Hydrobiologia **353:** 153–160, 1997.

The reproductive biology of *Lutjanus fulviflamma* (Forsskål,1775) (Pisces: Lutjanidae) in Kenyan inshore marine waters

B. Kaunda-Arara[1] & M.J. Ntiba[2]
[1]*Department of Fisheries, Moi University, P.O. Box 1125, Eldoret, Kenya*
[2]*Department of Zoology, University of Nairobi, P.O. Box 30197, Nairobi, Kenya*

Received 5 November 1996; in revised form 27 May 1996; accepted 3 June 1997

Key words: maturation cycle, spawning season, fecundity, oocyte recrudescene

Abstract

The testicular and ovarian maturation cycle of the dory snapper, *Lutjanus fulviflamma* (Forsskål) (Osteichthyes:Lutjanidae), a commercially valuable species in the western Indian Ocean, is described macroscopically. The ovary was also studied microscopically. *L. fulviflamma* has one prolonged spawning season which begins from November/December, lasting till April/May. Discontinuous spawning is established by (i) temporal variation in the relative weight of testis and ovaries, ii) a seasonal occurrence of various maturity stages and, iii) a seasonal occurrence of developing fish in the samples. Using volumetric and histological techniques, the fecundity of this species was determined at 51,000 to 460,000 (mean: 167,000) oocytes in fish of between 17 to 30 cm total length, respectively. Oocyte recrudescence in the ovary is asyncronous, but the number and size of batches of eggs released in a single spawning season are yet to be determined.

Introduction

Studies of gonadal maturation in teleosts have concentrated on species from temperate waters (Quasim, 1973; Macer, 1974); only few studies address tropical species (Marichamy, 1970; Cyrus & Blaber, 1984). In Kenya, studies into the reproductive biology of marine fish are only available for the monoclebream, *Scolopsis bimaculatus* Rüppell (Nzioka, 1981) and the rabbit fish, *Siganus sutor* Valenciennes (Ntiba, 1986). Other than information on the zoogeographical distribution and taxonomy of the family Lutjanidae (Smith & Heemstra, 1986), there is only scanty literature on the reproductive ecology of *Lutjanus fulviflamma* in the western Indian Ocean . In contrast other Lutjanidae such as the grey snapper, *L. griseus* from marine waters around Florida (Starck, 1971), the lane snapper, *L. synergris* L. (Druzhinin, 1970) and *L. analis* Valenciennes (Rojas, 1960) both from Caribbean waters, have been studied in detail. The object of this study is to shed some light on some aspects of the reproductive biology of the dory snapper, *L. fulviflamma*, the most important lutjanid of Kenyan inshore marine waters (Kenya Fish. Dept. Stat. Bull., 1995). Lutjanids form the third most abundant group of fish in artisanal catch of the local reef fisheries (Nzioka, 1984). Juveniles of *L. fulviflamma* constitute 60% of the catch in the mangrove-lined creeks which are their nursery area (Ntiba, et al., 1993).

Materials and methods

Sampling and treatment of fish

Fish were caught using baited traditional *dema* traps. They range in size from about 91 cm in height, with a mesh size of 3.6 cm^2, to about 2 m, with a mesh size of 20.5 cm^2. The *dema* trap has one door through which fish enters anteriorly. The traps were set at a depth of 8–14 meters during day-time low tide at the mouth of the creeks surrounding Mombasa Island, Kenya. Each trap was visited after 24 hours (next low tide). Sampling was done twice-monthly from October 1991 to September 1992. The total length (TL) of each fish was measured to the nearest millimeter and total wet body weight recorded to the nearest 0.1 g on a top-loading

balance. The fish were dissected and sex determined. Colour, texture and degree of vascularization of the sexed gonads were recorded, and the whole gonad weighed to the nearest 0.01 g on a Mettler analytical balance. The weight of the gonad relative to body weight, gonado-somatic index (GSI), was calculated:

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

Histological techniques

A maturity stage was assigned to each gonad following Ntiba & Jaccarini (1990). Preliminary oocyte counts and analysis of variance showed that there was no significant difference ($P>0.05$) in oocyte size distribution along the antero-posterior axis of the right and left lobes of the ovary. In subsequent analysis portions were cut from the mid-region of the ovary, weighed to the nearest 0.01 g, and fixed in Gilson's fluid for fecundity estimates. The remaining ovary was fixed in aqueous Bouin's solution for histological work. This material was dehydrated in graded alcohols, cleared in xylene and embedded in paraffin wax, sections cut at 4–12 μm and stained in iron hematoxylin and eosin (Steedman, 1960).

Gilson's material and counting of oocytes

The material for fecundity estimates was stored in Gilson's fluid for 3 months. The plastic bottles containing this material were vigorously shaken from time to time to aid in the release of oocytes from the ovarian walls. Before counting, the contents of each bottle were poured into a petri dish and those oocytes not liberated from the ovarian tissue removed by teasing. The oocytes were repeatedly washed in tap water.

The clean and separated oocytes were transferred to another 1 litre beaker containing a known volume of 1 molar sugar solution. A plastic ruler was used to stir vigorously the egg suspension to ensure an even distribution of oocytes in the suspension column. After 10 strokes of the ruler a subsample was taken by a Labsystem finelet pipette. One aliquot usually gave sufficient numbers of large and small oocytes to yield satisfactory counts and diameter distributions. The oocytes were pipetted into a zooplankton chamber, and their diameter measured along an horizontal axis using a calibrated eye-piece graticule under a standard dissecting microscope at a magnification of $\times 40$. The accuracy of the subsampling method was tested by taking 10 replicates

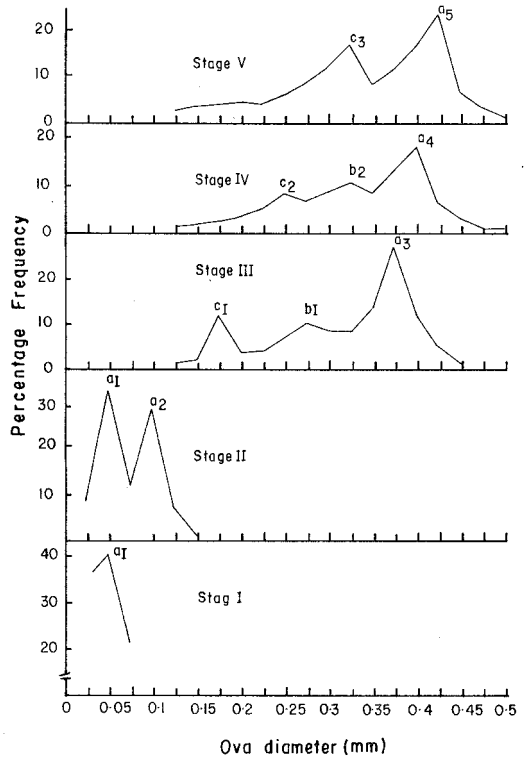

Figure 1. Size frequency distribution of the intra-ovarian oocytes in the ovary of *L. fulviflamma* of various maturity stages in Kenyan inshore marine waters. n = 20

and calculating the coefficient of variation which was found to be 5.8%. The fecundity (F) for each female fish was calculated as follows:

$$F = \frac{V}{V_1} n \times \frac{W}{W_1},$$

where, n = number of oocytes in the subsample; V = volume of the egg suspension; V_1 = volume of subsample; W = weight of whole ovary; W_1 = weight of portions of ovary fixed.

Oocytes to be spawned

One of the major problems in making accurate fecundity estimates is to identify the oocytes that will be spawned in the next spawning season. Oocyte size frequency distribution of Gilson's treated material for all maturity stages of *L. fulviflamma* (Figure 1) showed that at stages III, IV and V there are three types of oocytes in the ovary, meaning that this fish is a multiple spawner (Marza, 1938), releasing several batches of eggs in a single spawning season. The complica-

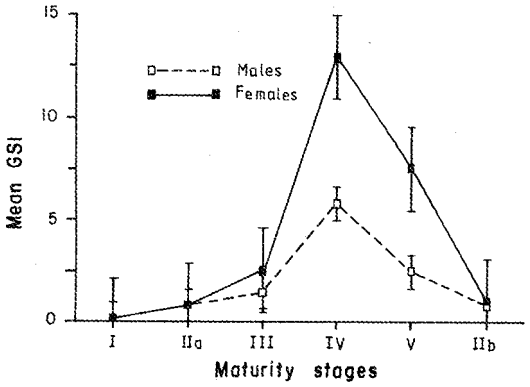

Figure 2. Variation of the gonadal-somatic index with the stage of maturation in male and female *L. fulviflamma* in Kenyan inshore marine waters. The vertical lines represent ± s.e.m.

Table 1. Monthly variation in sex ratio in *L. fulviflamma* in Kenyan inshore marine waters and their associated probabilities (F, females; m, males; Df, degree of freedom).

Month	No. of Males	No. of Females	M:F	DF	X^2
October 1991	38	30	1.3:1	1	0.941
November	50	35	1.4:1	1	2.647
December	89	23	3.9:1	1	38.892∗
January 1992	58	35	1.7:1	1	5.688∗
February	37	34	1.1:1	1	0.127
March	31	32	1.0:1	1	0.016
April	32	34	1.0:1	1	0.015
May	31	51	0.6:1	1	4.878∗
June	17	32	0.5:1	1	4.592∗
July	36	21	1.7:1	1	3.947∗
August	22	38	0.6:1	1	4.267∗
September	25	23	1.1:1	1	0.083
Total	466	388	1.2:1	1	7.124∗

∗ Significant at 5% level.

tion with multiple spawning fish is to make a decision on the smallest oocyte which will be released together with the larger ones in the last batch of oocytes to be spawned. Following Macer (1974) and Ntiba & Jaccarini (1990), we included those oocytes that had started to develop cytoplasmic vacuoles. The method of Ntiba & Jaccarini (1992) was followed to determine both the size at which oocytes with vacuoles in the cytoplasm started appearing in significant numbers, and the proportion of atretic oocytes in the ovary.

Table 2. Percentage occurence of males and females of *L. fulviflamma* in different size groups from Kenyan inshore marine waters.

Size group TL(cm)	n	% ♂♂	% ♀♀
12–13	25	64.0	36.0
14–15	53	58.5	41.5
16–17	136	69.1	30.9
18–19	194	61.3	38.7
20–21	188	50.5	49.5
22–23	127	43.3	56.7
24–25	84	51.2	48.8
26–27	36	36.1	63.9
28–29	11	9.1	90.9
30–31	5	0.0	100

Results

Sex ratio

A total of 891 fish were sampled, out of which 37 were juveniles. The sex structure of the population is shown in Table 1. During the months of December, January, May, June, July and August, there was significant difference from the expected sex ratio of 1:1. Males were dominant in December, January and July, while females were in May, June and August. In the other months the sex ratio was 1:1 as expected. The overall sex ratio for the population was significantly different from 1:1 ($X^2 = 7.124$, $P > 0.05$; df= 1). Further analysis showed more males than females in all size classes smaller than 21 cm TL, and more females in size classes above 21 cm TL (Table 2).

Description of gonad maturity stages

A description of the maturity stages is given in Table 3, and the histological organization of the ovary is shown in Plate 1 (a and b). Spent fish, which in the gonad staging schemes of Ntiba & Jaccarini (1990) and Nzioka (1981), would have been classified as stage VI, were difficult to identify in this study. Although the pattern of weight increases of the male and female gonads is the same throughout the maturation cycle, the ovaries are heavier than the testis in maturity stages III, IV and V (Figure 2). The gonads attain a peak weight at stage IV, and then a gradual decrease in weight through stages V to IIb. This decrease is indicative of the release of sev-

Table 3. Maturation stages of the gonads of *Lutjanus fulviflamma* from Kenyan inshore marine waters.

Maturity stage	Testis	Ovary	
	Macroscopic appearance	Macroscopic appearance	Microscopic appearance
Stage I (Immature)	Thin and thread-like running longitudinally along the body cavity extending for less than 50%. Sex can only be determined microscopically.	Thin and thread-like running longitudinally along the body cavity extending for less than 50%. Sex can only be determined microscopically.	Oocytes have maximum diameter of 45 μm. Have a thin densely staining cytoplasm and large rounded nucleus. No cytoplasmic vacuoles. Ovary wall less than 27 μm thick.
Stage IIa (Immature)	Grayish white, flattened and occupy about half of the body cavity with no milt.	Cylindrical with gradual tapering towards the distal end. No oocytes visible through ovary wall.	Largest oocyte between 39–45 μm in diameter. Cytoplasm densely staining. No cytoplasmic vacuoles. Rounded nucleus with about 8 nucleoli on the periphery. Ovary wall about 97 μm thick.
Stage IIb (Recovering)	Flattened, firm to feel, creamy white in colour. Running almost full length of body cavity.	Flattened, soft and flabby to feel. Colorless or pink.	Same as IIa but residual atretic oocyte present. Re-organization of ovigerous lamellae starting. High level of atretic oocytes.
Stage III (Active)	Thickened wall, exudes milt on cutting. Light yellow and extends 50% of abdominal cavity. Blood vessels conapicuous.	Opaque with large oocytes visible through ovary wall. Dense network of blood vessels visible internally.	Largest oocytes range from 91–219 μm in diameter. Cytoplasmic vacuoles (5 μm) appear in the largest oocytes. Oocytes arranged in well organized ovigerous lamellae.
Stage IV (Ripe)	At their maximum size opaque-whitis, soft and exudes milt on cutting. Extends upto 90% of abdominal cavity. Blood vessels become less conspicuous.	Fully swollen and translucent oocytes clearly visible through ovary wall. Blood vessels become less obvious.	Largest oocytes with maximum diameter of 420 μm. Stages I and II oocytes present in various proportions. Large oocyte filled with eosinophilic yolk-granules. Organization of ovigerous lamellae is disappearing. Ovary wall is about 182 μm thick.
Stage V (fully ripe)	Fully developed as in stage IV but exudes milt with slight pressure. Blood vessels absent. Extend upto 99% of the abdominal cavity.	Very soft to feel, yellow and often swollen. Some ovaries with a hollow central region. Rounded with granular surface.	Largest oocytes with a diameter of 522 μm with densely staining yolk granules. Some granules colesce to form large tightly packed granules and an occasional yolk mass (smooth yolk). Cell membrane is well defined. Ovary wall is 40 μm thick.

eral batches of eggs and sperm at different spawning times during a season.

The breeding cycle

The changes in the GSI of mature (stages III-V) male and female *L. fulviflamma* were calculated on a monthly basis (Figure 3). These plots indicate that although the magnitude of changes of GSI for males and females differ slightly in most months, they basically follow the same pattern of weight changes. There is a gradual fall in GSI from a peak in November for the females, and, December for the male, to low levels in June followed by gonad weight increase from July/August through September and October to a peak in November/December. The prolonged period of weight loss by the gonads indicates a prolonged spawning period, November/December to April/May, during which several batches of eggs are released.

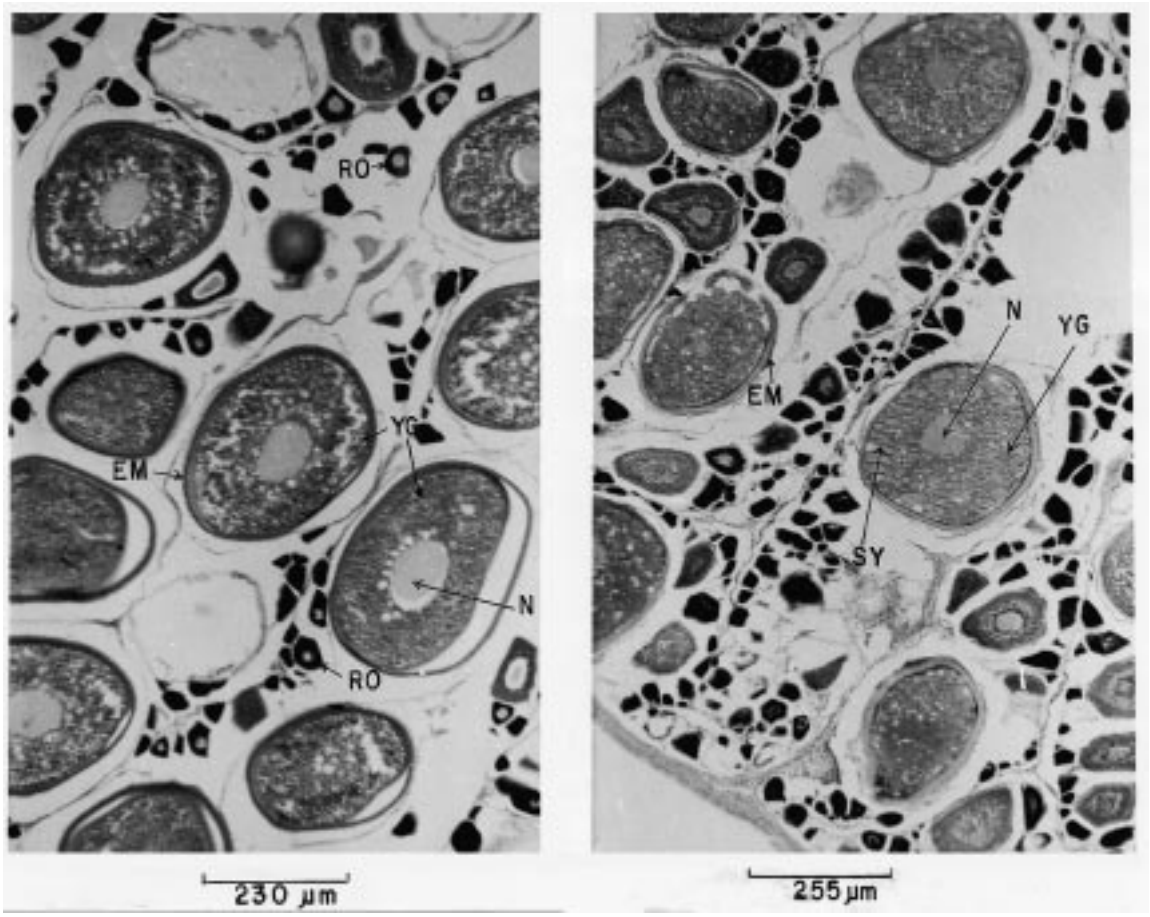

Plate 1. Histological characteristics of (a) stage IV ovary with many resting and yolky oocytes and (b) stage V ovary of *L. fulviflamma* where yolky granules coalesce to form smooth yolk. Key: N, Nucleus; RO, resting oocyte; YG, Yolk granules; EM, Chorion; Sy, Smooth yolk.

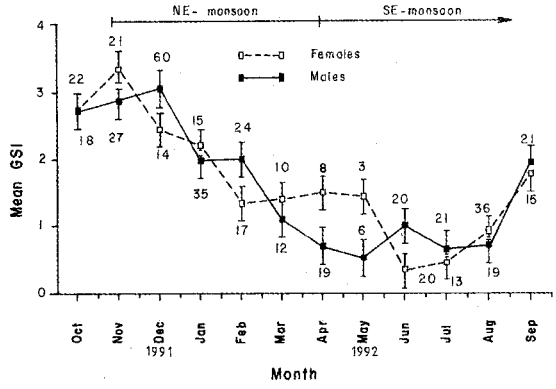

Figure 3. Temporal variation of the gonadal-somatic index in the male and female *L. fulviflamma* in Kenyan inshore marine waters. The vertical lines represent ± s.e.m. Numbers of fish used for analysis are shown for each month.

Immature fish (stages I and II) occur in nearly all months while recovering/spent fish significantly appeared in the samples from January onwards (Figure 4). There is a gradual increase in the number of early developing fish (stage III) from July onwards. Stage IV fish occur in the samples from August to March while stage V fish appear in the samples from October to March.

Oocyte recrudescence in the ovary

As was shown earlier, stage I fish have one type of oocytes in the ovary (Figure 1). As development proceeds, another type of slightly bigger oocytes appear as mode a_2 in stage II fish. In stage III, three types of oocytes appear in the ovary viz-axis c_1, b_1 and a_3 at 0.18 mm, 0.28 mm and 0.38 mm, respectively. At stage IV the leading peak (a_3) moves slightly to the right to become a_4 at 0.4 mm. The two other minor peaks, c_1 and b_1 seen at stage III now move to the

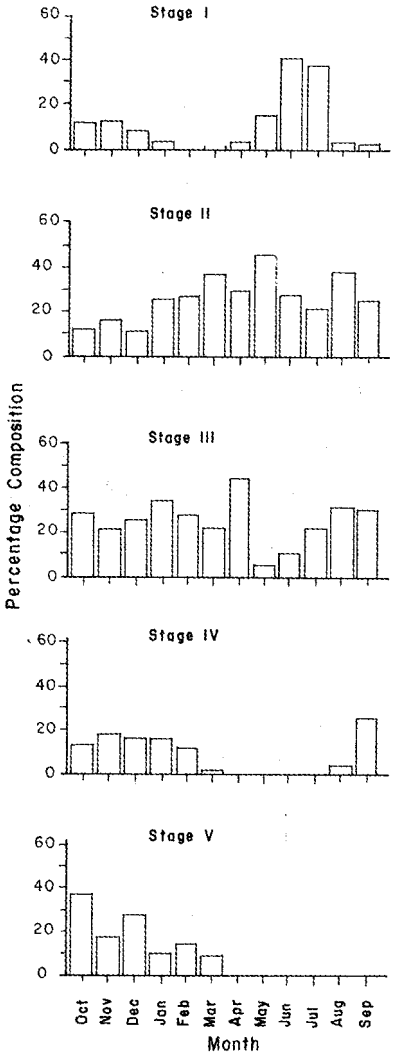

Figure 4. Monthly percentage occurrence of the maturity stages of *L. fulviflamma* in Kenyan inshore marine waters during the period 1992-93.

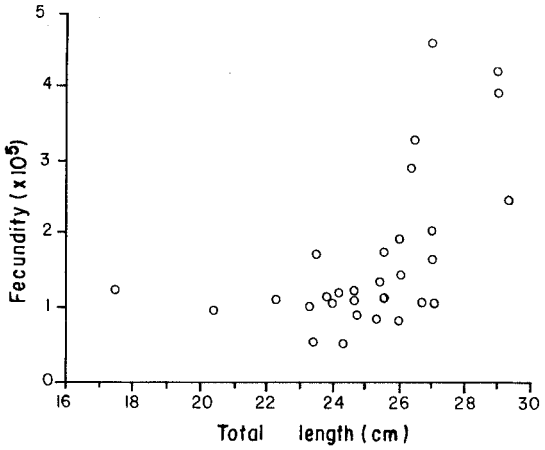

Figure 5. Relationship between total length and fecundity in *L. fulviflamma* samples from Kenyan inshore marine waters. n = 30

Fecundity estimates

Following Ntiba & Jaccarini (1992), it was determined that vacuoles started appearing in oocytes of *L. fulviflamma* at 0.2 mm. Therefore using the formula, $F = ((V/V_1)n)(W/W_1)$ on all oocytes $\geq$ 0.2 mm in diameter from the Gilson's preserved ovaries, an estimate of the total number of oocytes was made which represents the potential fecundity of each female fish. Thirty stage IV ovaries were used and the total number of oocytes to be released during the next spawning season plotted against the total body length (Figure 5). The fecundity (F) of the dory snapper approaches a cubic relationship with body length (L), $F = 27.69L^{2.64}$; r = 0.55, n=30, the relationship between fecundity and body weight in grammes was found to be, $F = 496\ W^{1.02}$; r = 0.60, n=30.

Fecundity ranged from 51,464 to 459,887 oocytes in fish of 17 cm to 30 cm TL, respectively. For the thirty fish examined, there was a mean of 166,985 oocytes to be spawned in the coming breeding season.

right at stage IV to become c_2 and b_2 at 0.25 mm and 0.33 mm, respectively. At stage V only two types of oocytes are present in the ovary. These are c_3 and a_5 at 0.33 mm and 0.43 mm, respectively. Oocyte type a_5 at stage V was most possibly made by a merger of oocytes c_2 and a_4 seen at stage IV. Histological examination showed that the largest oocytes at stage V were yolky and had a transparent peripheral zone (Cf: Plate 1).

Discussion

The observed difference in sex ratio in December, January, May, June, July and August during this study could be due to differential migration of the sexes during and before the spawning season (Thomson & Munro, 1983). The minimum size at first sexual maturity for *L. fulviflamma* is 20.5 and 24.3 cm TL for males and females respectively (Kaunda-Arara, Unpublished

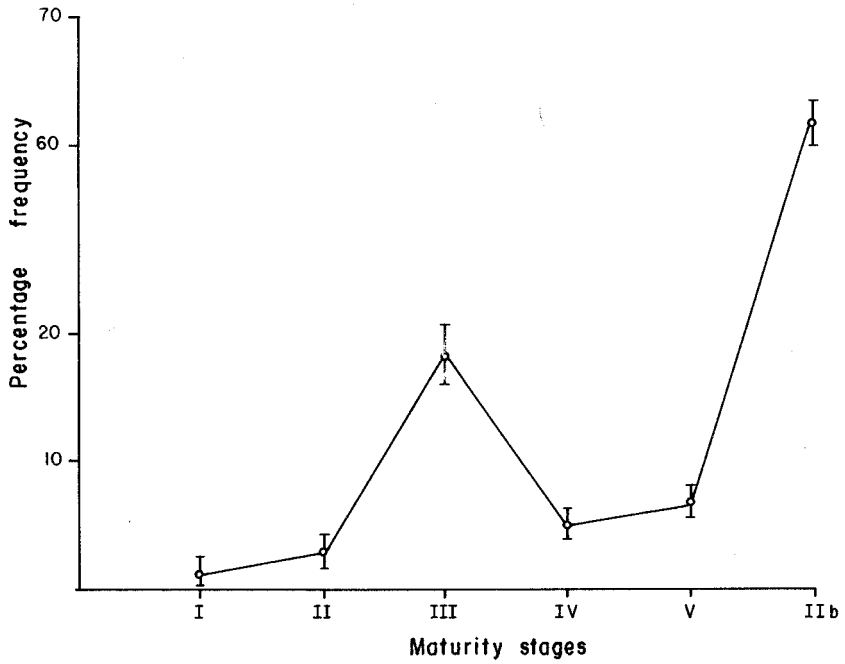

Figure 6. Relationship between number of atretic oocytes and stages of maturation in the ovaries of *L. fulviflamma* in Kenyan inshore marine waters. The data are from 4 ovaries from each of the maturity stage. The vertical bars are equal to ± s.e.m.

data). Therefore the appearance in almost equal numbers of both sexes in the size groups of between 20 and 25 cm in the population may suggest congregation for spawning, while the dominance of males in the lower size groups (Table 2) may be due to differential growth rates. The earlier maturation of males is probably what causes their preponderance in the smaller size classes since, no sex-reversal has been reported among the Lutjanidae (Thomson & Munro, 1983).

While immature fish (stages I and II) were available throughout the study period, mature fish (stages III, IV and V) occurred in considerable proportions during the October to March period. This may indicate a prolonged spawning season for the species extending from October to March. This conclusion is also supported by the pattern of variation of GSI. This means that the recruitment of oocytes in the ovary of *L. fulviflamma* is asynchronous and are released in several batches during the spawning season. This study also shows that intra-ovarian distribution of the oocytes is multimodal (Figure 1). Future work should try and estimate the time of release of these batches and their sizes. The spawning season for this species coincides with the northeast monsoon period, a time when, the East African coastal waters are calm thus favouring the survival of ichthyoplankton. The species spawns pelagic eggs as evidenced by the presence of smooth yolk in stage V ovary (Plate 1b).

Six gonad maturity stages (I–V and IIb) have been described for *L. fulviflamma* in the present study. Nzioka (1981) and Ntiba & Jaccarini (1990) defined seven stages for *Scolopsis bimaculatus* and *Siganus sutor*, respectively. In *L. fulviflamma* spent individuals (stage VI) were not obtained, probably because an individual fish would quickly release its eggs in several batches at the end of which the ovary quickly recovers to stage IIb. Quasim (1973) working with *Blennius pholi*, which also releases its eggs in batches, did not find any stage VI fish in his samples, Macer (1974) points out that the spawning process in fish is rapid and can obscure certain maturity stages.

The wide range of fecundity (51,000 to 460,000 eggs) estimated for different sizes of *L. fulviflamma* agrees with fecundity estimates made for other Lutjanidae (Rojas, 1960; Rodriguez, 1962). Kisselevitch (1923), Clark (1934) and Allen (1951) found that fecundity increases as the square of body length in the Caspian sea herring, the North sea plaice and in the brown trout, respectively. In *L. fulviflamma* fecundity has been found to approach a cubic relationship with body length and direct proportionality with body weight.

While several reasons have been given for the resorption of oocytes in the ovary after spawning (Macer, 1974; Cyrus & Blaber, 1984; Ntiba & Jaccarini, 1992), the reasons for pre-spawning atresia are unclear. Both pre- and post-spawning atresia have been shown to occur in *L. fulviflamma* in this study (Figure 6).

Acknowledgments

We thank Dr E. Okemwa, Director, Kenya Marine and Fisheries Research Institute, for laboratory facilities. This work was funded by the German DAAD programme.

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