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Maturation and spawning in the hill-stream loach *Noemacheilus triangularis* Day

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Abstract. Five stages of maturity have been delineated for *Noemacheilus triangularis* Day. Progression of the mode in each stage of maturity has been traced from ova-diameter studies. The size at first maturity for male is around 44·5 mm and for female around 52 mm. Spawning is asynchronous and prolonged. There may be successive spawning during the year. Sexes are present in almost equal numbers. Fecundity is dependent on length and weight of fish and on length and weight of ovary.

Keywords. Maturity stages; spawning season; spawning frequency; gonado-somatic index; sex ratio; fecundity; hill-stream loach; *Noemacheilus triangularis*.

1. Introduction

During the past decade, a large volume of information has accumulated on the breeding biology of commercially important fishes (Ibrahim 1956; Qasim and Qayyum 1961; Qayyum and Qasim 1964a, b, c; Bhatt 1968, 1970, 1971a, b; Parameswaran *et al* 1972; Sobhana and Nair 1974). A review of the literature reveals that very little has been done on the reproductive biology of any fish belonging to the family Cobitidae. The fact that these fishes are not of much economic importance may be the reason for this neglect. Though not important as a food fish, *Noemacheilus triangularis* is an interesting cobitid on account of its peculiar mode of life, habitat and wide geographical distribution. Therefore, it was thought worthwhile to examine in detail the breeding biology of the species.

2. Materials and methods

Samples were collected from the Kallar river during the first half of each month, over a period of 12 months, from October 1973. All specimens were measured and weighed. After noting the length, the gonad of each fish was dissected out, weighed and assigned proper stages of maturity. A few ovaries in the advanced stages of development were thereafter preserved in 5% formalin.

Test measurements of ova from anterior, middle and posterior parts of the ovary indicated no difference in the distribution of ova in different regions of the ovary. Hence 500 ova from the middle portion of each ovary of different stages of maturity were measured to study the maturation of the ova through the different stages.

The percentage occurrence of different stages (males and females separately) was noted and grouped into 5 mm interval length groups to indicate the minimum size at first maturity. A quantitative assessment of the condition of the gonad, employing the technique of gonad-index, expressing the gonad weight in terms of the percentage of the body weight, has been worked out. The gonad weight in the immature fish did not fluctuate much from season to season, hence their weights were excluded from the analysis. This data together with those drawn from different maturity stages help to delineate the spawning season of the species. For the study of development of ova, the method adopted by Prabhu (1956) was followed.

For fecundity studies, ripe specimens collected in March were used. The ova diameter studies have indicated that in ripe ovaries there are three groups of ova. The ripe ova in this species are shed in batches. It is therefore difficult to estimate the correct number of ova destined to be spawned in a year. However, it has not been possible to devise any other suitable alternative method of estimating fecundity in *N. triangularis*. Fairbridge (1951) in *Neoplatycephalus macrodon*, Karekar and Bal (1960) in *Polynemus indicus*, James (1967) in *Eupleurogrammus intermedius*, Kagwade (1970) in *Polynemus heptadactylus* have noticed similar conditions in the fecundity studies of the respective species. Bagenal (1970) also pointed out that some tropical fishes may contain eggs in a more or less continuous range of development stages. As followed by James (1967) in *E. intermedius* with three groups of ova in mature ovaries, the number of mature intraovarian eggs was, therefore, taken to be the fecundity of each individual. For each specimen used, the weight of the preserved ovaries was recorded, a portion of the ovary was, then, weighed separately and all mature ova contained in the latter were counted, from which the total number of ova in the pair of ovaries was computed. The relationships between fecundity and the total length and weight of the fish, and length and weight of the ovary were then estimated.

3. Observations

3.1. *Maturity stages*

The classification of gonads into five maturity stages, as suggested by Qasim (1973) for tropical and subtropical forms, and applied to several fresh water fishes by Qayyum and Qasim (1964a, b, c) and Bhatt (1968, 1970, 1971a, b) was found to be applicable for *N. triangularis*. In identification of the maturity stages of the gonad, its colour, general appearance and extent in the body cavity were taken into consideration.

3.1.1. *Stage I—Immature virgins*

The ovaries are very small and semitransparent. Ova are not visible to the naked eye. Under the microscope, they are irregular in shape, transparent with a nucleus in the centre. Yolk formation has not commenced. The diameter of the majority

of ova ranged from 32 μ to 144 μ, with the maximum size up to 272 μ. The ovarian wall is thick.

Testes are small and slender and occupy about the same space as the ovaries and can be distinguished only microscopically from the latter.

3.1.2. *Stage II—Maturing virgins and recovered spent*

The ovaries occupy about 1/4 of the body cavity and are pale yellow in colour. The maturing group of ova gets separated from the general stock. They are spherical and opaque. The mode of the largest group of ova falls around 550 μ, the maximum size of the ova being 640 μ. The ovarian wall is not thick.

Testes show a corresponding increase in size, occupying about 1/3 of the body cavity. The colour remains white.

3.1.3. *Stage III—Ripening*

Ovaries are elongated, yellowish in colour, fully packed with large eggs and occupy 3/4 of the space of the body cavity, extending almost to its entire length. The largest common egg diameter falls around 700 μ, the maximum size being 776 μ. The ovarian wall becomes thinner.

Testes show an ivory colouration and occupy about 1/2 the space of the body cavity.

3.1.4. *Stage IV—Ripe*

Ovaries are large, brownish yellow in colour and occupy the whole of the body cavity, enclosing the intestine by lateral extensions. Eggs are opaque and very distinct, the largest common egg diameter in this stage falls at 800 μ, the maximum size of the ova being 878 μ. The ovarian wall becomes distended, very thin, delicate and easily rupturable.

Testes are flabby and massive, whitish in hue and fill the entire available space of the body cavity.

3.1.5. *Stage V—Spent*

The ovaries are brownish yellow in colour and occupy about 1/5 of the space of the body cavity, *i.e.*, a little smaller than the maturing ovaries. In recently spawned fish, the ovary has a few remnants of mature ova, measuring a maximum diameter of 760 μ, as resorbing and disintegrating opaque structures. But the majority of the ova are small, transparent, invisible to the naked eye and belong to the immature stock.

This stage apparently resembles stage II but differs from it in the relatively smaller size and loosely packed nature of the mature ova. Further, such ovaries are often found with a few transparent areas scattered all over.

Testes appear shrunken and occupy only 1/4 of the body cavity with some unspawned milt.

3.2. *Development of ova to maturity*

Ova diameter frequency polygons of the different maturity stages are drawn in figure 1. The curves represent average frequencies from samples of specimens representing the same stage of maturity.

In stage I, only immature ova are present. The smallest ova measure 32 μ and the largest 272 μ, with a mode at 100 microns (figure 1A). Since ova less than 200 μ are invariably present in all stages of maturity, only those measuring above 200 μ are taken into consideration in the subsequent stages. In stage II, the first batch of developing ova gets separated from the general immature stock with a mode at 550 μ (figure 1B). The maximum size of the ova is 640 μ. The formation of a second batch of eggs is apparent at this stage, with a mode at 400 microns. In stage III, the maturing group of ova shows further increase in size. The largest ova measure 776 μ while larger groups show a mode at 700 μ. The second batch of maturing ova also shows evidence of growth and has taken up the mode at 600 microns. The tertiary batch of eggs also appear at this stage with a mode at 400 microns (figure 1C). In stage IV the mature group progresses in size, and the mode has been shifted to 800 microns. The largest ovum measured 875 microns. The maturing batches of ova show apparently no advancement in development (figure 1D). Thus the first group of eggs gets distinctly separated from the preceding batches.

3.3. *Minimum size at first maturity*

The percentage occurrence of males and females at different stages of maturity in various size groups is presented in tables 1 and 2 respectively.

Males with gonads in the maturing stage begin to appear from 35–39 mm length group upwards, higher stages of maturity appear from 45–49 mm length group. In females those up to a length of 45 mm includes only immature ones, and the higher stages of maturity are seen only when they reach more than 44 mm in length. Fish of 45–49 mm length group includes maturing forms too, in less numbers. Fully mature fish appear from 50–54 mm length group upwards. Fish of 55–59 and 60–64 mm length groups include 79% and 88% fully mature forms respectively whereas those of 65 mm and above are all in mature condition.

The percentage of mature (maturing, ripening and ripe) males and females at each length group are plotted in figures 2 and 3. It can be inferred from the maturity curve for male fish (figure 2) that none of the fish below 37 mm is found maturing. In the case of fish of 37 mm in length, 2% are mature, those of 42 mm 43%, those of 47 mm 77%, those of 52 and 57 mm 81·5% and 92% respectively are mature and practically all fish are mature when 62 mm long. The length at which 50% of the fish attain maturity is regarded as the length at first maturity (Kagwade 1968). Therefore, it is clear from figure 2 that the minimum size for the attainment of maturity for at least 50% of male *N. triangularis* is around 44·5 mm (TL). It may be seen from figure 3 that the percentage of mature fish below 52 mm was generally low when compared to fish above 52 mm indicating that a great majority of females reach sexual maturity at about 52 mm (TL).

It, therefore, appears that males mature at a length smaller than that of the females. Correspondingly the maximum size attained by males is also found to

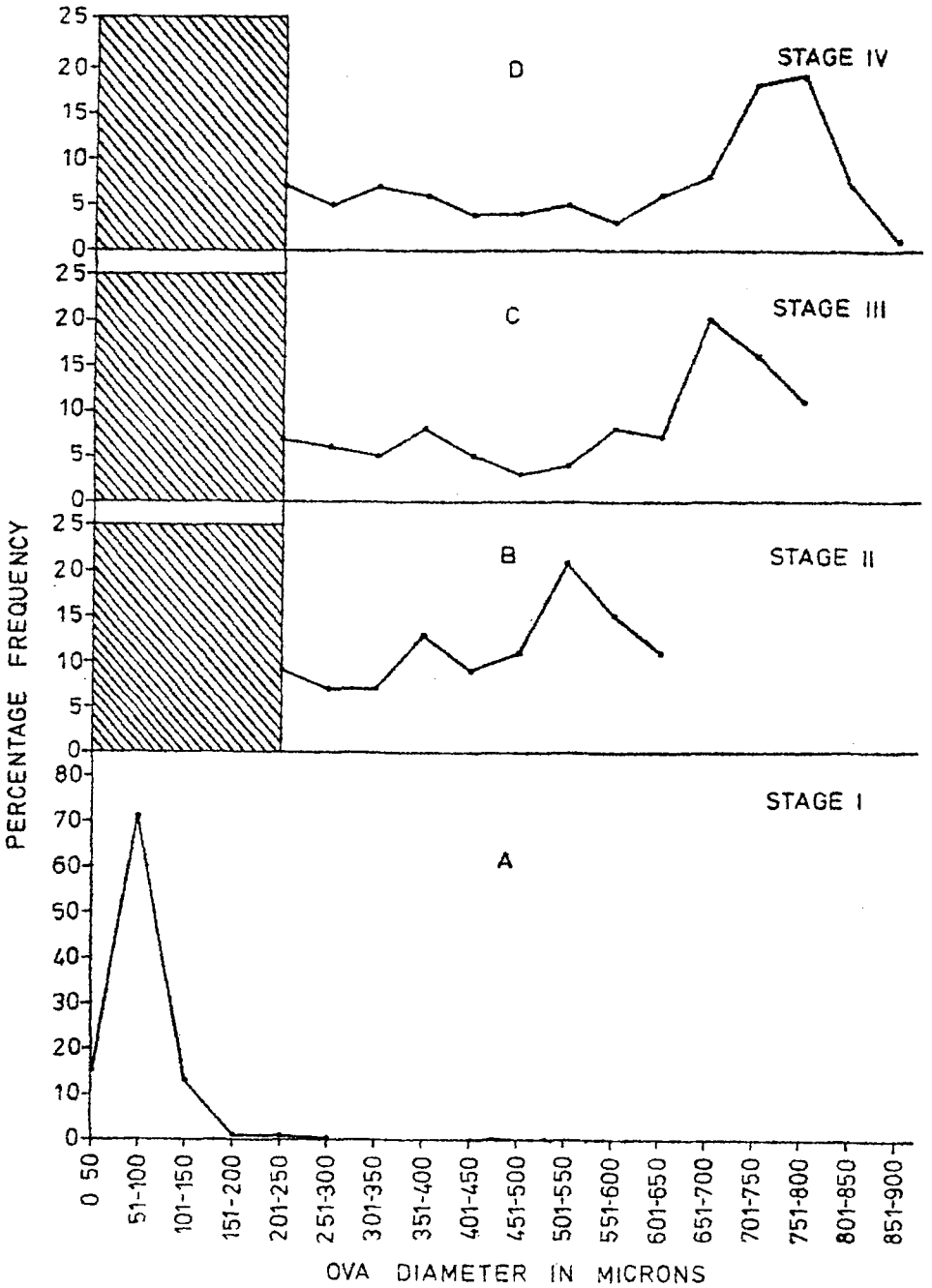

Figure 1. Ova diameter measurements from ovaries of different stages of maturity (Stages I to IV) of *N. triangularis*.

Table 1. Percentage occurrence of male *N. triangularis* in different stages of maturity in various size groups.

Total length in mm	No. of fish	Immature	Maturing	Ripening	Ripe
20–24	9	100	..	..	..
25–29	41	100	..	..	..
30–34	45	100	..	..	..
35–39	45	98	2	..	..
40–44	21	57	43	..	..
45–49	52	23	48	27	2
50–54	43	18·5	18·5	49	14
55–59	26	8	8	42	42
60–64	5	..	..	20	80
65–69	1	..	..	..	100
70–74	..	..	..	..	..
75–79	..	..	..	..	..
80–84	..	..	..	..	..

Table 2. Percentage occurrence of female *N. triangularis* in different stages of maturity in various size groups.

Total length in mm	No. of fish	Immature	Maturing	Ripening	Ripe
20–24	5	100	..	..	..
25–29	27	100	..	..	..
30–34	48	100	..	..	..
35–39	27	100	..	..	..
40–44	32	100	..	..	..
45–49	26	88	8	4	..
50–54	27	44	7·5	7·5	41
55–59	29	3·5	14	3·5	79
60–64	16	..	6	6	88
65–69	11	..	..	..	100
70–74	3	..	..	..	100
75–79	1	..	..	..	100
80–84	1	..	..	..	100

be less than that by females. The longest male measured during the present study was 69 mm whereas the longest female measured was 81 mm.

3.4. *Spawning season*

The monthly percentage occurrence of fish with gonads in the five stages of maturity was noted (females 50 mm or more and males 45 mm and above alone included) to delineate the spawning season (tables 3 and 4). From the data it would appear

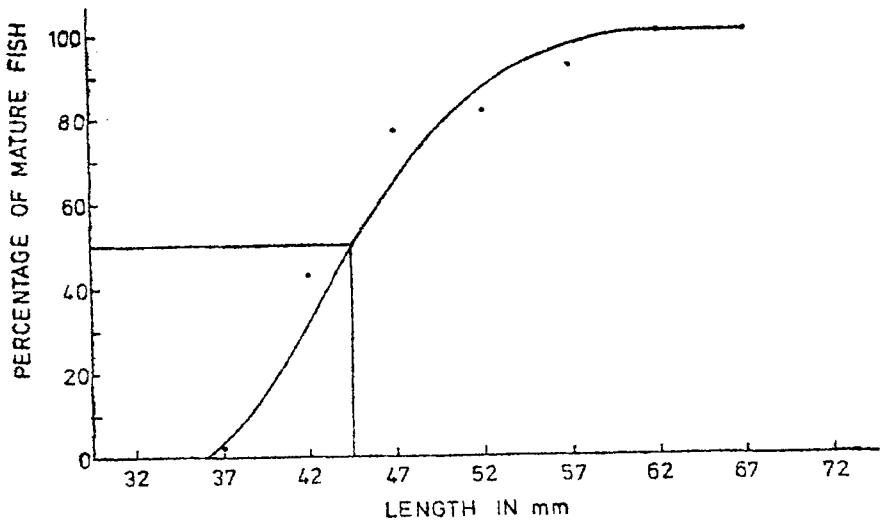

Figure 2. Percentage occurrence of mature males of *N. triangularis* at various length groups.

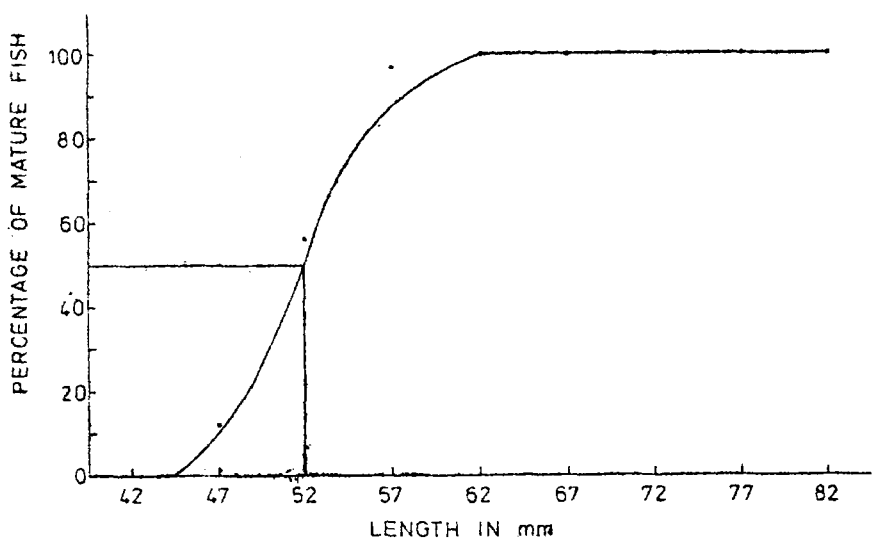

Figure 3. Percentage occurrence of mature females of *N. triangularis* at various length groups.

that fish with immature testes are seen only during certain months of the year whereas the other three stages are seen almost throughout the year. Thus it can be inferred that the testis is active almost throughout the year.

It would appear from the data presented in table 4 that fish with stage IV ovaries occur almost throughout the year (except in July and August) with peak in January, February, March, May and in September. The spent females occur in February,

Table 3. The monthly percentage occurrence of male *N. triangularis* with gonads in different stages of maturity [Males 45 mm and above only considered].

Months	No. of fish	Stages of maturity				
		I Immature	II Maturing	III Ripening	IV Ripe	V Spent
October	18	11	39	44·5	5·5	..
November	28	46·5	25	28·5	..	..
December	4	..	25	50	25	..
January	20	..	25	25	50	..
February	17	..	..	29·5	47	23·5
March	13	7·5	15·5	46·5	7·5	23
April	10	10	..	30	10	50
May	6	..	16·5	83·5	..	..
June	5	..	60	20	20	..
July	14	28·5	35·5	21·5	..	14·5
August	6	..	100	..	..	..
September	6	16·5	..	67·0	16·5	..

Table 4. The monthly percentage occurrence of female *N. triangularis* with gonads in different stages of maturity [Females 50 mm and above only considered].

Months	No. of fish	Stages of maturity				
		I Immature	II Maturing	III Ripening	IV Ripe	V Spent
October	4	25	50	..	25	..
November	7	..	43	..	57	..
December	4	25	..	..	75	..
January	11	9	9	..	82	..
February	13	8	..	..	77	15
March	23	9	..	4	87	..
April	9	22	..	11	11	56
May	7	..	..	14	86	..
June	11	18	..	18	55	9
July	7	43	14	..	..	43
August	5	80	20	..	..	..
September	4	..	..	20	80	..

April, June and July with peaks in April and July. Therefore the high incidence of ripe individuals in certain months and spent ones with residual eggs of previous spawning in subsequent months over a number of months indicate that individuals of this species may spawn in any of these months. Thus *N. triangularis* has a rather prolonged breeding season.

The gonado-somatic index—the gonad weight expressed as per cent of body weight—presented in figure 4, shows four peak periods represented by 4 modes of varying intensity in January, March, May–June and in September. The high average values reveal high gonadal activity. The testes seem to be active throughout the year especially from January to June and in September.

Spawning probably begins in February as is evidenced by a decrease in index value and by the presence of spent females in February. In March, again the index increases. Since this species did not apparently spawn as a unit, a large proportion of unspawned ripe females of February together with the spent ones by their maturation of oocytes contribute to this high value. The second wave of spawning apparently begins in late March and in early April since individuals in the spent condition and various stages of development are caught in April. A high percentage (56%) of the fish, caught during this month is spent. From the low index in April, the value steeply climbs to attain the peak for the year in May. Thereafter, the values show a downward trend with a steep fall in July. This shows that spawning again takes place during late May and apparently continues through June, to end in late June. Many spent individuals occur in early July. In these spent individuals, gonadal regression has been initiated and the resorption of yolky oocytes is in progress. From July, values show a slight rise through August and reach another peak in September. This peak in September may be formed as a result of the subsequent maturation of the ova left in the ovary of those fish which had spawned in the earlier phase of the spawning season.

3.5. *Spawning frequency*

Ova diameter frequency given in figure 5 indicates that there are three batches of eggs represented by modes a, b and c. The batch represented by mode 'a' is a

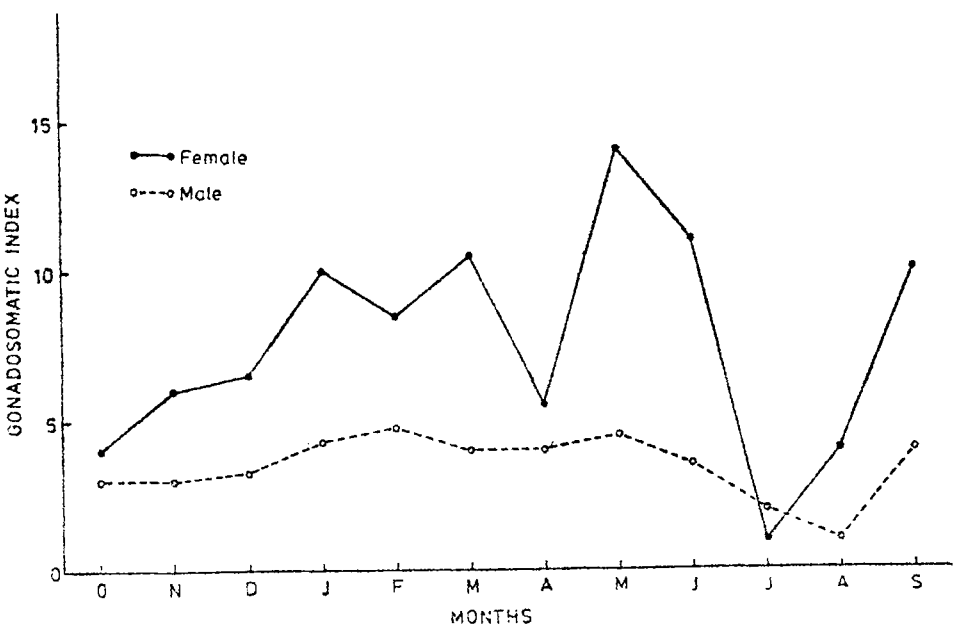

Figure 4. Monthly average gonado-somatic index of male and female *N. triangularis*.

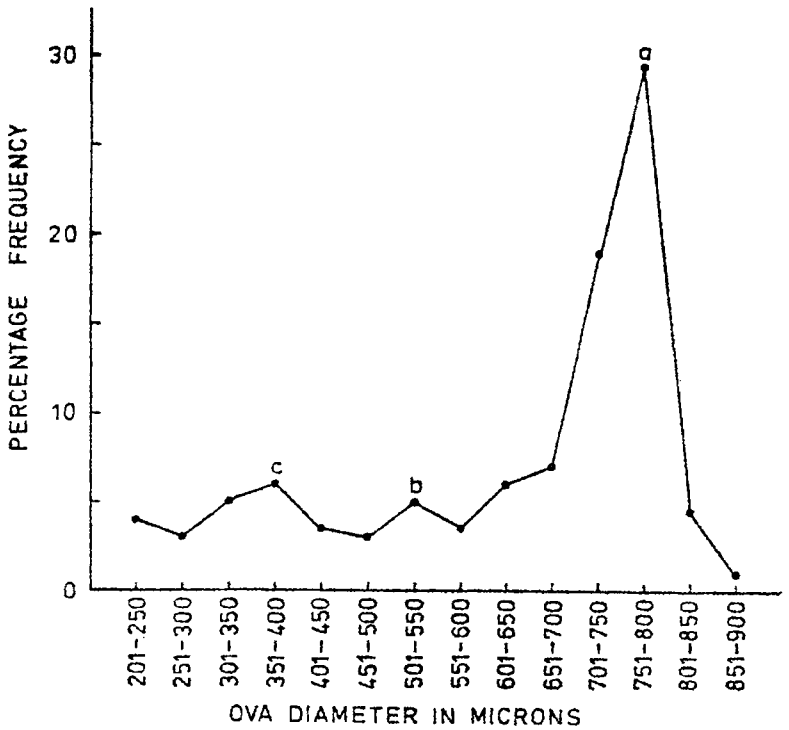

Figure 5. Ova-diameter measurements of 1000 ova from mature ovary of *N. triangularis*.

distinct group of mature ova, to be spawned first. The intermediate stages of ova represented by 'b' and 'c' are not distinctly separated from each other. As the mature group of eggs is eliminated in spawning, the batches represented in modes 'b' and 'c' undergo rapid growth and takes the position of modes 'a' and 'b' respectively. This shifting can be attained very soon since these batches of eggs have already undergone half the process of maturation and hence take only about half the time taken by an immature group to attain full maturity (de Jong 1939).

Clark (1934), Hickling and Rutenberg (1936), de Jong (1939) and Prabhu (1956) have stated that multiplicity of modes in the ova diameter of mature ovary of a fish is an indication that it spawns more than once in a year and the spawning season in the fish will always be a prolonged one. Since there is clearly more than one group of maturing oocytes in *N. triangularis*, it is reasonable to conclude that there are successive spawnings in the mature female and that the spawning season is prolonged.

The occurrence of large numbers of ova of intermediate size, between the small immature ova and the bigger yolky ones in mature fish itself, is an indication of a prolonged spawning period (Fulton 1899).

4. Sex ratio

The two sexes are present in more or less equal numbers as out of the total of 507 fish sexed, 269 are males and 238 females.

5. Fecundity

Fifteen specimens with stage IV ovaries were used for the study, the results of which are presented in figures 6–9.

The average number of ova in an ovary ranges from 800 to 2126. The average number of ova per gram body weight ranges from 5224/g body weight to 9442/g body weight.

The relation between fecundity (F) and total length of fish (L) can be expressed as:

$F = 4{\cdot}0568 \times 10^{-5} \times L^3 + 3{\cdot}7458$

coefficient of correlation $r = 0{\cdot}9690$.

The relationship between fecundity (F) and weight of fish (W) can be expressed as:

$\log F = 0{\cdot}7482 \log W - 0{\cdot}6244$

coefficient of correlation $r = 0{\cdot}8870$.

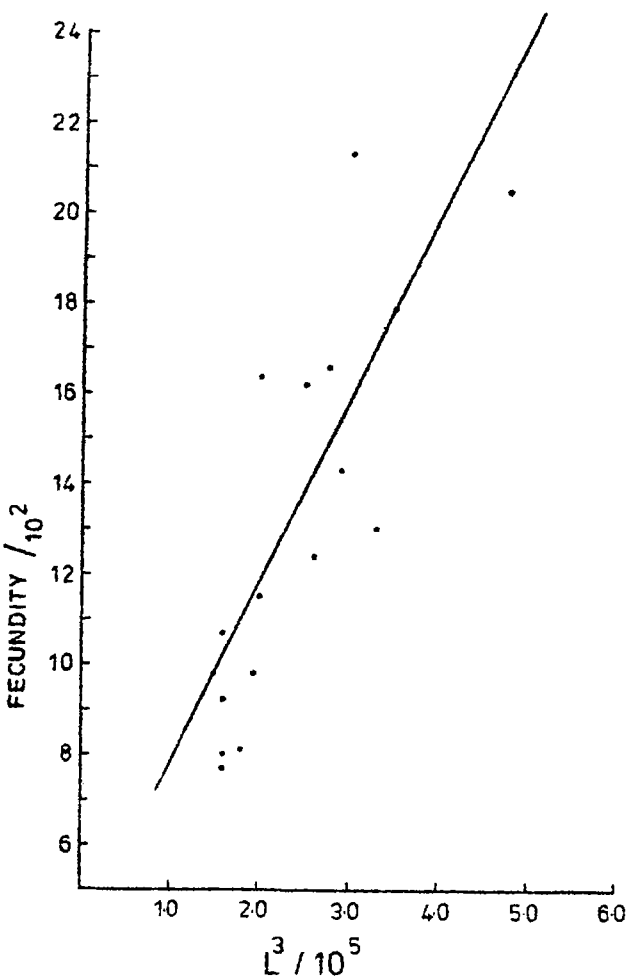

Figure 6. (*Caption in p.* 41)

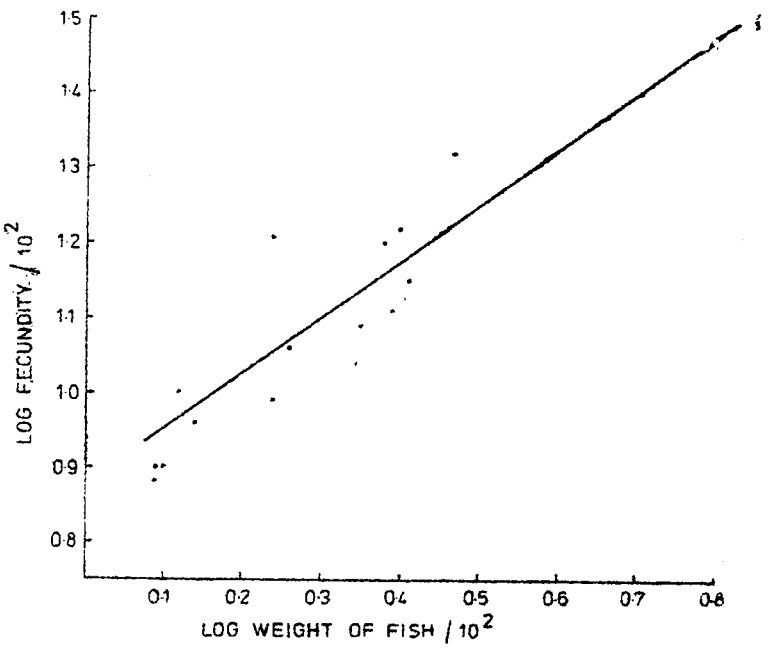

Figure 7. (*Caption in p.* 41)

The relationship between fecundity (F) and length of ovary (Lo) can be expressed as:

$\text{Log } F = 2{\cdot}5652 \text{ log } Lo - 2{\cdot}4500$

coefficient of correlation $r = 0{\cdot}8729$.

The relationship between fecundity (F) and weight of ovary (Wo) can be expressed as:

$\log F = 0{\cdot}6044 \text{ log } Wo - 0{\cdot}3897$

coefficient of correlation $r = 0{\cdot}9114$.

4. Discussion

The occurrence of stage IV individuals in large numbers in the months of January, March, May–June and September and the high values of the gonado-somatic index in these months coupled with the occurrence of spent specimens in the subsequent months suggest that spawning in *N. triangularis* takes place during these months. The presence of three distinct groups of ova in a fully mature ovary represents 3 distinct batches of eggs to be released, suggesting that the fish spawn at least three times successively during the prolonged spawning season. This leads to the inference that *N. triangularis* is an intermittent breeder with prolonged spawning, lasting for 6–8 months during the year. Thus *N. triangularis* belongs to the category ' D ' of Prabhu (1956) and Karekar and Bal (1960) or category II of Qasim and Qayyum (1961).

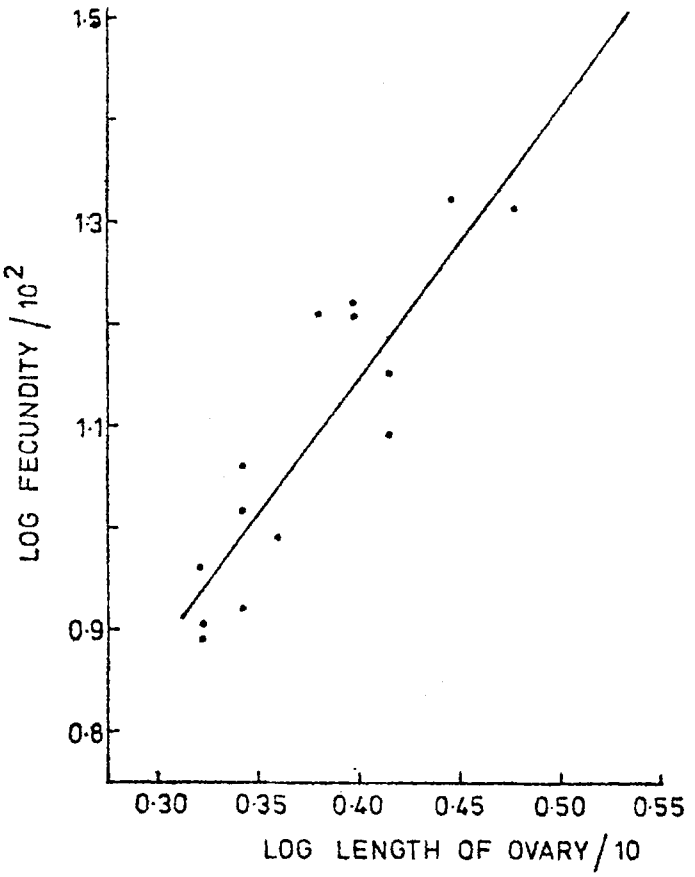

Figure 8. →

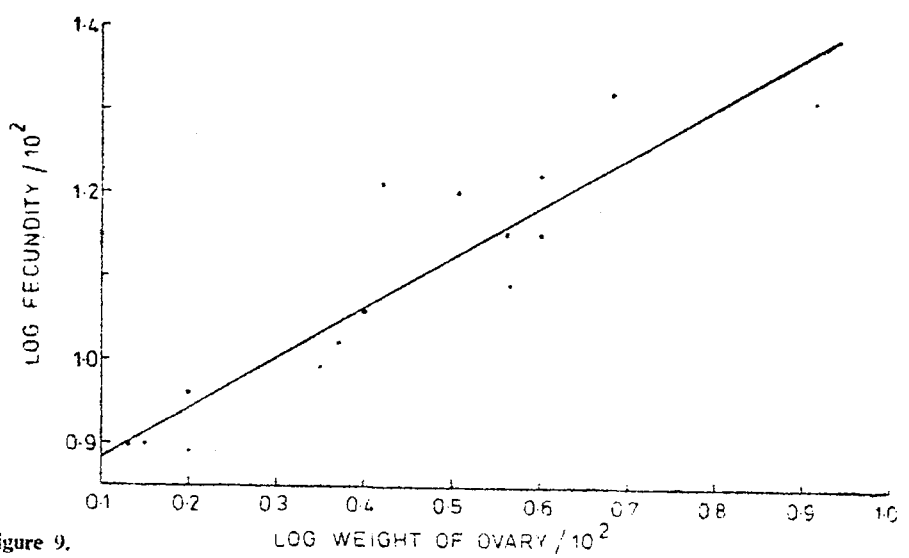

Figure 9.

Figures 6–9. Relationships of fecundity to length of fish, weight of fish, length of ovary, weight of ovary respectively in *N. triangularis*.

No reports are available on the number of ova produced in relation to length or weight of any of the cobitids. Hence there is no scope for comparison of the results. The results of the present study indicate that fecundity of *N. triangularis* is related to the length and weight of fish and length and weight of ovary. The coefficient of correlation between the fecundity and above mentioned characters are highly significant and all these show a linear relationship. The fecundity of the fish is therefore, dependent on the length and weight of fish and length and weight of ovary. Variations in the relation between fecundity and the above mentioned characters have been reported. Jhingran (1961) found a proportional increase in fecundity with increase in length and weight of the Gangetic anchovy *Setipinna phasa*. Mathur (1964) observed that egg production in *Hilsa ilisha* is somewhat more highly correlated with weight than with length of fish. The fecundity studies in *Herklotsichthys punctuatus* revealed a close relationship with weight of the ovary rather than with the length and weight of the fish (Marichamy 1971). The results of the present study conform to the general observation that the number of eggs increases with the size of the fish.

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