunae within the stroma, indicated that these fishes had functioned as males during the previous spawning season.

In medial and posterior sections of each TYPE V gonad sectioned, gonial cells were located within the thickened wall of the ovaries below the sterile zone (Fig. 26). These gonial cells represented a male remnant which had been evident in all TYPE V gonads up to this size.

Length classes:250-449mm

The four length classes between 250 and 449mm were combined for descriptive purposes to avoid repetition, as most of the gonads sectioned in these classes conformed to the basic types outlined in previous sections. Sections were examined from gonads of fish up to 650mm (R. sarba is reported to attain a length of 800mm, van der Elst 1989), but gonads of fish between 450-650mm (n=26) also conformed to the basic types outlined in previous sections and descriptions of these gonads are not included in the study.

Macroscopic investigation of gonads from fish measuring 250-449mm revealed a male to female (combined) sex ratio close to unity in each of the 50mm length classes (see Fig. 19), but there were notable changes in the ratios of the four gonadal types within and between classes. A major change in ratios occurred between 200-299mm. In the length class 200-249mm the majority of fish sampled were males (72%), whereas in the following length class they constituted only 51%. The proportion of TYPE II gonads remained relatively unchanged (23% to 19%) but TYPE III gonads which, up until this stage had represented the 'basic type' or main line of development, had dropped from 49% to 32%. This decrease coincided with a sharp increase in TYPE IV (predominantly female) gonads (13% to 39%) and a two-fold increase in TYPE V gonads. Thereafter the ratios of males remained relatively constant (after an initial further decrease in the number of TYPE III gonads), while the number of TYPE V gonads rose steadily with a corresponding decrease in numbers of TYPE V gonads (see Fig. 19).

Histological investigation of gonads within these length classes (n=34) revealed that the morphology of the four basic gonad TYPES remained unchanged from previous length classes (Fig. 27), but that there was a notable increase in the number of TYPE IV (predominantly female) gonads with 'compressed' male lobes similar to those noted for the first time in the previous length class (Figs 28,29). Some of these fish were sampled during the height of spawning activity and all stages of spermatogenesis were evident in the 'compressed' male elements (Fig. 28d). Gonads in post-spawning condition (traces of spermatozoa still evident within sinuses and in the sperm duct) were of two types. In the first, spermatogonia were proliferating and there was little evidence of degenerating tissue (Fig. 29a,b). In the second there was extensive degeneration of gonia and somatic tissue with numerous cells in phagocytic activity (Fig. 29c,d). A notable feature within this and the smaller length classes was the fact that all TYPE IV gonads were, or had been, functional males and that development of oocytes in the female elements of these gonads had not progressed past the perinucleolar stage (Fig. 30).

2.3.3 Gonadal development in A. berda

Early morphological development and sex differentiation:

Length class: 0-49mm

The smallest fishes investigated in this study measured between 20-30mm (fork length) (n=6). At these lengths the formation of the gonads had begun. Each gonadal primordium consisted of a small membrane-lined organ containing small numbers of somatic cells and prominent blood vessels, suspended from the dorsal abdominal wall by a thin mesovarium (Fig. 31a). No germ cells were evident in these primordia.

Anterior, medial and posterior sections of gonadal primordia from two fishes measuring 38mm and 45mm were prepared for electron microscopy. The primordia in these fishes were single structures without a central cavity and a caudo-cranial gradient of somatic development was evident. The anterior part of the primordia was small and was dominated by two prominent blood vessels which extended dorsally along the length of the newly forming gonad (Fig. 31b). Medially, the primordia were more elongate and several capillaries were evident amongst somatic cells (Fig. 31c). Growth was most extensive in the posterior region where the primordia extended further into the abdominal cavity (Fig. 31d).

At this stage there was no evidence of colonization of the gonadal primordia by primordial germ cells (PGC's), or the development of gonial cells. Gonadal primordia consisted primarily of somatic cells, loose connective tissue and fibrillar tissue (Fig. 32a). Small clusters of granulocytes were scattered throughout the stroma, but were more numerous in the dorsal region (Fig. 32b). Large clusters of very dense pigment granules were found in close proximity to blood vessels (Fig. 32c). In the posterior region a large number of vacuolated cells were evident in the ventral stroma (Fig. 32d).

Length class: 50-99mm

Formation of a central cavity occurred in the length class 50-99mm. In each gonadal primordium (n=13) it was formed by the growth of a second extension from the mesovarium ventrally alongside the first (Fig. 33a,b). The two extensions fused in the ventral region (Fig. 33c). The caudo-cranial gradient of somatic development noted in smaller fishes was reversed in the formation of the central cavity, the two elements fusing progressively from anterior to posterior. There was asymmetrical development of the left and right gonadal primordia (Fig. 33). This asymmetry was not consistent from one fish to another, either one of the lobes developing faster than the other.

Anterior, medial and posterior sections of gonadal primordia from four fishes (54,58,63,90mm) were prepared for electron microscopy. In anterior regions, the stroma consisted almost entirely of somatic cells, loose connective tissue and large aggregates of pigment granules (Figs 34a,35a & 36a). From the middle of the primordia through to the posterior region, however, the lateral (outer) element was composed mainly of fibrillar and loose connective tissue (Figs 34b,c, 35b,c & 36b) interspersed with somatic cells and aggregations of pigment granules (Fig. 36c). This tissue formed a sterile zone in which no development of sex cells occurred.

The development of a central cavity and the subsequent appearance of germ cells (gonia) signified the transition from primordium to gonad. In smaller fishes within this length class, cysts of less-electron-dense (LED) cells (Fig. 36d) were evident along the margins of the central cavity (presumptive female area) and in the stroma of the ventral part of the gonad (presumptive male area), but no PGC's or gonial cells were evident. The appearance of the cysts in the presumptive female area coincided with the initial development of lamellae which protruded into the central cavity. Dorsal to the central cavity, below the mesovarium, were concentrations of granulocytes and vacuolated cells amongst loose connective and fibrillar tissue.

In larger fishes within this length class, male and female presumptive areas and the sterile zone were easily recognized (Fig. 35). In anterior and medial sections large lacunae separated the presumptive female area from the stroma (Fig. 35a,b). In the posterior region the gonad was more compact (Fig. 35c). The stroma was composed mainly of granulocytes, vacuolated cells and fibrillar tissue (Fig. 36a,b). In the female area, a number of LED cysts were evident along the margins of the central cavity (Fig. 37a) and lamellae protruded further into the cavity.

A large number of LED cysts (Fig. 37c) were also evident in the presumptive male region. A few gonia and cysts of less-electron-dense cells (Fig. 37d) were evident near the major blood vessel and in the ventral region, but nowhere had gonial cells begun to proliferate and PGC's could not be found. In two fishes measuring 63mm and 90mm, no gonia were located in the former and only seven in the latter.

Length class: 100-149mm

Differentiation of male and female germinal tissue occurred in this length class. According to Kyle (1986), these fishes are in the early part of their second year of life (12-16 months). Development varied considerably between individuals in this length class and ranged from undifferentiated gonads with few gonial cells, to fully functional mature gonads.

Anterior, medial and posterior sections of three differentiating gonads (104,115,120mm) were prepared for electron microscopy. Apart from larger lacunae separating the female germinal tissue from the back stroma (Figs. 38,39,41), and more distinctive male and female elements, these gonads were similar in morphology and cellular composition to those described in the previous length class. Up to, and including, the time of differentiation, therefore, all gonads conformed to a 'basic type'.

In the gonads of smaller fishes within this length class (104, 115mm), cysts of less-electron-dense cells were more numerous (Fig. 40a). In the presumptive female area they were located along the margins of the central cavity and in the presumptive male area they were located throughout the stroma. In some of the cysts, one or two cells with prominent nucleoli were evident (Fig. 40b).

A few gonial cells were also evident in female presumptive areas (one in AM&P sections of A. berda 104mm and five in AM&P sections of A. berda 115mm). These gonial cells (Fig. 40c) were located amongst numerous cysts in loose connective tissue near the main blood vessel, and along the margins of the central cavity. They were surrounded by flattened cells similar in appearance to follicle cells which surround oocytes at a later stage of development (Fig. 40c,d).

Development of germ cells in the gonads of A. berda (120mm) was far more advanced than in those of A. berda (115mm) as a result of a simultaneous proliferation of both male and female tissue (Fig. 41b,c). Gonial cells were present throughout the female element (Fig. 41d), with most activity in the mid-region, and a number of early previtellogenic oocytes were also evident (Fig. 41a-c). In the male element, spermatogonia were evident within lobules in the posterior region and all stages of spermatogenesis were present, including spermiogenesis (Fig. 41e,f). Despite a careful search through all of the early developmental stages of A. berda, only one cell was seen which conformed to the descriptions of primordial germ cells (PGC's) in the literature. This cell, which lacked a prominent nucleolus but which had all the other features listed earlier, was found in a fish of 115mm in association with gonial cells in the female element (Fig. 42b). Similar cells could not be located in the male element and none were found in either element in the gonads of the fish of 120mm.

Leydig cells first became evident in the fish of 120mm (Fig. 42c). Their differentiation was simultaneous with the formation of spermatogenic lobules within the male element.

Gonadal development after differentiation:

Length class: 100-149mm (cont.)

In the Richards Bay estuarine sanctuary the majority of fish in the length class 100-149mm were classified macroscopically as immature (55%), but a number had developed into males with TYPE II (37%) and TYPE III gonads (8%) (Fig. 43). Microscopic investigation of male gonads (n= 12) showed that they conformed morphologically and cytologically to the basic types identified in A. berda and R. sarba (see above).

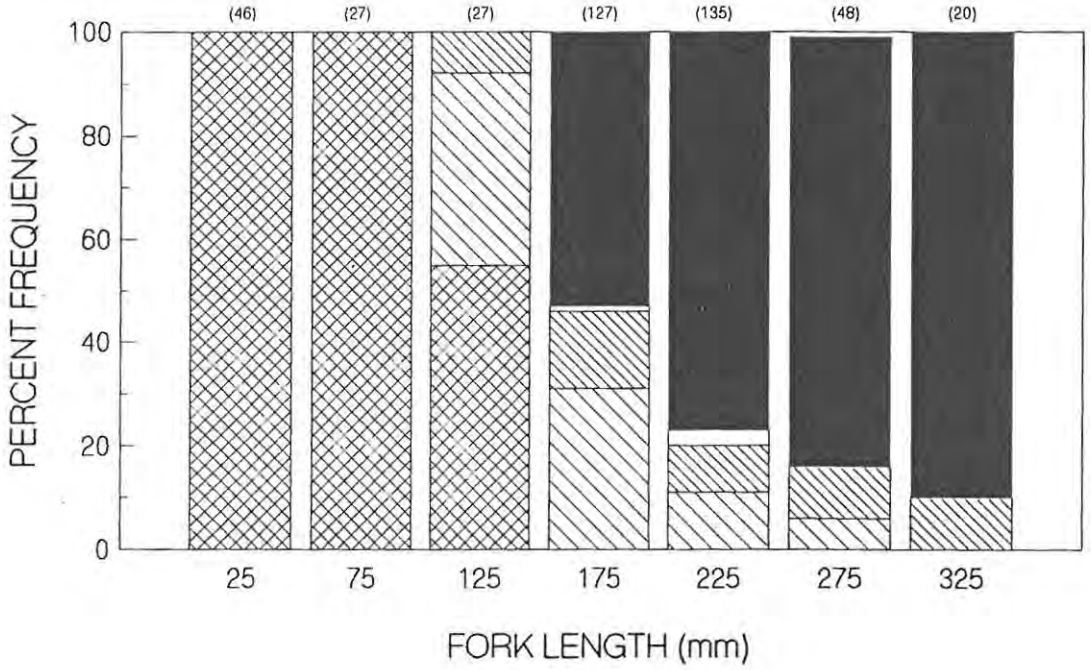

Figure 43. Percentage composition, in 50mm size classes, of gonad types in Acanthopagrus berda sampled from the Richards Bay estuary. ⊠ Immature (I), ▨ male (II), ▨ predominantly male (III), □ predominantly female (IV), ■ female (V). Sample sizes in parenthesis.

Male TYPE II gonads contained ovarian tissue consisting of a single (or at most a few) layer of previtellogenic oocytes along the margins of the former central cavity, opposite the sterile zone (Fig. 44a,b). These oocytes were not visible to the naked eye. In TYPE III gonads, previtellogenic oocytes were more numerous. They were located within lamellae which extended into the former central cavity opposite the sterile zone (Fig. 44c,d) and due to sufficient germinal, connective and somatic tissue they were easily discernible with the naked eye.

No females in this length class were sampled in the Richards Bay estuary (Fig. 43) although females between 100-149mm were sampled in Durban Bay and the Umgeni estuary. In all ovaries sectioned for analysis (n=7), development of oocytes had not proceeded past the previtellogenic stage (Fig. 45). In females with TYPE V gonads, there was either a male remnant on the ventro-lateral surface in mid- and posterior regions (Fig. 45c), or gonial cells could be detected in the tunica albuginia below the thickened sterile zone (Fig. 45d,e). In females with TYPE IV gonads (n=4), male elements were either inactive or undergoing spermatogenesis. In two females with TYPE IV gonads all stages of spermatogenesis were evident in the male elements (which constituted 10% & 14% of the gonads), indicating that they had been functional males, but both testes were undergoing extensive degeneration. They had become vacuolated and phagocytosis was evident in the interstitium (Fig. 45a,b).

Length class: 150-199mm

In this length class all fish sampled in the Richards Bay estuary (n= 127) had well developed gonads and were mature. Whereas no functional females were recognised in the smaller size classes, they constituted 53% of this size class. There were relatively fewer fish with TYPE II (male) gonads (31%), but the proportion of those with TYPE III (male) gonads had increased to 15%, resulting in a functional sex ratio close to unity (see Fig. 43).

Microscopic examination of 40 gonads in this length class revealed that the basic morphology of the four gonad types remained unchanged (Figs 46,47,48). The distinction between male TYPE II and TYPE III gonads was sometimes difficult to make from histological sections, however, as there was a continuum in the amount of ovarian tissue in these gonads.

In TYPE V gonads (n=12), gonial cells were evident in the tunica albuginia of most (n=8) active and inactive gonads (Fig. 47), in mid- and posterior sections. They were most abundant at the base of the thickened sterile zone, but in some gonads small groups were also found further along the tunica albuginia, in close association with blood vessels (Fig. 47b).

Females with TYPE IV gonads represented only 1% (n=2) of the total sample in this length class from the Richards Bay estuary. Since this type was considered the one most likely to indicate transition from male to female function, further samples were collected from Durban Bay for histological analysis. Of six gonads conforming to this type, active spermatogenesis was evident in the male element of two, while in the others the male element was degenerating (Fig. 48a-c). Scattered gonial cells were evident near the outer margins of these gonads, but nowhere were they abundant. The matrix had become disorganized and consisted almost entirely of interstitial tissue, vacuoles and brown bodies (Fig. 48b,c). Each of these gonads had well developed sperm ducts, suggesting that these fish had functioned as males.

Length class: 200-249mm

This length class was dominated by females with TYPE V gonads (77%) (see Fig. 43). Of twenty TYPE V gonads sectioned, sixteen had thickened sterile zones. Gonial cells were evident in the tunica albuginia of only a few of these gonads, generally close to the sterile zone. The other TYPE V gonads had testicular remnants within which gonia were evident along the entire length, even in the most anterior regions which were barely discernible as male tissue (Fig. 49).

The proportion of TYPE II males was considerably reduced (11%) in this length class and, collectively, males constituted only 20% of the sample of 135 fish (see Fig. 43). Apart from a number of gonads which became convoluted during the spawning season (Fig. 50a), male gonad morphology remained the same (Fig. 50b,c). In none of the 23 male gonads sectioned had oocytes in the female element developed past the previtellogenic stage. There were, however, signs of lamellar development in two gonads. Whereas there was little interstitial tissue in the ovarian lamellae of most male gonads (Fig. 50b), the lamellae of these gonads had filled and thickened with interstitial tissue (Fig. 50d). Sperm production was complete in both of these gonads, spermatogenic tissue consisting entirely of spermatozoa and gonial cells. Interstitial tissue and spermatogonia located amongst lobules of spermatozoa appeared to be degenerating (Fig. 50e). A number of cells had become vacuolated and a clusters of cells in phagocytic activity were evident amongst these cells. Spermatogonia along the distal margins of these testes appeared to be inactive, neither degenerating nor proliferating.

The remaining 3% of the fish in this length class consisted of females with TYPE IV gonads. There were no TYPE IV gonads in the samples collected for histological analysis.

Length classes: 250-299mm and 300-349mm

These length classes were composed almost entirely of females with TYPE V gonads (83% & 90% respectively) (see Fig. 43). Of the TYPE V female gonads sectioned for histological analysis, four had thickened sterile zones, four had no thickened sterile zones and one had a testis remnant. Gonial cells were evident in gonads with thickened sterile zones (Fig. 51) and in the testis remnant, but not in gonads without thickened sterile zones. No females with TYPE IV gonads were sampled.

The proportion of males with TYPE II gonads was 6% and 0% respectively, whereas the proportion of male TYPE III gonads remained at a constant 10% (see Fig. 43). With one exception, no changes in structural morphology of male gonads were noted. The exception was a mature male in which oocytes in the ovarian element were undergoing vitellogenesis (Fig. 52). This fish was sampled at the end of the spawning season and the testis was in a partially spawned condition. The gonads were large and, while there was little sperm in the sperm duct, all stages of spermatogenesis were evident throughout the gonad, including numerous sinuses of spermatozoa.

One specimen larger than 350mm was sampled. This fish was an active male with a TYPE II gonad (Fig. 53), showing that even though no males of this type were sampled in the length classes 250-349mm, they do occur throughout the size range.

2.3.4 Classification of gonad types - C. puniceus and C. nufar

Preliminary investigation of the gonads of C. puniceus and C. nufar showed that they were similar to one another but were different, in many respects, to those of R. sarba and A. berda. Gonads could be classified as follows: I - Indeterminate, II - Immature female, III - female, IV - male, V - bisexual. Each of these gonad types could be identified macroscopically to determine their frequency of occurrence in wild populations.

The five gonad types were defined as follows: TYPE I - INDETERMINATE where, macroscopically, the sex could not be determined. Microscopically these gonads ranged from gonadal primordia in which no germinal tissue was evident, to gonads in which gonial

cells had proliferated and the production of previtellogenic oocytes had commenced. TYPE II - IMMATURE FEMALE, macroscopically the gonads were typically ovarian in structure and colour, being tubular organs which were translucent and pink to yellow/orange in colour. Microscopically, numerous ovigerous lamellae projected from the tunica albuginea into the central cavity. Development of oocytes was arrested at the previtellogenic stage. The male element, which was present in all gonads investigated, consisted either of one or two layers of gonial cells in the tunica albuginea at the base of the sterile zone, or a slightly enlarged element with numerous gonial cells. Neither was visible to the naked eye. TYPE III - FEMALE, macroscopically the ovaries were larger and more opaque. Oocytes were visible throughout much of the year and all stages of oogenesis were evident during the spawning season. Microscopically, the ovaries were similar in structure to those of immature females, including the male element, but all stages of oogenesis were evident during the spawning season. TYPE IV - MALE, macroscopically the gonads were more dense than ovaries and were v-shaped in cross-section. They ranged in colour from white to grey-pink, according to their state of maturity. Microscopically, the testis consisted of numerous spermatogonial cysts and spermatogenic lobules which opened into minor sperm ducts and finally into the main sperm duct. The main sperm duct surrounded the remnants of a female central cavity. TYPE V - BISEXUAL, macroscopically both male and female elements were visible. The testis was ventro-lateral to the ovary, extending from the mid- to the posterior region. Oocytes in the ovary were arrested in the previtellogenic stage, but activity in the male element ranged from the proliferation of gonial cells to spermiogenesis.

2.3.5 Gonadal development in C. puniceus

Early morphological development and sex differentiation:

Length class: 0-49mm

Despite extensive sampling along the Natal coast over a period of eight years, no juvenile C. puniceus of this size were located in the wild. Scientist and sport divers searched for these fish on numerous reefs, along reef/sand interfaces and over sand from the shoreline to 60m depth from Kosi Bay in the north to the Aliwal shoal in the south. Trawl catches made by commercial and research craft were inspected and commercial linefishermen were asked to notify the Oceanographic Research Institute of any juveniles caught within this size range. None were reported.

In an effort to overcome this problem, attempts were made to culture juveniles from eggs stripped from ripe adults treated with HCG (human chorionic gonadotropin) and LHRHa (Leutenizing hormone-releasing hormone analogue) (Garratt et al. 1989). Juveniles were successfully raised through two critical feeding periods which occurred during the first two weeks but they could not be raised past the age of one month (+/-10mm) and the project did not produce the required samples for histological analysis.

Length class: 50-99mm

Of four fish sampled during this study, the gonadal primordia of two were prepared in wax (70mm, 76mm) and two were prepared for electron microscopy (95mm,96mm). In primordia removed from fish measuring 70mm and 76mm, the formation of the central cavity was complete, but there was no evidence of germ cells within the stroma or along the margins of the central cavity.

Anterior, medial and posterior sections of undifferentiated gonads from fish measuring 95mm and 96mm showed a number of less-electron-dense (LED) cysts of cells along the margins of the central cavity and a few gonial cells were evident amongst them. Proliferation of gonial cells had not commenced, but their appearance along the margins of the central cavity signified the transition from primordium to gonad (Figs 54,55). Left and right gonads were of similar size and structure in both fish, but cytological development was more advanced on the one side (Fig. 54). In the one lobe, only LED cysts were evident along the margins of the central cavity, while in the other there were a number of gonial cells amongst these cysts. A distinct sterile zone extended down the outside of each lobe, as in A. berda and R. sarba, but, unlike these species, a distinct male element was not evident. The only indication of a male element was the presence of a few LED cysts and gonia in the outer stroma below the sterile zone (Fig. 54d). Morphologically these gonads appeared to be developing into ovaries.

A distinctive feature of the gonads of both fish was the development of large lacunae between the germinal tissue situated along the margins of the central cavity and the tunica albuginea (Fig. 54). Lacunae were more abundant in lobes in which development was most advanced and their formation appeared to be related to the development of lamellae.

Anterior, medial and posterior sections prepared for electron microscopy revealed a cellular composition of the gonads similar to those of A. berda and R. sarba. Numerous

granulocytes and vacuolated cells were evident amongst the somatic cells, loose connective tissue and fibrillar tissue throughout much of the gonad (Fig 56a). The sterile zone was composed mainly of loose connective and fibrillar tissue with scattered somatic cells (Fig. 56b). Cysts lining the central cavity were composed of the same LED cells as those in A. berda and R. sarba (Fig. 56c). There were no apparent differences in the structure and cytology of gonial cells (Fig. 56d) between the former species and C. puniceus.

Length class: 100-149mm

Sex differentiation occurred within this length class, with all but one of the eleven gonads examined developing into ovaries. Within the gonads of these small fishes, gonial cells had proliferated along the margins of the central cavity and the first oocytes were evident (Fig. 57). In the larger specimens of this size class, there was a proliferation of oogonia and simultaneous production of previtellogenic oocytes which resulted in the formation of lamellae that extended further into the central cavity giving the gonads a typically ovarian appearance (Fig. 58). In none of these gonads was there an anti-posterior gradient of development similar to that found in A. berda and R. sarba. Initially, proliferation of gonia and previtellogenic oocytes appeared to be more rapid in the mid-region (Fig. 57), but further development appeared to be uniform throughout the gonad. Male tissue was difficult to locate in these gonads and consisted of only a few gonial cells in the tunica albuginea below the sterile zone in posterior sections.

As in the previous length class, all gonads investigated in this length class were characterised by large lacunae which had developed between the germinal tissue, situated along the margins of the central cavity, and the tunica albuginea (Figs 57,58). The formation of these lacunae appeared to be necessary to allow for the rapid proliferation of germ cells and the simultaneous folding of the central cavity membrane into lamellae (Fig. 58d). Continued proliferation of germ cells resulted in a progressive reduction of these lacunae.

Morphologically, the exceptional gonad was similar to the others in this length class and was typically ovarian in form but, within developing lamellae, both male and female germinal tissue were proliferating alongside each other (Fig. 59). All stages of spermatogenesis were evident within cysts situated along the margins of the forming lamellae (Fig. 60). Amongst these cysts there were a number of previtellogenic oocytes.

Sections prepared for electron microscopy showed that cell types and their configuration in normal gonads remained unchanged from those in the previous length class. Granulocytes and vacuolated cells were abundant in the dorsal regions and in the stroma backing the germinal tissue of each lobe (Fig. 61a-c), and the sterile zone consisted mainly of loose connective and fibrillar tissue (Fig. 61d).

Along the greater part of the central cavity, germinal tissue was connected to the back stroma by thin interconnecting strands of connective tissue and somatic cells (Fig. 62a). Up to this time there had been no evidence of cells conforming to PGC's described in other teleosts. Gonial cells appeared to develop from within LED cysts along the margins of the central cavity (Fig. 62b,c). The development of a single gonial cell within a cyst forced the other cells within that cyst to its outer margins. In this process these cells flattened and differentiated into myoid boundary cells. Once this process was complete, gonial cells proliferated by mitosis (Figs 61c,62d) and early previtellogenic oocytes (pre-perinucleolar) appeared soon thereafter (Fig. 63).

Gonadal development after differentiation:

Descriptions of gonadal development in C. puniceus larger than 200mm are based on fish sampled from the Leadsman shoal in the St Lucia marine reserve (see Fig. 1). Smaller fish were not resident on the shoal and those in the 150-199mm length class were sampled from various stations along the Natal coast between Richards Bay and Durban. Macroscopically, all fish sampled within this length class (n=223) were classed as immature (inactive) females. No males were recorded.

Length class: 150-199mm

All gonads sectioned within this length class (n=13) were ovaries with well developed ovigerous lamellae filling the central cavity. Male tissue was generally restricted to the mid-posterior region and was limited to a small band of gonial cells within the tunica albuginea below the sterile zone (Figs 64-67). In a gonad taken from a specimen of 182mm (FL), sufficient gonial cells had proliferated to form a small appendage in the posterior region (Fig. 65d), but there was no evidence of further development of male tissue in any of the other gonads. In most gonads, gonial cells in the tunica albuginea appeared to be inert while those along the margins of ovigerous lamellae were proliferating (Fig. 67). There was no evidence of Leydig cells in association with gonial cells in the tunica albuginea.

Length class: 200-249mm

Seventy six percent of the fish in this length class sampled from the Leadsman shoal (n=21) were immature females (TYPE II). The remainder were mature females with TYPE III gonads (Fig. 68) and one bisexual. No males were recorded.

Anterior, medial and posterior (AMP) sections of five gonads were prepared for histological investigation. Mid-sections were taken from the remainder (n=7). In the hermaphroditic gonad, male tissue was evident macroscopically as a thickening of the tunica albuginea in the ventro-lateral region. Microscopic investigation of this gonad revealed a small v-shaped appendage immediately below the sterile zone in mid- and posterior sections (Fig. 69). Gonial cells were evident in this appendage, and surrounding it, and were most numerous in the mid-region. In mid- and posterior sections lacunae were evident in the sterile zone. There was no evidence of male tissue in the anterior region of the gonad. In all other gonads male tissue was restricted to a thin band of gonial cells within the tunica albuginea (Figs. 70,71).

Length class: 250-299mm

All fish sampled in this length class (n=93) were sexually mature and 96% were TYPE III females (see Fig. 68). Two bisexuals and two males were also recorded, the latter measuring 272mm and 289mm being the smallest males recorded from the Leadsman Shoal.

Histological analysis was conducted on the gonads of twelve specimens, including four from which AMP sections were taken. As in the previous length class, the majority of gonads examined were ovaries with male elements consisting of a narrow band of gonial cells in the tunica albuginea below the sterile zone. Male elements were evident in all specimens examined, including ripe females (Fig. 72). In one fish, which was sampled at the beginning of the spawning season, gonial cells in the male element were proliferating and in the posterior region all stages of spermatogenesis were evident, including spermiogenesis (Fig. 73). Within the ovary a number of yolk vesicle oocytes amongst densely packed previtellogenic oocytes indicated that this fish would have spawned as a female during the forthcoming spawning season.

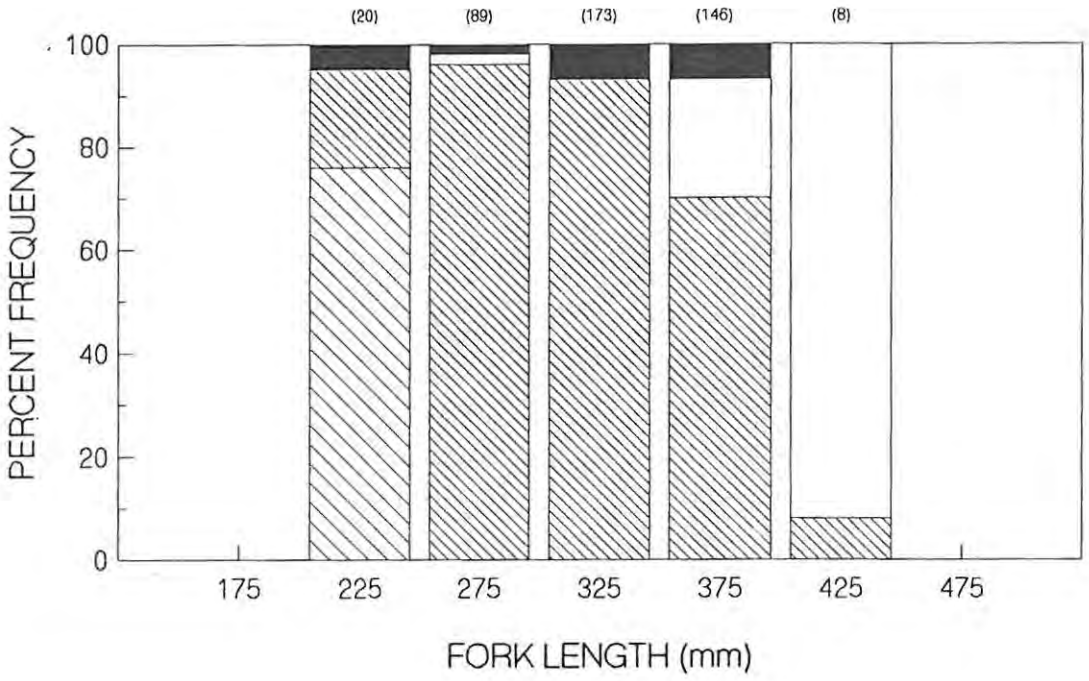

Figure 68. Percentage composition, in 50mm size classes, of gonad types in Chrysoblephus puniceus sampled from the Leadsman Shoal in the St. Lucia marine reserve. ▨ Immature female (II), ▨ female (III), □ male (IV), ■ bisexual (V). Sample sizes in parenthesis.

The gonads of both males consisted of testicular tissue which was v-shaped but which was compressed dorso-ventrally (Fig. 74). A conspicuous ovarian remnant, consisting of an empty central cavity around which the main sperm duct had developed, was dorsal to the testicular tissue.

Length class: 300-349mm

Of 186 fish sampled in this length class, 93% were females with TYPE III gonads (see Fig. 68). Several bisexuals were recorded (7%), but there were no males.

Histological investigation of the gonads of 10 fish, including three from which AMP sections were taken, showed that all TYPE III gonads conformed to the general description of these gonads in smaller length classes. Inactive and active gonads contained male tissue, in the form of gonial cells, in the tunica albuginea below the sterile zone (Fig. 75). In some gonads, especially those in ripe condition, gonial cells in the tunica albuginea were few in number and difficult to detect (Fig. 75d). In these gonads serial sections were necessary to locate the male element.

AMP sections of bisexual gonads showed typically ovarian gonads with a thickened tunica albuginea below the sterile zone in mid- and posterior sections. Gonial cells were proliferating within male elements (Fig. 76).

Length classes: 350-399mm and 400-449

These length classes were combined for descriptive purposes because most fish changed sex within this size range in the community on the Leadsman shoal. Macroscopic investigation showed that 70% of C. puniceus in the length class 350-399mm were females (n = 182) and that the remainder were either bisexuals (TYPE V) or males. In the length class 400-449mm, 92% of the fish examined (n = 100) were males (see Fig. 68).

In an earlier study Garratt (1986b) reported relatively few bisexuals in the population of C. puniceus in Natal (1%, n = 5310). Evidence of sex change in this species was based largely on population structure (size separation of the sexes) and evidence of degenerating female tissue in a small sample of gonads in which there was the simultaneous development of male tissue. These gonads were obtained during the months of February and March.

Suspecting that sex change in C. puniceus may occur only during these months, further effort to obtain material for descriptive purposes was directed at these months. Histological analysis of 44 gonads sampled from the Leadsman shoal and in southern Mozambique between February-April 1989-92, including 17 from which AMP sections were examined, confirmed that this species does change sex. Continued absence of sex-changing fish throughout the remainder of the year also confirmed that sex change is limited to the period mid-way between spawning seasons.

Transitional gonads ranged from those which were predominantly female (Fig. 77), through gonads with male and female elements of equal volume in which female germinal tissue was considerably reduced (Fig. 78), to those in which the female element consisted of a large central cavity within which ovigerous lamellae were further reduced in number and size (Fig. 79). In each of these gonads there was active spermatogenesis in the male element (Fig. 77d) and simultaneous degeneration of previtellogenic oocytes and ovigerous lamellae in the female element (Figs 78b, 79b). Degeneration of female germinal tissue was characterised by extensive formation of yellow-brown bodies within lamellae (Fig. 80).

The testes of males which had recently changed sex differed morphologically from those in older males. In these young males the testes were characterised by two lobes which developed laterally and dorsally around the former, but still relatively large, central cavity (Fig. 81). This gave the gonad a characteristically anchor-like outline. In these gonads and in transitional gonads which were in the final stages of sex change, the main sperm duct completely enclosed the central cavity (Figs 79, 81). In the latter, testes conformed to the basic v-shape found in sparids in which the central cavity is considerably reduced in size and is virtually enclosed by male tissue.

2.3.6 Gonadal development in C. nufar

Early morphological development and sex differentiation:

Length class: 0-49mm

As in C. puniceus, extensive searches for juveniles of this size, in the area between Kosi Bay and the Aliwal shoal, failed to produce any specimens and their whereabouts remains unknown. Juveniles were, however, successfully raised through to the age of eight months from eggs which were spawned in the main display tank at Sea World in Durban (Garratt

et. al. 1989). At one month the mean length of these juveniles was 10mm total length (TL), at two months 37mm (FL) and at three months 60mm (FL). Survival past two months was very low and only two specimens of 37, 44mm (FL) were preserved in formalin for histological analysis.

Sectioning of the smaller fish was unsuccessful and descriptions are limited to a specimen of 44mm at the light level. For the most part, the gonadal primordium of this specimen consisted of a single element suspended from the dorsal wall of the body cavity by a thin mesovarium (Fig. 82). Only in the anterior region was the central cavity fully formed (Fig. 82a). Along the remainder of the primordium, the second element consisted of a small appendage which had begun to extend down the side of the first element. Development of this second element was most advanced in the most anterior region of the mid-section (Fig. 82b), suggesting a cranio-caudal gradient of development of the central cavity similar to that recorded in the other species.

Cellular organization within the gonadal primordium also appeared to be similar to that of R. sarba, A. berda and C. puniceus of comparative size, the stroma primarily consisting of somatic cells, granulocytes and vacuolated cells. At this stage of development there were a few lacunae within the stroma but the primordium was relatively compact. No gonial cells were evident and there were no less-electron-dense (LED) cysts along the margins of the central cavity, which would have indicated the onset of germinal development.

Length class: 50-99mm

Prior to Cyclone Domoina in 1984 and the Natal floods in 1987, juvenile C. nufar in this size range were commonly caught off Durban's beaches by seine netters. Since these floods they have not been seen by the netters and only a few (n=6) have been caught in deeper water using SCUBA. Three juveniles of 53, 76 & 91mm (FL) raised in captivity were added to this sample. The descriptions of gonad development in this length class are based on these nine specimens, five of which were embedded in resin and four in wax.

Representative material from two fish measuring 76mm and 79mm (FL), which was prepared for electron microscopy, is presented (Figs. 83,84). In all specimens examined a central cavity had developed and in resin embedded sections a few cysts of LED cells were evident along the margins of the cavity. No gonial cells were evident at this stage of development and the structures were considered 'undifferentiated primordia'.

A characteristic feature of these primordia was the numerous lacunae in mid- and posterior

regions and columnar epithelial cells lining the cental cavity. Anterior, medial and posterior (AMP) sections prepared for electron microscopy revealed a similar cellular composition to the gonadal primordia of R. sarba, A. berda and C. puniceus. Numerous granulocytes and vacuolated cells were scattered amongst somatic cells and loose connective tissue throughout the stroma (Fig. 84d). The sterile zone, which extended halfway down the outside of each lobe, was more compact than the germinal region, being composed of more numerous granulocytes and vacuolated cells amongst loose connective and fibrillar tissue (Fig. 84e). Presumptive male and female elements could not be distinguished at this stage of development and the LED cysts lining the central cavity were similar to those described for the above species. Serial sections from AMP regions of these gonadal primordia did not reveal any cells conforming to primordial germ cells (PGC's) described in other teleosts.

Length class: 100-149mm

The majority (76%) of gonads in this length class were classified macroscopically as indeterminate (TYPE I) (Fig. 85). The remainder were TYPE II immature females. No males or bisexuals were recorded.

AMP sections were cut from ten gonads in this length class. Two of these gonads had differentiated into ovaries in which gonial cells were proliferating along the margins of the central cavity and in which the first previtellogenic oocytes were evident (Fig. 86a,b). Development in the remaining gonads was limited to a small number of LED cysts and gonial cells along the margins of the central cavity (Fig. 86c-e). All gonads were similar in morphology and appeared to be developing in a female direction. A presumptive male element was not evident in any of these gonads.

Anterior, medial and posterior (AMP) sections of two gonads were prepared for electron microscopy. In each of these gonads, germinal tissue was separated from the back stroma by large lacunae (Figs. 87,88). In the smaller specimen, only LED cysts were evident along the margins of the central cavity (Fig. 87d,e). The composition of these cysts was similar to those reported in R. sarba, A. berda and C. puniceus, consisting of a number of cells which appeared to be similar in morphology and size to surrounding somatic cells, but distinguishable from them by their less-electron-dense nature. In the larger specimen, several gonial cells were evident amongst LED cysts, but proliferation of gonial cells had not commenced (Fig. 88).

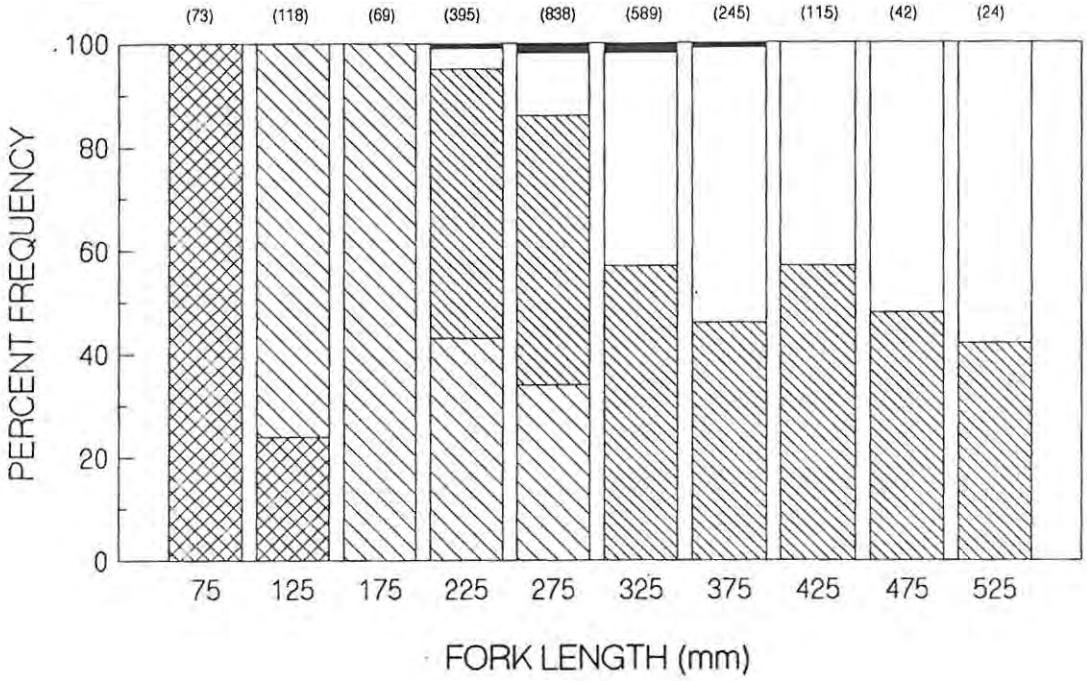

Figure 85. Percentage composition, in 50mm length classes, of gonad types in Cheimerius nufar sampled along the Natal coast. ⊠ Indeterminate (I), ▨ immature female (II), ▨ female (III), □ male (IV), ■ bisexual (V). Sample sizes in parenthesis.

Serial sections from AMP regions of each of these gonads were scanned for PGC's. No cells conforming to those reported for other teleosts were evident and, as suggested for C. puniceus, it appeared that gonial cells developed from within the LED cysts lining the central cavity (Figs 89,90).

Length class: 150-199mm

Development varied considerably in this length class, ranging from differentiating gonads with gonial cells proliferating along the margins of the central cavity (Figs 91,92), to well-developed ovaries in which numerous previtellogenic oocytes were evident in well-developed lamellae (Fig. 95). All the fish sampled in the length class (n=69) were classified macroscopically as immature females (TYPE II) and no males or bisexuals were recorded (see Fig. 85).

Anterior, medial and posterior sections of two differentiating gonads were prepared for electron microscopy. The morphology of these gonads was similar to those described in the previous length class (Figs 91,92). Gonial cells had proliferated along the margins of the central cavity and early previtellogenic oocytes were evident amongst them, but this germinal tissue was still separated from the back stroma by large lacunae. In some areas the proliferation of gonial cells along the margins of lacunae towards the back stroma, together with a simultaneous folding of the central cavity membrane, had resulted in the initial formation of lamellae (Fig. 91c,e). A similar pattern of development was noted in C. puniceus and it would appear that this is a mechanism whereby lamellae are formed in the ovaries of these species.

LED cysts appeared to decrease in number in the mid- and posterior regions and were less obvious amongst the proliferating gonial cells along the margins of the central cavity (Fig. 92d). Anterior regions of these gonads were, however, less developed and contained numerous LED cysts. Gonial cells were evident within several of these cysts (Fig. 92e) and, as serial sections of anterior, medial and posterior regions of both gonads failed to reveal any PGC's similar to those described in other teleosts (Bruslé & Bruslé 1978a,b, Bruslé 1986, Bruslé-Sicard et al. 1992), it appeared that these gonial cells developed from within the LED cysts and not from relatively large PGC's which migrated into the region. Similar evidence was also found in the ventral part of the sterile zone, in which the initial development of a male element had commenced (Figs 92c,93).

A further eleven gonads were prepared for histological analysis, including seven from which anterior, medial and posterior sections were cut. Two of these gonads were in an early stage of differentiation similar to that described above. The remainder were typically ovarian in structure with numerous previtellogenic oocytes developing in ovigerous lamellae which protruded progressively into the central cavity (Fig. 94). All gonads sampled were thus developing in a female direction. No fish with mature gonads were sampled in this length class and no males within this size range were recorded along the entire Natal coast.

Even though all gonads were developing into ovaries, there did appear to be differences in gonad structure within the sample. Gonads varied in the the extent of development of sterile zones and male elements. In some gonads the sterile zone extended no further than one third down the length of the gonad in all sections (Fig. 94a-c). In others it extended half to two thirds down the gonad in mid- and posterior regions (Fig. 94d-f). In the former, the male element consisted of relatively few gonial cells in the ventral region of the sterile zone (Fig. 95a,b). In the latter, gonial cells were more numerous, and in several gonads they appeared to be proliferating (Fig. 95c,d). These differences probably reflect different stages of development along a continuum.

Length class: 200-249mm

Ninety four percent of the fish sampled in this length class were classified macroscopically as females (TYPES II&III), 4% as males and 1% as bisexual (see Fig. 85). This was the first appearance of males in the population. A sample of 17 gonads was sectioned for histological analysis, including five from which anterior, medial and posterior sections were cut. Mid-sections were taken from the remainder. All but two of these gonads were TYPE II ovaries with previtellogenic oocytes in well-developed lamellae. In AMP sections a male element was evident as a small number of gonial cells in the tunica albuginea below the sterile zone in mid-posterior regions (Fig. 96).

Two bisexuals, identified macroscopically as such, were included in the sample. In the first, the male element was visible as a thin band of testicular tissue in the mid-region of the gonad close to the mesovarium (Fig. 97). Microscopic investigation of this gonad revealed that the male element extended along the length of the gonad, but that proliferation of gonial cells had commenced in the mid-region (Fig. 97e). In the second bisexual gonad the male element was clearly visible as a v-shaped appendage ventral to the ovary (Fig. 98). Within this male element all stages of spermatogenesis were evident, including small lobules of spermatozoa (Fig. 98b).

Length class: 250-299mm

As in previous length classes, the majority of fish in this length class were females (86%), but the proportion of males (12%) and bisexuals (2%) had increased (see Fig. 85).

Ten gonads were sectioned for histological analysis, including three from which AMP sections were taken. Most of these gonads (n=6) were inactive ovaries with a similar morphology to those described in the previous length class (Fig. 99). Gonial cells of the male element were evident in varying quantities in the tunica albuginea of each gonad and in some gonads they appeared to be proliferating (Fig. 99d). Gonial cells were also evident in the tunica albuginea of active ovaries, but they were few in number and more difficult to locate (Fig. 100a,b).

Both testes sectioned were similar in morphology. Each was composed of a male element which was compressed dorso-ventrally (Fig. 100c) and which appeared to have developed around the former ovary. The central cavity of the ovarian remnant was completely enclosed by the main sperm duct.

Length classes: 300-549mm

These length classes were combined for descriptive purposes because the majority of fish within them were males and females with gonads of similar morphology. A few bisexuals were sampled amongst fishes of 300-400mm, but thereafter the population consisted entirely of males and females in roughly equal proportions (see Fig. 85).

Between the length classes 250-299mm and 300-349mm there was a marked increase in the number of males so that in the latter class 41% of the fish sampled were males. In the sample of 15 gonads removed for histological investigation, nine were male and six were female. Ovaries of inactive and active females were similar to those described earlier (Fig. 101a,b), with one exception. In this gonad, all stages of spermatogenesis, including spermatozoa, were evident in the thickened tunica albuginea concomitant with the production of ripe eggs in the ovary (Fig. 101c,d).

All testes sectioned in these length classes no longer had the appearance of being flattened dorso-ventrally, but were of the typical v-shape found in gonochorists, in which the main sperm duct is situated dorsally within the 'v' (Sadovy & Shapiro 1987) (Fig. 102). In each testis, the main sperm duct completely enclosed an ovarian remnant.

2.4 DISCUSSION

Having studied gonadal development in a number of Mediterranean seabreams, including several protandrous hermaphrodites, D'Ancona (1949a) presented a schematic representation of early gonadal development in seabreams and suggested that this pattern of development may apply to all members of the family, irrespective of reproductive style (Fig. 103a). Since the descriptions of D'Ancona (1949a), little work has been carried out on early gonadal development in sparids and his descriptions have remained unchallenged. As most previous studies were restricted to the light level (eg. Kinoshita 1936, 1939, Pasquali 1941, Okada 1965a,b,c, Zohar et al. 1984), one of the aims of the present study was to determine, incorporating electron microscopy, if these descriptions were accurate and if they adequately described early gonadal development in local species. A further objective was to determine if there were any characteristics of early gonadal development which could be used to reliably distinguish protandrous hermaphrodites from protogynous and rudimentary hermaphrodites.

Gonadal development in R. sarba and A. berda

The present study has shown that gonadal development, from the formation of gonadal primordia to sexual maturity and beyond, is similar in R. sarba and A. berda. As there is good evidence to suggest that R. sarba is a protandrous hermaphrodite (Kinoshita 1939, Yeung & Chan 1987), the similarities in gonadal development presented in this study suggest that A. berda has the same capability of changing sex and that it, too, is protandrous. Further evidence supporting protandry in A. berda is given below.

Early gonadal development (the development of gonadal primordia and sex differentiation) follows the general pattern described by D'Ancona (1949a), but there are several aspects of morphological and cytological development in R. sarba and A. berda which differ from the Mediterranean species described. It is believed that these differences are related to differences in interpretation of events, including the timing thereof, rather than interspecific differences in gonadal development.

In the length class 0-49mm, initial development of gonadal primordia in R. sarba and A. berda had commenced in specimens of 20-32mm. Morphologically, these primordia consisted of a single element suspended from the dorsal wall of the body cavity by a thin mesovarium (Fig. 103b(A,B)). The cytological composition of these, and more advanced,

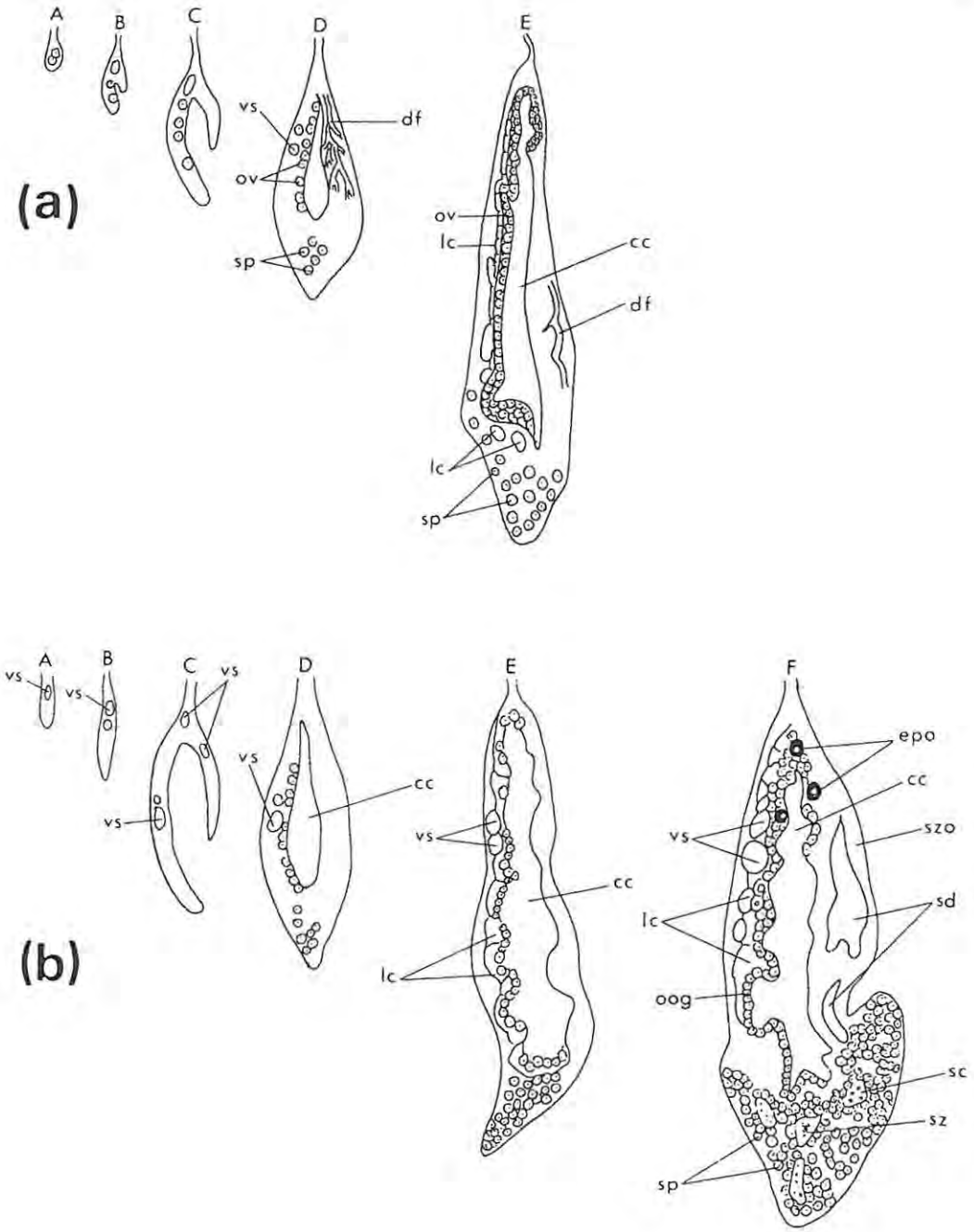

Figure 103. Schematic representation of early gonadal development in sparids. (a) redrawn from D'Ancona (1949a), (b) pathways proposed for Rhabdosargus sarba and Acanthopagrus berda in the present study. (A-B) each primordium starts as a single element. A second element forms later and extends down alongside the first (C). The two elements fuse ventrally forming a central cavity (D). Further development results in three distinct zones: a female element along the inner margins of the central cavity, a male element in the ventral stroma and a sterile zone opposite the female element in which the vas deferens (sperm duct) forms (E,F). df = vas deferens, cc = central cavity, epo = early previtellogenic oocytes, lc = lacunae, oog = oogonia, sc = spermatocytes, sp = spermatogonia, sz = spermatozoa, szo = sterile zone, vs = blood vascular system.

In the length class 50-99mm, three distinct regions became evident in the gonadal primordia of R. sarba and A. berda (Fig. 103b(E)); a germinal area along the inner margins of the central cavity (presumptive female element) extending down to a distinct ventral element (presumptive male element), and a sterile zone in the outer element which extended half to two thirds down its length. A similar pattern of development was reported by D'Ancona (1949a,b) for Mediterranean species (Fig. 103a(E)).

The development of the central cavity and the appearance of germinal tissue within distinct regions signified the transition from gonadal primordia to differentiating gonads. In A. berda, LED cysts and gonial cells were evident in both male and female presumptive areas. Proliferation of gonial cells had not commenced in either region, but there were more gonial cells in the female presumptive area at this stage of development. In R. sarba, LED cysts were evident along the margins of the central cavity and in the stroma of the ventral region. Gonial cells were, however, limited to the margins of the central cavity suggesting, as in A. berda, that initial colonization and development of germinal tissue occurs earlier in the presumptive female element than in the presumptive male element. Consistent with the descriptions of D'Ancona (op. cit.) was the development of lacunae which separated the germinal tissue surrrounding the central cavity from the stroma at this stage of development.

Between 100-149mm (FL), all gonads were bisexual and similar in structure. Up to, and including differentiation, therefore, all gonads had conformed to a 'basic type' in both species. In smaller fish within this length class (100-115mm), the gonads consisted of a thin band of tissue surrounding a large central cavity with a small male element forming the apex of the ventral region. Presumptive male and female elements could be distinguished from one another, but the development of germ cells was limited to a small number of LED cysts and gonial cells in both regions. In fish >115mm, there was a simultaneous proliferation of gonial cells in both male and female elements, but further development was more rapid in the male element. A few previtellogenic oocytes were evident amongst proliferating gonia in the female element, but development in the male element included all stages of spermatogenesis, including the production of sperm. Similar developments were reported in the sparids Mylio macrocephalus (Okada 1965a) and Boops boops (Michèle & Lafauri 1974). The timing of these developments was roughly the same in all the species, occurring in the second year of life. In Boops boops there was the simultaneous proliferation of gonial cells in both male and female elements, but spermatogenesis did not begin until the first oocytes had appeared.

In this regard it is important to note that the area of the gonad from which sections are taken can bias the results in several ways. Investigation of eight differentiating gonads from which anterior, medial and posterior sections were taken, revealed a simultaneous proliferation of gonial cells in both male and female elements. In the anterior regions of these gonads (extending almost half-way along the gonad), the female element was dominant and proliferation of oogonia appeared to precede development of spermatogonia in the male element. In mid- and posterior sections the opposite trend was evident. This can be further complicated by the fact that development can proceed at different rates in each lobe and not necessarily in the same sequence. Asymmetrical development of left and right gonads was not described by D'Ancona (1949a), yet it was characteristic of early gonadal development in both R. sarba and A. berda. Neither left nor right lobe appeared to be consistently larger than the other, but in each case development was more advanced in the larger lobe. Similar asymmetry of differentiation has also been noted in Micropterus (Johnston 1951), in Salmo (Ashby 1957) and in Oryzias (Yamamoto 1953, Gamo 1961).

Antero-posterior gradients of sexual differentiation have been noted in many species of fish (Johnston 1951, Yamamoto 1953, 1959, Yamamoto & Matsuda 1963, Takahashi & Takano 1971, Nakamura & Takahashi 1973, Takahashi 1974, Cala 1976, Yoshikawa & Oguri 1978, Mezhnin 1978, Roblin & Bruslé 1983) and, regardless of reproductive style, this phenomenon underlines the necessity to carry out observations throughout developing gonads in order to assess their state of differentiation (Roblin 1980, Sadovy & Shapiro 1987).

The timing of sexual differentiation in fish varies greatly between species. In some fish, differentiation occurs during prenatal development or during hatching (Satoh & Egami 1973). In others it occurs during the early larval stage (Nakamura & Nagahama 1993) or after an undifferentiated state which may last several months or years (Chan & Yeung 1983, Bruslé & Bruslé 1983). Differentiation of male and female tissue in R. sarba and A. berda occurred between 100-149mm (FL). Sex differentiation thus occurs relatively late in these species, taking place towards the end of the first year of life or early in the second year. Similar results have been suggested for the sparids Sargus vulgaris (van Oordt 1929), Sparus aries, Sparus longispinis (Acanthopagrus schlegeli) and S. latus (Kinoshita 1939), Boops salpa (Michèle & Lafaurie 1974), Pagrus ehrenbergi and P. orphus (Alekseev 1982), Diplodus sargus kotschyi (Abou-Seedo et al. 1990), Diplodus sargus capensis and D. cervinus (Mann 1992) and have been recorded in the seabass Dicentrarchus labrax (Roblin 1980, Roblin & Bruslé 1983), the flathead mullet Mugil cephalus (Stenger 1959) and the grey mullet Mugil (Liza) auratus (Bruslé & Bruslé 1978a,b, Bruslé 1982).

It has been shown in several species of teleosts that female differentiation occurs earlier than male differentiation (Ashby 1959, Nakamura & Takahashi 1973, Harrington 1975, Bruslé & Bruslé 1978a,b, Magomedov et al. 1979, Takashima et al. 1980, Davies & Takashima 1980, Bruslé 1983). Included are species with ovotestes (Harrington 1975, Bruslé 1983). In other species ovarian and testicular differentiation is synchronous (Roblin & Bruslé 1983). Morphological differentiation of the ovary and testis generally precedes cytological differentiation (Yoshikawa & Oguri 1979, van den Hurk et al. 1982, Bruslé & Bruslé 1983) and ovarian preponderance prior to germinal colonization has been noted by D'Ancona (1949a), Harrington (1975) and Bruslé (1982,1983).

Recently, Shapiro (1992) used the work of Zohar et al. (1978, 1984) on the protandrous sparid Sparus aurata to test whether the paradigm of 'primacy of female development' in mammals also applied to protandrous sparids. From the results of an investigation into the sequential development of S. aurata gonads, in which samples of ten fish were removed each month from a captive stock of juveniles, Shapiro (1992) showed that gonads developed initially in a predominantly ovarian direction. Testicular development was preceded and followed by ovarian growth (Fig. 104a). One must assume from this figure that 'rudimentary development' indicates that sex was not determined in 0-4 month old fish. Converting the lengths of juvenile A. berda investigated in the present study to age, using the growth curve of Kyle (1986) for fishes in their first year of life, and assuming that the rudimentary gonads of Shapiro (1992) represented undifferentiated gonads, a comparative sequence of gonadal development was constructed for A. berda (Fig. 104b).

This figure is based on a relatively small sample (n=119) and A. berda lengths could not be related to age throughout the range of ages presented by Shapiro (1992) but it does, nevertheless, show that early gonadal development in A. berda follows a similar trend to that in S. aurata. After an initial undifferentiated stage, which appears to be more prolonged in A. berda, ovarian growth predominates in all fishes for several months (to the end of the first year). Only during the period in which A. berda undergoes cytological differentiation is there a marked difference in development. Whereas in S. aurata all individuals enter a male phase of development after the initial female phase, in A. berda there is a period in which male and female elements develop at a similar rate. Thereafter all individuals enter a male phase and become sexually mature as males. After this phase the number of females in the population increases rapidly with a corresponding decrease in males.

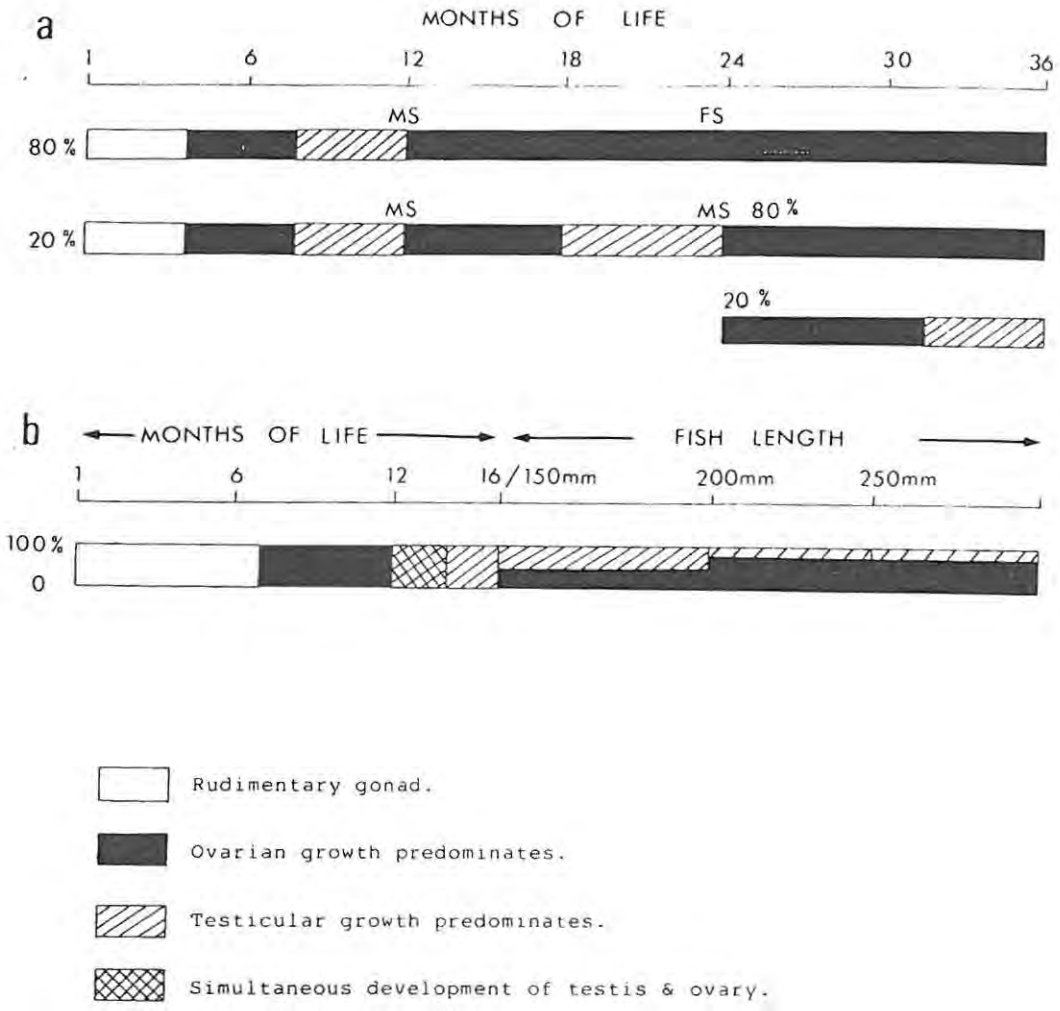

Figure 104. Sequences of gonadal differentiation and development in (a) Sparus aurata (from Shapiro 1992) and (b) Acanthopagrus berda (this study). In (a) bars show the pattern of development followed by 80% & 20% of the population. MS = spawning as a male, FS = spawning as a female. In (b) the single bar shows the pattern of development in a sample of A. berda from the Richards Bay estuary population. Age data are limited to juveniles (Kyle 1986) and beyond 16 months the proportion of gonad types in this population is related to size rather than age.

The results presented in this study are based on limited samples, but they can be considered as representative of age-related developmental patterns in A. berda, on the basis that samples of this species were removed primarily from a single system (Richards Bay estuary) into which juveniles recruit each year. There is no evidence of adult emigration from this system.

Shapiro (1992) states that some protandrous sparids, such as R. sarba (Yeung & Chan 1987), may not appear to follow the 'primacy of female development' paradigm, but argues that there are two difficulties with most studies which yield these counter examples. The first is small sample size (eg. Kinoshita 1936). The second concerns the collection of monthly specimens which include individuals of all ages and sizes. In such cases "it is not easy to separate annual patterns within an age or size class from age-related, developmental patterns (Lissia Frau et al. 1976, Michèle 1977). Patterns may also be confused by collections relying on commercial catches that sample multiple sites" (Shapiro 1992, p.200). A similar pattern of early development (up to sexual maturity) to that in A. berda suggests that the paradigm does, however, apply to R. sarba even though samples were taken on an opportunistic basis from the length of the Natal coast. A possible reason for this is that early gonadal development in this species is related to size rather than age.

Gonadal development in A. berda was related to age for this exercise because Shapiro (1992) did not furnish length data for S. aurata. It has been shown, however, that sexualization of fish from natural environments may not be related so much to their absolute age, but rather to the attainment of a certain size (Kuhlman 1975, Roblin 1980). For some species it has been suggested that the influence of size is more important than age (Kuhlman 1975, Lebrun 1977, Bieniarz et al. 1981, Dutta 1979 cited by Shelton et al. 1981), but for others it has been suggested that it is related more to length of life than size (Ashby 1957, Takahashi & Takano 1971, Davies & Takashima 1980).

From the results presented in this study, it is concluded that morphological differentiation of the gonads of R. sarba and A. berda initially proceeds in a female direction. Gonial cells first appear in the presumptive female element, but they appear in the male element soon thereafter. There is then a simultaneous proliferation of gonial cells in both elements followed by cytological differentiation in both elements. Further development (reduction division) is initially in the male direction and all fish appear to mature first as males. Thereafter, the proportion of females increases rapidly with a proportional decrease in males.

It is generally accepted that in all vertebrate groups primordial germ cells (PGC's) migrate from an extra-gonadal origin to the gonadal primordia, passing through various endodermal and mesodermal tissues (Nieuwkoop & Sutasurya 1979). As stated by D'Ancona (1956), the gonad is not the organ which gives birth to the germinal elements, it is only the organ which supplies the space for these elements to differentiate and multiply. In fish, it has been suggested that PGC's migrate from the sub-intestinal vitellus to the dorsal mesentary via the ventral mesoderm and the sphlenic mesoderm (Johnston 1951, Gamo 1961, Mezhnin 1978).

Much research has been carried out on gonadogenesis in teleosts, but cytological investigations at the ultrastructural level have been relatively few (Hogan 1973, Satoh & Egami 1973, Satoh 1974, Kanobdee 1975, Bruslé & Bruslé 1978a,b, Bruslé 1980, 1982, 1983, 1986, 1987, 1989, Hamaguchi 1982, van den Hurk et al. 1982, Bruslé-Sicard et al. 1992). Colonization of gonadal primordia by PGC's has not been described in seabreams and, in order to determine if colonization and subsequent differentiation of sex cells in seabreams conforms to the general vertebrate pattern, it was first necessary to identify PGC's within gonadal primordia and then determine the stage at which colonization and differentiation occurs.

According to Bruslé (1989) and Bruslé-Sicard et al. (1992), PGC's in teleosts are relatively large undifferentiated cells characterised by irregular outlines, a high nucleus to cell ratio, an equally high electron density in nucleus and cytoplasm and few membrane organelles. Dense fibrillar material found in close association with the nuclear membrane ('nuage') or in association with mitochondria ('ciment') are characteristics shared with gonial cells. "PGC's are generally referred to as the germ cells in undifferentiated gonads" (Bruslé-Sicard et al. 1992, p. 402). These authors have suggested that PGC's are present throughout sexual development and that they constitute a bipotential germ stock.

Extensive searches of serial sections of gonadal primordia of R. sarba and A. berda smaller than 100mm (FL) failed to produce evidence of such PGC's, even though a number of LED cysts and gonial cells were already evident in specimens within this size range. Only in the length class 100-149mm were cells identified which conformed to the above characteristics and they were found in only two specimens: an A. berda of 115mm and a R. sarba of 135mm. In the A. berda only one "PGC" was found in a nest of oogonia situated in the posterior region of the female element. In the R. sarba several "PGC's" were found in both

male and female elements, but they were more abundant in the female element amongst nests of oogonia. In the male element they were located amongst spermatogonia and lobules of spermatocytes and spermatozoa ie. relatively late in the developmental process.

The conclusion that one can draw from the evidence presented in this study is that either PGC's are very few in number throughout all developmental stages in these protandrous sparids, or that the cells identified by Bruslé (1978-89) and Bruslé-Sicard et al. (1992) are not necessarily PGC's. Numerous EM sections of R. sarba and A. berda have produced strong evidence to suggest that gonial cells arise and develop within LED cysts. These cysts are the first 'structures' to appear along the margins of the central cavity in both species and, soon after their appearance, gonial cells can be seen developing within them. The development of gonial cells in LED cysts at a time when there is no evidence of PGC's in the gonads suggests that gonial cells arise from one, or more, of the cells within a cyst which differentiate into gonial cells. Further evidence of this process is presented later in C. puniceus and C. nufar, where the significance of these results is discussed further.

The early separation of gonial cells in the sparid gonad made it possible to determine whether gonial cells in male and female elements of differentiating gonads could be distinguished from one another morphologically. It is not known if PGC's give rise to differentiated spermatogonia and oogonia in the early stages of gonad development, or if gonial cells remain undifferentiated (and thus bipotential) until later in life when they are masculinized or feminized (Reinboth 1970, 1982, Chan & Yeung 1983, Yeung & Chan 1987, Nakamura et al. 1989). There were no significant differences in morphology of gonial cells in male and female elements and, in the absence of direct evidence indicating early physiological sexual differentiation of gonial cells (Nakamura 1981), the results presented in this study support the theory that gonial cells remain undifferentiated bipotential cells until they are either masculinized or feminized in later development, under the influence of surrounding tissue. Bruslé & Bruslé (1978) have suggested that in the mullet (Mugil auratus) spermatogonia and oogonia can be distinguished from one another, indicating a possible early differentiation. Several studies at the light level have also suggested that spermatogonia are preformed and lie dormant in the ovaries of the protogynous species Coris julis (Reinboth 1962, Duchac & Buhler 1983), Labroides dimidiatus (Robertson 1972), Centropyge interruptus (Moyer & Nakazono 1978), Dascyllus (Sphigel & Fishelson 1986), Pimelometopon pulchrum (Warner 1973), Monopterus albus (Chan et al. 1972), Hemanthias vivanus (Hastings 1981) and in several Lethrinids (Young & Martin 1982). Flores et al. (1985), working on two species of the genus Xiphophorus

(fam. Poeciliidae) found that there was no significant difference in morphology of these cells and concluded that gonial cells were probably undifferentiated bipotential cells which developed according to masculinizing or feminizing effects of surrounding tissue within male and female elements. This view, proposed by D'Ancona (1949a), was consistent with the results of this study. Furthermore, the appearance of Leydig cells in the male element at the time of sexual differentiation suggests that these interstitial cells are involved in the masculinizing process (Christensen 1975).

Gonadal development after differentiation:

Rhabdosargus sarba became sexually mature between 150-199mm (FL) and most fish developed into TYPE III males. Although macroscopically 30% of the fish sampled in this length class appeared to be female, histological investigation revealed that a large proportion of the fish with TYPE IV gonads (which represented 17% of this length class) may, in fact, have been functional males. This was due to the fact that although the female element was relatively larger, only the male element was mature. Similar gonadal development has been documented in R. sarba (Yeung & Chan 1987) and Acanthopagrus australis (Pollock 1985). The percentage of males in this length class may thus have been as high as 75%. TYPE IV gonads in the 200-249mm length class also appeared to be functional males, increasing the percentage of males in that class to a possible 85%. In the 250-299mm length class the sex ratio was close to unity (see Fig. 19). This pattern persisted for several size classes and within these classes females with TYPE V gonads appeared to function as females.

The presence of fish within the population in which the most developed element of the gonad (in size) did not represent the functional sex, highlights one of the problems identified by Sadovy & Shapiro (1987) of using sex ratios determined macroscopically to identify functional hermaphrodites. These authors pointed out that difficulty and confusion easily arise in classifying the sexuality of individuals that have an initial bisexual juvenile stage, such as R. sarba and A. berda. In this case, the incorrect classification of these fish partially obscured the fact that all fish developed and functioned first as males.

Subsequent to the attainment of sexual maturity, the relative proportions of the various types of gonads in samples did not follow a similar pattern with increasing size in R. sarba and A. berda. Shortly after the attainment of sexual maturity the ratio of A. berda changed

from all males to predominantly females and thereafter the number of females increased steadily with a corresponding decrease in males (see Fig. 43). This resulted in a distinct bimodal size frequency distribution. Since the fish within the Richards Bay estuary can be considered a sub-population this bimodality, coupled to the fact that all fish appear to pass through a functional male phase, is strong evidence for protandrous sex change in A. berda. The similar bimodal length frequency distribution recorded by Kyle in the Kosi estuary population further supports this view (Fig. 105).

In R. sarba, each of the four types of mature gonad were evident throughout the size range, with the exception that there were no TYPE IV gonads in the size class 500-599mm (see Fig. 19). This may have been due to the small sample size (n = 14) and sampling technique. In the size classes 250-449mm, there was a clear decreasing trend in female TYPE IV gonads and a corresponding increasing trend in female TYPE V gonads, suggesting that functional females were changing progressively from one gonad type to the other. Similar trends were also evident in the proportion of male gonads between 200-350mm. Male TYPE III gonads showed a decreasing trend with a corresponding increase in the proportion of TYPE II gonads, suggesting that some males with TYPE III gonads may have been changing to TYPE II. Thereafter, the proportions of male gonads remained fairly constant up to the 450mm size class. Between 450-599mm, sex ratios of R. sarba became rather erratic, dominance alternating between males and females. Again, this may have been due to smaller sample sizes in the larger length classes and the opportunistic nature of sampling. Large fish were relatively few in number and samples were gathered over a longer period and a wider area than fish of smaller sizes. While sex ratios in the smaller size classes may have been fairly representative, this probability diminished with increasing size.

Yeung & Chan (1987) reported a distinct bimodal size frequency distribution in R. sarba, with males dominating the smaller size classes and females the larger size classes. They suggested that females arose by the transformation of males via a transitional intersexual phase and provided conclusive evidence of protandry from gonadal histology, population structure and biopsy.

In their overview of the criteria used to diagnose hermaphroditism in fishes, Sadovy & Shapiro (1987) have suggested that convincing diagnosis of sex change will generally require the use of several indicative features. They illustrate many problems associated with the use of sex ratios to diagnose sex change and suggest that bimodal size frequency

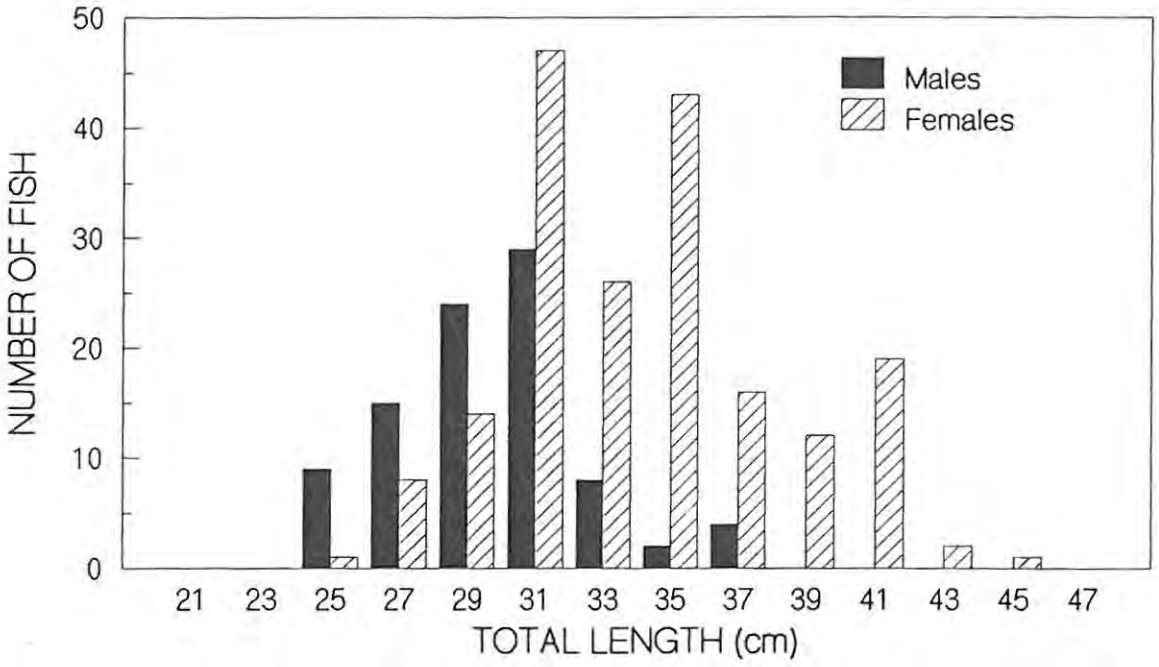

Figure 105. Length frequency distributions of male and female Acanthopagrus berda sampled by line from the Kosi estuary and lakes during 1984-86 (R. Kyle, KwaZulu Bureau of Natural Resources. Unpublished data).

distributions are weak indicators of sex change. If, however, size frequency distributions are supported by strong evidence from gonadal histology, the correct diagnosis may follow, especially if an attempt is made to exclude alternative explanations for bimodality.

Alternative explanations which could produce bimodal size-frequency distributions independently of hermaphroditism are: a) differential growth rates and maturation, b) differential mortality rates, c) differential migration or spatial segregation by sex and d) selective capture (Reinboth 1970, Sadovy & Shapiro 1987).

Currently there are insufficient data on A. berda to consider some of these mechanisms (R. sarba is not considered as it did not have a similar bimodal distribution) and the possibility of differential growth rates and mortality rates cannot be ruled out. The fact that all fish appear to pass through a functional male phase suggests, however, that differential maturation is not a factor. Even though a large percentage of the fish within this size class (100-149mm, see Fig. 43) were classified as immature (and thus potentially capable of maturing into females in the following length class), all gonads investigated histologically were of the same basic type and were developing in a male direction. With regard to differential migration, spatial segregation and selective capture, there is no evidence to suggest that A. berda leave an estuary once they have recruited to it. Estuaries can thus be considered as virtually closed systems. Within the Richards Bay estuary there did not either appear to be spatial segregation of the sexes or selective capture, catches in all areas consisting of a wide range of sizes and the full range of gonad types.

Sex change in the Sparidae is difficult to diagnose (Yeung & Chan 1987, Sadovy & Shapiro 1987), due mainly to the fact that male and female elements are separate and there are seldom traces of a previous functional sexuality (Reinboth 1962, Atz 1964). Sex change is especially difficult to diagnose in protandrous species because there are few (if any) reliable diagnostic features which separate those testes which will function normally in the following spawning season from those which will not. Indicators of degenerating testicular tissue proposed by Yeung & Chan (1987) include: i) phagocytosis in the interstitium, ii) a decrease in size and cytoplasmic contents of gonial cells, and iii) sometimes an increase in vascularization of the testicular lobe. Apart from (ii), which would be difficult to diagnose, these features may be common to both 'types' of testes and a distinction between the two may be limited to the extent of degeneration, rather than to the features themselves.

Sadovy & Shapiro (1987) have suggested that the strongest individual indicators of protandry are transitional individuals whose gonads contain degenerating testicular tissue and developing ovarian tissue. While numerous ovotestes with degenerating male germinal tissue were sampled throughout the adult size ranges of both R. sarba and A. berda, a major stumbling block in the diagnosis of protandrous sex change in these species was the absence of a simultaneous development of oocytes in the female element of nearly all gonads. Detailed inspection of TYPE III & TYPE IV gonads of A. berda and R. sarba in the length classes 150-199mm and 200-249mm respectively, (these fish were considered the most likely candidates to change sex), collected between spawning seasons, revealed some gonads with male elements in which there was active spermatogenesis and others in which there was extensive degeneration of germinal tissue in the male element. The latter may have been in the process of changing sex but the absence of simultaneous development in the ovary precluded a diagnosis of sex change according to the criteria of Sadovy & Shapiro (1987). Yeung & Chan (1987) diagnosed protandry in R. sarba in an earlier study even though they were faced with the same problem. Descriptions of gonad structure and development in that study were similar to those presented above, even though their classification of gonad types differed slightly. In their study, oocytes in the female element of TYPE III males (their TYPE II) and TYPE IV females (included in their TYPE II males) were also arrested in the perinucleolar stage throughout all seasons.

Similarly, in the ovotestes of the protandrous sparids Acanthopagrus schlegeli and Rhabdosargus sarba (Kinoshita 1936, 1939), Mylio macrocephalus (Okada 1965b,c), Acanthopagrus latus and A. cuvieri (Abu-Hakima 1984), oocyte development never passed the previtellogenic stage until sex separation was almost complete and the testis had completely regressed. There are conflicting reports concerning protandry in Diplodus sargus capensis. Coetzee (1986) reported that the previtellogenic stage was not passed until sex separation was almost complete, but Mann (1992) has recently reported a few (n=4) ovotestes in which there was the simultaneous development of oocytes and degeneration of testicular tissue. During the present study only two such ovotestes were sampled. The low numbers of these gonads in both studies suggests that they were exceptional cases and that during sex change in protandrous sparids, oogenesis generally does not proceed past the previtellogenic stage until the male element has been reduced to a remnant.

Notwithstanding the above, the investigation of histological sections prepared from mature fish throughout the size ranges of both R. sarba and A. berda revealed both cytological and morphological changes which were indicative of sex change. Degeneration of testicular tissue in both species appeared to be of two types. In the first, empty sperm sinuses became flattened and, in the process, the large central sperm sinus filled with connective tissue. There were areas in which active phagocytosis was evident, but gonial cells were still numerous throughout the testis (especially in the distal areas), and nowhere was there extensive degeneration of germinal and interstitial tissue. These gonads are thought to represent those which would function as males in the following spawning season. In the second type of gonad, there was a total degeneration of testicular space. Degenerating germinal cells were surrounded by spongy lacunar tissue, in which there were numerous phagocytes and detritic masses. Such extensive degeneration of these testes suggested that they would not function again.

In summary, morphological changes in gonad structure which were indicative of protandrous sex change were: a) testes which consisted of reduced lobes (on either side of a reduced central sperm sinus) which had become compressed against the ovary and b) a sperm duct which was reduced first to a thin cavity, then to several small lacunae and which finally closed. Within these gonads spermatogonia became increasingly rare within a spongy-lacunar tissue. These morphological and cytological characteristics have also been reported in the sparids Sparus auratus and Diplodus annularis (D'Ancona 1949b), Sparus longispinis (Acanthopagrus schlegeli) (Okada 1965b,c) and Boops salpa (Michèle & Lafourie 1974). The eventual disappearance of gonial cells in the lining of ovaries of large R.sarba (also reported in some individuals by Yeung & Chan 1987) and A. berda suggests that sex change may be irreversible in these species later in life.

Also included in this study was an attempt to produce conclusive evidence of protandrous sex change in A. berda using mark-recapture. Having established the spawning pattern of A. berda in the Kosi estuary (see section 3.2.2), 315 fish were removed from the spawning aggregation at the mouth during the peak spawning seasons of 1990-91, sexed and tagged. The majority of these fish were ripe running males. In June 1992 and 1993, 74 of these tagged fish were recovered from the spawning aggregation by spear and six were recovered from artisanal fish traps. Only one A. berda was recovered which had changed sex from a functional male to a functional female (the fish was ripe-running when tagged and recovered). While not conclusive this does, nevertheless, provide further evidence of protandrous sex change in this species.

The descriptions of gonadal development presented in this study were used to construct possible pathways of sexuality in R. sarba and A. berda and to relate them to the results of previous work on protandrous sparids. Early gonadal development in R. sarba and A. berda has been compared to the descriptions of D'Ancona (1949a). Comparisons with more recent works are limited to post-differentiation gonadal development, as there are no recent detailed descriptions of pre-differentiation development.

In order to construct possible pathways of gonadal development in wild populations of sparids, which are difficult if not impossible to observe in their natural habitats, one has no recourse but to relate the relative frequencies of gonad types within samples to the size (or age) of the fish within those populations. In isolation these results may remain purely speculative. In conjunction with results obtained elsewhere, they become more robust.

Histological and demographic evidence presented in this study suggests that R. sarba in Natal waters follow similar patterns of gonadal development to this species in the waters of south east Asia (Yeung & Chan 1987), but that sexuality may be more plastic than previously suggested (Fig. 106a&b). In both studies it was found that immature and early mature gonads (Type I) are composed of roughly equal volumes of male and female tissue. The ovarian element contains oogonia or oocytes which are arrested in the previtellogenic stage, whilst in the male element there is active spermatogenesis. All fish function first as males (Types II,IV in Yeung & Chan (1987) and Types II,III,IV in this study). Yeung & Chan (1987) suggest two possible pathways of development. The first involves the development of typically male gonads (TYPE IV) directly from the young ovotestes TYPE I (Fig. 106a(i)). In TYPE IV gonads the female element becomes vestigial and contains a small number of gonial cells. The occurrence of intersexes throughout the size range (TYPE II), in which both male and female elements are prominent but only the male is functional, led them to suggest a second pathway along which functional females develop from these individuals through protandrous sex change (Fig. 106a(ii)).

The present study suggests that there are three possible pathways. A small number of fish appeared to develop directly from immatures (TYPE I) to males with TYPE II gonads (Fig. 106b(i)), as in Yeung & Chan (1987), but the majority developed into functional males with TYPE III (predominantly male) gonads (Fig. 106b(ii)). Changes in the relative proportions of TYPES II,III & IV gonads with increasing size thereafter (see Fig. 19) suggested that some males with TYPE III gonads later developed TYPE II gonads, while others changed sex to females with TYPE V gonads (Fig. 106b (iii & iv resp.)). The

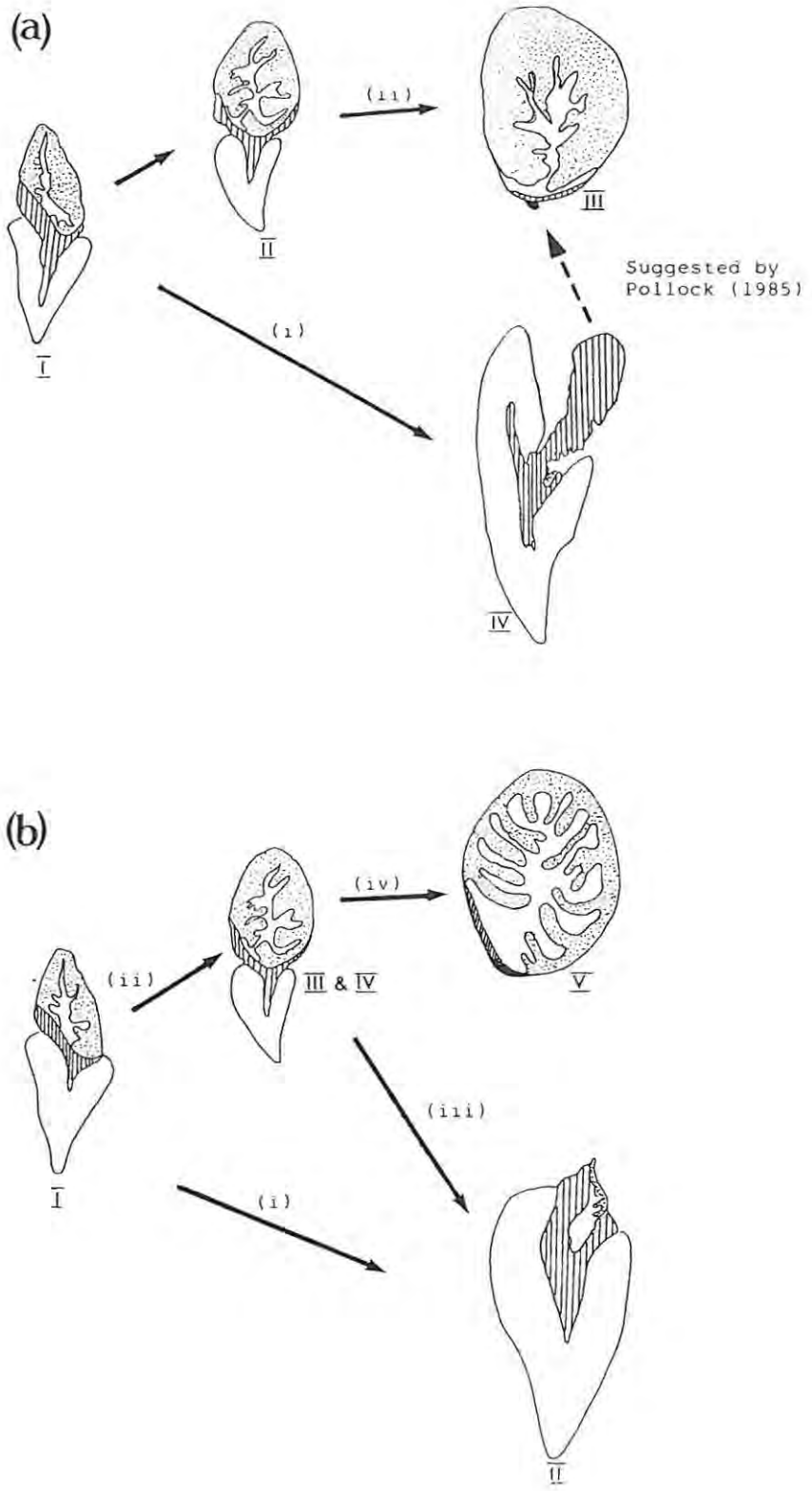

Figure 106. Pathways of sexual development proposed for Rhabdosargus sarba: (a) Yeung & Chan (1987) (redrawn from Yeung & Chan 1987), (b) this study. (a) includes a pathway of development proposed for Acanthopagrus australis by Pollock (1985). In (a) I= immature/early mature - male dominant, II= intersex, III= functional female, IV= functional male. In (b) I= immature/early mature - male dominant, II= male, III= predominantly male, IV= predominantly female, V= female. ☐ dominant/functional male lobe, ■ male remnant, ▥ sperm duct/sterile zone, ▩ female element/remnant.

development of functional females through protandrous sex change (via pathway ii & iv) is the same as that proposed by Yeung & Chan (1987). These authors did not relate their findings to population structure, but they did state that their TYPE IV males (TYPE II in this study) increased in frequency with increasing size, indicating the possibility of a similar pattern of development to R. sarba in Natal (via pathway iii). The difference in interpretation between the two studies may stem from the fact that Yeung & Chan (1987) reported that the female rudiment in their TYPE IV male gonads contained only oogonia, suggesting that the female element had never developed past this stage. In the present study, however, all gonads of this type (TYPE II in this study) contained previtellogenic oocytes in the former ovarian cavities, suggesting that the female element had developed further than the initial gonial stage. In both studies, however, the basic pattern of protandrous sex change remains the same.

The schematic representation of gonadal development in the yellowfin bream Acanthopagrus australis (Pollock 1985) is similar to that presented for R. sarba by Yeung & Chan (1987) in all respects but one. Pollock (1985) also indicates two pathways of development (Fig. 107). In the first, functional males with reduced female elements are derived from young fish with ovotestes similar to those described above. In the second, females are derived from these young fish with ovotestes, passing through a stage in which only spermatozoa are evident in the ovotestes. Pollock (1985) suggests that testes in which there is no other spermatogenic tissue but spermatozoa are indicative of sex change. Apart from the fact that similar testes were not described by Yeung & Chan (1987) or the present study, his interpretation differs only in the suggestion that females can be derived from both types of males (Fig. 106a - dashed arrow).

The evidence presented for the congeneric A. berda in the present study appears to support such a development. Unlike R. sarba, most A. berda appeared to mature and function first as males with 'typically male' TYPE II gonads (see Fig. 43). The proportion of fish with these gonads dropped steadily thereafter with a corresponding increase in the proportion of female TYPE V gonads. As the proportion of males with TYPE III (predominantly male) gonads remained fairly constant over the entire size range, it would appear that females with TYPE V gonads were derived from males with TYPE II gonads. A change of this nature would, of necessity, pass through stages conforming to TYPES III & IV depicted in Figure 106b.

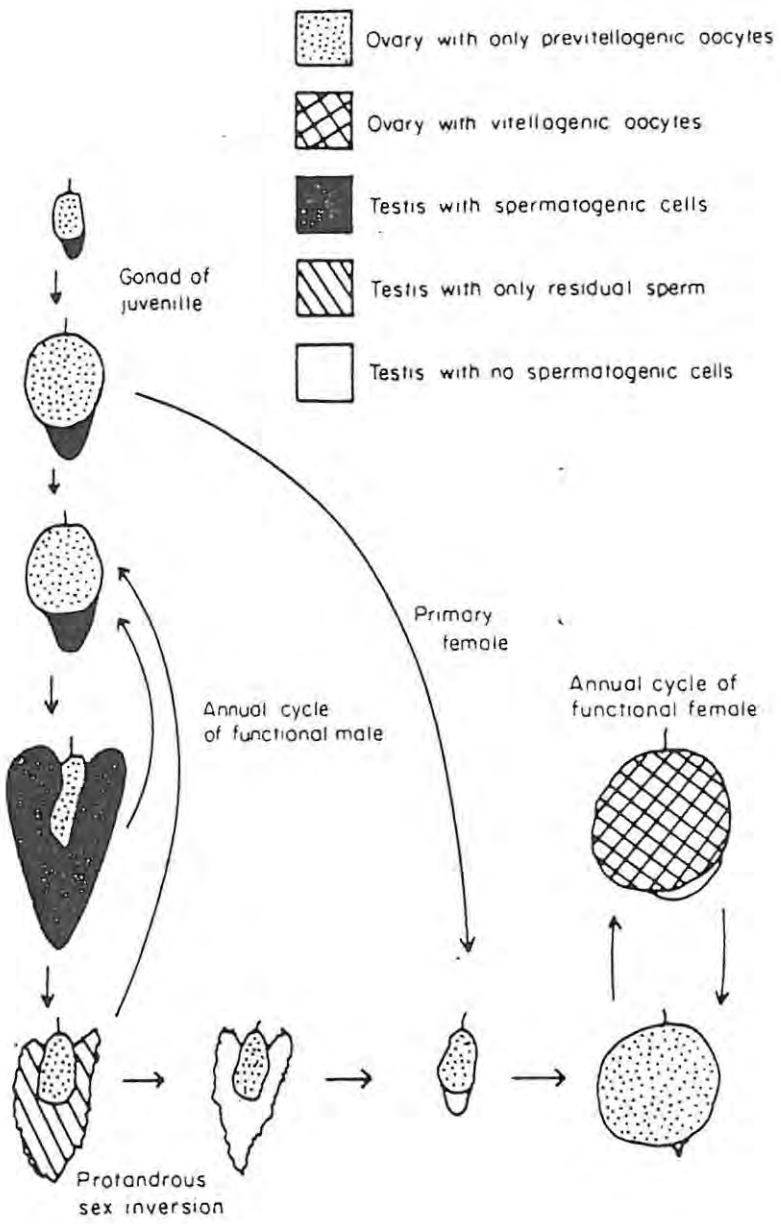

Figure 107. Schematic representation of gonadal development and reproductive cycle in the yellowfin bream Acanthopagrus australis (from Pollock 1985).

Intuitively, a developmental pathway in which gonads initially develop with male and female elements well represented, then pass through a stage in which the female element appears to become vestigial before changing sex to females with typically ovarian gonads is unlikely to exist. Yet the results of both studies have indicated such a pathway, suggesting perhaps that males with TYPE II gonads (TYPE IV in Yeung & Chan 1987) should not be considered as terminal phase males.

Gonadal development in C. puniceus and C. nufar:

From the results presented in this study it is evident that there are similar patterns of gonadal development in C. puniceus and C. nufar and that these differ from those of R. sarba and A. berda. Early gonadal development is similar in the four species with respect to the formation of the central cavity, the formation of germinal and sterile zones and the cytological composition of the gonadal primordia. Thereafter, morphological and cytological differentiation differs.

In C. puniceus and C. nufar the gonad remains typically ovarian in morphology and proliferation of gonial cells is limited to the female element (Fig. 108). The male element consists of a small number of dormant gonial cells located in the tunica albuginea below the sterile zone. There is no development in this element until sexual maturity is approached in C. nufar and sex change occurs later in life in C. puniceus. A similar pattern of gonadal development was also recorded in the protogynous hermaphrodites Pagrus pagrus, P. orphus and P. ehrenbergi (Alekseev 1982), Pachymetopon aeneum (Buxton & Clarke 1986), Chrysoblephus laticeps and C. cristiceps (Buxton 1989) and the rudimentary hermaphrodite Pachymetopon grande (Clarke 1988). In the present study a clear distinction could thus be made between protandrous hermaphrodites on the one hand and protogynous and rudimentary hermaphrodites on the other using morphological and cytological characteristics of early gonadal development. One can conclude from the above that D' Ancona's (1949a) descriptions of early gonadal development may describe development in protandrous species fairly accurately, but that they do not adequately describe early gonadal development in all species of sparids.

In C. nufar >44mm (FL), initial development had commenced but the central cavity had not formed and there was no evidence of germinal tissue in the stroma (see Fig. 82). This level of development corresponded to that recorded in R. sarba and A. berda of similar

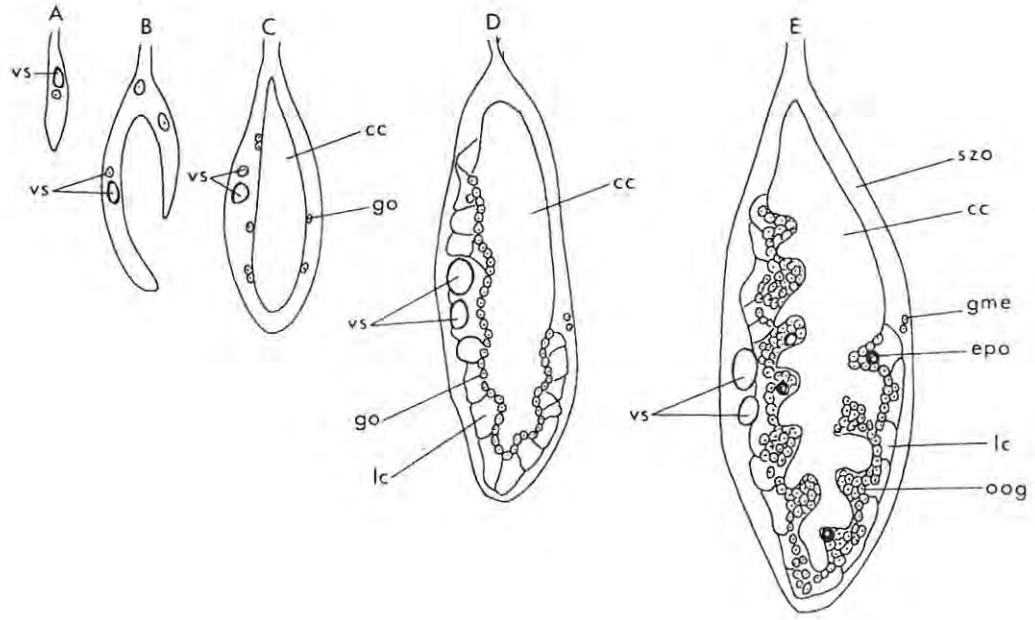

Figure 108. Schematic representation of early gonadal development in Chrysoblephus puniceus and Cheimerius nufar. Each primordium starts as a single element. A second element forms later and extends down alongside the first (A-B). The two elements fuse ventrally forming a central cavity (C). Germinal tissue develops along the margins of the central cavity (female element) and is separated from the back stroma by a network of lacunae (D). Gonial cells proliferate along the margins of the central cavity and lamellae begin to intrude into the cavity. The male element, situated below the sterile zone, consists of only a few dormant gonial cells separated from the female element by connective tissue (E). cc = central cavity, epo = early previtellogenic oocyte, gme = gonia in male element, go = gonia, lc = lacunae, oog = oogonia, szo = sterile zone, vs = blood vessels.

size, suggesting that initial formation of the gonadal primordia progresses at a similar rate in these sparids. This trend continued in the subsequent length classes, with formation of the central cavity occurring progressively in a caudal direction and being complete in specimens of 76-99mm. All C. puniceus and C. nufar less than 100mm (FL) possessed undifferentiated gonads in which two distinct regions were evident, a germinal area along the margins of the central cavity and a sterile zone in the outer element extending half to two thirds down its length. As in R. sarba and A. berda, LED cysts and gonial cells were evident along the margins of the central cavity of larger gonads within this length class, but serial sections failed to reveal any evidence of PGC's anywhere in the developing gonads.

Between 100-149mm (FL) all C. puniceus and C. nufar gonads were typically ovarian in morphology, unlike those of R. sarba and A. berda which were distinctly bisexual at this stage of development. Apart from one fish in which male and female germinal tissue was mixed and developing simultaneously (considered abnormal but nevertheless ovarian in morphlolgy), no males or fish with distinctly bisexual gonads were evident in either species within this size range. The male element remained dormant, being composed of a small number of gonial cells in the tunica albuginea. Differentiation of C. puniceus gonads occurred in this size range, as in R. sarba and A. berda, but it did not take place in C. nufar until the fish had reached a size of 150-199mm (FL). Sex differentiation thus occurs relatively late in all the sparids investigated in this study.

While different in detail, lacunae separating germinal tissue from the stroma were present in undifferentiated gonads of all the species studied and in C. puniceus and C. nufar there was further evidence of gonial cells developing within LED cysts along the margins of the central cavity and within the thin strands of connective tissue between lacunae. Successive stages of this process were evident in numerous sections (see Figs. 88-92). Personal communications with prominent workers in this field (S. Bruslé-Sicard, University of Perpignan; L. Fishelson, University of Tel Aviv; H. Grier, Florida Marine Research Institute) have failed to shed any light on this matter. There is scepticism that a relationship exists between these LED cysts and developing gonial cells (Bruslé-Sicard), even though this study has shown quite clearly that such a relationship does exist. The reason for the scepticism is that this pattern of development does not fit into the modern theory of sex differentiation in fishes (or vertebrates in general).

The present view is that gonial cells arise from PGC's which migrate into gonadal primordia and that these PGC's share the same characteristics in all vertebrate species,

including teleosts. PGC's should, therefore, be the first germ cells to appear in the gonadal primordia and only thereafter should the production of gonial cells commence. Yet cells conforming to the PGC's described for other teleosts were located in only two gonads (R. sarba and A. berda) after extensive searches of serial sections from a wide range of fishes from each species investigated in this study. These cells were located in gonads from relatively large fish in which male and female germinal tissue was already well developed. Bruslé & Bruslé (1978a,b), Bruslé (1980, 1982, 1983, 1987, 1989) and Bruslé-Sicard et al. (1992) have conducted extensive research on these cells in several gonochorist and hermaphroditic teleosts, yet they too have failed to report the appearance of PGC's in gonadal primordia before the appearance of gonial cells.

In all the sparid gonads investigated in this study, LED cysts first appeared along the margins of the central cavity, followed by gonial cells, and a number of these gonial cells were clearly developing within LED cysts. As gonial cells proliferated, LED cysts became fewer and less easily distinguishable from surrounding somatic cells. As stated earlier, the conclusions that one can draw from this are either that the number of PGC's in sparid gonads is extremely low and insufficient sections have been studied in this investigation to locate them, or that the identification and description of PGC's in other teleosts is not necessarily correct. The development of LED cysts immediately prior to the appearance of gonial cells in gonadal primordia, and the development of gonial cells within these cysts, has not been previously reported in teleosts. Further work on this aspect of germ cell development did not fall within the bounds of the present study, but it is clearly an avenue of research which requires further detailed investigation.

No previous studies of gonadal development in protogynous sparids have included investigations of sex differentiation at the electron microscope level and there is thus no comparative material. Even at the light level there are few descriptions of early gonadal development and, within these descriptions, there appear to be many inconsistencies. These inconsistencies appear to have arisen through a combination of insufficient resolution at the light level and superficial observations. In a study on the red sea bream Pagrus major, for instance, Huang et al. (1974) suggest that this species is protogynous, all fish developing first into females (< 1 yr) and that hermaphroditic fish only become evident in the second year of life. Matsuyama et al. (1988), on the other hand, have suggested that P. major is a rudimentary hermaphrodite in which bisexuality is confined largely to the juvenile stage. Adults with well-developed bisexual gonads are believed to be sporadic cases in the population. Such diametrically opposed results must have arisen from

superficial observation in one, or possibly both studies. In the latter study, for instance, it is suggested that testes develop from immature gonads in which testicular tissue completely surrounds the ovarian tissue which borders the central cavity (Fig. 109).

This type of development has not been recorded previously in the Sparidae. It was not recorded in the study of Huang et al. (1974) and it does not conform to the descriptions of gonadal development presented in this study. Similar gonads to those described by Matsuyama et al. (1988) were evident in both C. puniceus and C. nufar (see Fig. 86). At the light level the spongy tissue surrounding the female element in these gonads also had the appearance of testicular tissue, but detailed investigation at the electron microscope level showed that this tissue was composed largely of loose connective tissue and scattered somatic cells and granulocytes. There was no evidence of testicular tissue surrounding the female element. Based on the results of the present study, it is suggested that close inspection of the tunica albuginea at the base of the sterile zone in the gonads of Pagrus major (Matsuyama et al. 1988, Fig. 2(4)) would have revealed a male element consisting of a few strings of dormant gonial cells. Conflicting descriptions such as these underline the need for more detailed work on early gonadal development in sparids.

In the single study in which gonadal development in sparid protogynous hermaphrodites has been traced from the formation of gonadal primordia to sex change, Alekseev (1982) assumed that early development in protogynous species followed the same pattern described by D'Ancona (1949a). The present study contradicts this view.

Gonadal development after differentiation:

In the size range 150-249mm (FL), all C. puniceus and C. nufar gonads were typically ovarian in structure, but in C. nufar there appeared to be two types of ovary. In the first, the male element consisted of a few inert gonial cells in the tunica albuginea. In the second, gonial cells were proliferating in the male element which had thickened ventrally (see Fig. 98). The latter gonads may have represented the first stages of prematurational sex change in C. nufar.

The fact that all C. nufar develop first as females indicates that sex change occurs in some individuals in this species. In this regard it is important to determine if this sex change occurs before or after sexual maturity is attained in individual fish. If it occurs before sexual maturity, the species is not a functional hermaphrodite and its classification as a

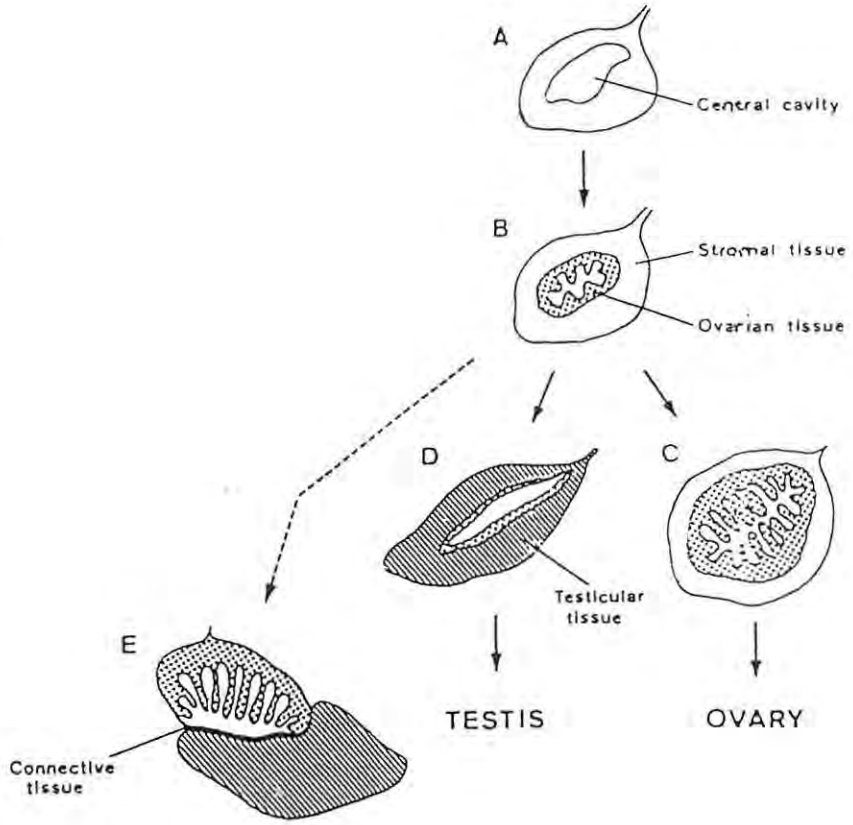

Figure 109. Schematic representation of sex differentiation and gonadal development in the red sea bream Pagrus major (from Matsuyama et al. 1988).

rudimentary hermaphrodite is correct. If, however, sex change occurs after sexual maturity and fish spawn first as females (even for one spawning season) then the species must be considered a protogynous hermaphrodite.

Sexual maturity of C. nufar is based on the length class in which 50% of the individuals within that length class reach maturity. This length class is 240-260mm (FL) (Garratt 1985b) and mean length at sexual maturity is 250mm (FL). Males first appear in the population in the length class 200-249mm, suggesting that some individuals may change sex before maturity. However, at least 25% of the fish in this size class are mature (Garratt 1985b) and it is possible that even these small males have first functioned as females. The number of males in this size class is very low (4%) and, even though their numbers increase substantially in the following length class (250-299mm, 12%), only after they have attained a size greater than 300mm are males equally represented in the population. As the majority of fish in the length class 250-299mm are female, it is possible that individuals first function as females in this species.

In this study it could not be determined if sex change occurs before or after sexual maturity and the reproductive style of C. nufar remains uncertain. Sex ratios in the adult population (size frequencies of males and females) and ovaries and testes of equitable size strongly suggest that this species is a functional gonochorist (Buxton & Garratt 1990). It should be noted, however, that Larranéta (1964) and Rijavec & Zupanovic (1965) reported a similar pattern of development in the protogynous Pagellus erythrinus. In this species, virtually all fish developed initially as females (5% as primary males) whereafter approximately 50% of the population changed to males and the rest remained females throughout their lives. The possibility, therefore, that some C. nufar undergo protogynous sex change should not be ignored. The plasticity of sex determination in this family (Michèle 1977, Alekseev 1982, Pollock 1985, Krug 1990, Mann 1992) may allow some individuals to pass through a functional female stage before changing sex to males, whilst in others sex change may occur before maturity. This pattern of development would indicate diandric protogynous hermaphroditism.

In an earlier study on the reproductive biology of C. nufar, Coetzee (1983) concluded that this species is a rudimentary hermaphrodite which functions as a gonochorist. His work was based on fish which were caught by anglers and his sample did not include immature fish. Observing a number of hermaphroditic fish in the smaller size classes, he assumed that all immature fish possess hermaphroditic gonads in which male and female elements are well represented (similar to those described in R. sarba and A. berda) and that these fish undergo sex separation to give rise either to functional males or functional females.

The present study has shown that this interpretation is invalid. All C. nufar develop initially in a female direction and only later do some fish change sex and become functional males. This work highlights the need for more critical studies on early gonadal development in fishes in order to produce accurate assessments of reproductive styles (Sadovy & Shapiro 1987, Matsuyama et al. 1988, Chang & Yueh 1990).

Whereas the number of male C. nufar had increased to 12% in the length class 250-299mm (FL), only two male C. puniceus within this size range were sampled from the Leadsman Shoal. In both species, however, testes in this size class were similar in morphology. They were compressed dorso-ventrally and appeared to be developing around the former ovarian cavity which was situated dorsally. In each gonad the sperm duct completely enclosed the former ovarian cavity. This pattern of development has also been reported in the protogynous sparid Dentex tumifrons (Nakazono 1987) and the conspecific protogynous hermaphrodites Chrysoblephus laticeps and C. cristiceps (Buxton 1989) and it is suggested that these characteristics may be reliable morphological indicators of recent sex change in protogynous sparids.

In all C. nufar larger than 300mm, testes were of the normal v-shape with the remnant of an ovarian lumen situated dorsally. The retention of an ovarian lumen is considered a reliable diagnostic feature of sex change amongst protogynous hermaphroditic fishes (Reinboth 1967, 1980) and the retention of such a lumen in C. nufar is considered additional evidence that this species changes sex.

Within the C. puniceus community on the Leadsman Shoal sex change occurred most frequently in the size range 350-450mm (FL). Fish smaller than 350mm were generally females, whereas between 350-450mm the sex ratio changed from 70% female to 92% male (see Fig. 68). Having established the period in which sex change takes place in C. puniceus (Feb-April), further sampling during these months produced a number of gonads from which it was possible to trace the developmental processes involved. Development of the testis appears to commence only after all vitellogenic oocytes have undergone atresia and only previtellogenic oocytes remain in the ovary. Gonial cells proliferate in the tunica albuginea and lacunae develop within the sterile zone, forming the sperm duct (see Fig. 77c). Initial proliferation of germinal tissue results in a thickening of the tunica albuginea in the ventral region. Spermatogenesis begins soon thereafter, so that all stages of spermatogenesis are evident even in newly forming testes (see Fig. 77d). Morphological

development is initially lateral (in both directions) along the tunica albuginea, resulting in newly formed testes which appear to be dorso-ventrally flattened (see Fig. 78). At this time extensive atresia of previtellogenic oocytes and somatic tissue begins in the ovary and lamellae are reduced in number (see Fig. 79). This process continues until there is no longer evidence of any female germinal tissue (including gonial cells) along the margins of the former central cavity. A similar pattern of gonadal develoment has been recorded in the protogynous sparids Pagrus pagrus, P. ehrenbergi, and P. orphus (Alekseev 1982). The thoroughness of this process in C. puniceus and C. nufar suggests that sex change is irreversible in both species. Once the dorso-ventral lobes of the testis have formed, growth continues ventrally and the testis finally forms into the typical v-shape common to members of this family (Reinboth 1962, Atz 1964, Alekseev 1982, Buxton & Garratt 1990).

Yellow-brown bodies have generally been associated with the atresia of yolky oocytes (Chan et al. 1967, Rastogi 1968, Moe 1969, Hastings 1981) and the presence of these bodies in the testes of some species has been used as evidence for protogynous sex change (McPherson 1977, Young & Martin 1982). Sadovy & Shapiro (1987) caution the use of this evidence on the grounds that similar pigment-containing bodies are also found in the liver, adrenals, germinal epithelium, heart muscle and other organs. A number of explanations for their origin in testes have been proffered that do not involve oocyte atresia (Reinboth 1962, Atz 1964, Smith 1965, Moe 1969, Roede 1972, Warner 1975, Roberts 1978). They do state, however, that "the production of yellow-brown bodies from vitellogenic oocytes, through atresia, is a well described and common occurrence" (Sadovy & Shapiro 1987, p. 146).

In an earlier study, Garratt (1986b) suggested that degenerating previtellogenic oocytes and yellow-brown bodies in ovigerous lamellae of bisexual C. puniceus gonads signified sex change in this species. The inclusion of yellow-brown bodies as a criterion for sex change in that study has recently been criticised by Bruslé-Sicard et al. (1992) on the grounds given above. Further investigation of transitional C. puniceus gonads in the present study has shown, however, that yellow-brown bodies were numerous during the degeneration of previtellogenic oocytes and ovigerous lamellae (see Figs 78-80). In all gonads atresia of previtellogenic oocytes and lamellae did not take place until after all yolked oocytes had been reabsorbed, indicating that the yellow-brown bodies were associated with this atresia. While this is also contrary to the findings of Reinboth (1962) and Hastings (1981), who suggest that degeneration of previtellogenic oocytes involves no phagocytosis but rather the loss of organization within the nucleii and the fragmentation of the cytoplasm, it is suggested that in the Sparidae, in which male and female elements are separate, extensive yellow-brown bodies in degenerating ovigerous lamellae can be considered a reliable

diagnostic feature of sex change in these fish.

The descriptions of post-differentiation gonadal development presented in this study were used to construct possible pathways of sexuality in C. puniceus and C. nufar and to relate these to the results of previous work on protogynous and rudimentary hermaphrodites. Gonadal development in C. puniceus is fairly simple, as there are no alternative pathways. The species is monandric with all males being derived from females through sex change. The gonads of all individuals differentiate into ovaries with a reduced sterile zone and testicular primordium (Fig. 110a(A&B)). All individuals mature as females (Fig.110a(C). Sex change occurs relatively late in life and it is characterised by the total degeneration of the ovarian element and proliferation of testicular tissue in the ventral region (Fig. 110a(D-F). Development in C. puniceus thus follows the general pattern proposed for protogynous sparids (Buxton & Garratt 1990).

In C. nufar there are three possibilities. The first is protogynous sex change as described for C. puniceus above, in which all individuals mature as females, and males are derived from mature females thereafter (Fig. 110b (i)). The second is pre-maturational sex change in which individuals initially develop in a female direction but some fish change sex to males before they reach maturity (Fig. 110b (ii)). This would represent rudimentary hermaphroditism, as adults would function either as males or females. The third is protogynous sex change by some individuals before sexual maturity and by others after sexual maturity (Fig. 110b (ii & iii)). In this case, most individuals would function as gonochorists, but the ability of females to change sex later would remain.

Histological and demographic evidence presented in this study suggests that C. nufar is a rudimentary hermaphrodite which follows the second pathway of development in which there is pre-maturational sex change. However, as stated earlier, it is possible that some C. nufar undergo protogynous sex change. A similar pattern of development to the third alternative given above has been proposed for the protogynous sparids Pagrus pagrus, P. orphus and P. ehrenbergi (Alekseev 1982). At this time, with our limited knowledge of mating systems in the Sparidae, it may be difficult to perceive how a reproductive strategy of this nature (third alternative) would be maintained. However, as pre-maturational sex change in some individuals has been documented in several protogynous scarids (Choat & Robertson 1975), labrids (Hoffman 1983) and gobies (Cole 1983), the possibility that this pathway exists cannot be ignored.

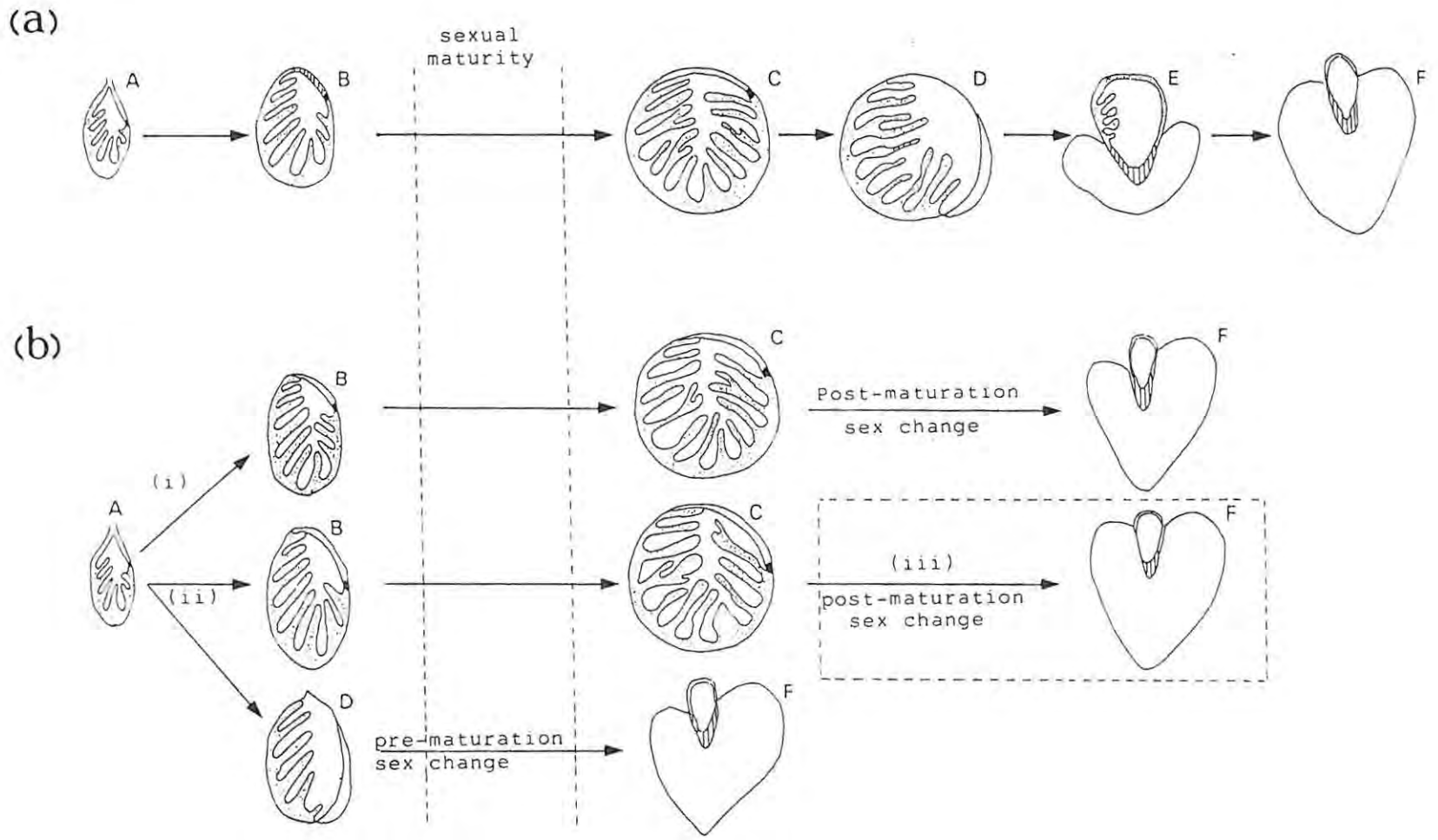

Figure 110. Pathways of sexual development proposed for (a) Chrysoblephus puniceus and (b) Cheimerius nufar. A = newly differentiated gonad, B = immature ovary, C = mature ovary, D = testicular tissue proliferating on ovary, E = intersex, F = functional male with ovarian remnants. ■ inert male element, ▒ female element, □ male element, ▥ sperm duct/sterile zone.

CHAPTER 3 - SPAWNING SITES, TIME OF SPAWNING AND SPAWNING BEHAVIOUR

3.1 INTRODUCTION

Studies of the spawning behaviour of marine fishes have generally been undertaken in shallow tropical waters where, in relatively calm conditions and excellent visibility, divers can spend long periods observing fishes in their natural environment. Most seabreams are found in temperate seas where conditions are less conducive to underwater observations and, for this reason, little is known about their spawning behaviour. Our knowledge is limited to brief, and rather superficial, observations on the behaviour of three species in captivity, Spondyliosoma cantharus (Wilson 1958), S. emarginatum (Van Bruggen 1965), Chrysoblephus laticeps (Buxton 1989) and two in the wild Chrysophrys auratus (Cassie 1956) and Lagodon rhomboides (Caldwell 1957). Two of these, Spondyliosoma emarginatum and S. cantharus, are the only nest builders with demersal eggs known in the family. The other species are presumed to produce pelagic eggs.

The study of sub-tropical and temperate deep water species in their natural environments poses several problems, including safe, no decompression diving limits and increased diving risk due to strong currents and reduced visibility. This research is also expensive. Offshore diving operations in deep water which extend into the hours of darkness require a considerable amount of ship's time. Such problems limited the hours spent searching for spawning sites and observing C. puniceus and C. nufar in their natural habitat.

The study of the estuarine dependent riverbream, A. berda, poses a different set of problems. High turbidity in most of the estuaries of southern Africa in which this species lives and, in some cases, strong tidal flow make it virtually impossible to observe the behaviour of fishes in these systems. Fortunately, the lower reaches of the Kosi estuary in northern Natal (see Fig. 1) has sufficiently clear water for such a study and the behaviour of fishes in the annual aggregation of this species at the mouth during the months of May and June has been observed by divers for three consecutive years.

To describe the spawning behaviour of the species under investigation, it was necessary to establish where and when spawning in each species took place. Following this, it was possible to describe the spawning behaviour in detail, or alternatively to describe behaviour in captive animals. This information was then used to test Warner's (1975, 1988a,b) hypotheses that:

(i) In shoaling species, or species which aggregate for spawning, protandrous hermaphroditism (eg. A. berda) should occur where there is random pairing of single males and females.

(ii) Protogynous hermaphroditism (eg. C. puniceus) is characterised by haremal or lek mating systems in which there is male dominance and territoriality. Reproductive success is related to size and sex change is regulated by behavioural cues within communities.

(iii) Rudimentary hermaphrodites (eg. C. nufar) are functional gonochores in which reproductive success is not size-related. Spawning is random, either in pairs matched by size or in group spawning, and males compete with each other to fertilize eggs.

3.2 SPAWNING IN A. BERDA

The spawning season of A. berda, based on macroscopic gonad staging and GSI values, extends from May-August, with peak spawning in May and June (Wallace 1975b, Kyle 1986). Wallace noted an increase in abundance of A. berda near the mouth of the St Lucia estuary between April and July and suggested that spawning occurs close inshore in the vicinity of the mouth. Kyle (1986) recorded increased trap catches in the Kosi estuary which coincided with an annual migration to the mouth during the spawning season and, using tagging, estimated the number of fish moving to the mouth each year to be in the region of 45,000. As recruitment from the sea into the estuarine systems of the east coast is well documented (Begg 1978, Kyle 1986, van der Elst 1988), it was assumed that spawning occurred in the sea and that mature adults moved out into nearshore waters to spawn.

Whereas similar migrations prior to spawning have been noted in the congeneric A. australis (Pollock 1982, Pollock & Weng 1983, Pollock 1984), spawning migrations have not been described in the other members of the genus (Kuronuma & Abe 1972, Hussain & Abdullah 1977, Etessami 1983, Abu-Hakima 1984, Chao & Liao 1984, Masuda et al. 1984) and the spawning sites and behaviour of these fishes are unknown.

The aggregation of large numbers of A. berda in spawning condition at Kosi mouth in June, coincidental with activity at the surface, and the collection of large volumes of eggs in plankton nets deployed for prawn larvae (Forbes, A.T. University of Natal, pers. comm. 1989), prompted an investigation into the possibility that A. berda spawn in the mouth of this estuary and not out at sea.

3.2.1 Methods

Surface plankton nets were used to determine if spawning occurred inside the mouth of the Kosi estuary. Sampling extended from one neap tide through a spring tide (full moon) to the following neap tide in June 1990. In order to identify A. berda eggs, eggs and sperm were removed from ripe fish which were speared by divers and the eggs were artificially fertilized. The larvae were raised through to the first feeding stage and were described at 4, 30, 54 & 78h after hatch.

To determine the time and location of spawning, plankton samples were taken at set intervals on outgoing and incoming tides (half hour and one hour respectively) from two dinghies, one moored below the reef at the mouth (station A) and the other above (station B) (Fig. 111). Sampling was sometimes staggered, reducing the interval between samples to 15min. Samples were collected by streaming the plankton nets behind the moored dinghies, allowing tidal flow to maintain them horizontally. Flowmeters were used to determine the flow of water passing through the 200μm plankton nets. Three plankton sampling regimes were selected. The first consisted of 10min samples every 20min for the duration of both outgoing tides (day and night) every third day. The second consisted of 10min samples every 20min for the first two hours after the turn of the tide on all other outgoing tides. The third consisted of a) five 10min samples taken at 20min intervals on incoming tides (day and night) immediately prior to slack water at high tide on every third day, and b) 10min samples taken at 50min intervals during an entire incoming tide.

The volume of eggs collected in each sample was recorded and the percentage contribution of each species was determined from subsamples once the species had been identified. The number of A. berda eggs per millilitre was determined and a subsample from each sample was placed in an incubation jar to check their identification.

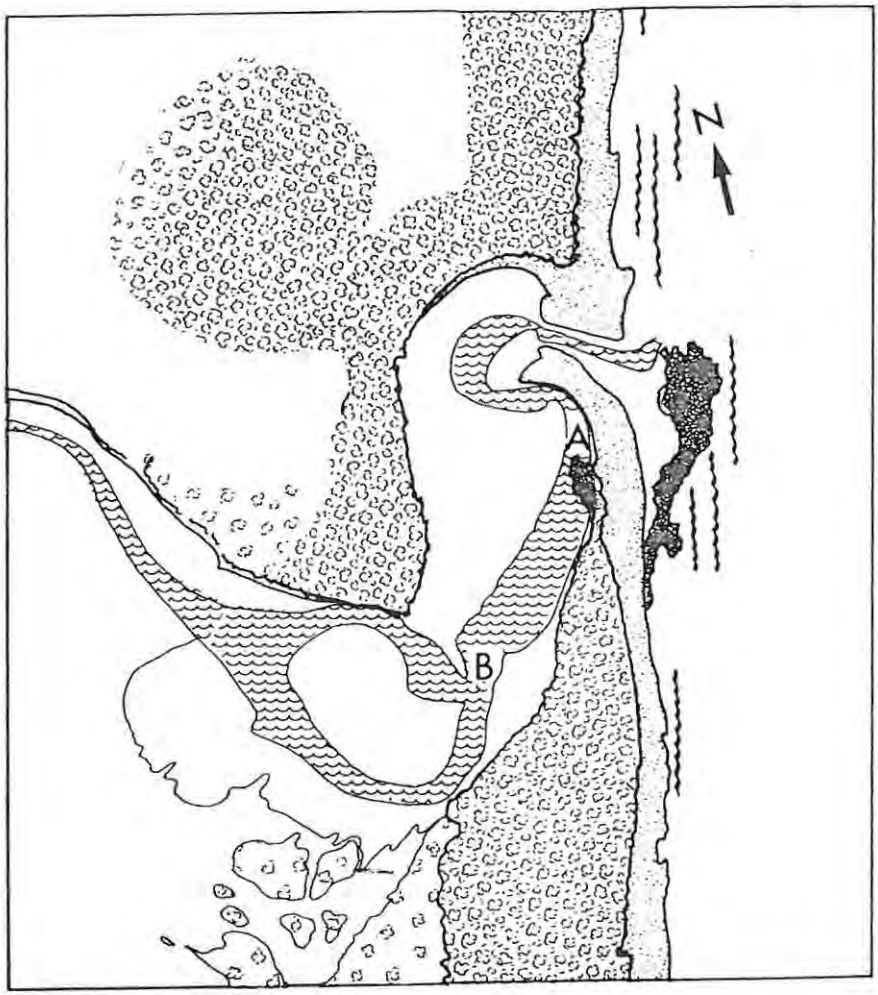

Figure 111. Study site at Kosi Mouth, showing the reef inside the mouth and the two sampling stations (A & B) at which plankton samples were taken. beach; reef; main channel; sand banks exposed at low tide; shallow water at low tide.

Once it had been established that the aggregation of A. berda at the mouth of the Kosi estuary represented a spawning aggregation and that spawning occurred in the mouth and not out at sea, it became possible to investigate oocyte development during spawning (to determine spawning periodicity) and spawning behaviour of this species.

Spawning periodicity was investigated by comparing oocyte composition in the ripe ovaries of A. berda to that in C. nufar and other sparids. Cheimerius nufar is a serial spawner known to spawn several batches of eggs per day over an extended period. Chrysoblephus puniceus and C. nufar have spawning seasons of similar duration. Similar patterns of oocyte development in the three species may indicate similar spawning periodicity and conversely, dissimilar patterns may indicate different spawning strategies.

Ripe ovaries, each at the same stage of development, were removed from three fish of each species. Sections were cut from the mid-region of each gonad and three random samples of equal area were photographed. The number of oocytes in each developmental stage on the photographs were counted and their relative percent composition determined. The ratio of tertiary yolk ($3^{O}YO$) oocytes to empty follicles was also included in the analysis.

The fact that spawning in A. berda occurred only at night and was timed to coincide with peak tidal flow out of the estuary posed several problems, not the least of which was the periodic movement into and out of this mouth, during high tides, of the Zambezi Charcharinus leucas and other species of sharks. Notwithstanding this potential danger, a series of anchors were laid in the mouth with ropes attached which enabled divers to pull themselves into the centre of the channel and remain there without exerting too much energy.

Three divers, two with cameras (video and still) and the third with lights, entered the water on several occasions timed to coincide with peak spawning activity. These attempts to observe and film spawning behaviour failed. The divers could not find the shoal, even though they left the ropes and searched in the vicinity of the reef at the mouth. It is possible that the fish were evading the bright lights and, as it had previously been noted that the air exhaled from the demand valves of SCUBA sets also disturbed them, it was concluded that observations on the spawning behaviour of A. berda were not possible.

During the following spawning season a team of divers visited Kosi mouth to spear fish which had been tagged in previous years in an attempt to produce conclusive evidence of sex change in this species. During these dives spawning was observed and social interactions before and during spawning were noted.

Recovery of tagged fish was timed to coincide with a spring tide (new moon) during the peak spawning season. Conditions at the mouth were best for spearing fish three days before to three days after spring tides. Divers were in the water for several hours each day and used the time to observe behaviour within the shoal while hunting tagged fish. Noting colour changes in several individuals in the late afternoon on the first day, these fishes were observed closely and two were speared to establish the significance of the colour changes. Both were ripe-running females with large volumes of ovulated eggs. Observations on the following day were continued until dark.

Spawning was observed the following evening and, as the tide on the next day turned too late to allow further observations, a second trip was undertaken on the following spring tide (full moon) to gather more data on the spawning behaviour of this species. In total, behaviour within the shoal was observed for approximately 26 hours. Behaviour patterns relating to spawning were observed over a period of 11 hours.

3.2.2 Results

Spawning sites

Two divers, who made repeated dives at Kosi mouth throughout the study period, estimated the number of A. berda in the vicinity of the reef at the mouth to be in the region of 1000-1500 fish. No juveniles were present. Originally it was thought that there may be two discrete shoals, the second being situated some 300m up the estuary. Colour-coded tags showed, however, that there was mixing amongst these fish and that the aggregation could be considered as one shoal.

The live eggs and early larvae of A. berda are distinctive from all other seabreams because of the presence of an oil globule which is heavily pigmented with yellow melanophores around its entire surface (Fig. 112a,b,c) (Connell, A.D., CSIR Durban, pers. comm.). Another distinctive larval feature is four clusters of yellow melanophores in the head region, two in front of the eyes and two behind (Fig. 112b).

The successful artificial fertilization of A. berda eggs, and the rearing of their larvae, was critical to this study for identification purposes, especially as a second species, the large-scale mullet (Liza macrolepis), was also spawning in the estuary at the time, producing large quantities of eggs. The eggs and early developmental stages of A. berda have not

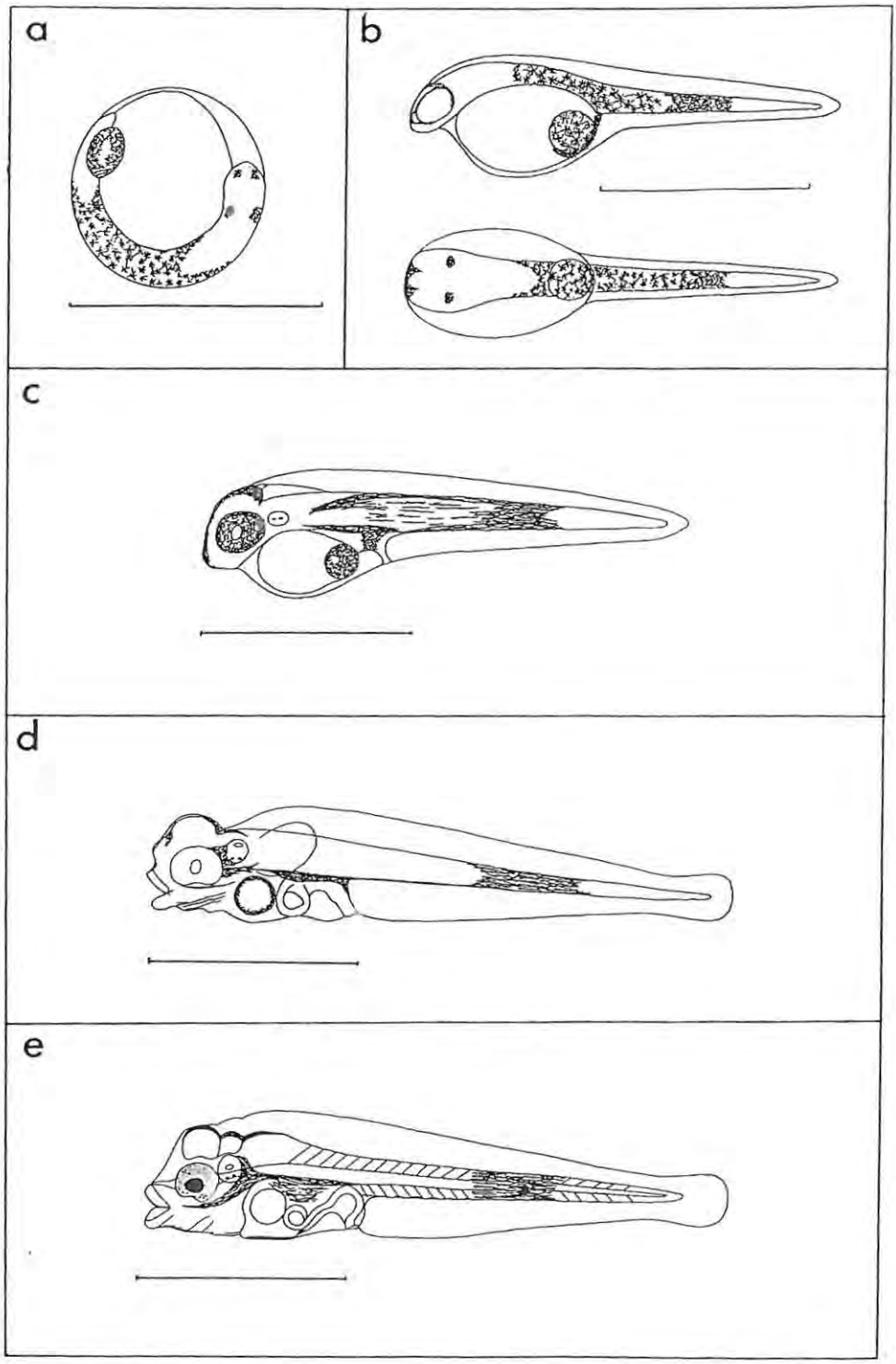

Figure 112. Egg and early larval development of Acanthopagrus berda showing characteristic yellow melanphore patterns: a = egg 29 h after fertilization; b, c, d, e = larvae 4, 30, 54 & 78 h post-hatch. (Scale bar = 1 mm).

been previously described and, while the descriptions presented in this paper are of live specimens which are not directly comparable to fixed specimens normally used for identification purposes, they are necessary for studies of this nature. These early stages are, in any case, not normally included in ichthyoplankton studies. Fixed eggs cannot be identified as they are denatured and post-hatch larvae are usually fragmented beyond recognition during the netting process.

The first plankton sampling regime revealed that A. berda spawn in the estuary above the reef and in its immediate vicinity. Spawning occurs only at night (Table 2, Fig. 113) on outgoing tides for 1-3h, beginning 1h after the turn of the tide. The flow meter at station B malfunctioned, so the results are not directly comparable with those at station A, but the pattern was clearly the same (Fig. 114). Generally the volume of eggs collected at station A was greater than that at station B (Table 2), indicating that more spawning occurred between the two stations than above station B. One day after the spring tide, however, greater volumes of eggs were collected at station B.

The larger volume of eggs collected at station B one day after the spring tide indicates that spawning occurred closer to station B on that occasion and that the eggs had dispersed before they reached station A. Assuming that flow rates were similar at both stations, this suggests that the spawning shoal had shifted slightly upstream with stronger incoming tidal flow. As the dinghy at Station B was positioned immediately downstream from a deep hole situated near the first bend of the estuary above the reef, some spawning probably occurred in this hole. Larger volumes of eggs at station A on other occasions, however, suggest that most spawning occurred in the immediate vicinity of the reef.

Time of spawning

The second sampling regime showed that A.berda spawned virtually every night between, and including, spring and neap tides on waxing and waning moons. Spawning was suspended only when outgoing tides at night occurred 2-3h before sunrise (Fig. 113) and the second outgoing tide occurred before sunset. The third sampling regime showed that insignificant numbers of eggs enter the estuary on incoming tides and that no A. berda eggs are amongst them. It was also determined from these samples that A. berda do not spawn earlier in the tidal regime, which would allow the eggs to be transported up the estuary and back down again.

Table 2. The occurrence and mean numbers of Acanthopagrus berda eggs sampled at each station on both outgoing tides every third day (- = no sampling/data).

Date	Ebb tides	Eggs present */absent 0		Mean # eggs / sample		Mean # eggs.m^{-3}	# samples	
		Stn A	Stn B	Stn A	Stn B	Stn A	Stn A	Stn B
03.06.90	1410–2013	0	–	0	–	0	7	–
	0130–0800	*	–	338	–	160	6	–
06.06.90	0350–1000	0	0	0	0	0	12	12
	1630–2220	*	*	165	135	25	11	12
08.06.90			SPRING	TIDE				
09.06.90	0530–1130	0	0	0	0	0	9	8
	1800–2400	*	*	511	2594	81	10	10
12.06.90	0710–1321	0	0	0	0	0	9	8
	1930–0130	*	*	1465	561	363	9	10
15.06.90	0930–1530	–	–	–	–	–	–	
	2200–0320	*	–	1800	–	207	7	–

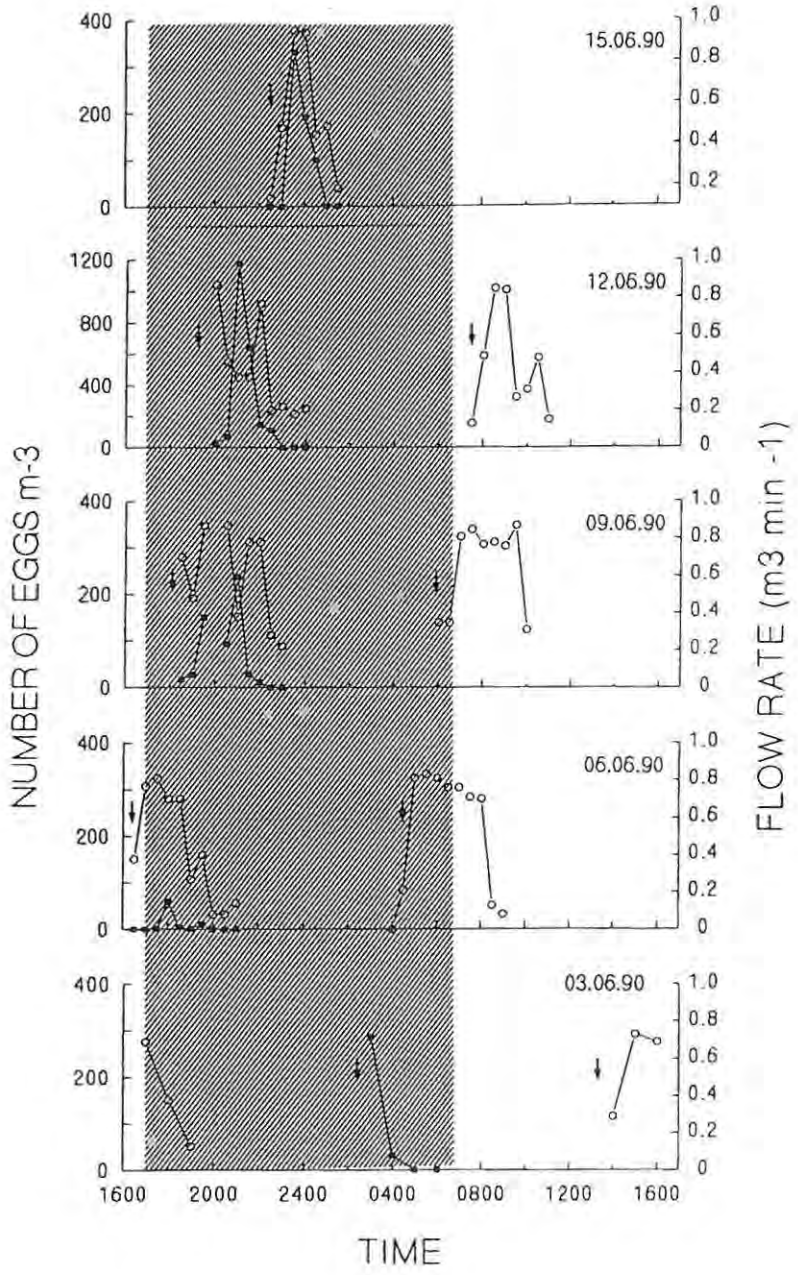

Figure 113. The number of Acanthopagrus berda eggs. m^{-3} sampled at Station A (■—■) and the duration of spawning in relation to the turn of the tide (↓), flow rates through the nets during outgoing tides (o—o) and hours of darkness (hatched).

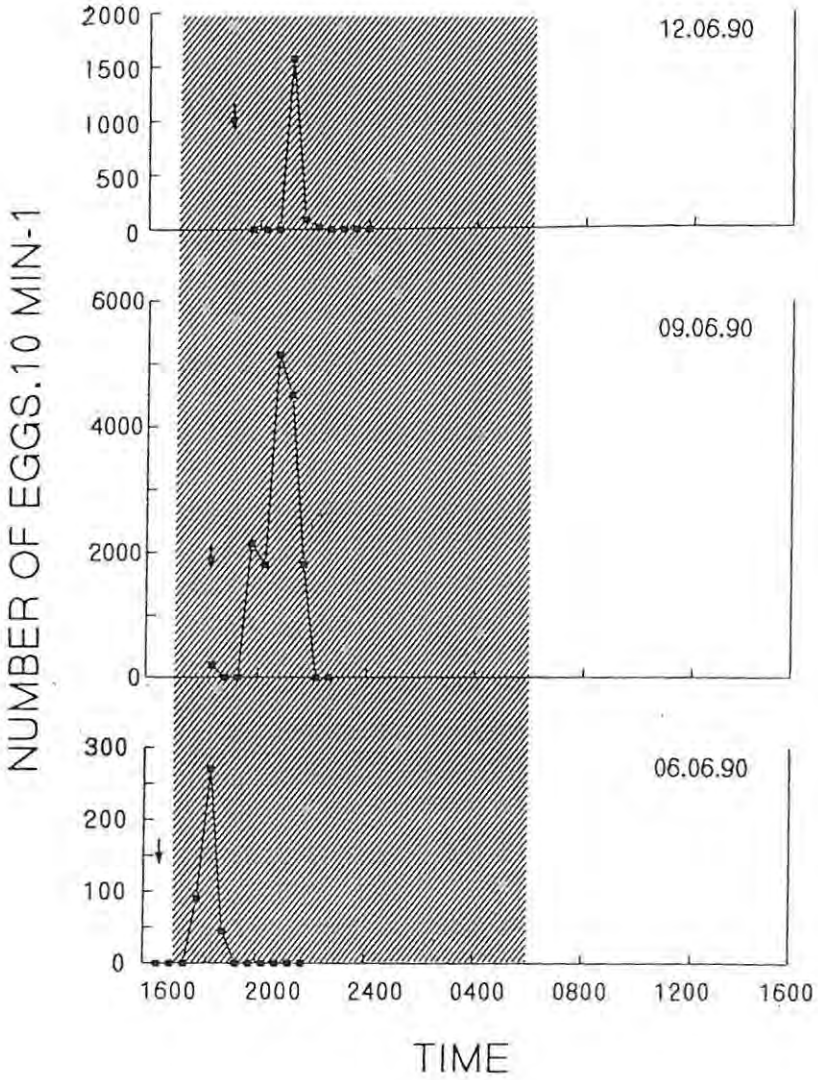

Figure 114. The number of Acanthopagrus berda eggs. 10 min^{-1} in each plankton haul at Station B and the duration of spawning in relation to the turn of the tide (↓) and hours of darkness (hatched).

Using the number of eggs per unit of water passing through the nets (first sampling regime) it was determined that spawning was more intense after the spring tide (Table 2, Figs. 113 & 114).

Colour changes

Acanthopagrus berda is a silver-grey fish with a white belly. The third anal spine, which is prominent, is also white and a yellow flash on the anal fin varies in intensity from one individual to another. The margin of the caudal fin is black.

During the day, all individuals within the shoal at Kosi mouth were a uniform colour. Prior to spawning, a large black patch developed on the flanks of ovulating females (Fig. 115). This black marking was faint and hardly noticeable in the late afternoon, but it intensified towards dusk and became prominent at sunset. It was accentuated by the simultaneous development of bright white markings on the belly, pelvic fins and part of the operculum. Prior to spawning females swam with their pelvic fins fully extended, thus increasing the contrast between black and white markings. Males remained silvery-grey.

Females with black markings were observed on the reef and along the southern bank in close proximity to the reef. There were no more than ten of them at any time in this small area and there were at least 200 individuals (presumably males) in their immediate vicinity. None of the females with black markings were big fish, being approximately 220-250mm (FL) in length.

Behavioural patterns

During the incoming tide, most of the fish in the aggregation were very active, swimming back and forth along the banks, around the reef and in the main channel. Females in spawning condition became discernible before the turn of the tide. They were accompanied by several males which ranged in size from 200-270mm and which maintained a position immediately behind them (Fig. 116a). Within the group of males the largest fishes maintained positions closest to the female. There was no contact between males and females and no overt aggression between males. The females remained in roughly the same area, finning into the incoming current. Occasionally they would sweep around in a wide circle with the accompanying males in close attendance (Fig. 116b), but they never moved more than 10-12m away and would always come back to appproximately the same position.

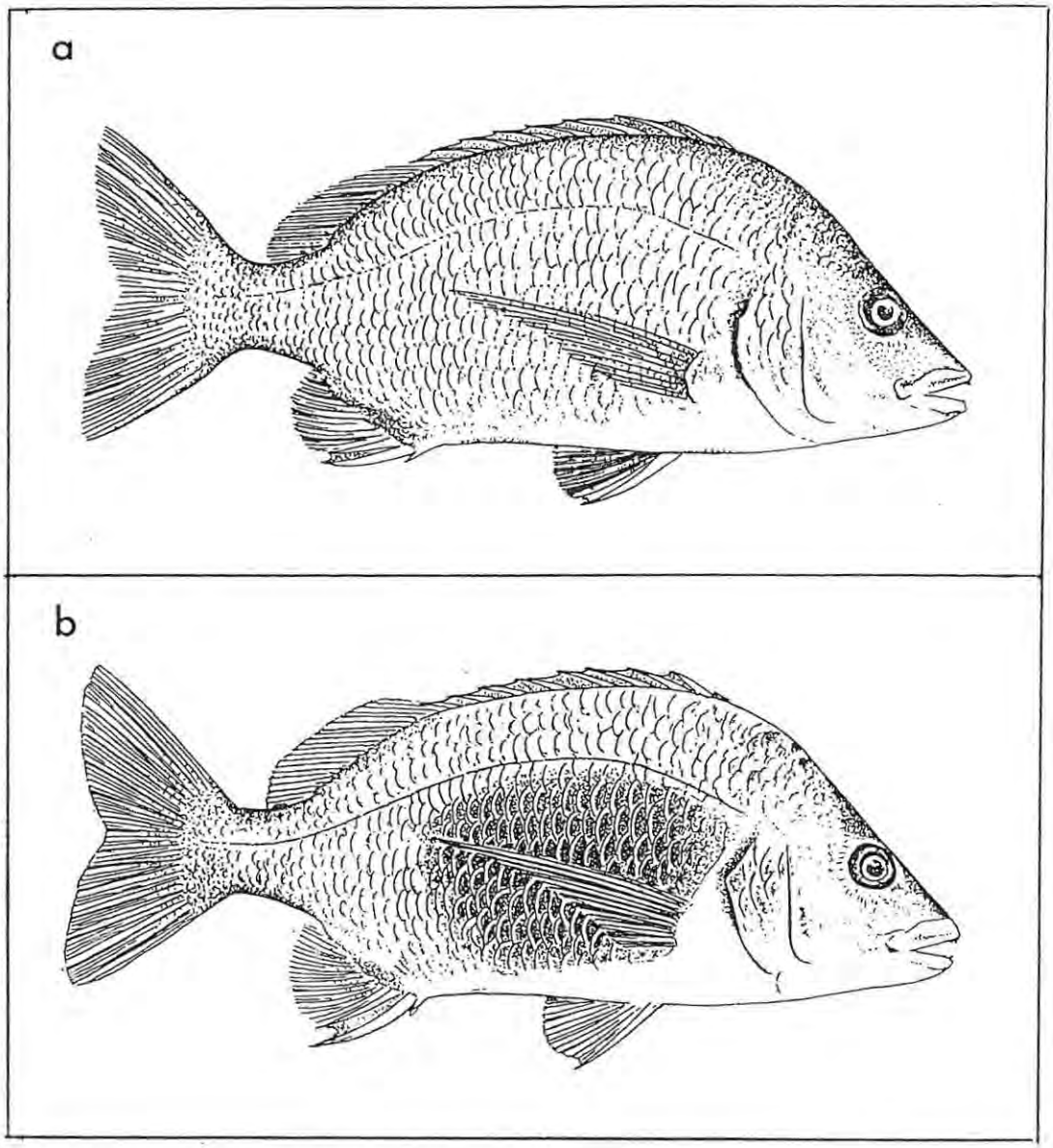

Figure 115. Male (a) and female (b) Acanthopagrus berda during spawning. Males remain silver-grey. Ovulating females develop a large, black patch on their flanks.

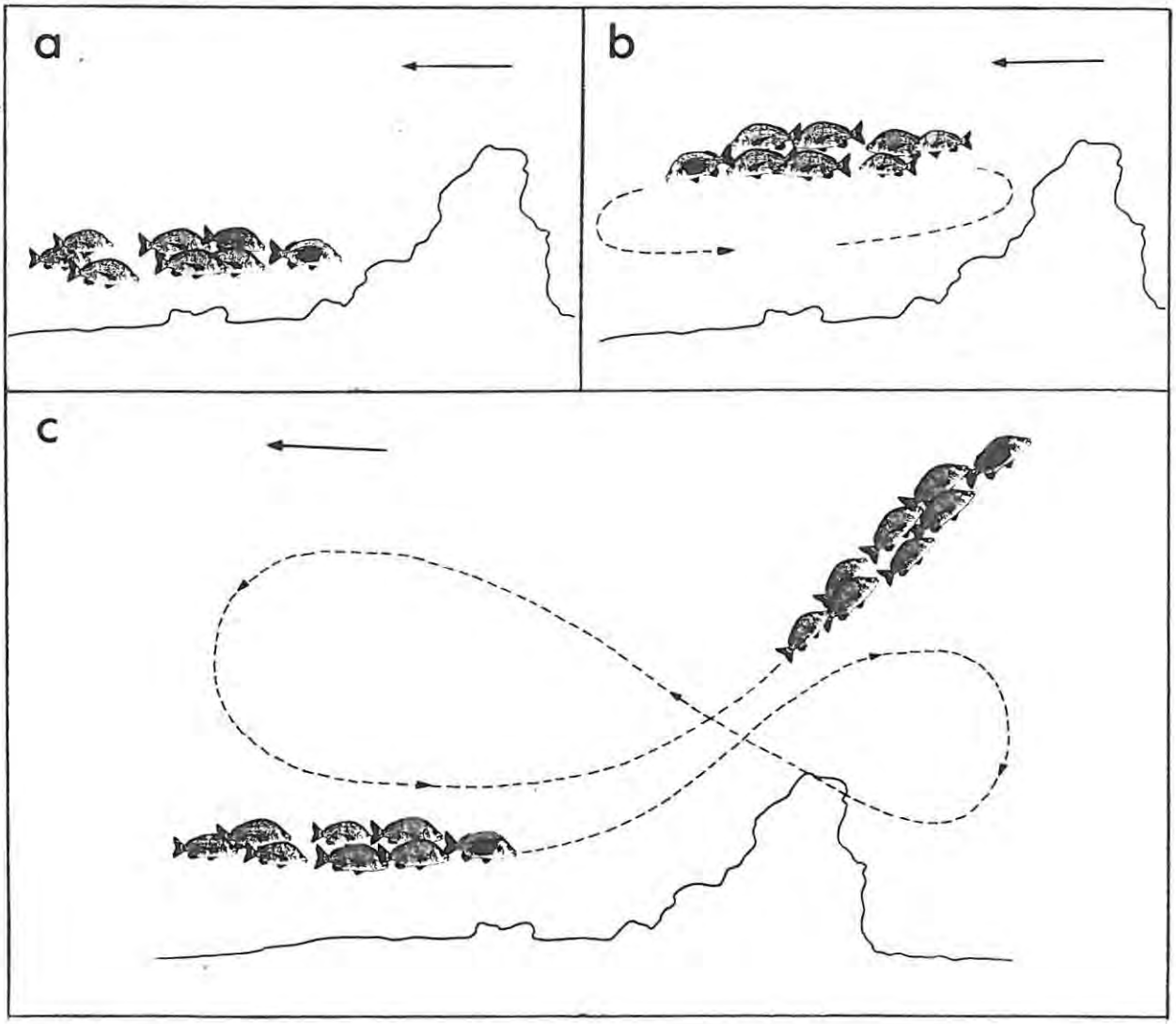

Figure 116. Reproductive behaviour in Acanthopagrus berda based on visual observations: a - female maintains position near reef with males trailing behind; b - female occasionally sweeps around in a circle with males following closely; c - when the female is ready to spawn she circles and weaves around in the same area increasing her pace. Males follow closely and spawn with her in a final rush near the surface. ← indicates direction of current.

Spawning behaviour

A marked decrease in activity was noted throughout the aggregation at the turn of the tide. This lasted for approximately 10-15 minutes until the current had reversed and the fish were facing upstream. At this time males became more active, constantly manouvering for better positions behind females. Males closest to the females at this time would continually veer left and right thus preventing others from coming closer to the female. At sunset the females became more active but still remained roughly in the same area. This activity attracted more males and a single female would have between 5-30 males accompanying her. Immediately before spawning the female started swimming faster, weaving back and forth and up and down in the water column. The group of males streamed out behind her (Fig. 116c) and increased their pace as she did. Spawning occurred in a final rush near the surface. The female released a large number of eggs and the males released sperm while the egg cloud was still small. The mass of gametes was clearly visible even in poor light.

Spawning occurred only after sunset and, in the limited period between sunset and total darkness, this spawning sequence was witnessed six times.

Spawning periodicity

The data showed that oocyte development in C. nufar and C. puniceus was similar and that this development was significantly different from that in A. berda. In the former species there was a uniform decrease in the number of oocytes in each developmental stage with increasing maturity, indicating a continual production of oocytes from the primary germ stock (Table 3, Fig. 117). Scott et al. (1993) presented similar data for the New Zealand snapper (sparid) Pagrus auratus. This pattern of development, also recorded in the Japanese snapper Pagrus major (Matsuyama et al. 1988, Kagawa et al. 1991), is typical of species with multiple spawning episodes during the spawning season (Wallace & Selman 1981, de Vlaming 1983) and it is considered indicative of a daily production of batches of mature oocytes.

In A. berda there were few yolk vesicle oocytes and virtually no primary and secondary yolk oocytes in the ovaries (Fig. 117). Volumetrically, the ovaries consisted almost entirely of 3°YO oocytes. ANOVA showed that there were significant differences in the proportions of yolk vesicle (YO), primary and secondary yolk vesicle (1°&2°YO) and tertiary yolk vesicle (3°YO) oocytes in the ovaries of A. berda compared to those of C. nufar and C. puniceus (Table 4).

Table 3. The number of oocytes in each developmental stage, number of empty follicles and ratio of tertiary yolk ($3^{o}y$) oocytes to empty follicles in Acanthopagrus berda, Cheimerius nufar and Chrysoblephus puniceus. PP = pre-perinucleolar, P = perinucleolar, YV = yolk vesicle, 1^{o} & 2^{o} y = primary and secondary yolk, $3^{o}y$ = tertiary yolk, EF = empty follicles.

ACANTHOPAGRUS BERDA							
Length	PP	P	YV	1°& 2°y	3°y	EF	3°/EF ratio
200mm	273	32	10	0	27	7	3.86
	331	33	9	0	27	5	5.40
	342	17	12	0	33	4	8.25
$\bar{x}$	315	27	10	0	29	5	5.80
177mm	274	24	9	4	38	3	12.67
	402	17	9	10	38	4	9.50
	460	30	12	4	37	6	6.17
$\bar{x}$	379	24	10	6	38	4	9.50
276mm	514	104	8	0	46	8	5.75
	158	25	0	0	67	11	6.09
	262	11	0	0	77	10	7.70
$\bar{x}$	311	47	3	0	63	10	6.30
CHEIMERIUS NUFAR							
525mm	336	60	46	21	12	12	1,00
	350	48	40	21	14	8	1.75
	312	29	33	29	11	10	1.10
$\bar{x}$	333	46	40	24	12	10	1.20
438mm	458	50	40	30	11	12	0.92
	526	60	36	29	10	18	0.56
	556	32	32	26	18	11	1.64
$\bar{x}$	513	47	36	28	13	13.7	0.94
388mm	466	44	46	26	12	11	1.09
	360	48	38	18	12	8	1.50
	300	76	29	19	12	10	1.20
$\bar{x}$	375	56	38	21	12	10	1.20
CHRYSOBLEPHUS PUNICEUS							
308mm	666	74	34	18	9	11	0.82
	582	116	30	15	9	11	0.82
	618	126	38	15	8	8	1.00
$\bar{x}$	622	105	34	16	9	11	0.90
312mm	312	80	48	16	11	8	1.38
	264	80	47	28	16	15	1.07
	250	68	62	25	12	10	1.20
$\bar{x}$	275	76	52	23	13	11	1.18
367mm	442	62	36	20	12	6	2.00
	470	52	30	24	8	10	0.80
	556	56	27	25	12	9	1.33
$\bar{x}$	489	57	31	23	11	8	1.38

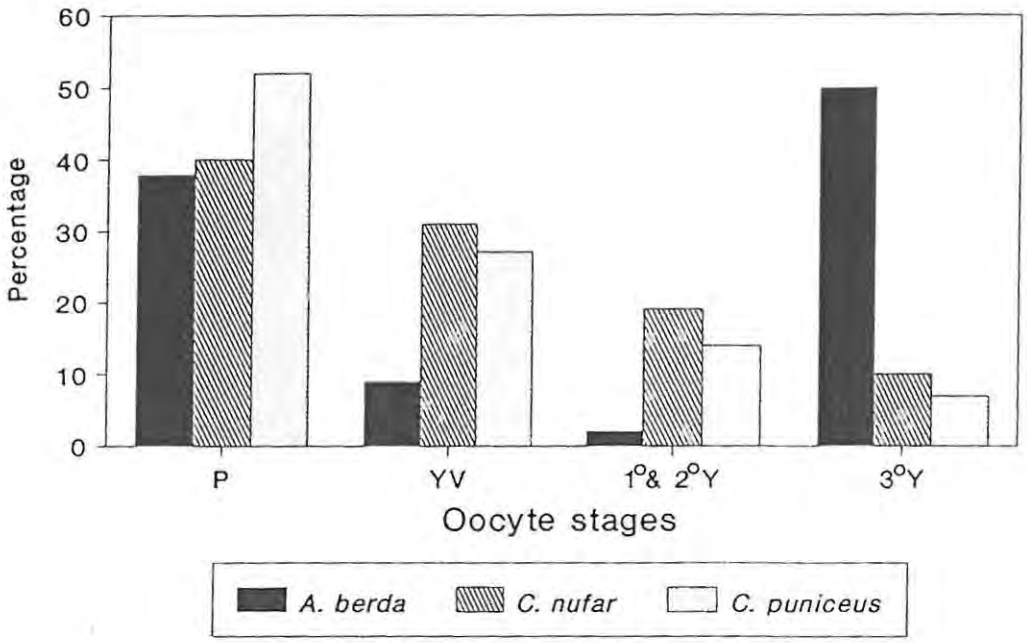

Figure 117. Percentage composition of oocyte stages in ripe gonads of Acanthopagrus berda, Cheimerius nufar and Chrysoblephus puniceus. P = perinucleolar, YV = yolk vesicle, 1^{o} & 2^{o}y = primary and secondary yolk, 3^{o}y = tertiary yolk.

Table 4. Test of significant differences (ANOVA) in the number of oocytes in each developmental stage, number of empty follicles and ratio of tertiary yolk ($3^{o}y$) oocytes to empty follicles in Acanthopagrus berda (AB), Cheimerius nufar (CN) and Chrysoblephus puniceus (CP). (p = 0.05)

OOCYTE STAGES	P-VALUE	DIRECTION OF DIFFERENCES
pre-perinucleolar (PP)	0.4583	
perinucleolar (P)	.0314*	AB < CN and CP
yolk vesicle (YV)	.0025*	AB << CN and CP
1° and 2° yolk (1° & 2°)	.0006*	AB << CN and CP
3° yolk (3°)	.0136*	AB >> CN and CP
empty follicles (EF)	0.1097	
3° yolk/empty follicle ratio (3° /EF)	0.0010*	AB>> CN and CP

*Significant

These results suggest that, unlike C. nufar and C. puniceus, A. berda produces a single batch of oocytes which are to be spawned each season. The ratio of 3^{o}YO oocytes to empty follicles (EF) also suggests that this batch of oocytes is released in several spawnings (Table 3). In C. nufar and C. puniceus the steady production of oocytes over an extended period suggests the release of smaller batches of mature eggs each spawning over an extended period, all mature eggs being spawned each morning (Table 3).

3.2.3 Discussion

In A. berda, a spawning strategy has developed in which spawning occurs in a relatively sheltered environment and the eggs are transported out into the marine environment during peak ebb tides. Spawning also occurs only at night and is curtailed when outgoing tides approach dawn. This behaviour suggests that the spawning strategy may have evolved in response to predation. As predation on the adults at the mouth can be considered minimal (pers. obs.), the development of nocturnal spawning is most likely a response to predation on their eggs by large numbers of planktivorous teleosts which also aggregate in the mouth during the spawning season. It appears, therefore, that A. berda has developed

a spawning strategy which has overcome the necessity to move out into the marine environment to spawn and that the assumption made to this effect by previous workers (Wallace 1975b, Kyle 1986, van der Elst 1988) is incorrect. A similar transportation of eggs by ebb tides into the marine environment has been assumed, but not shown, for the congeneric A. australis (Pollock and Weng 1983).

The spawning strategy outlined above may be common to the major estuarine systems of Natal and possibly the smaller systems which still function as estuaries (Begg 1978). Analysing monthly gill-net catches in the St Lucia estuary and narrows, Wallace (1975a) showed an increase in abundance of A. berda at the mouth during April-July. The presence of gravid fish in these samples and in anglers catches, and the absence of such fish further up in the system, led him to suggest that spawning occurs close inshore in the vicinity of estuary mouths.

The suggestion that A. berda spawns at sea may not have been accurate, but angler catch returns from St Lucia mouth during 1992-93 support the earlier data, showing that the increase in abundance of this species at the mouth during this period is an annual phenomenon (Fig. 118a). Further samples collected from the St Lucia estuary in the present study (May-June 1992) showed that the aggregation at the mouth was composed almost entirely of males and that the majority of fish were in spawning condition (Table 5). The similarities in the St Lucia and Kosi systems strongly suggest that the reproductive strategy described in the Kosi estuary is not unique to that system, but that it is common to all estuaries in Natal which still function as such. The fact that A. berda eggs have been collected in plankton samples taken at night on outgoing tides in the Durban harbour entrance channel during two successive spawning seasons (Connell, A.D., CSIR Durban, pers. comm.), further supports this hypothesis.

Table 5. Mean length, sex ratio and reproductive state of Acanthopagrus berda sampled at the mouth of the Kosi (June - July 1990-91) and St Lucia (1992) estuaries.

LOCALITY	SAMPLE SIZE	MEAN LENGTH (mm)	% MATURE	SEX RATIO (m/f)
Kosi	152	261 (SD = 28.3)	100	8.8 : 1
St Lucia	56	214 (SD = 36.0)	91	19 : 1

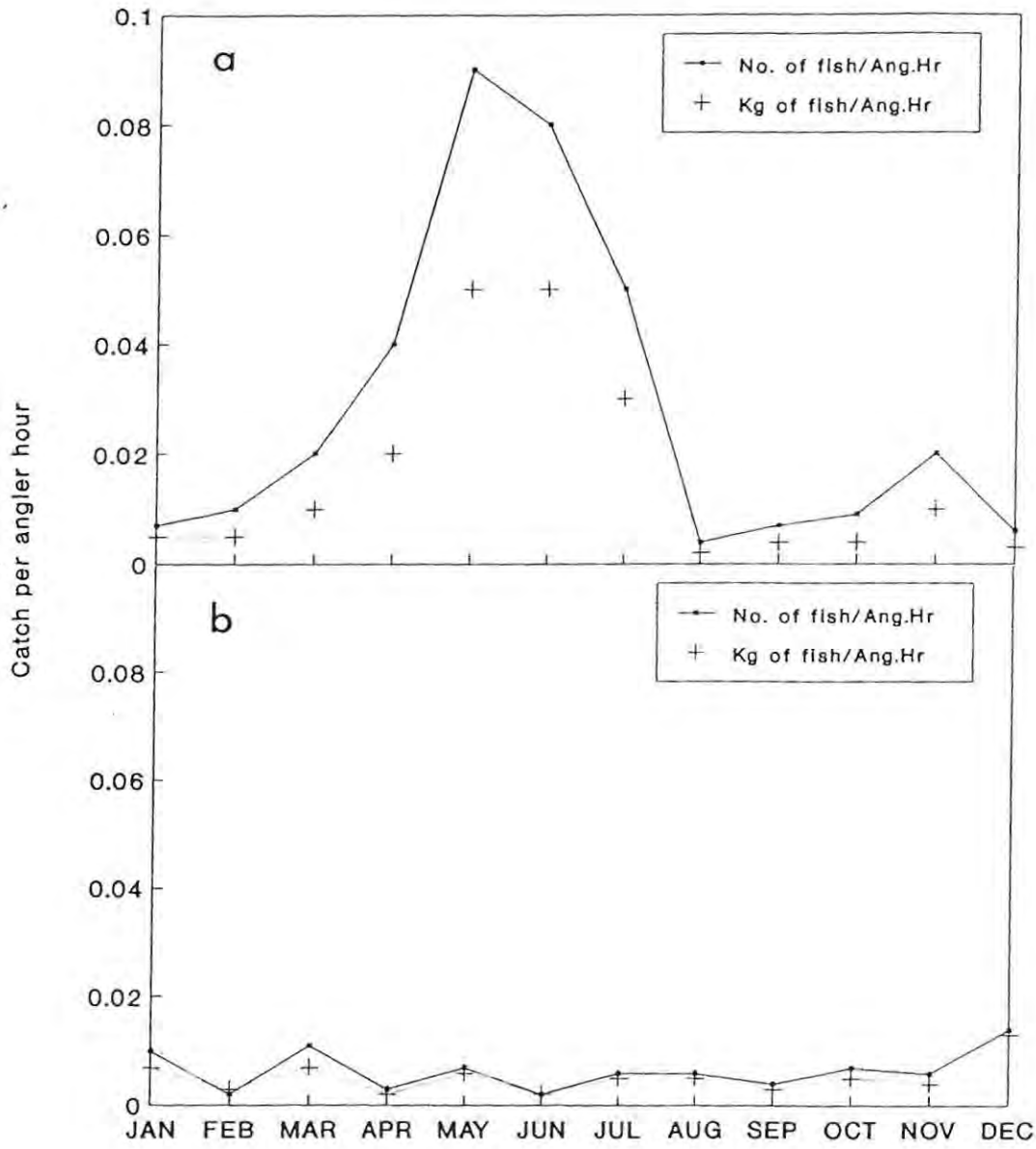

Figure 118. Catch per unit effort (CPUE) of Acanthopagrus berda in the St. Lucia estuary during 1992. (a) = CPUE at the mouth, (b) = CPUE in the lakes above the narrows (B. Mann, Oceanographic Research Institute, unpublished data).

In all probability, one of the most important points which has arisen from this study, from a fisheries management point of view, is that the degradation of estuarine habitats could have serious consequences for an estuarine-dependent species which has developed a spawning strategy of this nature. To this must be added the problem of increasing angling pressure during the peak spawning season, when large numbers of this species form dense shoals which are easily accessible to anglers.

Acanthopagrus berda featured prominently in estuarine anglers catches in earlier years, but several workers have noted a marked decline (Wallace 1975a, Begg 1978, Grindley & Heydorn 1979, van der Elst 1988). Whilst it has been suggested that excessive exploitation has been instrumental in this decline there is a strong case for estuarine degradation as a major factor as well (Begg 1978). Support for this comes from the Kosi estuary itself. This is the least disturbed estuary along the Natal coast (Begg 1978) and in this system angler's catches and those made in traditional fish traps have remained fairly constant from one year to the next over a prolonged period (Kyle 1986). Acanthopagrus berda has been, and remains, the third most important fish caught in traps in the system.

Estimations of the size of the aggregation at Kosi mouth each year have been made by several people over a number of years and they have been confirmed by observations made in this study. The fluctuating size of the aggregation (200-1500 individuals) suggests that it does not represent a discrete shoal, as such, but rather that individuals are continually recruiting to it, whilst others are leaving it, throughout the spawning season. Estimates (made from tagging) of the total number of adult fish which move down to the mouth during the months April-July each year support this hypothesis. Trap catches of A. berda are fairly constant from one year to the next and estimates of the number of fish moving down to the mouth each year range from 32 000 - 76 915 (95% confidence limits) (Kyle 1986). Even if this is an overestimate, the fact remains that thousands of A. berda appear to move down to the mouth each year, yet the aggregation seldom consists of more than 1500 fish at any one time. This suggests the possibility that both sexes recruit to the aggregation randomly or in groups, remain at the mouth for a limited period, and move back up into the lakes once they have spawned.

However, the overwhelming predominance of males in the aggregation; the size range of the fish in the aggregation; the size of the aggregation itself and the results of the tagging exercise included in the present study suggest a second, and more tenable, scenario: one in which a proportion of the males within the Kosi population (above a certain age/size) migrate to the mouth and remain there for the greater part of the spawning season.

Females within the population migrate to the mouth throughout the spawning season (as they become fully mature) and migrate back into the lakes once they have spawned. The very low proportion of females in the spawning aggregation during the day suggests either that the majority of females migrate to the mouth on a daily basis (joining the spawning aggregation at night), or that the sexes remain separate until spawning and that there is another aggregation consisting of females elsewhere in the lower reaches of the system. Artisanal fishermen, who know the system intimately, are not aware of such an aggregation and, as searches for other A. berda in the lower reaches of the system did not either reveal such an aggregation, it is unlikely to exist.

Data which may lend support to the hypothesis that females only remain at the mouth for a short period to spawn are found in the developmental stages of oocytes in ripe ovaries. The pattern of oocyte development in A. berda is quite different from species, such as C. nufar, which are known to spawn each day over an extended period. Acanthopagrus berda produce a single batch of oocytes each season. The ratio of tertiary yolk oocytes to empty follicles in ripe ovaries (from which a batch of oocytes has recently been released), has shown that these oocytes are released in several spawnings and, while it is not possible to determine the period over which these eggs are released, it is likely to be fairly short.

Previous histological investigations of oocyte development in the genus Acanthopagrus have indicated that some species are semelparous (single) spawners (A. latus and A. cuveiri, Abu-Hakima 1984, A. australis, Pollock 1982), whilst others are iteroparous (serial) spawners (A. schlegeli, Chao & Liao 1984). I suspect that these conflicting theories stem from differences in definition of these terms, rather than real differences in spawning strategies, and that all members of the genus spawn in a similar way. In the ripe ovaries of A. berda, for instance, the relative numbers of oocytes in each stage of development, and the volume of 3°YO oocytes in these ovaries, indicated that this species was a single spawner. However, the investigation into the ratio of 3°YO oocytes to empty follicles indicated that it is a serial spawner which lays several batches of oocytes, possibly over a short period. As it has been shown that spawning in A. berda occurs at 24h intervals, spawning periodicity appears to be similar to that of hatchery A. schlegeli, which spawn several batches of eggs at intervals of 8-24h (Chao & Liao 1984). It is possible that more detailed investigation of the gonads of A. latus, A. cuveiri and A. australis, especially into the relationship between the number of ripe oocytes and empty follicles, will reveal that they too are serial spawners which release their single batch of mature oocytes in several spawnings.

Results of the tagging exercise conducted in this study suggest that a large proportion of the aggregation at the mouth consists of the same males which return each year (those which have survived through the preceeding years). Of 315 fish which were sexed and tagged during the spawning seasons of 1990-91, 74 were recovered in June 1992 and 1993 by spear and a further six were recovered from artisanal fish traps. At the end of the spearing operations it was estimated that there were less than five tagged fish left in the aggregation. The high percentage (25%) of tag returns from fish which had been at large for 1-2 years indicated that a large proportion of the aggregation consisted of the same fish as previous years.

In a number of species which undertake annual spawning migrations, it has been shown that a large proportion of the adult population does not take part in the migration each year e.g the mullet Mugil cephalus (Thomson 1955), pacific salmon Oncorhynchus spp. (Harden Jones 1968), barramundi Lates calcarifer (Moore & Reynolds 1982) and the yellowfin bream Acanthopagrus australis (Pollock 1982, 1984). Pollock (1982) suggested that approximately half of the adult population of A. australis in Moreton Bay, Queensland, did not participate in the spawning migration. Later, he was able to show that successful reproduction occurred only in migratory fish (Pollock 1984). Gonads developed to a similar size in both migratory and non-migratory fish, but in non-migratory females oocytes which had developed to the yolk stage became atretic and were resorbed. He also showed that the majority of fish undertaking the spawning migration were males and that there was a decreasing tendency for females to participate in the migration with increasing age. The present study did not investigate all of these aspects of the spawning migration of A. berda, but there is sufficient indirect evidence to suggest a similar scenario in A. berda, with an alternative hypothesis.

In the St Lucia estuary, Wallace (1975b) recorded A. berda in spawning condition (ovulating) only at the mouth and noted that fish higher up in the system were not in spawning condition. He did not indicate the state of maturity of the fish higher up in the system and it is not clear if the gonads of these fish were maturing. However, the fact that A. berda were caught in the system at the time of spawning at the mouth, suggests that only a proportion of the overall population takes part in the annual spawning migration to the mouth, as in A. australis (Pollock 1984). Further evidence in support of this hypothesis was collected during this study from anglers' catches in the St Lucia lakes (National Marine Linefish Catch Statistics programme). Angler catch per unit effort (CPUE) remained low but fairly constant throughout the year (see Fig. 118b). There was a decrease in CPUE in June 1992 (the spawning season extends from May-July inclusive), but there were similar

decreases in February and April. As CPUE remained steady during the other two months of spawning, it is unlikely that the drop in June was related to spawning activity. It appears, therefore, that we may assume a similar spawning strategy in A. berda as the one described for A. australis (Pollock 1984).

However, whereas in A. australis it was postulated that the gonads of females which did not take part in the spawning migration developed to the same size (eggs developing to the yolk stage) but regressed thereafter, indicating that these fish did not take part in spawning, an alternative explanation in A. berda is that these females do migrate to the mouth to spawn. If, as suggested above, females 'filter' down to the mouth on a daily basis when they are ready to spawn, a part of the population (young males and maturing females) would always be in the system, giving the impression that this proportion of the population does not take part in spawning. Further work will be needed to determine the reproductive state of females higher in the system and their movements during the spawning season, if this hypothesis is to be validated.

Having established the time and place of spawning, the serial nature of spawning, spawning behaviour and the size and sex composition of the spawning aggregation at Kosi mouth, it was possible to compare the reproductive strategy of A. berda to other protandrous fishes and to relate these findings to current sex change theory.

Functional hermaphroditism is now known in over 100 fish species of 15 families and is thought to have evolved independantly on at least 10 occasions (Lowe-McConnell 1987). Whereas many reef fish are protogynous, protandry appears to be less common (Warner 1984) and is more usual in species which are not associated with reefs (Warner 1978), such as the polynemids on the west African shelf (Longhurst 1963), the platycephalids (Fujii 1971), the catadromous Lates calcarifer in Australia (Lowe-McConnell 1987) and sparids such as Pagellus acarne (Reinboth 1962, Alekseev 1967), Sparus aurata (Zohar et al. 1978), Rhabdosargus sarba (Yeung & Chan 1987), Acanthopagrus australis (Pollock 1984) and A. berda (this study). Many of these fish occur in large shoals in relatively shallow inshore waters and estuaries.

Apart from work conducted on several anemonefishes (fam: Pomacentridae), which live in obligate association with anemones and are monogamous (Moyer 1976, 1980, Moyer & Nakazono 1978, Fricke & Fricke 1977, Fricke 1979, Fricke 1983, Ochi 1989a,b, Ochi & Yanagisawa 1987, Hattori & Yanagisawa 1991), there is virtually no information on the mating systems of protandrous fish species and the selective advantages of protandry in

shoaling species are poorly understood. The anemonefishes are unique in the sense that the social system/environment limits the reproductive group size to two (Fricke & Fricke 1977, Moyer & Nakazono 1978, Charnov & Bull 1989). This has led to an environmentally induced monogamy and the selective advantages of protandry in these species are easily understood. Sex change and maturation are socially controlled and, even though the mechanisms regulating sex change and social structure have been shown to be more complex than was originally thought (Hattori & Yanagisawa 1991) they are, nevertheless, fairly well understood.

A number of studies have attempted to elucidate the evolutionary and ecological significance of the different types of piscine sexuality (Smith 1967, 1982, Ghiselin 1969, 1974, Warner et al. 1975, Warner 1975, 1978, 1988a,b, Charnov 1982, Fischer & Petersen 1987, Shapiro 1987b, 1989, Fishelson 1989). To be successful, such studies must be based on extensive knowledge of the social structures, behaviour and ecology of the species involved (Fishelson 1992). While considerable information is available for many protogynous species, nothing is known about these aspects of the life history of shoaling protandrous species.

Current sex change theory, based on the original size-advantage model of Ghiselin (1969), suggests that selection will favour protandry if female success/fertility increases with age (size) faster than male success/fertility (Charnov & Bull 1989). The model predicts that protandrous species should have a mating system such as monogamy, which reflects a limited influence of fighting ability on male success (Fischer & Petersen 1987), or random pairing of single males and females (Warner 1988a,b). Warner (1988a,b) has proposed that the strongest selection for protandry should occur in species in which there is random pairing of single males and females and that protandry should be more common in species with relatively small mating groups.

The present study has produced convincing evidence of protandry in A. berda, yet the spawning strategy of this species does not conform to the predictions given above, neither does it support the suggestion of Krebs & Davies (1981) that protandry will be favoured if male-male competition is not intense. The spawning aggregation at Kosi mouth was large and predominantly male and spawning was characterised by intense sperm competition, several to many males competing with each other to spawn with a single female. In effect, this was the antithesis to protogynous species in which territorial males spawn with a number of females.

What adaptive advantage does protandry confer on A. berda and what are the mechanisms which regulate sex change in this species? According to the size-advantage model of Ghiselin (1969), females must gain in relative reproductive success with increasing age (size) for protandry to be an evolutionary stable strategy (ESS) in A. berda. Gains in reproductive success may be related either to the mating system of the species, or to other factors not related to the mating system, such as differential growth and mortality between the sexes with increasing size (Charnov 1982).

Size-related differences in expected reproduction between the sexes can arise directly from the mating system (Warner 1988a) and the evolution of a mating system which has overcome the necessity to move out into the marine environment to spawn, such as that of A. berda, may have led to strong selection pressure favouring protandry in this species. Spawning in a strong and turbulent current, in which dispersal of eggs must be rapid, would necessitate the development of a mating system in which sufficient sperm is produced to ensure adequate fertilization. Intense competition among males to fertilize the eggs of single females may be the mechanism by which adequate fertilization is ensured and, where there is such intense competition, investment in male function may carry little return beyond a certain point (age/size). Warner (1988b) used this argument in the context of species in which spawning is random, but it may apply equally (or better) to the mating system of A. berda in which there is intense competition among males to fertilize individual females. Under these circumstances, there may be a relative decline in male function with size which is proportional to the number of other males with which a particular individual is competing, similar to that proposed for some protandrous plant and mollusc species (Lloyd & Bawa 1984, Charnov 1980 and Wright 1988).

The ability of bisexual fish to recognise the sex in demand, and to act accordingly, is extensively documented (e.g. Fishelson 1970, 1975, Robertson 1972, Popper & Fishelson 1973, Moyer & Nakazono 1978, Shapiro 1984, Shpigel & Fishelson 1986, Ross et al. 1990) and the average ratio of males to females during spawning sequences may be a proximate factor influencing sex change in A. berda. This ratio can be likened to the 'threshold value' suggested by Ross et al. (1983) for the saddleback wrasse Thalassoma duperrey, in which the proportion of larger and smaller fish within the home range is believed to trigger the initiation of sex change in individual females.

The results of work conducted on mating systems have explained so much about the mechanisms and selective advantages of sex change in fishes that very little attention has been given to factors such as differential growth and mortality, which can also result in

differential gains in reproductive success (Warner 1988b). A life history characteristic shared by several protandrous sparids, including A. berda, which may indicate that factors other than the mating system have played a role in the development of protandry in these species, is a juvenile phase which is spent in unstable and often harsh environments (lagoons and estuaries in which salinities and temperatures can vary considerably).

There is substantial evidence showing that in both plants and animals, including fishes, stressed individuals tend to function as males (Atz 1964, Freeman et al. 1980). For juvenile sparids entering unstable and perhaps harsh environments, it may be essential to direct most metabolic energy into somatic growth in order to survive. In such circumstances it would be better to be male first and change to female only when and if a large size is attained, or remain male if stressed (Warner 1988b). This strategy would be favoured if it increased the probability of reaching a highly fecund size. Further support for this hypothesis is found in the concluding statements made by Ghiselin (1969 p.200): "The size advantage model explains sequential hermaphroditism as occurring where an individual reproduces most efficiently as a member of one sex when small or young, but as a member of the other sex when it gets older or larger; it predicts proterogyny where there is sexual selection for larger males, and protandry where the young stages must hunt for a suitable environment".

In conclusion, the data collected during this study have indicated that the mating system of A. berda includes intense competition amongst males to fertilize individual females in a large spawning aggregation and, as such, it does not conform to current theory on protandrous sex change in the literature. There is evidence to suggest that protandrous sex change in this species has evolved through differential gains in reproductive success arising from the mating system, but it is possible that factors other than the mating system, such as differential growth and mortality, may also have played a role.

3.3 SPAWNING IN C. PUNICEUS

The spawning season of C. puniceus extends from July-November with peak spawning occurring in August-September. Samples obtained from commercial catches between 1979-1983 showed that C. puniceus did not spawn on the lower south coast of Natal (Garratt 1985b). Along the remainder of the coast spawning appeared to occur wherever there were shoals of this species. However, spawning sites of C. puniceus have not been identified and spawning behaviour is unknown.

Little is known about social structure and spawning behaviour in species which occur in relatively deep water on continental shelves. A brief description of spawning behaviour, in captivity, in the congeneric C. laticeps is given by Buxton (1990). Chrysoblephus laticeps, a protogynous hermaphrodite, has been shown to pair spawn on a daily basis, releasing gametes well above the reef after an elaborate courtship routine. In their natural environment large males are often found together with a number of females as discrete groups over extended periods of time. Spawning has not been observed in the wild, but from these observations Buxton (1987) suggested that this species was polygamous.

The aim of this study was to identify spawning sites of C. puniceus, determine the time of spawning and describe spawning behaviour.

3.3.1 Methods

Since the major concentrations of this species occur at depths of 50m (Garratt 1985b) and diving time is extremely limited at such depths, attempts were made to locate spawning sites in relatively shallow water. Observations were confined to the St Lucia marine reserve because poor visibility and rough sea conditions along most of the Natal coast during the peak spawning months of August, September and October made diving difficult.

During the period 1983-1986, nine research cruises were undertaken into the St Lucia marine reserve to locate shoals of C. puniceus and C. nufar. These cruises were primarily aimed at estimating stock size and residency of these species using mark and recapture methods. In addition, general biological data were routinely collected to examine the spawning condition of the fish in these shoals.

Shoals of C. puniceus in the reserve were located using echo-sounders. Linefishing was used to positively identify the shoals, to obtain samples for tag and release, to remove samples for population structure analysis and to assess reproductive state. The positions of all reefs with resident shoals of C. puniceus were marked on navigation charts using Decca navigation equipment, radar distances offshore and, later, GPS co-ordinates. During tag and release operations divers were sent down at regular intervals during the day to assess the effectiveness of the tagging exercise and to estimate mortality rates from the combined effects of tagging and barotrauma. In this manner the divers were able to note the size of the shoals, and the behaviour of the fish in them.

Tagging was discontinued in 1987 and on four subsequent cruises during the peak spawning seasons of 1987-1991, diving on shoals of C. puniceus was aimed exclusively at recognising spawning sites and describing spawning behaviour.

The majority of marine fishes, which spawn pelagic eggs, shed their gametes during the late afternoon and evening, over several weeks or months (Thresher 1984). Some restrict their spawning activities to periods which coincide with spring tides (Johannes 1978, Barlow 1981, Ross 1983, Foster 1987). Observations on C. nufar showed that this species spawns at sunrise, hence observations on C. puniceus were conducted throughout the day and into the night during spring and neap tides, concentrating effort in the early morning and evening. Shoals in the wild were observed by divers during the peak spawning seasons of 1987-1991 inclusive. Successive pairs of divers entered the water allowing for continual observations of up to three hours, depending on the depth of the reef. Dives started approximately 30min before sunrise and ended a few hours after sunset. Before entering the water, divers were briefed on the spawning behaviour of protogynous species which had been documented in the literature. They were instructed to note any interactive behaviour within the shoal, any colour changes of individual fish or any seemingly unusual behaviour. Divers were debriefed after each dive and the number of fish in the shoal was estimated by concensus.

In 1987, shoals of C. puniceus in spawning condition were observed by three pairs of divers on reefs at Leven point, the Redsands area of Leadsman shoal and Seven-mile-reef, Sodwana Bay. Duration of combined dives was 3 hours and 13 minutes and they were conducted between 14h55-19h05. Sunset was at 17h26.

In 1988, C. puniceus were observed in the wild and in captivity. In the wild, efforts were concentrated on the shoal at Redsands (Leadsman shoal) as the presence of ragged tooth sharks, Carcharhinus taurus, on the shallow reef at Leven Point during the peak spawning

season of the previous year had been un-nerving for the divers, especially at sunset. At Redsands dives were undertaken in the mornings from 05h44 to 10h48 (sunrise at 06h15) and again in the afternoons/evenings from 14h10 to 20h40 (sunset at 17h33). Thirty one dives, amounting to 22 hours 14 minutes, were made.

In 1988 a shoal of 92 fish which had been introduced into the main display tank of Sea World ($800m^3$) were also observed. These fish had been sexed by biopsy on capture. All were mature and sizes ranged from early maturity (+/- 280mm) through to maximum size (460-480mm). Part of the lower lobe of the caudal fin of all males was removed for identification purposes. The fins had grown out within 6-8 months, but the lobes remained thinner and more transparent for up to 18 months, allowing identification during this time. There were twelve males in the shoal at the outset. The study was conducted for a period of four months over the peak spawning season (July-October). Observations were made during early mornings and evenings for periods ranging from 1-3 hours, alternating (on a daily basis) with observations made on C. nufar. Plankton nets were deployed at other times during the day and night to ensure that spawning was not taking place at any other time.

In 1989 C. puniceus were again observed in the wild and in captivity. In the wild, diving was undertaken on the shoal at Redsands to establish if the pattern observed during the previous year continued on this reef and to see if males had taken up positions on the same part of the reef. Four dives, totalling 1 hour 37 minutes were made between 06h37 and 13h55. Sunrise was at 06h12.

The number of captive animals surviving from the previous year had been reduced to approximately twenty. A limited number of observations was made on these fish (1-2 mornings every second week for two months during the peak spawning period). A screen was placed in the overflow from the tank to monitor egg production during the day and night on these days.

By 1990 the number of C. puniceus at Sea World had dwindled to four fish and no attempts were made to observe spawning behaviour in captivity or in the wild. Efforts were, however, made to deploy remote video cameras on the shoal at Redsands in 1991. A research vessel was anchored over the shoal using three anchors and cameras were positioned directly below, on the reef, during the late afternoon so that behaviour of the fish in the shoal could be monitored throughout the night.

3.3.2 Results

Spawning sites

Shoals of C. puniceus were located in relatively shallow water at Leven Point (10-14m), Leadsman Shoal - Gipsy Hill (25-28m), Leadsman Shoal - Redsands (24-30m), Four-mile reef (26-30m), Seven-mile reef (14-20m) and Nine-mile reef (12-16m) at Sodwana Bay. The integrity and resident nature of these shoals was apparent from the proportion of tagged fish and the ease with which they were located on subsequent cruises. Tag shedding rates were high, most fish having shed their tags in eight months (Garratt, unpublished data). Scars from tagging were, however, evident on these fishes for up to one year. During successive spawning seasons a proportion of ripe and ripe-running fish removed from each of these shoals, indicated that spawning occurred on, or near, these reefs.

A feature common to all of these shoals was their location on the northern outer edge of each reef (Fig. 119). Current recordings made by divers indicated that the current alternated on an irregular basis from north to south on these shallow reefs, but that it was predominently north flowing at Leadsman/Redsands, Four-mile and Seven-mile reefs at Sodwana Bay during the spawning season. There are no data for Nine-mile-reef.

Spawning was not detected during any observations, but divers did note several changes in the behaviour, social structure and colour of the fish at Redsands during the spawning season of 1988. A group of approximately 30 large fish (ca. 350-420mm FL) had assumed positions on the northern outer edge of the reef and remained relatively close to the reef. Samples removed by spear identified these fish as males in spawning condition. These fish were aggressive towards one another and appeared to maintain positions on small contiguous areas of the reef. The remainder of the shoal, consisting almost entirely of smaller individuals and numbering about 200 fish, stayed in close proximity to this group but maintained a position higher in the water column throughout much of the day.

The establishment of positions by large males on a particular part of the reef, and changes in the colouration of these large fish, similar to those noted in the previous year in territorial male C. nufar (see below), suggested that this area of the Leadsman Shoal was a spawning site of C. puniceus. This group of male C. puniceus was observed again in 1989 and 1991.

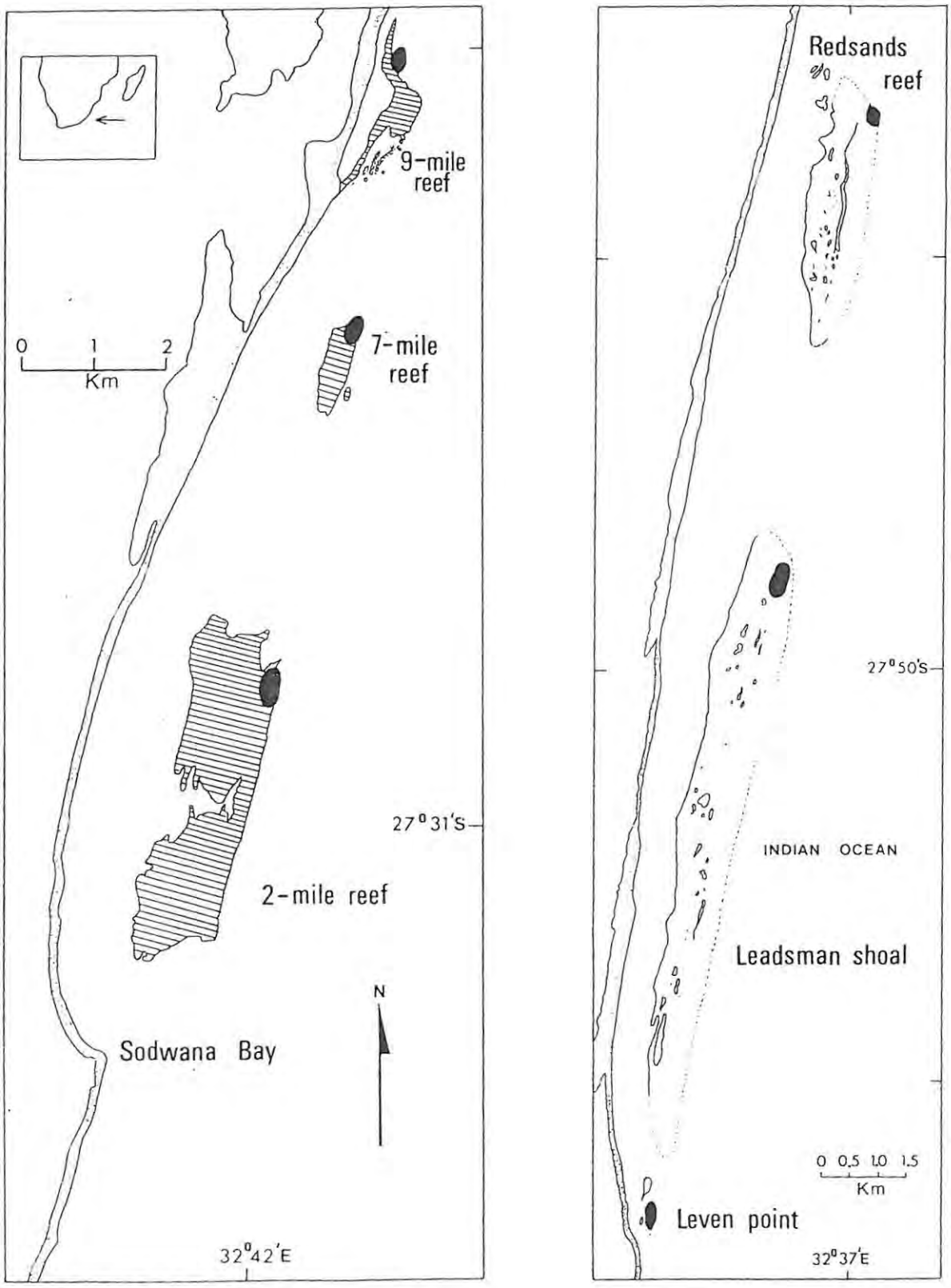

Figure 119. Inshore reefs in the St Lucia marine reserve with resident shoals of Chrysoblephus puniceus. Preferred sites of the shoals are on the northern ends of each reef (black ovals) (charts re-drawn from Ramsey & Mason 1990).

Similar colour changes and segregation of size classes were noted at seven-mile-reef in 1988, but diving effort between 1988-1991 was concentrated on the shoal at Redsands in an effort to describe the spawning behaviour of this species.

Time of spawning

Spawning was not observed in C. puniceus, but changes in the brightness of male colouration during the day, similar to that recorded in C. nufar, suggested that spawning was most likely to occur during the early morning (see below).

Colour changes

During the spawning season, each member of the group of males at Redsands had a prominent broad white vertical bar on their flanks which extended from the dorsal fin to the belly (Fig. 120a). During aggressive interactions between these males this colour pattern was reversed, the white band changing to a dull red and the rest of the body becoming lighter. This colouration had not been recorded previously in this species.

Observations made in 1988, 1989 and 1991 on the shoal at Redsands showed that the white markings of territorial males were most pronounced in the early mornings and faded during the day. The same pattern was evident in captive fish. In captivity and in the wild, females remained a uniform silvery-pink throughout all seasons (Fig. 120b).

Behavioural patterns

A considerable amount of effort was directed towards describing spawning behaviour in C. puniceus in the wild, and in captivity. Spawning was not observed in either environment, but changes in behaviour and social organization within shoals during the spawning season indicated the type of spawning strategy which this species may have. The observations made during the period 1988-1991 are reported below.

Most of the cruises aimed specifically at tagging C. puniceus in the reserve, between 1983-86, were scheduled between November-June, months in which weather conditions are fairly stable. These cruises did not coincide with the spawning season of C. puniceus and allowed divers to compare behaviour and social structure within the shoals during this inactive period to that during the spawning season. During dives undertaken on these cruises, no outwardly visible social structure was observed within any shoals of C. puniceus. No aggressive behaviour was noted nor were there any signs of territoriality. All fish were a uniform silvery pink colour and males could not be distinguished from females, other than by size.

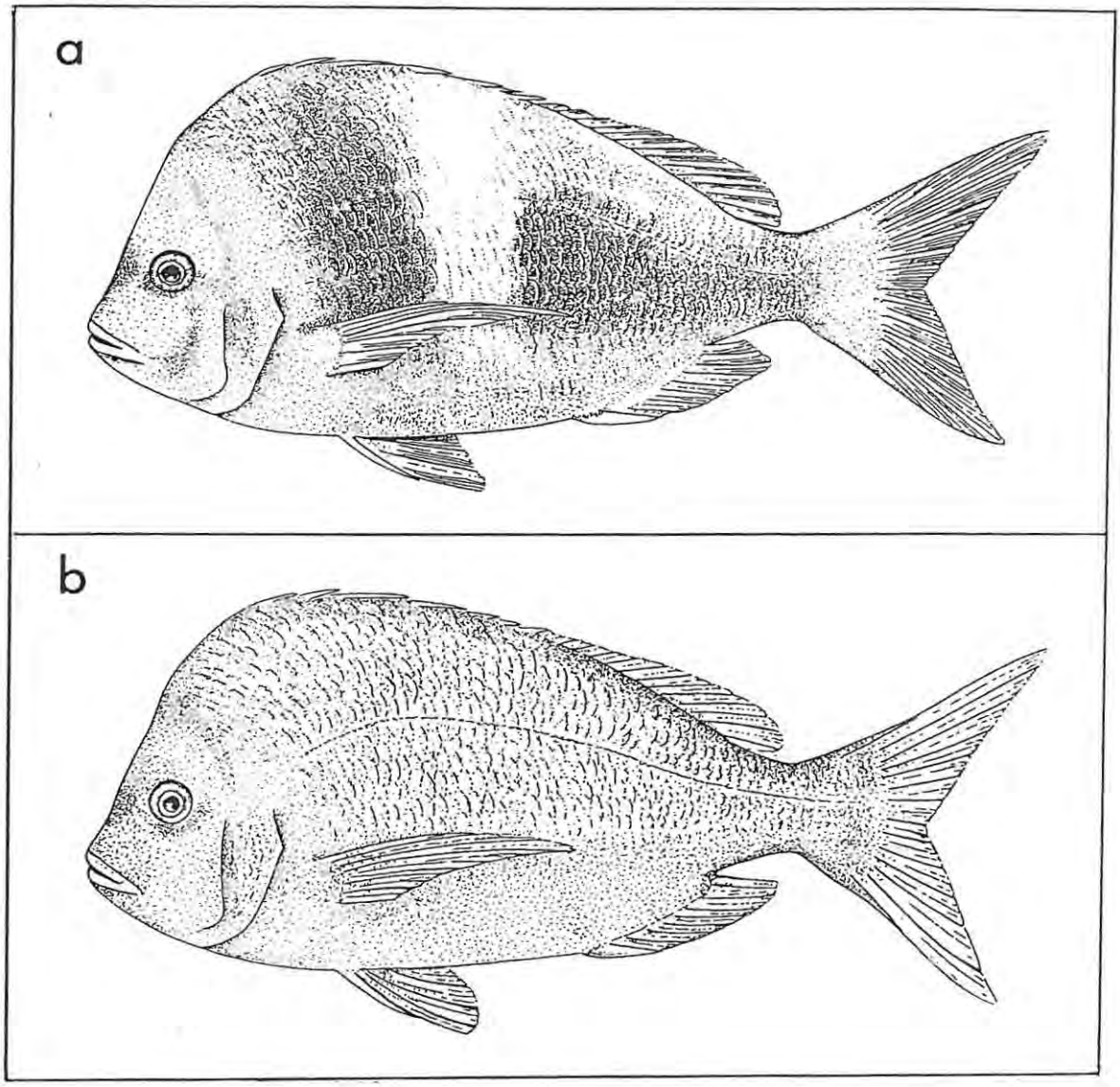

Figure 120. Male (a) and female (b) Chrysoblephus puniceus during the spawning season. Females remain silvery-pink. Males develop a prominent vertical white bar on their sides which extends from the dorsal fin to the belly.

Several changes occurred in the social structure and behaviour of the shoal at Redsands during the spawning season. The group of approximately 30 large males maintained their position on the northern outer edge of the reef. They remained relatively close to the reef and were aggresssive towards one another, but not to the extent of leaving their positions. Each position appeared to be no larger than $16m^2$. A behaviour which was common to all these males was a vertical movement up and down in the water column within their limited areas (Fig. 121a). This behaviour appeared to be random within the group, but it was carried out by all males when the remainder of the shoal periodically moved into the area directly above them, *en masse*.

The remainder of the shoal, consisting almost entirely of smaller individuals and numbering about 200 fishes, stayed in close proximity to the group of large fish, but generally higher in the water column (Fig. 121a). During the early morning, before and after sunrise, these fishes sometimes mixed with the group of large fish (Fig. 121b). It was difficult to determine if any spawning took place during encounters at this time, due to insufficient light. Careful observation after sunrise did not reveal any signs of spawning. Males would move up and down through the females, displaying, but there were no spawning rushes.

Males in captivity displayed the same behaviour patterns and colouration during the spawning season as those observed at Redsands. They developed a pronounced white bar on their flanks which was brightest during the early morning and faded during the day. They were very aggressive, maintained positions in the tank throughout the spawning season and periodically swam vertically up and down in the water column.

Males appeared to be far more aggressive in captivity than in the wild. Confined to the main display tank at Sea World, less dominant males could not move away from dominant males at will, but could only move from one area into another. As a result, they were continually chased and became severly stressed. Aggressive behaviour also was not limited to displays and brief chases, but often involved physical contact. This eventually resulted in severe lacerations to those individuals being chased and eventual death. Before the end of the spawning season, several males had been killed (n=7). There were five males remaining.

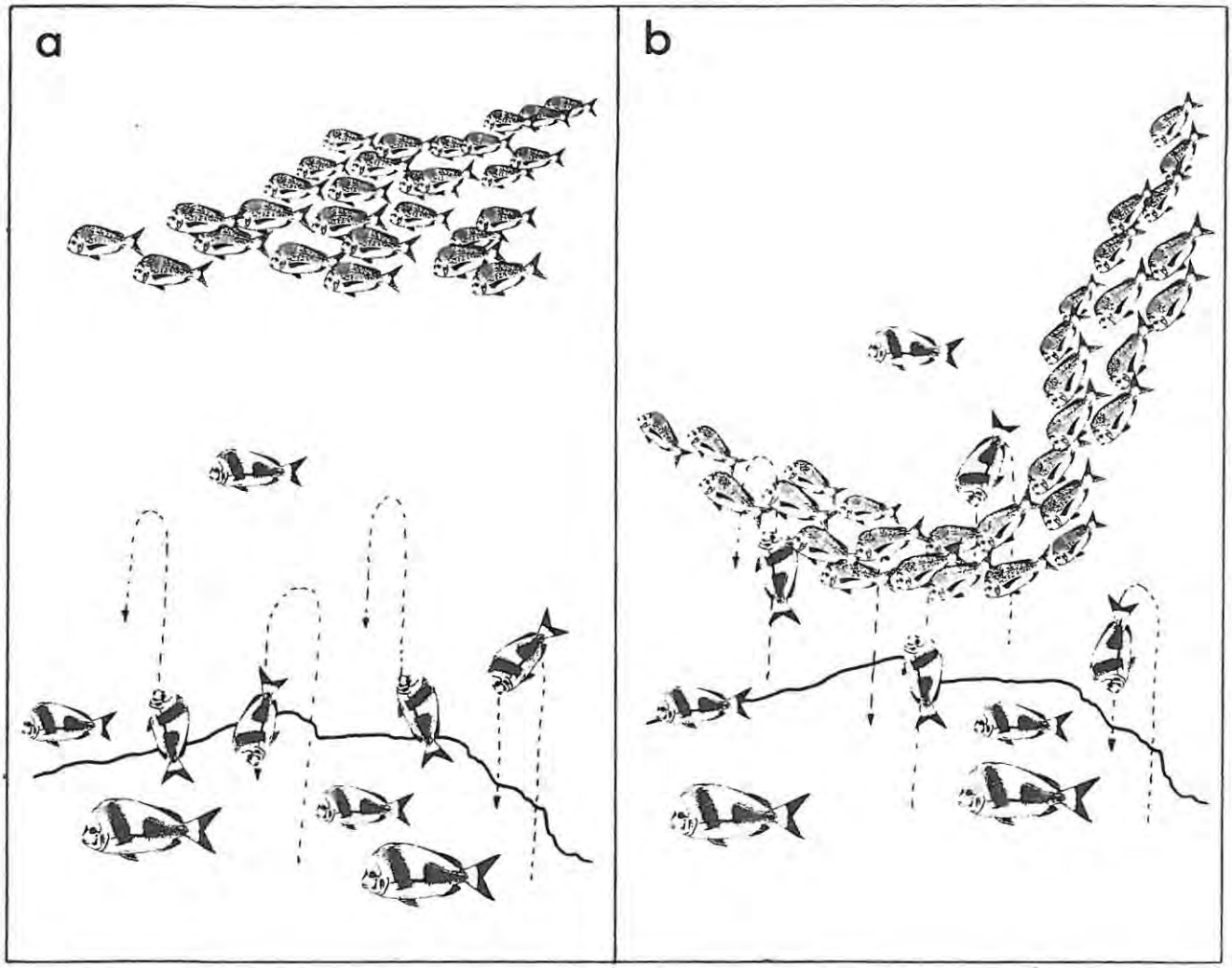

Figure 121. Male Chrysoblephus puniceus (vertical white bars) establish contiguous positions on the outside northern edge of Redsands reef. They periodically swim vertically upwards and downwards within these areas (a). During the day, females generally remain separated from the males. Near sunrise, females sometimes mixed with the males (b).

This intense aggression was then directed towards large females, and several more fish were killed before the end of the spawning season. One of these fishes was removed for histological investigation of its gonads before it was killed by the territorial males. It was a female measuring 310mm FL. The gonad contained a high percentage of yolked oocytes. Most of these oocytes were, however, in various stages of atresia. This may have been a direct result of stress from continual harassment but, as these fish did not spawn in captivity, it may also have been a result of the captive environment.

In November all aggressive behaviour had ceased, but some of the large fish retained faint bands on their bodies through to December. At this time it was noticed that one of these fish did not appear to have its caudal fin clipped, indicating that it was a female. This fish was sacrificed to investigate the state of its gonad. The specimen measured 372mm fork length. Histological investigation revealed a hermaphroditic gonad with male and female elements of similar size. The female element contained only pre-vitellogenic oocytes and numerous brown bodies, indicating degeneration of this element. Active spermatogenesis was taking place in the male element and the evidence suggested that this fish was in the process of changing sex.

The final effort in 1991 to observe spawning behaviour using remote controlled video cameras failed due to equipment failure, followed by severe storms at sea which made further work impossible. The cameras were successfully placed on the patch of reef normally occupied by males at Redsands by 16h50 on 22 September and the fish were observed on the monitor for about 50 minutes before water penetrated the housing. During this time a large number of fish within the shoal carried out the behaviour, described below, in which individuals followed each other in quick succession into a cave or under an overhang, but an opportunity to observe this shoal throughout the night and the following morning was lost.

Spawning behaviour

Nil

Social interactions other than spawning behaviour

A behaviour which was common to all the shoals during afternoons and evenings, and which was initially thought to be related to spawning, was the movement of a large number of fish following each other down to the reef and under an overhang or cave. This behaviour was witnessed on eleven dives and occurred up to six times during a dive. The fish followed either one after the other in quick succession or in pairs. Divers observed this

behaviour at several locations and captured it on video. The behaviour was related to a particular overhang or cave on the reef and divers could position themselves close to the feature without disturbing the fish or preventing them from continuing the behaviour. At this time of day, the white band on the sides of males was dull and there was no apparent pairing of these large males with smaller females during the behaviour pattern. Divers investigated the possibility that these fish were spawning in this manner by sweeping the area with fine-mesh hand nets to determine if eggs were released by these fish. No eggs were sampled and the significance of this behaviour remains unknown.

3.3.3 Discussion

The presence of shoals of C. puniceus in spawning condition on each of the reefs investigated in the St Lucia reserve during this study supports earlier data (Garratt 1985b) which indicated that spawning occurs wherever there are resident shoals of this species, with the exception of the southern region of distribution where no spawning takes place. Spawning also appears to occur at all depths. The tagging exercise indicated that shoals were fairly resident on reefs and the consistant size and location of these shoals, during and between spawning seasons, indicated that these fish do not migrate to specific spawning grounds, or reefs, to spawn.

The results of this study have shown that there are preferential residency sites on the northern ends of individual reefs and that these sites are occupied throughout the year. There may also be preferred spawning sites within the home ranges of these fish, similar to those described in many inshore reef fishes (e.g. Robertson & Hoffman 1977, Robertson 1981,1983, Thresher 1984, Choat & Robertson 1975, Warner et al. 1975, Moyer & Yogo 1982, Clavijo 1983, Ross 1986, Warner 1990). Only two sites were identified, one on the northern outer end of Seven-mile reef and another on the northern outer edge of the Redsands reef. Diving effort was concentrated on the Redsands reef and territorial males were observed on the same part of the reef during three spawning seasons (observations were not made in the other years), suggesting that it was a preferred spawning site.

Prevailing currents are north flowing over the Leadsman shoal during the spawning season and the location of the males on the Redsands reef indicated that spawning takes place on the down-current outer edge of this reef. This is a well documented strategy among reef fishes (Warner 1990) and one which has been assumed, in the past, to have evolved in response to high predation levels of eggs by planktonic feeders associated with reefs (Johannes 1978, Ross 1986, Foster 1987), or to maximize dispersal (Barlow 1981), or to

provide the best opportunity for pelagic larvae to survive in waters with patchy and irregualr distribution of food (Doherty et al. 1985). Shapiro et al. (1988) have recently questioned the validity of these hypotheses, however, on the grounds of insufficient evidence. No studies have evaluated spawning and non-spawning times and sites with respect to current regimes and these authors suggest that there is a critical need for studies to determine whether spawning at particular times and locations offers advantages over spawning at other times and locations, in terms of egg predation or movement off the reef. Without such studies, present theories remain purely speculative.

Changes in male colouration during the day, similar to those observed in C. nufar, both in captivity and in the wild, and the fact that no spawning was observed during daylight hours, strongly suggest that spawning in C. puniceus occurs during the early morning, most probably before sunrise.

Spawning was not observed in C. puniceus and, while changes in behaviour, social structure and colour of males during the spawning season may suggest a polygynous mating pattern similar to that reported in several protogynous hermaphrodites, there are insufficient data to support this hypothesis. It is possible that C. puniceus has a lek-like mating system similar to that of the parrotfish Scarus vetula (Clavijo 1983) and the wrasses Thalassoma bifasciatum (Warner et al. 1975, Warner & Robertson 1978), Halichoeres melanochir (Moyer & Yogo 1982) and Thalassoma duperrey (Ross 1986), in which males tend to concentrate at traditional sites, each defending a small area against the intrusions of other males (Warner & Robertson 1978), but further detailed observations are needed to clarify this aspect of reproduction in C. puniceus.

Without evidence of spawning behaviour, it is difficult to postulate the mechanisms whereby sex change is regulated within the population. In captivity, a female assumed male spawning colouration immediately after the spawning season and histological investigation of its gonads indicated that it was in the process of changing sex. This suggests that social structure during spawning may regulate sex change in this species. However, in wild populations, sex change appeared to take place much later, midway between spawning seasons, suggesting that social structure during the remainder of the year may be an important cue for sex change (D.Y. Shapiro, University of Puerto Rico, pers. comm. 1988).

Social control of sex change in C. puniceus was questioned by Garratt (1985a) on the grounds that fishing had significantly altered the population size structure along the Natal coast and this had resulted in a skewed sex ratio of 1:18.8 males to females. Unlike the exploited congeneric, C. cristiceps, in which size at sex change appeared to be related to sex

ratio (exploited stocks had a significantly lower mean size at sex change compared to protected stocks) (Buxton 1993), there did not appear to be a compensatory shift (Smith 1982) in the size at sex change in C. puniceus along the Natal coast. Garratt (1985a) concluded that either the population had not yet reached a 'critical' level after which males would be produced at an earlier age, or that C. puniceus is incapable of adjusting its sex ratio and that sex change is not controlled socially but, possibly, by endogenous factors. As sex change in C. puniceus occurs over a wide range of ages (sizes) (Garratt et. al. 1993), it is unlikely that the latter hypothesis is valid. If sex change is regulated socially, however, the mechanisms involved remain unclear.

Buxton (1993) noted a shift in the size (age) at sex change in C. cristiceps but not in another conspecific, C. laticeps. He attributed this to the fact that more reproductively active C. cristiceps females were vulnerable to fishing (some mature age classes of C. laticeps were not recruited to the fishery). Based on the 'sex ratio threshold' theory of Shapiro (1981), he suggested a similar scenario to that of C. puniceus, stating that: "If the ratio of males to females had not surpassed this threshold, then the size at sex change would remain unaltered" (Buxton 1993, p. 60). The data presented in this study suggest that the 'sex ratio threshold' hypothesis of Shapiro (1981) may also apply to C. puniceus.

To summarise, the evidence presented in this study shows that there are resident shoals of C. puniceus on reefs in the St Lucia marine reserve and that these shoals prefer the northern ends of these reefs. Temporary areas are occupied by large males during the spawning season, suggesting that there may be preferred spawning sites within the home ranges of these fish. Spawning was not observed during the study, but indirect evidence suggests that spawning takes place in the early morning, most probably before sunrise. The mechanisms regulating sex change, in this species, remain unclear.

3.4 SPAWNING IN C. NUFAR

Cheimerius nufar has a wider distribution than C. puniceus, occurring in tropical and subtropical waters of the western Indian Ocean (Smith & Heemstra 1986), including the entire east coast of Africa, Madagascar and Mauritius (Fischer & Bianchi 1984). Work conducted on reproductive seasonality in the eastern Cape (Coetzee 1983), Natal (Garratt 1985b) and the Gulf of Aden region (Druzhinin 1975) suggests that this species may spawn throughout its distribution.

Surveys of commercial catches in Natal have indicated that C. nufar spawns along the length of the coast and that most fish in spawning condition are found at depths greater than 50m (Garratt 1985b). Nowhere along the Natal coast were shoals of mature C. nufar seen in shallow water. Dives to 50m conducted during this study showed that members of this species generally occurred in small groups and were most often associated with reef margins. Attempts to locate shoals of spawning C. nufar in the wild were unsuccessful and the study of spawning in this species was limited to captive fish.

3.4.1 Methods

During the spawning season of 1988, a group of seven C. nufar, three males and four females, were observed in the circular 800 m^3 main display tank at Sea World Durban over a period of four months. These fish were all mature and ranged in length between 350-450 mm FL. The tank had a diameter of 13.8 meters and an approximate depth of 5.5 meters. It was exposed to natural light, but artificial light was used from sunset to 21h00, after which the facility was closed to the public. Water was pumped to the tank directly from the surf through well-points at a rate of one cubic meter per minute. The temperature in the tank remained within one degree celcius of surf temperatures throughout the year.

During this spawning season (1988), spawning was observed each day for the duration of the spawning sequences during the first month (July). Observations began 30 minutes before sunrise and the total number of spawnings made by males and females was recorded. Most spawning occurred within the first hour and it was assumed, for the purpose of determining frequency of spawning, that spawning had ceased when there had been no activity for 20 minutes.

Having established a pattern of spawning activity, observations were made on alternate days during August 1988 and thereafter on every third day. Sequences were photographed

and filmed on video. During all observations all fish were observed (group sampling) and spawnings were considered successful if mating continued through to the final rush in which eggs and sperm were released.

Observations to determine the time and duration of spawning were conducted on 16 days during the spawning season. These observations extended to 2h before and after spawning. Spawning activity recorded in two 24h observations to determine periodicity of spawning was used to supplement these data.

The number of sexually mature fish observed in the main display tank at Sea World during 1988 was low and, while they offered an ideal opportunity to record spawning behaviour within a small group, there was the possibility that spawning in larger shoals may be quite different. In order to investigate this possibility, a further 33 sexually mature fish were introduced into the main display tank during 1989 and 1990 and spawning behaviour was again observed in 1992.

In 1992 observations were conducted on five consecutive days every second week for two months (Aug-Sept.). The fish were observed for one hour each day beginning approximately 15 minutes before sunrise. Similar recordings to those of 1988 were made, but particular attention was given to the territories set up in the tank, the interactions of territorial males, the behaviour of females within these territories and the behaviour of subordinate 'streaker' males and inactive fishes.

3.4.2 Results

Time of spawning

In May 1988, one of the group of seven fish in the main display tank became darker and began tight circling movements throughout the day in the vicinity of a rock outcrop. This fish was observed closely during the following weeks and, on 24 June 1988, spawning of *C. nufar* in captivity was witnessed for the first time. Spawning continued within the group for a period of four months.

Spawning began within half an hour before sunrise throughout the spawning season (Fig. 122). Males became active each day as soon as there was sufficient light for them to recognize females and, simultaneously, their white markings became pronounced. Time of first spawning varied between 27 minutes before to two minutes after sunrise and continued for up to 105 minutes. Average duration of the spawning period was 60 minutes.

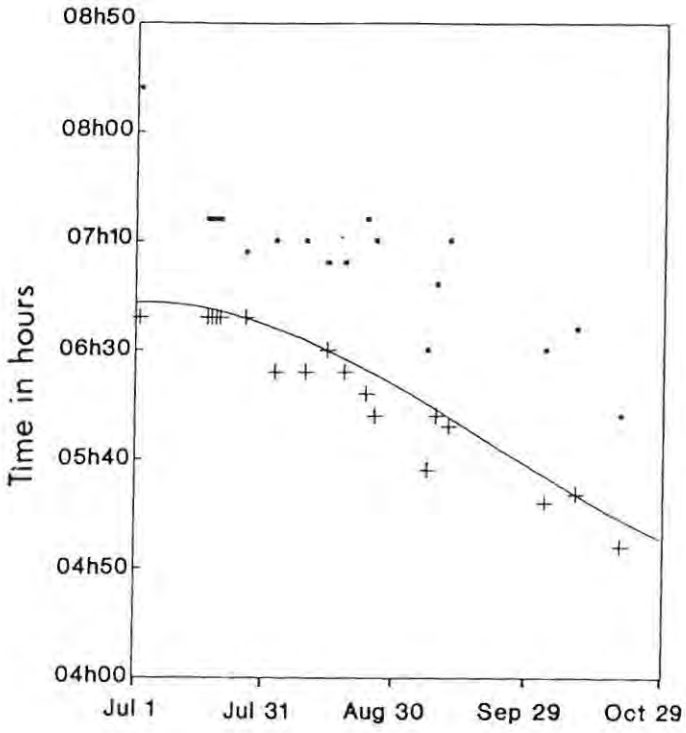

Figure 122. Time and duration of spawning sequences in Cheimerius nufar related to sunrise over a period of four months: + = commencement of spawning: ■ = termination of spawning. Solid line = sunrise.

Twenty-four-hour observations revealed that spawning was confined to this limited period at sunrise. During the remainder of the day territorial males maintained their positions and would occasionally inspect a female, but there was no response from any females. Male colouration also faded after the spawning period, to the extent that it was sometimes difficult to distinguish males from females during the remainder of the day. As intensity of male colouration was related to intensity of spawning behaviour (see below), this was considered further evidence that spawning was limited to a short period each morning.

Colour changes

Adult C. nufar are normally silvery-pink while juveniles are characterised by a series of prominent vertical red bands on the body (Fig. 123a) which fade with age and are only occasionally seen on adults.

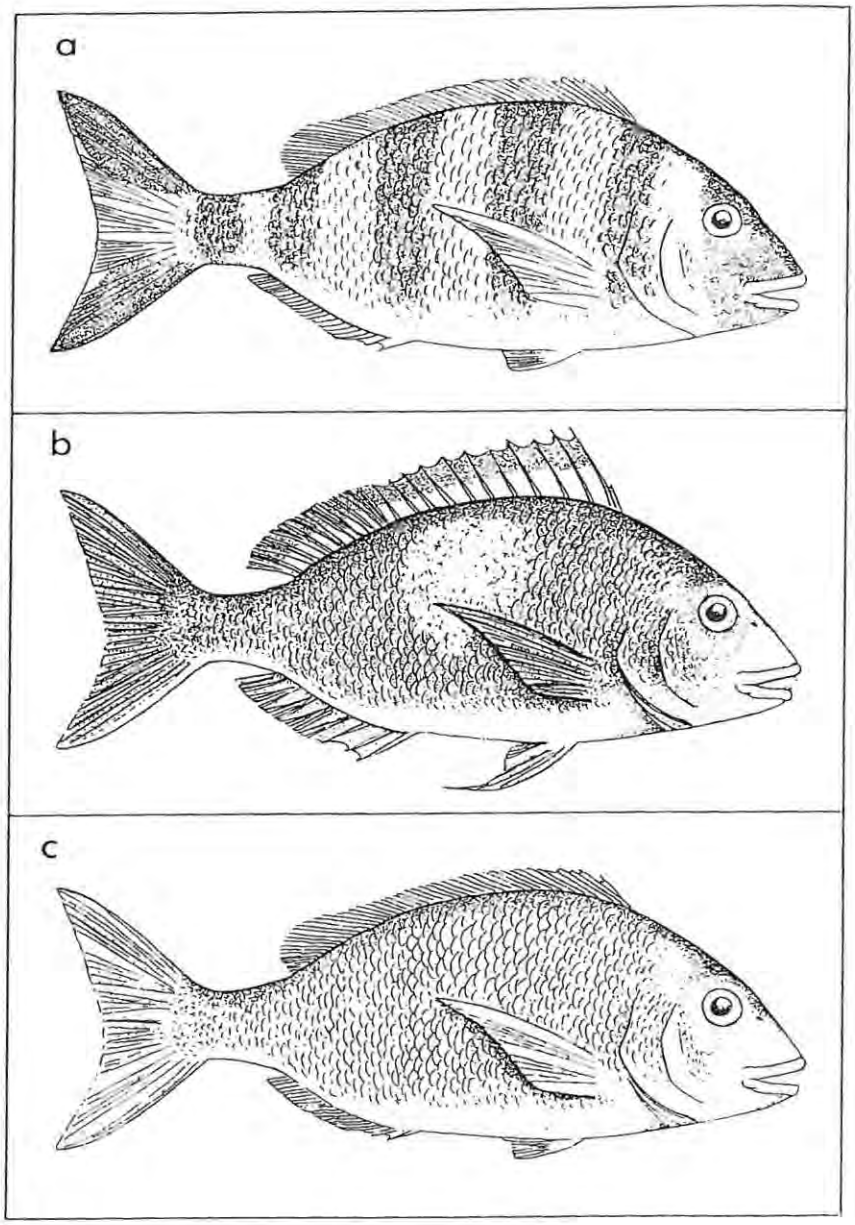

Figure 123. Juvenile (a), male (b) and female (c) Cheimerius nufar during the spawning season. Juveniles (and some inactive females and streakers) have several broad vertical red bands on their bodies. Males develop a large white patch on their flanks and the areas around the mouth, in front of the eyes, the base of the dorsal fin and the lower lobe of the caudal fin whitens. Spawning females remain silvery-pink.

At the beginning of the 1988 spawning season, male C. nufar darkened to a dull grey. Soon after this colour change, a large white patch developed on each flank and the area above the eyes, around the mouth, the base of the dorsal fin, and the lower caudal fin whitened (Fig. 123b). These white markings varied in intensity, becoming very pronounced at the height of the spawning activity and fading during the remainder of the day. Females remained a uniform silvery-pink throughout the spawning season (Fig. 123c).

In the 1992 spawning season, males were classified either as territorial males (T-males) or 'streakers' (S-males). The colours of territorial males (T-males) and active females remained the same as in previous years. The most successful S-male (see below) developed the same white markings as T-males during the spawning season but, unlike T-males, it's overall colour remained pink, similar to that of females. The white markings became prominent only for a short period each morning during peak spawning activity. A second S-male also developed white markings during spawning encounters, but these were very faint and faded quickly after a spawning rush in which it streaked.

Several fish developed prominent red bands on their bodies, similar to those described earlier for juveniles (Fig. 123c). Two S-males displayed these markings permanently while other fish, presumably mature but inactive females, displayed them briefly when they were approached by territorial males.

Spawning behaviour

Spawning behaviour of captive fish was observed during the spawning seasons of 1988 and 1992. During 1988 the following observations were made on the group of seven fish:

Territorial and courtship behaviour

The behaviour of males remained unchanged throughout most of the spawning season. Each male set up a territory within which it would swim in circles until either a female or a male approached (Fig. 124a). Males were very aggressive towards one another and an encroaching male would be chased out of the area immediately. Males continued to encroach on each other's territories throughout the season, however, and on many occasions they were able to join the mating sequence and successfully release their sperm when the female released her eggs. In this study these males are called 'streakers', as they represent an alternative mating strategy similar to that described by Warner et al. (1975).

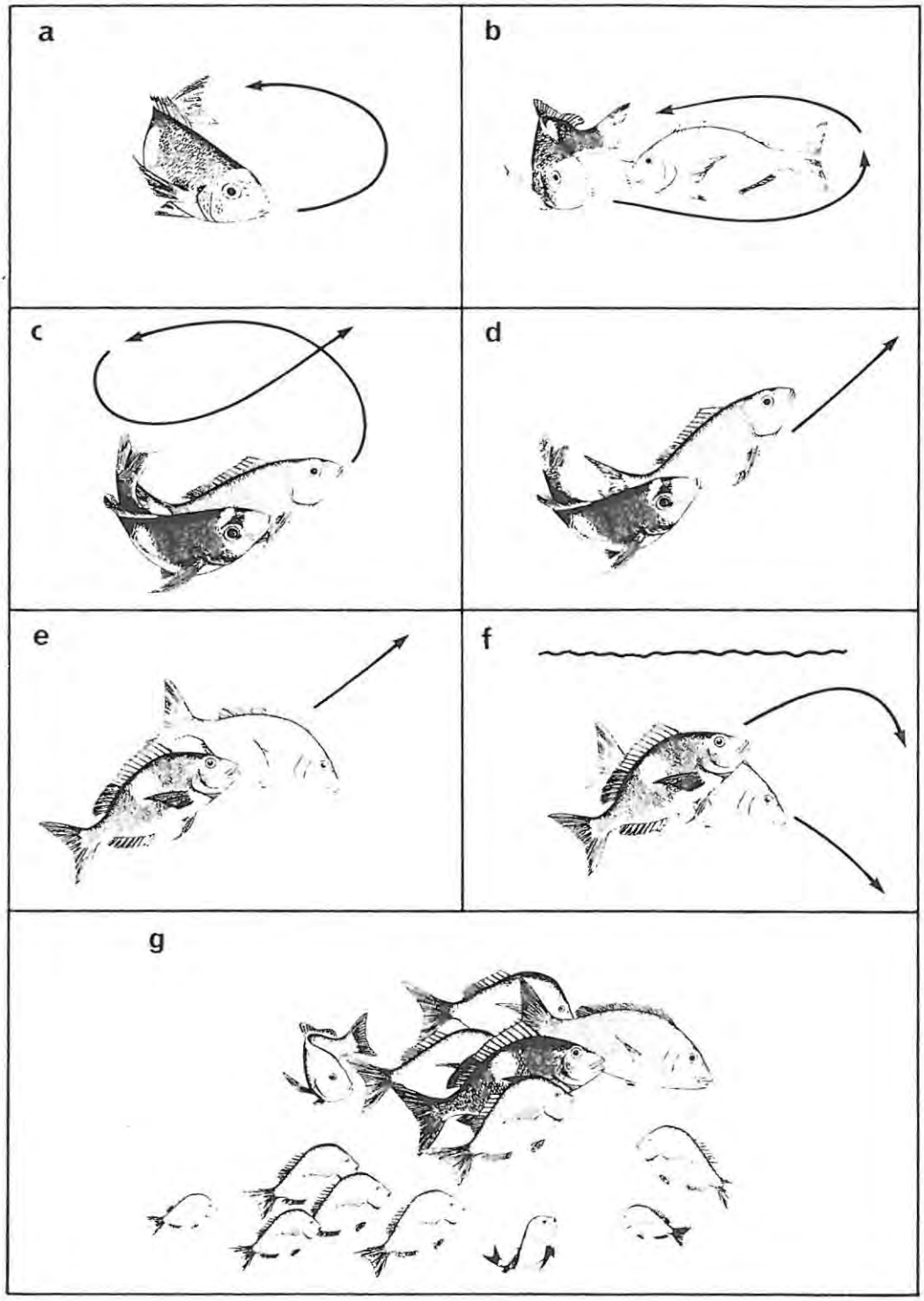

Figure 124. Reproductive behaviour in Cheimerius nufar based on visual observations and filmed sequences: a - male in tight circling movement; b - male rushes in front of female and circles; c - receptive female stays within the circle made by the male and both begin ascent; d - male drops back and begins nuzzling female; e - female becomes rigid and allows male to push her up towards the surface; f - female quivers and releases eggs and male swims past her releasing sperm (the pair separates at this stage); g - interference in the spawning sequence by several Chrysoblephus puniceus.

All females entering a territory were rapidly approached by the male with fins erect and mouth slightly open. The male would rush across the path of the female and attempt to keep her within a tight circle (Fig. 124b) and, depending on the females' receptiveness, a spawning sequence would begin. Spawning generally occurred between males and females of similar sizes. A receptive female would turn within the circle made by the male (Fig. 124c) and both would immediately begin ascending at a moderate pace towards the surface. Initially the male would swim alongside the female on the outside of the circle but, as they rose in the water column, he would fall back slightly and begin nuzzling her abdomen close to the vent (Fig. 124d). When the female was ready to release her eggs, she stiffened her body in a head-down position (Fig. 124e) and allowed herself to be pushed upwards by the male. The female's body would quiver on releasing the eggs and the male would swim rapidly past her, releasing sperm (Fig. 124f). 'Streakers' would sometimes join the sequence midway in the water column when the male was nudging the female and carry out the same behaviour on the opposite side of the female.

Non-receptive females ignored the approaches made by males and swam out of the territory without altering course. Males would remain in their territories throughout each day and would continue to be aggressive towards other males. However, to the passing disinterested females they would only make an occasional and less vigorous approach. Throughout the spawning season all other species in the tank were ignored.

Towards the end of the spawning season males would sometimes abort a mating sequence after the initial approach had been made and both fish were swimming towards the surface. The male would move away after briefly nuzzling the female on her abdomen and the sequence was terminated. On two such occasions, the female still elected to release her eggs. On other occasions there was interspecific interference at this time (see below).

Frequency of mating and the number of fish spawning

The frequency of mating and the number of fish spawning each day varied throughout the spawning season of 1988, with the total (all fish) daily number of successful matings ranging from 0-17. On many days some of the spawning activity occurred too rapidly and/or too far from the observer to identify which fish were spawning on each occasion. On these days recordings were limited to the number of males and females which were active (Table 6), and the total number of spawnings. There were days, however, on which it was possible to record the number of spawnings made by each fish. These ranged between 1-14 (Table 7). Mean spawning rates of males and females in 1988 were 3.04 (SD = 4.33) and 1.84 (SD = 3.31) per hour respectively. There was a significant difference (ANOVA, $p = < 0.05$, F ratio = 3.90) in spawning success rates between males M1 and M2, with male M2 obtaining

more spawns per hour of observations (Table 8). Because male M3 was killed shortly after the study began, the variance about the mean was much higher and confidence intervals (CI's) overlapped with those of M1 and M2.

Table 6. The number of male and female Cheimerius nufar spawning each week during the 1988 spawning season.

Month	Week	No in tank	Number of fish spawning		
			male	female	total
Jul	1	7	2	3	5
	2	7	2	3	5
	3	7	2	2	4
	4	7	3	2	5
Aug	1	7	2	2	4
	2	7*	2	2	4
	3	6	2	3	5
	4	6	1	1	2
Sep	1	6	1	3	4
	2	6	1	1	2
	3	6	no data		–
	4	6	0	0	0
Oct	1	6	1	2	3
	2	6	1	1	2
	3	6	1	1	2
	4	6	no data		–
Nov	1	6	1	2	3
	2	6	no data		–
	3	6	0	0	0
	4	6	0	0	0

* = largest of three males killed by predator.

Interspecific behaviour and aborted sequences

There are over 60 species of fishes in the main display tank at Sea World. Many of these are offshore reef species, yet the slinger, C. puniceus, was the only one which showed any interest in the spawning activities of C. nufar. From the very first day, slinger moved in rapidly on pairs of spawning fishes, jostling for positions close to the female (Fig. 124g). A large percentage of the eggs were seen to be consumed by these fishes, yet they were totally ignored by both male and female C. nufar throughout the season, even when it became difficult for the spawning fishes to continue their ascent. A shoal of Sarpa salpa (an inshore sparid) could be seen feeding on released eggs in the water column, but at no time did they interfere or venture near the spawning fish.

Table 7. The number of successful spawnings made by each fish on the days on which these data could be recorded during the 1988 spawning season (symbols indicate pairings).

	Males			Females				
Size	Small (350 mm →		Large 450 mm)	Small (350 mm →				Large 450 mm)
Date	M1	M2	M3	F1	F2	F3	F4	Total
3 July	?	?	0	11	0	0	0	11
17 July	0	0	2★	0	0	0	2★	2
18 July	0	0	3★	0	0	0	3★	3
19 July	2▼	0	0	0	2▼	0	0	2
20 July	2▼	0	0	0	2▼	0	0	2
21 July	0	0	1★	0	0	0	1★	1
22 July	0	0	2★	0	0	0	2★	2
27 July	1▼	5♦	0	0	6▼♦	0	0	6
15 Aug	2▼	10♦	+	1♦	9▼♦	0	2▼♦	12
17 Aug	2▼	12♦	–	3♦	9▼♦	0	2♦	14
19 Aug	6▼	11♦	–	4▼♦	13▼♦	0	0	17
22 Aug	4▼	0	–	0	4▼	0	0	4
24 Aug	2▼	0	–	0	2▼	0	0	2
26 Aug	8▼	0	–	0	8▼	0	0	8
5 Sept	0	4♦	–	0	0	0	4♦	4
7 Sept	0	14♦	–	4♦	9♦	0	1♦	14
9 Sept	3▼	0	–	3▼	0	0	0	3
12 Sept	4▼	0	–	4▼	0	0	0	4
4 Oct	0	9♦	–	0	0	3♦	6♦	9
11 Oct	0	4♦	–	0	0	4♦	0	4
14 Oct	0	5♦	–	0	0	5♦	0	5
17 Oct	0	7♦	–	0	0	7♦	0	7
21 Oct	0	6♦	–	0	0	6♦	0	6
2 Nov	0	3♦	–	2♦	0	0	1♦	3
Total	36	90	8	32	64	25	24	145
Mean (per spawning day)	3.27	7.50	2.0	4.00	6.40	5.00	2.40	6.04
S.D.	2.10	3.61	0.82	3.02	3.81	1.58	1.58	4.53

+ Fish killed by predator.

Table 8. Means and 95% confidence intervals of the spawning rates of territorial male C. nufar (M1-M3) in 1988

MALE	COUNT	MEAN	95% TUKEY HSD INTERVALS FOR MEAN	
M1	24	1.82	0.38	3.251
M2	24	4.81	3.38	6.24
M3	8	1.41	-1.07	3.89
Total	56	3.04	2.10	3.98

Observations conducted on captive fish in 1992 were of equal duration and all behaviour rates were recorded per hour. Similar patterns of behaviour in 1992 suggested that spawning behaviour of C. nufar reported in 1988 reflected normal behaviour in wild populations. The establishment of a larger shoal in the tank at Sea World resulted in additional information on social structure and success rates in such circumstances, but spawning behaviour remained the same with respect to colour changes in males, territorial and courtship behaviour, pair spawning and 'streaking'.

Territorial and courtship behaviour

Even though there were many more individuals in the tank during the spawning season of 1992 (approximately 30, including no less than seven males), three big males (ca 520-580mm fork length) again established territories (Fig. 125). Two of these fishes may have been those which set up territories in 1988, but the third had died during that year, indicating that the third territory in 1992 was occupied by one of the newly-introduced fish.

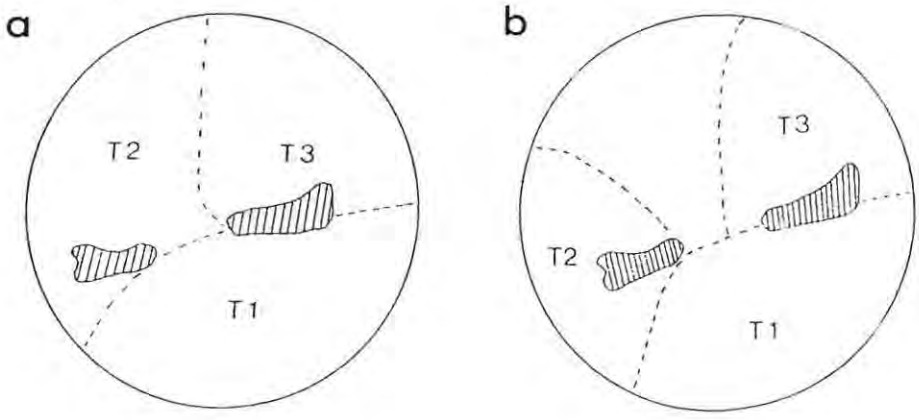

Figure 125. Territories established by male Cheimerius nufar in the main display tank at Sea World Durban, in 1988 (a) and 1992 (b). Hatched areas indicate rock formations.

Territories were similar in size to each other and remained the same throughout the two months of observations. They were, however, smaller than those maintained in 1988 because of a fourth area in which several (large and small) inactive fishes maintained position (Fig. 125b). This area separated territorial males T2 and T3, both of which made repeated forays into the area to investigate the reproductive state of its occupants. No spawnings took place with individuals in this area, each fish remaining almost motionless in the water during times of spawning and only moving to evade males T2 and T3. Male T1 never entered this area.

Territorial males circled and patrolled within their territories at an accelerated pace in the early mornings, swimming from one female to another while maintaining their territories. The majority of encounters with other T-males occurred when one male encroached on a territory, or swam close to another territory while attempting to mate with a female. Most encounters resulted in displays (Tables 9-11) in which males swam back and forth along two sides of an imaginary line with fins erect and white markings very prominent. These displays ranged in duration from a few seconds to almost one minute, after which both fish moved back into their respective territories to continue courting females. On other occasions encounters were more aggressive, with T-males chasing intruders out of their territories well into other territories before turning back to resume patrolling in their own. There were significantly more border displays among T-males than chases (t test, $p << 0.01$).

ANOVA showed that there was a significant difference in spawning success rates between T-males ($T2 > T1 > T3$, $p = < 0.01$) and there was a correlation between success rate and aggression, the most aggressive males (T2 & T1) being the most successful spawners (Table 12).

Males T2 and T3 did not allow any S-males to stay within their territories throughout the spawning season, yet all four S-males were allowed to remain in the territory of T1 from where they were able to 'streak' with all T-males. Territorial males T2 and T3 were more aggressive towards these S-males than T1 ($p = < 0.05$) and often entered the territory of T1 to chase them. Most of this aggression was directed at two fish with red bands, both of which had to be removed from the tank with severe lacerations within the study period. Both were young males with maturing gonads and one had attempted to spawn by 'streaking' on several occasions (S3, Table 13).

Table 9. The number of interactions between Cheimerius nufar territorial male (T1), other territorial males, females and 'streaker' males during observations made in 1992.

		T1 INTERACTIONS WITH:												
		FEMALES							TERRITORIAL MALES			'STREAKER' MALES		
DATE	DAYS	*L	M	S	NO RESPONSE	ASCENT ABORTED (LOST INTEREST)	ASCENT ABORTED (INTER-FERENCE)	SPAWNED	BORDER DISPLAY	BORDER CHASE	CHASE DURING/ AFTER SPAWNING	CHASE OUT OF TERRITORY	CHASE WITHIN TERRITORY	THREAT APPROACH
17.8.92	1	1			-		1		2	1				3
	2	2		1	-	1		2	1	1				
	3	5	1		-		3	3	2				1	7
	4		1		-			1	4		1			
	5		1	2	-		1	2				2	1	4
31.8.92	1	2	3	4	-		2	7	3		2	1	1	2
	2			4	-	1	1	2	2	1	1	1	5	3
	3	-	-	-	-	-	-	-	-	-	-	-	-	-
	4		1	1	-		1	1					5	5
	5	2			-		1	1					2	2
14.9.92	1	1		2	-			3	3				2	4
	2	3	4	3	-	1	4	5	1				3	3
	3	1	1	1	-			3	2				6	3
	4	1		2	-			3	1				10	2
	5	4		4	-		2	6	2		2		2	4
28.9.92	1	1	3	1	33			5	4			3	3	2
	2	3	1	3	53		2	5	7		1			4
	3		1	1	23		1	1	4			3	2	1
	4		2	7	44		3	6	3				4	4
	5			7	47		2	5	5		2			1
TOTAL		26	19	43		3	24	61	46	3	9	10	47	54
MEAN:		1.37	1.0	2.26		0.16	1.26	3.21	2.42	0.16	0.47	0.53	2.47	2.84
SD:		1.50	1.20	2.16		0.37	1.19	2.07	1.84	0.37	0.77	1.02	2.61	1.74

*LMS = Number of spawning ascents initiated with large, medium and small females,
- = no data

Table 10. The number of interactions between Cheimerius nufar territorial male (T2), other territorial males, females and 'streaker' males during observations made in 1992.

		T2 INTERACTIONS WITH:												
		FEMALES							TERRITORIAL MALES			SUBORDINATE MALES ("S")		
DATE	DAYS	*L	M	S	NO RESPONSE	ASCENT ABORTED (LOST INTEREST)	ASCENT ABORTED (INTER-FERENCE)	SPAWNED	BORDER DISPLAY	BORDER CHASE	CHASE DURING/ AFTER SPAWNING	CHASE OUT OF TERRITORY	CHASE WITHIN TERRITORY	THREAT APPROACH
17.8.92	1	2			-			2						
	2	4			-	2		2			1			
	3	6			-	2		4	1	1	1			
	4	5	1		-			6	4		3	1		
	5	7	3		-	2	3	5			1	1		
31.8.92	1	7			-	2	1	4	1	2	1	4		
	2	7	1		-	2	1	5	2	1		2		
	3	-	-	-	-	-	-	-	-	-	-	-	-	-
	4	4	1		-	2		3	1	1		2		
	5	2			-	2				3				
14.9.92	1	6			-			6		2	1	3		
	2	6			-			6			1			
	3	2	5		-	1		6	1	1	1	2		
	4	7	1		-			8	1		1	2		
	5	9			-		1	8	1	1	4	3		
28.9.92	1	9			26		2	7	2	1	5	1		
	2	7			49	1		6	2		1	3		
	3	2	1		27		1	2	1		1	2		
	4	4	2		47			6	1	2	1	4		
	5	8	1		45		3	6	3		4	1		
TOTAL		104	16	0		16	12	92	21	15	27	31	0	0
MEAN:		5.47	0.84	0		0.84	0.63	4.84	1.10	0.79	1.42	1.63	0	0
SD:		2.34	1.30	0		0.96	1.01	2.19	1.10	0.92	1.46	1.34	0	0

*LMS = Number of spawning ascents initiated with large, medium and small females,
- = no data

Table 11. The number of interactions between Cheimerius nufar territorial male (T3), other territorial males, females and 'streaker' males during observations made in 1992.

		T3 INTERACTIONS WITH:												
		FEMALES							TERRITORIAL MALES			SUBORDINATE MALES ("S")		
DATE	DAYS	*L	M	S	NO RESPONSE	ASCENT ABORTED (LOST INTEREST)	ASCENT ABORTED (INTER-FERENCE)	SPAWNED	BORDER DISPLAY	BORDER CHASE	CHASE DURING/ AFTER SPAWNING	CHASE OUT OF TERRITORY	CHASE WITHIN TERRITORY	THREAT APPROACH
17.8.92	1	1			-			1	2					
	2	2			-	2			1					
	3	2			-	1		1	1	1		4		
	4	2			-			2		1		1		
	5	2			-			2				2		
31.8.92	1	3			-		2	1	3		1	2		
	2	4			-			4	1					
	3	-	-	-	-	-	-	-	-	-	-	-	-	-
	4		1		-			1	1		1			
	5	1			-			1				1		
14.9.92	1	2			-			2	3	1	2	2		
	2	1			-			1	1	1		3		
	3	1			-			1	1			4		
	4				-							4		
	5	1	1	1	-			3	1	2	1			
28.9.92	1	2			-			2	2			2		
	2	5			-		1	4	7	1	1	1		
	3				-				1			1		
	4	2		1	-			3	2					
	5	1			-			1	2			1		
TOTAL		32	2	2		3	3	30	29	7	6	28	0	0
MEAN:		1.68	0.11	0.11		0.16	0.16	1.58	1.53	0.37	0.32	1.47	0	0
SD:		1.29	0.32	0.32		0.50	0.50	1.22	1.61	0.60	0.58	1.43	0	0

*LMS = Number of spawning ascents initiated with large, medium and small females,
— = no data

Table 12. Mean spawning rates (A) and border chases (B) per hour (+95% CI's) made by each territorial male C. nufar during observations in 1992.

(A)

MALE	COUNT	MEAN	95% TUKEY HSD INTERVALS FOR MEAN	
T1	19	3.21	2.48	3.94
T2	19	4.84	4.11	5.58
T3	19	1.58	0.84	2.31
Total	57	3.21	2.79	3.63

(B)

MALE	COUNT	MEAN	95% TUKEY HSD INTERVALS FOR MEAN	
T1	19	0.16	-0.10	0.42
T2	19	0.79	0.53	1.05
T3	19	0.37	0.11	0.63
Total	57	0.44	0.29	0.59

The behaviour of T1 towards the S-males in its territory differed from that of T2 and T3 in two respects. Firstly, while it periodically chased all S-males, the majority of these chases (82%) were less aggressive and did not extend further than the boundaries of its territory (Table 9). S-males remained within the territory. Secondly, it also made periodic threat approaches to reinforce its dominance over S-males. The latter were very brief, with S-males immediately responding by turning away.

Within the small group of S-males there appeared to be a peck order. There was no overt aggression between these fishes, but the dominant S-male often made threat approaches at the others which turned away when threatened. The number of 'streaks' made by each active male is given in Table 13, from which it is evident that the dominant S-male (S1) was by far the most successful 'streaker', spawning 55 times during observations with an average of 2.75 (SD = 1.80) 'streaks' per day. The number of 'streaks' made by the two most active S-males were significantly higher than streaks made by any of the T-males (t-test, $p < 0.05$).

A characteristic behaviour of all S-males was to swim slowly and maintain a position high in the water column until there was an opportunity to 'streak'. This behaviour reduced the number of confrontations between T-males and S-males, as T-males were most active nearer the floor of the tank. Most of the approaches made towards active females by S1 were also confined to the upper waters of the tank but, even so, T1 chased or threatened S1 on virtually every attempt S1 made to spawn individually with a female. No attempts made by S1 ($n=51$), or any of the other subordinate males, to spawn (not 'sneak') with active females was successful (Table 13).

Table 13. The number of 'streaks' made by each Cheimerius nufar male and the number of spawning attempts (not streaking) made by 'streaker' (S) males during observations made in 1992.

		Number of streaks - all males						Number of attempts to spawn by 'Streaker' males					
Date	Day	T1	T2	T3	S1	S2	S3	S1		S2		S3	
								N/R*	Sp	N/R	Sp	N/R	Sp
17.8.92	1	2			1			-	-	-	-	-	-
	2			2				8					
	3	1	1	2	3	1	1	7					
	4	2		2	3	3		2		1			
	5				3	2		4		1			
31.8.92	1			1	5	2		3					
	2				2	2	2	6		1			
	3	-	-	-	-	-	-	-	-	-	-	-	-
	4	2		1	3	1		5		1			
	5				1	1		1					
14.9.92	1			1	4	1		3					
	2			1	4	3	2						
	3				4	1	2	3					
	4	2			4	2		2					
	5	1		4	7	5		6					
28.9.92	1	2		2	2	2		1		1			
	2			1	2	2							
	3		1										
	4	1			4	4							
	5	2	1	1	3	3							
TOTAL:		15	3	18	55	35	7	51	0	5	0	0	0
MEAN:		0.75	0.15	0.9	2.75	1.75	0.35	2.55	0	0.25	0	0	0
SD:		0.91	0.37	1.07	1.80	1.37	0.75	2.65	0	0.44	0	0	0

*** N/R = no response from female, Sp = spawn, - = no data**

Courtship behaviour was the same as in previous years, with T-males (and S1&S2) rushing accross the paths of females and attempting to keep them within a tight circle and begin ascending. Females sometimes responded to the first rush, but on most occasions several attempts were made by T-males before females responded and started the ascent. Success rates of males T1 and T2 were estimated during the last week of observations (29.9.92 - 3.10.92).. The number of approaches made by each male which were ignored by females during that week ranged from 23-53 per day (Tables 9,10). Success rates of males T1 and T2 during the week were 11% and 14% respectively. Male T2 was thus most successful not only in the total number of spawnings (see below) but also per spawning attempt.

Even though there were many more individuals in the tank in 1992 and there were times when there were up to 12 fish spawning, there were never any indications that group spawning occurs in this species. All spawning was in pairs with T-males and S-males 'streaking' whenever possible. In the 1988 study, it was suggested that there was some form of mate selection, with possible size-matched pairing. Mate selection by females was very evident in 1992. During that year it was noted that active females stayed within preferred territories. There were too many females in the tank to identify them individually from day to day and it was not possible to determine if females remained in their chosen territories for long periods but, as there were never more than three active females in any territory on any morning (Tables 9-11), it was possible to determine that these females generally remained within territories for the duration of spawning on any one day. Size-matched pairing was not evident in 1992. The three T-males were all big fish of similar size (520-580mm FL). Two of them (T2 and T3) generally spawned with large females which were of a similar size or larger (Tables 10,11), but they also occasionally spawned with 'medium' and 'small' females. Male T1 also spawned with females throughout the size range, but was most successful with small females (Table 9).

Mate selection by females was most obvious in their response to advances made by S-males. Of the four active S-males in the tank only two attempted to court and spawn with females. S1 and S2 made 51 and 5 attempts respectively. Although attempts were made to spawn with females of all sizes, including females their own size, not once were they successful (Table 13). It would appear, therefore, that territorial males obtain a disproportionate number of matings.

Frequency of mating and the number of fish spawning

Females generally remained in one territory each day, but there was movement between territories and it was not possible to accurately determine the number of spawnings made by each female. Mean spawning rate of females was 3.21 (SD = 3.07) per hour of observations. The daily number of successful matings made by each male ranged between

1-8 per hour. The mean number of successful spawnings made by each territorial male varied considerably, with the most successful male (T2) spawning on average 4.84 (SD= 2.19) times per hour and the least successful (T3) only 1.58 (SD= 1.22) times per hour (Tables 9-11). Mean spawning rate of males was 3.04 (SD= 4.33) per hour.

Interspecific behaviour and aborted sequences

There were no C. puniceus in the main display tank at Sea World during the spawning season of 1992 and there was no interest or interference made by any other species during spawning of C. nufar.

A similar pattern of aborted spawning sequences to those reported in 1988 was evident in 1992. In the latter study the number of ascents aborted through 'loss of interest' on the part of the male and through interference from other males, was noted (Tables 9-11). The most successful territorial male (T2) aborted most often through 'lack of interest' in the female, while the second most successful male (T1) aborted most often through interference from other males. This interference was almost entirely from the (T2) male.

3.4.3 Discussion

Since May 1988, spawning in C. nufar has been observed at Sea World Durban for six consecutive years. Each year, the time and duration of spawning has followed the same pattern, irrespective of the number of individuals in the main display tank. Spawning in captivity has continued throughout the spawning seasons of wild fish (Garratt 1985b), and each year it has been confined to a few hours around sunrise. In a similar manner to fishes that track the tides (such as A. berda - this study), presumably to release eggs when they are most likely to be swept quickly away from the spawning area (Choat & Robertson 1975, Robertson & Hoffman 1977, Robertson 1983), C. nufar tracks a time of day with reduced visibility over a period of four months.

Studies on the reproductive behaviour of shallow water reef fishes have shown that those species which produce pelagic eggs generally rise in the water column to release them. The evolution of this behaviour, often in conjunction with high tides, is thought to be a mechanism whereby predation on gametes by filter feeders and planktivores is reduced (Johannes 1978, Ross 1983, Thresher 1984). A similar behaviour is evident in deep water reef fishes (Buxton 1987) and, in C. nufar, spawning well above the reef during semi-darkness may have resulted in a further reduction of predation on gametes by dominant reef fishes, such as C. puniceus. During the spawning season, C. nufar generally started

spawning in semi-darkness and only when there was sufficient light to see the eggs released by females, did C. puniceus interfere with spawning. Spawning during semi-darkness may also be a mechanism which reduces predation of the spawners themselves, while the development of distinct white markings may allow for mate recognition at this time.

As in A. berda and C. puniceus, colour changes in C. nufar associated with spawning were unknown prior to this study. In C. nufar and A. berda, these markings intensified immediately prior to, and during, spawning. Spawning was not observed in C. puniceus, but the same phenomenon occurred early each day in territorial males throughout the spawning season.

The study in 1992 showed that colour changes were not restricted to territorial male C. nufar. Two of the subordinate 'streaker' males retained the silvery pink colour of females, but developed a white patch on their sides during spawning. The white patch was most pronounced on the dominant (most successful) 'streaker' S1, and only became evident on S2 during spawning rushes. The remaining streakers retained the barred colouration of juveniles. The brightness and duration of white markings on these 'streakers' corresponded to their level in the peck order that was apparent among them and, as markings were brightest on territorial males, these markings appear to be not only a mechanism whereby females recognise males, but one which allows them to guage the best prospective territorial males with which to mate. This may account, in part, for the inability of 'streakers' to spawn (not streak) with any females, even those of similar size.

Spawning behaviour of territorial males and behaviour associated with the maintenance of their territories was the same in both studies, suggesting that these patterns reflect natural behaviour in wild populations. The establishment of the same number of territories in the tank each year may indicate that there is an optimum or preferred size of territory in the wild, the volume of water in the tank, or the surface area of the bottom of the tank (150 m^2), restricting the number to three. Territories were slightly smaller in 1992 because of a fourth area in which a number of inactive fish maintained positions. Similar aggregations of inactive fish may occur in the wild, but it is unlikely that their presence would restrict the activities of territorial males in a similar manner. The decrease in the size of territories in 1992 is, therefore, assumed to be the result of insufficient space for the number of fish in the tank.

In 1992 special attention was given to aggressive interactions between territorial males, between these males and 'streakers', and to the spawning success rates of both 'types' of males. The results showed that the most successful (obtained most spawnings) territorial males (T2 & T1) were the most aggressive fish in the tank. T1 allowed all the 'sneakers'to stay and operate within and from it's territory, and its behaviour towards them was quite different to that of T2 and T3. Whereas T2 and T3 chased 'streakers' out of their territories whenever they saw them, and also often entered the territory of T1 to chase them, T1 spent considerable effort reinforcing its dominance over the 'streakers' with threat approaches and short chases within its territory (Table 14). 60% of its interactions with other males was directed at the 'streakers' in this manner. As T1 did not appear to benefit in any way whatsoever in allowing the 'streakers' to remain within its territory, the reasons for allowing them to do so remain unclear. From the 'streakers' perspective, they may have gained some measure of protection from T1 against T2 and T3, whereas if they had operated from the fourth "inactive zone", they may have been continually harassed.

Table 14. The number of successful spawnings made by each territorial (T) male Cheimerius nufar and their interactions with other males during observations made in 1992.

Behaviour	T1	T2	T3
No. of successful spawnings	61	92	30
Interactions with T males	58	63	42
Chase S-male out of territory	10	31	28
Chase S-male within territory	47	0	0
Threat approach to S-male	54	0	0
Total interactions with males	169	94	70

Spawning rates of territorial males did not differ significantly between 1988 and 1992 (ANOVA, $p = >0.05$, F ratio = 0.07) (Table 15), even though there appeared to be a considerable range in success rates between T-males each year. Success rates are, however, difficult to determine, especially if spawning is often accompanied by 'streaking'. To obtain a more accurate estimate of individual success rates, including those of 'streakers', it would be necessary to determine the number of eggs fertilized by each fish during a spawning episode.

Fertilization rates have been shown to vary considerably (Fischer 1981, Nakatsuru & Kramer 1982) and it would be impossible to determine fertilization rates of 'streakers' versus T-males. However, assuming that variations in fertilization rates are the same for all males, that the number of eggs released by females during each spawn is fairly constant and that the number of streaks made on each T-male was proportional to the number of successful spawns made by that male, one may assess the relative success rates of all the males (Appendix 1). The results of this exercise suggested not only that territorial males obtain a disproportionate number of matings (relative to 'streakers'), but also that success rates among territorial males vary considerably, with the most aggressive males obtaining most spawnings and fertilizing the most eggs.

In terms of the number of courtship attempts made by territorial males to the number of successful spawns, success rates of the two most successful males in the 1992 study were surprisingly low (T1 & T2= 11% & 14% respectively), indicating that males generally have to expend a considerable amount of time and energy to entice females to spawn.

Table 15. Mean spawning rates (+95% confidence intervals) of territorial male C. nufar in 1988 and 1992.

YEAR	COUNT	MEAN	95% TUKEY HSD INTERVALS FOR MEAN	
1988	56	3.04	2.40	3.69
1992	57	3.21	2.57	3.85
Total	113	3.13	2.67	3.58

The results of the 1988 study suggested that size-matched pairing may occur in C. nufar, as smaller females generally spawned with smaller males (Table 16). There were times when two or even three females spawned successively with the same male, or the reverse (see Table 7), but only on one occasion was the smallest male seen to mate with the biggest female and it never mated with the second largest female. The removal of the biggest male, which was killed seven weeks after spawning had started, was unfortunate. During this time it spawned only with the biggest female, a further indication of size-matched pairing as there were other fish spawning at the time. Had it survived, however, it may have spawned later in the season with a few, if not all, of the females in the tank. In this regard it is interesting to note the success of the larger of the two remaining males after the removal of the largest male (Tables 7,16). It was almost three times more successful than

the smaller male, in terms of the number of successful matings, and it spawned with all four females. This may have been due to its size in relation to the females in the tank but it is also possible that it reflects dominance over the other male.

The results of the 1992 study indicated that there was mate selection by females, but even though there appeared to be a similar pattern to that in the previous study, the small size range of the three males made it difficult to establish if size-matched pairing occurs naturally in C. nufar. Males T2 and T3 were large fish of similar size (+/- 580mm FL) and male T1 was only slightly smaller (+/- 520mm FL). There were too many females in the tank to identify them individually and, for the investigation into size-matching, females were classified either as small, medium or large.

Table 16. The frequency of spawnings made by each female (F) Cheimerius nufar with each male (M) during 24 days of observations in 1988, presented as a percentage of their total spawnings.

(±350mm → ±450mm)					
	F4	4%	63%	33%	(n = 24)
	F3	0%	100%	0%	(n = 25)
	F2	41%	59%	0%	(n = 64)
	F1	43%	57%	0%	(n = 21)
		M1	M2	M3	
		(±350mm → ±450mm)			

The spawning rate of large females was significantly higher than medium and small females in 1992 (ANOVA, p= <0.01) (Table 17) and T2 obtained 66% of their spawnings (Table 18). Male T2 only spawned with large and medium size females. The large size of T2 may have influenced females to spawn preferentially with him but, the poor success rate of T3 with both large and medium females (20% & 6% respectively) suggests that other factors may play an equal or greater role in mate selection. These may include levels of aggression, the manner in which females are approached (learned behaviour?) and the intensity of spawning colours. Medium females spawned equally with males T2 and T1, but small females spawned almost exclusively with T1. As T1 was the smallest male in the tank, this may represent the best possible size-matching considering the limited number and size of males in the tank.

Table 17. Mean spawning rates of large, medium and small female C. nufar (+95% confidence intervals) in 1992

FEMALES	COUNT	MEAN	95% TUKEY HSD INTERVALS FOR MEAN	
LARGE	19	6.26	5.40	7.12
MEDIUM	19	1.58	0.72	2.44
SMALL	19	1.79	0.93	2.65
Total	57	3.21	2.71	3.71

Table 18. The frequency of spawnings made by large, medium and small female (F) Cheimerius nufar with each territorial (T) male during observations made in 1992, presented as a percentage of their total spawnings.

(±290mm → 620mm)					
	F(Large)	13%	66%	20%	(n = 119)
	F(Medium)	47%	47%	6%	(n = 30)
	F(Small)	94%	0%	6%	(n = 34)
		T1	T2	T3	
		(±520 → 580mm)			

To summarise, the most significant aspects of the spawning behaviour of C. nufar to have emerged from this study are: i) that male C. nufar establish temporary territories during the spawning season, ii) that large territorial males obtain a disproportionate number of matings, iii) that there is mate selection by females, iv) that large females appear to spawn more often than smaller females, v) that mating is by pair spawning and there is no evidence of group spawning, and vi) 'streaking' represents an alternative mating strategy for males until they attain a sufficient size to establish and defend territories.

Whereas the social structures and mating systems of many protogynous species, and some protandrous species, have received a great deal of attention in recent years, prior to the present study there had been no work of a similar nature on rudimentary hermaphrodites. Based on morphological and indirect behavioural characteristics, Buxton & Garratt (1990) suggested that rudimentary hermaphrodites would have a different mating behaviour. These characteristics included: little or no difference between the mean size of functional

males and females, a relatively large testis in functional males compared to protogynous species, and shoaling during the breeding seasons (Ahrens 1964, Garratt 1988, J.R. Clarke, Port Elizabeth Museum, pers. comm.) Similar characteristics have been observed in tropical labrids (Choat & Robertson 1975). In these fishes the size of the testis was correlated with spawning behaviour, group spawners having much larger testes than pair spawners. The advantage of a large testis is seen in terms of sperm competition, where the number of eggs fertilized is a function of the amount of sperm released in a group spawning sequence (Warner & Robertson 1978). On these grounds, Buxton & Garratt (1990) suggested that mating of rudimentary hermaphrodites, such as C. nufar, should take place either in pairs matched by size, or in a group spawning sequence.

The results of the present study suggest that group spawning does not occur in this species, and that spawning takes place in pairs which may, or may not, be size-matched. The results have also revealed some plasticity in spawning behaviour and an interesting similarity with protogynous species - viz. territoriality. The temporary nature of this territoriality, the spawning behaviour of territorial males and 'streakers', and mate selection by females, are characteristics described in the 'lek-like' mating systems of the protogynous wrasses Thalassoma bifasciatum (Warner et al. 1975, Warner & Robertson 1978) and Thalassoma duperrey (Ross 1983, 1986). Both of these wrasses are "open society" species which live in extensively overlapping home ranges and mate promiscuously rather than in a harem.

The similarities in the mating systems of C. nufar and these wrasses, suggests three possibilities: i) C. nufar is a gonochorist which does not conform to current theoretical predictions (Warner 1975, 1988), ii) it is a protogynous hermaphrodite which has been incorrectly diagnosed as a rudimentary hermaphrodite, iii) protogyny in the family Sparidae is an ancestral condition and C. nufar represents a species whose reproductive strategy is in the process of evolutionary change from a protogynous form to a gonochoristic form (or the reverse).

With respect to the first two possibilities, further work on this species is highly recommended. The fact that this species appears to share characteristics of both gonochorists and protogynous hermaphrodites, suggests that it may offer a unique opportunity to test predictions concerning mating systems and the evolution of sex change in fishes.

The possibility that C. nufar is a protogynous hermaphrodite was raised earlier in this study. Histological analysis of gonads showed that all individuals start life as females and males only begin to appear in the population in the size range in which sexual maturity is

attained. Considering the spawning behaviour of C. nufar described above, there is even more reason to suspect protogyny in this species. Gonadal development in C. nufar is similar to that of C. puniceus throughout the size range but, as there was no size separation of the sexes in the adult population, few hermaphrodites had been reported (Coetzee 1983, Garratt 1985a) and functional males had relatively large testes compared to protogynous species (such as C. puniceus and C. laticeps), it was suggested that C. nufar was more likely to undergo pre-maturational sex change and function as a gonochorist. However, sex separation may not be evident in species such as C. nufar, in which the mating system includes an alternative mating strategy for smaller males, such as 'streaking'. With regard to the relative size of the testis in C. nufar, this may be seen as an advantage in a mating system in which small males compete with territorial males to fertilize eggs by 'streaking'. While C. nufar males may have relatively large testes compared to other protogynous species, there may be relative differences in the size of testes between territorial and 'streaker' males, similar to those recorded in tropical labrids (Choat & Robertson 1975). This aspect was not investigated in the present study.

The first and third possibilities are not mutually exclusive. If C. nufar is in the process of changing from protogyny to gonochorism (or the reverse), it is unlikely that it will conform to current theoretical predictions. The possibility that C. nufar is in the process of evolutionary change should not be ignored, even though there are no means of testing the hypothesis or even knowing the direction in which the species may be evolving.

Whether hermaphroditism is a more primitive condition from which gonochorism has arisen, or a specialization derived from the gonochoristic form, is still debated. Accumulating evidence suggests that functional hermaphroditism is a derived, specialized condition which has evolved independently among fishes at least ten times (Smith 1975, Lowe-McConnell 1987). Yet Smith (1975) considers synchronous hermaphrodites of the family Serranidae to be the most primitive members of the family, from which protogynous groupers must have evolved, and he suspects that gonochoristic synodontids and harpadontids are derived from hermaphrodites.

With regard to sparids, the early work of D'Ancona (1949b) suggested that gonochorism in this family may be derived from hermaphroditism. Investigation of gonadal structure and development revealed that species identified as gonochorists were, in fact, rudimentary hermaphrodites. Acording to D'Ancona (1949b) this strongly suggested a functionally hermaphroditic ancestry. Later, in his review of hermaphroditism in fishes, Atz (1964, p. 181) supported this view with the statement: "the Sparidae seemingly provide a step-wise

progression from species in which all the individuals are protandrous or protogynous hermaphrodites, through rudimentary hermaphrodites of different forms and frequencies, to species that are structural as well as functional gonochorists".

The most recent evidence of ancestral protogyny is found in studies on gobies (Fam. Gobiidae). In this family, as in the Sparidae, there are a number of gonochorists and protogynous hermaphrodites. On the basis of similarities in gonad morphology in protogynous gobies (Fam. Gobiidae) of the genus Coryphopterus, which have been separated geographically for millions of years, Cole & Shapiro (1990, 1992) have recently suggested that protogyny in this genus is an ancestral, rather than a recently derived, condition.

CHAPTER 4 - SUMMARY AND GENERAL CONCLUSIONS

Initial development of gonadal primordia, including the formation of a central cavity, the formation of germinal and sterile zones, and their cytological composition, was similar in all the species investigated in this study. Once the formation of the central cavity was complete, germinal tissue became evident and presumptive male and female elements could be distinguished from one another. In all species, initial cytological development (appearance of germinal tissue) was in a female direction, but proliferation of gonial cells in the male elements of R. sarba and A. berda soon overtook that in the female elements. In these species, gonadal primordia differentiated morphologically into distinctly bisexual organs and all fish developed and functioned first as males.

Early gonadal development in C. puniceus and C. nufar differed from that of A. berda and R. sarba. In C. puniceus and C. nufar the male element remained dormant and gonadal primordia differentiated into ovaries. There was no further development of the male element at this stage and the newly differentiated gonads of protogynous (and possibly rudimentary) hermaphrodites could easily be distinguished from those of protandrous hermaphrodites. If these findings apply to all sparids, further investigations of early gonadal development may resolve much of the confusion and uncertainty surrounding sexuality in this family.

This study has shown that D'Ancona's (1949b) descriptions of early gonadal development in sparids fairly accurately describe development in the protandrous species investigated in this study. Inconsistencies included the process of formation of the central cavity and the developmental stage at which germinal tissue became apparent in gonadal primordia. In the present study, the central cavity of all gonadal primordia was formed by the extension of two elements ventrally from the mesovarium which fused in the ventral region. It was not formed from a furrow which progressively deepened on the first element, as suggested by D'Ancona (1949b). Germinal tissue did not either become apparent until the central cavity was fully formed. Prior to this, gonadal primordia consisted of somatic cells, granulocytes, fibrillar and connective tissue. These processes were common to all the species investigated in this study and it is suggested that the present findings reflect, more accurately, initial gonadal development in all members of the family. D'Ancona's (1949b) descriptions of early gonadal development do not adequately describe development in the protogynous (and possibly rudimentary) hermaphrodite included in this study.

Cells conforming to primordial germ cells (PGC's) described in other teleosts were identified in only two specimens. In both instances sexual differentiation was complete and all stages of spermatogenesis were evident in the male element. These results suggest that either PGC's are very few in number throughout early developmental stages in sparids, or that the cells described as such in other teleosts are not necessarily PGC's. In all specimens investigated in this study, less-electron-dense (LED) cysts of cells, and gonial cells, were the first indications of germinal development. The development of gonial cells within LED cysts has not been reported previously in teleosts and this aspect of early gonadal development requires further detailed investigation.

It is not known if gonial cells become differentiated in the early stages of gonadal development, or if they remain undifferentiated until later in life when they are masculinized or feminized. One study at the electron microscope level and several at the light level, have suggested early differentiation. In this study, gonial cells in presumptive male and female elements could not be distinguished from one another morphologically and the results lend support to the theory that they remain undifferentiated, bipotential cells until they are either masculinized or feminized in later development, under the influence of surrounding tissue.

Morphological and cytological development of all gonads proceeded initially in a female direction, irrespective of reproductive style. This evidence suggests that the paradigm of "primacy of female develoment" in mammals applies to all sparids, including protandrous hermaphrodites, and it lends further support to Shapiro's (1992) hypothesis that the paradigm may apply to all fishes.

Sexual differentiation occurred relatively late in all the sparids investigated, occurring in individuals of 100-150mm (FL) i.e. at the end of the first year of life or early in the second year. It appears that this is a characteristic shared by all sparids. The gonads of R. sarba and A. berda differentiated into distinctly bisexual organs. All fish passed through a predominantly male phase and functioned first as males. Thereafter the number of females in the population increased, with a corresponding decrease in males. This trend was short lived in R. sarba but continued in A. berda, with the result that there was a distinct bimodal length frequency distribution in the latter species. Previous studies have shown that R. sarba is a protandrous hermaphrodite. Evidence of protandry in A. berda presented in this study includes: i) a similar pattern of gonadal development to that of R. sarba, ii) bimodal size frequency distributions in two 'semi-closed' systems (Richards Bay estuary and Kosi estuary), iii) histological evidence of sex change and iv) conclusive evidence of sex change using mark-recapture.

A major stumbling block in the histological diagnosis of protandrous sex change in R. sarba and A. berda was the absence of a simultaneous development of oocytes in the female element during degeneration of the testis. In these, and several other protandrous sparids, oocyte development did not pass the previtellogenic stage until sex separation was almost complete and the testis had regressed and become vestigial. There was, nevertheless, cytological and morphological evidence of sex change in these fishes. Total degeneration of testicular space in some gonads suggested that these fish would not function again as males. Morphological evidence of sex change included ovotestes with dorso-ventrally 'compressed' testes and the disappearance of sperm ducts in the sterile zone.

Schematic representations of early gonadal development in C. puniceus and C. nufar are presented. It is proposed that these schematic representations apply to all sparid protogynous (and possibly rudimentary) hermaphrodites. The gonads of C.puniceus and C. nufar differentiated into ovaries in which the male element was restricted to a few strings of gonial cells in the tunica albuginea below the sterile zone. The male element remained dormant until sexual maturity was approached in C. nufar and sex change occured later in life in C. puniceus. Sex change thus occurs in both species. In C. nufar it was not clear if sex change occurred before or after sexual maturity and the reproductive style of this species remains uncertain. Protogynous sex change was reported previously in C. puniceus (Garratt 1986b) and further histological evidence of sex change was presented in this study. In both species morphological evidence of sex change included dorso-ventrally 'compressed' testes and the development of a sperm duct which completely encircled the former ovarian cavity. In C. puniceus numerous yellow-brown bodies were associated with the degeneration of previtellogenic oocytes and somatic and connective tissue within lamellae. Contrary to recent objections in this regard (Sadovy & Shapiro 1987, Brusle-Sicard et al. 1992), these yellow-brown bodies appear to be a reliable diagnostic feature of sex change in this species.

Pathways of gonadal development proposed for R. sarba and A. berda in the present study are similar to those proposed for R. sarba by Yeung & Chan (1987) and Acanthopagrus australis by Pollock (1985) respectively. Pathways of development proposed for C. puniceus and C. nufar share several similarities with those presented by Buxton & Garratt (1990) and Alekseev (1982).

The investigation into the spawning habits of the estuarine-dependent A. berda revealed that this species spawns inside the mouth of the Kosi estuary at night. Spawning tracks peak flows through the night, ensuring the rapid transport of eggs to sea. Evidence

obtained from other estuaries in Natal suggests that this spawning strategy may be common to all functional estuaries. It is proposed that adult male A. berda migrate to the mouth and remain there for the greater part of the spawning season. Females filter down to the mouth throughout the season, spawn, and return to the lakes.

The spawning strategy of A. berda does not conform to predictions made from the size advantage model (Ghiselin 1969), which predicts that protandrous species should have mating systems such as monogamy, or random pairing of single males and females. Based on this model, Warner (1988b) proposed that the strongest selection for protandry should occur in species in which there is random pairing of single males and females and that protandry should be more common in species with relatively small mating groups. Spawning in A. berda occurred in large, predominantly male, aggregations and it was characterised by intense sperm competition, several to many males competing to spawn with a single female. It is proposed that protandrous sex change in A. berda has evolved through differential gains in reproductive success arising from the mating system, but that it is also possible that factors other than the mating system, such as differential growth and mortality, may have played a role.

Spawning was not observed in C. puniceus, but there was some evidence to suggest that males may establish temporary territories during the spawning season. In captivity and in the wild, intense aggression among males and colour changes during the spawning season, suggested that this species could have a lek-like mating system similar to several protogynous labrids and scarids, in which males gather at traditional sites and defend small areas against intrusions of other males.

The mechanisms whereby sex change is regulated in the C. puniceus population are not clear. Sex change in captive fish immediately after the spawning season suggests that the social structure during spawning regulates sex change. Timing of sex change in wild populations, however, suggests that social structure during the remainder of the year may be more important.

The investigation into the spawning habits of C. nufar revealed that this species spawns at sunrise over a period of +/-4 months. Spawning in semi-darkness may be a mechanism which reduces predation on eggs and on the spawners themselves, while development of distinct white markings may allow for mate recognition at this time.

Observations on spawning in C. nufar were limited to captive fish and spawning sites in the wild have not been identified. Characteristics of spawning behaviour in C. nufar included: i) the establishment of temporary territories by males during the spawning season, ii) large territorial males obtained a disproportionate number of matings, iii) there was mate selection by females, iv) large females spawned more often than smaller females, v) mating was by pair spawning and there was no evidence of group spawning and vi) 'streaking' represented an alternative mating strategy for males until they attained a sufficient size to establish and defend territories. All the above characteristics are shared by protogynous labrids and scarids which have lek-like mating systems described above. The similarities in these mating systems suggest three possibilities: i) C. nufar is a gonochorist which does not conform to current theoretical predictions, ii) it is a protogynous hermaphrodite which has been incorrectly diagnosed as a rudimentary hermaphrodite, iii) protogyny in the family Sparidae is an ancestral condition and C. nufar represents a species whose reproductive strategy is in the process of evolutionary change from a protogynous form to a gonochoristic form (or the reverse).

With respect to the above, further work on C. nufar is highly recommended. The fact that this species appears to share characteristics of both gonochorists and protogynous hermaphrodites, suggests that it may offer a unique opportunity to test predictions concerning mating systems and the evolution of sex change in fishes.

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APPENDIX I

RELATIVE SUCCESS RATES (FERTILISED EGGS) OF TERRITORIAL (T) AND "STREAKER" (S) MALES

Number of spawnings and 'streaks' made by each male.

Male	No. Spawnings	No. "Streaks"
T1	61	15
T2	92	3
T3	30	18
S1	0	55
S2	0	35
TOTAL	183	126

The number of eggs fertilised by territorial males (T) is represented by the formula:

$$Te = (s_1 \times f_1) + (s_2 \times f_2) + (s_3 \times f_3)$$

where s_1 = number of spawns made without interference from "streakers".
s_2 = number of spawns in which "streakers" took part.
s_3 = number of streaks made by the territorial male.
f_1 = number of eggs fertilized without interference from streakers.
f_2 = number of eggs fertilized in spawns in which "streakers" took part.
f_3 = number of eggs fertilized by a territorial male when it "streaked" on another territorial male.

The number of eggs fertilized by "streaker" males is represented by the formula:

$$Se = (S \times g)$$

where Se = number of eggs fertilized by a "streaker" male.
S = number of streaks made by a "streaker" male.
g = number of eggs fertilized by a "streaker" male when it streaked on a territorial male.

Assumptions:

i) variations in fertilization rates are the same for all males.
ii) the number of eggs released by females during each spawn is constant.
iii) the number of streaks made on each T-male was proportional to the number of successful spawns made by that male.

Example:

Assuming streakers obtain 33% of fertilization and each spawn consists of 100 eggs:

$T1,e = (s_1 \times f_1) + (s_2 \times f_2) + (s_3 \times f_3)$
$= (19 \times 100) + (42 \times 66) + (15 \times 33)$
$= 5167$

Hence:

$T_2e = 7157$
$T_3e = 2914$

Similarly:

$S_1e = (S \times g)$
$= (55 \times 33)$
$= 1815$ and
$S_2e = 1155$

Relative number of eggs fertilized by territorial (T) and 'streaker' (S) males, assuming 'streakers' obtain 33%, 40% and 50% fertilization and each spawn consists of 100 eggs.

Males	33%	40%	50%
T1	5167	5020	4750
T2	7157	6800	6200
T3	2914	2920	2900
S1	1815	2200	2750
S2	1155	1400	1750

COMPARATIVE ASPECTS OF THE REPRODUCTIVE BIOLOGY OF SEABREAMS (PISCES: SPARIDAE)

Volume 2
Histological study of gonadal development.
Photomicrographs for Chapter 2

THESIS
Submitted in fulfilment of the
requirements for the Degree of
DOCTOR OF PHILOSOPHY
of Rhodes University

by

PATRICK ASHWORTH GARRATT

December 1993

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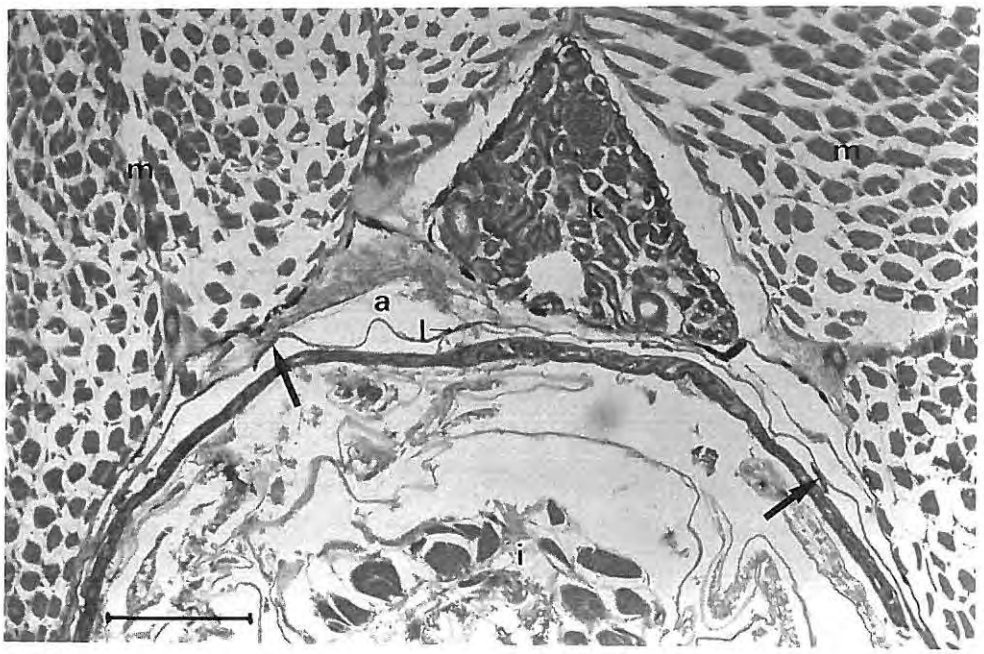
GONADAL DEVELOPMENT IN RHABDOSARGUS SARBA

Figure 2. Transverse section through the mid-region of the body cavity of a 32mm (fork length - FL) Rhabdosargus sarba, showing developing gonadal primordia (arrows) suspended from the dorsal abdominal wall. a = air bladder, i = intestine, k = kidney, m = muscle, l = lining of body cavity. Scale bar = 500μm.

Figure 3. Anterior (**a**), medial (**b**) and posterior (**c**) sections of the undifferentiated gonadal primordium of a 39mm (FL) Rhabdosargus sarba, showing early stages of central cavity formation. A second element (s) has started to develop alongside the first to which it will fuse in the ventral region. bv = major blood vessels, l = lining of body cavity, m = mesovarium. Scale bars = 50μm.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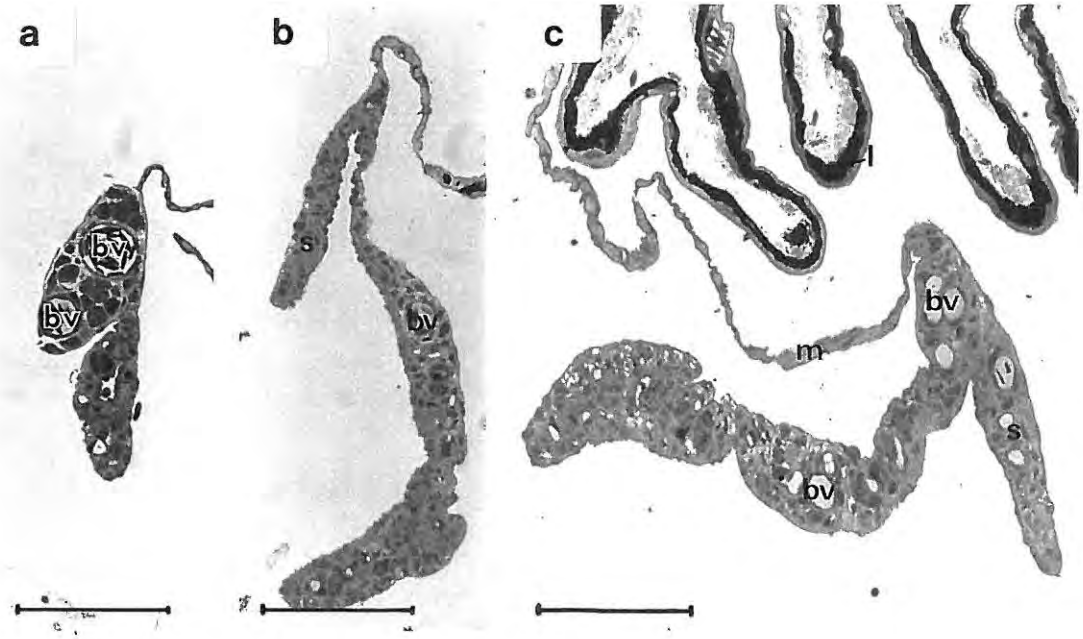
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Figure 4. Electron micrographs of cells comprising the gonadal primordia of Rhabdosargus sarba ranging in length from 39-49mm (FL). **(a)** - granulocytes (g) and fibrillar tissue (f) encompassing the major blood vessel (bv) in the anterior region. **(b&c)** - somatic cells (s), granulocytes (g) and loose fibrillar tissue (f) forming the matrix of both elements in medial and posterior regions. **(d)** - cyst of less-electron-dense cells (cs) in the stroma. n= nucleus. Scale bar = 1µm.

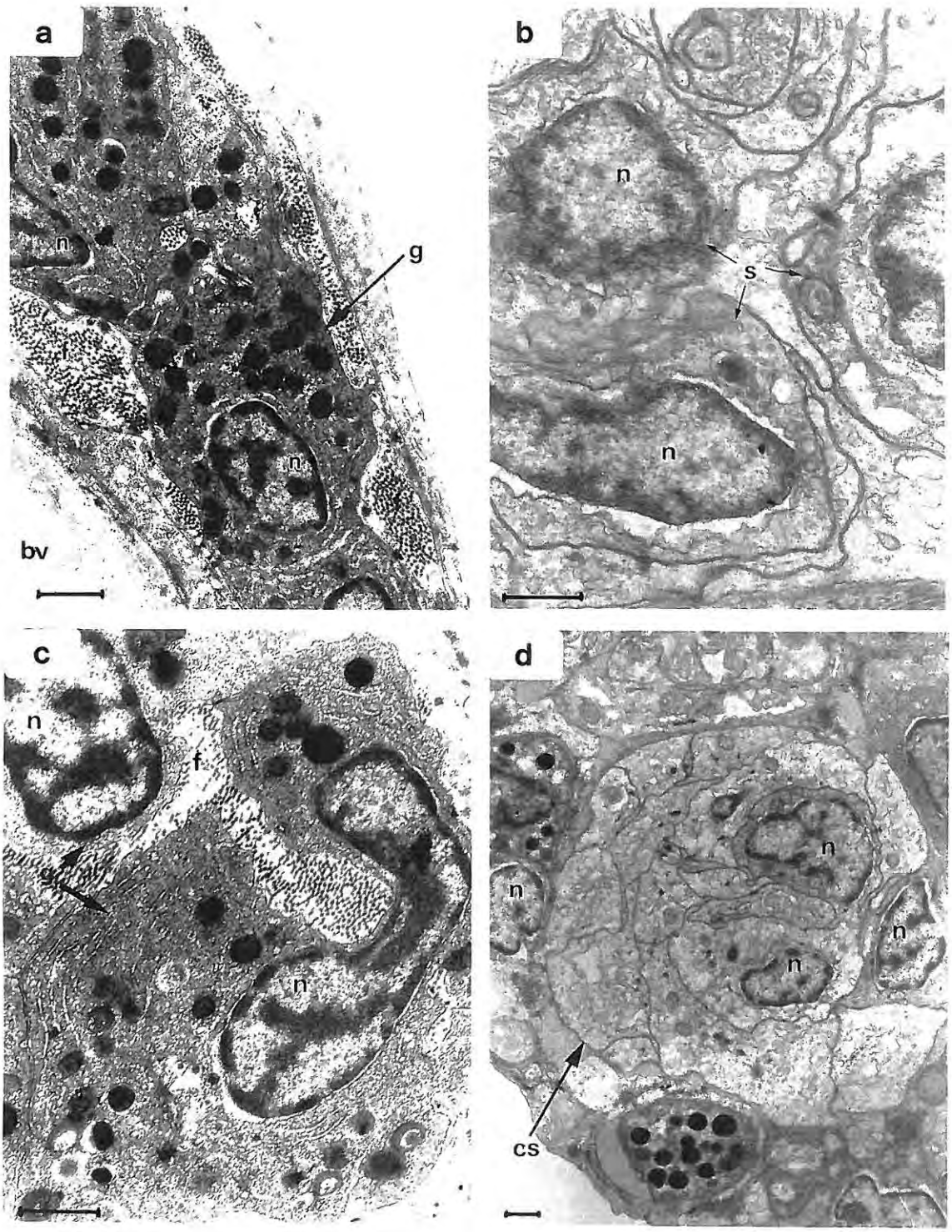
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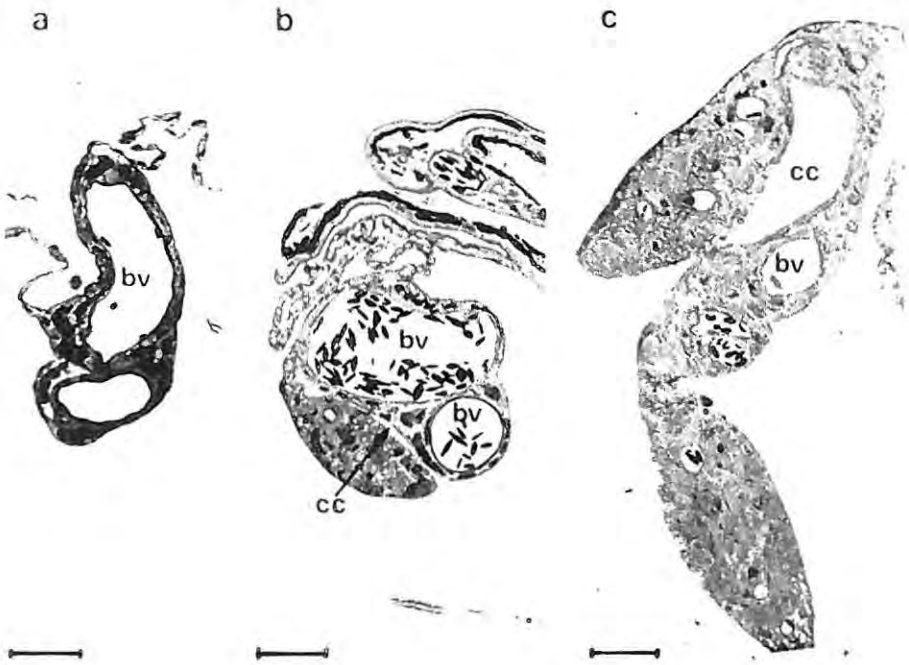
Figure 5. Anterior (**a**), medial (**b**) and posterior (**c**) sections of an undifferentiated gonadal primordium from a Rhabdosargus sarba measuring 55mm, showing incomplete formation of a central cavity (cc) in medial and posterior regions. bv = blood vessel. Scale bar = 25μm.

Figure 6. Anterior (**a**), medial (**b**), and posterior (**c**) sections of an undifferentiated gonadal primordium from a Rhabdosargus sarba of 79mm (FL), showing the progressive formation of a central cavity (cc) from anterior to posterior, and the formation of a distinct ventral apex (va). Scale bar = 100μm.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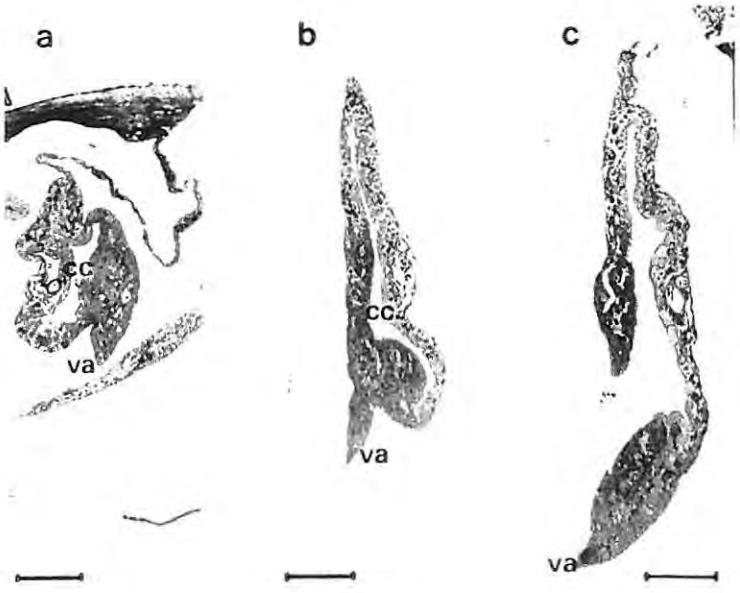
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Figure 7. Electron micrographs of cells comprising the gonadal primordia of Rhabdosargus sarba measuring 55-79mm (FL). **(a)** - clusters of granulocytes (g) in the dorsal region below the mesovarium; **(b)** - somatic cells (s) and scattered granulocytes (g) comprising the outer elements of each primordium and **(c)** - a distinct ventral apex; **(d)** - epithelial cells (e) protruding into the central cavity, and lamellae starting to form. Scale bar = 1μm.

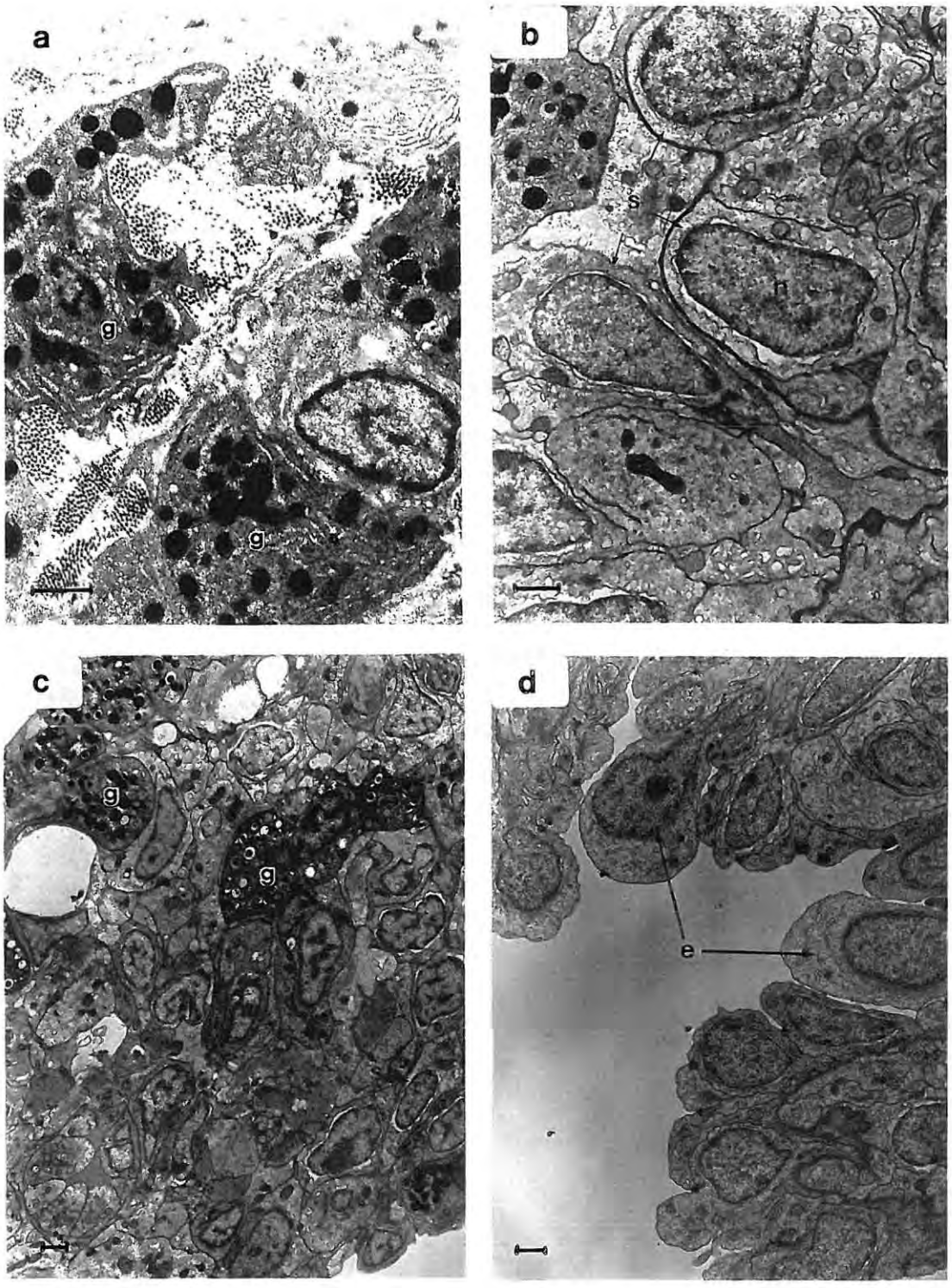
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Figure 8. Anterior (a), medial (b) and posterior (c) sections of differentiating gonads of a Rhabdosargus sarba of 92mm (FL), showing the completed formation of a central cavity along the length of both gonads and the first appearance of gonial cells (go) along the margins of the central cavity. me = male element. Scale bars = 100μm.


a
go
go
b
go
c
go
me

Figure 9. Electron micrographs of early germ and associated cells in the gonads of a Rhabdosargus sarba of 92mm (FL). **(a)** - a cyst of less-electron-dense cells (cs) within a newly forming lamella; **(b&c)** - gonial cells (go) developing within these cysts (cs); **(d)** - somatic cells (s), granulocytes (g) and vacuolated cells (v) in close association with the germinal tissue. ci = 'ciment', m = mitochondria, n = 'nuage'. Scale bars = 2μm.

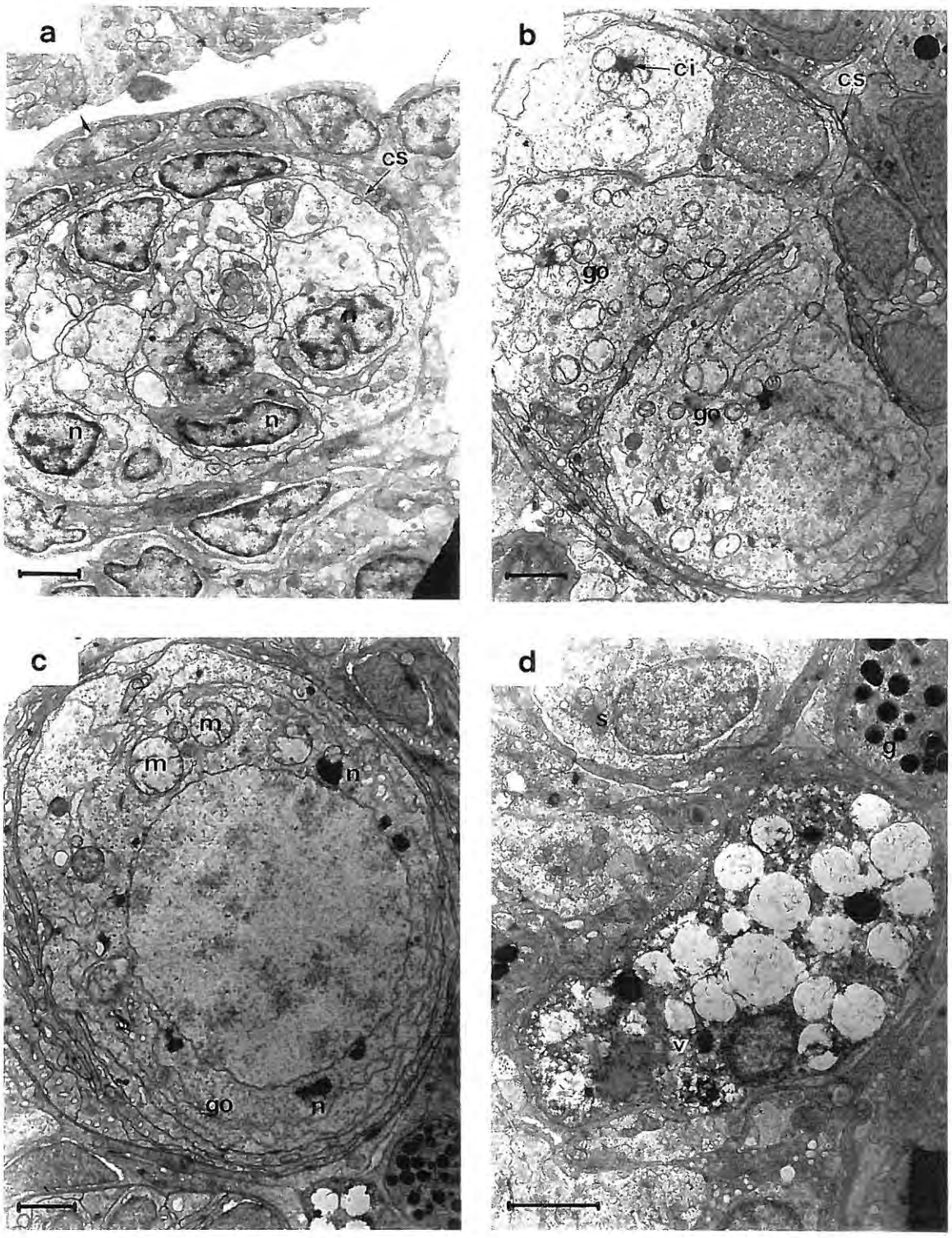
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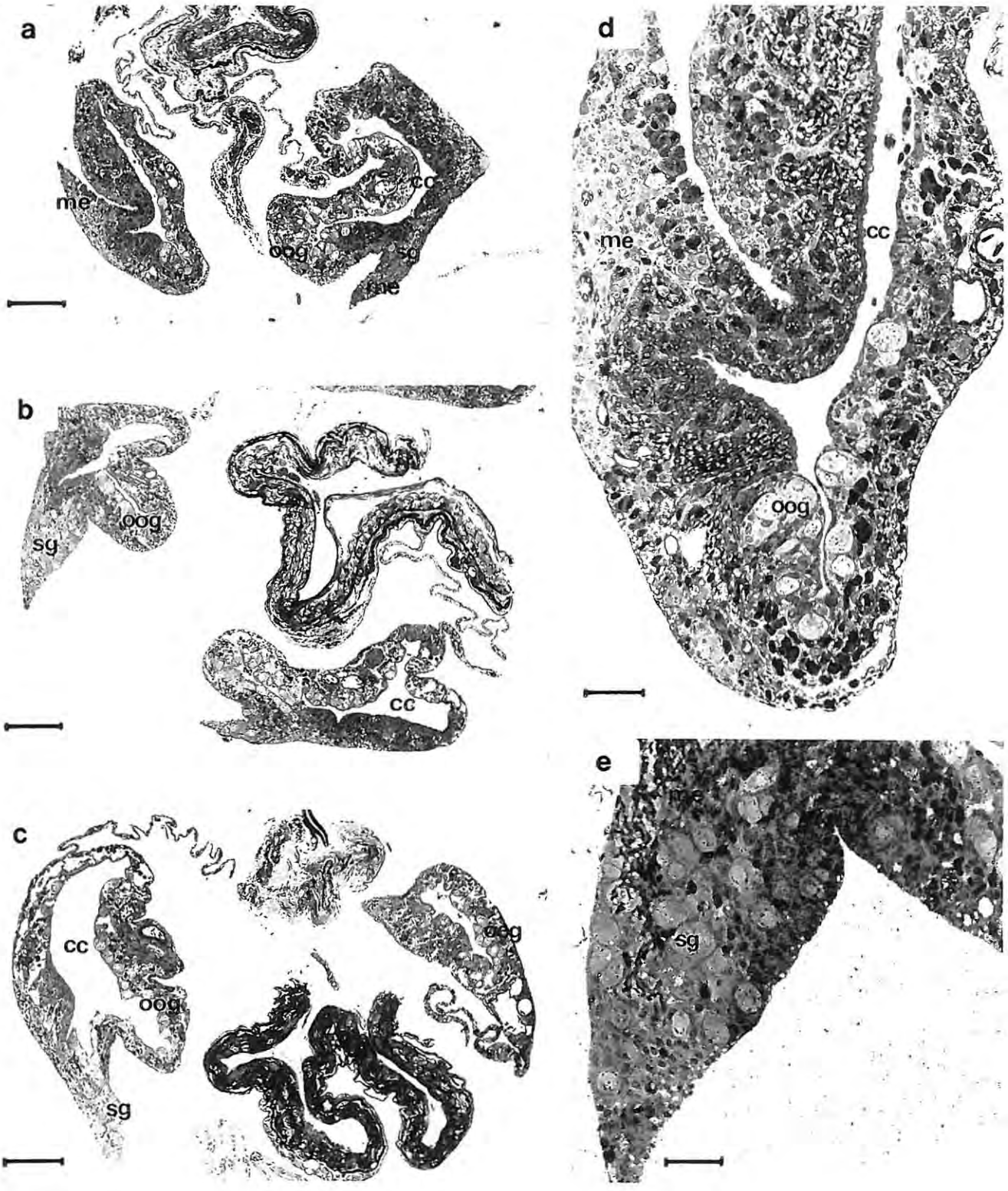
Figure 10. Anterior (**a**), medial (**b**) and posterior (**c**) sections of differentiating gonads from a Rhabdosargus sarba of 100mm(FL), with higher magnifications of male elements in anterior and medial regions (**d&e**), showing no development of male germinal tissue in the anterior region, but the simultaneous proliferation of gonial cells in both male and female elements in the mid-region. cc = central cavity, me = male element, oog = oogonia, sg = spermatogonia. Scale bars in (**a-c**) = 100µm, in (**d-e**) = 25µm.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me
sg

Figure 11. Anterior (**a**), medial (**b**) and posterior (**c**) sections of gonads from a Rhabdosargus sarba of 135mm (FL), showing the simultaneous proliferation of gonial cells in male and female elements, and early development of the sperm duct. In the male element (**d**), all stages of spermatogenesis are evident. In the female element (**e**), early previtellogenic oocytes are evident amongst cysts of mitotic oogonia. cc = central cavity, epo = early previtellogenic oocytes, m.oog = mitotic oogonia, psc = primary spermatocytes, oog = oogonia, sd = sperm duct, sc = spermatocytes, sg = spermatogonia, ssc = secondary spermatocytes, st = spermatids, sz = spermatozoa, szo = sterile zone. Scale bars in (**a-c**) = 100µm, in (**d-e**) = 25µm.

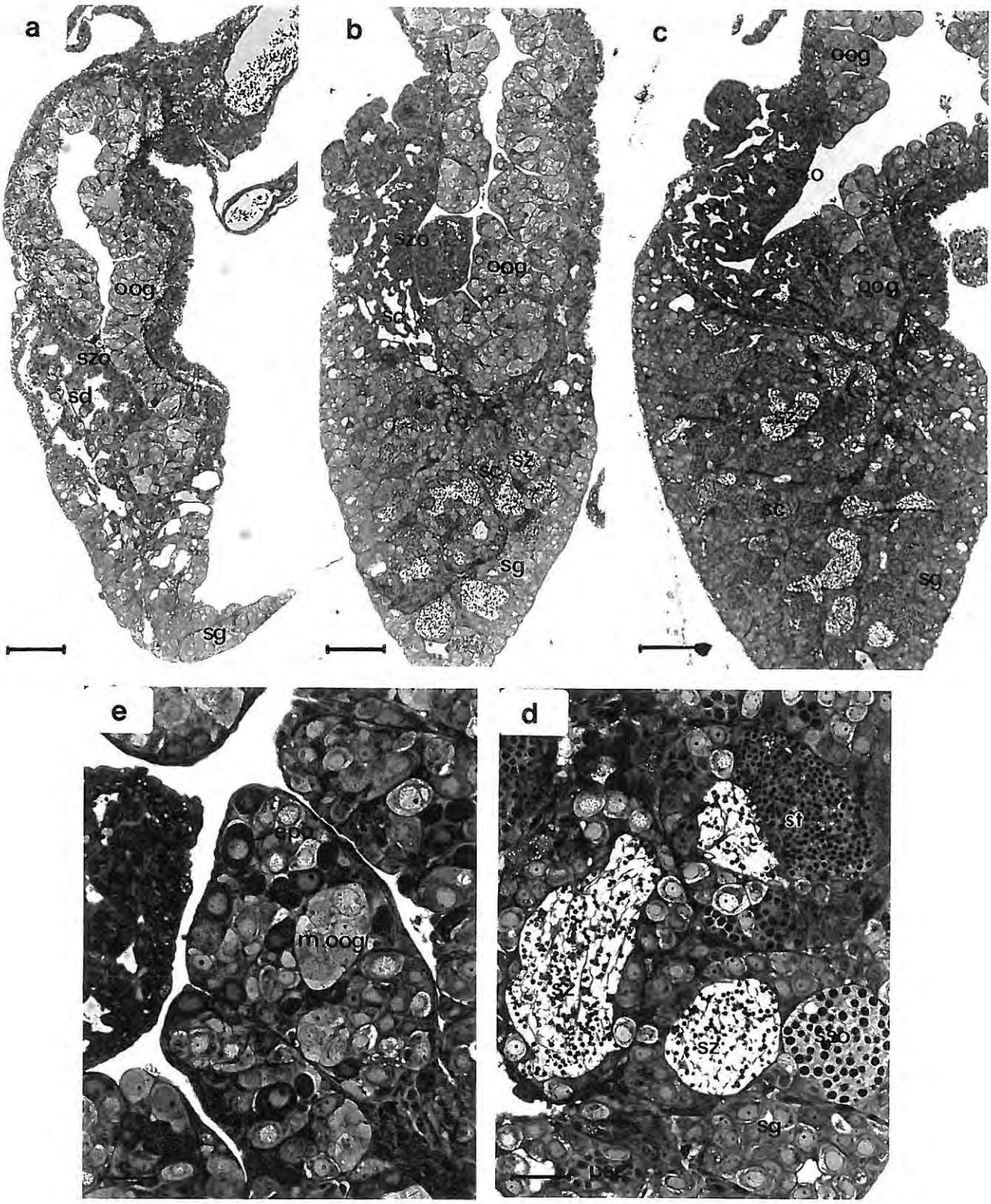
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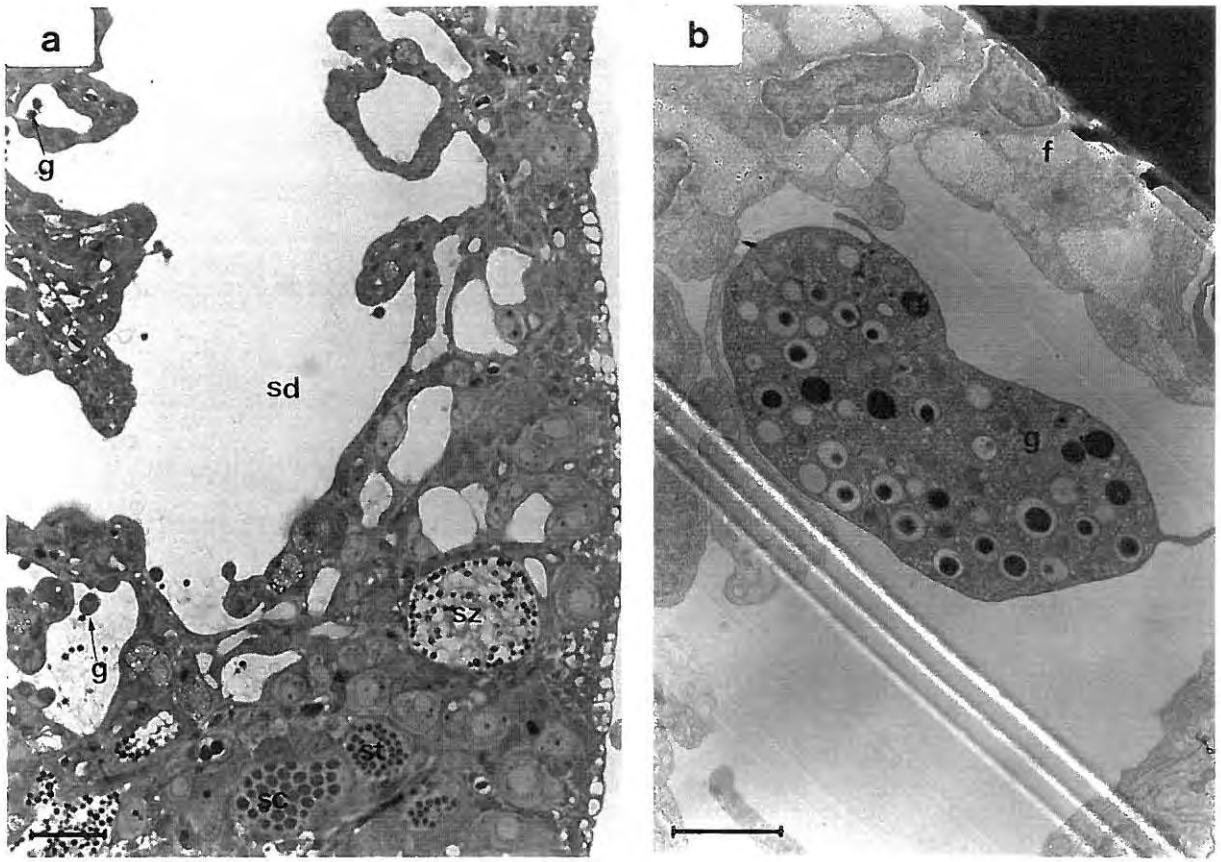
Figure 12. **(a)** - Formation of the sperm duct in Rhabdosargus sarba appeared to be a direct result of extensive atresia of somatic and connective tissue in the sterile zone. **(b)** - numerous granulocytes in phagocytic activity were present at the time. f = fibrillar tissue, g = granulocyte, sc = spermatocytes, sd = sperm duct, st = spermatids, sz = spermatozoa. Scale bar in **(a)** = 100µm, in **(b)** = 2µm.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g

Figure 13. Electron micrographs of gonial cells in presumptive female (**a,b**) and male (**c,d**) elements of a Rhabdosargus sarba of 135mm FL. c = 'ciment', cm = cell membrane, g = gonial cell, Lc = Leydig cell, m = mitochondria, ma = mitochondrial aggregate, n = 'nuage', nu = nucleus, nuc = nucleolus, pfc = pre-follicle cell, psc = primary spermatocyte, ser = smooth endoplasmic reticulum, sz = spermatozoa. Scale bars = 2µm.

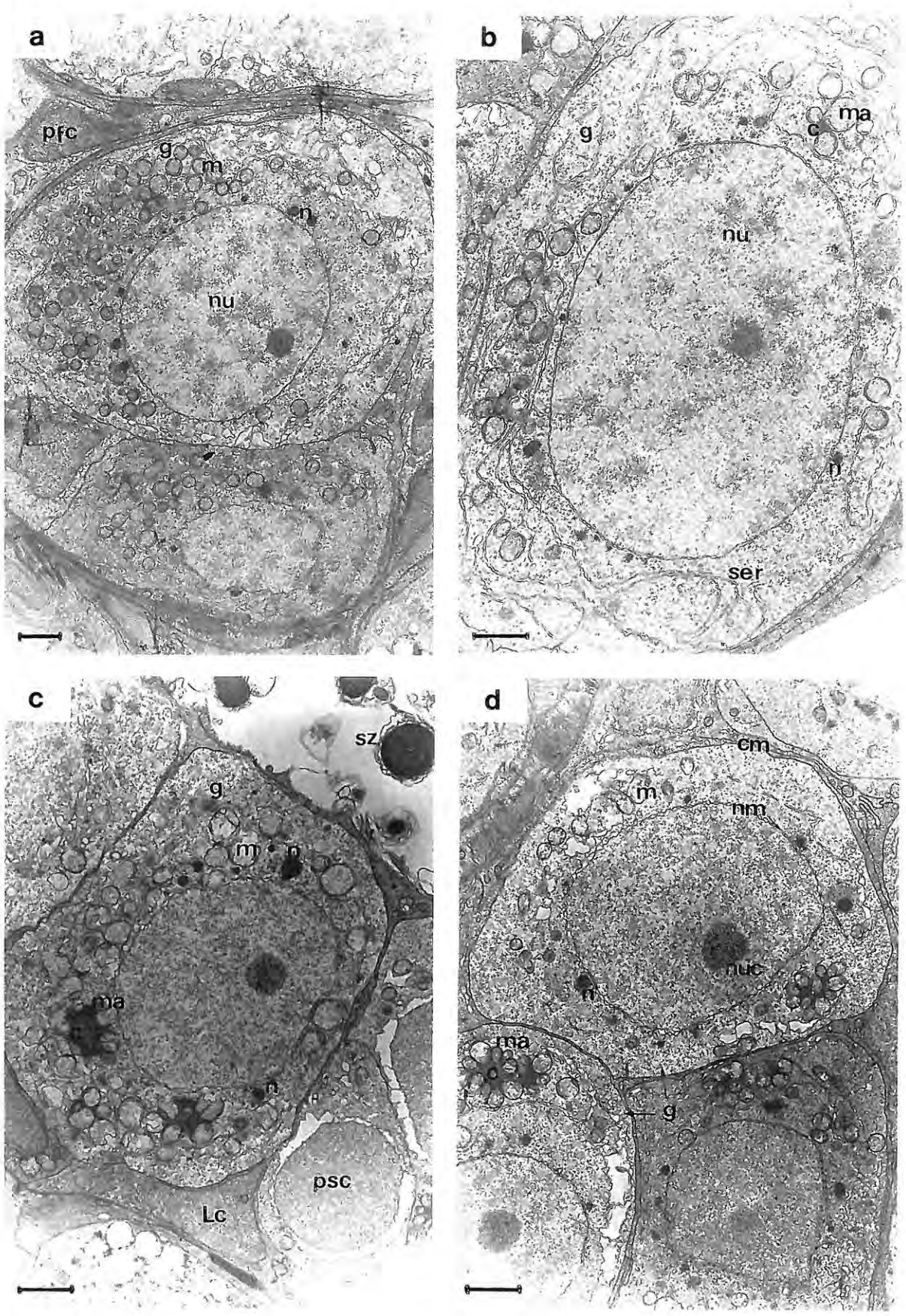

a
pfc
g
m
n
nu
b
g
ma
c
nu
n
ser
c
sz
g
m
n
ma
n
psc
Lc
d
cm
m
nm
nuc
n
ma
g

Figure 14. Electron micrographs of cells surrounding the germinal tissue of the female elements of Rhabdosargus sarba in the 100-149mm size class, and developmental stages of early oocytes in these gonads. **(a&b)** - somatic cells (sc) and dense clusters of granulocytes (gr) and vacuolated cells (v) surrounded the female germinal tissue; **(c)** - early previtellogenic oocyte with undulating nuclear envelope (une) and pre-follicle cells (pfc); **(d)** - later stage previtellogenic oocyte with regular outlines, surrounded by flattened follicle cells (fc). Scale bars = 2μm.

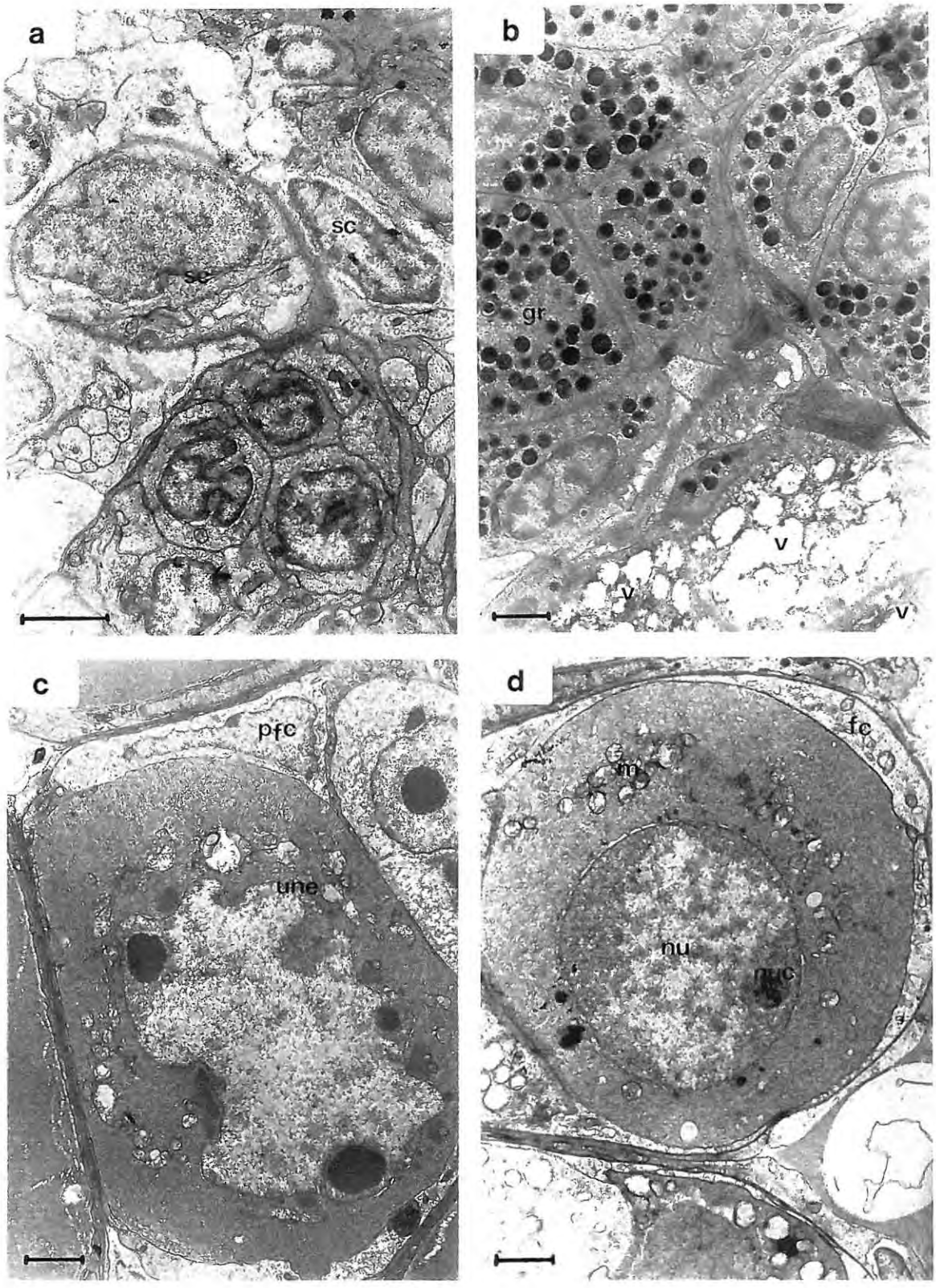

a
sc
sc
b
gr
v
v
v
c
pfc
une
d
fc
m
nu
nuc

Figure 15. Electron micrographs of primordial germ cells (PGC's) in female **(a,b)** and male **(c,d)** elements of a Rhabdosargus sarba of 135mm (FL). fm = dense fibrillar matrix, ma = mitochondrial aggregate, n = 'nuage', nu = nucleus, nuc = nucleolus, oog = oogonium, spg = spermatogonium, sz = spermatozoa. Scale bars = 2μm.

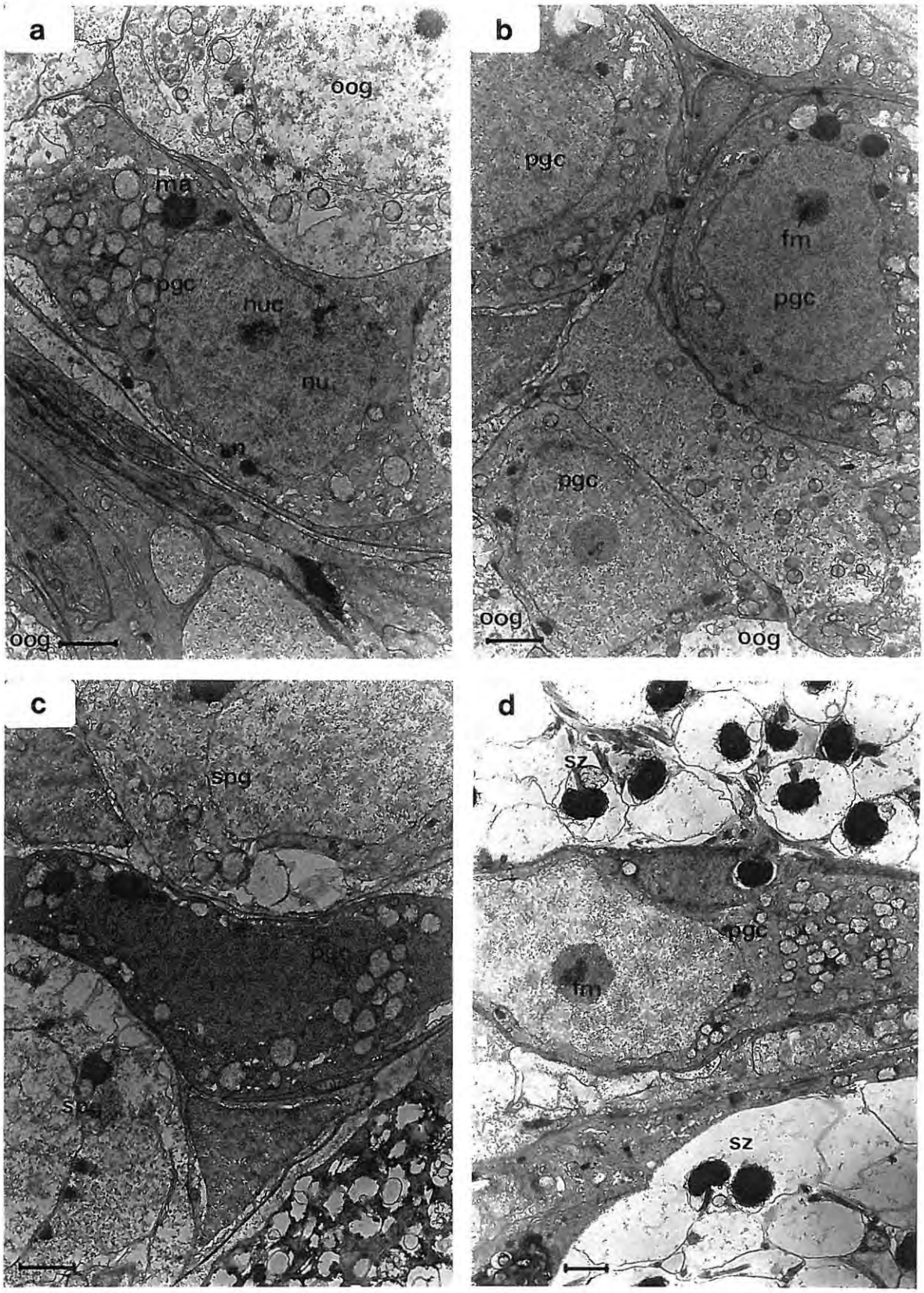

a
oog
mc
pgc
huc
nu
n
oog
b
pgc
fm
pgc
pgc
oog
oog
c
spg
spg
d
sz
pgc
fm
sz

Figure 16. Electron micrographs of germinal and interstitial tissue in the male elements of Rhabdosargus sarba of 120-135mm (FL) in which spermatogenesis has commenced. **(a)** - spermatocytes in the leptotene stage of meiotic prophase, with thickened chromosomes (ch) surrounded by a fibrillar network; **(b)** - spermatogonial cyst (scy); **(c,d)** - Leydig cells (Lc) between spermatogonial cysts. bm = basement membrane, m = mitochondria, sc = Sertoli cell, ser = smooth endoplasmic reticulum, spg = spermatogonium, tc = tubular cristae. Scale bars = 2µm.

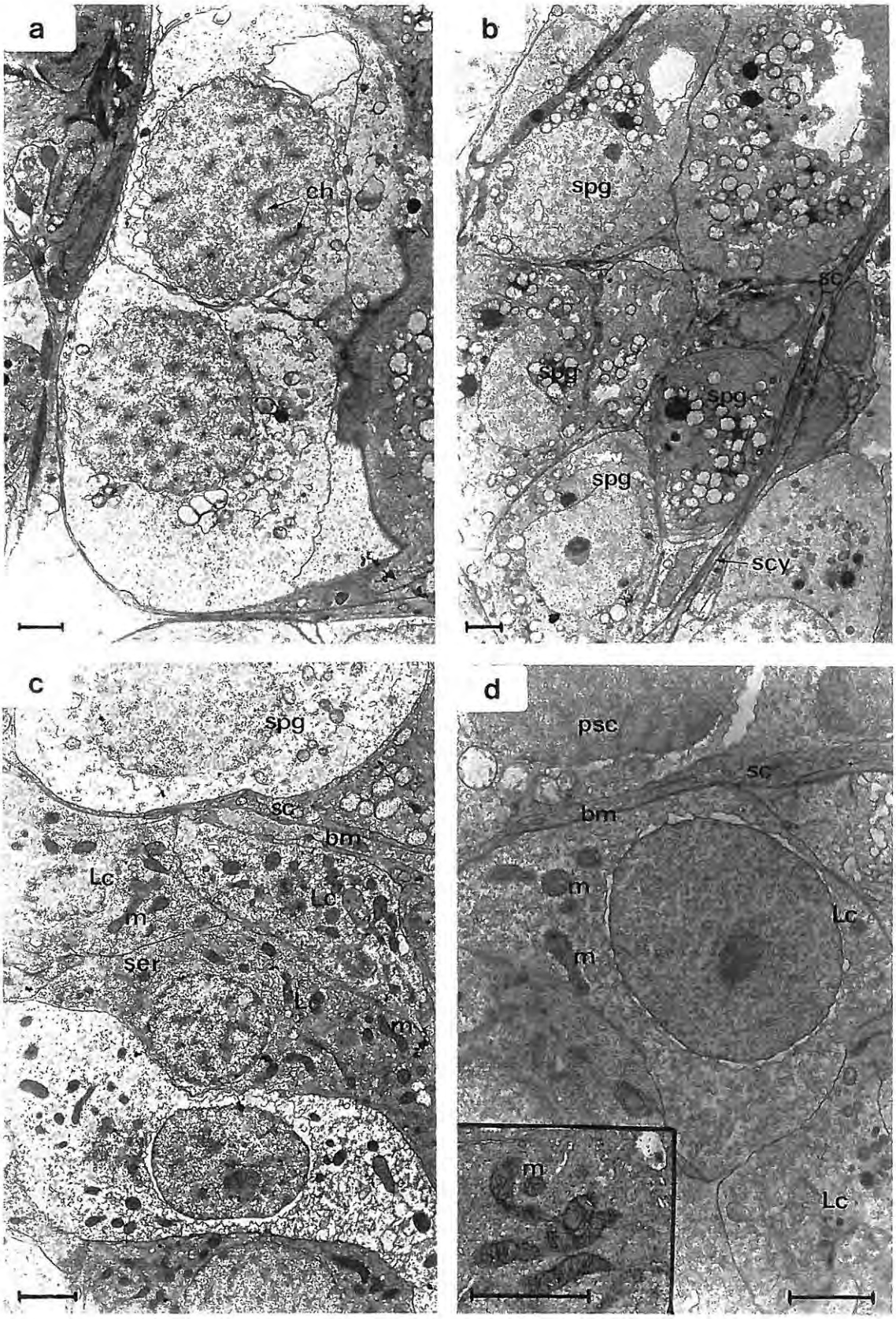

a
ch
b
spg
sc
spg
spg
spg
scy
c
spg
sc
bm
Lc
Lc
m
ser
Lc
m
d
psc
sc
bm
m
Lc
m
m
Lc

Figure 17. Electron micrographs showing synchronous spermatogenesis within cysts in male elements of 120-135mm (FL) Rhabdosargus sarba. **(a)** -spermatogonia (spg), primary (psc) and secondary (ssc) spermatocytes in spermatogonial cysts; **(b)** - spermatogonia (spg) and secondary spermatocytes (ssc) in cysts bounded by Sertoli cells (Sc); **(c)** - secondary spermatocytes (ssc) in a cyst alongside another containing spermatids (st) in a late stage of spermiogenesis. Scale bars = 2µm.

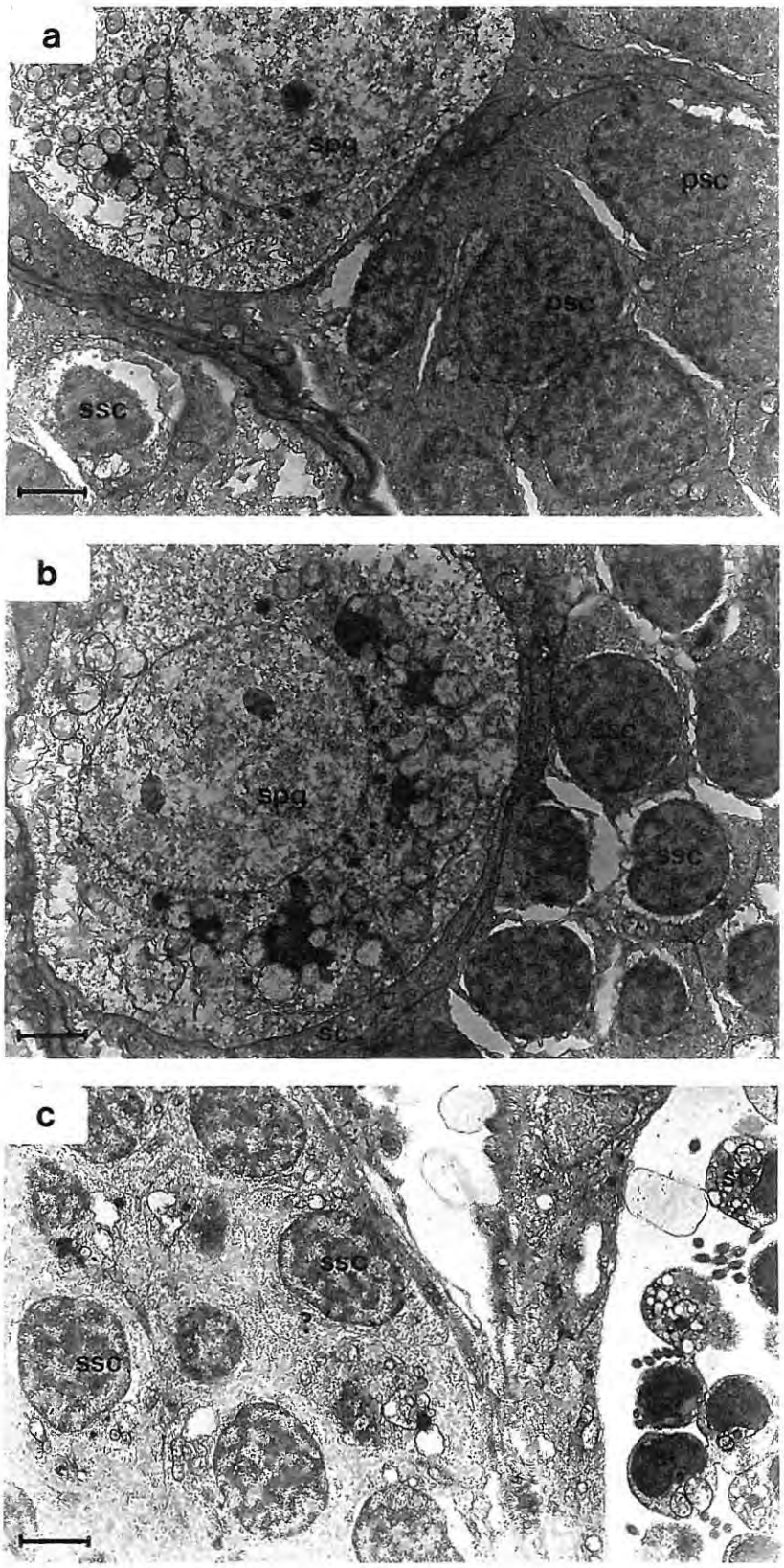

a
spg
psc
psc
ssc
b
spg
ssc
ssc
Sc
c
ssc
ssc

Figure 18. Electron micrographs showing spermiogenesis in male elements of 120-135mm (FL) Rhabdosargus sarba. **(a)** spermatids (st) in early stage of spermiogenesis; **(b)** - spermatids in late stage spermiogenesis and spermatozoa (sz) within a spermatogonial cyst. Scale bars = 1μm.

(**Figure 19** in volume 1)

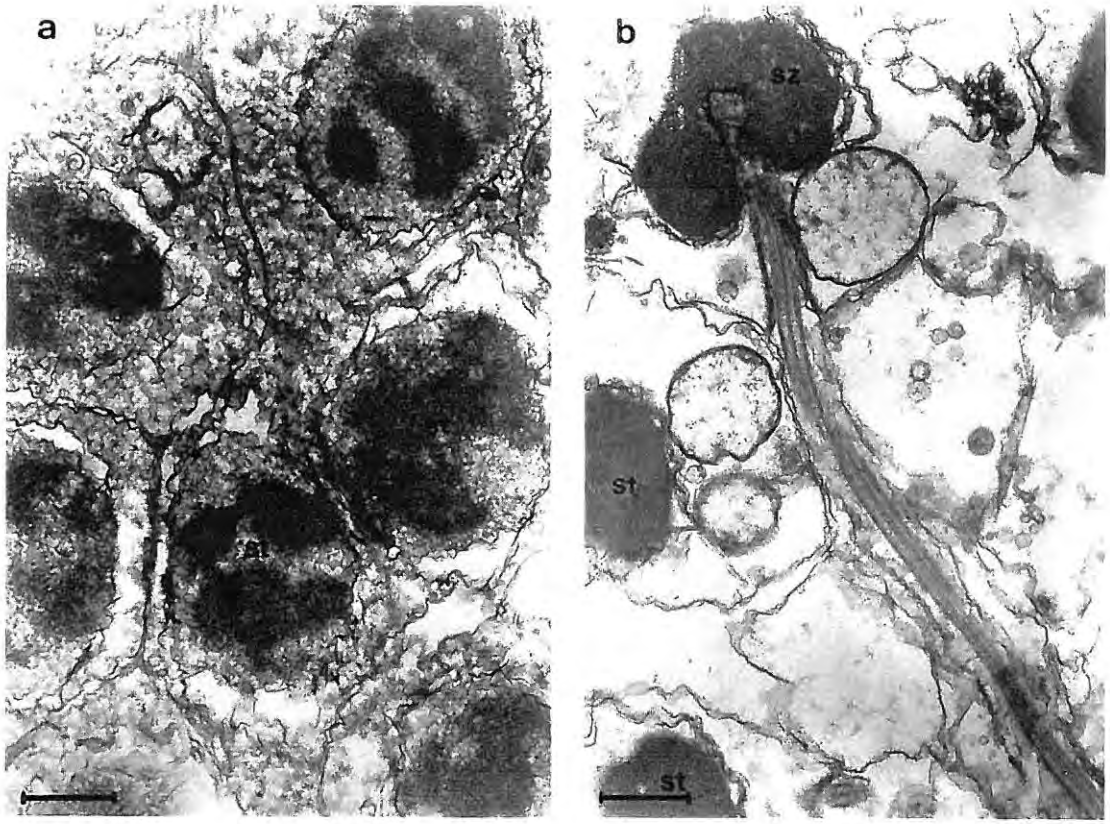

a
st
b
sz
st
st

Figure 20. Light micrographs of functional TYPE III (predominantly male) Rhabdosargus sarba gonads. **(a,b,c)** anterior, medial and posterior sections. Spermatogenic activity is limited in anterior regions of male elements **(d)** as compared to posterior regions **(e)**. cc = central cavity, l = ovigorous lamellae, sc = spermatocytes, sd = sperm duct, sg = spermatogonia, sz = spermatozoa, ss = sperm sinuses. Scale bar in **(a-c)** = 500μm, in **(d,e)** = 25μm.

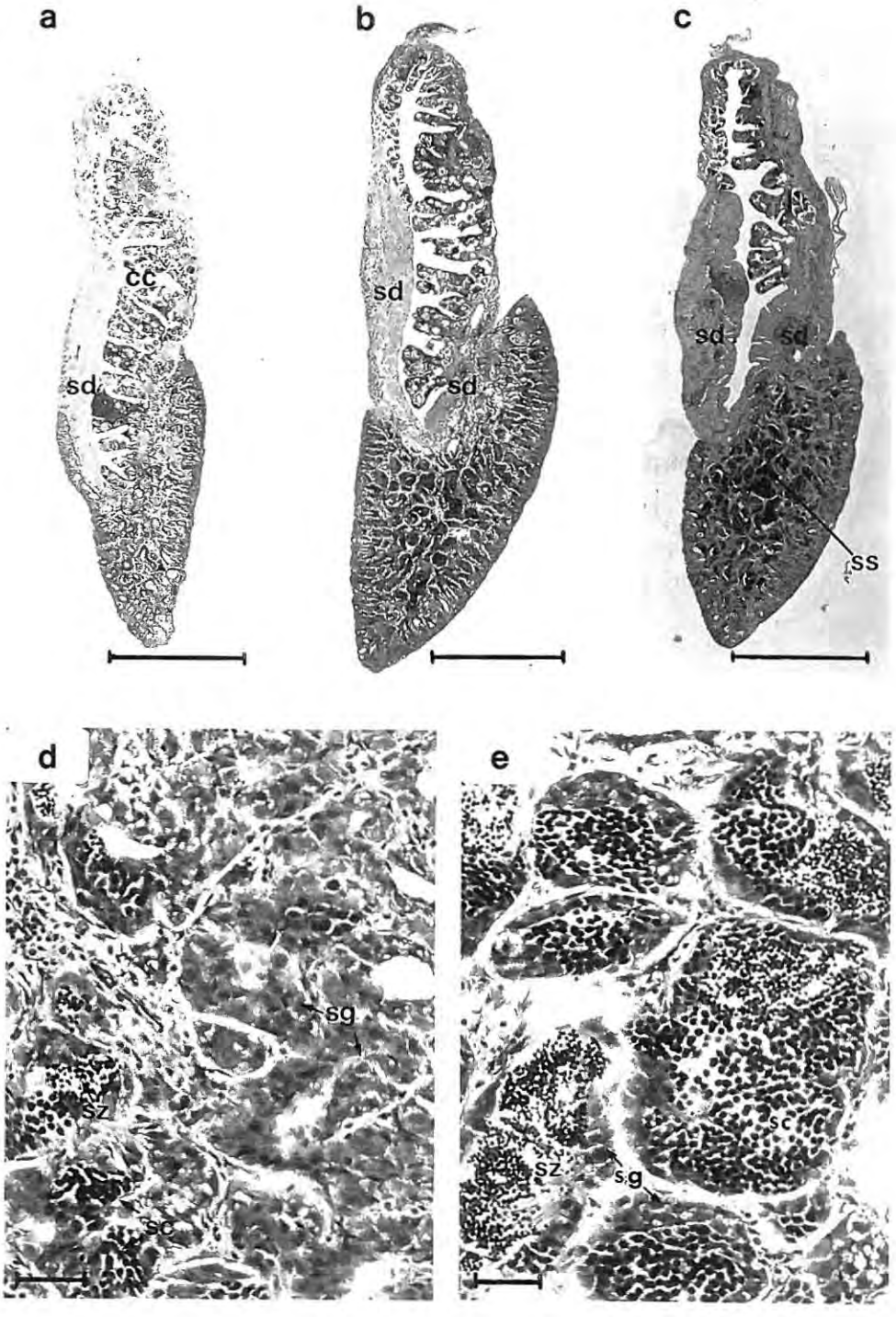

a
b
c
cc
sd
sd
sd
sd
sd
ss
d
e
sg
sz
sc
sz
sg
sc

Figure 21. (**a-d**) - Medial sections of Rhabdosargus sarba TYPE III (predominantly male); (**e**) - TYPE V (female); and (**f,g**) - TYPE IV (predominantly female) gonads showing typical configurations in the length class 150-199mm. (**h**) all stages of spermatogenesis were evident in the male elements of TYPE IV (predominantly female) gonads. cc = central cavity, g = gonia, l = ovigerous lamellae, m = mesovarium, ov = ovary, p.ooc = previtellogenic oocytes, sd = sperm duct, sc = spermatocytes, sg = spermatogonia, ss = sperm sinuses, sz = spermatozoa, t = testis. Scale bars in (**a-d**) & (**f,g**) = 250µm, (**e**) = 100µm, (**h**) = 25µm.

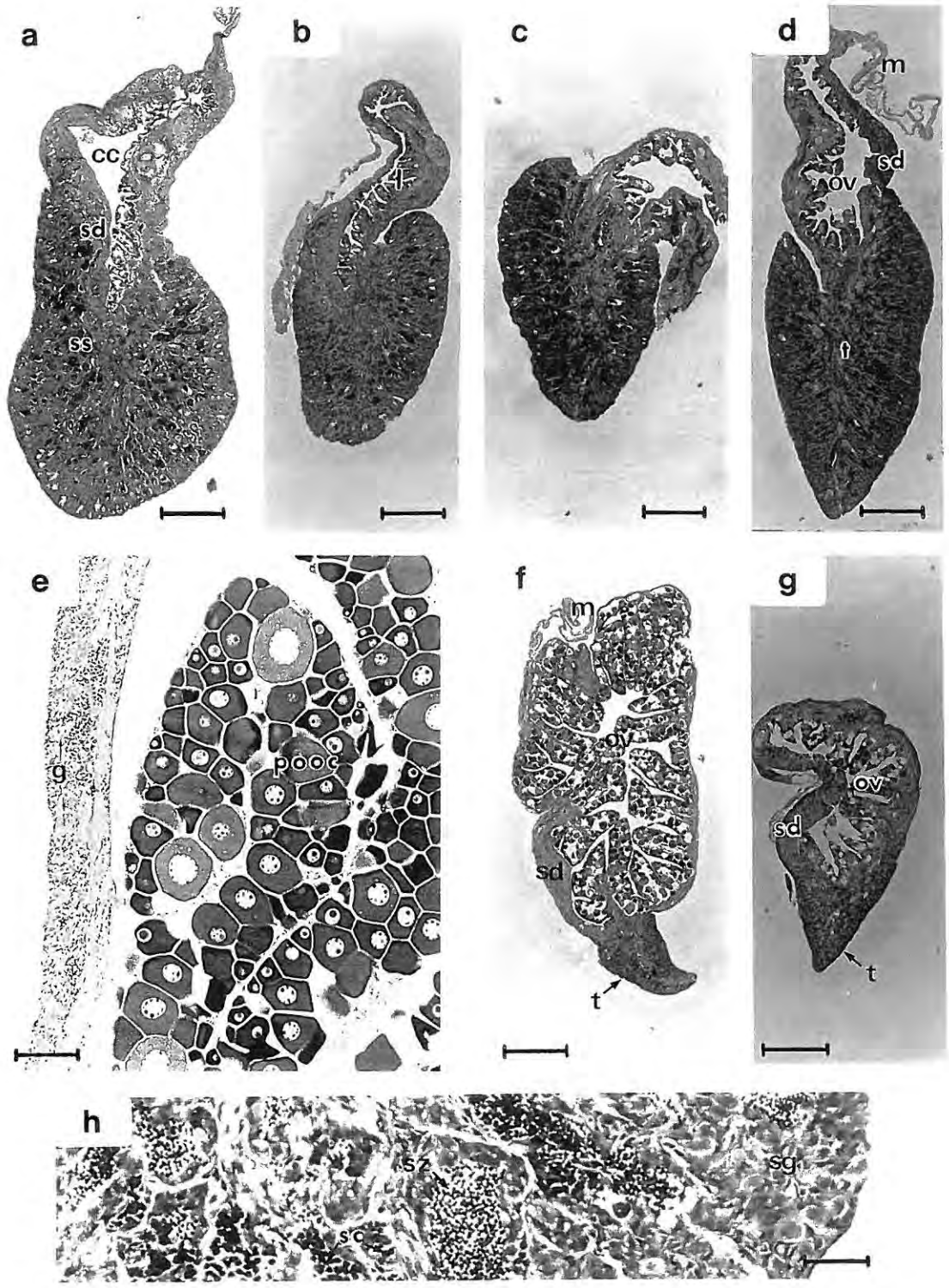

a
cc
sd
ss
b
c
d
m
sd
ov
f
e
g
pooc
f
m
ov
sd
t
g
ov
sd
t
h
sz
sg
sc

Figure 22. Anterior (**a**), medial (**b**) and posterior (**c**) sections of a TYPE IV (predominantly female) gonad from a Rhabdosargus sarba of 177mm (FL), showing the relative size of male and female elements and extensive degeneration of spermatogenic tissue (with increased vascular supply) in anterior (**d**) and posterior (**e**) regions of the male element. bv = blood vessels, dsg = degenerating spermatogenic tissue, ov = ovary, pvo = previtellogenic oocytes, sd = sperm duct, t = testis. Scale bars in (**a-c**) = 250μm, (**d,e**) = 25μm.

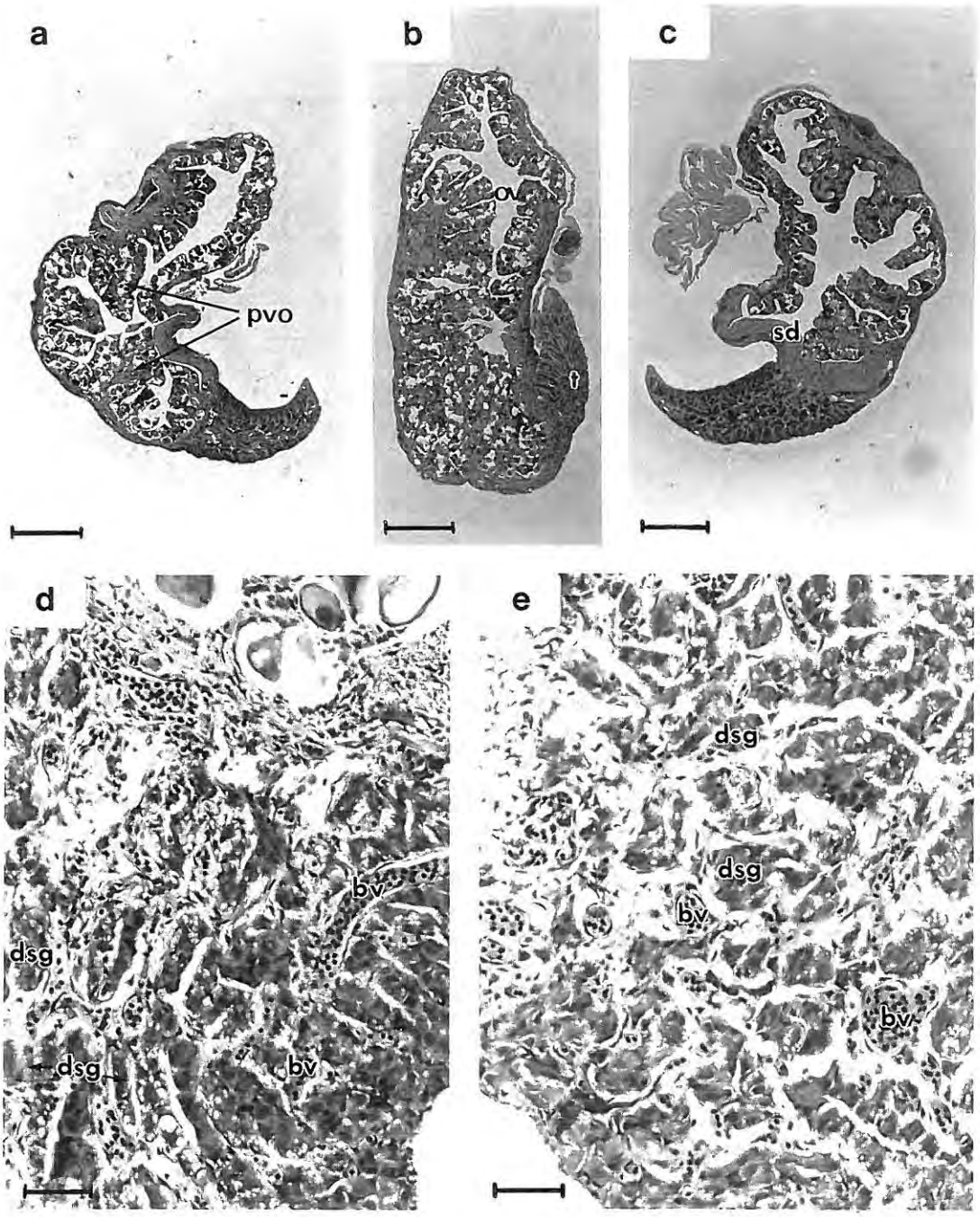

a
pvo
b
ov
c
sd
d
bv
dsg
dsg
bv
e
dsg
dsg
bv
bv

Figure 23. Anterior (**a**), medial (**b**) and posterior (**c**) sections of a TYPE III (predominantly male) gonad from a Rhabdosargus sarba of 215mm (FL). (**d**) - extensive degeneration of male tissue after spawning; (**e**) - 'normal' degeneration of testicular tissue after spawning. dsg = degenerating spermatogenic tissue, ov = ovary, ph = cells in phagocytic activity, sc = spermatocytes, sd = sperm duct, sg = spermatogonia, sz = spermatozoa. Scale bars in (**a-c**) = 250μm, in (**d,e**) = 25μm.

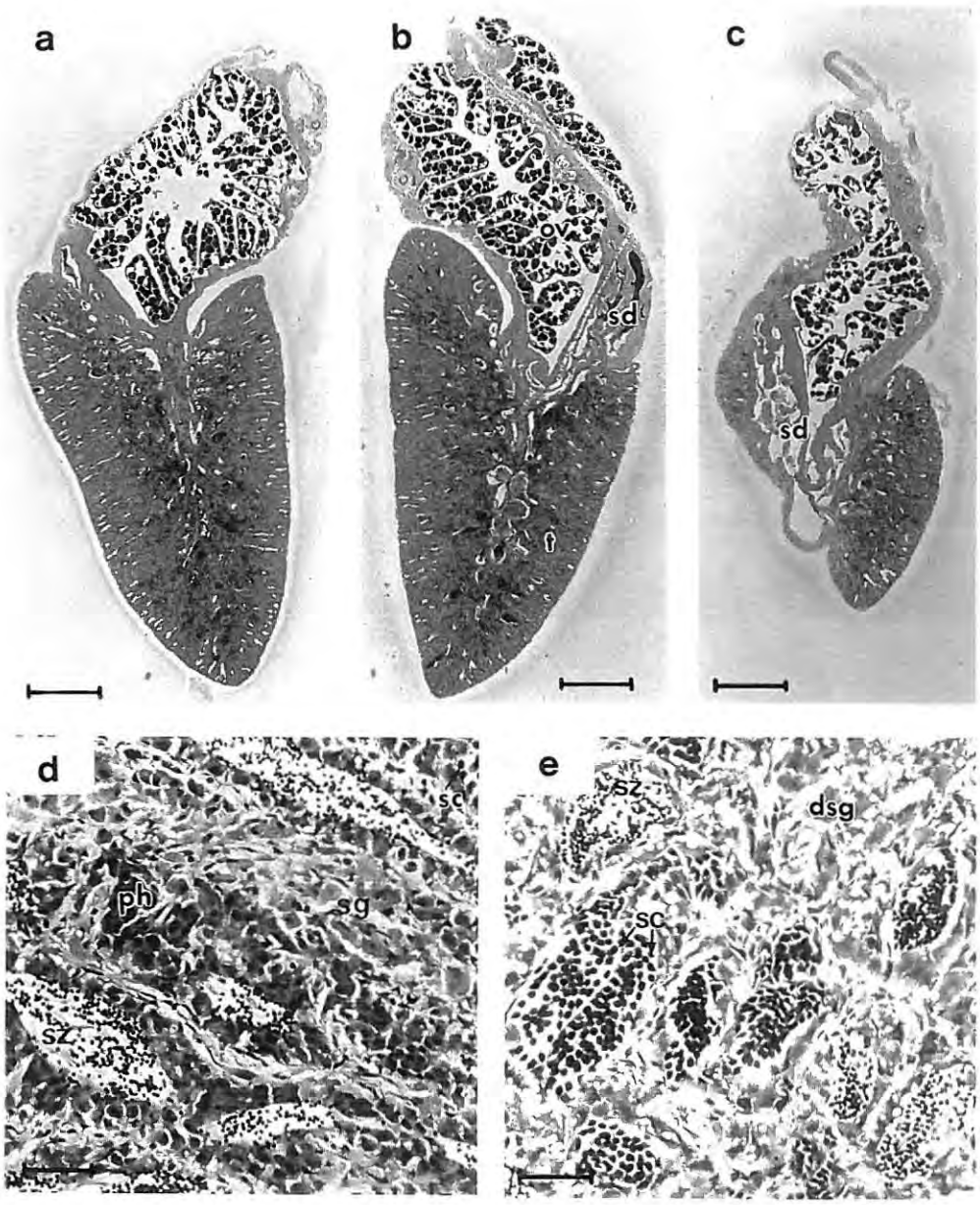

a
b
ov
sd
c
sd
d
sc
ph
sg
sz
e
sz
dsg
sc

Figure 24. Anterior (**a**), medial (**b**) and posterior (**c**) sections of a TYPE IV (predominantly female) gonad from a Rhabdosargus sarba of 229mm (FL) in post-spawning condition, showing a reduced and convoluted male element. (**d**) - development of oocytes in the female element restricted to the previtellogenic stage. (**e**) - recovering male element with spermatogonia proliferating. ov = ovary, pno = previtellogenic oocytes, s = sperm duct, sg = spermatogonia, t = testis. Scale bars in (**a-c**) = 250µm, in (**d,e**) = 50µm.

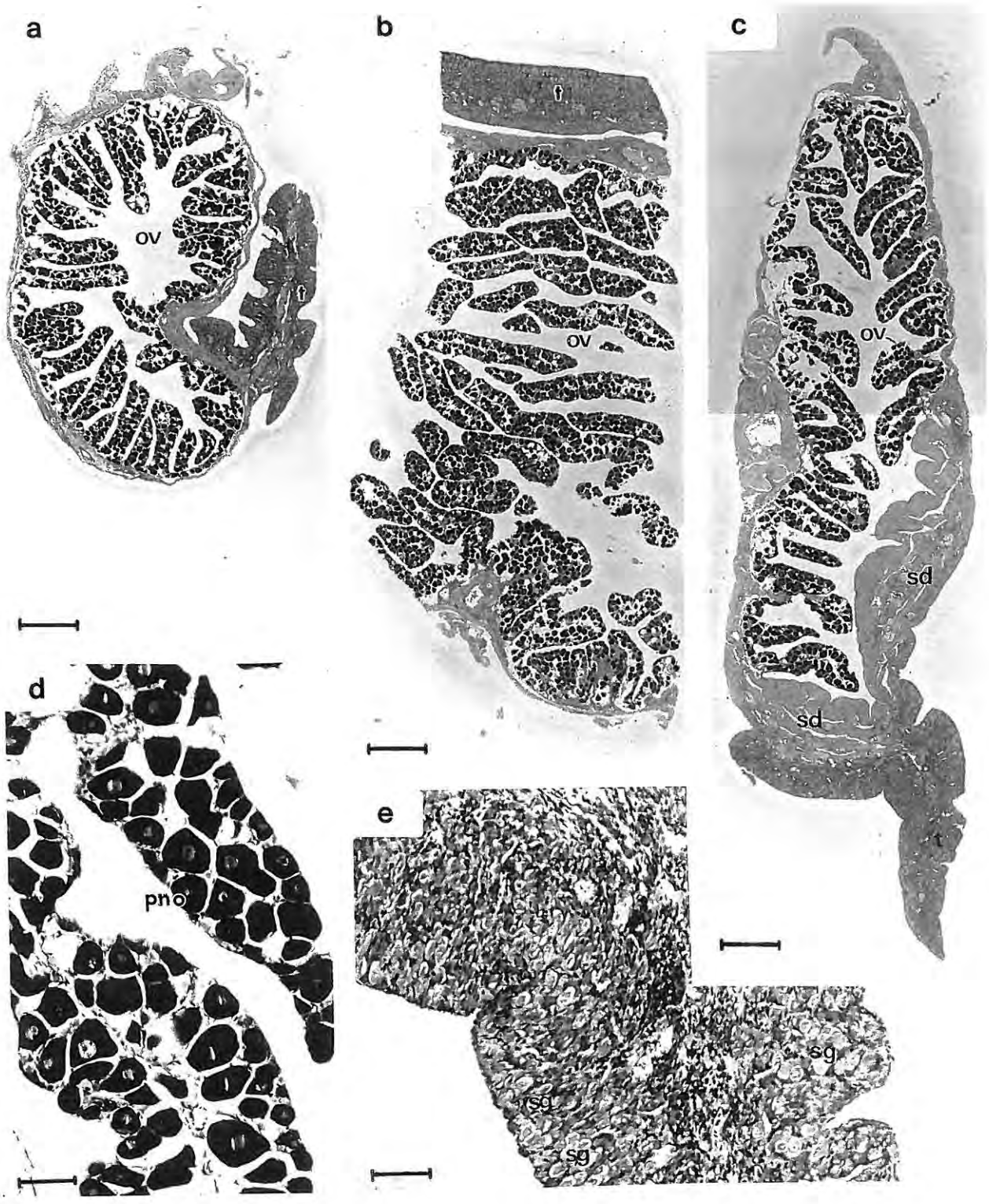
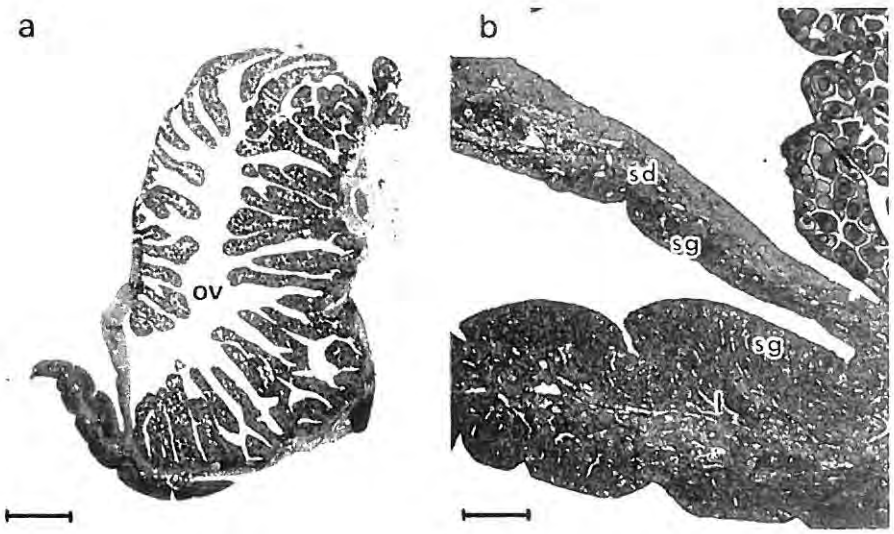

a
ov
b
ov
c
ov
sd
sd
d
pno
e
sg
sg
sg

Figure 25. Medial section of a TYPE IV (predominantly female) gonad from a Rhabdosargus sarba of 241mm (FL), showing (**a**) a male remnant with two 'compressed' lobes and (**b**) a sperm duct which is closed and which contains gonial cells. l = lacunae, ov = ovary, sd = sperm duct, sg = spermatogonia, t = testis. Scale bar in (**a**) = 500µm, in (**b**) = 100µm.

Figure 26. Medial section of a TYPE V (female) gonad from a R. sarba of 246mm (FL), showing gonial cells in the thickened tunica albuginia below the sterile zone. g = gonial cells, p.ooc = previtellogenic oocytes, ta = tunica albuginea. Scale bar = 50µm.


a
ov
b
sd
sg
sg

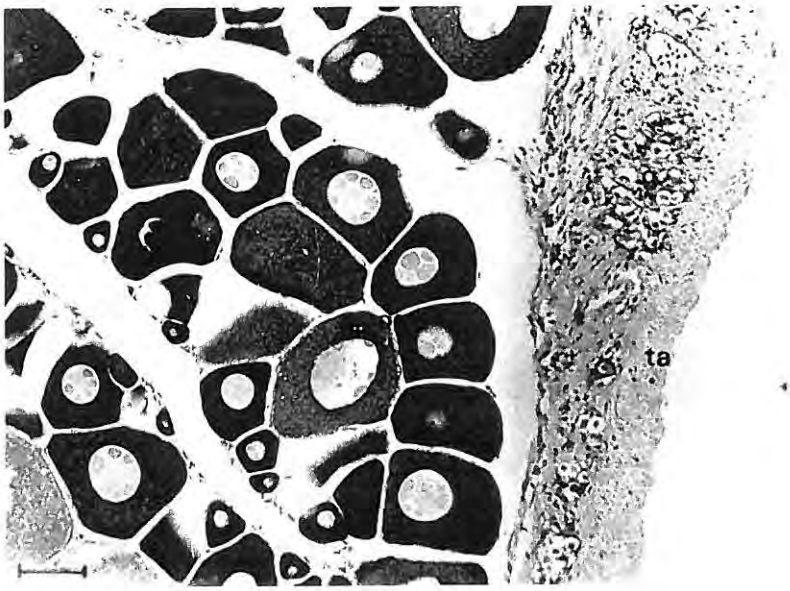

ta

Figure 27. Medial sections of: **(a)** Rhabdosargus sarba TYPE III (predominantly male) and **(b)** TYPE IV (predominantly female) gonads showing typical morphology in the length classes 250-449mm. **(c)** - Gonial cells in the tunica albuginia of a ripe TYPE V (female) gonad containing oocytes in all stages of vitellogenesis **(d)**. cao = cortical alveolar oocyte, g = gonial cells, ov = ovary, pvo = previtellogenic oocyte, sd = sperm duct, ta = tunica albuginea, t = testis, ta = tunica albuginea, cao = cortical alveolar oocyte, 2°YO = secondary yolk oocyte, 3°YO = tertiary yolk oocyte. Scale bars in **(a)** = 250µm, **(b)** = 500µm, **(c)** = 25µm, **(d)** = 100µm.

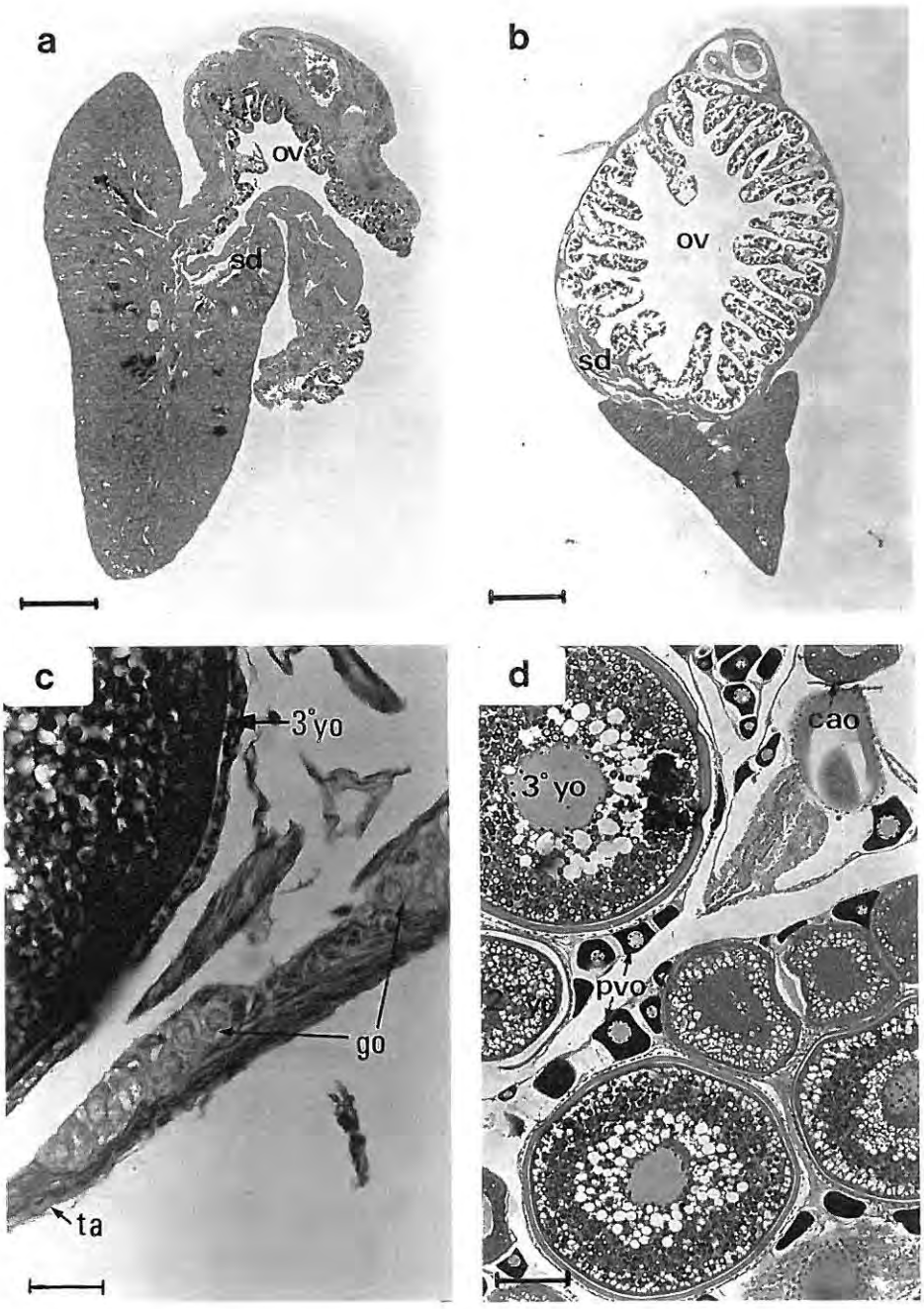

a
ov
sd
b
ov
sd
c
3°yo
go
ta
d
cao
3°yo
pvo

Figure 28. Anterior (**a**), medial (**b**) and posterior (**c**) sections of a TYPE IV (predominantly female) gonad from a Rhabdosargus sarba of 385mm (FL), showing a 'compressed', but functional, male element along the length of the gonad. (**d**) all stages of spermatogenesis were evident in the male element. ov = ovary, pno = previtellogenic oocytes, sc = spermatocytes, sd = sperm duct, sg = spermatogonia, sz = spermatozoa, tf = 'flattened testis'. Scale bars in (**a-c**) = 500µm, (**d**) = 25µm.

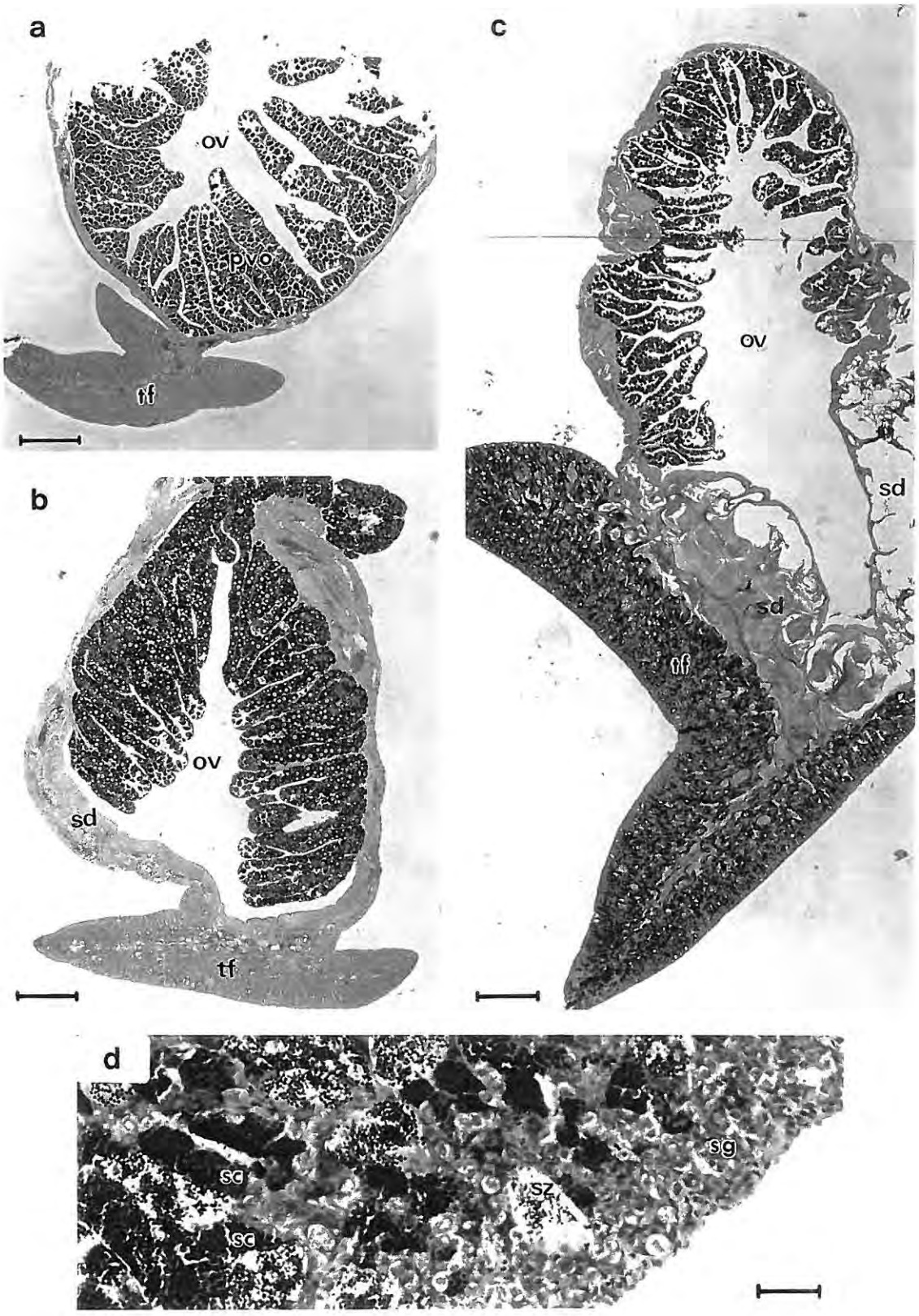

a
ov
pvo
tf
b
ov
sd
tf
c
ov
sd
sd
tf
d
sc
sc
sz
sg

Figure 29. Medial sections of TYPE IV (predominantly female) gonads from two Rhabdosargus sarba, both of 340mm FL, in post-spawning condition, showing gonial cells proliferating in the 'compressed' male element of one **(b)** and extensive degeneration in the other **(d)**. bv = blood vessel, l = lacunae, ov = ovary, ph = cells in phagocytic activity, pvo = previtellogenic oocytes, sd = sperm duct, sg = spermatogonia, sz = spermatozoa, tc = 'compressed' testis. Scale bars in **(a&c)** = 500µm, **(b)** = 25µm, **(c)** = 50µm.

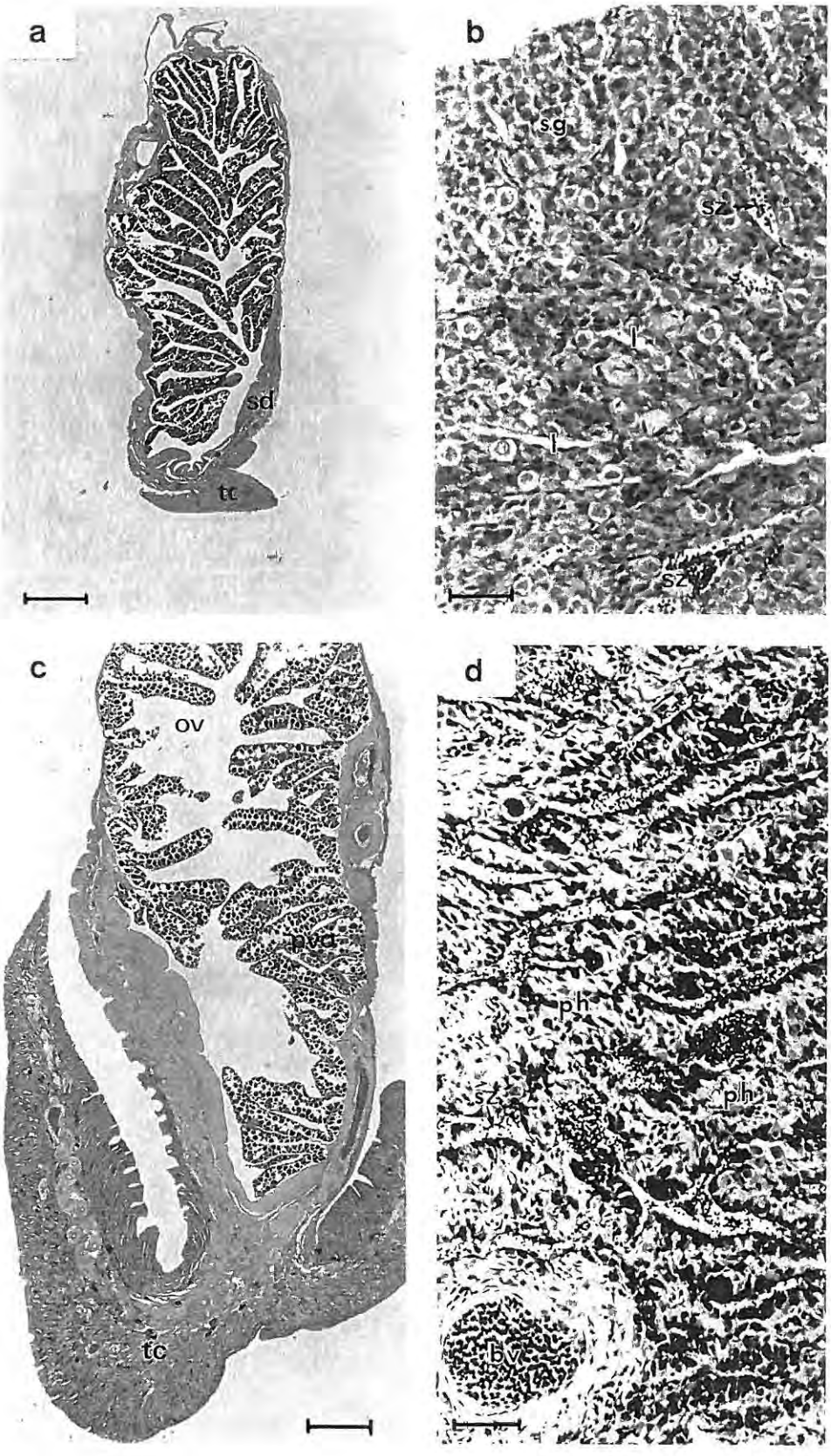
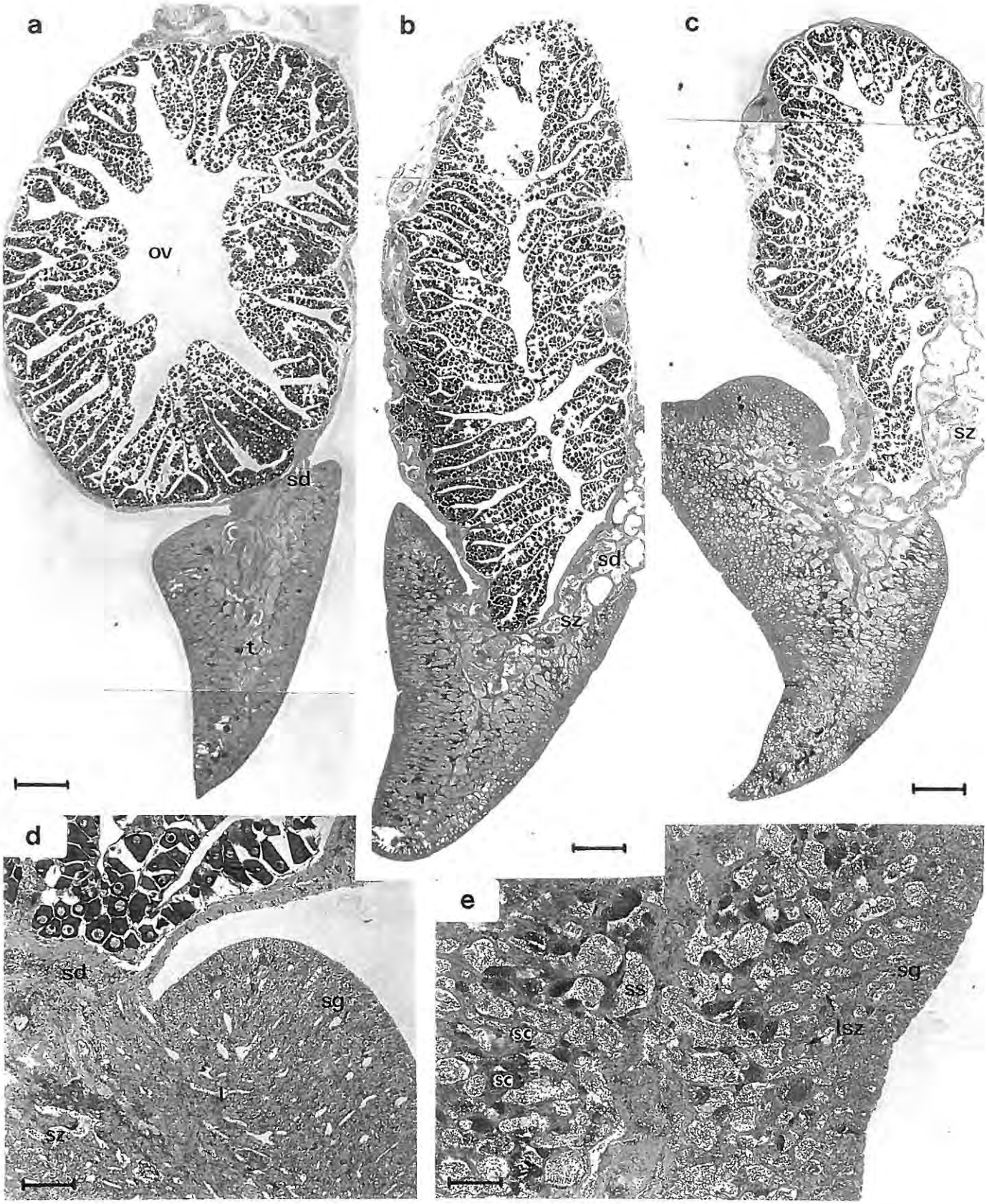

a
sd
tc
b
sg
sz
l
l
sz
c
ov
pvd
tc
d
ph
sz
ph
bv

Figure 30. Anterior (**a**), medial (**b**) and posterior (**c**) sections of a TYPE IV (predominantly female) gonad from a Rhabdosargus sarba of 415mm (FL), showing a functional male element beginning to 'flatten' in the posterior region. Oocytes throughout the female element are arrested in the previtellogenic stage. (**d**) - empty lacunae in the anterior region suggests a partially spawned condition. (**e**) - all stages of spermatogenesis are evident in the posterior region. l = lacunae, lsz = lobules of spermatozoa, ov = ovary, pno = previtellogenic oocytes, sc = spermatocytes, sd = sperm duct, sg = spermatogonia, ss = sperm sinuses, sz = spermatozoa. Scale bars in (**a-c**) = 500µm, (**d&e**) = 100µm.


a
ov
sd
t
b
sd
sz
c
sz
d
sd
sg
t
sz
e
ss
sc
sc
sg
lsz

GONADAL DEVELOPMENT IN ACANTHOPAGRUS BERDA

Figure 31. Transverse sections of developing gonadal primordia in Acanthopagrus berda: **(a)** - the mid-region of the body cavity of a 20mm (FL) fish, showing a developing primordium suspended from the dorsal abdominal wall. **(b-d)** anterior, medial and posterior sections of an undifferentiated primordium from a fish of 45mm (FL). ab = air bladder, bv = blood vessel, i = intestine, l = lining of body cavity, m = muscle, me = mesovarium, p = gonadal primordium. Scale bar in **(a)** = 50μm, in **(b-d)** = 25μm.

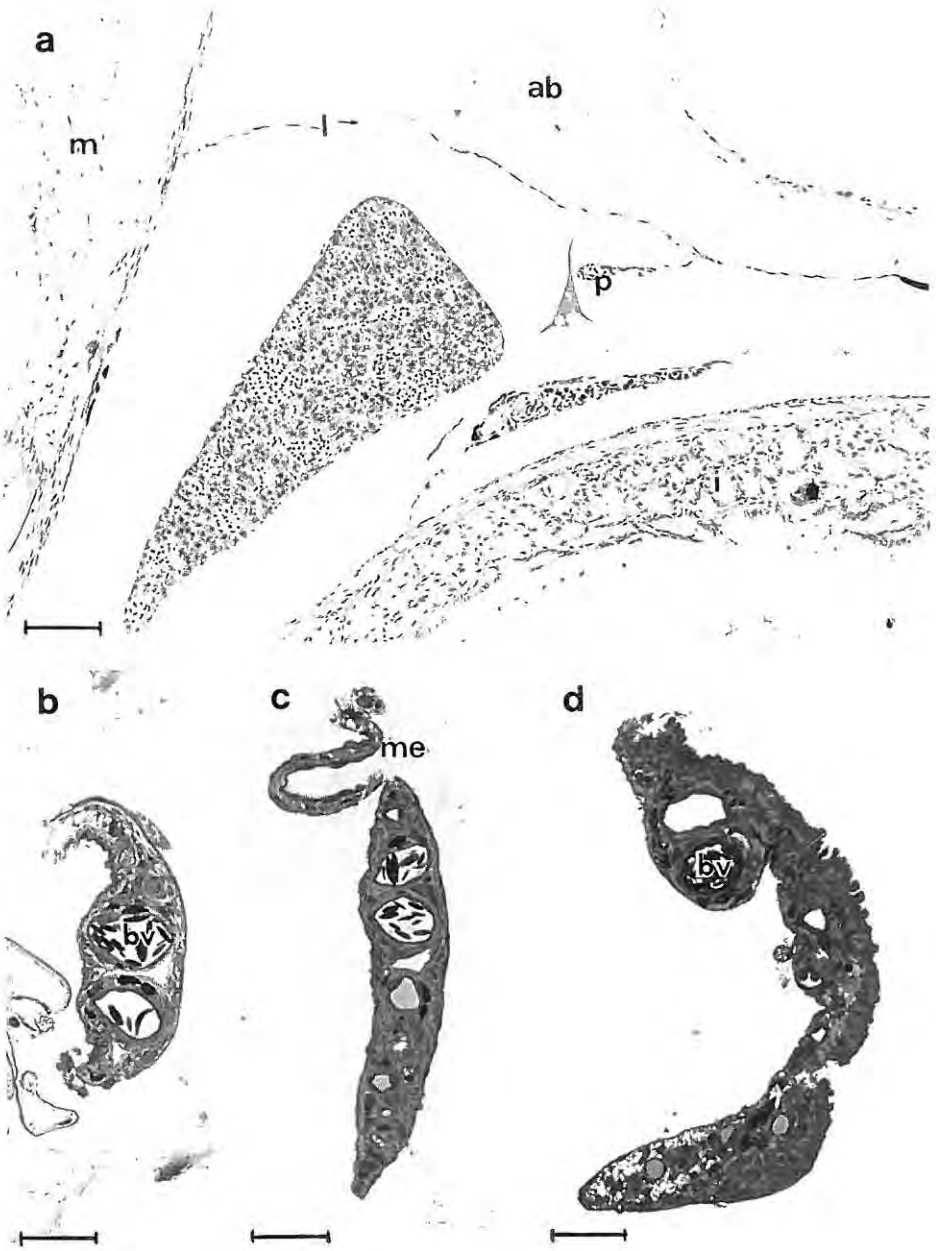

a
ab
m
p
l
b
bv
c
me
d
bv

Figure 32. Electron micrographs of cells comprising the gonadal primordia of Acanthopagrus berda in the length class 0-49mm (FL). **(a)** somatic cells, loose connective tissue and fibrillar tissue formed the matrix of the early gonadal primordia. **(b)** somatic cells and clusters of granulocytes in dorsal regions. **(c)** cluster of pigment granules near the major blood vessel. **(d)** vacuolated cell in the ventral region. ct = connective tissue, f = fibrillar tissue, g = granulocytes, p = pigment granules, s = somatic cells, v = vacuolated cell. Scale bar = 2µm.

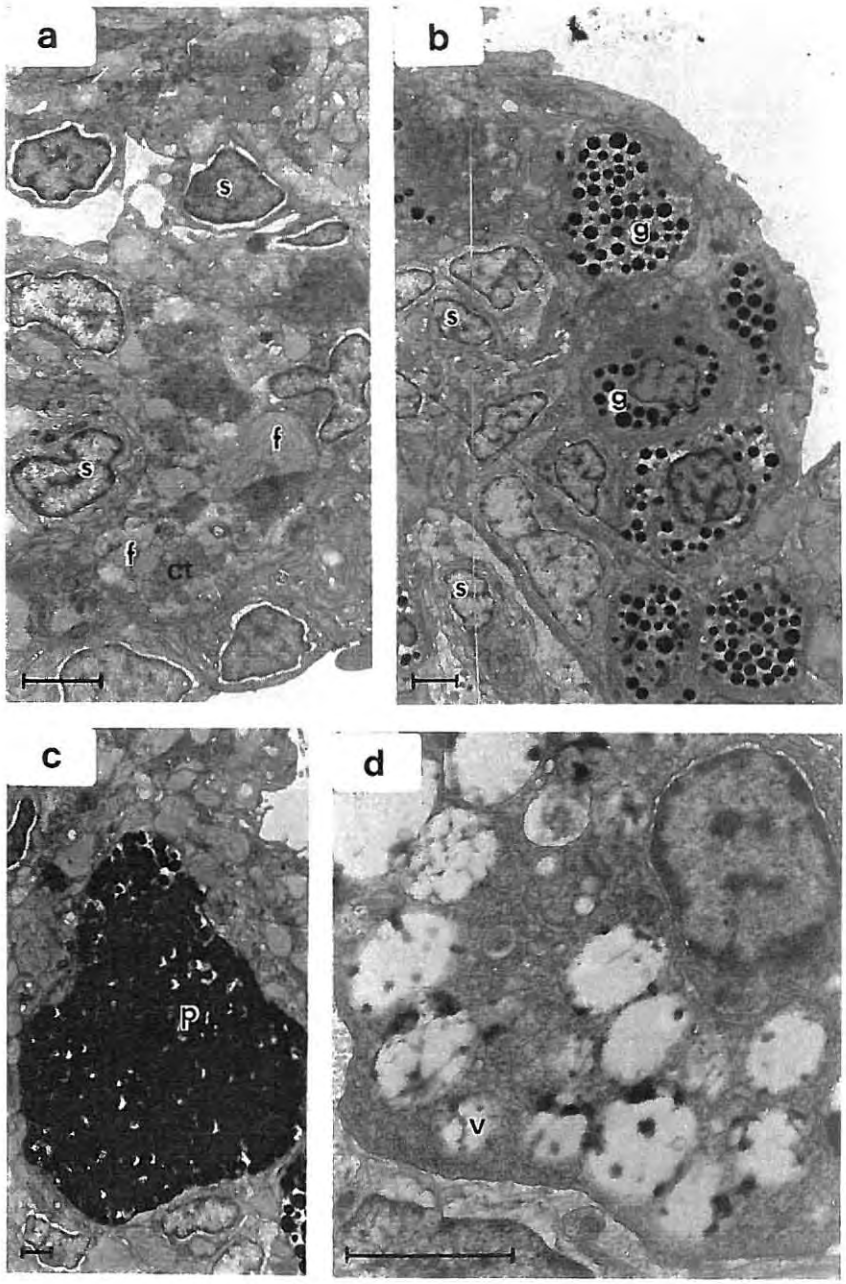

a
s
f
s
f
ct
b
g
s
g
s
c
P
d
v

Figure 33. Transverse sections of pairs of undifferentiated Acanthopagrus berda gonadal primordia, showing formation of the central cavity through the growth of a second (lateral) extension ventrally alongside the first (medial) element, and the asymmetric development of left and right lobes. **(a)** A. berda 56mm. **(b)** A. berda 64mm, and **(c)** A. berda 78mm. cc = central cavity, 1st e = first extension of gonadal primordium, 2nd e = second extension, l = lining of body cavity, m = mesovarium. Scale bar in **(a)** = 25μm, in **(b,c)** = 50μm.

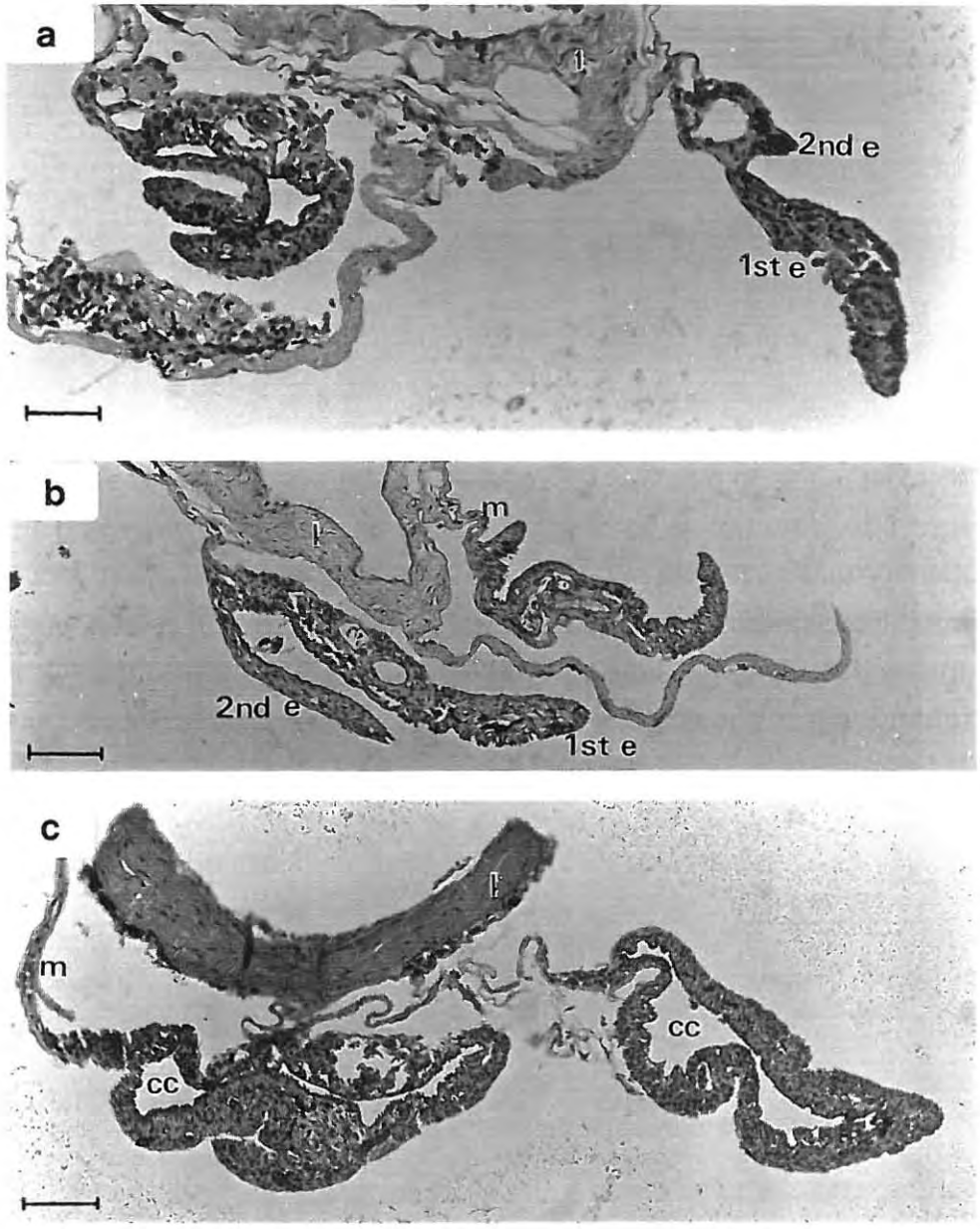
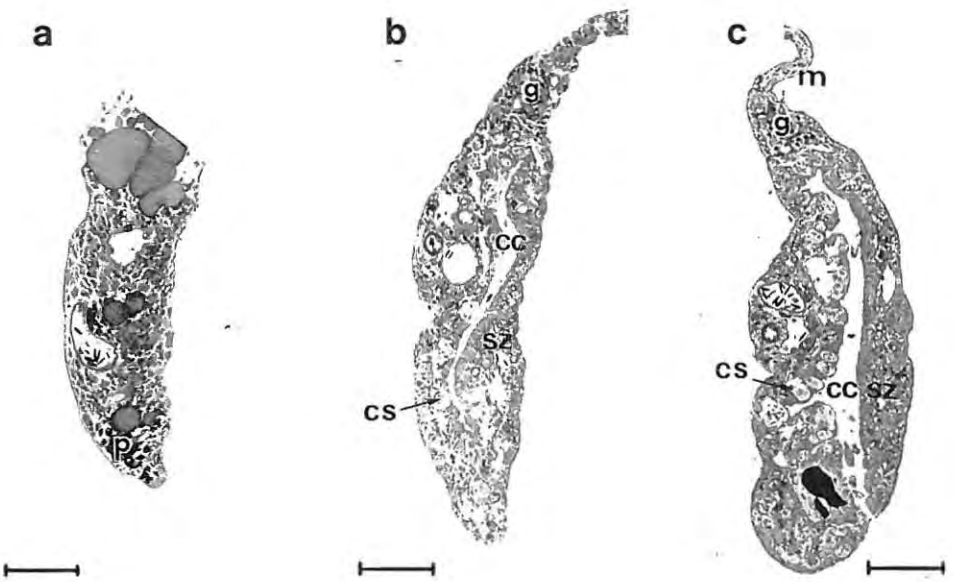

a
l
2nd e
1st e
b
m
l
2nd e
1st e
c
l
m
cc
cc

Figure 34. Anterior **(a)**, medial **(b)** and posterior **(c)** sections of an undifferentiated gonadal primordium from an Acanthopagrus berda of 63mm (FL), showing a newly formed central cavity, sterile outer element, and cysts of less-electron-dense cells forming along the margins of the central cavity, opposite the sterile zone. cc = central cavity, g = granulocytes, m = mesovarium, p = pigment granules, cs = cysts of less-electron-dense cells, sz = sterile zone. Scale bar = 50µm.

Figure 35. Anterior **(a)**, medial **(b)** and posterior **(c)** sections of a differentiating gonad from an Acanthopagrus berda of 90mm (FL), showing a fully formed central cavity. Male and female presumptive areas with a number of less-electron-dense cysts of cells and the first gonial cells. In anterior and medial regions female germinal tissue is separated from the stroma by lacunae. cc = central cavity, cs = cyst of less-electron-dense cells, fe = female element, go = gonial cells, l = lacunae, me = male element. Scale bar = 100µm.


a
b
c
g
cc
sz
cs
m
g
cc
sz
cs
p

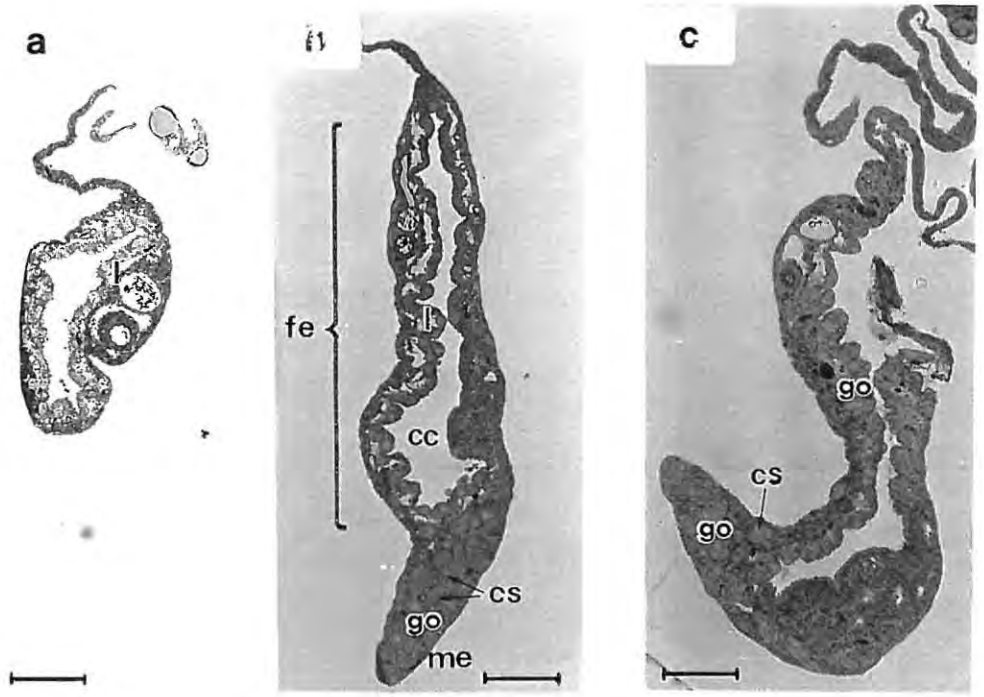

a
b
c
l
fe
l
cc
cs
go
me
go
cs
go

Figure 36. Electron micrographs of cells comprising undifferentiated gonadal primordia of Acanthopagrus berda in the length class 50-99mm. **(a)** - anterior regions were comprised of somatic cells, loose connective tissue and large aggregates of pigment granules, **(b)** - the sterile zone consisted mostly of fibrillar and loose connective tissue, **(c)** - a cluster of pigment granules amongst somatic cells and fibrillar tissue in the sterile zone, **(d)** - a cyst of less-electron-dense cells on the margin of the central cavity. cs = cyst of less-electron-dense cells, ct = connective tissue, f = fibrillar tissue, nu = nucleus, p = pigment granules, s = somatic cell. Scale bar = 2μm.

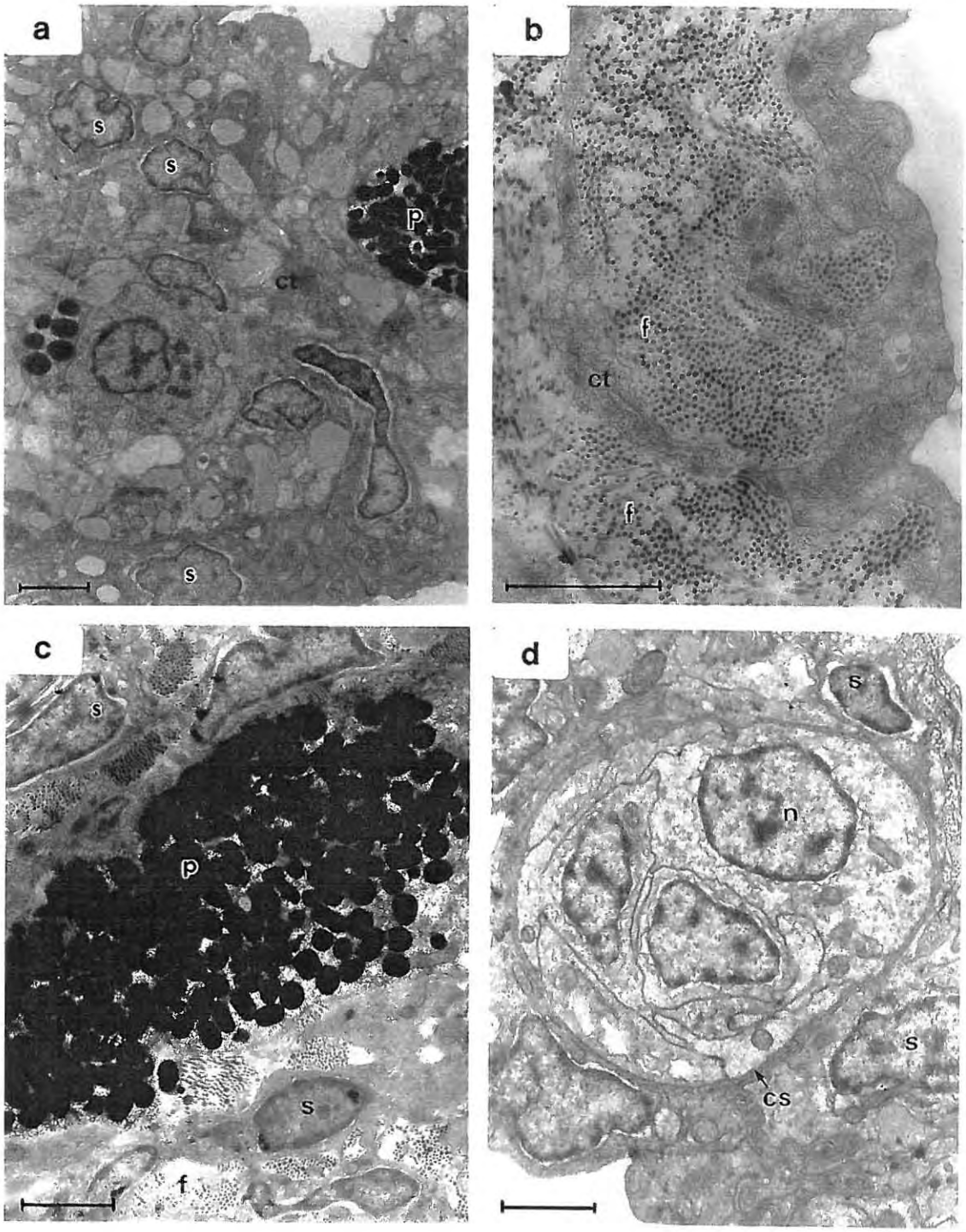

a
s
s
p
ct
s
b
f
ct
f
c
s
p
s
f
d
s
n
s
cs

Figure 37. Electron micrographs of cells found in early differentiating gonads of Acanthopagrus berda in the length class 50-99mm. **(a)** - cysts of less-electron-dense cells along the margins of the central cavity, **(b)** - the stroma in this region consisted mainly of granulocytes, vacuolated cells and fibrillar tissue, **(c)** - a cyst of less-electron-dense cells in the presumptive male region, **(d)** - a gonial cell in the presumptive female region. bv = blood vessel, c ='ciment', cs = cyst of less-electron-dense cells, f = fibrillar tissue, g = granulocyte, go = gonial cell, m = mitochondria, n ='nuage', nm = nuclear membrane, nu = nucleus, s = somatic cells, v = vacuolated cell. Scale bar = 2μm.

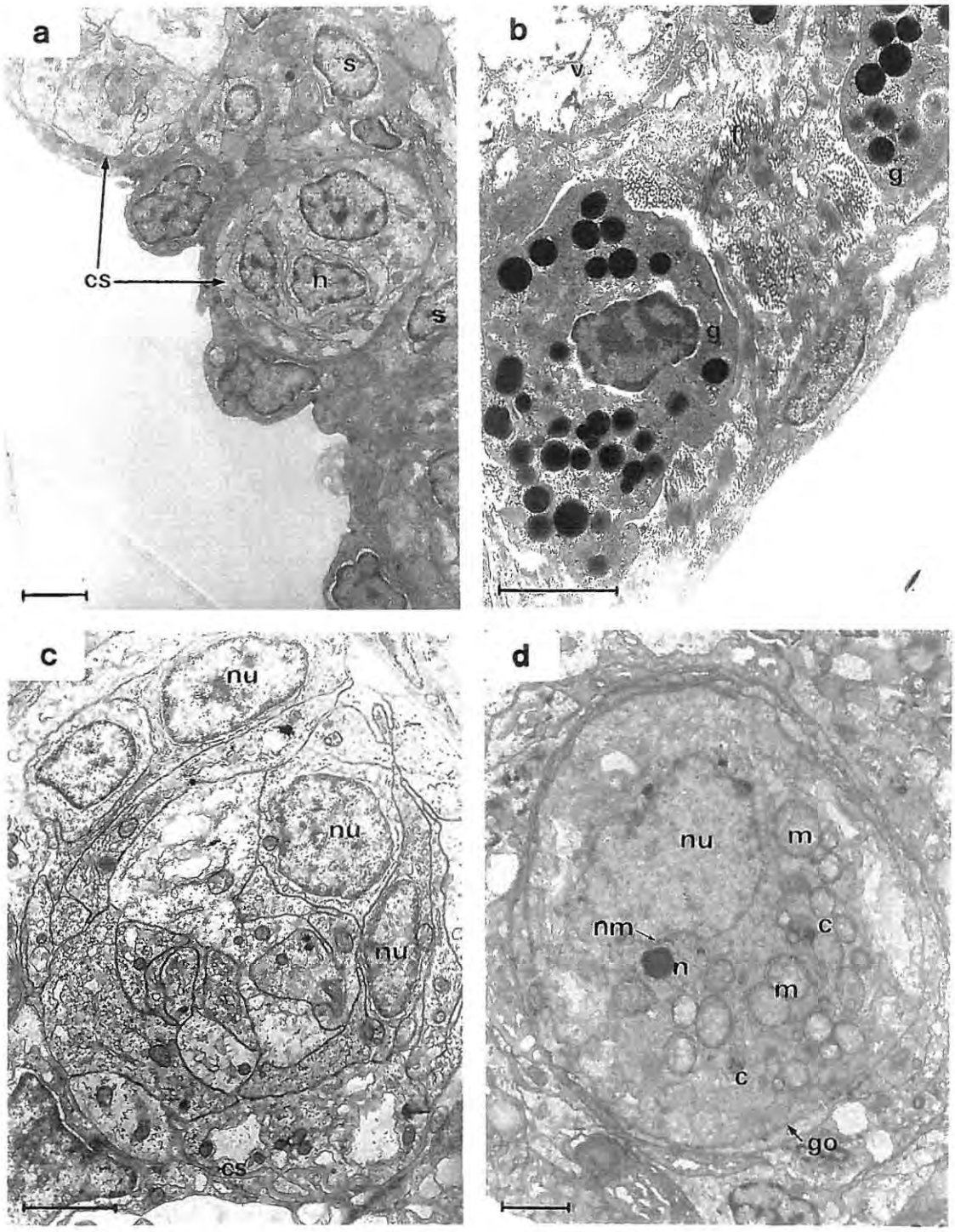
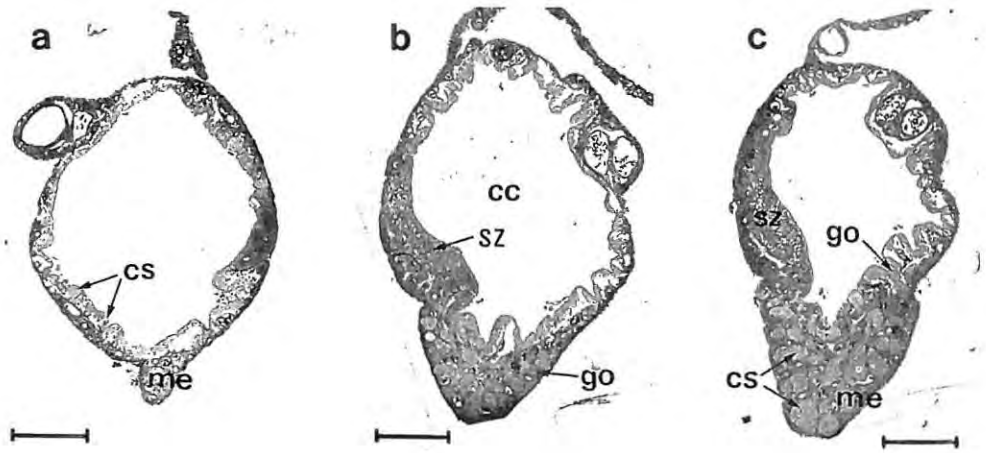

a
s
cs
n
s
b
v
f
g
g
c
nu
nu
nu
cs
d
nu
m
nm
c
n
m
c
go

Figure 38. Anterior (a), medial (b) and posterior (c) sections of an undifferentiated gonad from an Acanthopagrus berda of 104mm (FL), showing a large central cavity bordered by a sterile zone on one side and lamellae with large lacunae on the other, and a distinct presumptive male element in the ventral region. Numerous cysts of less-electron-dense cells are evident along the margins of the central cavity and in the stroma of the presumptive male element and the first gonial cells have appeared. cc = central cavity, cs = cyst of less-electron-dense cells, go = gonial cells, l = lacunae, me = male element, sz = sterile zone. Scale bar = 100µm.

Figure 39. Anterior (a), medial (b) and posterior (c) sections of an undifferentiated gonad from an Acanthopagrus berda of 115mm (FL), showing a more distinct male element but otherwise similar features to undifferentiated gonads of smaller fish. bv = blood vessel, cc = central cavity, cs = cysts of less-electron-dense cells, l = lacunae, me = male element, sz = sterile zone. Scale bar = 2µm.


a
b
c
cs
me
cc
sz
go
sz
go
cs
me

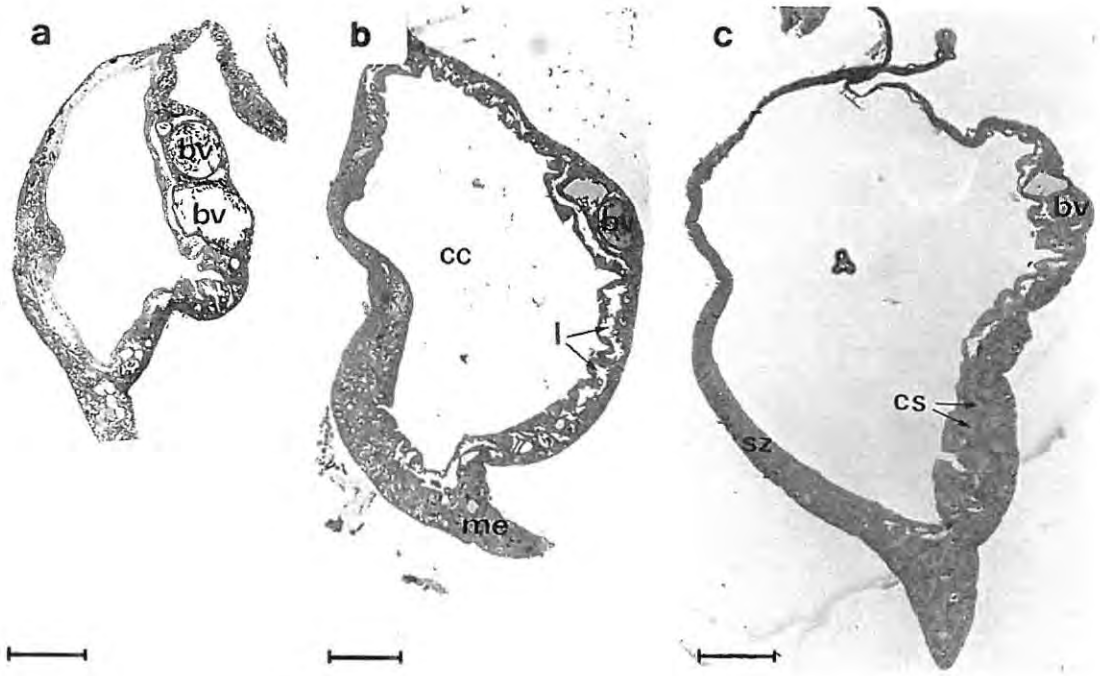

a
b
c
bv
bv
cc
bv
l
me
bv
cs
sz

Figure 40. Electron micrographs of cells comprising undifferentiated gonads of Acanthopagrus berda in the length class 100-149mm (FL). **(a)** - cysts of less-electron-dense cells along the margins of the central cavity, **(b)** - cell with prominent nucleolus in a cyst of less-electron-dense cells, **(c)** - gonial cell in loose connective tissue near the major blood vessel, **(d)** - follicle cells surrounding a gonial cell in presumptive female region. c = 'ciment', cc = central cavity, cs = cyst of less-electron-dense cells, ct = connective tissue, fc = follicle cell, go = gonial cell, m = mitochondria, ma = mitochondrial aggregate, nu = nucleus, nuc = nucleolus. Scale bar = 1µm.

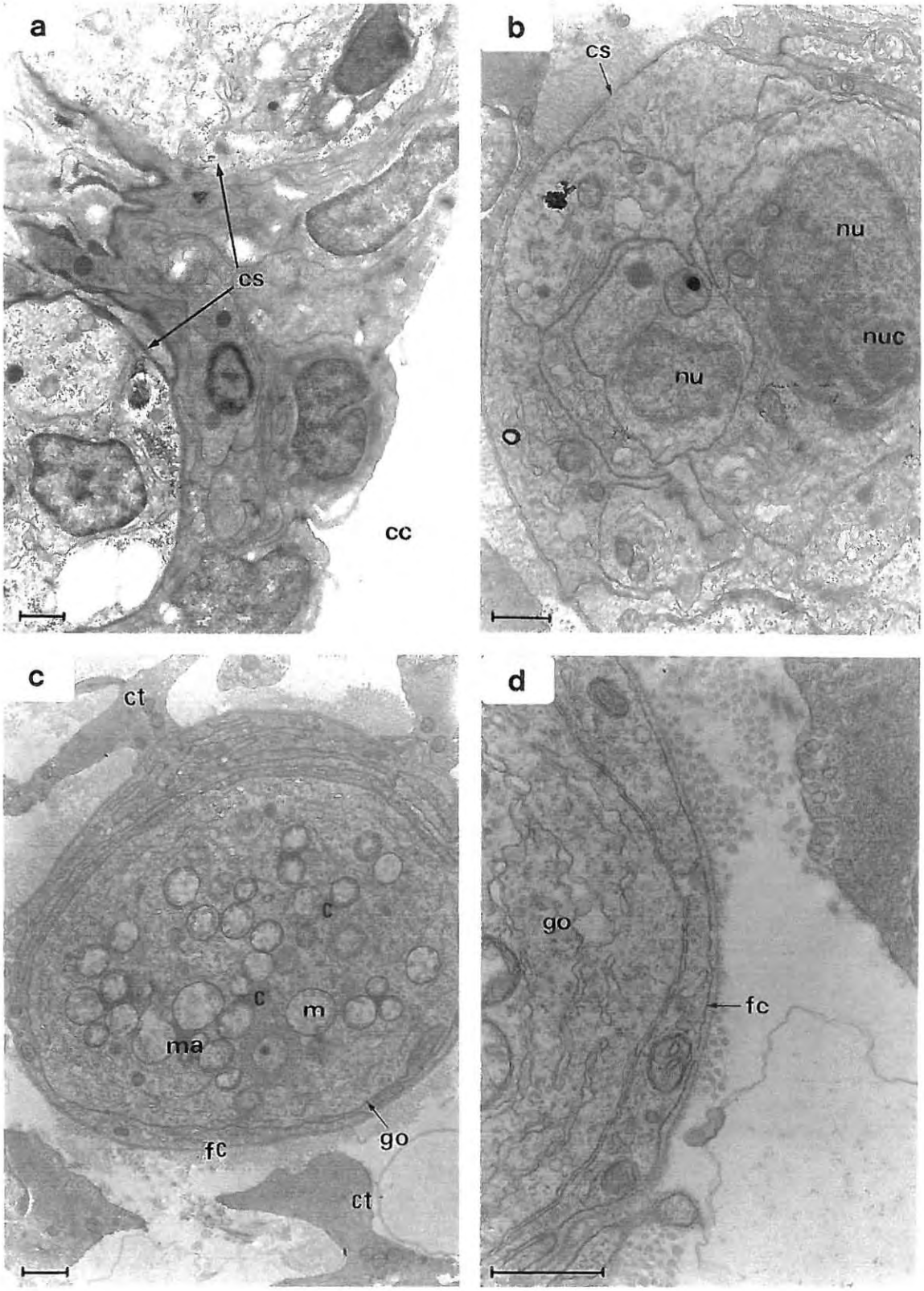

a
cs
cc
b
cs
nu
nuc
nu
c
ct
c
c
m
ma
fc
go
ct
d
go
fc

Figure 41. Anterior (**a**), medial (**b**) and posterior (**c**) sections of a differentiated gonad from an Acanthopagrus berda of 120mm (FL), showing the simultaneous proliferation of germ cells in male and female elements. (**d**) - gonial cells proliferating within nests in the female element, (**e**) - spermatozoa within a small lobule in the male element, (**f**) - primary spermatocytes and a spermatogonium. bv = blood vessel, cc = central cavity, epo = early previtellogenic oocyte, l = lacunae, lo = lobule, oog = oogonia, sc = spermatocytes, sd = sperm duct, sg = spermatogonia, sz = spermatozoa, szo = sterile zone. Scale bar in (**a-c**) = 100µm, in (**d-f**) = 2µm.

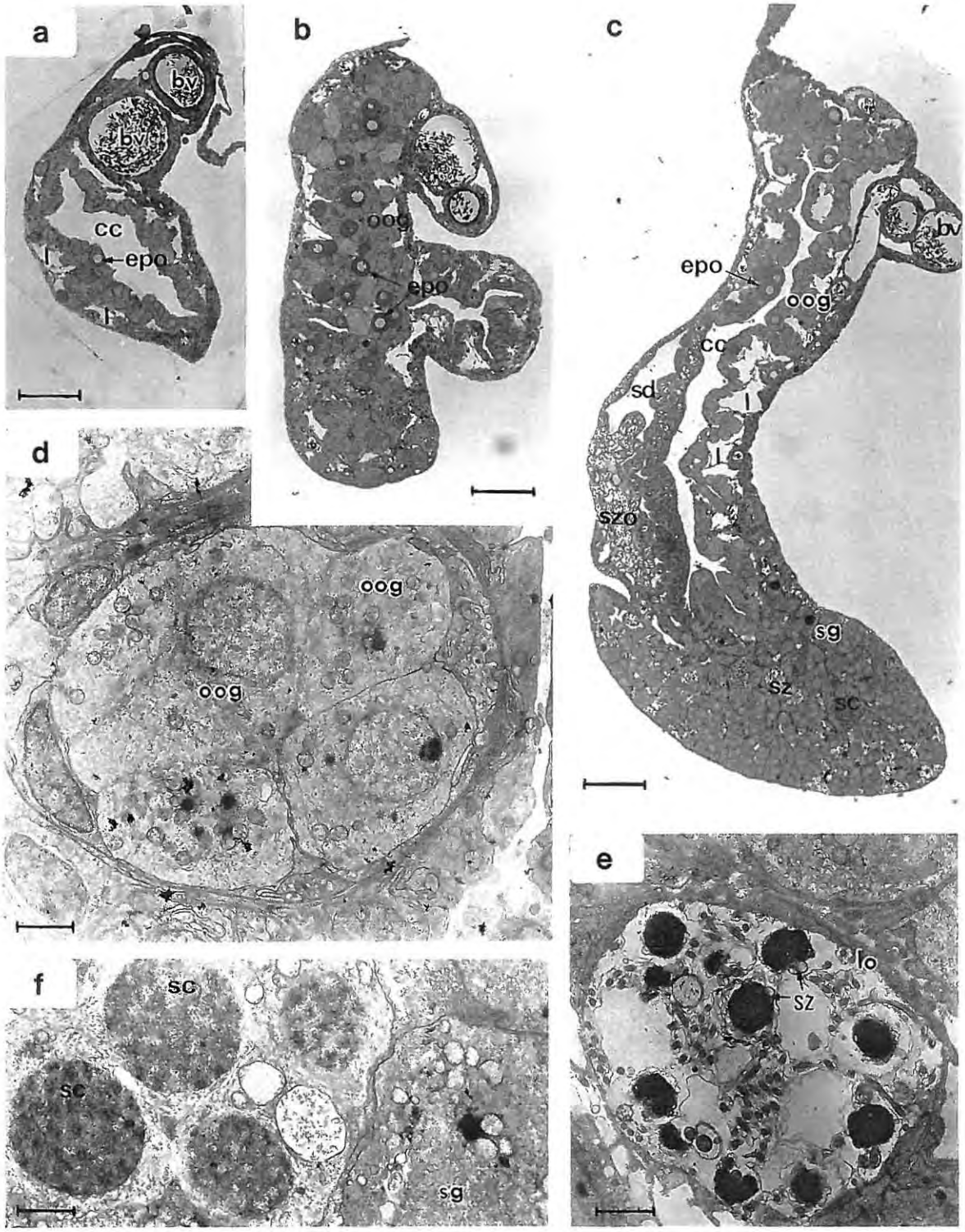

a
bv
bv
cc
epo
l
l
b
oog
epo
c
bv
epo
oog
cc
sd
l
lo
szo
sg
sz
sc
d
oog
oog
e
lo
SZ
f
sc
sc
sg

Figure 42. Electron micrographs of germ cells and associated Leydig cells in the differentiated gonad of an Acanthopagrus berda of 120mm (FL). **(a)** - oogonia along the margins of the central cavity proliferate, forming protrusions into the central cavity, **(b)** - a cell conforming to the general features of primordial germ cells, within a nest of oogonia, **(c)** - Leydig cells between spermatogenic lobules in the male element. bm = basement membrane, cc = central cavity, lc = Leydig cell, m = mitochondria, ne = nuclear envelope, nu = nucleus, nuc = nucleolus, oog = oogonia, pgc= primordial germ cell, ser = smooth endoplasmic reticulum, sg = spermatogonium. Scale bar = 2µm.

(**Figure 43** in volume 1)

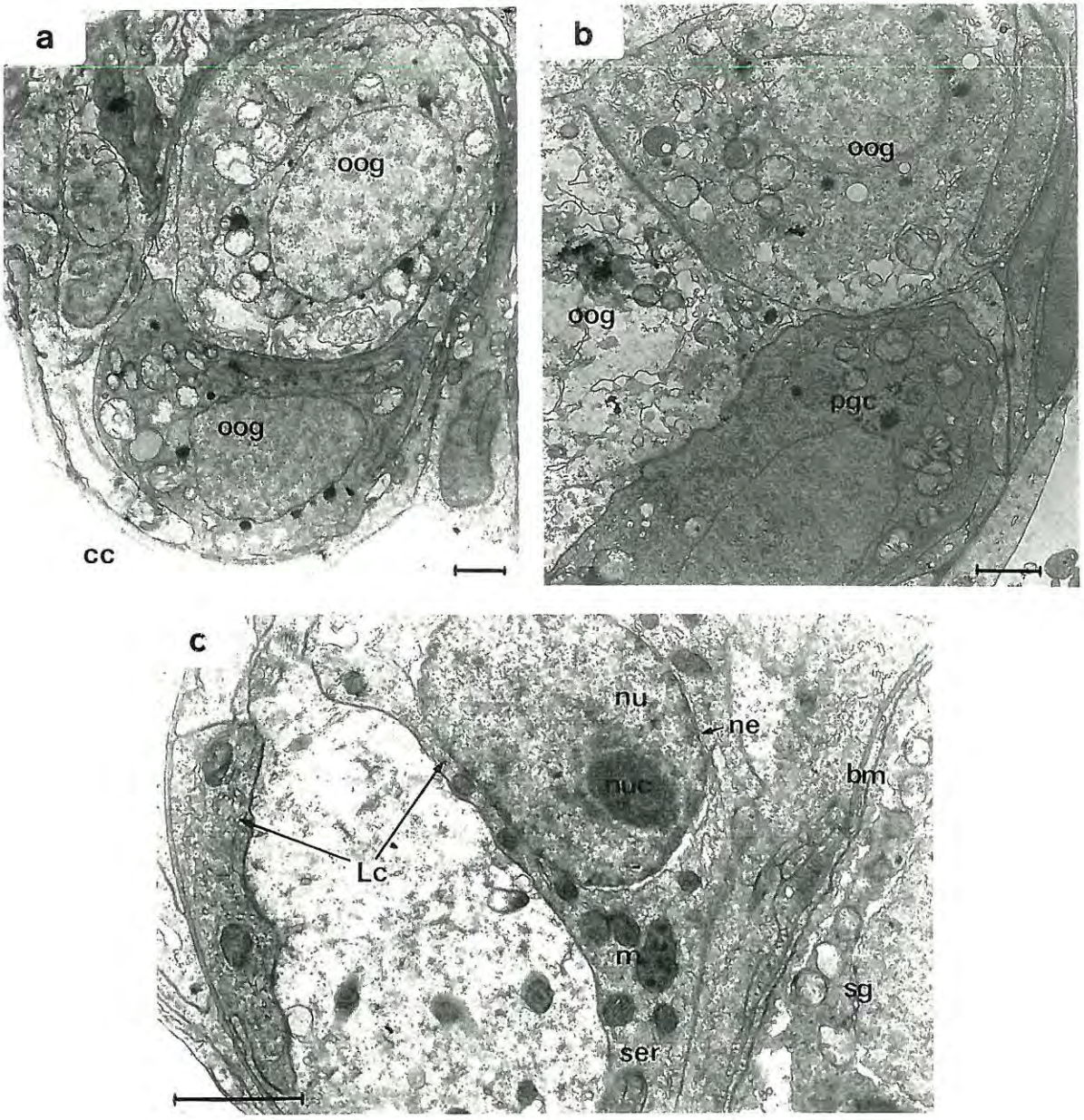

a
oog
oog
cc
b
oog
oog
pgc
c
nu
ne
bm
nuc
Lc
m
sg
ser

Figure 44. Medial sections of male TYPE II and TYPE III Acanthopagrus berda gonads showing typical morphology in the length class 100-149mm. **(a)** - ripe TYPE II gonad with much reduced female element, **(b)** - female tissue within this gonad consists of a single row of previtellogenic oocytes opposite the sterile zone, **(c)** - ripe TYPE III gonad, **(d)** - enlargement showing lamellae with previtellogenic oocytes along their margins and sparse connective tissue. cc = central cavity, ol = ovigerous lamellae, pvo = previtellogenic oocyte, sd = sperm duct, sz = spermatozoa, szo = sterile zone. Scale bar in **(a)** = 500µm, in **(b,d)** = 100µm, in **(c)** = 250µm.

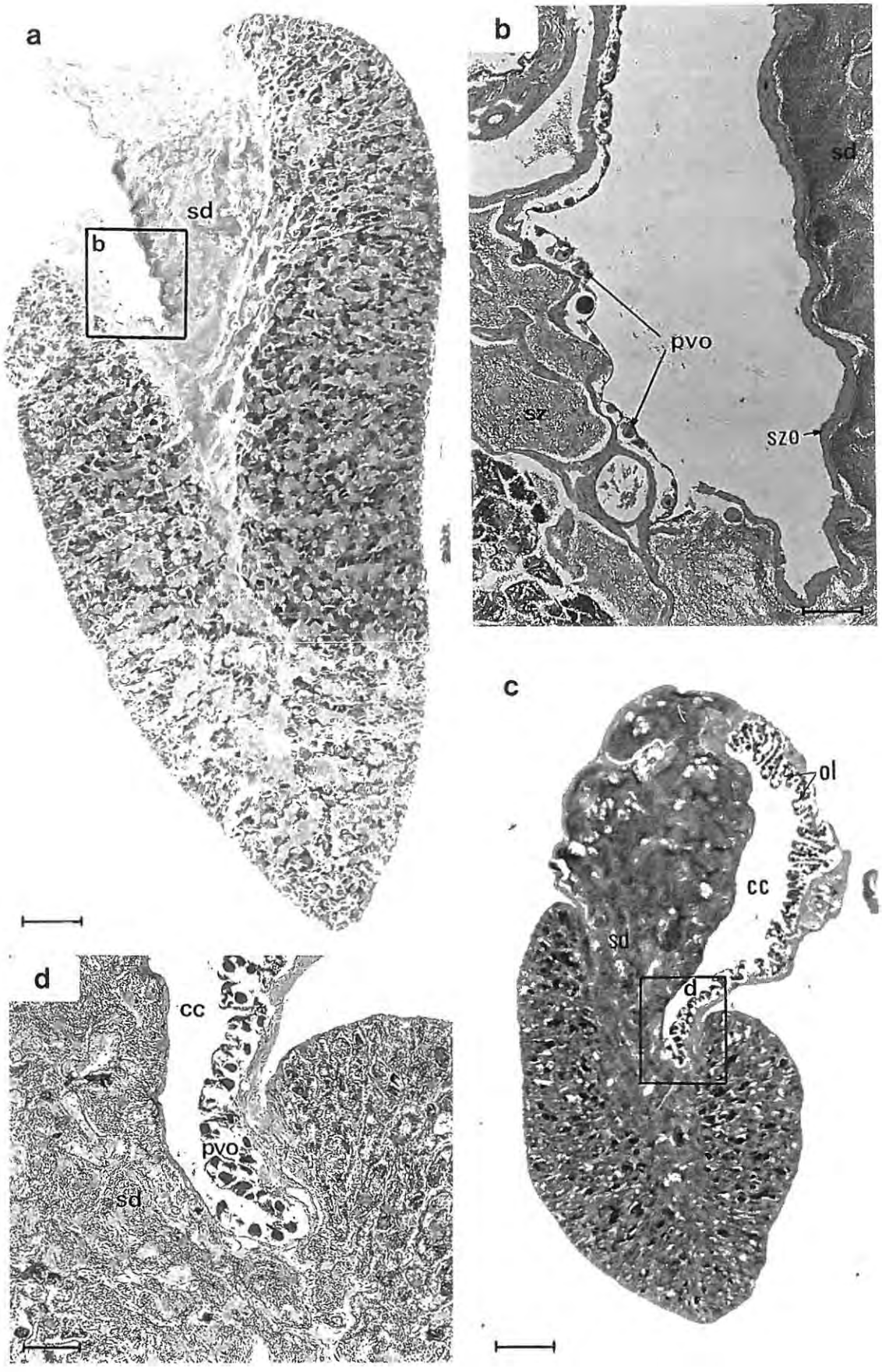

a
sd
b
b
sd
pvo
sz
szo
c
ol
cc
sd
d
d
cc
pvo
sd

Figure 45. Medial sections of female TYPE IV and TYPE V Acanthopagrus berda gonads showing typical morphology in the length class 100-149mm. **(a,b)** - Type IV gonad with active male element. Oocytes in female element arrested at previtellogenic stage, **(c)** - TYPE V female with male remnant, **(d)** - TYPE V female with thickened sterile zone, **(e)** - gonial cells in tunica albuginia of gonad in **(d)**. cc = central cavity, fe = female element, go = gonial cells, me = male element, mr = male remnant, pvo = previtellogenic oocytes, sc = spermatocytes, sd = sperm duct, sg = spermatogonia, sz = spermatozoa, szo = sterile zone, va = vacuolated area. Scale bar in **(a,c,d)** = 500µm, in **(b,e)** = 50µm.

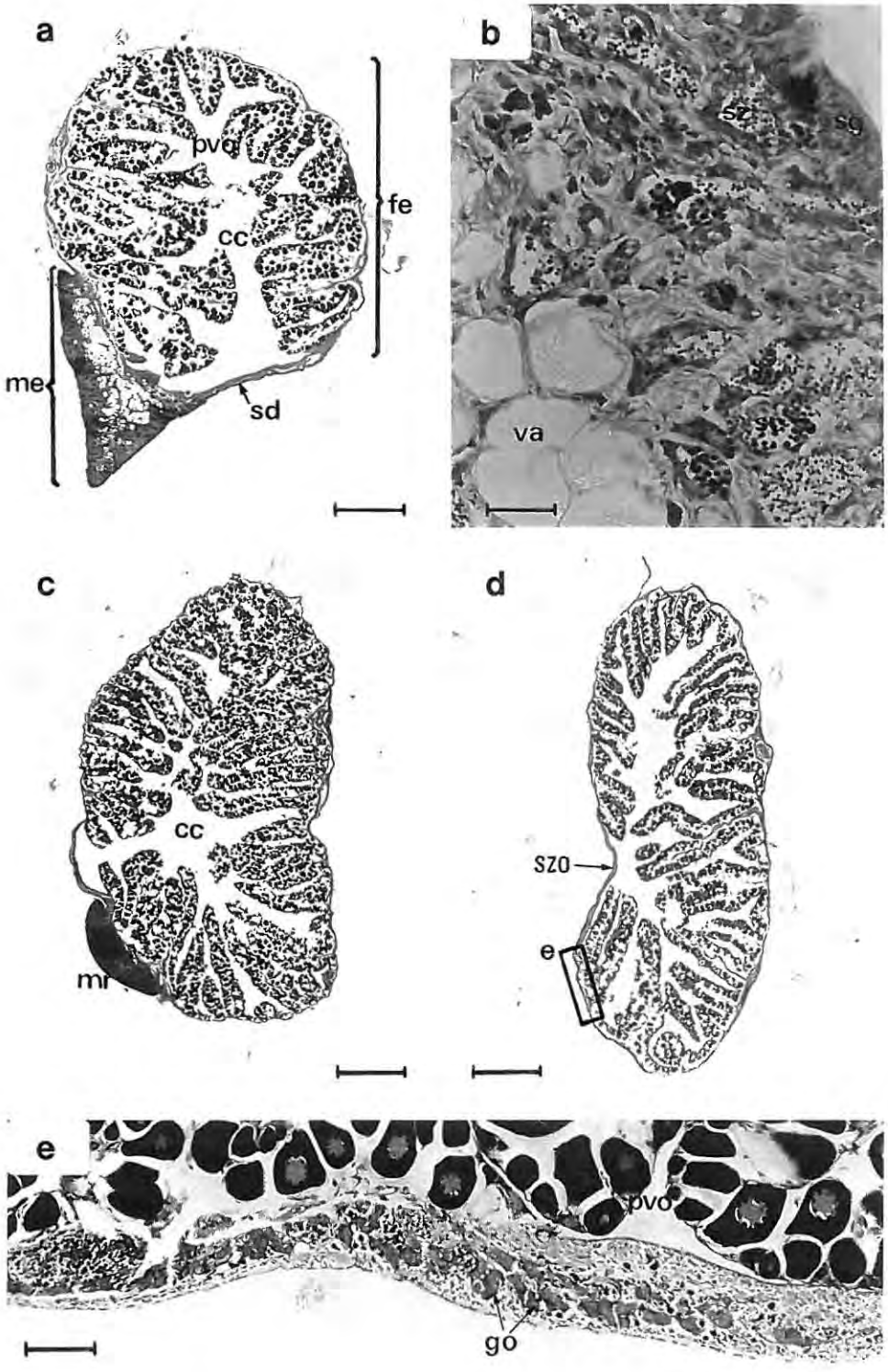

a
pvo
fe
cc
me
sd
b
sz
sg
va
c
cc
mi
d
SZO
e
e
pvo
go

Figure 46. Medial sections of male Acanthopagrus berda TYPE II and TYPE III gonads showing typical morphology in the length class 150-199mm. **(a)** - ripe TYPE II gonad from an A. berda of 188mm, with reduced female tissue enclosed by male tissue, **(b)** - female tissue consisting of a few previtellogenic oocytes scattered along the margins of the former central cavity, **(c)** - ripe TYPE III gonad from an A. berda of 150mm, showing a conspicuous female element opposite the sperm duct. cc = central cavity, pvo = previtellogenic oocytes, sd = sperm duct, sz = spermatozoa. Scale bar in **(a)** = 500µm, in (b) = 100µm, in **(c)** = 250µm.

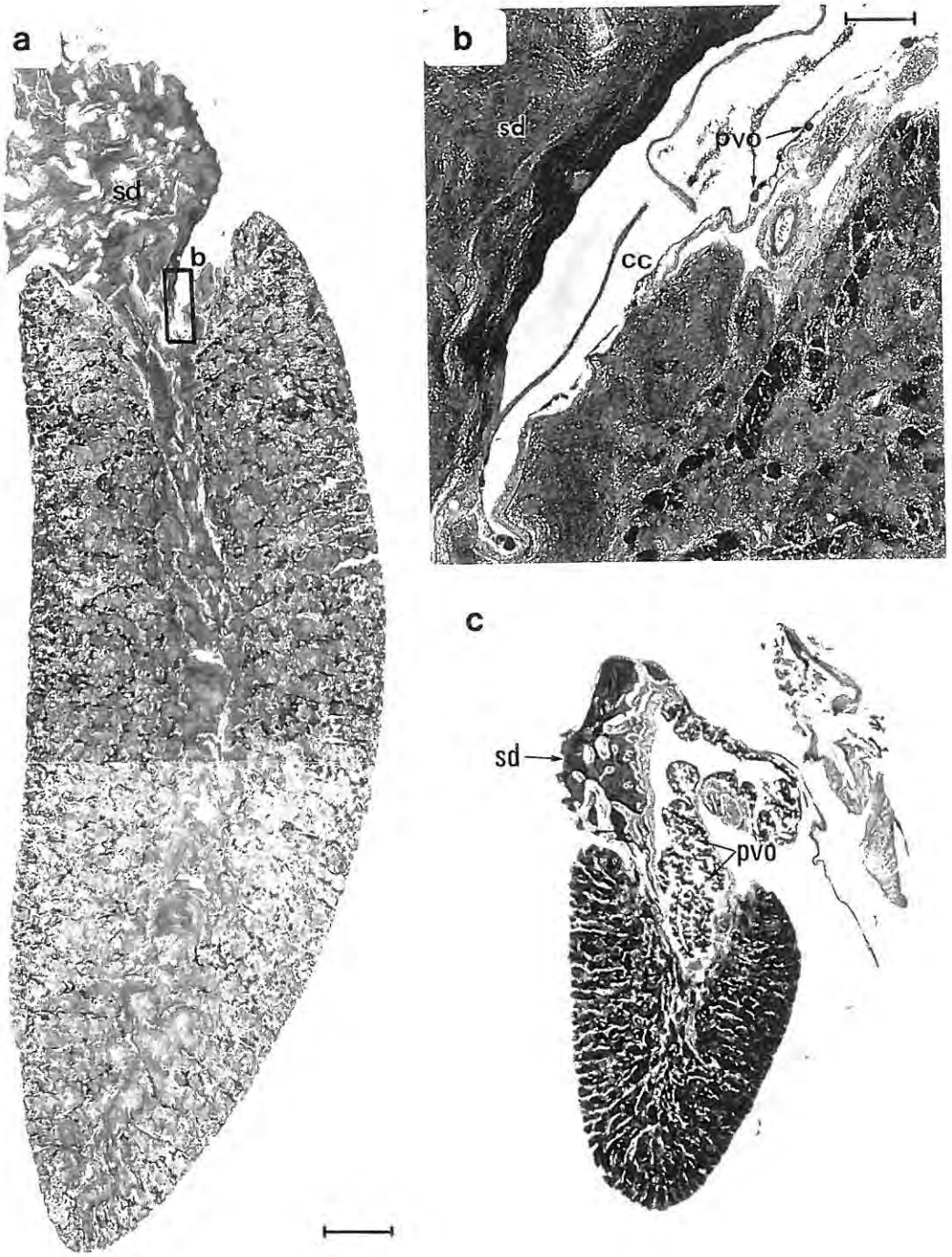

a
sd
b
b
sd
pvo
cc
c
sd
pvo

Figure 47. Medial sections of TYPE V female gonads from Acanthopagrus berda of 167mm **(a,b)** and 182mm **(c)**, showing typical morphology in the length class 150-199mm and gonial cells in the tunica albuginia of both inactive and active gonads. bv = blood vessel, cc = central cavity, go = gonial cells, pvo = previtellogenic oocytes, ta = tunica albuginia, 3°YO = tertiary yolk oocyte. Scale bar in **(a)** = 500µm, in **(b)** = 25µm, in **(c)** = 50µm.

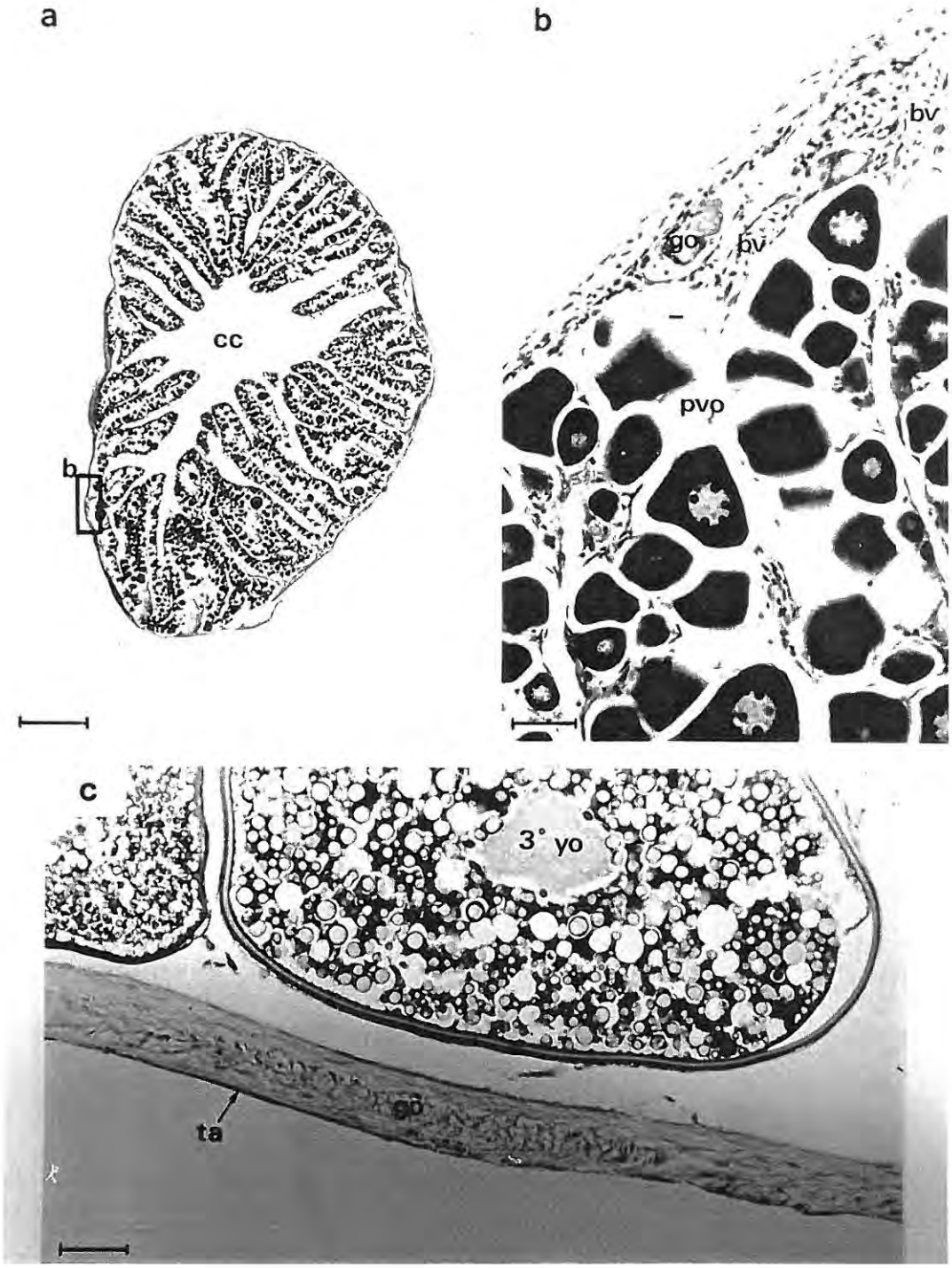

a
cc
b
b
bv
go
bv
pvp
c
3° yo
go
ta

Figure 48. Medial section of a TYPE IV female gonad from an Acanthopagrus berda of 155mm showing degenerating tissue in the male element. bb = brown bodies, cc = central cavity, me = male element, pvo = previtellogenic oocytes, sd = sperm duct. Scale bar in **(a)** = 500µm, in **(b)** = 100µm.

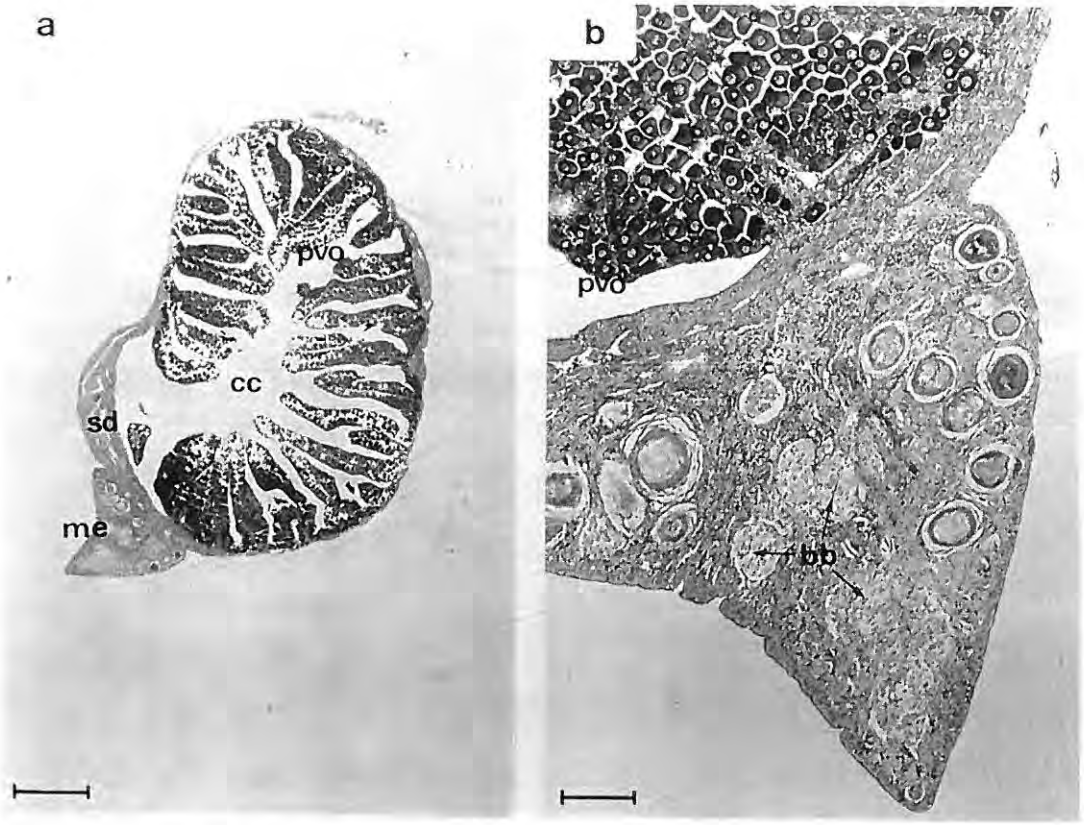

a
pvo
cc
sd
me
b
pvo
bb

Figure 49. (a) - A TYPE V female gonad from an Acanthopagrus berda of 200mm, showing typical morphology in this length class. Anterior **(b,c)** medial **(d)** and posterior **(e)** sections show gonial cells scattered throughout the length of a reduced male element. pvo = previtellogenic oocytes, go = gonial cells, 2°YO = secondary yolk oocytes. Scale bar in **(a)** = 500µm, in **(b,e)** = 100µm, in **(c,d)** = 50µm.

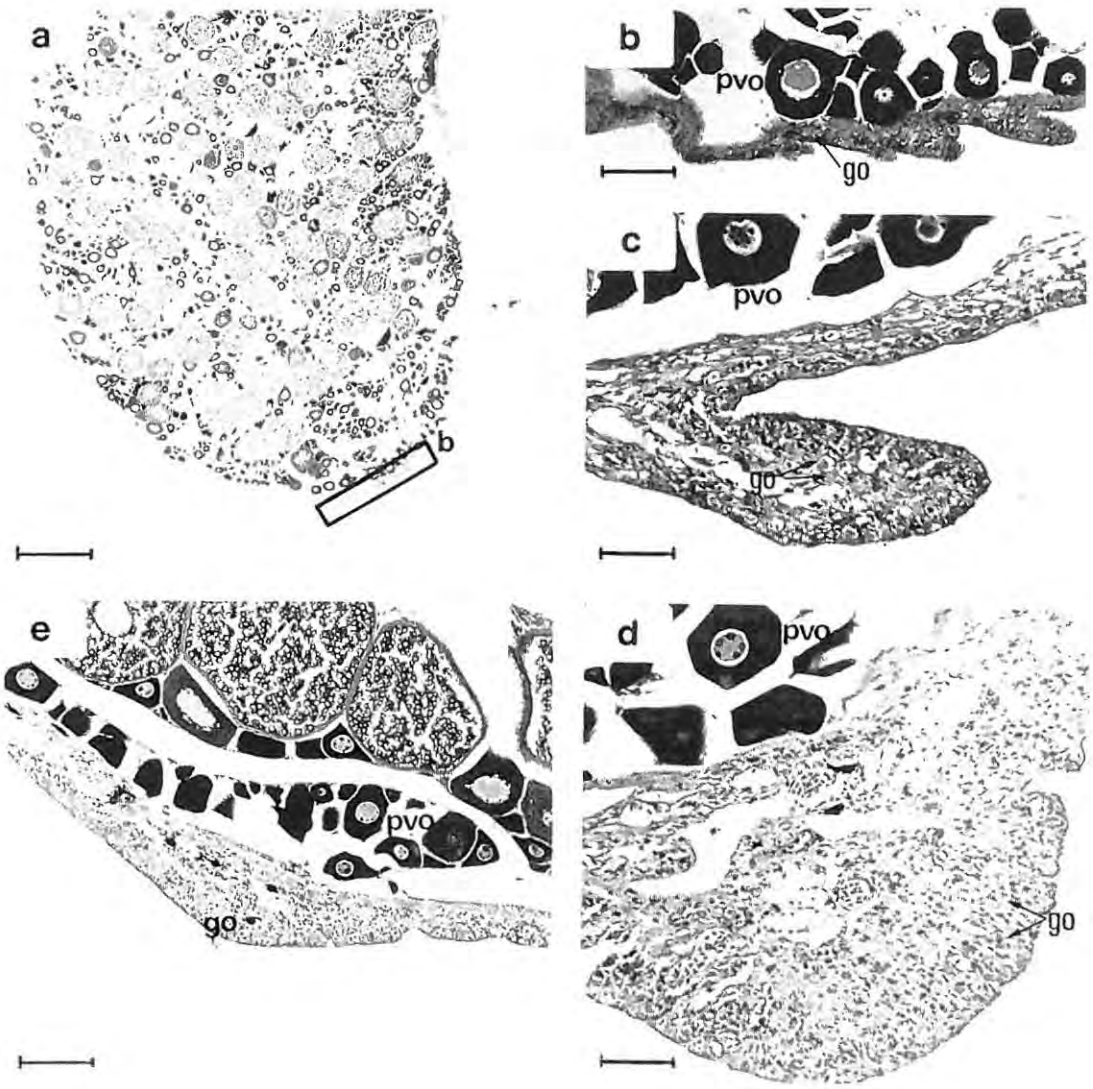

a
b
b
pvo
go
c
pvo
go
e
pvo
go
d
pvo
go

Figure 50. Medial sections of male Acanthopagrus berda TYPE II and TYPE III gonads in the length class 200-249mm. **(a)** - a convoluted, ripe male TYPE III gonad, **(b)** - a 'normal' ripe male TYPE III gonad, **(c)** - a male TYPE II gonad with male tissue enclosing female tissue, **(d)** - lamellae within the convoluted gonad have thickened and filled with connective tissue, **(e)** - spermatogenesis is complete within the male element of the convoluted gonad and interstitial tissue and spermatogonia amongst lobules of spermatozoa appear to be degenerating. cc = central cavity, ct = connective tissue, ph = cells in phagocytic activity, pvo = previtellogenic oocytes, sd = sperm duct, sg = spermatogonia, sz = spermatozoa, v = vacuolated cells. Scale bar in **(a-c)** = 500µm, in **(d)** = 100µm, in **(e)** = 25µm.

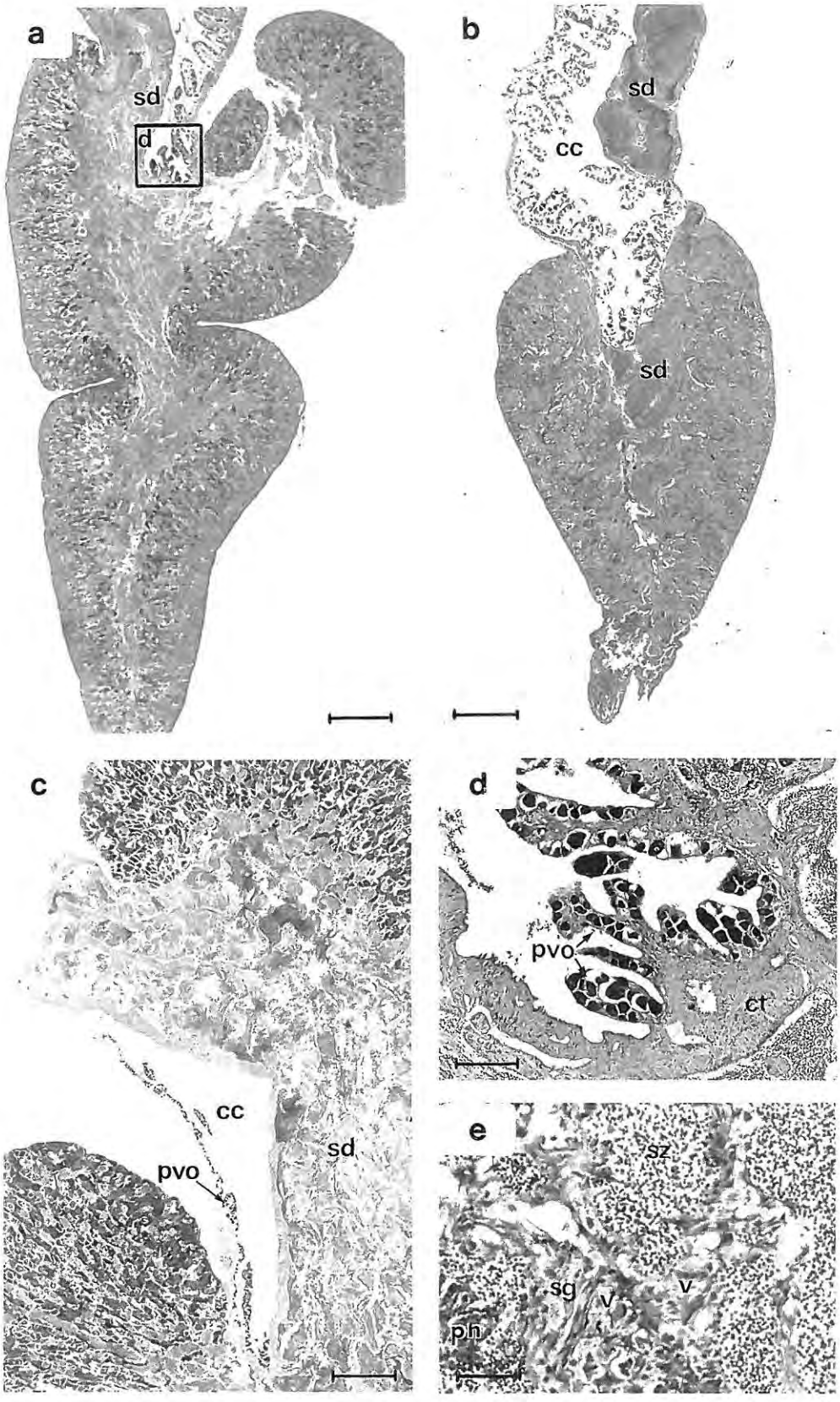

a
sd
d
b
sd
cc
sd
c
cc
sd
pvo
d
pvo
ct
e
sz
sg
v
v
ph

Figure 51. Medial sections of female Acanthopagrus berda TYPE V gonads, showing typical morphology **(a)** in the length classes 250-349mm. Gonial cells are evident in the tunica albuginia of both inactive **(b)** and active **(c)** gonads. bv = blood vessel, go = gonial cells, pvo = previtellogenic oocytes, tta = thickened tunica albuginia, 3°YO = tertiary yolk oocyte. Scale bar in **(a)** = 500µm, in **(b)** = 25µm, in **(c)** = 50µm.

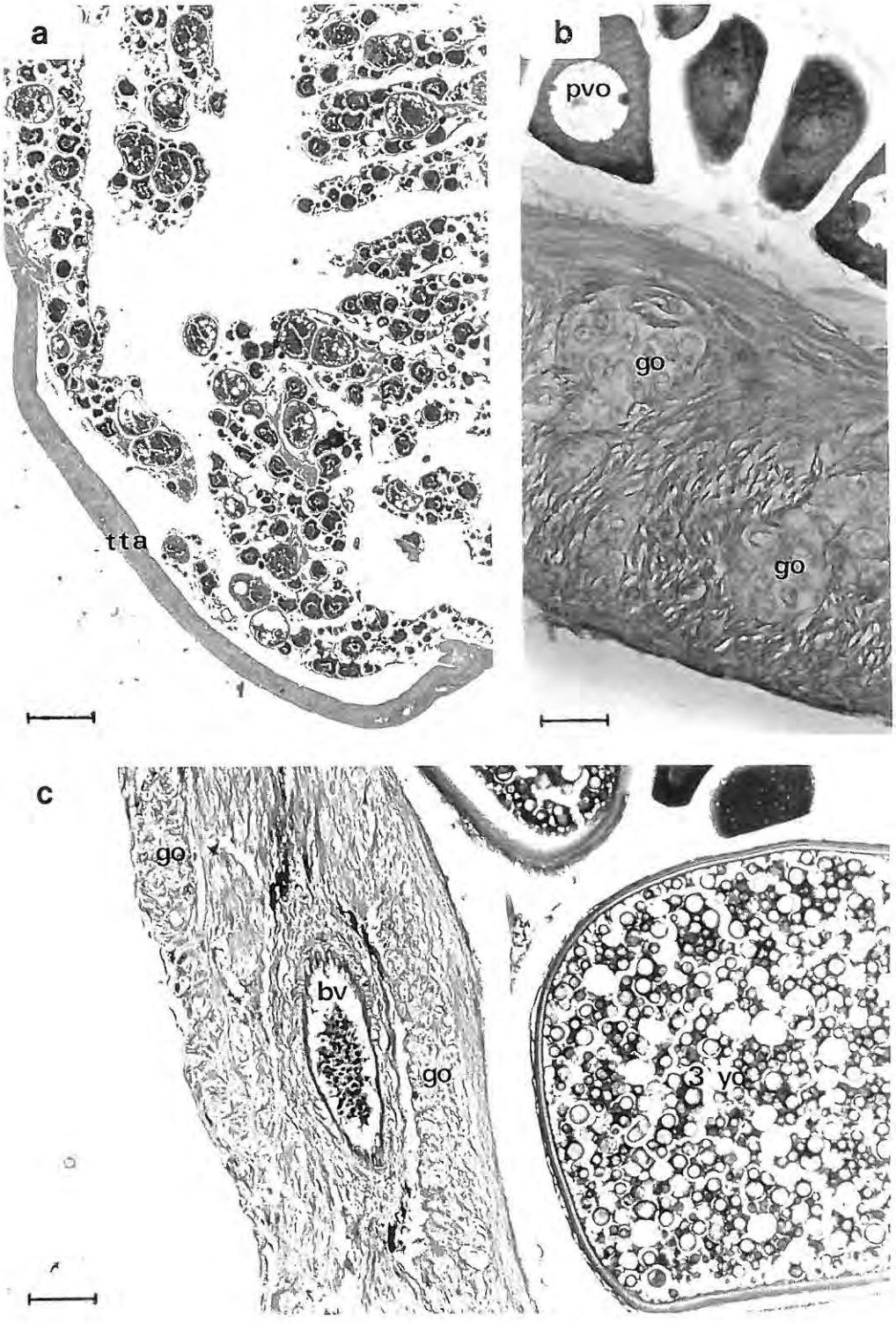

a
tta
b
pvo
go
go
c
go
bv
go
3
yo

Figure 52. A male TYPE III gonad from an Acanthopagrus berda of 254mm in which oocytes in the female element were undergoing vitellogenesis. pvo = previtellogenic oocytes, sd = sperm duct, sz = spermatozoa, yvo = yolk vesicle oocytes. Scale bar = 500μm.

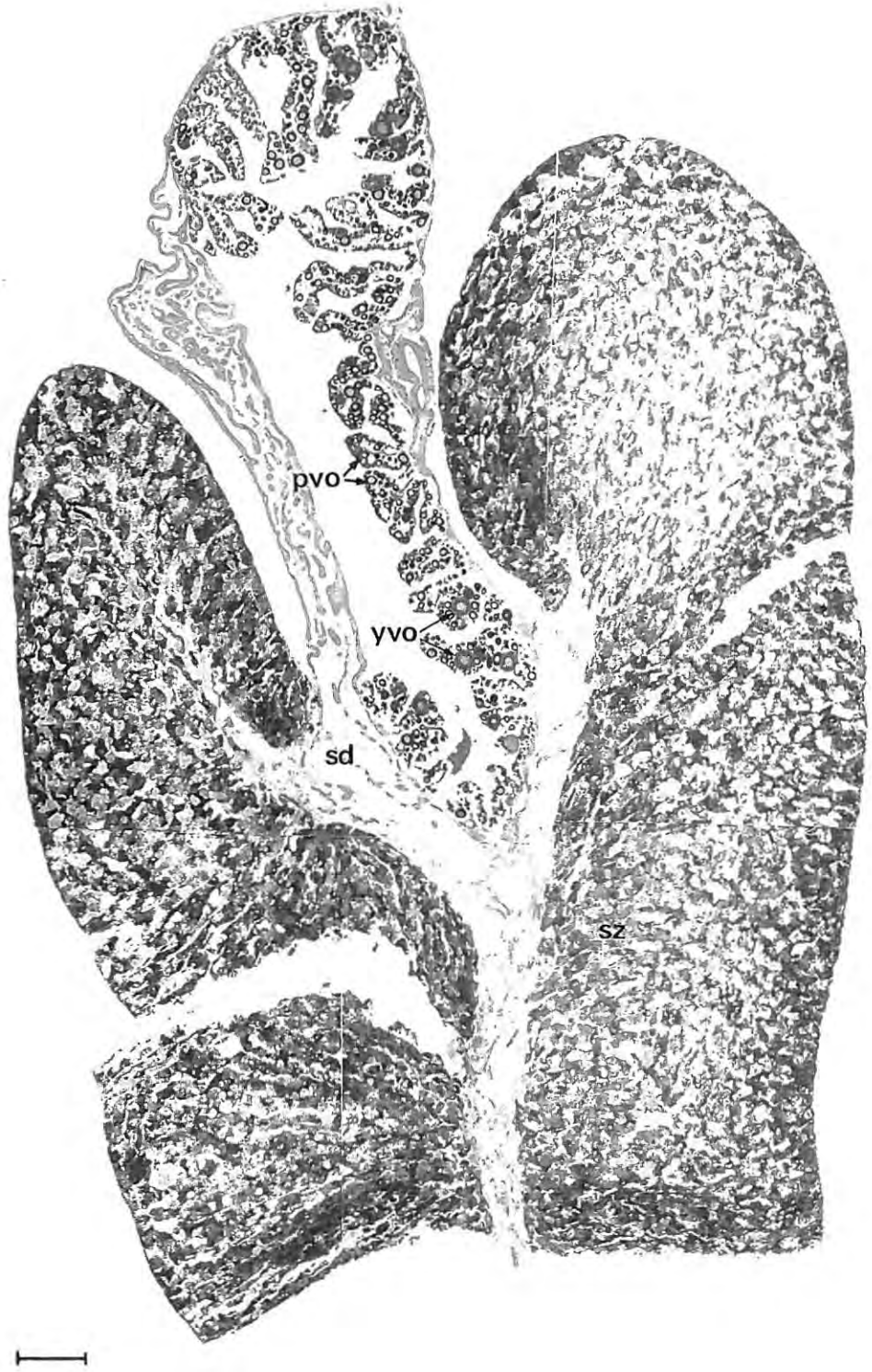
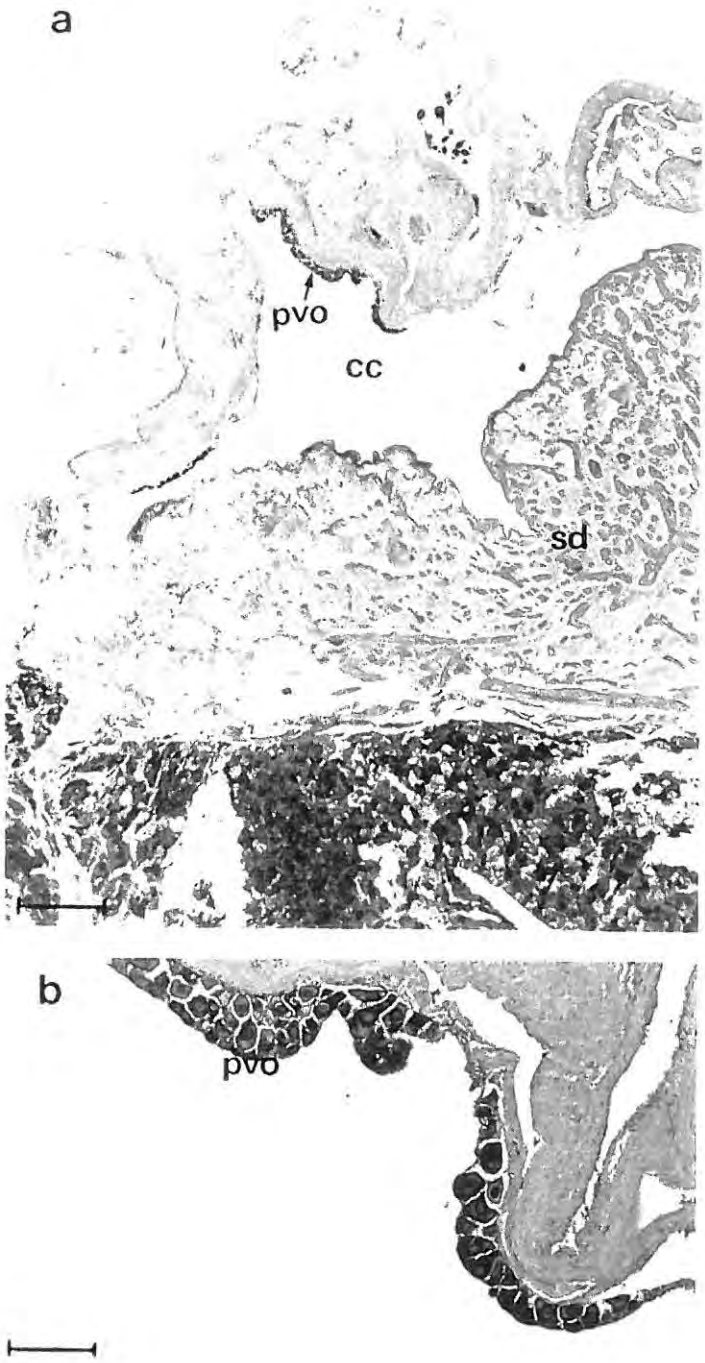

pvo
yvo
sd
sz

Figure 53. A male TYPE II gonad from an Acanthopagrus berda of 373mm. **(a)** - female tissue is limited to a thin strip along the margins of the central cavity, opposite the main sperm duct, **(b)** - this tissue consists of one to two layers of previtellogenic oocytes. cc = central cavity, pvo = previtellogenic oocytes, sd = sperm duct. Scale bar in **(a)** = 500µm, in **(b)** = 100µm.


a
pvo
cc
sd
b
pvo

GONADAL DEVELOPMENT IN CHRYSOBLEPHUS PUNICEUS

Figure 54. Medial (a,b) and posterior (c,d) sections of undifferentiated gonads (left and right) of a Chrysoblephus puniceus of 95mm (FL), showing the development of less-electron-dense cysts and gonial cells along the margins of the central cavity and more advanced development in the one lobe. bv = blood vessel, cc = central cavity, cs = less-electron-dense cyst, go = gonial cells, l = lacunae, me = male element, szo = sterile zone. Scale bar = 25μm.

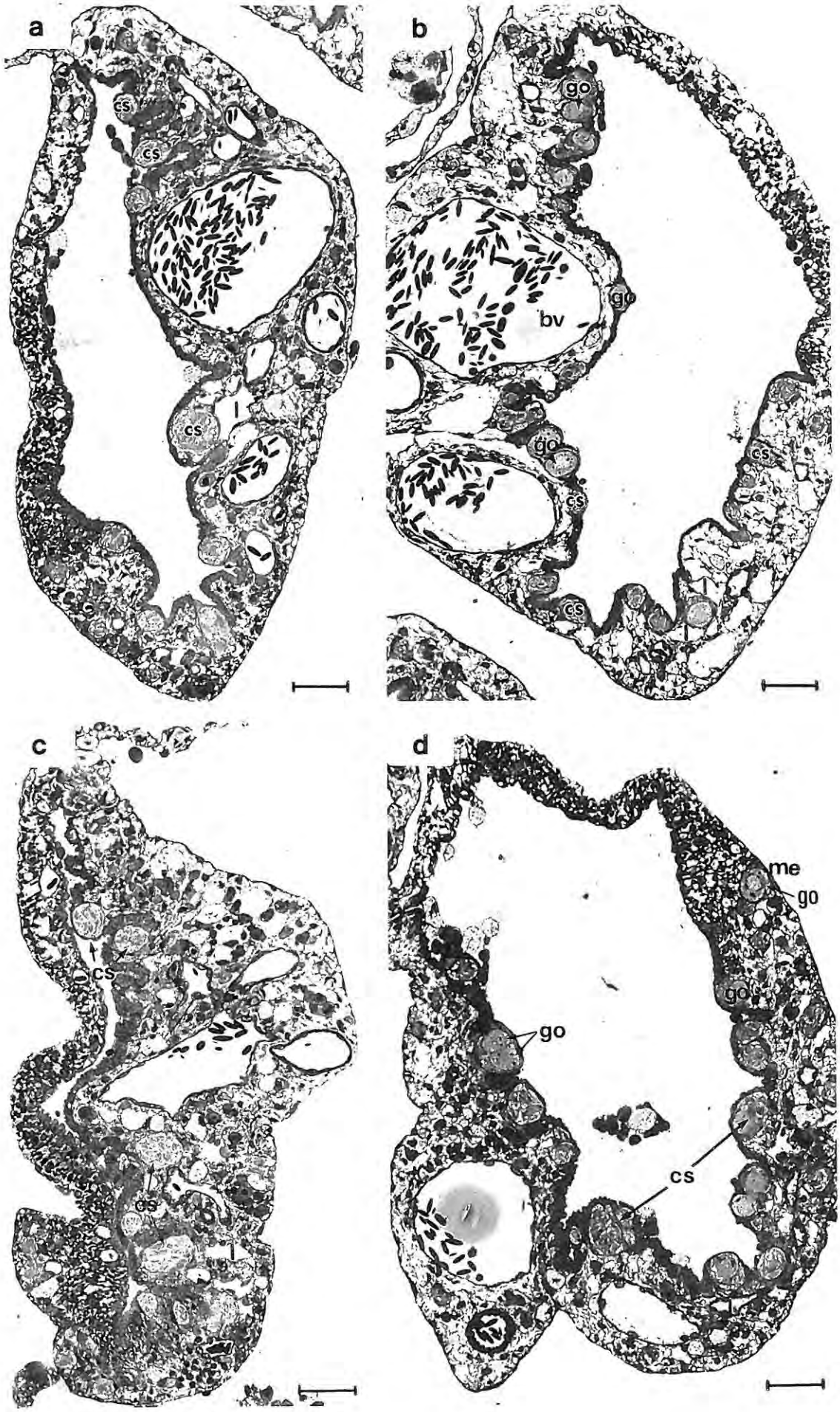

a
cs
cs
cs
b
go
go
bv
go
cs
cs
cs
c
cs
cs
d
me
go
go
go
cs

Figure 55. Anterior (**a**), medial (**b**) and posterior (**c**) sections of undifferentiated gonads of a Chrysoblephus puniceus of 96mm (FL), showing the development of less-electron-dense cysts and gonial cells along the margins of the central cavity in mid- and posterior regions. bv = blood vessel, cc = central cavity, cs = less-electron-dense cyst, go = gonial cells, szo = sterile zone. Scale bar in (**a**) = 25μm, in (**b,c**) = 50μm.

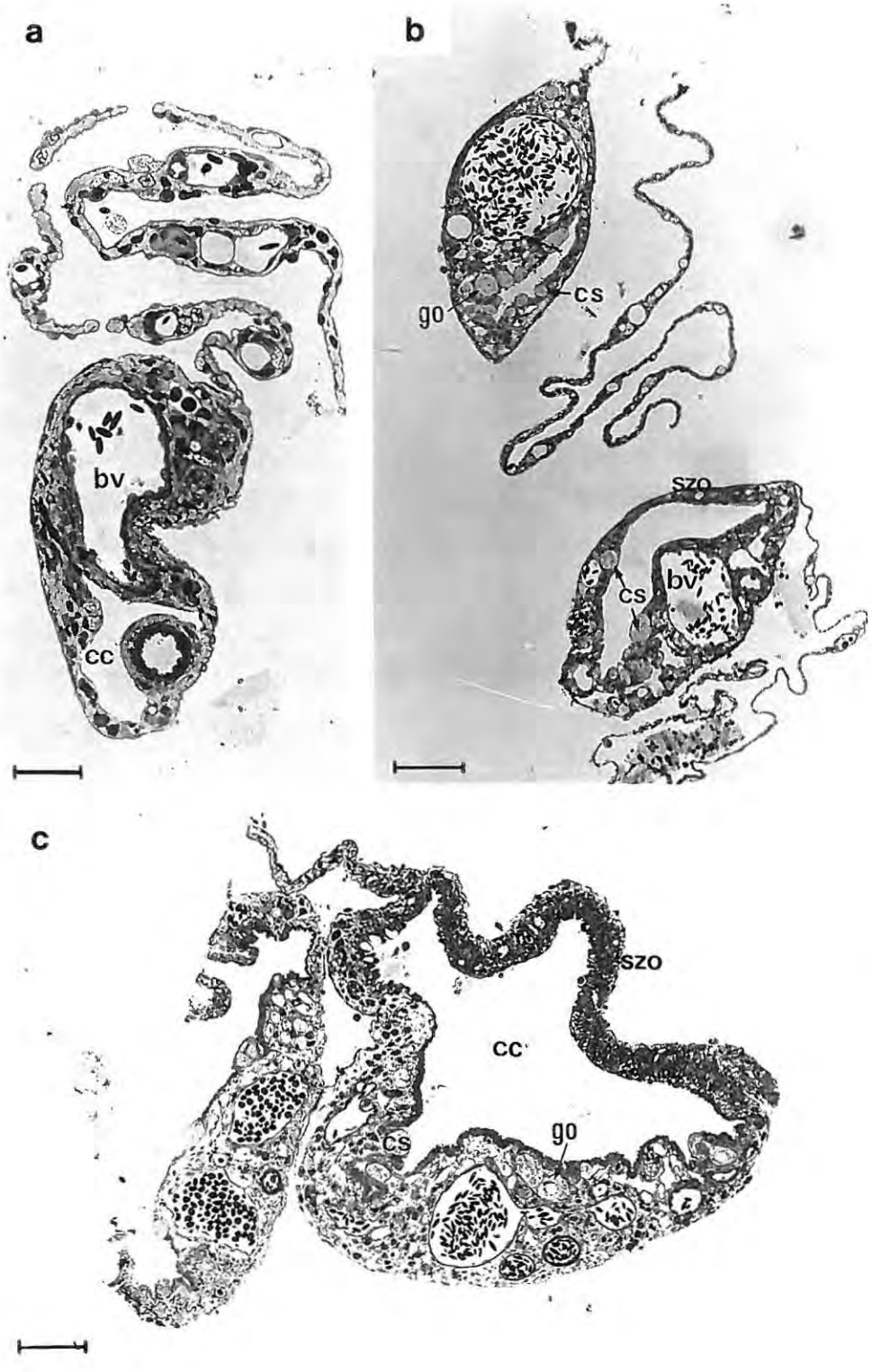

a
bv
cc
b
go
cs
szo
cs
bv
c
szo
cc
cs
go

Figure 56. Electron micrographs of cells in undifferentiated gonads of Chrysoblephus puniceus in the length class 50-99mm (FL). **(a)** - granulocytes and vacuolated cells amongst somatic cells, loose connective and fibrillar tissue adjacent to the major blood vessel, **(b)** - sterile zone of fibrillar and loose connective tissue bordered by columnar epithelial cells, **(c)** - cyst of less-electron-dense cells adjacent to the central cavity, **(d)** - gonial cells developing along the margins of the central cavity. bv = blood vessel, cs = less-electron-dense cyst, e = epithelial cells, f = fibrillar tissue, g = granulocytes, go = gonial cells, l = lacunae, m = mitochondria, n ='nuage', nu = nucleus, nuc = nucleolus, pfc = pre-follicle cell, s = somatic cells, v = vacuolated cells. Scale bar = 2µm.

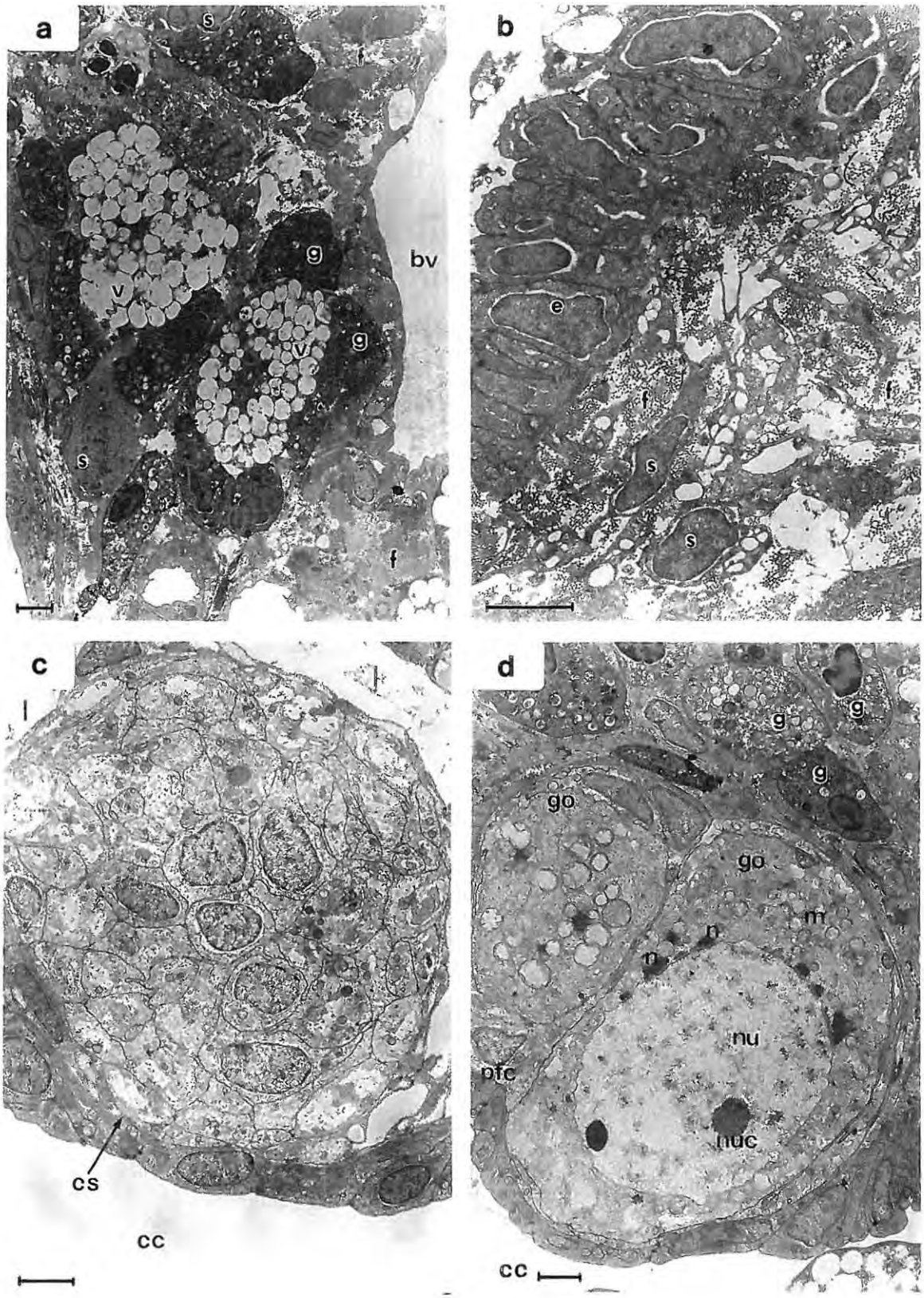

a
s
g
bv
v
v
g
s
f
b
e
f
f
s
s
c
cs
cc
d
g
g
g
go
go
m
n
n
nu
ptc
nuc
cc

Figure 57. Anterior (**a**), medial (**b,d**) and posterior (**c,e**) sections of a gonad from a Chrysoblephus puniceus of 100mm(FL) differentiating into an ovary, showing the development of gonia and previtellogenic oocytes along the margins of the central cavity. bv = blood vessel, cc = central cavity, g = gonial cells, l = lacunae, m.oog = mitotic oogonia, pvo = previtellogenic oocytes. Scale bar in (**a-c**) = 50μm, in (**d,e**) = 25μm.

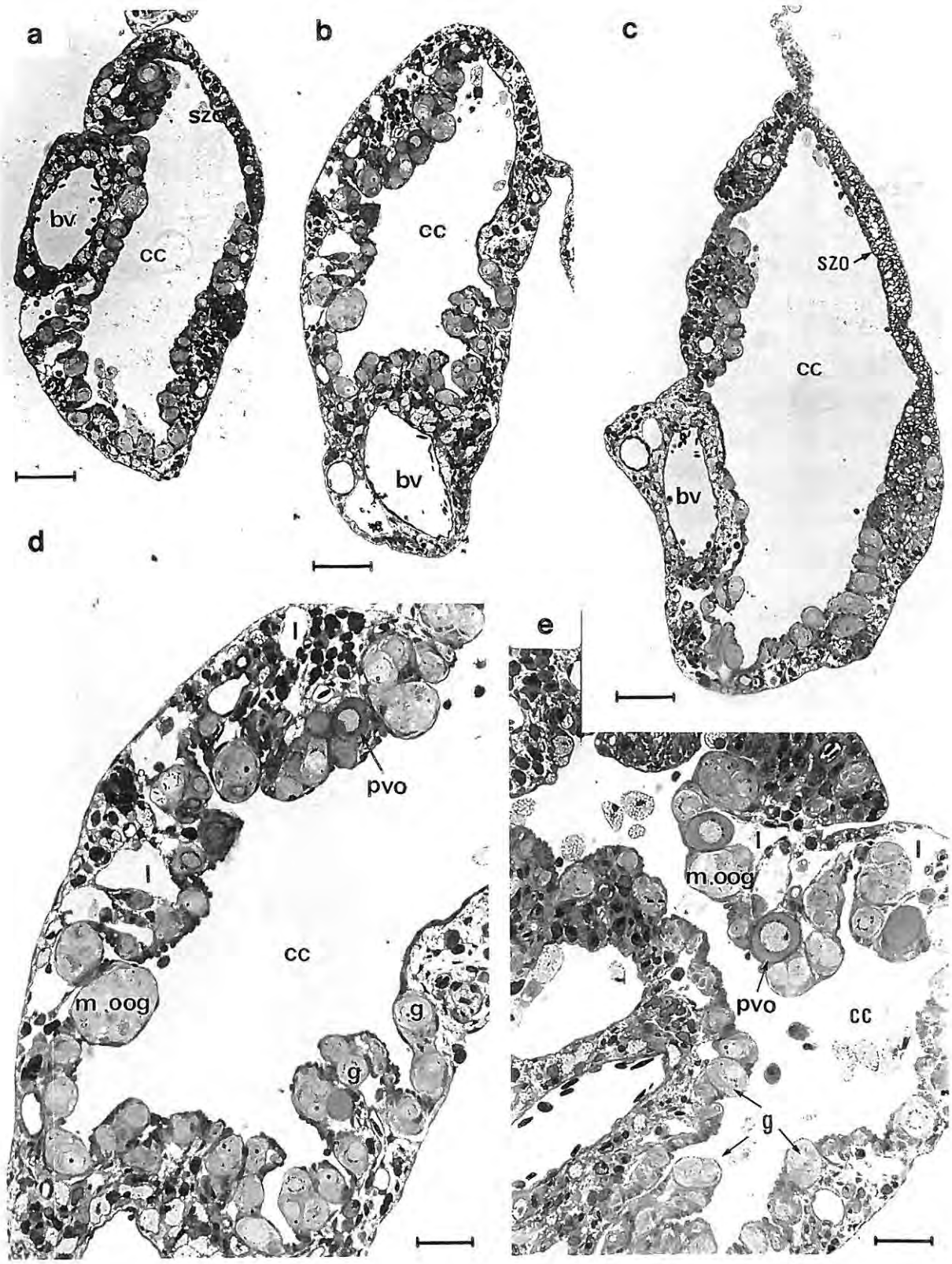

a
szo
bv
cc
b
cc
bv
c
szo
cc
bv
d
l
pvo
l
cc
m oog
g
g
e
l
l
m oog
pvo
cc
g

Figure 58. Anterior (a), medial (b) and posterior (**c,d**) sections of an ovary from a Chrysoblephus puniceus of 135mm (FL), showing proliferation of oogonia and previtellogenic oocytes along margins of lamellae protruding into the central cavity. bv = blood vessel, cc = central cavity, l = lacunae, m.oog = mitotic oogonia, oog = oogonia, pvo = previtellogenic oocyte, szo = sterile zone. Scale bar in (**a-c**) = 100µm, in (**d**) = 25µm.

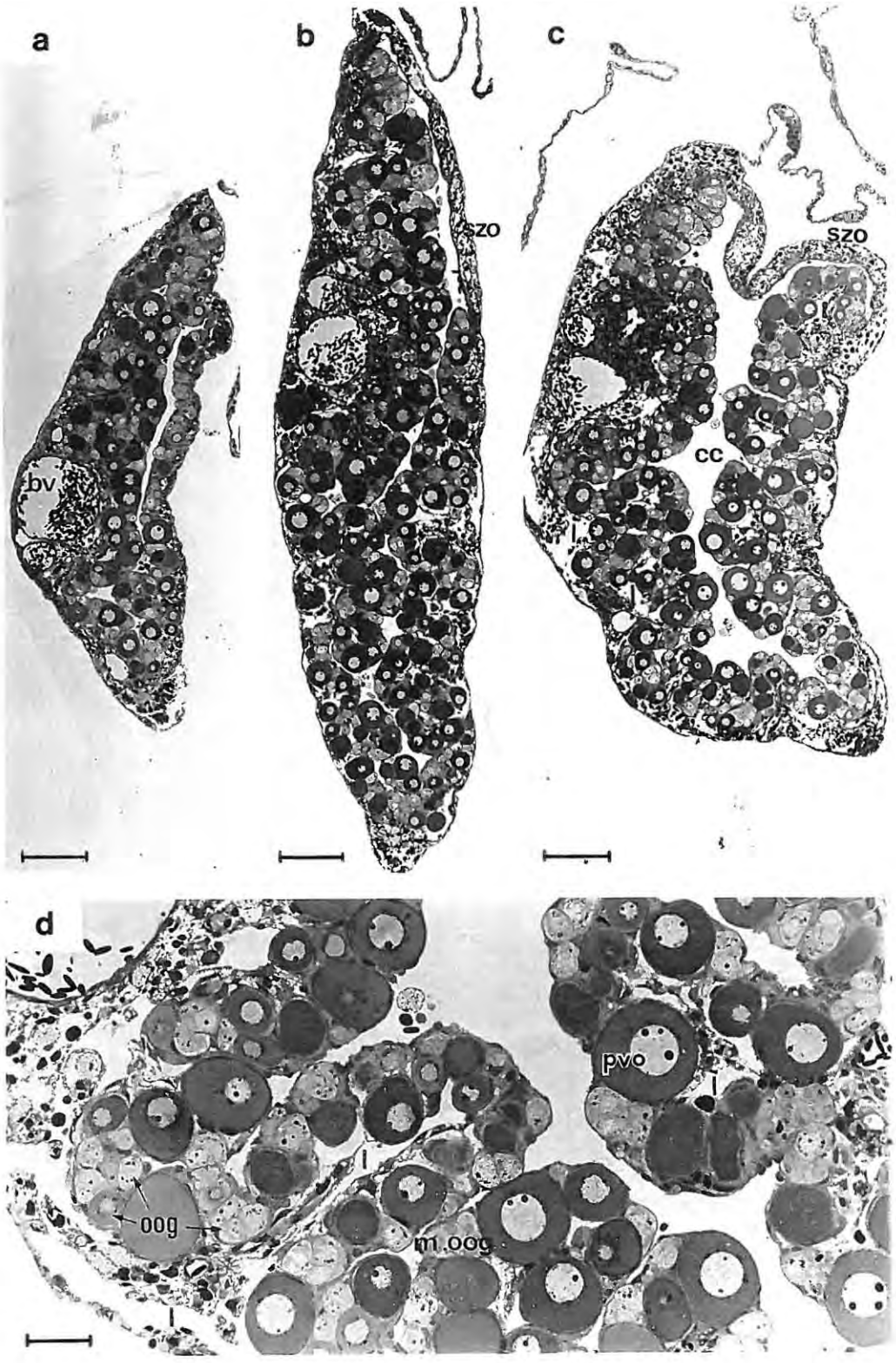

a
b
c
bv
szo
szo
cc
d
pvo
oog
m.oog

Figure 59. Medial (**a,c**) and posterior (**b**) sections of a gonad from a Chrysoblephus puniceus of 144mm (FL), showing typical ovarian morphology but with male and female germinal tissue proliferating alongside one another in lamellae. bv = blood vessel, cc = central cavity, l = lacunae, psc = primary spermatocytes, pvo = previtellogenic oocytes, ssc = secondary spermatocytes, st = spermatids, sz = spermatozoa, szo = sterile zone. Scale bar in (**a,b**) = 100μm, in (**c**) = 25μm.

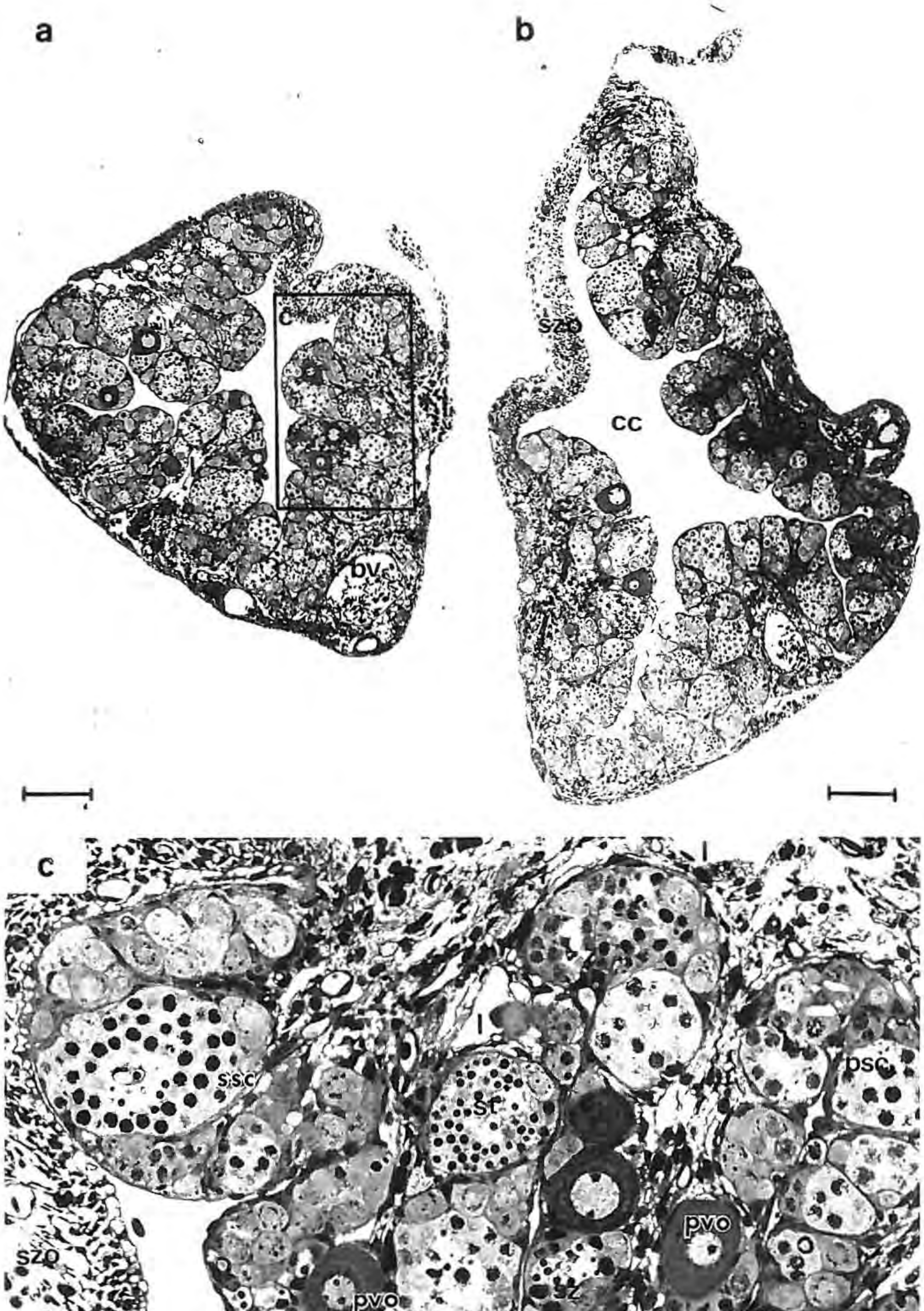

a
b
c
bv
szo
cc
c
ssc
l
st
bsc
pvo
szo
pvo
sz

Figure 60. Electron micrographs of male and female tissue developing alongside one another in the ovary of a Chrysoblephus puniceus of 144mm (FL). **(a)** - previtellogenic oocyte adjacent to a cyst of primary spermatocytes, **(b)** - spermatogonia, within cysts, developing into primary spermatocytes, **(c)** - primary and secondary spermatocytes in cysts along the margins of a lamella, **(d)** spermatozoa developing within cysts in the lamellae. cc = central cavity, cy = cysts in which spermatogonia are developing into primary spermatocytes, psc = primary spermatocytes, ssc = secondary spermatocytes, sz = spermatozoa. Scale bar in **(a-c)** = 5µm, in **(d)** = 1µm.

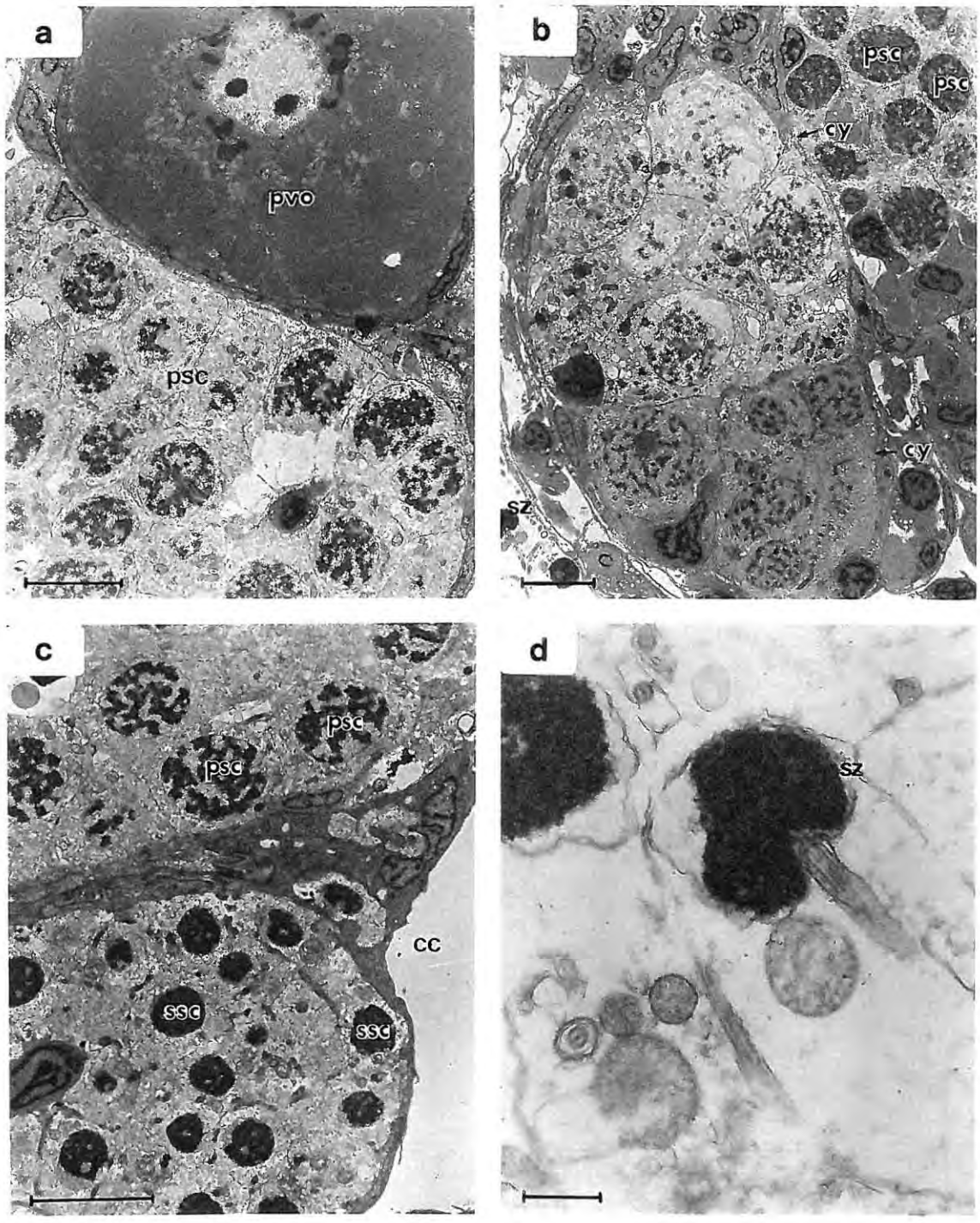

a
pvo
psc
b
psc
psc
cy
cy
sz
c
c
psc
psc
cc
ssc
ssc
d
sz

Figure 61. Electron micrographs of cells comprising differentiating gonads of Chrysoblephus puniceus in the length class 100-149mm (FL). **(a)** - granulocytes and vacuolated cells in the stroma backing the germinal tissue, **(b)** - granulocytes and vacuolated cells in loose fibrillar and connective tissue surrounding the major blood vessel, **(c)** - oogonia proliferating along margins of the central cavity, separated from the stroma by lacunae, **(d)** - sterile zone composed mainly of loose connective and fibrillar tissue. bv= blood vessel, ct = connective tissue, f = fibrillar tissue, g = granulocyte, l = lacunae, oog = oogonia, pfc = pre-follicle cell, s = somatic cells, v = vacuolated cells. Scale bar = 2µm.

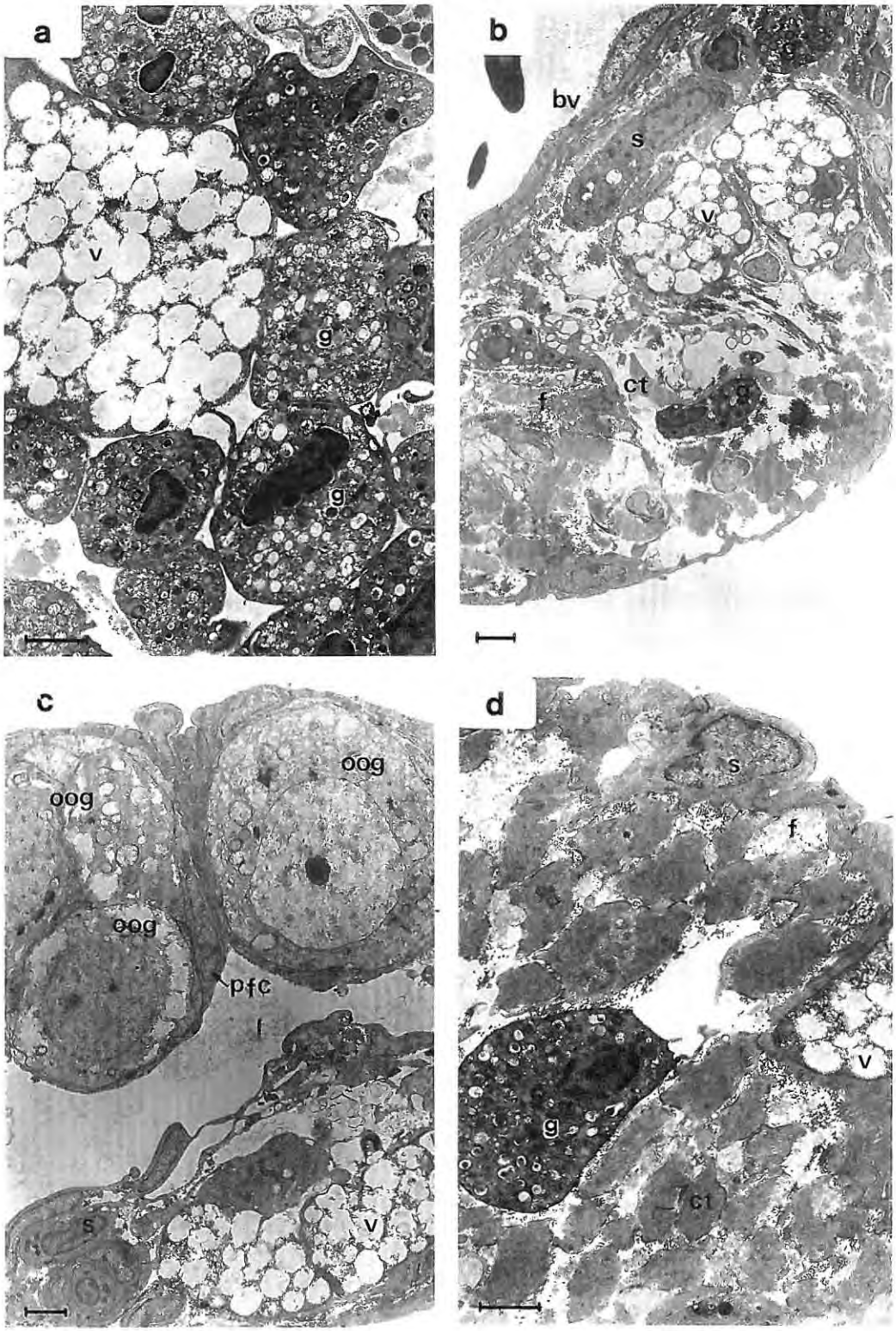

a
v
g
g
b
bv
s
v
ct
f
c
oog
oog
oog
pfc
f
s
v
d
s
f
v
g
ct

Figure 62. Electron micrographs of germinal tissue developing along the margins of the central cavity in the gonads of Chrysoblephus puniceus from the length class 100-149mm (FL). **(a)** - cyst of less-electron-dense cells developing in a thin interconnecting strand of connective tissue and somatic cells between lacunae, **(b)** - a differentiating gonial cell within a cyst forces the other cells to the outer margins of the cyst, **(c)** - further development and growth of the gonial cell forces the remaining cells in the cyst to flatten around it, **(d)** - once differentiation of the gonial cell is complete, mitosis commences and gonial cells proliferate within cysts. c ='ciment', cc = central cavity, cs = cyst of less-electron-dense cells, dgo = differentiating gonial cell, flc = flattened cyst cells, go = gonial cell, l = lacunae, m = mitochondria, ma = mitochondrial aggregation, m.oog = mitotic oogonium, n ='nuage', nu = nucleus, oog = oogonium. Scale bar = 2µm.

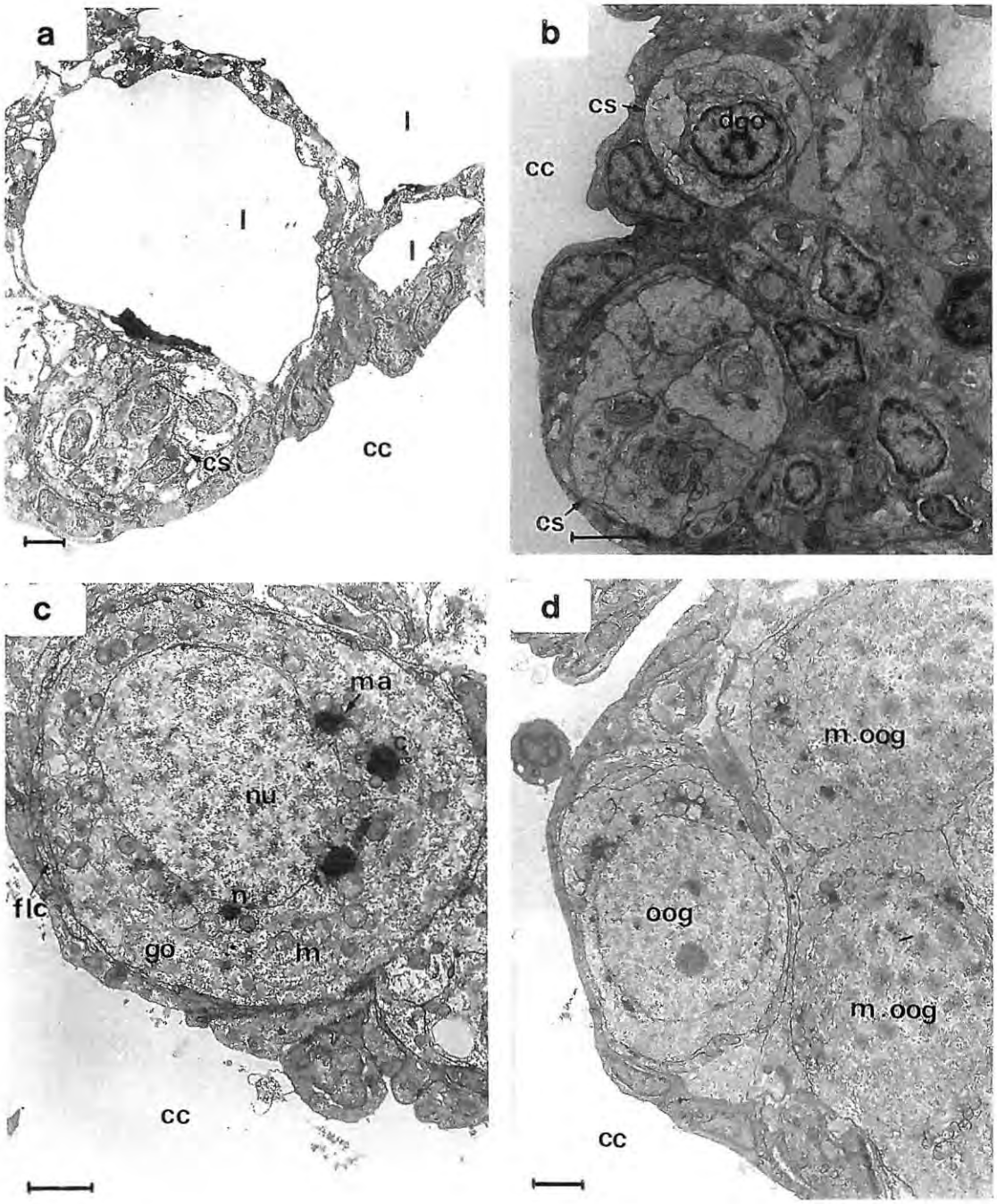

a
l
l
l
cs
cc
b
cs
dgo
cc
cs
c
ma
nu
n
flc
go
m
cc
d
m.oog
oog
m.oog
cc

Figure 63. Electron micrographs of early previtellogenic oocytes developing along the margins of the central cavity in the gonads of Chrysoblephus puniceus from the length class 100-149mm (FL). (a,b) - oocytes are surrounded by follicle cells which separate them from adjacent gonial cells. cc = central cavity, epo = early previtellogenic oocyte, fc = follicle cell, go = gonial cell. Scale bar = 5µm.

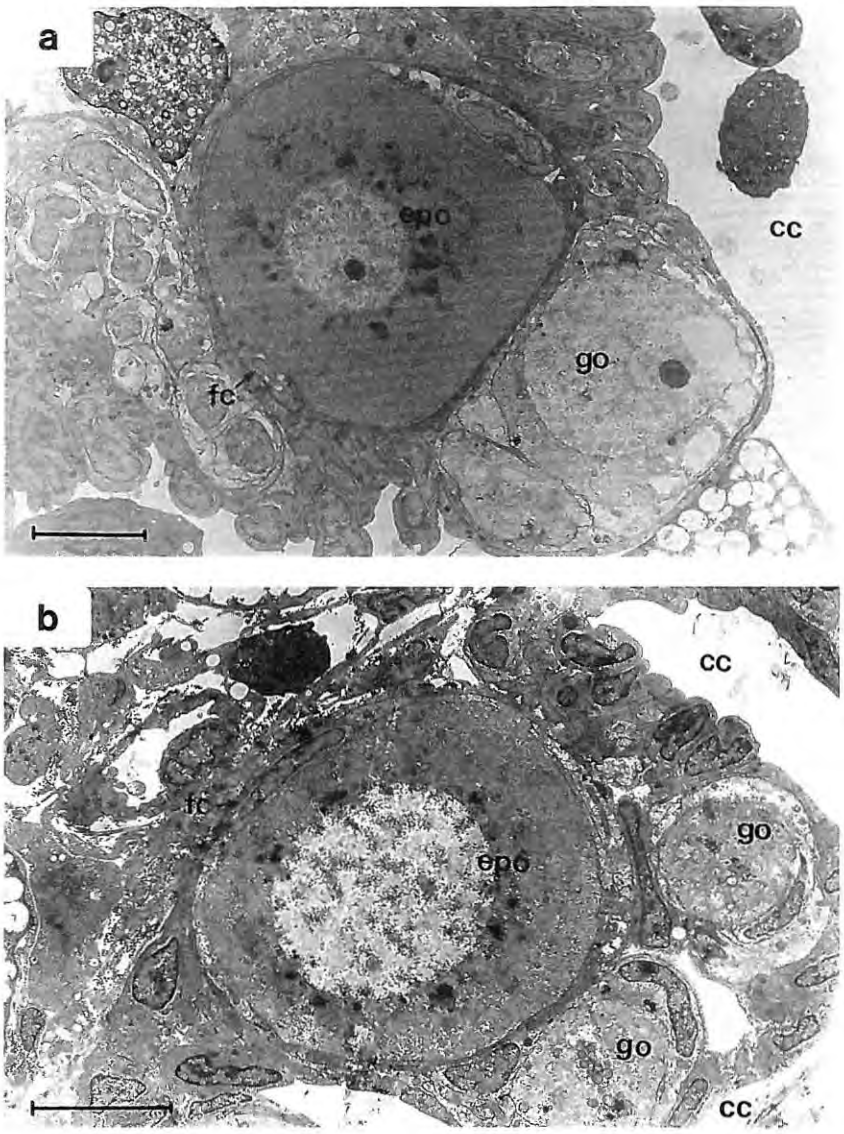
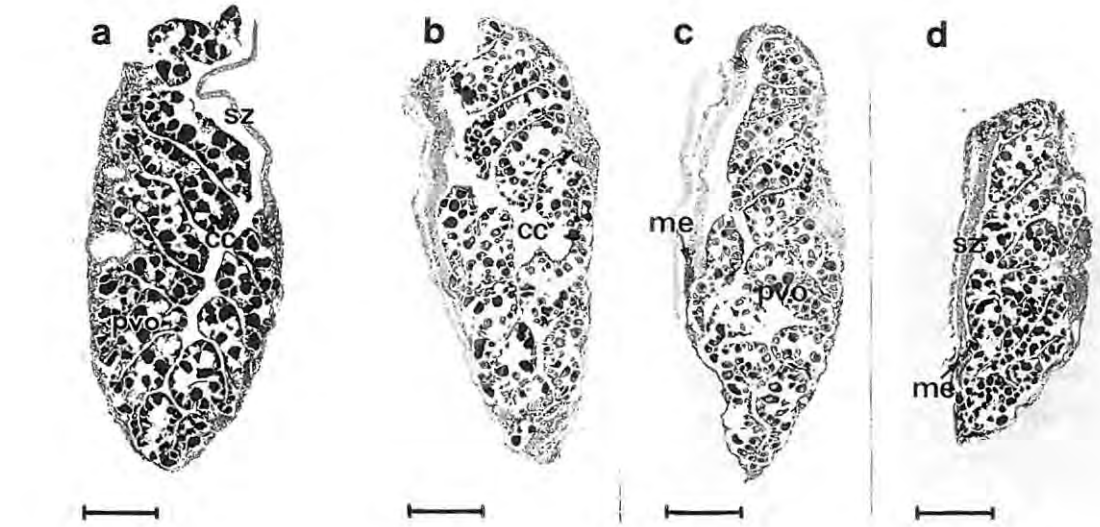

a
epo
cc
go
fc
b
cc
fc
go
epo
go
cc

Figure 64. Light micrographs of Chrysoblephus puniceus ovaries from the length class 150-199mm (FL). **(a)** - mid-section showing ovigerous lamellae extending into the central cavity and male tissue restricted to a few gonial cells below the sterile zone. **(b)** - anterior, **(c)** - medial and **(d)** - posterior sections from a fish of 168mm, showing male tissue restricted to the mid- and posterior regions. cc = central cavity, me = male element, pvo = previtellogenic oocytes, sz = sterile zone. Scale bar = 250μm.

Figure 65. Light micrographs of Chrysoblephus puniceus ovaries from the length class 150-199mm (FL). **(a)** - mid-section from a fish of 180mm showing typical morphology, **(b)** anterior, **(c)** medial and **(d)** posterior sections from a fish of 182mm, showing the development of a small male appendage in the posterior region. cc = central cavity, me = male element, pvo = previtellogenic oocytes, sz = sterile zone. Scale bar = 250μm.


a
sz
cc
pvo
b
cc
c
me
pvo
d
sz
me

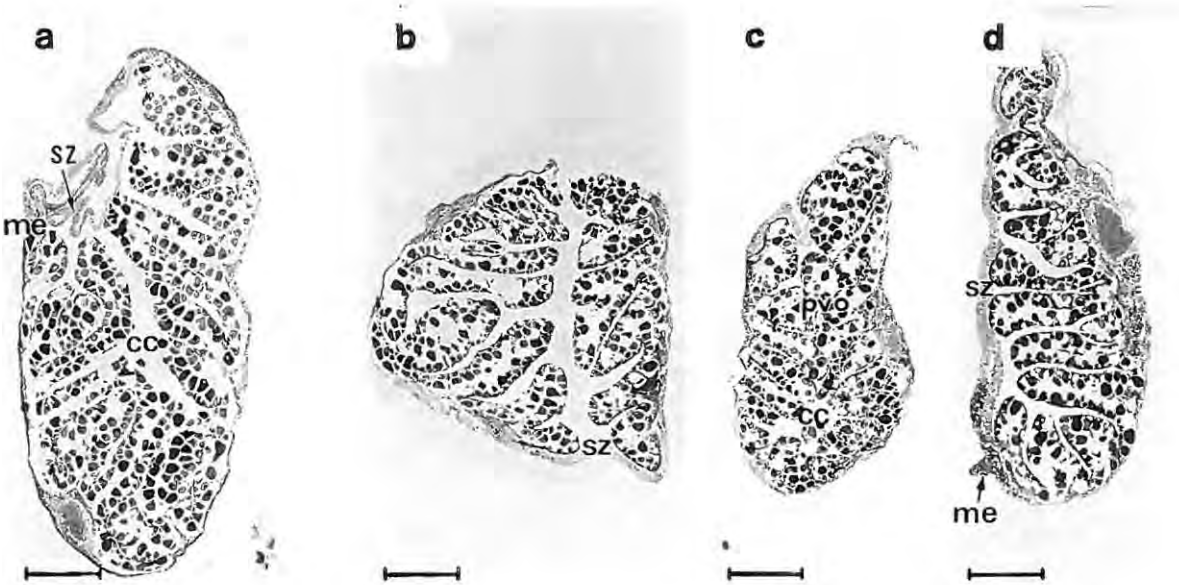

a
sz
me
cc
b
sz
c
pvo
cc
d
sz
me

Figure 66. Medial (**a**) and posterior (**b**) sections of a Chrysoblephus puniceus ovary taken from a fish of 155mm (FL), showing typical morphology of the ovary (inserts), with previtellogenic oocytes proliferating throughout the gonad. The male element is restricted to a few gonial cells in the tunica albuginea below the sterile zone in the posterior region. me = male element, oog = oogonia, pvo = previtellogenic oocytes, sz = sterile zone. Scale bar in inserts = 500µm, in higher magnifications = 25µm.

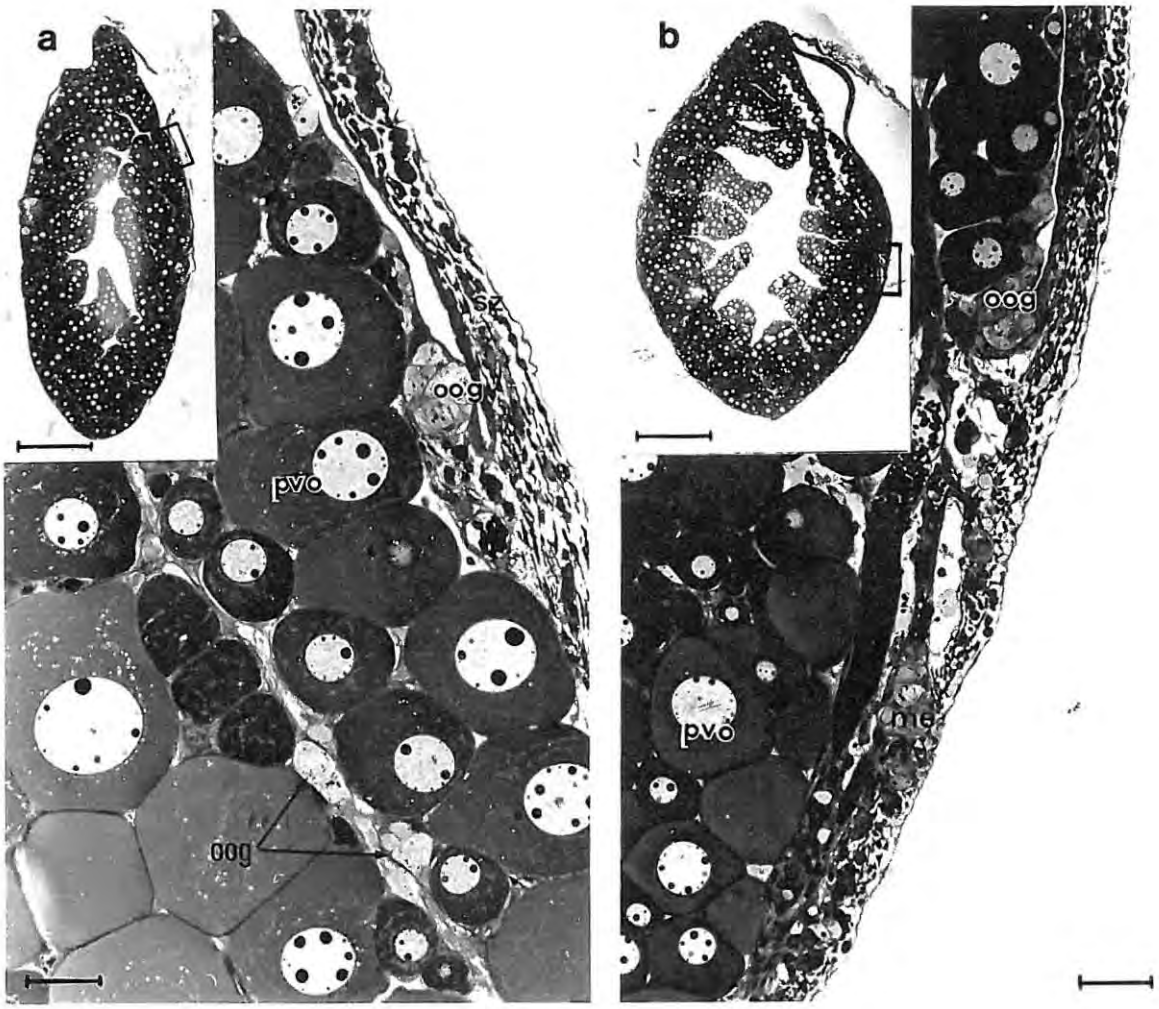

a
b
oog
pvo
oog
pvo
oog

Figure 67. Medial sections of a Chrysoblephus puniceus ovary from a fish of 160mm (FL). **(a)** - gonial cells proliferating along margins of the lamellae and undergoing meiosis to form previtellogenic oocytes, **(b)** - inactive gonial cells in the male element. cc = central cavity, epo = early previtellogenic oocytes, me = male element, m.oog = mitotic oogonia, oog = oogonia, pvo = previtellogenic oocytes. Scale bars = 25µm.

(**Figure 68** in volume 1)

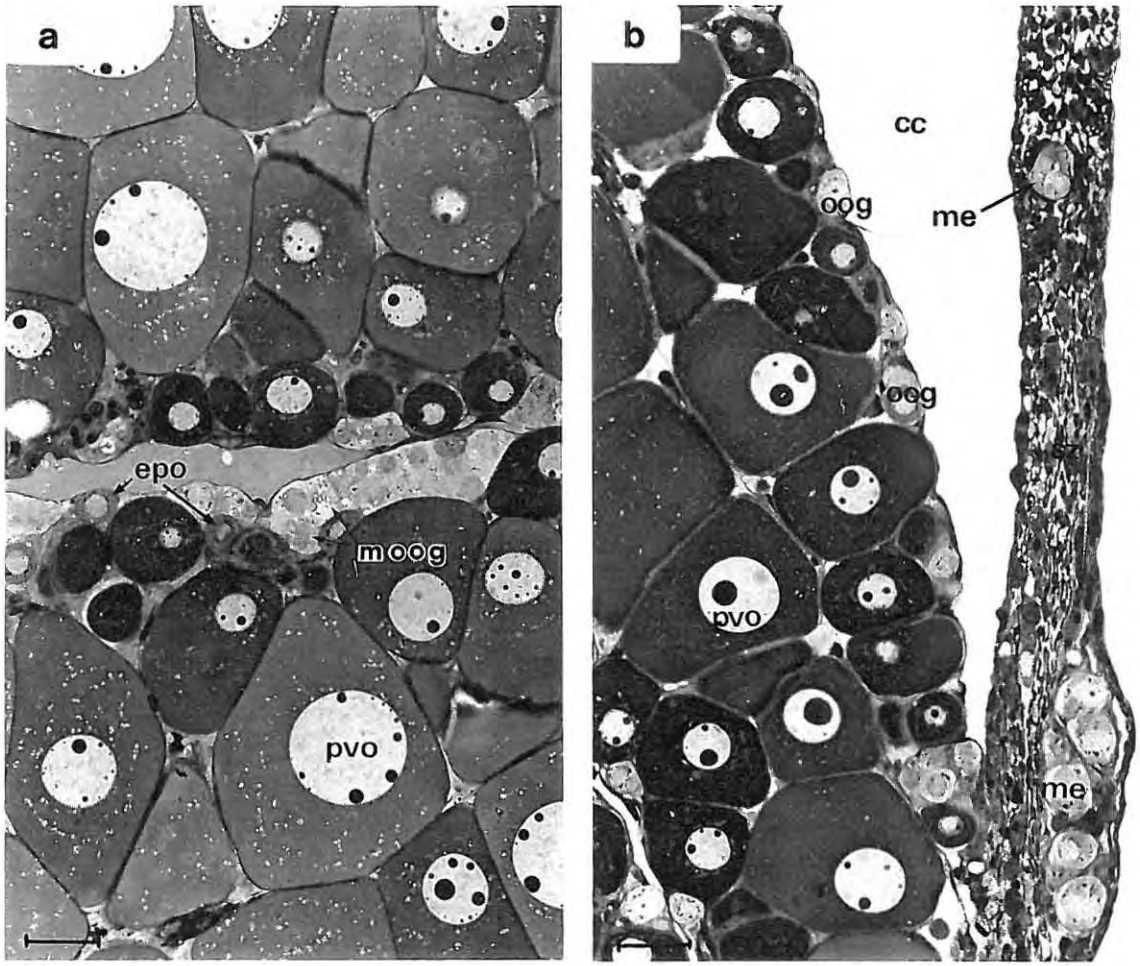

a
epo
moog
pvo
b
cc
oog
me
oog
pvo
me

Figure 69. Anterior (**a**), medial (**b**) and posterior (**c**) sections of a Chrysoblephus puniceus gonad, identified macroscopically as a hermaphrodite, from a fish of 203mm (FL). The male element consisted of a small v-shaped appendage in the mid-posterior region of the gonad in which there were numerous gonial cells. bv = blood vessel, cc = central cavity, l = lacunae, me = male element, pvo = previtellogenic oocytes, sz = sterile zone. Scale bar = 250µm.

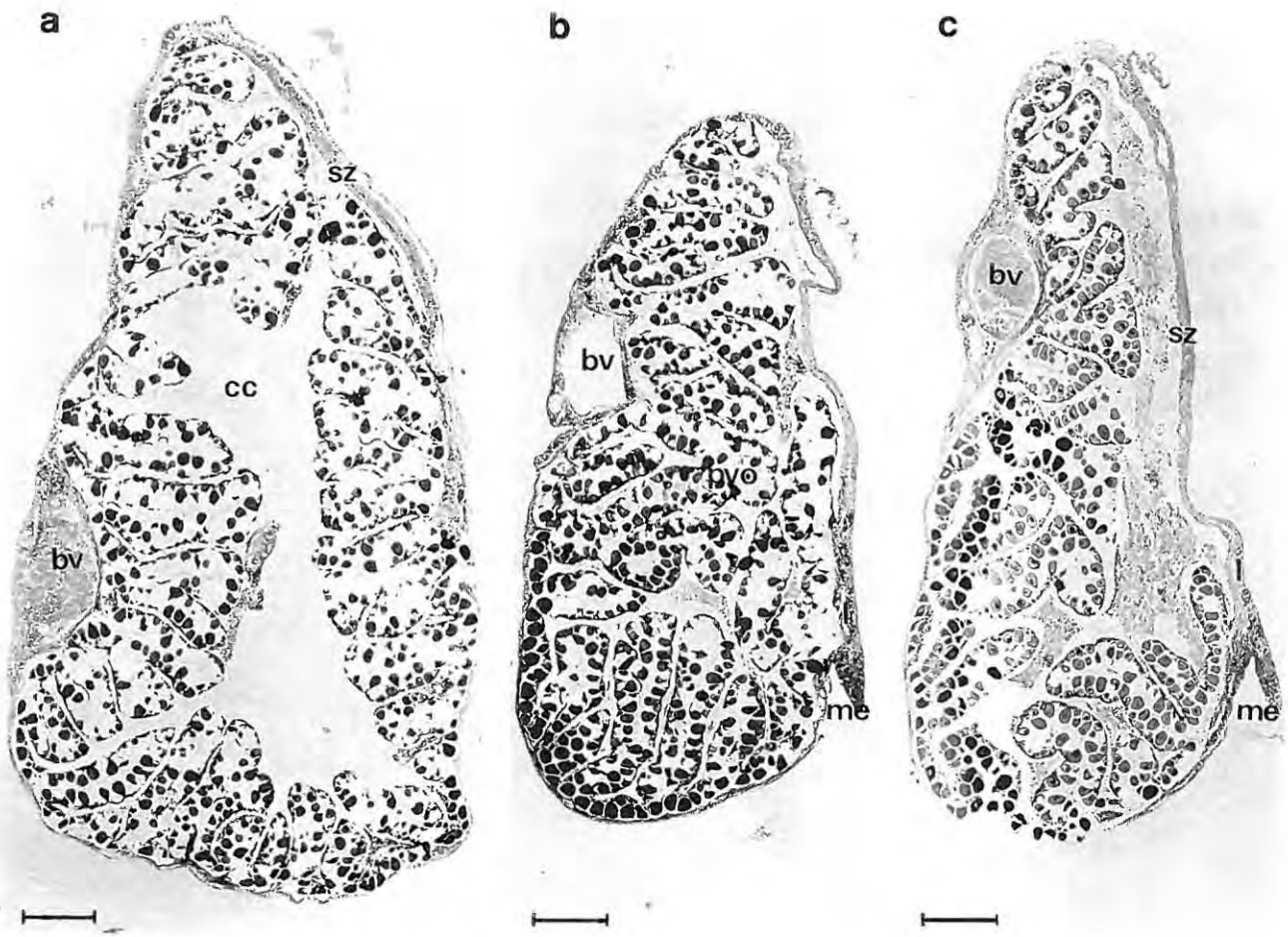

a
sz
cc
bv
b
bv
pvo
me
c
bv
sz
l
me

Figure 70. Medial section of a Chrysoblephus puniceus gonad from a fish of 225mm (FL), showing (**a**) - typical morphology of ovaries in this length class. The male element (**b**) is evident as a thin band of gonial cells within the tunica albuginea below the sterile zone. cc = central cavity, me = male element, pvo = previtellogenic oocytes, sz = sterile zone, ta = tunica albuginea. Scale bar in (**a**) = 500µm, in (**b**) = 25µm.

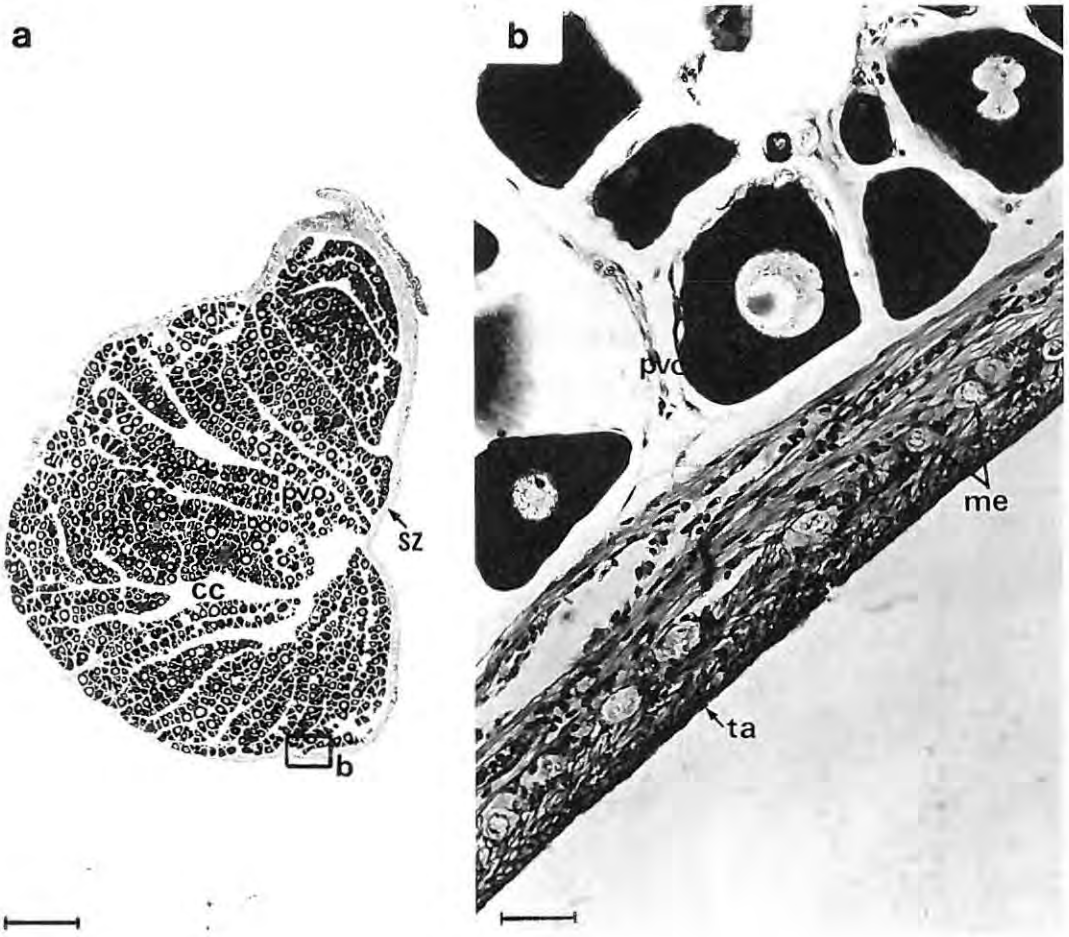

a
b
pvp
SZ
CC
b
pvc
me
ta

Figure 71. Anterior (**a**), medial (**b**) and posterior (**c**) sections of a Chrysoblephus puniceus gonad from a fish of 230mm (FL), showing typical morphology of ovaries in the length class 200-249mm. The male element (**d**) was further developed in this gonad, consisting of numerous gonial cells within a thickened tunica albuginea. cc = central cavity, go = gonial cells, me = male element, pvo = previtellogenic oocytes, sz = sterile zone. Scale bar in (**a-c**) = 500µm, in (**d**) = 100µm.

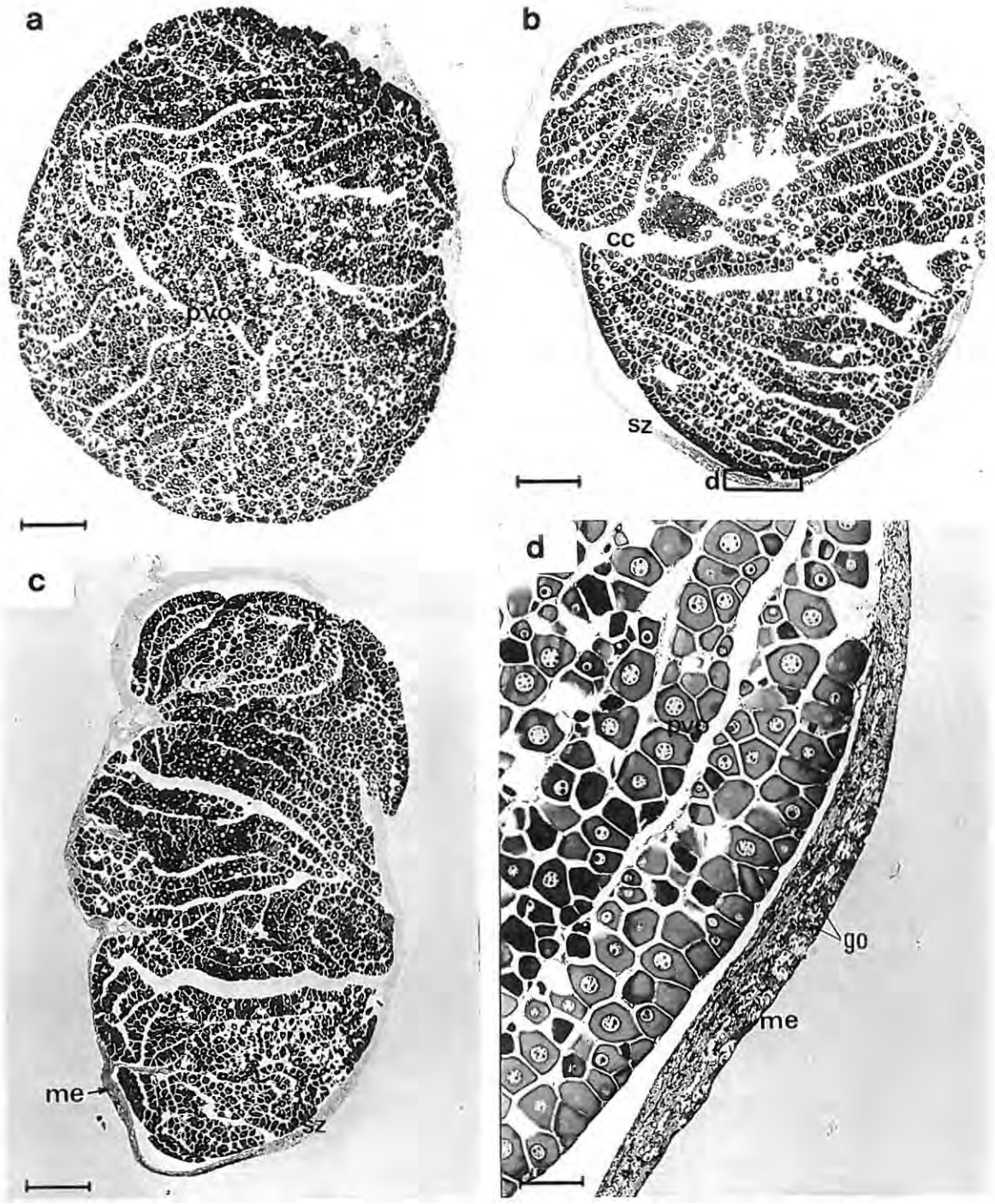

a
pvo
b
cc
sz
d
c
me
sz
d
pvo
go
me

Figure 72. Medial sections of Chrysoblephus puniceus ovaries in the length class 250-299mm (FL), showing **(a)** - typical morphology. Male elements varied from a large number of gonial cells in thickened tunica albuginea **(b)** to a few isolated gonial cells **(c,d)**, in both active and inactive gonads. bv = blood vessel, cc = central cavity, go = gonial cells, me = male element, pvo = previtellogenic oocytes, re = ripe egg, sz = sterile zone, yvo = yolk vesicle oocyte, 3°YO = tertiary yolk oocyte. Scale bar in **(a,c)** = 500µm, in **(b)** = 100µm, in **(d)** = 25µm

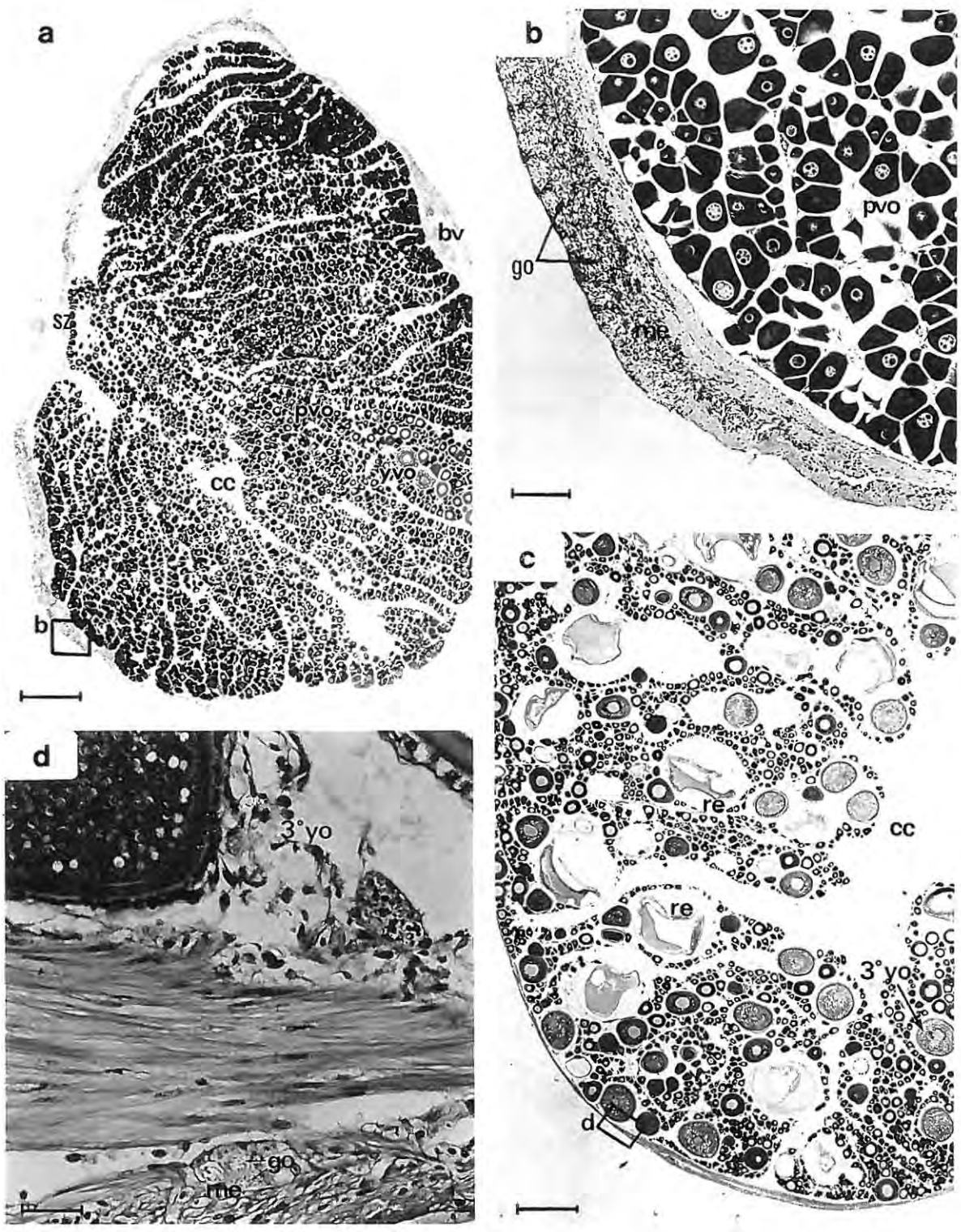

a
bv
sz
pvo
yvo
cc
b
b
go
me
pvo
c
re
cc
re
3° yo
d
d
3° yo
go
me

Figure 73. Medial **(a,b)** and posterior **(c,d)** sections of a predominantly female hermaphroditic gonad from a Chrysoblephus puniceus of 255mm (FL). All stages of spermatogenesis were evident in the male element and a number of oocytes in the ovary had developed to the yolk vesicle stage. cc = central cavity, pvo = previtellogenic oocytes, sc = spermatocytes, sg = spermatogonia, sz = spermatozoa, YVO = yolk vesicle oocytes. Scale bar in **(a,c)** = 50µm, in **(b,d)** = 500µm.

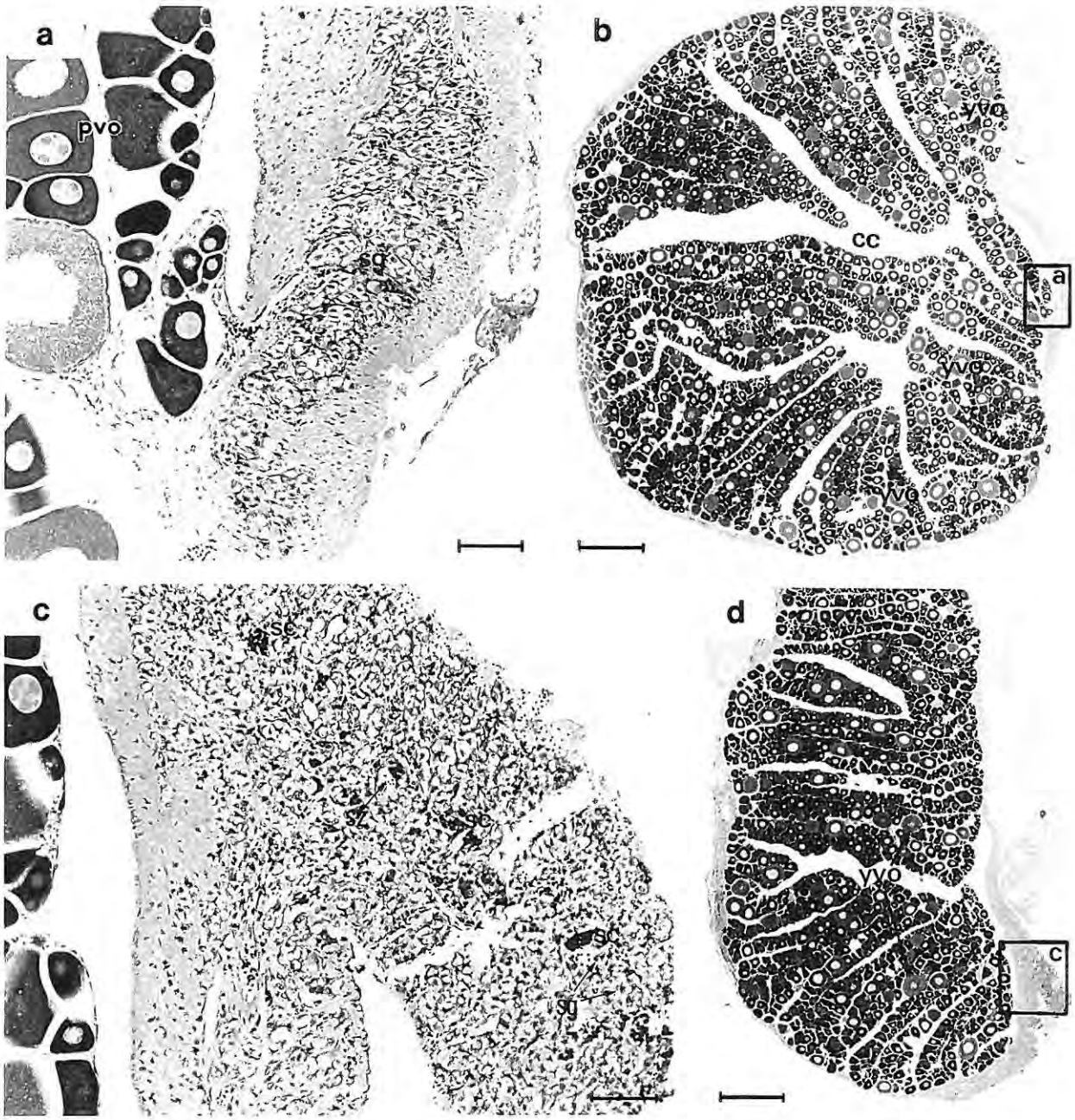

a
pvo
sg
b
vvo
cc
a
vvo
vvo
c
sc
sz
sc
sc
sg
d
vvo
c

Figure 74. Medial sections of the testes of two small male Chrysoblephus puniceus, showing v-shaped testicular tissue which is compressed dorso-ventrally. In the smaller fish of 272mm FL (**a**), the ovarian remnant is relatively large and only partially enclosed by the testis. It is completely surrounded by the sperm duct. In the larger fish of 289mm FL (**b**), the ovarian remnant is reduced in size and is not enclosed by the testis, but it remains surrounded by the sperm duct. cc = central cavity, or = ovarian remnant, sd = sperm duct, t = testis. Scale bar = 500µm.

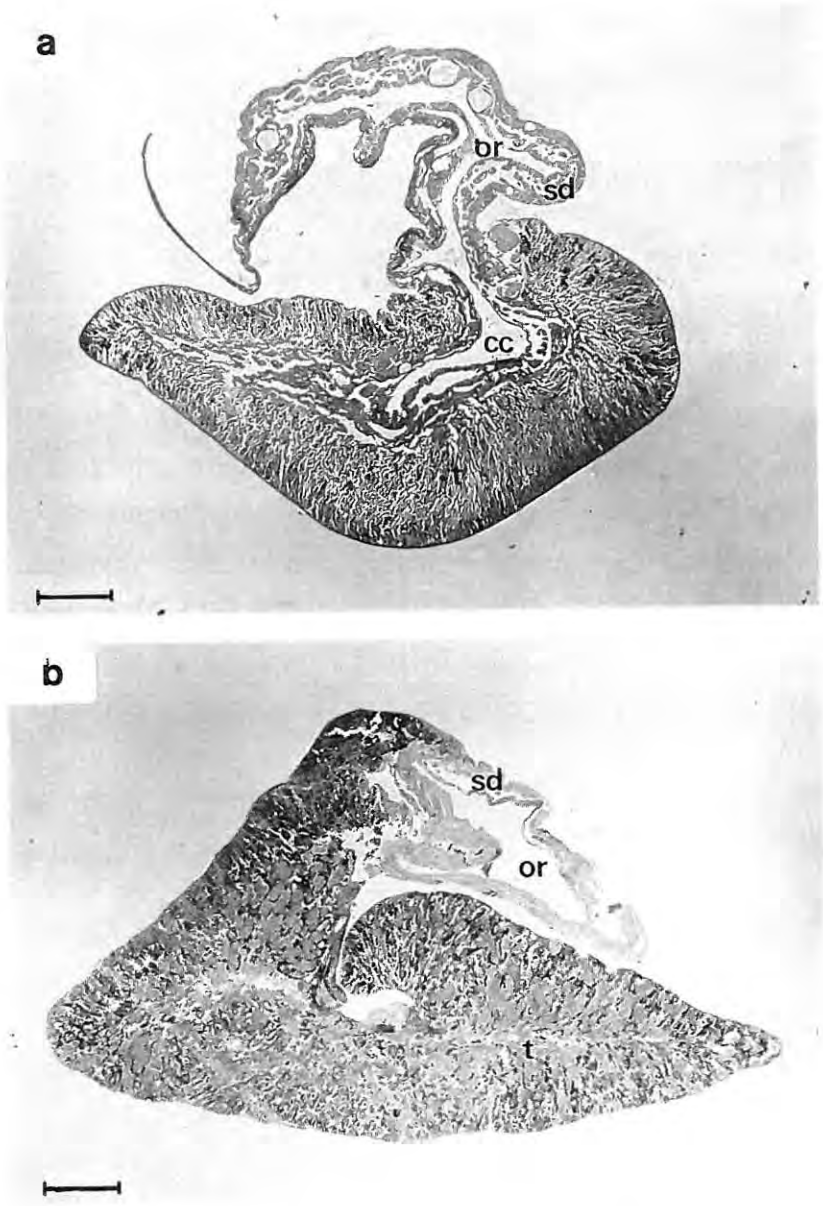

a
br
sd
cc
t
b
sd
or
t

Figure 75. Medial sections of inactive (**a**) and active (**b**) Chrysoblephus puniceus ovaries showing typical morphology in the size range 300-349mm (FL). The male element varied from a thickened tunica albuginea with numerous gonial cells (**c**) to a few isolated gonia which were difficult to locate (**d**), especially in ripe gonads. cc = central cavity, me = male element, pvo = previtellogenic oocytes, sg = spermatogonia, sz = sterile zone. Scale bar in (**a,b**) = 500µm, in (**c**) = 100µm, in (**d**) = 25µm.

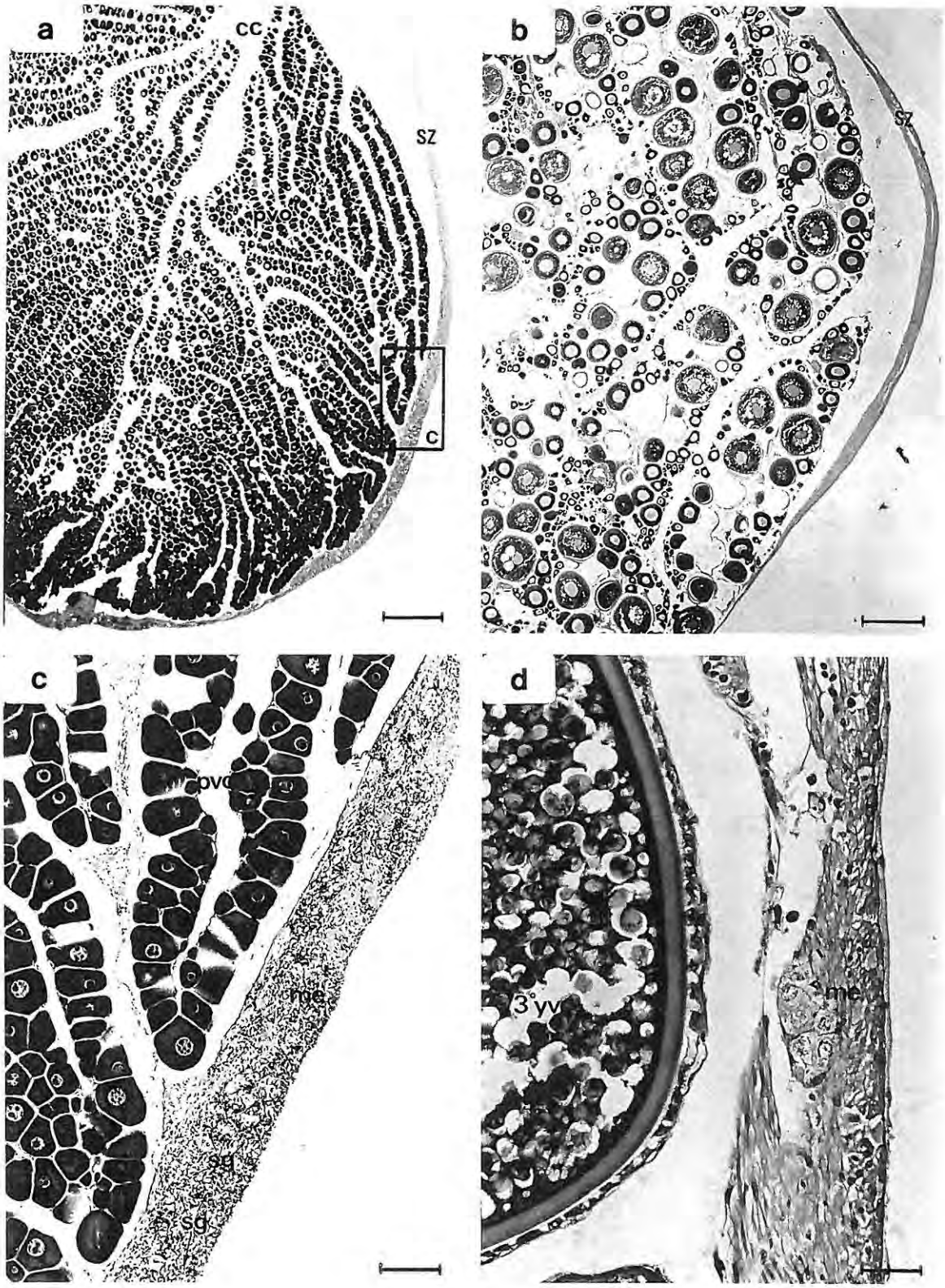

a
cc
SZ
pvc
c
b
SZ
c
pvc
me
sg
sg
d
3°yv
me

Figure 76. Anterior (a), medial (b) and posterior (c) sections of a predominantly female hermaphroditic Chrysoblephus puniceus gonad from a fish of 325mm (FL), showing a male element in mid- and posterior regions in which gonia are proliferating and spermatogenesis has commenced **(d)**. cc = central cavity, me = male element, pvo = previtellogenic oocytes, sc = spermatocytes, sg = spermatogonia, sz = sterile zone, szo = spermatozoa. Scale bar in **(a-c)** = 500µm, in (d) = 100µm.

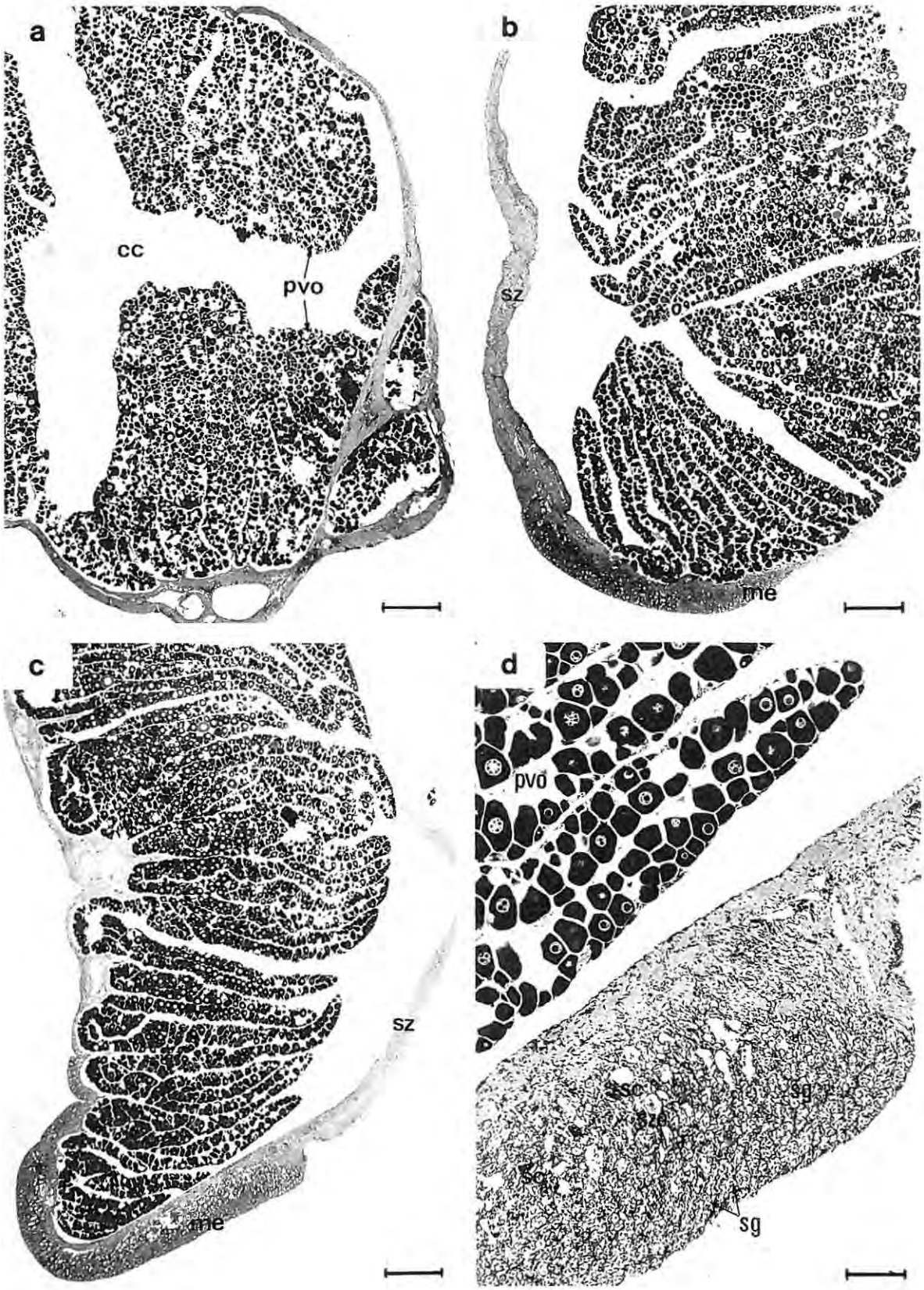

a
cc
pvo
b
sz
me
c
sz
me
d
pvo
sg
sg

Figure 77. Anterior **(a)**, medial **(b)** and posterior **(c)** sections of a predominantly female transitional Chrysoblephus puniceus gonad, showing a thickened tunica albuginea around the entire ovary and much connective tissue within ovigerous lamellae. In the male element **(d)**, which is restricted to the posterior region, all stages of spermatogenesis are evident, and the formation of a sperm duct has commenced in the sterile zone **(c)**. ct = connective tissue, me = male element, pvo = previtellogenic oocytes, sc = spermatocytes, sd = sperm duct, sg = spermatogonia, sz = sterile zone, szo = spermatozoa, tta = thickened tunica albuginea. Scale bar in **(a-c)** = 500µm, in **(d)** = 25µm.

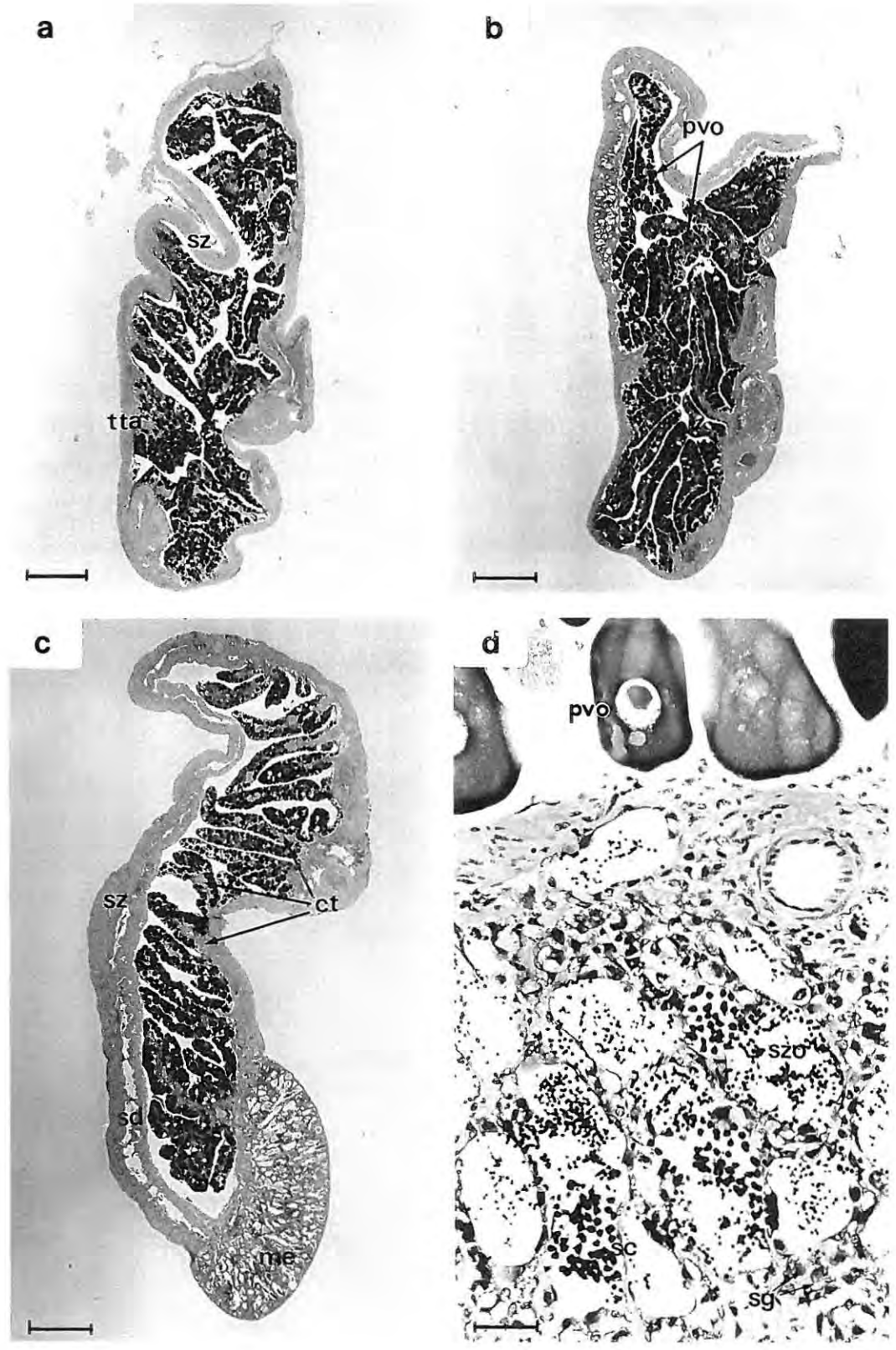

a
sz
tta
b
pvo
c
sz
ct
sd
me
d
pvo
szo
sc
sg

Figure 78. Medial section of a transitional Chrysoblephus puniceus gonad from a fish of 372mm (FL). **(a)** - male and female elements are of equal volume. The male element is active and there is extensive degeneration of germinal tissue in the female element. Ovigerous lamellae are reduced in number and size. **(b)** - degeneration of female germinal tissue is characterised by the presence of extensive yellow/brown bodies amongst previtellogenic oocytes in reduced lamellae. cc = central cavity, pvo = previtellogenic oocytes, sd = sperm duct, t = testis, yb = yellow/brown bodies. Scale bar in **(a)** = 500µm, in **(b)** = 50µm.

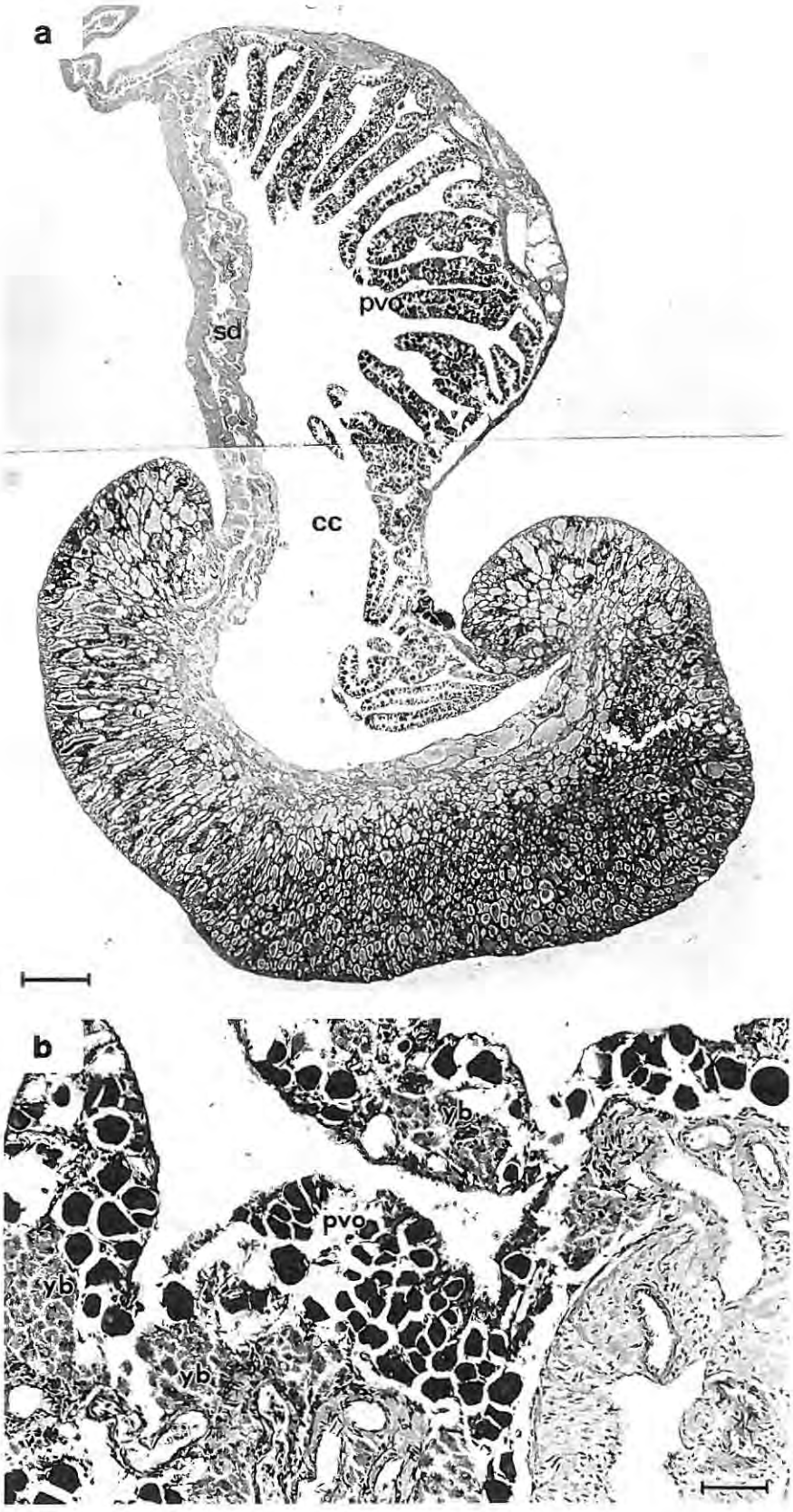

a
pvo
sd
cc
b
yb
pvo
yb
yb

Figure 79. Medial section of a predominantly male transitional Chrysoblephus puniceus gonad from a fish of 390mm (FL). **(a)** - female germinal tissue is further reduced in volume and much of the central cavity, which is surrounded by the sperm duct, is empty. In the male element all stages of spermatogenesis are evident, including numerous lobules of spermatozoa. **(b)** - degenerating previtellogenic oocytes in the female element. cc = central cavity, dpvo = degenerating previtellogenic oocytes, lsz = lobules of spermatozoa, sd = sperm duct, t = testis. Scale bar in **(a)** = 500µm, in **(b)** = 100µm.


b
a
cc
cc
sd
sd
dpvo
dpvo

Figure 80. Sections from two transitional Chrysoblephus puniceus gonads, showing extensive yellow/brown bodies in degenerating female germinal tissue. **(a)** - C. puniceus of 373mm, **(b)** - C. puniceus of 393mm. pvo = previtellogenic oocytes, yb = yellow/brown bodies. Scale bar in **(a)** = 100µm, in **(b)** = 50µm.

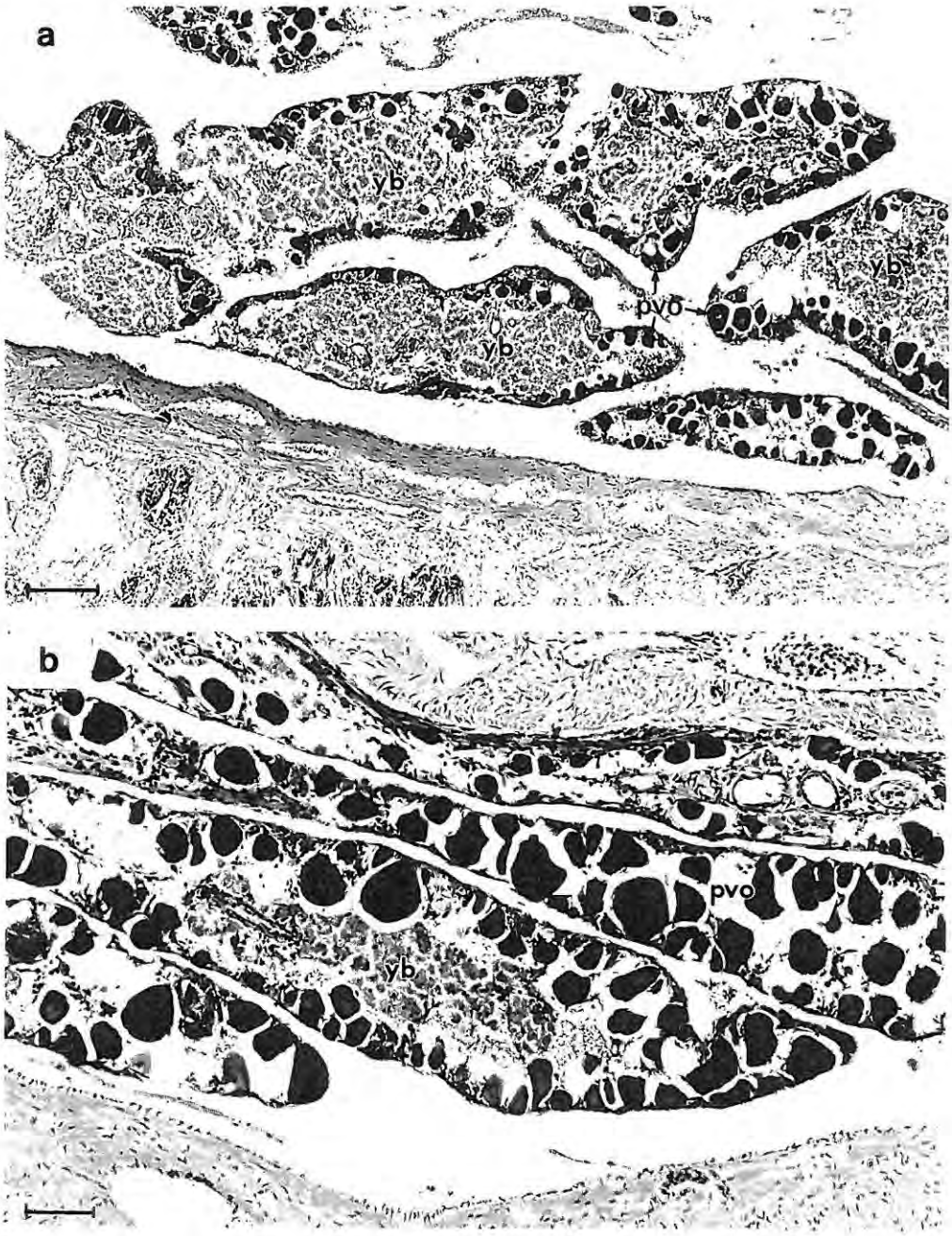
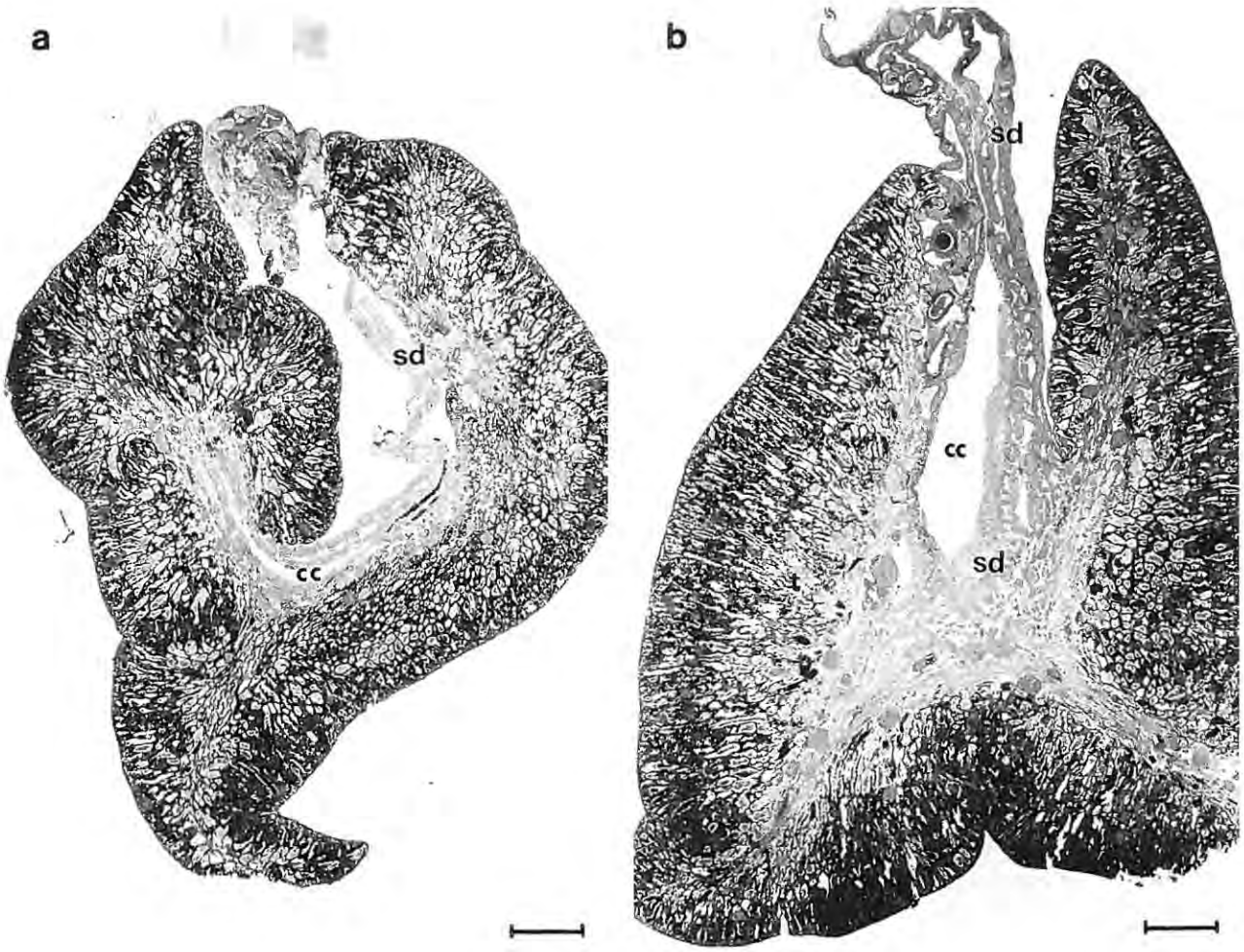

a
yb
yb
yb
pvo
b
pvo
yb

Figure 81. Sections of two recently formed Chrysoblephus puniceus testes, showing characteristic 'anchor shape' of testicular lobes around the former central cavity of the female element. **(a)** - C. puniceus of 350mm, **(b)** - C. puniceus of 370mm. cc = central cavity, sd = sperm duct, t = testis. Scale bar = 500µm.


a
sd
cc
b
sd
cc
sd

GONADAL DEVELOPMENT IN CHEIMERIUS NUFAR

Figure 82. Anterior **(a)**, medial **(b,c)** and posterior **(d)** sections of the undifferentiated gonadal primordium of a 44mm (FL) Cheimerius nufar, showing a cranio-caudal gradient of development in the formation of the central cavity. Formation is complete in the anterior region **(a)** but along the remainder of the primordium the second element has only begun to extend down alongside the first. bv = blood vessel, cc = central cavity, e1 = first element, e2 = second element, l = lacunae, m = mesovarium. Scale bar = 25μm.

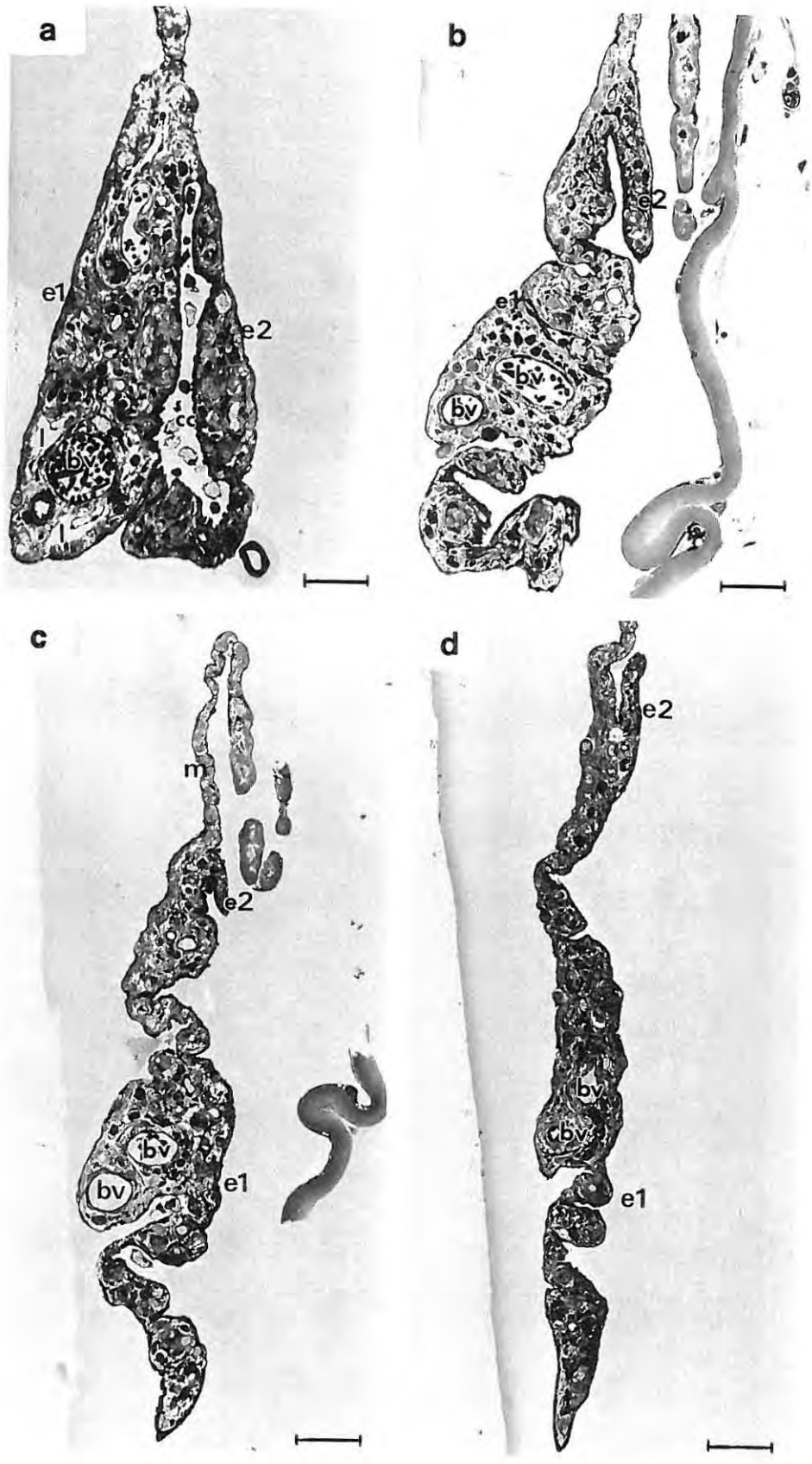

a
e1
e2
cc
l
bv
l
b
e2
e1
bv
bv
c
m
e2
bv
bv
e1
d
e2
bv
bv
e1

Figure 83. Anterior (a), medial (b) and posterior (c) sections of the undifferentiated gonadal primordium of a 76mm (FL) Cheimerius nufar, showing a fully formed central cavity along the length of the primordium, a distinct sterile zone in mid- and posterior regions and numerous lacunae within the stroma. bv = blood vessel, cc = central cavity, l = lacunae, m = mesovarium, sz = sterile zone. Scale bar = 25μm.

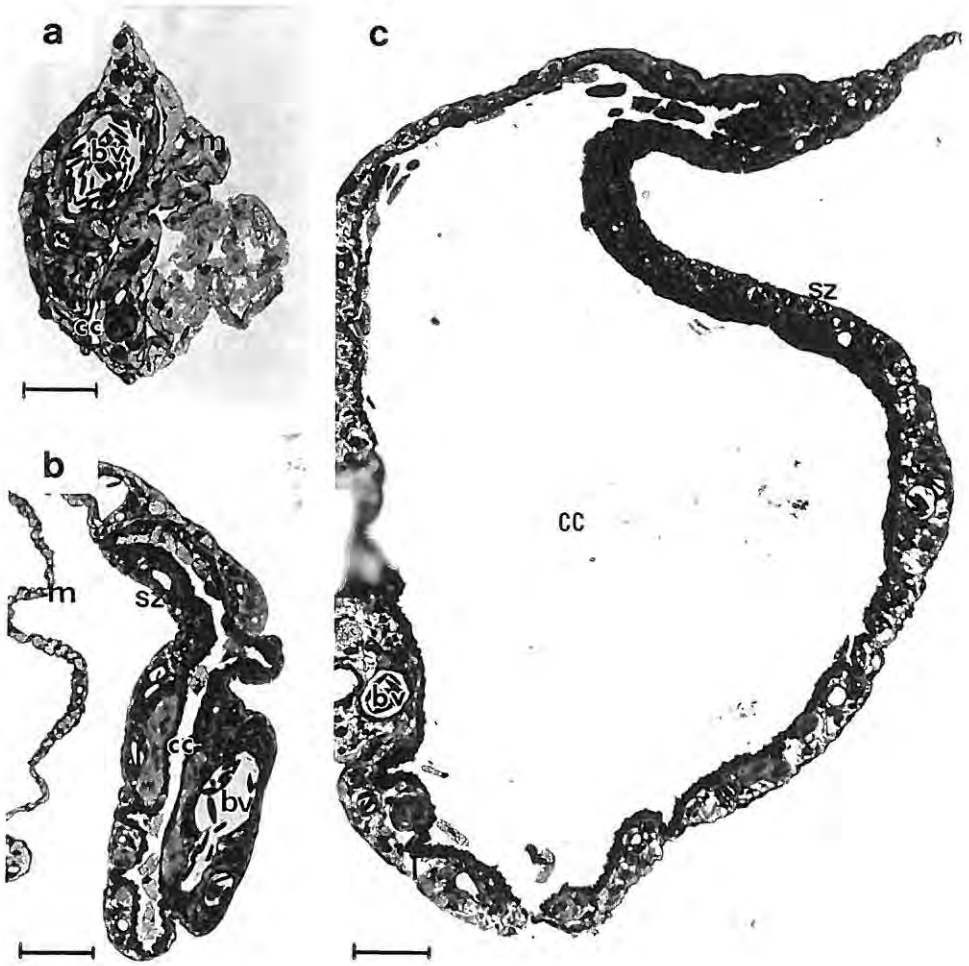

a
bv
m
cc
b
m
sz
cc
bv
c
sz
cc
bv

Figure 84. Anterior **(a)**, medial **(b)** and posterior **(c)** sections of the undifferentiated gonadal primordium of a 79mm (FL) Cheimerius nufar, showing several cysts of less-electron-dense cells along the margins of a fully formed central cavity, a distinct sterile zone and the development of extensive lacunae throughout the stroma. **(d)** - electron micrograph of cells comprising the sterile zone, **(e)** - electron micrograph of cells comprising the germinal zone. bv = blood vessel, cc = central cavity, ce = columnar epithelium, cs = cysts of less-electron-dense cells, ct = connective tissue, f = fibrillar tissue, g = granulocytes, l = lacunae, m = mesovarium, s = somatic cells, v = vacuolated cells. Scale bar in **(a-c)** = 25µm, in **(d,e)** = 5µm.

(**Figure 85** in volume 1)

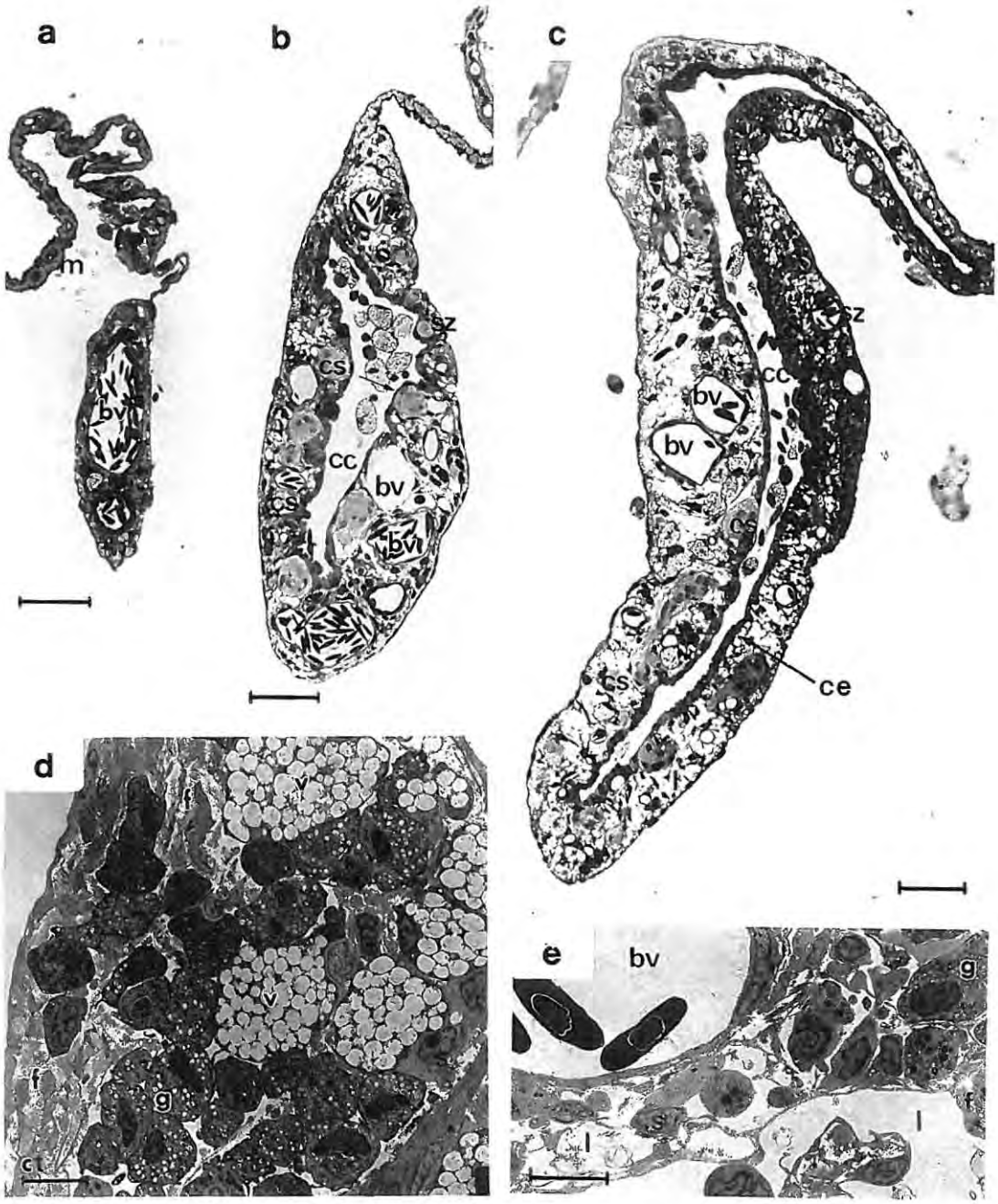

a
b
c
m
bv
cs
cc
bv
bvi
sz
cs
cc
bv
bv
cs
ce
d
v
v
f
g
cl
e
bv
g
s
l
l
f

Figure 86. Medial sections of undifferentiated and differentiated gonads of Cheimerius nufar in the size class 100-149mm (FL). **(a,b)** - young ovaries in which initial formation of lamellae has commenced and gonial cells and previtellogenic oocytes are proliferating along the margins of the central cavity. **(c-e)** - undifferentiated gonads in which germinal tissue is limited to small numbers of less-electron-dense (LED) cysts and gonial cells. cc = central cavity, cs = cyst of less-electron-dense cells, go = gonial cells, l = lacunae, m = mesovarium, pvo = previtellogenic oocytes, sz = sterile zone. Scale bar = 100µm.

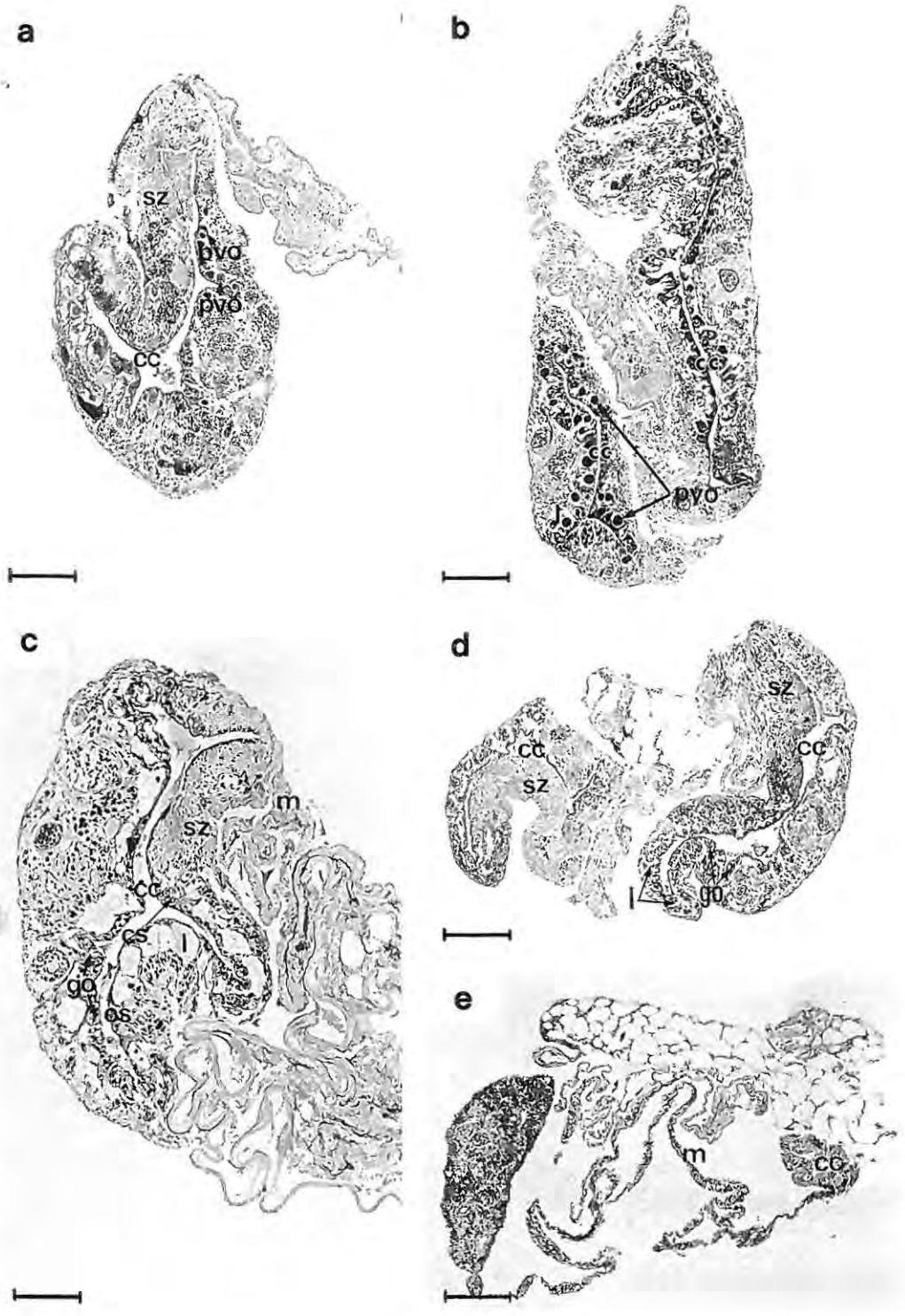

a
sz
pvo
pvo
cc
b
cc
c
pvo
l
c
sz
m
sz
cc
cs
l
go
os
d
sz
cc
cc
sz
l
go
e
m
cc

Figure 87. Anterior (**a**), medial (**b**) and posterior (**c**) sections of the undifferentiated gonadal primordium of a 118mm (FL) Cheimerius nufar, with higher magnifications of the anterior region (**d**) showing the development of LED cysts along the margins of the central cavity which are separated from the stroma by large lacunae. (**e**) - electron micrograph of a LED cyst showing typical cellular composition. bv = blood vessel, cc = central cavity, cs = cyst of less-electron-dense cells, l = lacunae, m = mesovarium, nu = nucleus, sz = sterile zone. Scale bar in (**a-c**) = 50µm, in (**d**) = 25µm, in (**e**) = 1µm.

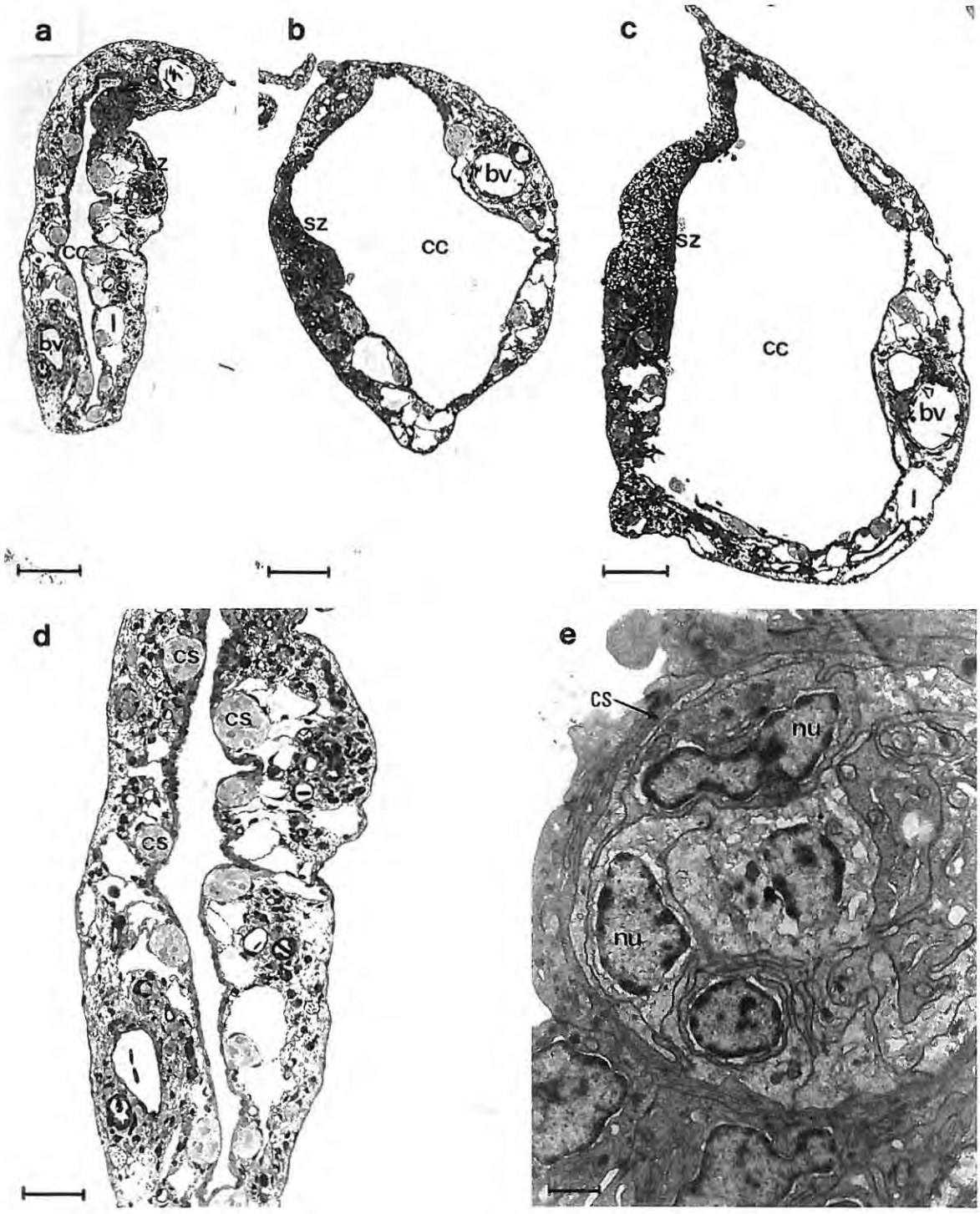

a
b
c
sz
cc
bv
l
d
cs
e
nu

Figure 88. Medial section (**a**) of the undifferentiated gonad of a 137mm (FL) Cheimerius nufar, with higher magnifications of dorsal (**b**) and ventral (**c**) regions. LED cysts and gonial cells developing along the margins of the central cavity are separated from the stroma by large lacunae. cc = central cavity, cs = cyst of less-electron-dense cells, go = gonial cells, l = lacunae, m = mesovarium, sz = sterile zone. Scale bar in (**a**) = 100µm, in (**b,c**) = 25µm.

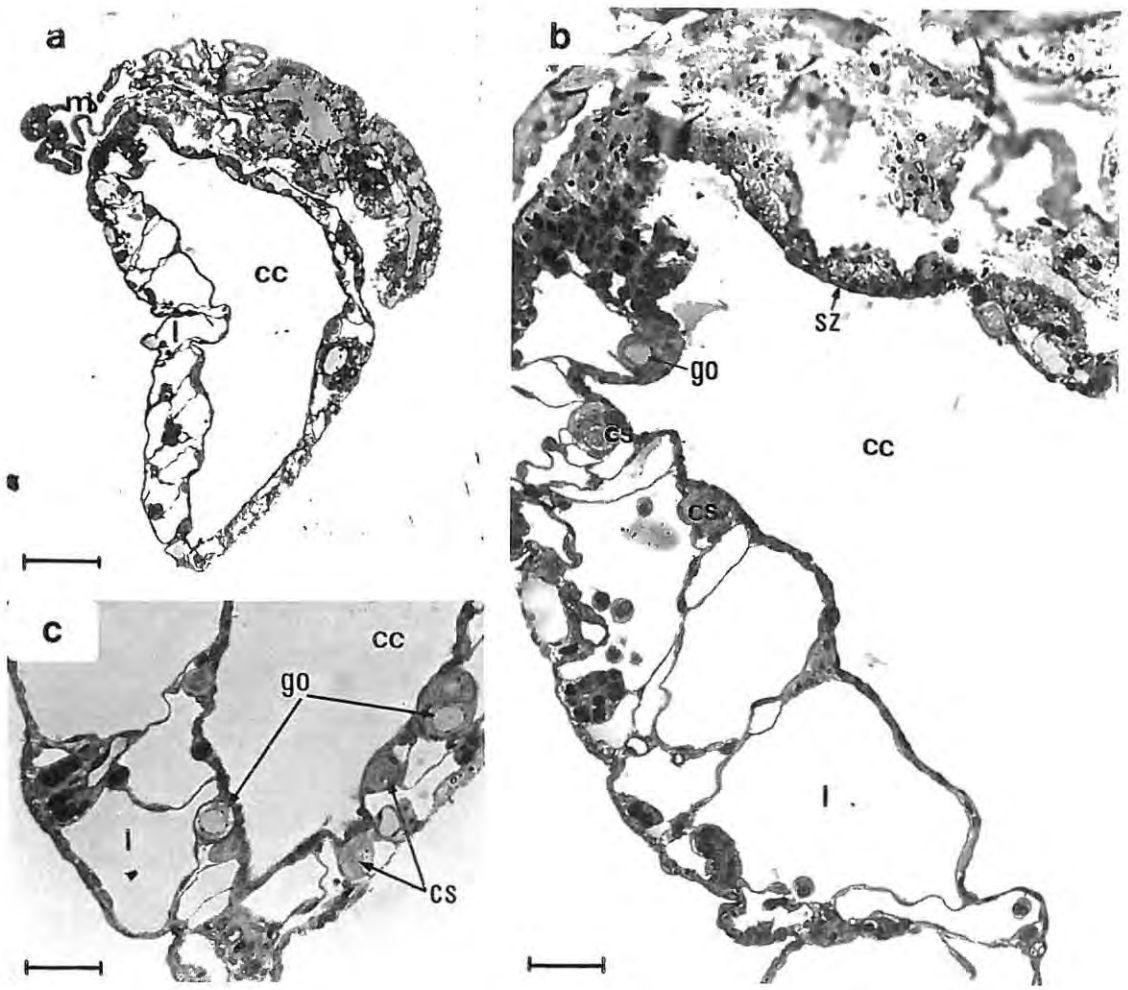

a
m
cc
l
b
sz
go
cs
cc
cs
l
c
cc
go
l
cs

Figure 89. Electron micrographs of LED cysts which have formed along the margins of the central cavity of an undifferentiated gonad from a Cheimerius nufar of 137mm (FL). LED cysts are separated from the stroma by large lacunae. **(a,b)** - cysts in early stages of development in which no differentiation of cells has occurred. **(c)** - a gonial cell developing within a cyst. cc = central cavity, cs = LED cyst, e = epithelial cells, go = gonial cell, l = lacunae. Scale bar = 3µm.

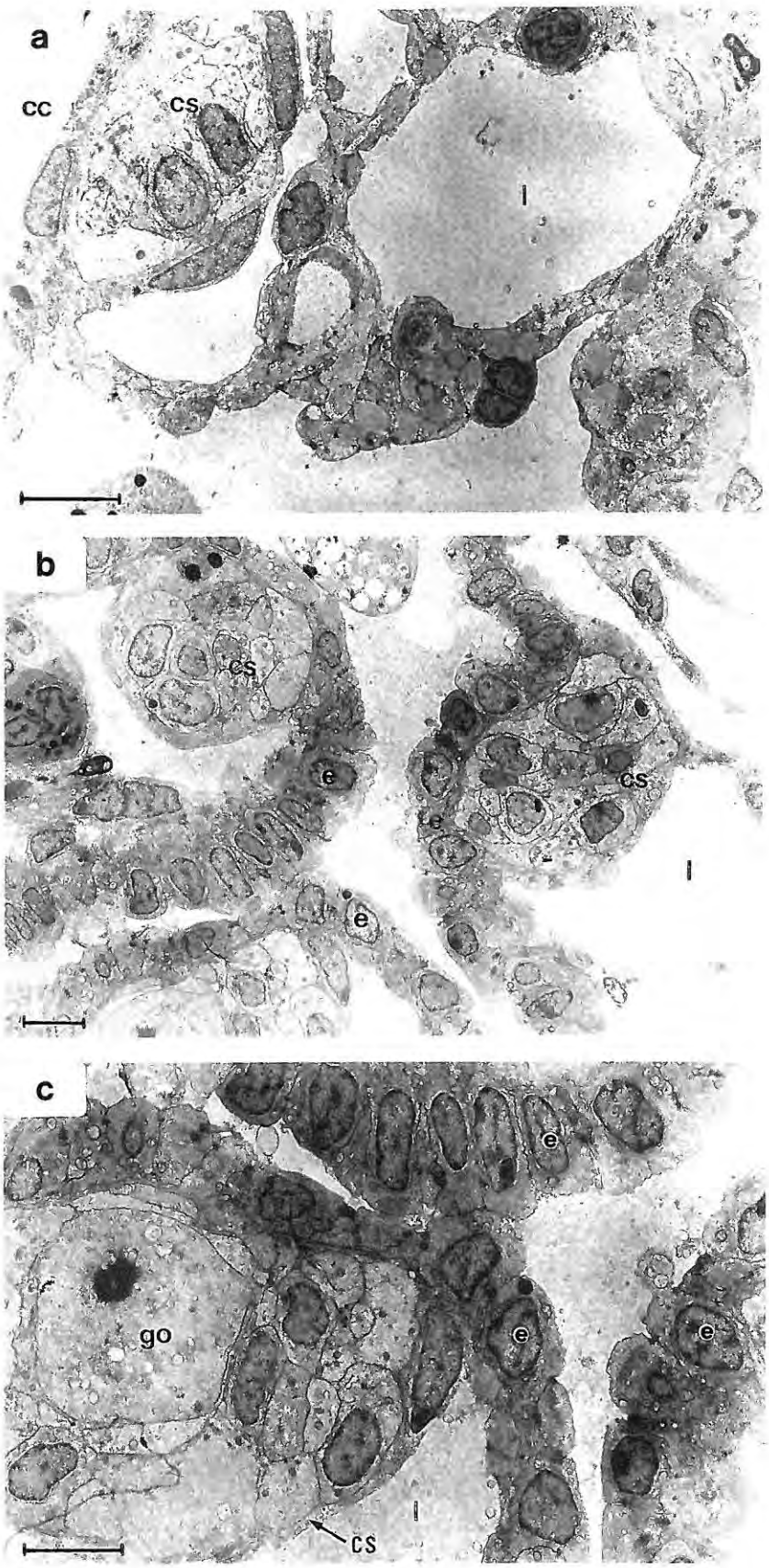

a
cc
cs
l
b
cs
e
cs
e
l
c
e
go
e
e
l
cs

Figure 90. Electron micrographs showing a series of developmental stages within LED cysts situated along the margins of the central cavity in a Cheimerius nufar of 137mm (FL). (**a**) - a single gonial cell developing within a cyst of LED cells, (**b**) - as the gonial cell develops it forces associated cells to the outer margins of the cyst, (**c**) further development results in associated cells becoming flattened around the gonial cell, (**d**) the end result is a single gonial cell surrounded by flattened myoid boundary cells. cs = LED cyst, go = gonial cell, mb = myoid boundary cells, n ='nuage', nu = nucleus, nuc = nucleolus. Scale bar = 2μm.

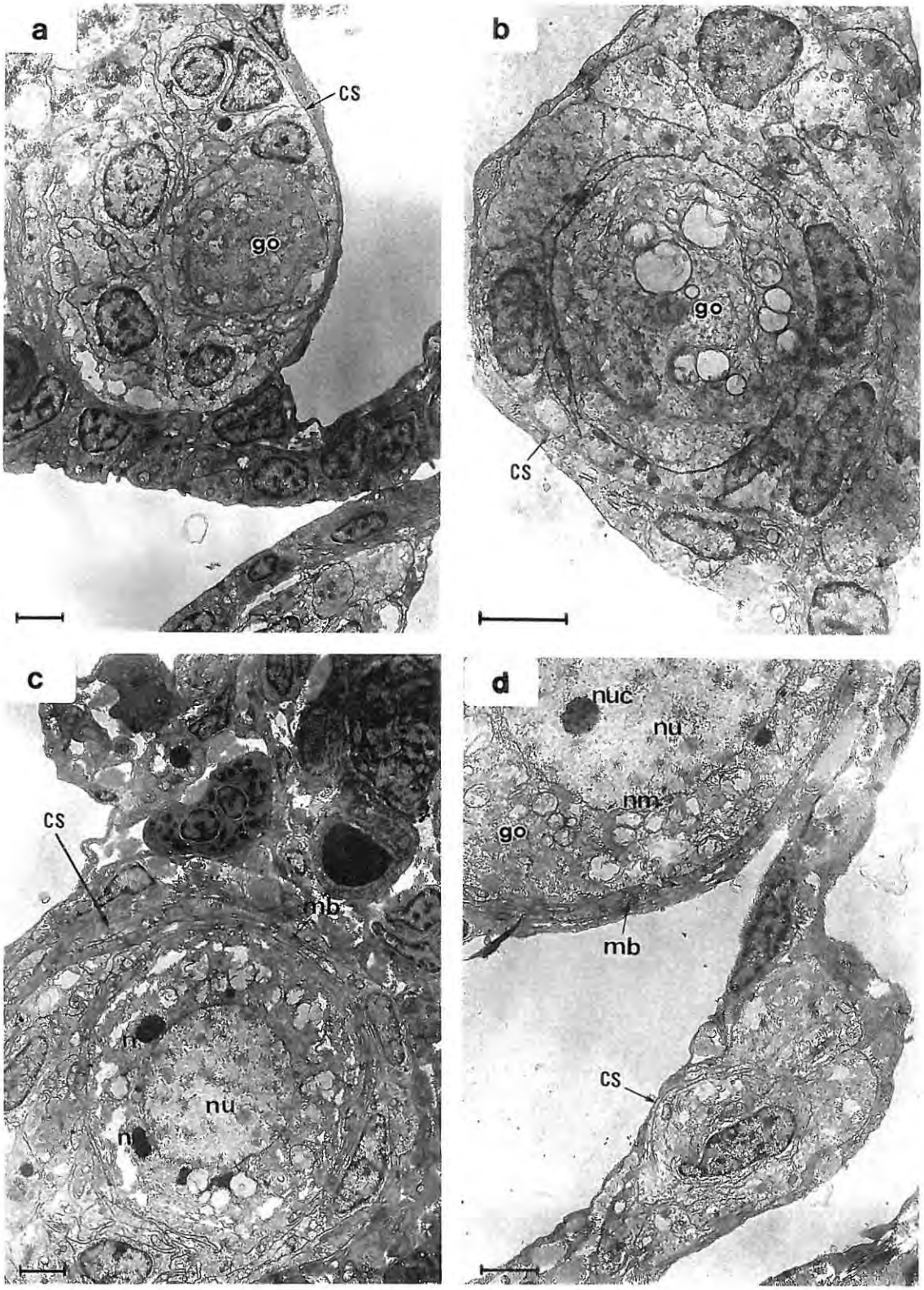

a
cs
go
b
go
cs
c
cs
mb
n
nu
n
d
nuc
nu
nm
go
mb
cs

Figure 91. Anterior (**a**), medial (**b**) and posterior (**c**) sections of the differentiating gonad of a 150mm (FL) Cheimerius nufar, with higher magnifications of mid-and posterior regions showing (**d**) - an early previtellogenic oocyte amongst proliferating gonial cells and (**e**) - the commencement of lamellar formation along the margins of the central cavity. bv = blood vessel, cc = central cavity, l = lacunae, la = lamella, m = mesovarium, m.oog = mitotic oogonia, pvo = previtellogenic oocyte, sz = sterile zone. Scale bar in (**a-c**) = 50µm, in (**d,e**) = 25µm.

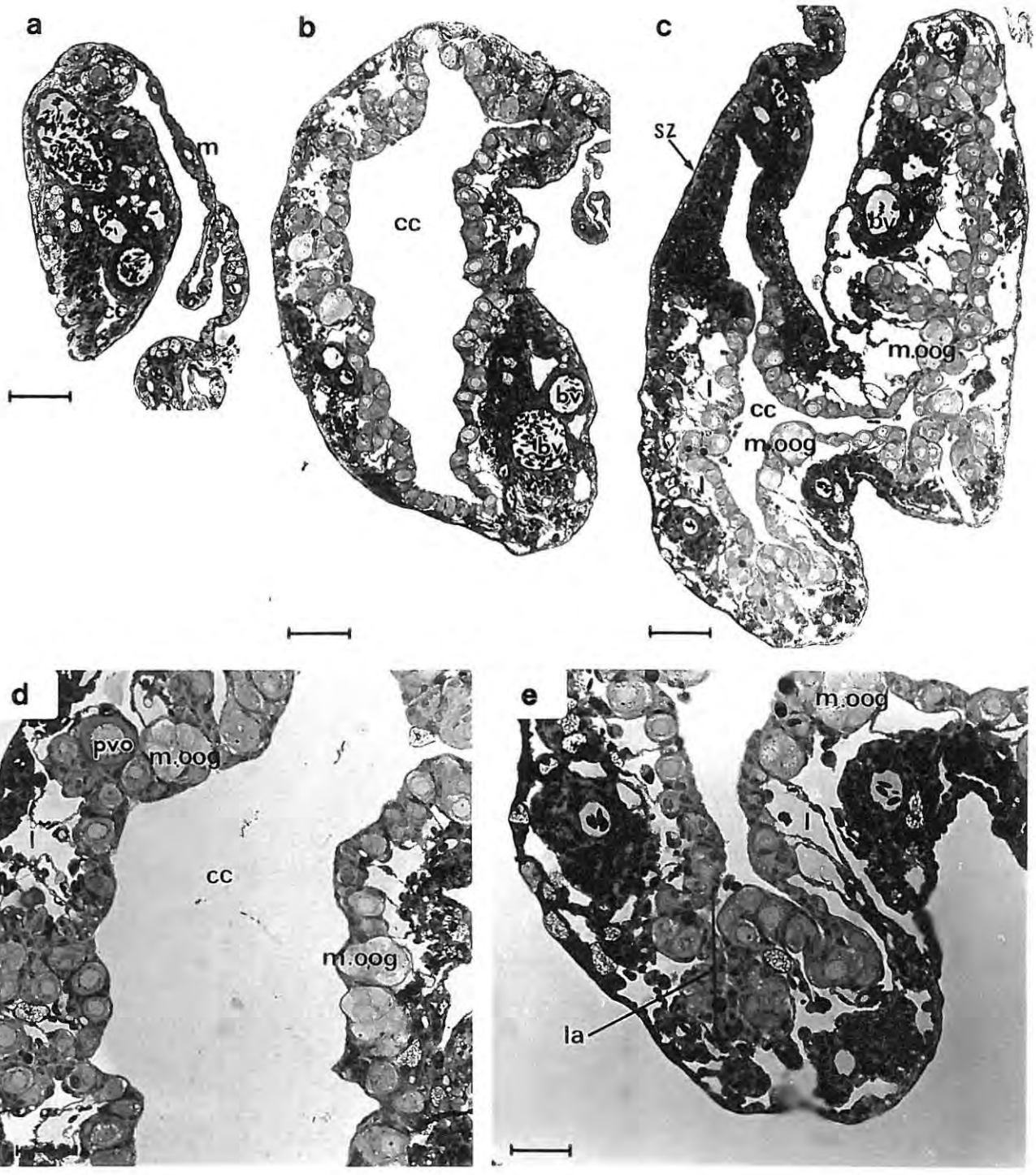

a
m
cc
b
cc
bv
bv
c
SZ
bv
m.oog
l
cc
m.oog
l
d
pvo
m.oog
l
cc
m.oog
e
m.oog
l
la

Figure 92. Medial (a) and posterior (b) sections of a differentiating gonad of a 168mm (FL) Cheimerius nufar, with higher magnifications of LED cysts and gonial cells along the margins of the central cavity (**c-e**). (**c**) - LED cysts and a single gonial cell within the ventral region of the sterile zone indicate the initial development of the male element, (**d**) - proliferation of gonial cells in the posterior region results in a reduction of LED cysts, (**e**) - several gonial cells developing within LED cysts in the less-developed anterior region. bv = blood vessel, cc = central cavity, cs = LED cysts, go = gonial cells, l = lacunae, sz = sterile zone. Scale bar in (**a,b**) = 100µm, in (**c-e**) = 25µm.

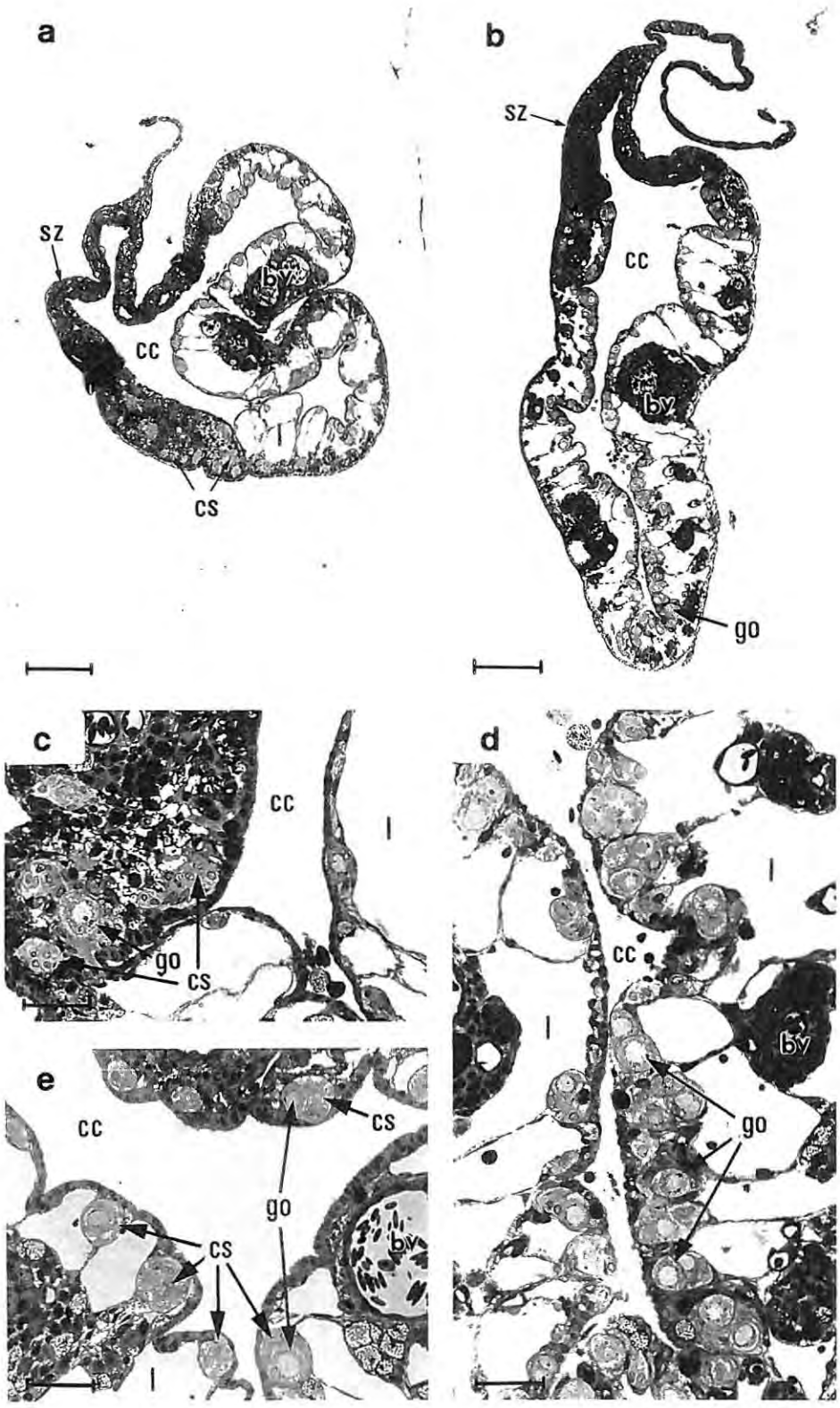

a
SZ
by
cc
l
cs
b
SZ
cc
by
go
c
cc
l
go
cs
d
l
cc
l
by
go
e
cc
cs
go
cs
by
l

Figure 93. Electron micrographs of: **(a)** - a LED cyst at the base of the sterile zone of the differentiating gonad of a 168mm (FL) Cheimerius nufar, **(b)** - a gonial cell in the same region which has developed within a cyst. Associated cells surrounding the gonial cell have become flattened to form myoid boundary cells. cs = LED cyst, ct = connective tissue, f = fibrillar tissue, go = gonial cell, mb = myoid boundary cells, n = 'nuage', nu = nucleus, s = somatic cells. Scale bar = 2µm.

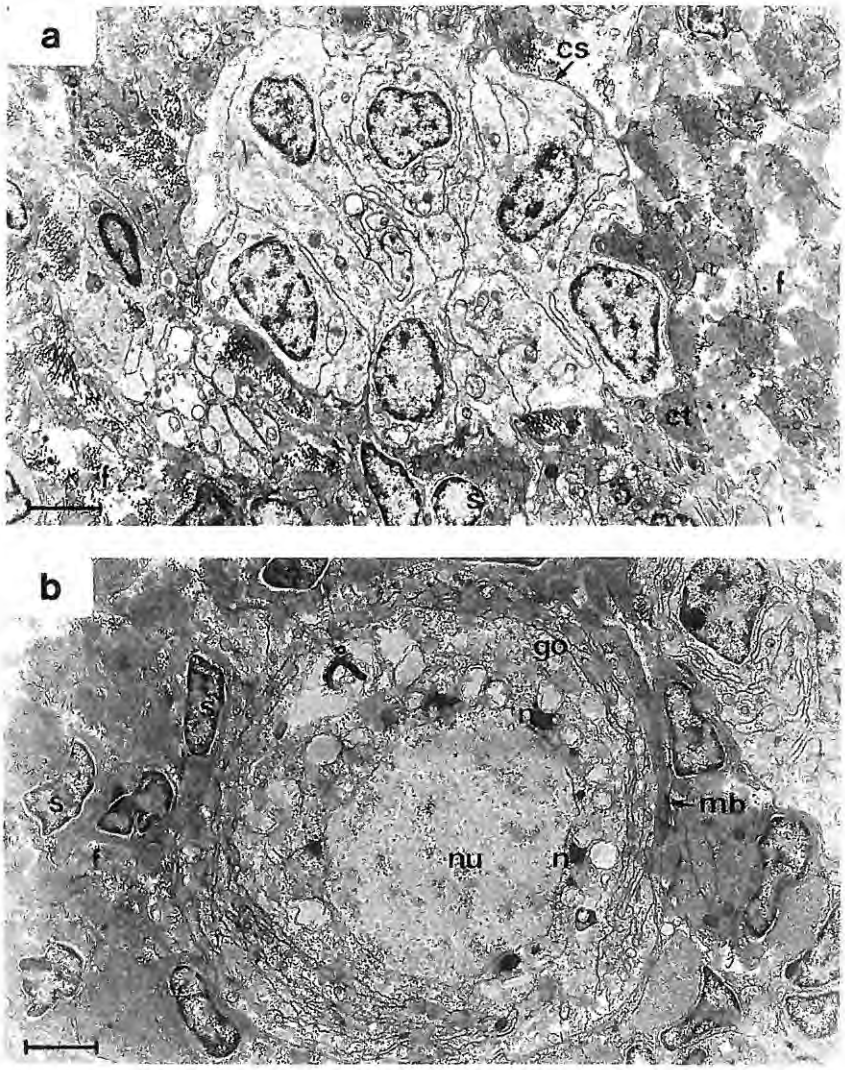

a
cs
f
ct
f
s
b
go
n
mb
s
f
nu
n

Figure 94. Anterior (**a,d**), medial (**b,e**) and posterior (**c,f**) sections of young ovaries from Cheimerius nufar of 165mm & 167mm (FL), showing two types of development. In the first (**a-c**), the sterile zone extends no further than one third down the length of the gonad in all regions. In the second (**d-f**), it extends one half to two thirds (or more) down the length of the gonad in mid- and posterior regions. bv = blood vessel, cc = central cavity, m = mesovarium, pvo = previtellogenic oocytes, sz = sterile zone. Scale bar = 250μm.

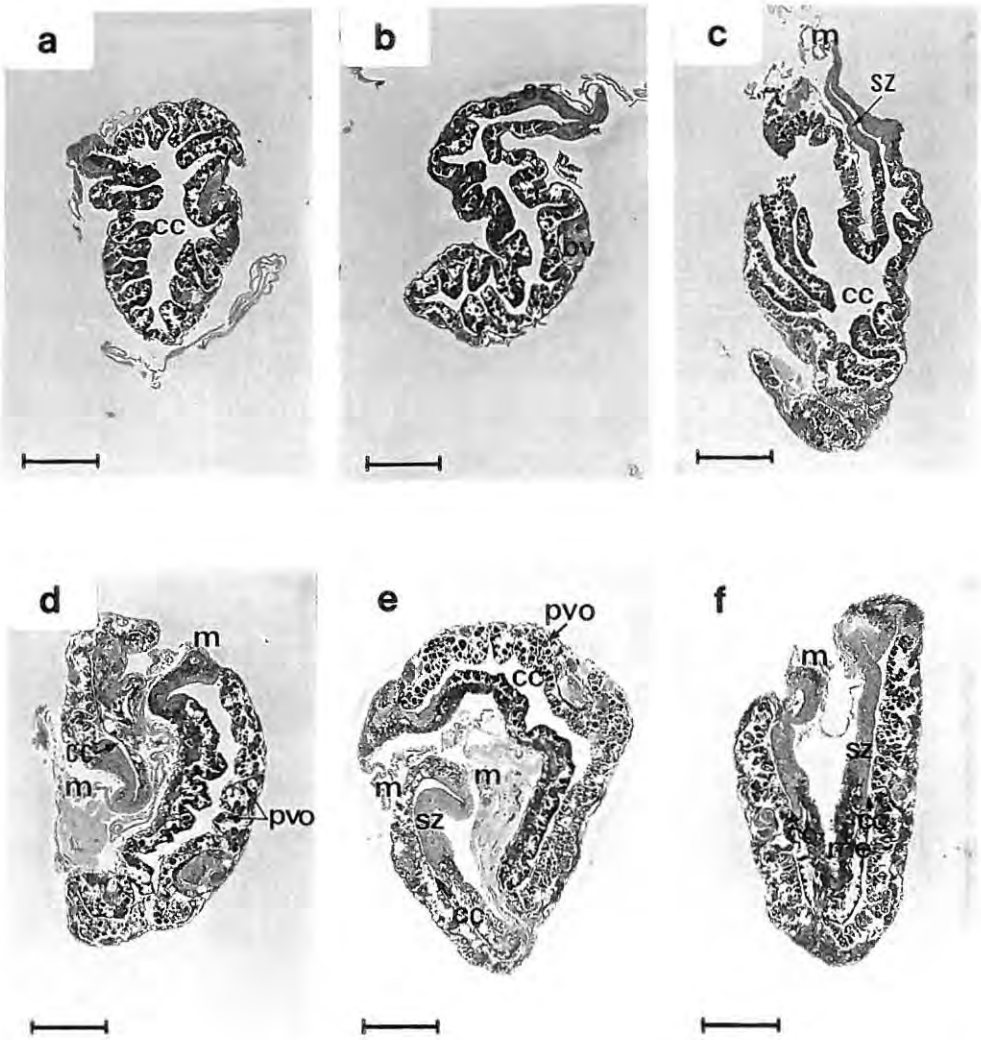

a
cc
b
sz
ov
c
m
sz
cc
d
m
cc
m
pvo
e
pvo
cc
m
m
sz
cc
f
m
sz
cc

Figure 95. Medial sections of ovaries from Cheimerius nufar of 189mm & 197mm (FL), showing: **(a,b)** - a reduced sterile zone in the dorsal region below which there is a small, inactive male element (few gonial cells), **(c,d)** - a male element which extends half way down the side of the ovary and in which gonial cells are proliferating. cc = central cavity, go = gonial cells, m = mesovarium, me = male element, pvo = previtellogenic oocytes, sz = sterile zone. Scale bar in **(a)** = 100µm, in **(b,d)** = 50µm, in **(c)** = 250µm.

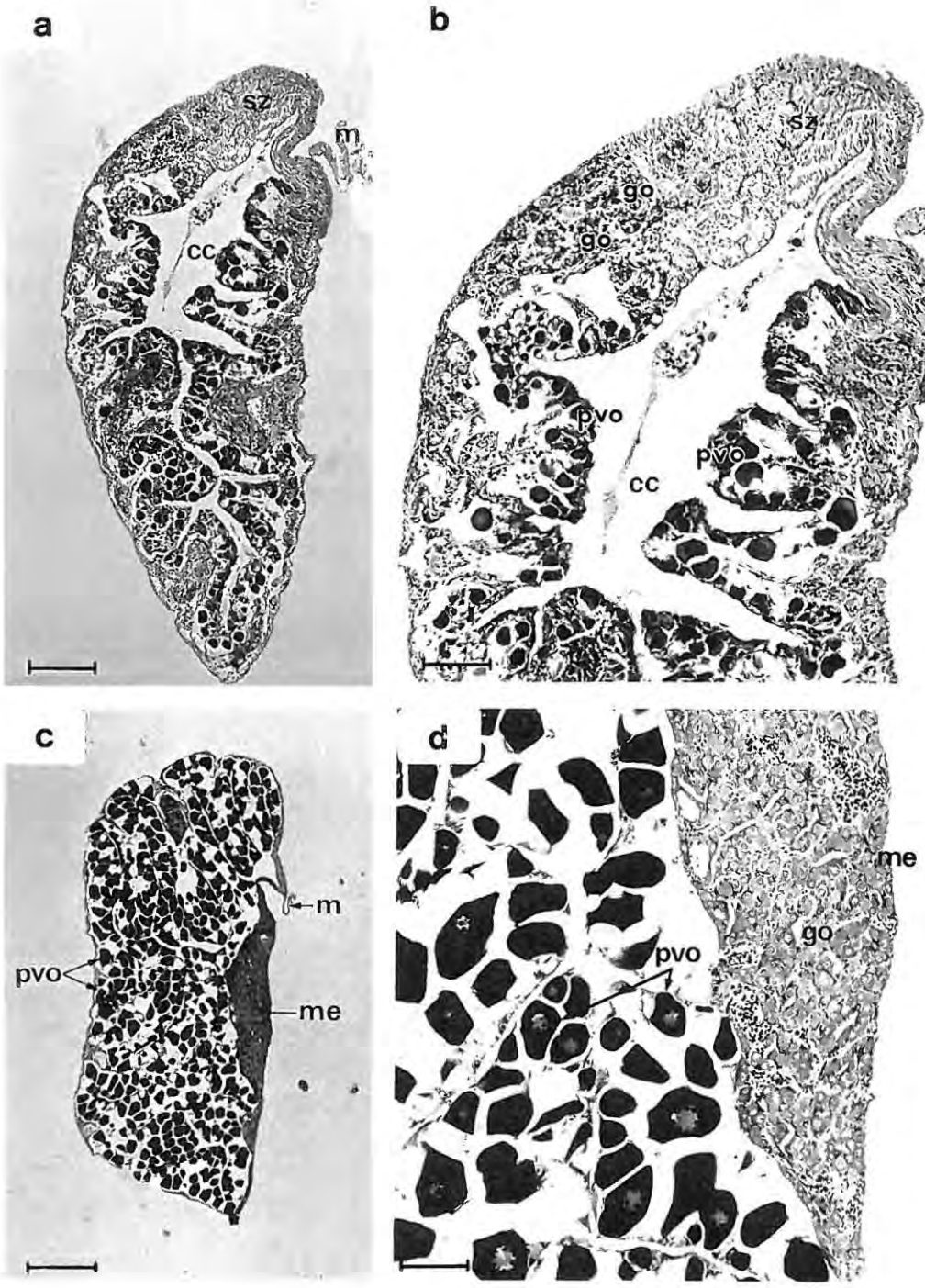

a
sz
m
cc
b
sz
go
go
pvo
pvo
cc
c
m
pvo
me
d
me
go
pvo

Figure 96. Anterior (**a**), medial (**b**) and posterior (**c**) sections of a gonad from a Cheimerius nufar of 200mm (FL), showing typical morphology of TYPE II (immature female) ovaries. (**d,e**) - higher magnifications of mid- and posterior sections showing a male element consisting of a small number of gonial cells within the tunica albuginea. cc = central cavity, go = gonial cells, m = mesovarium, me = male element, pvo = previtellogenic oocytes, sz = sterile zone. Scale bar in (**a-c**) = 500µm, in (**d,e**) = 25µm.

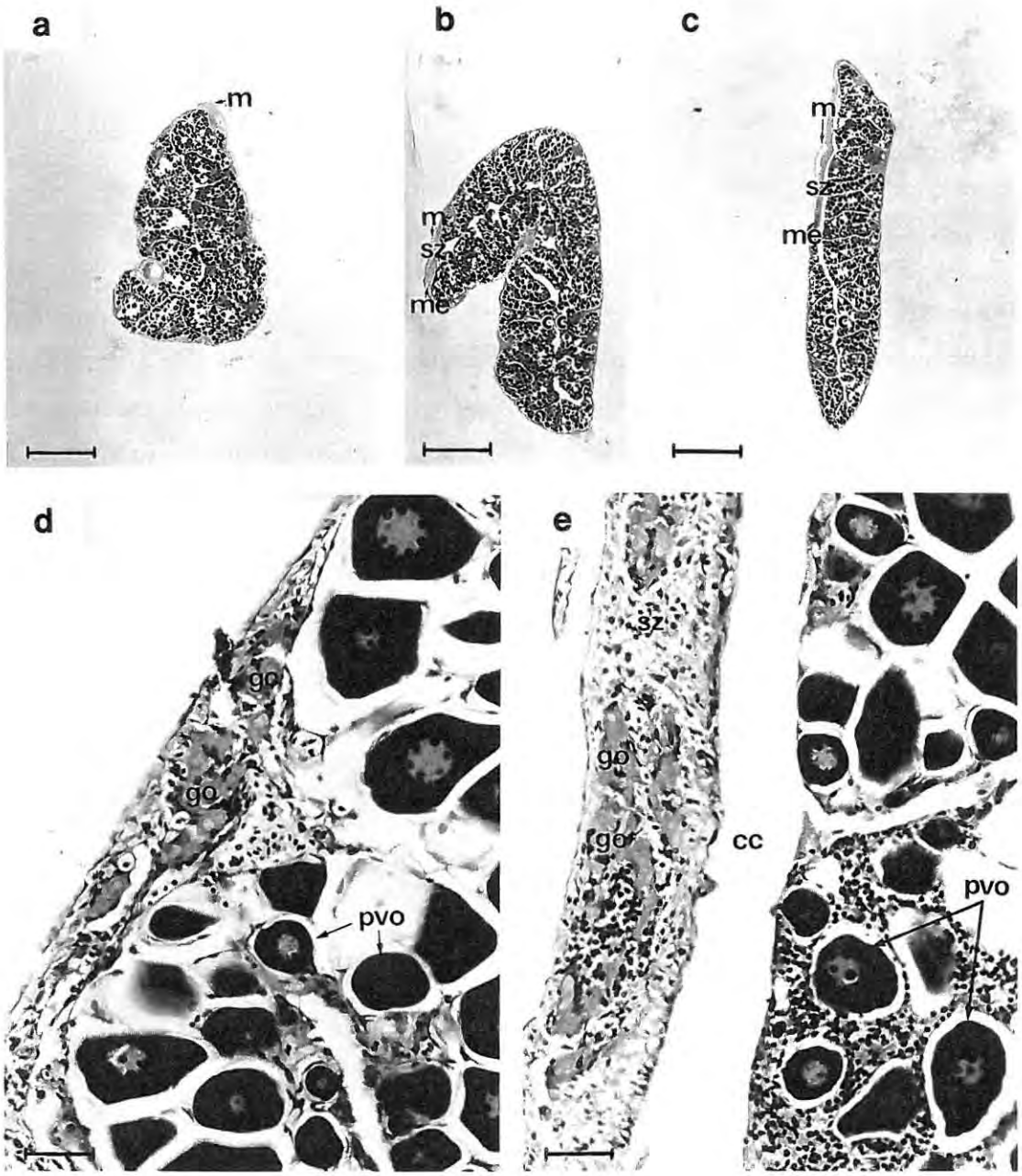

a
m
b
m
sz
me
c
m
sz
me
d
go
go
pvo
e
sz
go
go
cc
pvo

Figure 97. Anterior (**a**), medial (**b**) and posterior (**c**) sections of a predominantly female hermaphroditic gonad from a Cheimerius nufar of 235mm (FL), with higher magnifications of these regions (**d-f**) showing a male element which extends along the length of the gonad but in which proliferation of gonial cells is limited to the mid-region (**e**). go = gonial cells, m = mesovarium, me = male element, pvo = previtellogenic oocytes. Scale bar in (**a-c**) = 500µm, in (**d,f**) = 50µm, in (**e**) = 25µm.

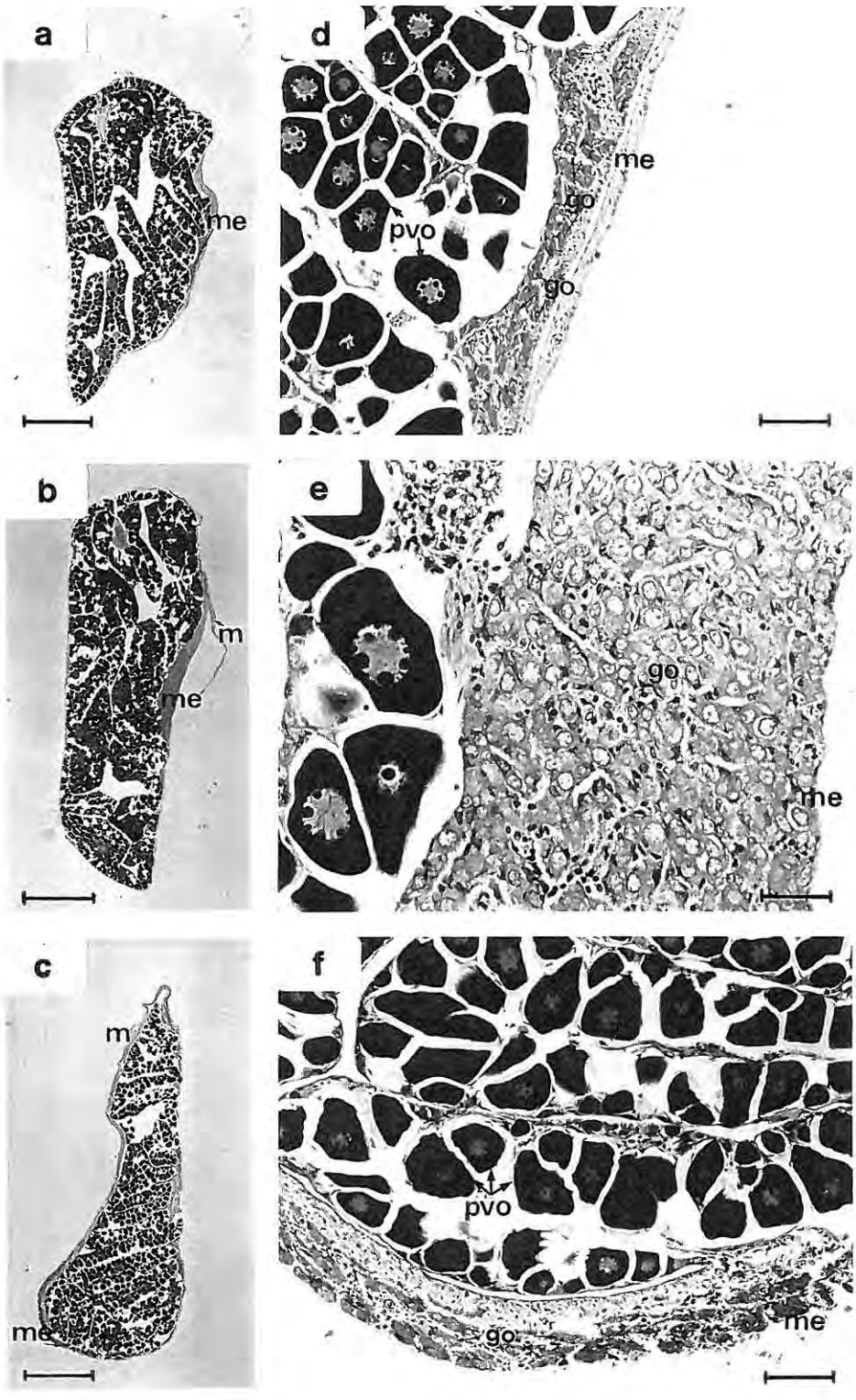

a
me
d
me
co
pvo
go
b
m
me
e
go
me
c
m
me
f
pvo
go
me

Figure 98. Posterior section **(a)** of a predominantly female hermaphroditic gonad from a Cheimerius nufar of 230mm (FL), showing a v-shaped male element situated ventral to the ovary. **(b)** - all stages of spermatogenesis were evident in the male element. cc = central cavity, go = gonial cells, me = male element, pvo = previtellogenic oocytes, sc = spermatocytes, szo = spermatozoa. Scale bar in **(a)** = 250µm, in **(b)** = 25µm.

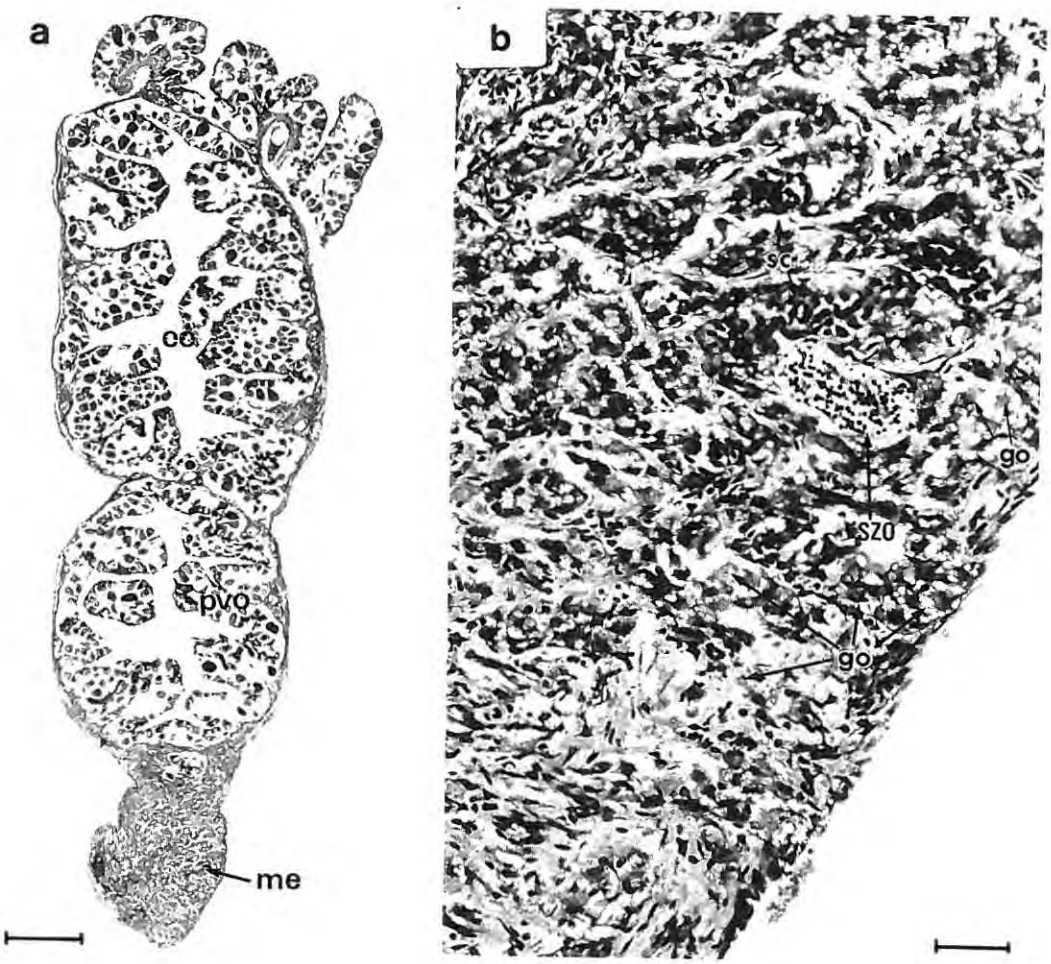

a
cg
pvo
me
b
sc
go
SZO
go

Figure 99. Anterior (**a**), medial (**b**) and posterior (**c**) sections of an inactive (resting) ovary from a Cheimerius nufar of 270mm (FL) in which the sterile zone extends less than one third down the length of the ovary. The male element (**d**) is restricted to the dorsal region in this specimen. cc = central cavity, go = gonial cells, m = mesovarium, me = male element, pvo = previtellogenic oocytes, sz = sterile zone. Scale bar in (**a-c**) = 500µm, in (**d**) = 25µm.

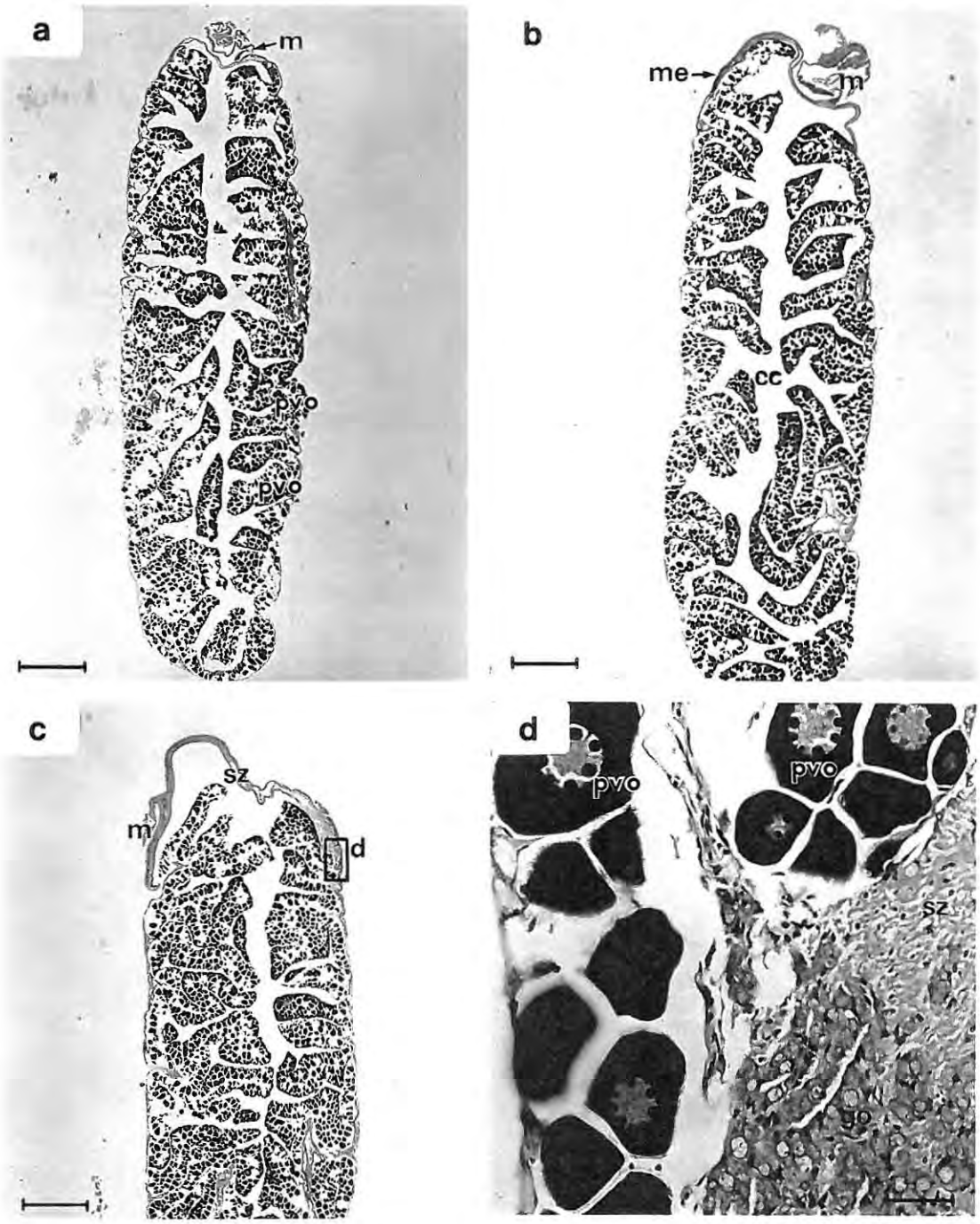

a
m
pvo
pvo
b
me
m
cc
c
sz
m
d
d
pvo
pvo
sz
go

Figure 100. Medial sections of an active ovary **(a,b)** and a newly formed testis **(c)** from Cheimerius nufar of 280mm and 290mm (FL) respectively. The male element in active ovaries **(b)** consists of relatively few gonial cells and is often difficult to locate. Newly formed testes **(c)** develop around the former ovary and have the appearance of being flattened dorso-ventrally. In these testes, the main sperm duct completely encloses the ovarian remnant. go = gonial cells, me = male element, or = ovarian remnant, pvo = previtellogenic oocyte, sd = sperm duct, sz = sterile zone, t = testis, yvo = yolk vesicle oocyte. Scale bar in **(a,c)** = 500µm, in **(b)** = 25µm.

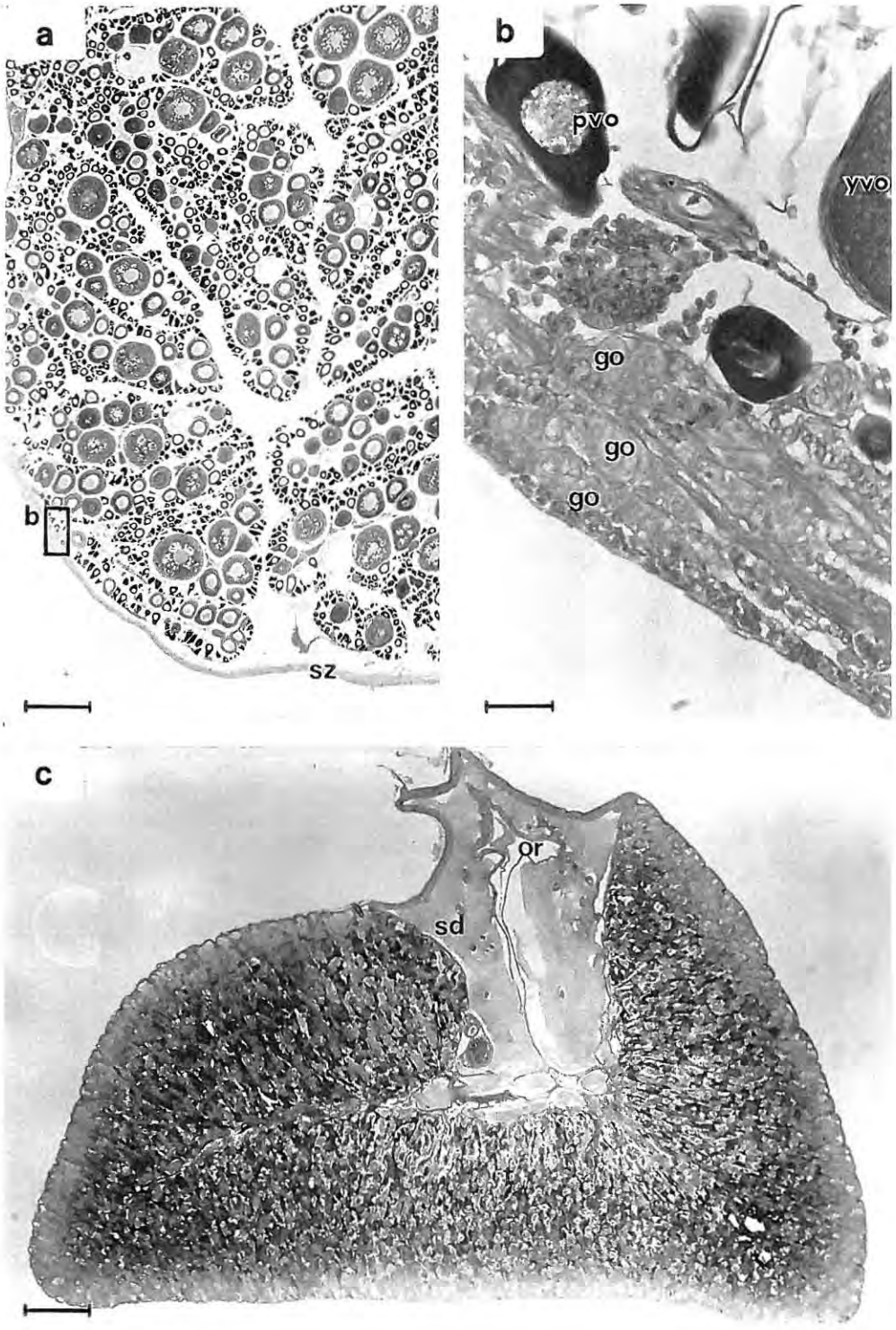

a
b
sz
b
pvo
yvo
go
go
go
c
or
sd

Figure 101. Medial sections of ripe ovaries from Cheimerius nufar of 408mm (**a,c**) and 307mm (**b,d**), showing the male element at the base of the sterile zone in each gonad. (**c**) - in the first gonad (408mm) the male element consists of a few layers of gonial cells in the tunica albuginea. (**d**) - in the second, all stages of spermatogenesis are evident in the male element, even though it consists of only a slightly thickened tunica albuginea. go = gonial cells, me = male element, re = ripe egg, sc = spermatocytes, szo = spermatozoa, ta = tunica albuginea, 3°YO = tertiary yolk oocyte. Scale bar in (a) = 250µm, in (**b**) = 500µm, in (**c**) = 25µm, in (**d**) = 50µm.

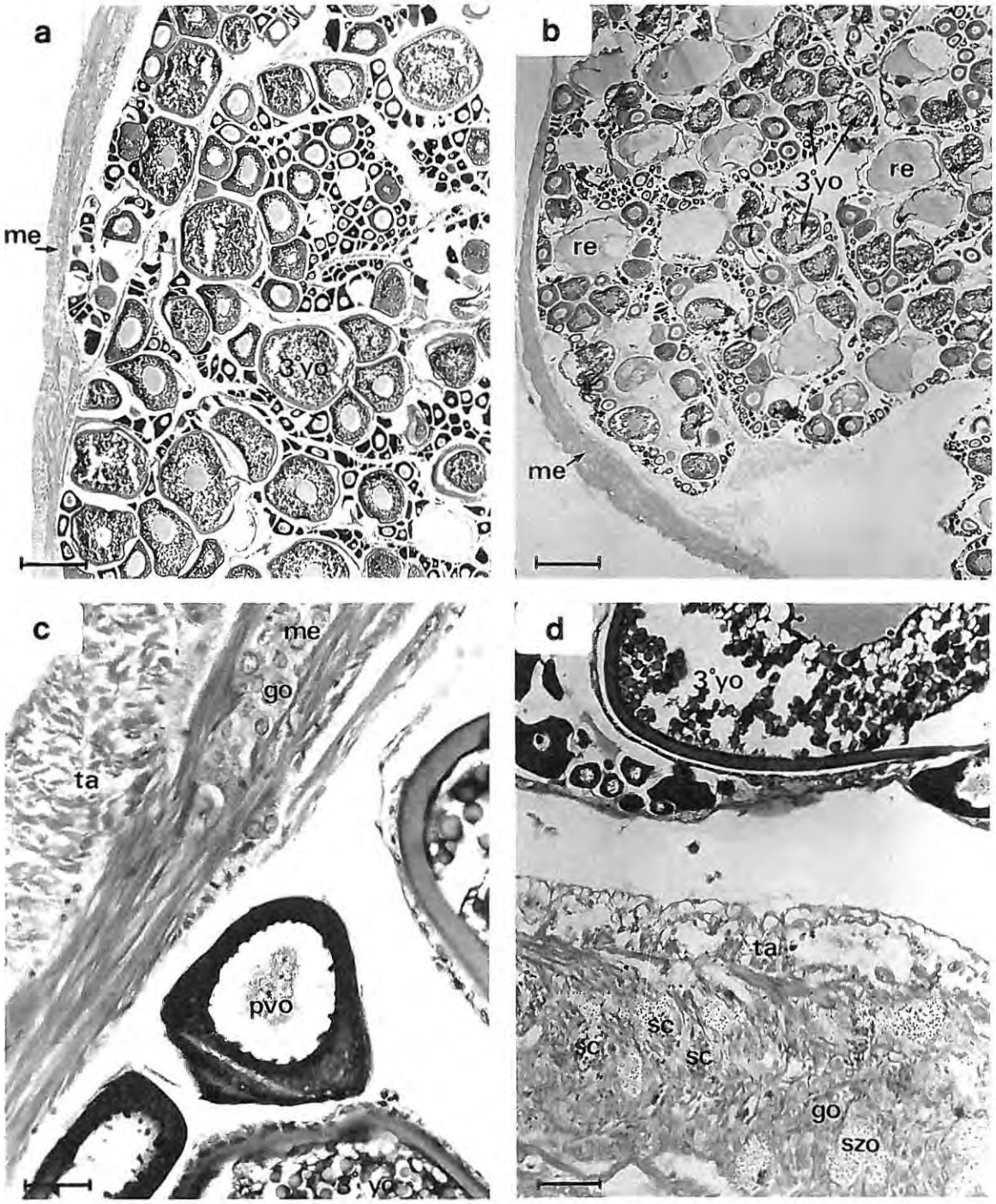
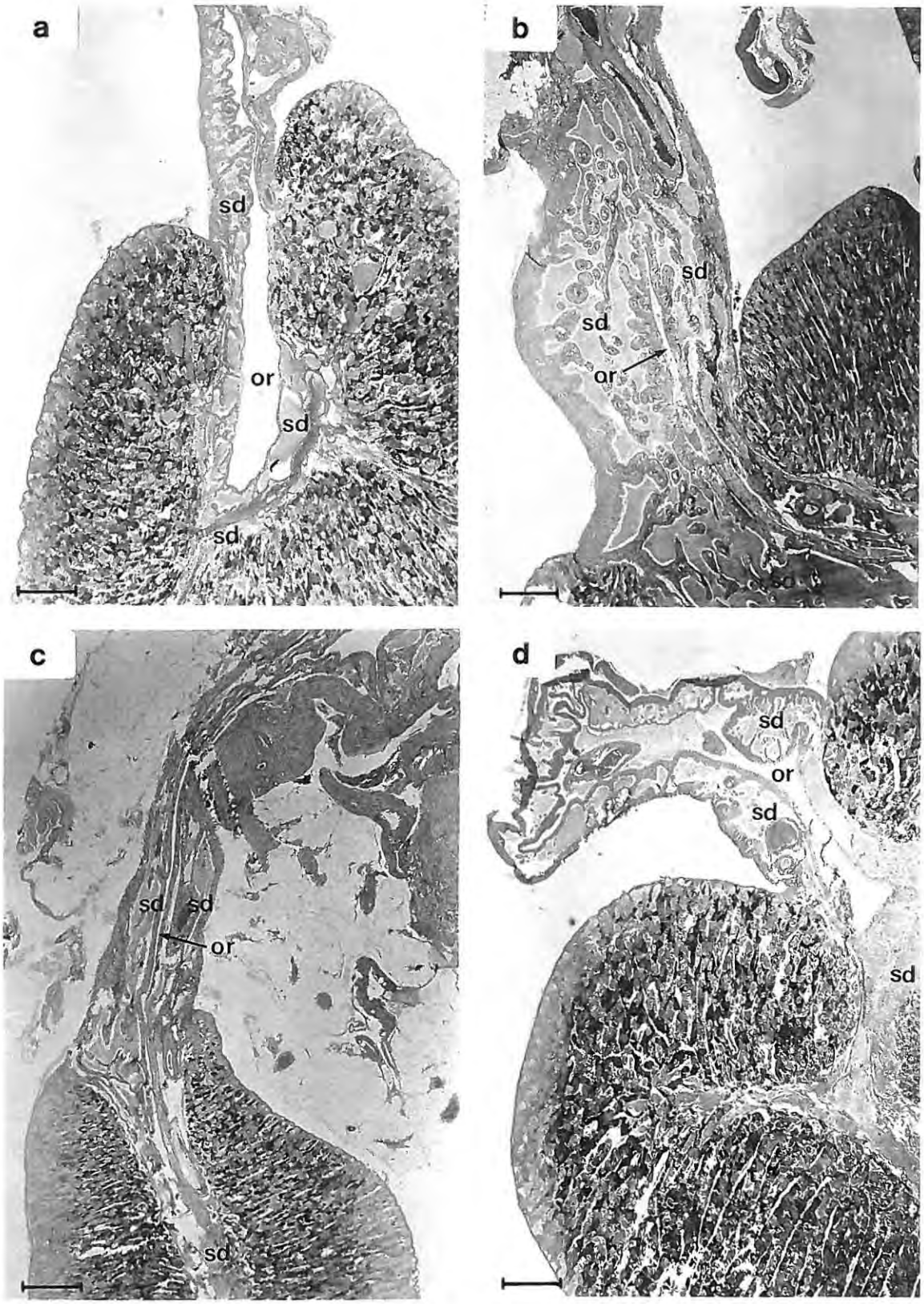

a
me
3°yo
b
3°yo
re
re
me
c
me
go
ta
pvo
d
3°yo
ta
sc
sc
sc
go
szo

Figure 102. Medial sections (**a-d**) of active testes removed from mature Cheimerius nufar ranging in size from 312-523mm (FL), showing typical morphology. In each testis, the main sperm duct is dorsal and completely encloses a former ovarian cavity. or = ovarian remnant, sd = sperm duct, t = testis. Scale bar = 500μm.


a
sd
or
sd
sd
b
sd
sd
or
sd
c
sd
sd
or
sd
d
sd
or
sd
sd