Hydrobiologia **247**: 215–222, 1992.
V. Jaccarini & E. Martens (eds), The Ecology of Mangrove and Related Ecosystems.

The effect of oocytic atresia on fecundity estimates of the rabbit fish *Siganus sutor* (Pisces: Siganidae) of Kenyan marine inshore waters

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Key words: oocytic atresia, fecundity, *Siganus sutor*, Kenya coast

Abstract

In the strongly group-synchronized oocyte development of *Siganus sutor* (Valenciennes, 1835) the group of oocytes to be released in the following spawning, is identified. The smallest size of oocyte belonging to this group was identified by the presence of cytoplasmic vacuoles in oocytes in histological sections. These vacuolated oocytes corresponded to oocytes of 150 μm diameter obtained by treatment with Gilson's fixative. The mean number of such oocytes in stage 4 (late developing) ovaries was found to be 638000. The proportion of these oocytes removed by atresia before spawning was determined on histological sections to be 5%. The corrected estimate of mean fecundity was thus 606000 oocytes per spawning.

Introduction

A major problem in fecundity studies of fish is how to identify those oocytes which are potentially capable of release in the coming spawning season (Macer, 1974). Some workers count the actual number of oocytes which are released in a single spawning season. The difficulty with this method is that it is often not easy to determine the number of spawning seasons for a female in a year. So most other workers use the presence of yolk in the oocytes as the criterion for determining the oocytes to be spawned. However, Macer (*op. cit.*), working with the carangid *Trachurus trachurus*, argued that the presence of vacuoles in the cytoplasm is an earlier indication of active maturation of the oocytes than the presence of yolk. He pointed out that using the acquisition of yolk as the sole criterion of oocyte development could seriously underestimate fecundity by omitting a later batch of oocytes which are actively developing but have not yet laid any yolk and which may be spawned together with the more advanced yolked oocytes.

Another complicating factor in the estimation of fecundity is the phenomenon of atresia (Yamamoto, 1956; Macer, 1974; Guraya *et al.*, 1975; Monaco *et al.*, 1978; Wallace & Selman, 1981; Cyrus & Blaber, 1984). This has mostly been disregarded in the estimation of fecundity, in spite of the fact that it may affect a considerable proportion of the oocytes at all stages of development (Macer, 1974; Wallace & Selman, 1981; Cyrus & Blaber, 1984). Most studies of atresia in the teleost ovary have failed to investigate its causes, and no work has so far quantified its role in regulating the numbers of oocytes as they mature in tropical fishes. Yet it is clear that atresia can have an important effect on recruitment into the fishery.

The object of this study is to determine fecundity in *S. sutor*, which is by far the most important siganid of the Kenyan inshore water (Ntiba, 1986), taking into account the effect of atresia.

The siganids contribute 50% of the total artisanal catch of the reef fisheries of Kenya (Nzioka, 1984). The mangrove-lined creeks provide nursery grounds for these economically important fish (Grove *et al.*, 1986).

Materials and methods

Fish were caught using traditional bottom traps in shallow water (< 14 m depth) in the vicinity of Mombasa, Kenya. Sampling was done fortnightly around neap tides throughout 1985, with an occasional additional sample taken on spring tides. The total number of fish caught was 904. All fish were sexed and maturity stages assigned according to a modified Nikolsky (1963) scheme, which has been described elsewhere (Ntiba & Jaccarini, 1990). In this scheme six maturity stages are distinguished, *viz.* (1) virgin, (2a) developing virgin, (2b) resting and recovering, (3) early developing, (4) late developing, (5) ripe, sometimes running, (6) spent.

Histological techniques

For histology, portions of ovaries were either fixed for 24 h in Smith's formal dichromate, washed in running tap water for another 24 h, and stored in 10% formalin, or fixed and stored in Bouin's fluid. The stored material was dehydrated in graded alcohols, cleared in xylene and embedded either in paraffin wax or in ester wax (Steedman, 1960). Sections were cut at 4–10 μm and stained in iron haematoxylin and eosin.

Gilson's treatment and counting of oocytes

Pieces of ovary were cut longitudinally and turned inside out and treated with Gilson's fluid in 50 ml specimen bottles for three months. The bottles were vigorously shaken from time to time to aid in the release of oocytes from the ovarian walls. Before counting, the contents of the bottles were poured into a petri dish and those oocytes which had not been liberated from the ovarian tissue were released by teasing. The debris of the ovarian walls was then removed and the remaining fluid containing the oocytes poured into 1 l beakers. The oocytes were washed in tap water which was changed several times by decanting after allowing the oocytes to settle. Each time the water was decanted, the supernatant was checked under a dissecting microscope for any small oocytes.

For counting, the clean oocytes were poured into a 1 l beaker and water made up to a known volume. To ensure the even distribution of oocytes in the water column, a plastic ruler was used to stir the oocytes vigorously with a to and fro motion. A subsample was taken after 10 strokes of the ruler by a Labsystem finelet 1 ml pipette. One aliquot usually gave sufficient numbers of the large and small oocytes to yield satisfactory counts and diameter distributions.

The oocytes were pipetted into a solid watch glass and their diameter measured along a horizontal axis, regardless of their shape, under a standard microscope using a calibrated eye-piece graticule using a total magnification of × 40. The accuracy of the subsampling method was tested by taking seven replicates and calculating the coefficient of variation, which was found to be 6.2%. The total number (N) of oocytes in any size class in the ovary was calculated as follows:-

$$N = \frac{V}{V_1} n \times \frac{W}{W_1}, \quad \text{where}$$

n = number of oocytes of a given size class in the subsample;

V = volume of sample;

V_1 = volume of the subsample;

W = weight of whole ovary;

W_1 = weight of portion of ovary preserved.

Distribution of oocytes within the ovary

To establish whether there were any differences in the distribution of oocytes of different stages within the ovary, counts were made on portions

of the material treated with Gilson's taken from the anterior, mid- and posterior regions of the ovary and from the right and left lobes. An analysis of variance showed that there were no significant differences in oocyte diameter distribution among the different regions of the ovary ($p > 0.05$). Therefore portions for oocyte counts could be cut from any region of the ovary. Throughout this work, however, portions were cut from the mid-region of left or right lobes.

The oocytes to be spawned

In order to make accurate fecundity estimates, it is necessary to identify the oocytes that will be spawned in the season following capture. This was done in the first place by examining the oocyte frequency distribution of Gilson's treated material for all the maturity stages of *S. sutor* (Fig. 1). It is seen that stage 4 and 5 ovaries have an oocyte diameter distribution that is strongly bimodal, with small and large oocytes. In some ovaries intermediate-sized oocytes are completely missing. In contrast, stage 1, 2, 3, and 6 ovaries have only one mode, that of small oocytes.

With such a strongly bimodal distribution of oocytes in stage 4 and 5 ovaries, it is reasonable to assume that the mode of larger oocytes will be spawned in the season following capture. The question now arises: what is the smallest size to include in the mode of large oocytes? Following Macer (*op. cit.*), it was decided that the most reasonable answer is to include those oocytes which have started to form cytoplasmic vacuoles.

The size of such oocytes was determined on histological sections. Eight stage 4 ovaries were examined at $\times 40$, and the size frequency distribution of all oocytes in randomly selected sections was determined. The sections were moved along the y-axis by means of a mechanical stage and all the oocytes cut through the nucleus and falling within the graticule were measured. Care was taken to avoid counting the same oocyte twice by calculating how many sections had to be skipped to avoid revisiting even the largest oocytes.

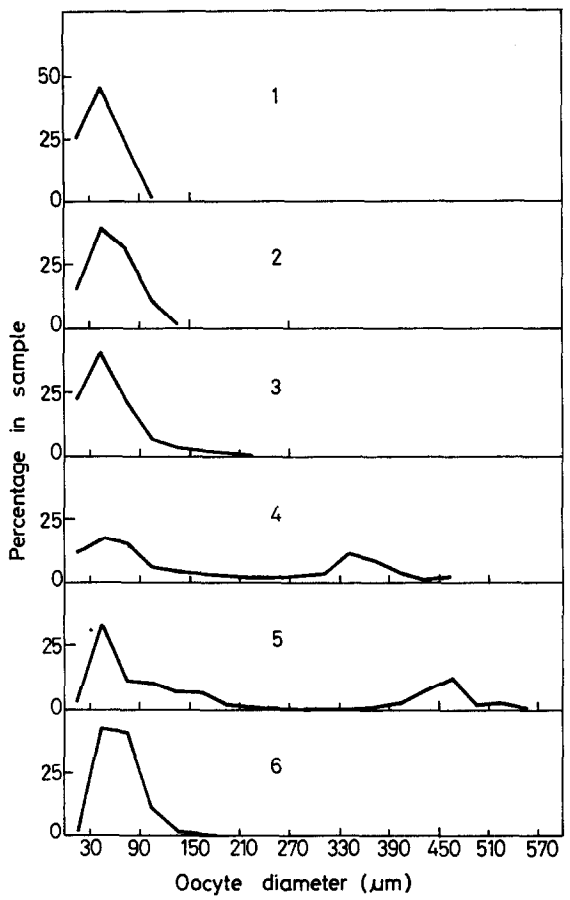

Fig. 1. Oocyte frequency distribution in Gilson's treated ovaries of *Siganus sutor* in various maturity stages, indicated by number in each polygon. Total counts of oocytes from 6 ovaries were made from each maturity stage.

Results

Figure 2 shows the percentage of oocytes containing vacuoles in each oocyte size class. Since the smallest size of oocyte to show vacuoles was, of necessity, determined on sectioned material, account had to be taken of the possible effect of differential shrinkage between material fixed in Bouin's for sectioning and Gilson's treated material used for the isolation of oocytes. To measure this effect the mean diameters of oocytes of the same stage of development were determined using six stage 4 ovaries, portions of each of which were fixed in Bouin's and Gilson's, respectively. It was found that Bouin's fixative shrunk material 9.3% more than Gilson's fluid. All measurements

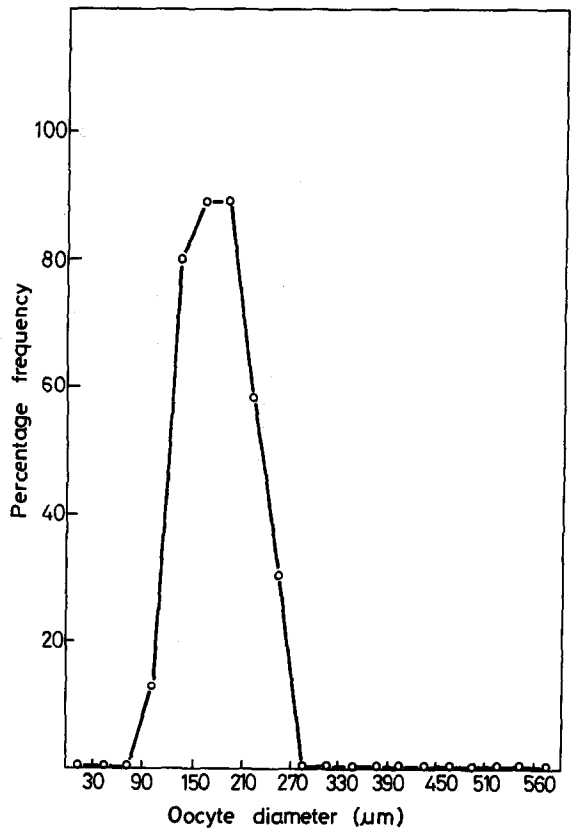

Fig. 2. Percentage of oocytes containing cytoplasmic vacuoles in histological sections of eight stage 4 ovaries of *Siganus sutor.*

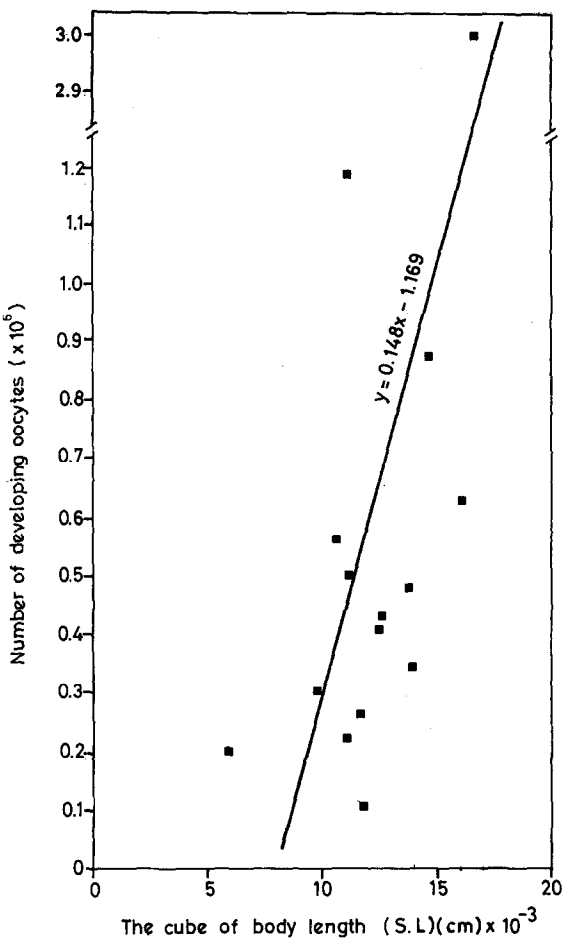

Fig. 3. Number of developing oocytes against the cube of standard body length in cm in fifteen stage 4 ovaries of *Siganus sutor.*

done on histological material were thus increased by this percentage to obtain Gilson's equivalents, which are used for plotting Fig. 2. This plot shows that vacuoles start appearing in the cytoplasm of the oocytes on their reaching a diameter of 90 μm, and that, at 150 μm, about 87% of the oocytes have a vacuolated cytoplasm. From about 200 μm the cytoplasm fills with yolk granules and the vacuoles are obliterated, so that at about 290 μm no vacuolated oocytes remain. Therefore, for fecundity estimates, all oocytes isolated by Gilson's treatment which were ≥ 150 μm were counted.

Counting the oocytes to be spawned

Applying the equation $N = (V/V_1)n \times (W/W_1)$ on the numbers of oocytes ≥ 150 μm in diameter obtained by Gilson's treatment, an estimate of the total number of such oocytes is obtained and hence the potential fecundity of each female determined. Fifteen stage 4 ovaries were used and the total oocytes to be released at the next spawning plotted against the cube of body length in cm (Fig. 3). A correlation was found between fecundity and cube of body length ($r = 0.546$, d.f. 13, $P < 0.05$). A mean of 638000 oocytes to be released in the next spawning was determined.

Effect of egg atresia on fecundity estimates

Atretic oocytes have a chorion which initially becomes less distinct, then disintegrates and sinks into the cytoplasm of the oocyte. At the same time

the yolk is resorbed and the oocytes collapse. Associated with such oocytes is a multitude of squamous follicular cells and many blood vessels which invade the atretic oocytes. These observations are in close agreement with those of Cyrus & Blaber (1984) working with *Gerres* species.

Figure 4 gives the percentage frequency of atretic oocytes for each maturity stage of the ovary. It is apparent from this figure that the degree of oocyte atresia is lowest in stage 1 (< 20%), rises to a minor peak of 45% in stage 3, and a major peak of 58% in stage 6. Stages 4 and 5 have a rate of atresia of 27 and 32%, respectively.

While some stages 4 and 5 ovaries treated with Gilson's fluid had the intermediate-sized oocytes missing altogether, this was never the case with histological sections of stage 4 ovaries, which always showed developing oocytes of all sizes including an appreciable proportion of oocytes of intermediate size. This apparent discrepancy can be explained by the observation that many of the intermediate-sized oocytes (as well as some smaller and larger ones) were atretic and had a disintegrated chorion and were already invaded by a number of squamous follicular cells. One would expect these oocytes to disintegrate under Gilson's treatment. A comparison of oocyte diameter frequency distributions from portions of the same ovaries fixed in Bouin's and in Gilson's fluid separately supports this interpretation. Figure 5 shows the proportion of atretic oocytes in different size classes in 10 ovaries in histological sections of material fixed in Bouin's and the size frequency distribution for the same 10 ovaries fixed in Gilson's fluid. It is clear that the batch of the intermediate-sized oocytes (210–330 μm), present in the histological sections of stage 4 ovaries, is almost completely missing from the oocyte population derived from Gilson's treated material. As seen from histological sections, atresia peaks precisely in the oocyte-size class missing from Gilson's treated material.

It follows that the estimate of 638000 as the mean fecundity of *S. sutor* in Kenyan waters derived from stage 4 ovaries must be corrected by the proportion of oocytes lost through atresia by

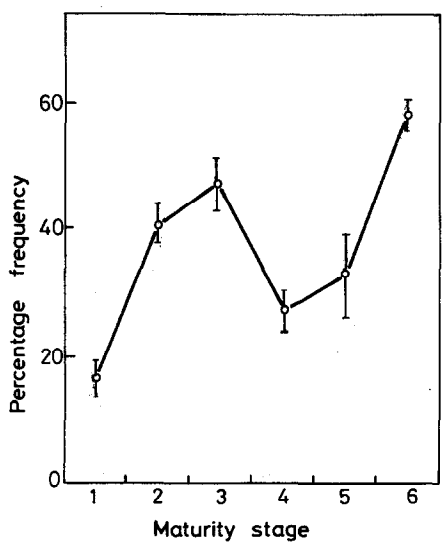

Fig. 4. The mean (± sem) percentage of atretic oocytes in six ovaries of *Siganus sutor* in each maturity stage.

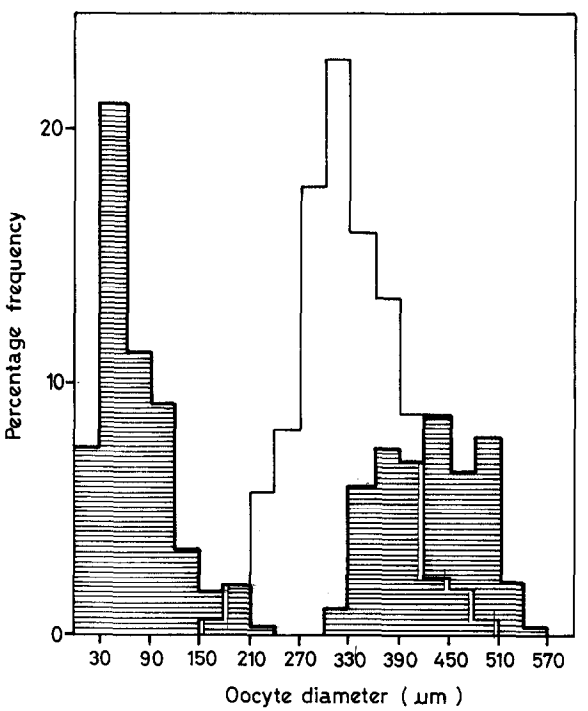

Fig. 5. Cross-hatched histogram gives the percentage frequency distribution of the various oocyte classes (*non-atretic*) from 10 ovaries of *Siganus sutor* isolated with Gilson's fluid. Open histogram gives the percentage frequency distribution of the *atretic oocytes* in histological sections of the same ovaries. One lobe of each ovary was treated with Gilson's fluid, while the other lobe was fixed in Smith's fluid.

the time they reach stage 5, i.e., before they are spawned. From Fig. 4, it is seen that this amounts to 5% over the stage 4 level. Applying that correction factor the estimated resultant fecundity becomes 606000 oocytes per spawning.

Though there are two spawning peaks per year in the population of *S. sutor* (Ntiba & Jaccarini, 1990), it is not clear from the evidence available whether individual fish spawn once or twice a year. On the latter assumption, the mean corrected fecundity estimate would be about 1.2 million.

Discussion

Fecundity

The strongly bimodal size frequency distribution of oocytes in stage 4 and 5 ovaries of *S. sutor* suggests that oocyte development in this species is group-synchronized. In this type of development two populations of oocytes are distinguished: a more advanced, fairly synchronous, population of large oocytes, and a less advanced heterogeneous population of small oocytes from which the larger ones are recruited (Wallace & Selman, 1981). Taking the fecundity for the immediately following spawning season to be the total number of all developing oocytes in the ovary at stage 4 minus what will be lost to atresia by stage 5, the estimated mean for *S. sutor* in Kenyan coastal waters is 606000. De Souza (1988) obtained a total fecundity of 700000 eggs for *S. sutor* at the Kenya coast by counting only yolked eggs from stage 4 and 5 ovaries treated with Gilson's fluid. His counts therefore involved only non-atretic oocytes. De Souza's estimate agrees numerically fairly well with ours, but this is hard to explain since we counted both yolked oocytes and the earlier vacuolated ones to obtain our estimate. Monacop (1937, cited in Lam, 1974) working with *S. canaliculatus*, a species closely related to *S. sutor*, reports 300000–500000 oocytes in its ovary. In the ovaries of artificially spawned *S. canaliculatus* individual variation ranging from 50000 to over 2 million eggs were found (Bryan *et al.*, 1975), while Hasse *et al.* (1977) reported a mean per female of 300000 eggs with a mean diameter of 0.5 mm. This wide range agrees with our findings in which the fecundity ranges from 200000 in a fish of 18.0 cm to well over 3 million in one of 25.5 cm SL. A similar situation was found for *Trachurus trachurus* of the North Sea (Macer, *op. cit.*). Macer found a correlation between fecundity and the cube of body-length ($P < 0.001$). Though we also see such a correlation ($P < 0.05$) in *S. sutor* inspection of residuals shows that the linear regression model was not a good fit. But Fisher's z transform shows no significant difference ($z = 1.31$) between Macer's and our correlation coefficients. So more data are required to test the apparent discrepancy with the model. A possible explanation of our findings is that *S. sutor* is short-lived, e.g. the largest fish in a sample of over 900 was less than two years old (Ntiba & Jaccarini, 1988). One perhaps might not expect a well-defined trend in fecundity to be established in the short time available.

Some workers use already running fish obtained from commercial fishermen (Hasse *et al.*, 1977; De Souza, 1988). But the uncertainties introduced by this procedure are obvious, since such fish may have already shed some eggs.

It is certain that *S. sutor* populations spawn twice a year in East Africa waters (Ntiba & Jaccarini, 1990 and this Symposium). This is indicated by the temporal variation in the weights of the gonads, the seasonal appearance of juveniles, the temporal changes in the relative condition factor, K_n, and especially by the seasonal occurrence of spent males and females in the catches. This is in agreement with results of other workers studying different species of siganids in Palau, Singapore, and the Philippines (Lam, 1974; Kami & Ikehara, 1976; Hasse *et al.*, 1977). However whether the individual *S. sutor* spawn once or twice a year it is impossible to decide on the present evidence.

Functional significance of atresia

In group-synchronous ovaries, a variety of oocyte recruitment strategies exist (Wallace & Selman,

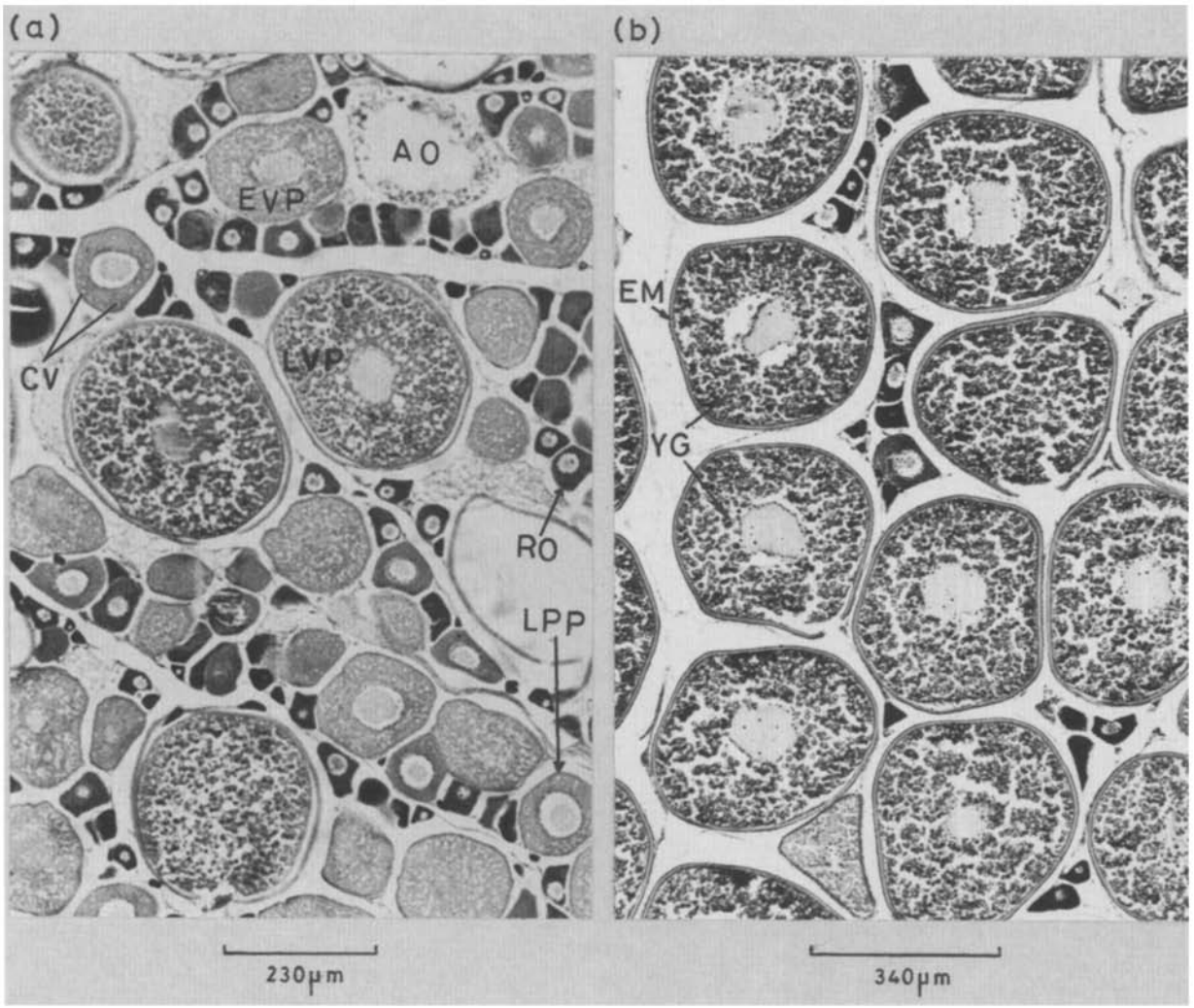

Fig. 6. Sections from stages 4 (a) and 5 (b) ovaries of *Siganus sutor*. Iron haematoxylin and eosin. AO: atretic oocyte; RO: resting oocyte; LPP: late protoplasmic phase; EVP: early vitellogenic phase; LVP: late vitellogenic phase; CV: cytoplasmic vacuoles; EM: egg membrane; YG: yolk granules.

1981). Recruitment can be (a) directly from oogonia, at the end of the gonadotropin-independent stage, (b) from pre-vitellogenic to vitellogenic stages, or (c) from oocytes which have terminated the vitellogenic stage. In *S. sutor* recruitment seems to be at the end of the gonadotropin-independent stage, since oocytes in this stage are always present in the ovary. This type of oocyte recruitment was shown in the herring, *Clupea harengus* (Bowers & Holliday, 1961). Considering *S. sutor* (Fig. 1), it is clear that at stage 4 there is a considerable number of the intermediate-sized oocytes, while at stage 5 there are two distinct modes separated by a gap. These results suggest that during development from stages 4 to 5, the group of largest oocytes is augmented in numbers by the later development of the smaller ones. This is further supported by the histological appearance of stages 4 and 5 ovaries (Figs 6a and b) in which the former present a wide variety of oocyte stages, from early resting ones, through vacuolated oocytes of a wide range of sizes, to advanced vitellogenic oocytes; while stage 5 ovaries present an almost uniform distribution of ad-

vanced vitellogenic oocytes, with some scattered groups of much smaller resting oocytes situated in the interstices.

Atresia of oocytes is prevalent in both pre- and post-spawning stages of *S. sutor*. While in the latter atresia aids in the removal of unwanted material from the ovary and helps to re-utilize valuable material, the functional significance of pre-spawning atresia is not so clear. Wallace & Selman (1981) suggest that poor nutrition and hormonal imbalances, especially of the gonadotropins, may cause involution of developing oocytes in the pre-spawning phase. The ovaries may function as storage organs. The occurrence of atresia at all oocyte maturation stages may suggest that this storage function is a finely-tuned process and that this fish may be responding to one or more physiological and environmental cues (Mommsen & Walsh, 1988).

Acknowledgements

We thank Mr. Allela, Director, Kenya Marine and Fisheries Research Institute, Mombasa, for use of facilities. This work was supported by a German DAAD research grant and another grant from the National Council for Science and Technology, Kenya. We thank Mr. Kigondu and Mr. Oswago, Department of Zoology, University of Nairobi, for help with the illustrations. We also thank the Botany Department for help with the micrographs.

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