MARDI Res. Bull. 1, 1:55 — 68 (1973)

THE REPRODUCTIVE PATTERNS AND MATURATION OF THE GONADS IN AN AQUARIUM FISH, BETTA PUGNAX (CANTOR)*

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Accepted for publication: 18 Dec. 1972.

Ringkasan

Corak pembiakan dan kematangan gonad sejenis ikan akuarium, *Betta pugnax* telah dikaji. Jenis ini menjadi matang apabila ukuran panjangnya 4 cm. dan 110 hingga 120 hari umurnya. Ikan ini jenis maun, sebahagian besar makanannya terdiri dari serangga-serangga di daratan. Di musim hujan makannya lebih nyata. G.S.I. dan keadaan pembiakan ikan ini menunjukkan bahawa pembiakan jenis ini ialah sepanjang masa tetapi terlebih jelas di musim hujan. Musim pembiakan jenis ikan ini lebih dipengaruhi oleh hujan daripada bekalan makanan.

Introduction

This paper deals with the reproductive patterns and maturation of the gonads of an aquarium fish, *Betta pugnax* (Cantor). Our knowledge of the reproductive patterns of fishes inhabiting the tropics (Nair 1958, Sathyanesan 1959 and 1962, Lehri 1967) is scanty. Thus these studies were undertaken in order to assess the annual and seasonal reproductive patterns in the field. The maturation of the gonads and food habits of this fish were also studied to elucidate any relationship with the breeding cycle.

According to Bullough (1963) breeding of fishes is governed by two factors. These are the internal gonadal rhythm which is characteristic of the species, and the external enviromental factor which determines the precise time of spawning. In the tropics, factors such as rain and food should be more critical than photoperiod or temperature as there is little seasonal variation in the latter. Whether food or rain or both factors act together to trigger the time of breeding season is, however, the critical question.

The few published literature on the reproductive patterns of tropical species (Das 1927, Soong 1948, Nair 1958) suggests that rain is one of the important factors which influences the breeding of many tropical fishes. Some investigators in the tropics suggest that breeding is aseasonal in many fishes (Deraniyagala 1929, Tan 1968) and reptiles (Berry and Lim 1967).

Materials and Methods

The specimens used for the study of gonadal maturation were bred in the aquarium while those for the reproductive pattern studies and gut content analysis were obtained from a stream at 9 m/s Kuala Lumpur — Damansara Road, Selangor. The gut contents were examined with a binocular microscope and the results are given in table I.

Sections of the gonads were prepared following Gurr's techniques (1956). The gonads were classified to stages according to James (1946). Breeding conditions of the fish were determined by the following criteria: The gross enlargement of the gonals and thats the germ cells consisted of mainly mature sperms (fig. 9) and stage IV oocytes (fig. 4)

The gonadosomatic index (G.S.I.) of *Betta pugnax* was computed and expressed as a percentage ratio of the weight of the gonad to that of the body weight. The individual G.S.I. of the gonads and the means for each period was computed. Because of the relatively low numbers in each monthly sample, the specimens were grouped into bimonthly classes for analysis of the reproductive patterns (table II and III).

* Part of a thesis accepted by the University of Malaya, Kuala Lumpur in partial fulfilment of the requirements for the degree of Master of Science.

TABLE I. *Betta pugnax*: Percentage occurance of diet items in the gut during the different months of the year.

Date	1	2	3	4	5	6	7	8	9	10
Aug. 24,66	14	71.6	28.4	45.7	10.5	5.2	0.0	5.1	5.1	10.0
Sep. 14,66	13	84.5	15.5	45.5	0.0	12.0	12.0	12.0	1.5	1.5
Oct. 19,66	20	90.0	10.0	39.5	6.0	10.5	13.0	10.5	4.5	6.0
Nov. 23,66	11	91.0	9.0	82.8	4.1	0.0	0.0	0.0	4.1	0.0
Dec. 66	36	72.0	28.0	33.0	2.0	6.9	6.9	6.9	9.4	6.9
Jan. 1,67	18	67.0	33.0	43.0	14.6	0.0	4.7	0.0	4.7	0.0
Feb. 1,67	18	61.5	38.5	30.5	4.0	3.0	6.0	3.0	3.0	12.0
Mar. 15,67	11	72.7	27.3	51.0	6.5	0.0	6.5	0.0	0.0	8.7
Apr. 15,67	16	87.5	12.5	76.6	0.0	0.0	0.0	2.7	2.7	5.5
May 15,67	8	62.5	37.5	54.7	0.0	0.0	0.0	0.0	0.0	7.8
Jun. 26,67	12	58.3	41.7	43.9	0.0	0.0	4.8	0.0	0.0	9.6
Jul. 25,67	8	62.5	37.5	31.3	0.0	10.4	0.0	10.4	10.4	0.0
Aug. 27,67	13	69.6	30.4	48.3	5.2	0.0	0.0	5.2	0.0	10.9

Results

Maturation of the Ovary

Stage I Ovary

Specimens measuring 1.5 — 2.4 cm in standard length and 30 — 45 days old already possessed stage I ovary. In cross-sections, the ovary is a sac-like structure with a central cavity surrounded by ovarian wall. The ovarian wall is composed of several lamellae, each separated by an ovacoel (fig. 1) The mature oocyte (stage I oocyte) measures 15 — 75 microns in diameter.

Stage II and III Ovary

Specimens measuring 2.5 — 4.0 cm. in standard length and 50 — 105 days old possess stages II and III ovaries. Stage II ovary has a reduced ovarian cavity due to the enlargement of the ova (figs. 2 and 3). Stage II cocytes measure 160 — 280 micron in diameter (fig. 2) and their cytoplasm are vacuolated. The vacuoles are probably the original lipoid vesicles as in *Eucalia* sp. (Braekevelt and McMillan 1967) which were dissolved during histological preparation.

Stage III ovary bearing stage III oocytes (fig. 3) measure 320 — 430 microns in diameter. This stage is characterised by the initiation of yolk deposition. The cytoplasm becomes more vacuolated (fig. 3).

Stage IV Ovary

Stage IV ovary with stage IV oocytes occur in fish measuring about 4.5 cm. in standard length and 4 months old. These are mature ovary. The oocytes are rounded and translucent in appearance and measure 610 — 900 microns in diameter (fig. 4). Though stage IV oocytes are the dominant oocytes in a mature ovary, there are also nests of immature oocytes at different developmental stages.

Stage V Ovary

These are the spent ovary. In *Betta pugnax*,, there are no truly spent ovary since in a newly spawned fish, the oocytes are already in stage III (fig. 5).

Maturation of the Testis.

Stage I Testis

Stage I testis occurs in fish measuring 1.2 — 2.4 cm. in standard length and 30 — 45 days old. It appears as small, paired, slender and thread-like at first and ribbon-like later (fig. 6). The lobules of immature testis contain many spermatogonial cells arranged in separate cysts. The nucleus of the spermatogonium measures 6.6 — 8.3 microns in diameter (fig. 7). Besides the spermatogonia, there are also primary germ cells which occur singly, in twos or threes (fig. 7).

Stage II Testis

Stage II testis occurs in fish measuring 2.5 — 4.0 cm. in standard length and 50 — 105 days old. The oldest germ cells in this stage are the spermatids. The disposition of the various cysts in the lobules are at random (fig. 7). The nucleus (4.9 — 5.5 microns) of the spermatocytes in crescentic while the spermatids have round nuclei (3.3 microns).

Stage III Testis

Stage III testis in *Betta pugnax* corresponds to stages C and D of James (1946). Specimens over 4 cm. in standard length are already in the mature stage. The testis appears swollen and the lobules are filled with spermatozoa (fig. 9). In early stage III testis, there is a considerable reduction in the number of cysts of actively dividing germ cells and an increase in the spermatozoon masses (fig. 8). The mature sperm has a round head (2.2 — 2.5 microns) and a long tail (12.5 microns).

Feeding

There is a high percentage (73.4%) of fish with food items in the guts throughout the year (table I and fig. II). Ants form the bulk of the diet while termites, chironomid larvae and others are minor constituents. Though algae are part of the diet, they might have been ingested accidently.

Although food items and rainfall are abundant throughout the year, there are periods where more fish possess food in the gut (fig. II). The absence of food in the guts of some male fish may be due to the incubation of eggs by these males as they do not feed during incubation period (Aug 1971).

Reproduction

In the Damansara Road stream, two incubating males were found in July, three in August and September, and young larvae were present throughout the sampling period.

The number of fish examined for reproductive study is given in table II. Their gonads did not show any marked change in gross appearance, weight or gonadal activity.

The G.S.I. is 0.39 — 0.18 for the testis and 2.60 — 0.87 for the ovary (table III). There is a positive correlation ($r = 0.392$ for testis and $r = 0.565$ for ovary) between the G.S.I. of the gonads and the percentage of fish with food in their guts (table III and fig. 12). The correlation between the G.S.I. of the gonads and rainfall is positive and significant ($P < 0.05$).

Histological examination of the gonads indicates that varying proportion of fish are in breeding condition at different times of the year (table IV). However, more fish are in breeding condition at two periods in the year, October — November and February — May. The correlation of the G.S.I. of the gonads and breeding condition of the fish is highly significant, fig. 13).

TABLE IV. *Betta pugnax*: Percentage of adult fish in breeding condition as determined by the histological studies of the gonads.

Bimonthly periods	*No. of fish*	*Males*	*Females*
Aug. — Sept.	26	31.25	40.00
Oct. — Nov.	26	58.33	64.28
Dec. — Jan.	37	42.10	22.22
Feb. — Mar.	16	75.00	62.50
Apr. — May.	15	77.78	50.00
Jun. — Jul.	12	40.00	28.57

Discussion

Betta pugnax becomes sexually mature at about 4 cm. in standard length and in about 110 — 120 days. The rapid attainment of sexual maturity in this species supports Nikolsky's (1963) view that small species especaliy in the tropics become mature early.

This species is an omnivore, as noted by Alfred (1958), feeding largely on surface insects (table I). More fish tend to be feeding on this diet during rainy seasons (fig. II), presumably because more food items are washed into the stream by the rains, in the months of October — November and March — April.

Betta pugnax is a fractional spawner (Aug 1971). Fractional spawning and prolonged spawning periods are characteristic of many tropical species (Nikolsky 1963). This spawning pattern is related to the absence of marked seasonal variation in the abundance of food supply or in other ecological factors, in the tropics. This is confirmed by the histological studies of the gonads (fig. 5 and 10) which show the presence of various stages of gametogenesis at any one time. Spent ovary or the resting stage testis are completely lacking. Also, incubating males and newly hatched larvae occur throughout the year. Although this fish breeds throughout the year, there are periods when breeding is more pronounced: October — November and February — May. These are indicated by the G.S.I. of the gonads (fig. 13) and their breeding condition (table IV). The narrow range of the G.S.I. is related to the occurrence of gonads at various stages of development throughout the year, to part of te population always being in breeding condition (table IV). This is in contrast to the wide range found among temperate species (James 1946, Ahsan 1966).

Environmental factors such as rain (Das 1927, Soong 1948, Nair 1958), temperature (Mathews 1938, Bullough 1939), food (Marshall and Hook 1960) and light (Harrington 1959, Henderson 1963) have been attributed to control breeding in vertebrates including fishes. From the data of the present study, one may conclude that food and rain are important variables in regulating breeding in *Betta pugnax*. Whether light and temperature play any role in triggering reproduction in fishes in the tropics can only be speculative at the present level of knowledge in this field. Perhaps the quality and intensity of these two factors may be important in triggering reproduction in fishes in the tropics.

Food is an important factor in the breeding of *Betta pugnax* (table III, fig. 13), mainly because of its relationship to rainfall (fig. II). There is conflicting report regarding the importance of food in the breeding of vertebrates (Baker 1938, Marshall 1947, Church 1960 and Berry 1964). Evidently more detail investigations in this field should be undertaken.

Rainfall seems to be vital in the breeding of *Betta pugnax*, not only to its relationship to food abundance but mainly because of its relationship to the condition of the gonads (table IV, fig. 13). The precise role of rainfall is uncertain: namely whether the physical effect of rain itself, the physiological and psychological effects of flooding caused by rain, the abundance of food due to rain (Baker 1938), the increased turbidity due to rain (Nair 1958), the change in water temperature due to rain (Nair 1958), or a combination of these, is the critical factor. From the data of the present study one may conclude that rain plays a vital role in the breeding of *Betta pugnax* and possibly other tropical species. The aseasonal or acyclic breeding pattern in this species may be explained on the basis of the climate where this species occurs. There is no truly dry season around Kuala Lumpur, since rain falls every month of the year. In the tropics where there is a distinct wet and dry season, it has been reported that some species breed during the wet season (Nair 1958). However, the precise role played by rain and food in triggering reproduction in fishes are still open for further investigation.

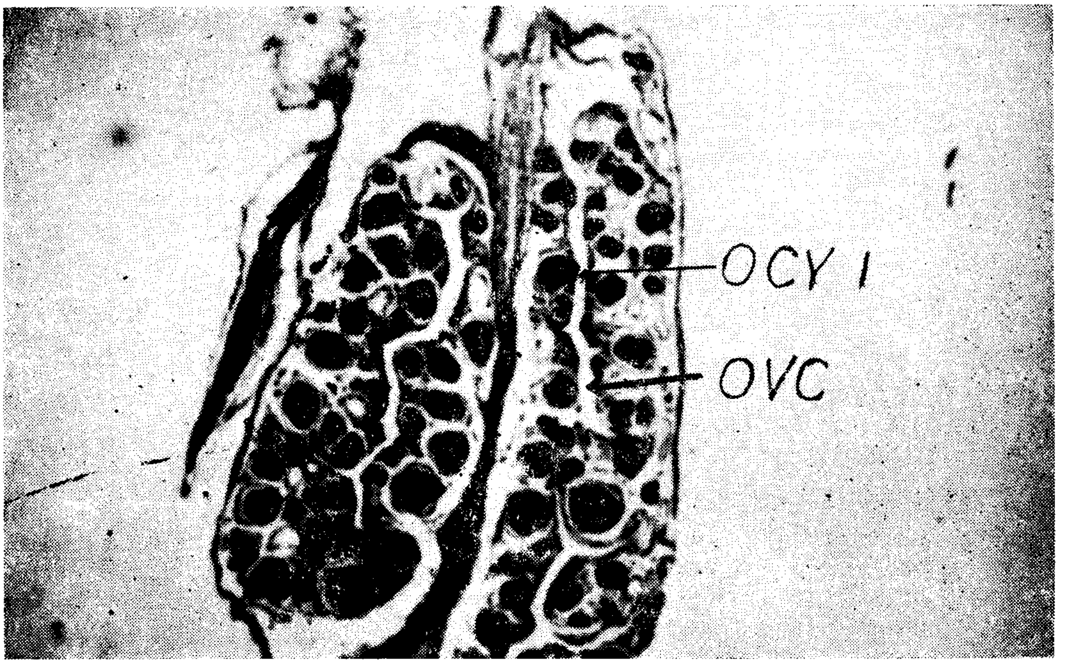

Fig. 1
Betta pugnax: L.S. of immature ovary from a 1.6 cm. fish showing the lamellae, ovocoel (OVC) and stage 1 oocytes (OCY 1). (25X). Haematoxylin-eosin.

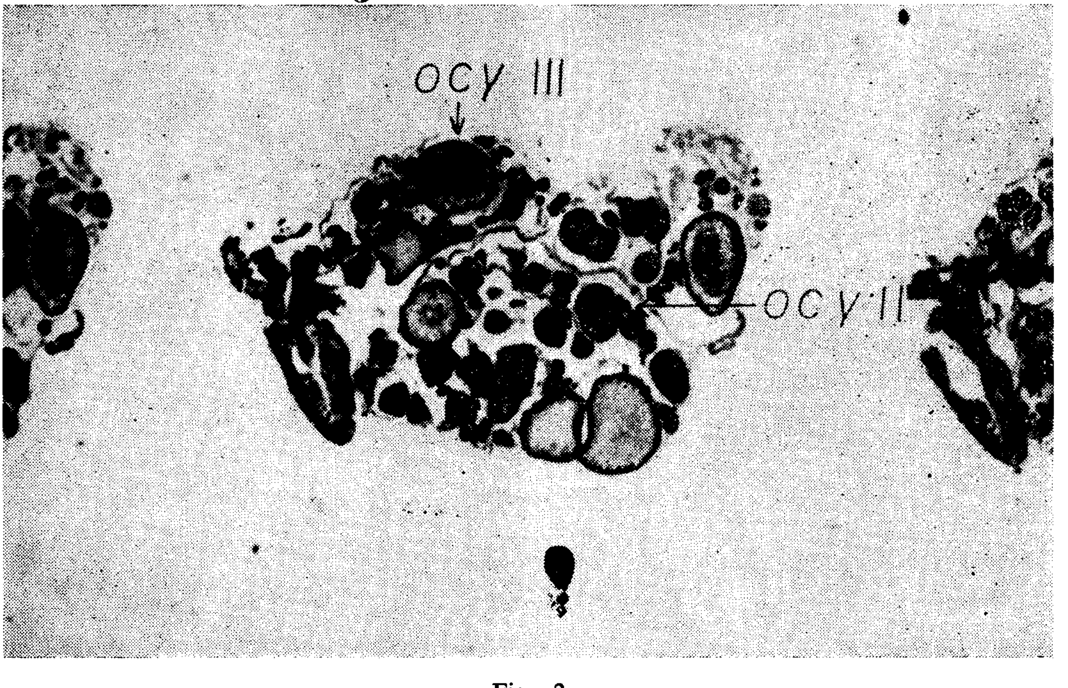

Fig. 2
Betta pugnax : L.S. of late stage II ovary from a 2.5 cm. fish showing stages I, II and III oocytes (10X). Haematoxylin-eosin.

Fig. 3
Betta pugnax: L.S. of stage III ovary from a 3.4 cm. fish showing stages I-III oocytes (10X). Haematoxylin-eosin.

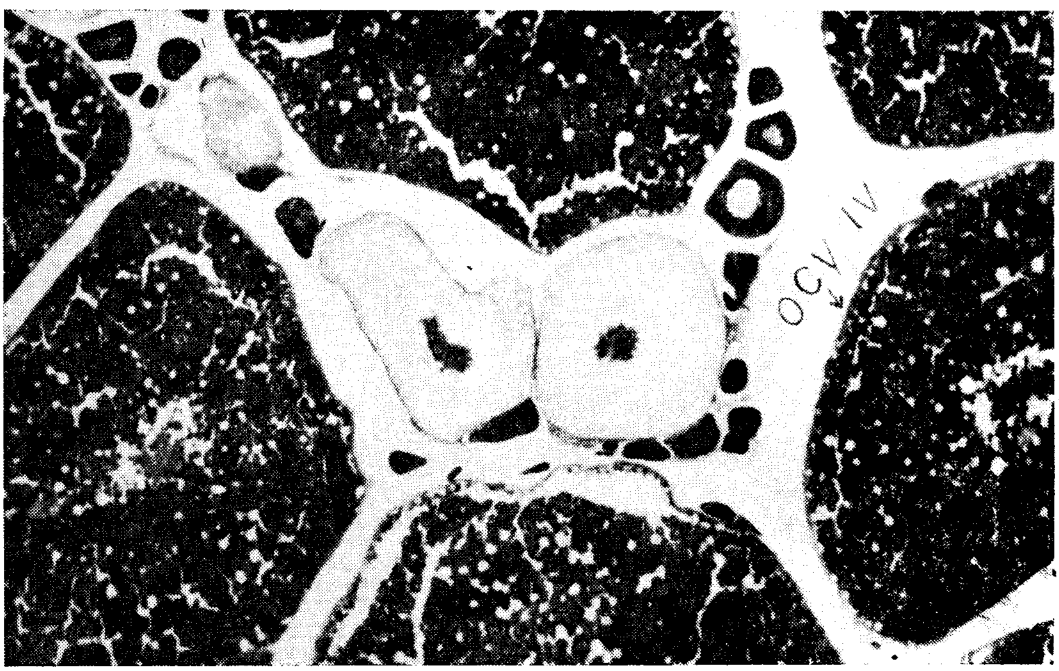

Fig. 4
Betta pugnax: L.S. of stage IV ovary from a 4.5 cm. fish showing many stage IV oocytes, and few stages I and III oocytes (10X). Haematoxylin-eosin.

Fig. 5
Betta pugnax: L.S. of ovary showing empty follicles, primary oocytes, and maturing oocytes. (10X). Haematoxylin-eosin.

Fig. 6
Betta pugnax: L.S. of stage I testis. (15X). Haematoxylin-eosin.

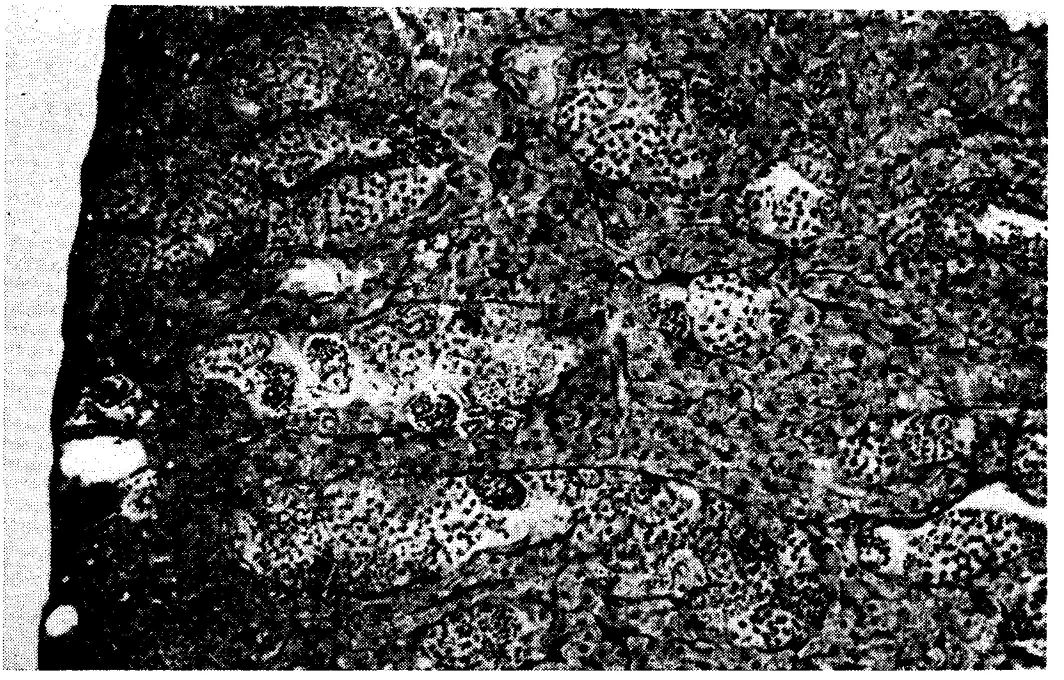

Fig. 7
Betta pugnax: L.S. of the testis showing the primary germ cells (Pgc), spermatogonia, spermatocytes (SPC, and spermatids (SPD). (100X). Haematoxylin-eosin.

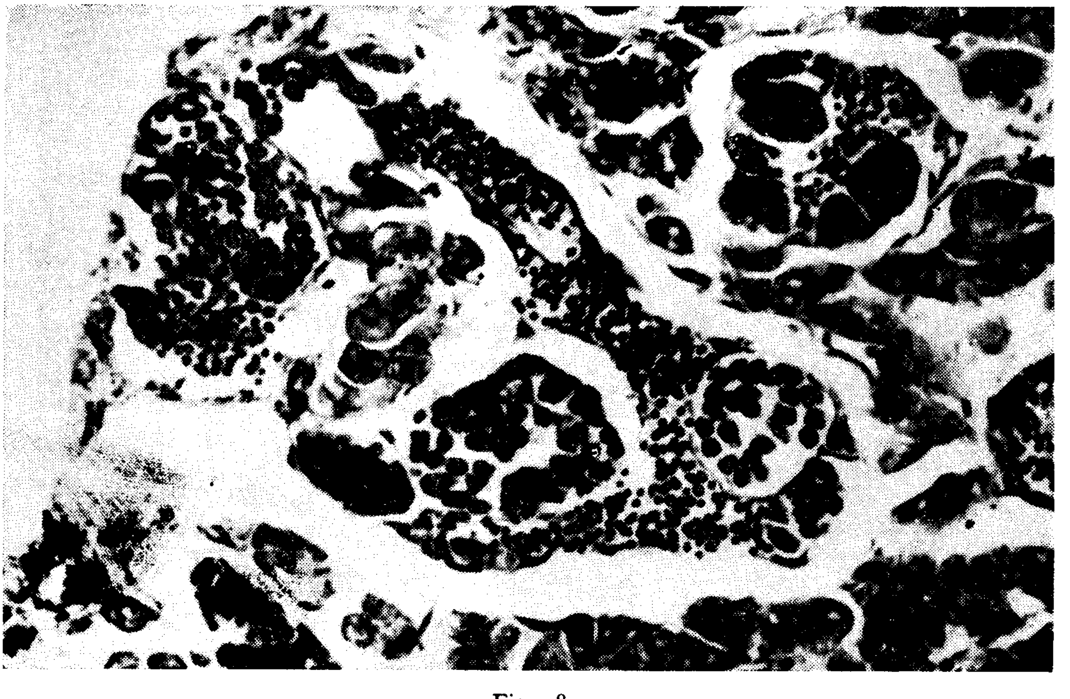

Fig. 8
Betta pugnax: L.S. of the testis showing a lobule enclosing a number of cysts of actively dividing germ cells and sperm masses. (160X). Haematoxylin-eosin.

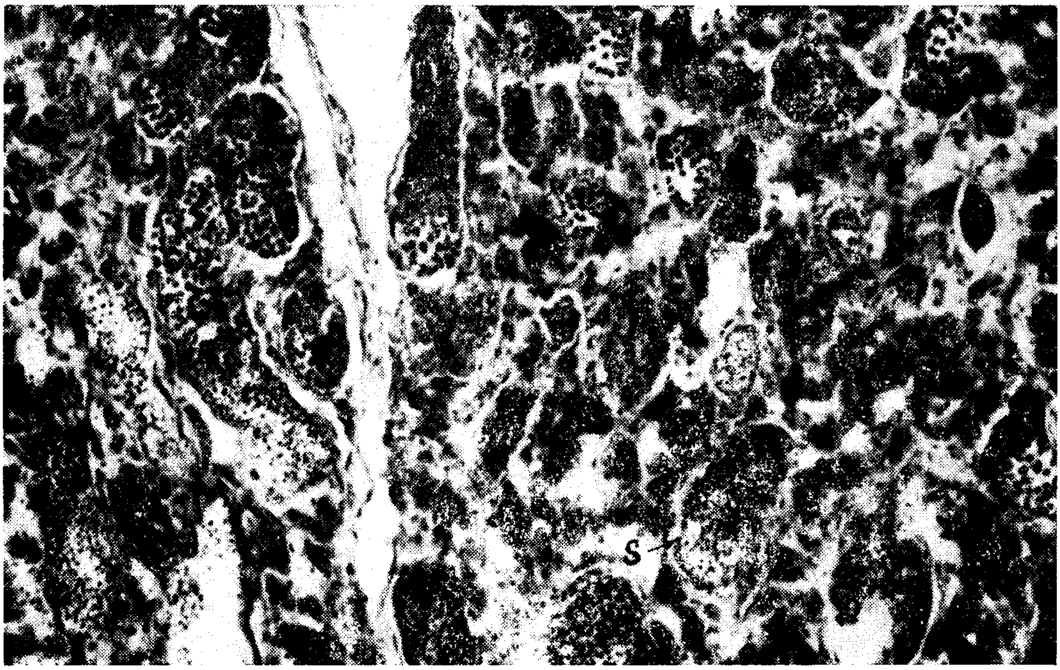

Fig. 9
Betta Pugnax: L.S. of testis showing mature spermatozoa in the lobules. (160X). Haematoxylin-eosin.

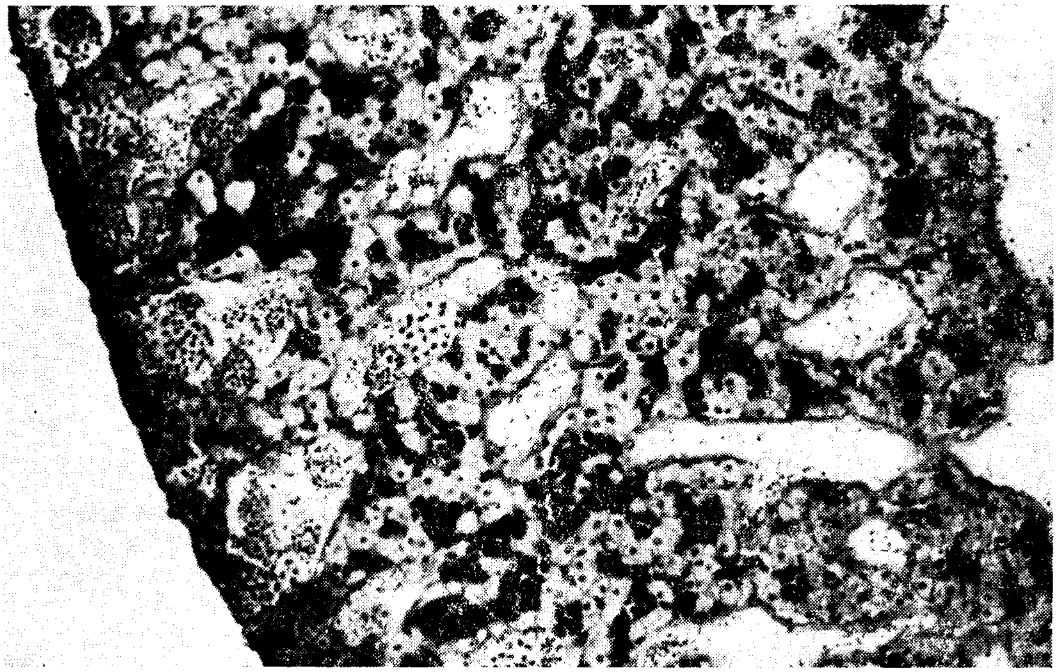

Fig. 10
Betta pugnax: L.S. of testis showing empty lobules towards the median region, and the primary and secondary spermatocytes, spermatogonia and spermatids towards the preiphery. (100X). Haematoxylin-eosin.

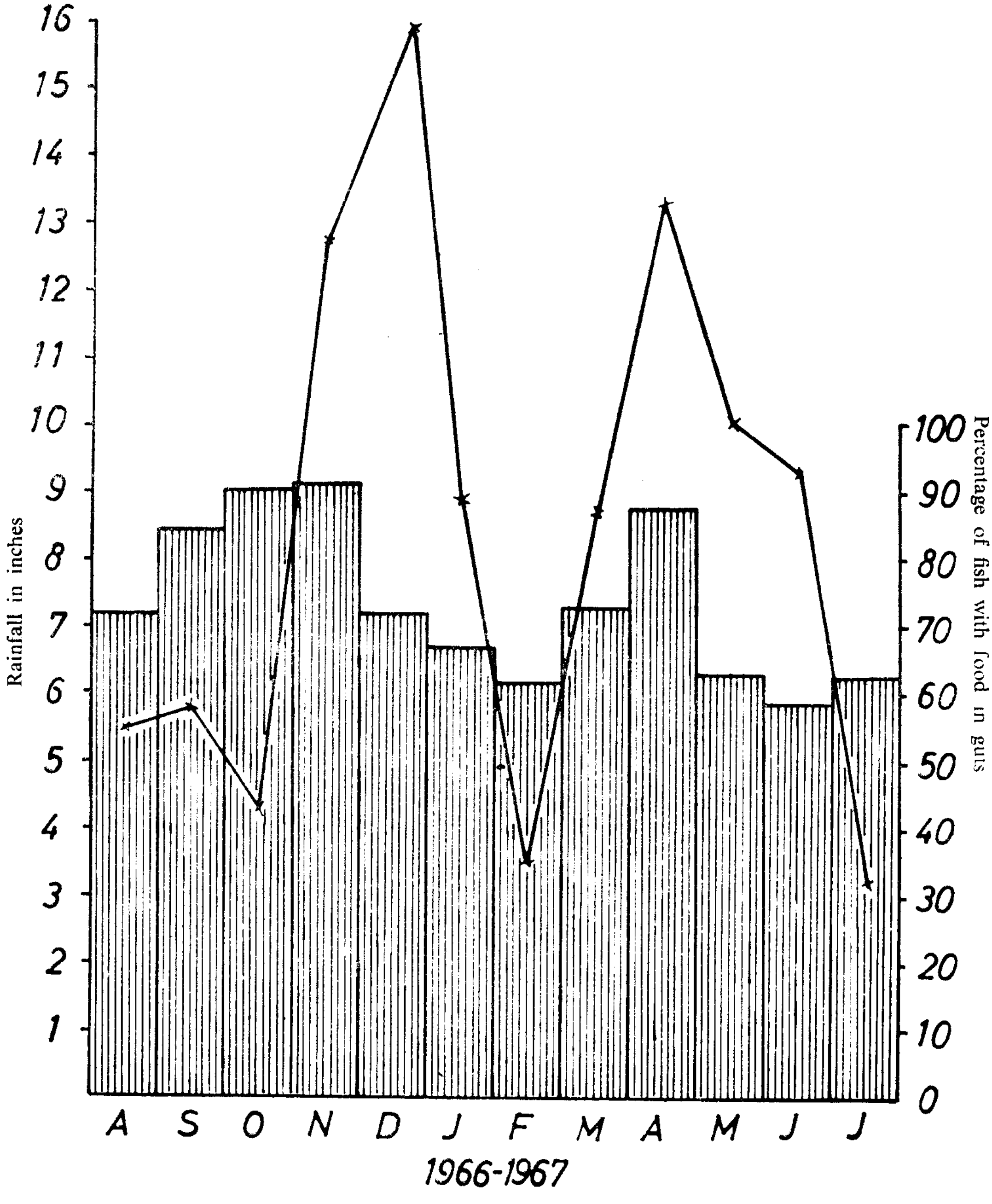

Fig. 11
Betta pugnax: Percentage of fish with food in guts per month (histogram) in relation to rainfall (linear).

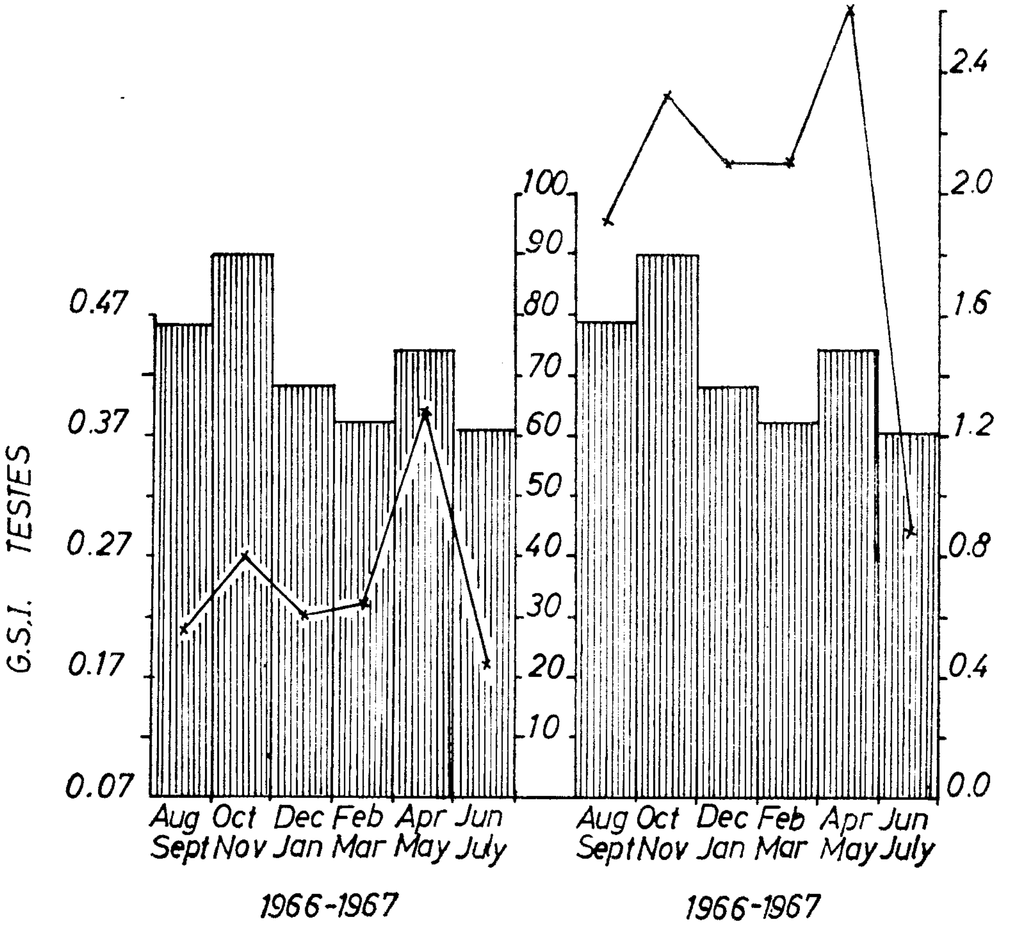

Fig. 12

Betta pugnax: Mean G.S.I. (linear) of gonads in relation to fish with food in guts expressed as percentage (histogram), at bimonthly periods.

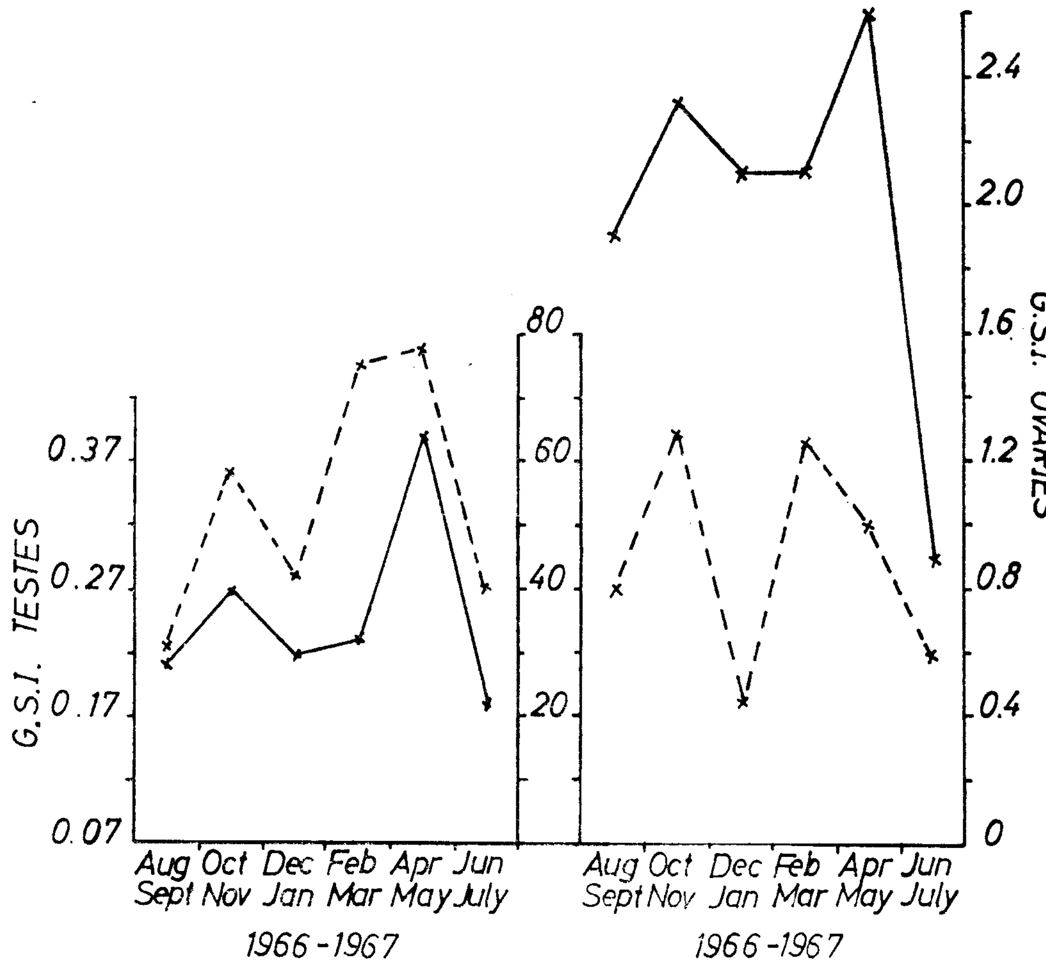

Fig. 13

Betta pugnax: Mean G.S.I. of the gonads (solid line) in relation to fish with breeding condition (broken line) expressed as percentage, at bimonthly periods.

Acknowledgements

This investigation was conducted during the tenure of a Tutorship award in the School of Biological Sciences, University of Malaya, Kuala Lumpur and with financial assistance from the University of Malaya. The author expresses thanks to Dr. Khoo Soo Ghee for supervision of this work and Dr. J.I. Furtado and other members of the School for their helps during this investigation, to Encik Mohd. Jaafar bin Daud and Encik Lim Teck Jin.

Summary

The reproductive pattern and maturation of the gonads of *Betta pugnax* were investigated. This species matures at 4 cm. standard length and 110 — 120 days old. It is an omnivore, feeding largely on terrestrial insects. Feeding is more pronounced during rainy seasons. The G.S.I. and breeding condition of the fish indicate that *B. pugnax* breeds throughout the year, but more predominantly during rainy seasons. Seasonal predominance of breeding seems to be influenced more by rainfall than food supply.

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