

## **Gonad maturation and spawning times of *Siganus sutor* off the Kenya coast: evidence for definite spawning seasons in a tropical fish**

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The gonad maturation cycle of *Siganus sutor* (Valenciennes, 1835) (Osteichthyes-Siganidae) is described for both males and females using macroscopic criteria for the testes and both macroscopic and microscopic ones for staging the ovaries. *Siganus sutor* has two major spawning seasons: one in January/February and the other in May/June. The presence of these seasons is established by (a) the temporal variations in the condition factor and in the relative weight of the gonads, (b) the progression of peaks of maturity stages with seasonal occurrence of spent fish in the samples, and (c) the seasonal appearance of juveniles. This is a significant result for a tropical marine fish.

Key words: maturation cycle; spawning seasons; *Siganus sutor*.

### **I. INTRODUCTION**

The reproductive biology of siganids in Kenya has received little attention (Nzioka, 1979, 1981). Indeed this applies to tropical teleosts in general, most studies being concerned with temperate species (Abu-Hakima, 1984; Cyrus & Blaber, 1984). Work on the siganids does not provide much information on gonad maturation (George, 1972; May *et al.*, 1974; Bryan *et al.*, 1975; von Westernhagen & Rosenthal, 1976; Hasse *et al.*, 1977; Gundermann *et al.*, 1983). In the present study the scheme of ovarian maturity stages based on visual inspection and the relative dimensions of the gonads is validated by histological analysis. Ntiba and Jaccarini (unpublished results) further support this by an analysis of size frequency distribution of the isolated oocytes. The spawning seasons are determined by a combination of various lines of evidence.

### **II. 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.

The standard length (S.L.) was read on a measuring-board to the nearest millimetre. The total body weight was taken to the nearest gramme using a top-loading balance while the gonads were weighed to the nearest 0.01 g. The width and length of the sexed gonads were also taken. When the sample size exceeded 50, a subsample of 50 using random numbers was taken for analysis.

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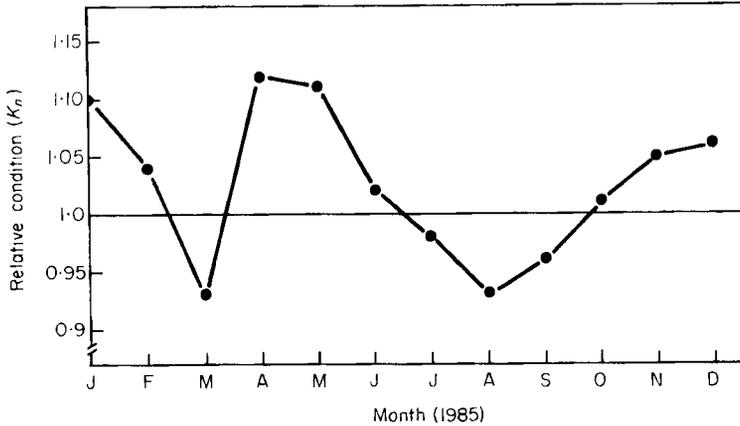


FIG. 1. Monthly variation in the relative condition factor of *Siganus sutor*.

The colour and texture of the gonads were recorded and a maturity stage assigned following a modified Nikolsky scheme (Nikolsky, 1963). Oocyte counts for the different size classes from left and right lobes of the ovaries and from various regions along the antero-posterior axis were made on isolated oocytes obtained from ovarian tissue treated with Gilson's fluid. A preliminary analysis of variance showed that there is no significant difference ( $P > 0.05$ ) in oocyte size distribution from the different regions of the ovaries. In all subsequent analyses a small section of the ovary was cut from the mid-region, weighed to the nearest 0.01 g, and preserved in Gilson's fluid for fecundity counts. The rest of the ovary was preserved in Smith's formol-dichromate or in Bouin's fixative for histological study. This material was dehydrated in graded alcohols, cleared in xylene and embedded in either paraffin wax or ester wax (Steedman, 1960). Sections were cut at 4–10  $\mu\text{m}$  and stained in iron haematoxylin and eosin. The oocyte diameters given for the various gonad maturity stages described in Table I were measured both on the isolated oocytes and on the histological preparations. In the latter the oocytes measured were those sectioned as close as possible through the largest plane of the nucleus. Since the average size of the oocytes in the histological sections was 9.3% less than that of the isolated oocytes, a correction was applied to the former.

The weight of the gonad relative to the body weight, the gonadosomatic index (GSI), was calculated using the formula:

$$\text{GSI} = \frac{\text{weight of ovary}}{\text{weight of fish} - \text{weight of ovary}} \times 100.$$

### III. RESULTS

#### LENGTH-WEIGHT RELATIONSHIP AND CONDITION FACTOR

The length-weight relationship was first determined separately for each sex by the method of least squares using log-log transformed data. An analysis of covariance showed no significant differences between the regressions for the two sexes ( $F = 1$ ; d.f. 38, 38;  $P > 0.05$ , Snedecor & Cochran, 1967). Since there was homogeneity of residual variances for the two sexes, the two regression lines were tested for any differences and were found not to differ significantly ( $F = 1.89$ ; d.f. 1, 76;  $P > 0.05$ ). The data for the two sexes were therefore pooled. A similar analysis on the pooled data for each month showed that there was no significant difference between the monthly slopes ( $F = 1.14$ ; d.f. 11, 8;  $P > 0.05$ ). It was therefore decided

TABLE I. The maturity stages of the gonads of *Siganus sutor*

Maturity stage	Ovary		
	Macroscopic appearance of testis	Macroscopic appearance	Microscopic criteria
1 Virgin	Small and flat, smooth translucent; colourless to light grey. No blood vessels visible. Testis length: width ratio of 10, extends for 35% of the abdominal cavity.	Small, rounded, surface rough, translucent. No oocytes visible through ovary wall. No blood vessels. Ovary length: width ratio of 8; extends for less than 50% of the abdominal cavity.	Few oocytes larger than 90 $\mu\text{m}$ , have a thin densely staining cytoplasm; large rounded nucleus with many small nucleoli; no cytoplasmic vacuoles; oocytes are irregularly shaped with no defined cell membrane and arranged in ovigerous lamellae (Stage 1 oocytes). Ovary wall > 15 $\mu\text{m}$ thick.
2a Developing virgin	Small, flat, smooth and soft in texture as compared to 2b fish. Tiny blood vessels start forming. Testis length: width ratio at a mean of 3.5; gonad extends for 44% of the abdominal cavity.	Small, rounded with a rough surface and soft in texture. Translucent with tiny blood vessels forming internally. No oocytes visible through ovary wall. Ovary length: width ratio of 3; gonad extends c. 50% of the abdominal cavity.	Few oocytes larger than 120 $\mu\text{m}$ ; oocytes have a densely staining cytoplasm. No cytoplasmic vacuoles, cells have a large rounded nucleus on the periphery of which are several nucleoli, two being quite large. Oocytes are irregularly shaped but few are rounded. Oogonia visible between resting oocytes which are arranged on ovigerous lamellae. Ovary wall about 50 $\mu\text{m}$ thick.
2b Resting and recovering (mature fish)	As 2a but tough in texture, deep brown in colour and slightly longer than 2a; an apparent cavity in the centre of gonad.	Soft and flabby with a cavity at the centre of the gonad. Greyish in colour and no oocytes visible through ovary wall.	Same as 2a but residual atretic oocytes present and the septum not very organized. Reorganization of ovigerous lamellae starting.

TABLE I. (Continued)

		Ovary	
Maturity stage	Macroscopic appearance of testis	Macroscopic appearance	Microscopic criteria
3 Early developing	Becoming broader, smooth, light brown. Watery milt exudes on cutting. Blood vessels (internal) visible through testis wall. Gonad length: width ratio of 2.8, gonad extends for 53% of the abdominal cavity.	Becoming broader with a rough texture, whitish. Tiny oocytes visible through ovary wall. Dense network of blood vessels visible internally through the ovary wall. Ovary length: width ratio of 2.5, gonad extends for c. 50% of the abdominal cavity.	Many oocytes larger than 120 $\mu\text{m}$ to a maximum diameter of 210 $\mu\text{m}$ ; large oocytes have cytoplasmic vacuoles and some have started acquiring the yolk; larger cells are rounded and have a small nucleus relative to the size of the cytoplasm; some oocytes have an outer layer of deeply staining cytoplasm and an inner lighter layer (Stage 3 oocytes). Oocytes are arranged in well organized ovigerous lamellae. Large oocytes start forming a definite chorion. Stage 1, 2 and 3 oocytes present. Ovary wall is about 360 $\mu\text{m}$ thick, richly supplied with blood vessels.
4 Late developing	Broad and firm in texture; smooth, flat, light grey; exudes thick bloody milt on cutting. Lobulation of the right and left testis starts. Length: width ratio of 2.4, gonad extends for 92% of the abdominal cavity.	Becoming broader, firm, granular and rounded. A heavy network of blood vessels now appears externally on the surface of the ovary wall. Large yellow oocytes visible through the ovary wall. Ovary length: width ratio of c. 2 and gonad extends for about 90% of the abdominal cavity.	Many oocytes between 210–450 $\mu\text{m}$ . A mode of large oocytes appears at 370 $\mu\text{m}$ in Gilson's counts. Stage 2, 3 and 4 oocytes present in various proportions. Many cells with cytoplasmic vacuoles; largest oocytes (Stage 4) filled with eosinophilic yolk granules; chorion of the largest cells is well-defined and starts becoming striated. Organization of ovigerous lamellae still apparent although disappearing and some yolky oocytes undergoing active atresia. Ovary wall c. 120 $\mu\text{m}$ thick.

5 Ripe and sometimes running

Broadest, mostly firm but some were flaccid (perhaps have already lost some milt). Flat, smooth and highly lobulated. Completely white but the posterior tips sometimes grey with white speckled appearance. No blood vessels and thick milt exudes on slight pressure. Testis length: width ratio of 2:2 and it extends for 99% of the abdominal cavity.

Broadest and firm, where shedding of eggs has not yet commenced, otherwise soft. Rounded and with a rough granular surface. Blood vessels coalesce to form larger ones on the external surface of the ovary wall. Yellowish in colour possibly due to the large yellow oocytes that are visible through ovary wall. Ovary length: width ratio *c.* 2 and ovary extends for 99% of the abdominal cavity.

Many oocytes between 210–560  $\mu\text{m}$ . A mode of large oocytes at 460  $\mu\text{m}$ . Many oocytes are at stages 2 and 5 a few others are at stages 3 and 4. Many of the largest cells have densely staining yolk granules in the cytoplasm and have a well defined striated cell membrane. There are no blood vessels internally but some of the yolk oocytes are atretic. Ovary wall about 90  $\mu\text{m}$  thick.

6 Spent

Reduced in size and sometimes very small, flaccid and walls hard in texture. Flat, lobules disappearing. Dark brown in colour and no blood vessels visible; no milt. Testis length: width ratio of 3:2 and gonad extends for 56% of the abdominal cavity.

Reduced in size, flaccid but ovary wall is tough and smooth (no granulation). Round to ovoid in shape. Reddish in colour. Residual oocytes are visible through flabby wall. Ovary length: width ratio is 2:5 and gonad extends for 50% of the abdominal cavity.

Many oocytes between 30–90  $\mu\text{m}$ , and the largest oocytes are 180  $\mu\text{m}$  in diameter. Small oocytes have a thin densely staining cytoplasm. A few atretic residual oocytes present. Invasion of oocytes by follicular cells. A dense network of blood vessels indicates a high level of oocyte atresia. Septum disorganized; no definite empty follicular coats. Ovary wall 300  $\mu\text{m}$  thick.

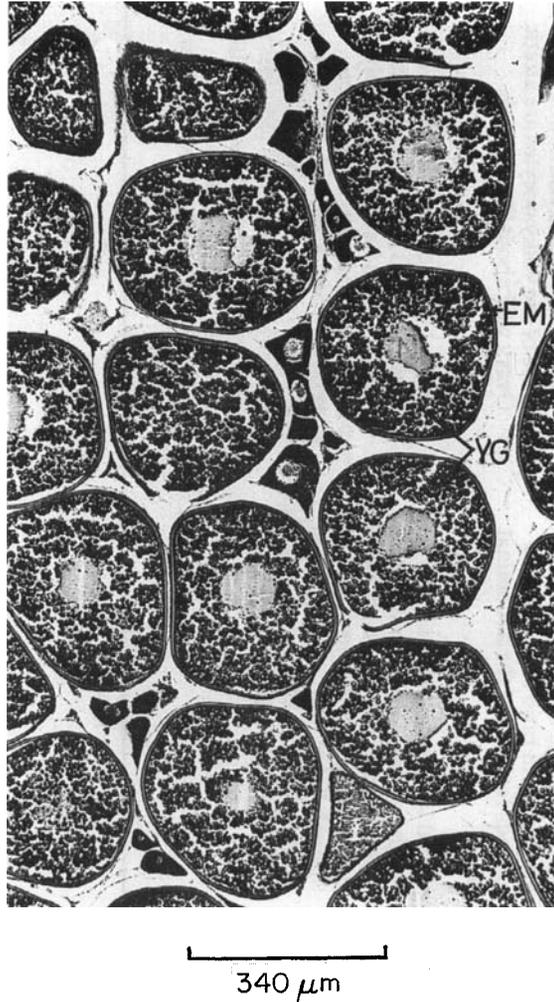


FIG. 2. Section from a stage 5 ovary of *Siganus sutor* packed with final vitellogenic stage oocytes with numerous yolk granules (YG) and highly developed egg membrane (EM). Iron haematoxylin and eosin.

to pool all length-weight data. The overall length-weight relationship is described by the equation

$$\log_{10} W = 2.96 \log_{10} L - 1.56 \quad (r = 0.97, \text{d.f. } 902, P < 0.01)$$

where:  $W$  is the weight in g and  $L$  is the length in cm.

Using the expected weight,  $\hat{W}$ , for each fish derived from the overall length-weight regression, the relative condition factor,  $K_n$ , was obtained employing the formula:

$$K_n = W/\hat{W}$$

where:  $W$  is the observed weight and  $\hat{W}$  is the expected weight (Bagenal & Tesch, 1978). The monthly relative condition factor is shown in Fig. 1.

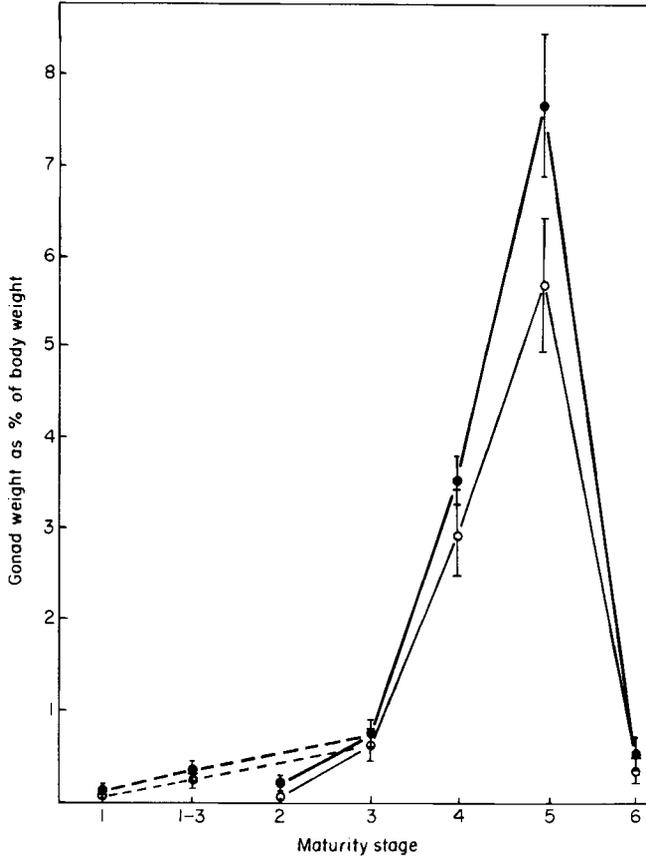


FIG. 3. Relative gonad weight at the various maturity stages of testes (○) and of ovaries (●) of *Siganus sutor*. The dashed line shows the values for the developing virgins. Vertical bars show  $\pm$  S.E.M.

### GONAD MATURITY STAGES AND THE BREEDING CYCLE

The scheme of gonad maturity is given in Table I, the histological appearance of a stage 5 ovary is illustrated in Fig. 2.

Figure 3 shows the gonad weight relative to the body weight at each maturity stage for males and females. These plots confirm the validity of the gross morphological criteria used for determining the maturity stages of the gonads.

The weights of the testes and ovaries as a percentage of the weight of the body exclusive of gonads were calculated on a monthly basis. These are plotted in Figs 4(a) and (b). The plots for males and females are congruent and point to January/February and May/June as peak spawning times. Though we were forced to change the sampling site at the end of March because of climatic factors, it is clear from the plots for both males and females that we are dealing either with the same population of fish, or with populations whose maturity cycles are synchronous.

### SEASONAL OCCURRENCE OF GONAD MATURITY STAGES

The proportion of each maturity stage occurring as a percentage of the total number of gonads for each month for males and females respectively is plotted in

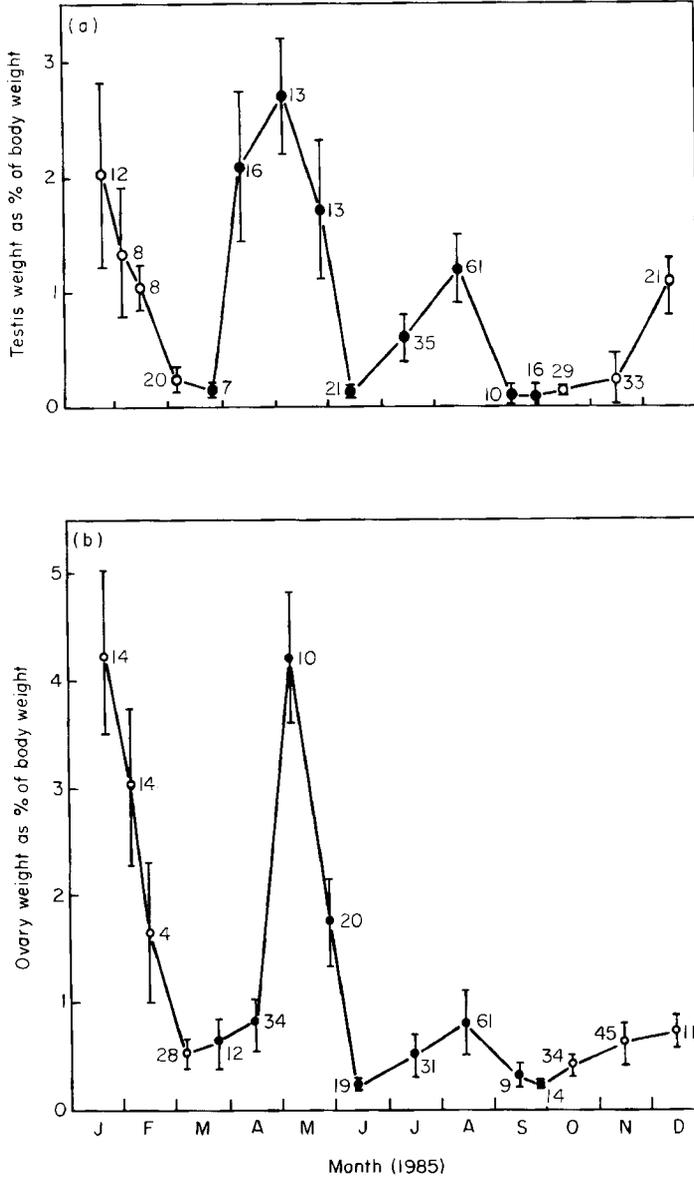


FIG. 4. The temporal variation in the relative weight of (a) testes and (b) ovary of *Siganus sutor* excluding immature (stage 1) fish. Vertical bars show  $\pm$  S.E.M. Samples from mouth of Tudor Creek (○), samples taken outside reef edge (●).

Fig. 5. A gradual progression of maturity stages is discernible in this figure pointing once more to peak spawning in January/February and May/June. Major testicular and ovarian activity, as shown by a rapid increase in the proportion of stage 3 ovaries and testes following a sharp drop in stages 1 and 2, starts in November after a relatively long quiescent period. The start of this activity thus coincides with the beginning of the north-eastern monsoon. An appreciable proportion of females reach stage 5 in January and then again in May and June. Spent fish are abundant in the catches in March and then again in June and July.

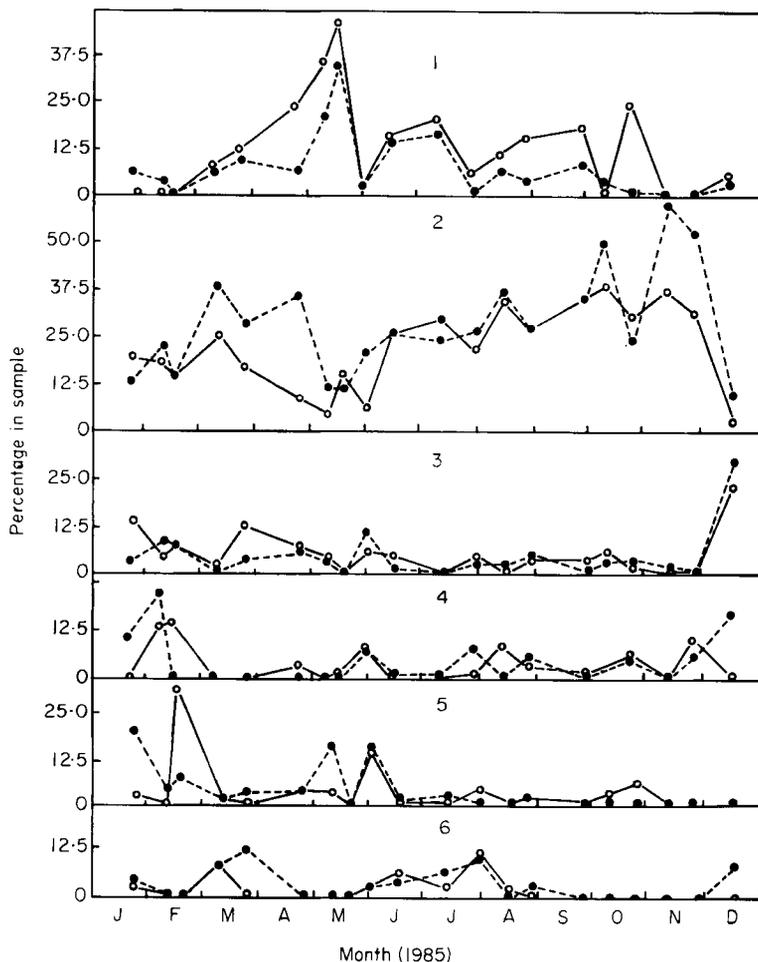


FIG. 5. The percent occurrence of maturity stages in monthly samples for male (○) and female (●) *Siganus sutor*. Maturity stage of the gonads is indicated by the number above each polygon.

APPEARANCE AND ABUNDANCE OF JUVENILES IN INSHORE WATERS

Table II gives the numbers and size ranges of juveniles taken each month in one single beach seine haul.

IV. DISCUSSION

The most important result of this work is the clear demonstration of a pattern of spawning which has not been normally associated with tropical fish. *Siganus sutor* of inshore Kenyan waters has two sharply defined spawning seasons occurring one to two months after the beginning of each monsoon. Besides this population characteristic, the histological analysis of the individual ovaries further points to a highly synchronous development of oocytes which seems to suggest that their release takes place over a short interval of time. This analysis is strongly supported by the size frequency distribution of Gilson isolated oocytes, which is very strongly

TABLE II. Appearance of juveniles of *Siganus sutor* in the Tudor Creek, Mombasa, Kenya

Month	No. in a single seine trawl		Size range, s.L. (cm)
Jan.	—	—	—
Feb.	6	557	2.2-4.5
	20	261	1.9-8.7
Mar.	6	164	2.2-6.0
	22	15	3.2-5.2
Apr.	—	—	—
May	—	—	—
Jun.	—	—	—
Jul.	—	—	—
Aug.	—	—	—
Sep.	1	3	4.3, 4.6, 6.5
	15	3	3.0, 4.5, 4.7
Oct.	7	5	3.6, 3.8, 4.7, 5.6, 6.0
Nov.	—	—	—
Dec.	—	—	—

bimodal in stages 4 to 5 ovaries, and unimodal in stages 1 to 3 and stage 6 (our unpublished results). Our work provides strong grounds for treating any reference to an absence of pronounced seasonality in tropical marine organisms with caution. McClanahan (1988) has reviewed the literature documenting distinct seasonal patterns in physical, chemical, and biological oceanographic parameters in East African coastal waters and has shown a strong relationship of this seasonality with the monsoon regimes.

The gross morphological criteria (Table I) used for assigning the ovaries to specific maturity stages were validated by histological analysis of the different stages. Moreover the weight changes in both ovaries and testes over the maturity stages assigned followed an expected and logical sequence which gave further support to the gross morphological criteria used (Fig. 3).

Since the relative condition factor depends to a large extent on the maturity stage of the gonads, the monthly variation in the two variables must be considered as providing only one line of evidence on the breeding pattern of *S. sutor*. The pattern of this variation points to two well defined spawning seasons: January/February and May/June [Figs. 1 and 4(a) and (b)]. The slight recovery of the gonads in July/August is most probably not significant since it does not approach the values shown at the start of the two spawning seasons. The existence of the two spawning peaks is supported by the presence of spent females in the samples taken immediately afterwards; and also by the occurrence of juveniles in February and March and again in September and October (Table II).

Our finding of two spawning seasons is supported by evidence from related siganids. In Palau, juveniles of *S. canaliculatus* appear in March/April and November/December. In Singapore, they first appear from February to May and again from August to October (Lam, 1974). In Guam, juveniles of *S. spinus* and *S. argenteus* appear in April/May, June, and October, always after the last lunar

quarter (Kami & Ikehara, 1976). De Souza (in press) working on *S. sutor* in the same general area of our study reported a spawning peak in December/January and a second one in May. This agrees well with our findings. In the light of this general agreement as to the presence of two definite spawning seasons for *S. sutor* and *S. canaliculatus*, it is difficult to explain the report of Bwathondi (1981) working off the Tanzanian coast that siganids breed throughout the year.

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