



Studies on some aspects of Reproductive biology of *Amblypharyngodon mola* (Hamilton-Buchanan, 1822)

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Abstract

Study of reproductive biology of any fish species is important to get information for successfully continuing its culture. *Amblypharyngodon mola* is a popular food fish as well as at present a good ornamental fish in India; but except the work of Ravi Shankar and Sarala (1986) no such detail work has been performed till date on reproductive biology of this fish species in the context of agro-climatic condition of India. So, the present work has been conducted to study some aspects of reproductive biology of *Amblypharyngodon mola*. Sex-ratio, length at first sexual maturity, cycle of gonadal maturation and spawning periodicity has been studied as part of this study. Monthly variation in sex ratio has been studied after counting the total number of two sexes in the monthly collected samples. Length at first sexual maturity has been determined following the length class in which at least 50% of the fish specimens have been observed to be matured. Cycle of gonadal maturation has been studied by macroscopic and microscopic observation of the different maturation stages of gonad. Spawning periodicity has been determined studying the monthly Gonado-Somatic-Index, Condition Factor and mean monthly ova-diameter. Frequency distribution of different size groups of intra ovarian oocytes has been studied on monthly basis to get the information on the type of oocyte development. The results of the study have revealed female dominance over male in the population but males have shown earlier maturation than females. The breeding season for this fish species has been observed to extend from April to December with two spawning months in June and November respectively. Fractional spawning behaviour along with asynchronous oocytes development has also been observed.

Key words: *Amblypharyngodon mola*, reproductive biology, sex ratio, length at first sexual maturity, spawning periodicity, cycle of gonadal maturation

Introduction

Amblypharyngodon mola, commonly known as Mola Carplet or Pale Carplet is a freshwater species; a natural inhabitant of ponds, streams, ditches, beels, baors, reservoirs and inundated fields. The species is distributed in India, Bangladesh, Pakistan and Myanmar¹. In West Bengal it is locally known as mourala, mowa or mowka. It is a popular food fish here in West Bengal due to its good taste as well as high nutrient value with presence of good amount of vitamin A, protein and mineral contents^{2,3}. In recent times, it has also got its entry in ornamental fish trade and has been reported to be available in ornamental fish markets with moderate demand and availability⁴.

Study on reproductive biology of any fish species is essential for assessing commercial potentialities of its stock, life history, culture practice and actual management of its fishery⁵. Reproductive potential of a population is one of the basic exigencies to designate the individuals of that population in respect to their gonadal conditions⁶. In order to make success in fish culture, it is important to assess the yearly breeding cycle of culturable fishes⁷. Spawning of fish occurs during a particular phase of the reproductive cycle; some of them breed once annually while others at regular intervals throughout the year. Knowledge of gonadal development and the spawning season of

a species allow subsequent studies on spawning frequency of its population, which is important for its management⁸. Study of sex-ratio, length at first sexual maturity, cycle of maturation and spawning periodicity etc. are important aspects of reproductive biology study of any fish species^{9,10}.

Earlier different aspects of reproductive biology of *Amblypharyngodon mola* have been carried out in Bangladesh^{3,11-15} but except the work of Ravi Shankar and Sarala¹⁶ no such detail work has till been performed on reproductive biology of this fish species here in India. So, the present work has been conducted to study some aspects of reproductive biology of *Amblypharyngodon mola*.

Material and Methods

Monthly samples of *Amblypharyngodon mola* have been collected from September, 2009 to August, 2010 from an undisturbed wetland near Baruipur, South-24-Paraganas, West Bengal (Latitude N 22°34', Longitude E 88°43'). In total, 753 specimens of *Amblypharyngodon mola* have been collected during the entire study period for studying the reproductive biology.

After collection, fish specimens have been transferred to ice box and morphometric study has then been performed after reaching

laboratory. Total Length (TL) in cm has been measured for each of the individual fish from tip of the mouth to the tip of the caudal fin using a measuring scale to the nearest of 0.1 cm and the Total Body Weight (TBW) in grams has been measured for each individual fish species to the nearest of 0.01 gm using an electronic balance (Sartorius, Model No. BT 223S). Before weighing, each of the specimens has been washed with water and left exposed to air and the excess of moisture has been dried off with the help of a blotting paper for taking accurate weight. Then fish specimens have been dissected out carefully ventrally; then gonads have been taken out cautiously and the surface moisture of the gonads has been removed using the blotting paper. The weight and length of the gonads have been measured to the nearest of 0.01 gm and 0.1 cm respectively.

In *Amblypharyngodon mola*, it is very difficult to distinguish sex externally except for a small duration of the breeding season by observing the swollen belly of the female fishes; so in the present study sexes have been confirmed after examination of the gonads. Monthly variation in sex ratio has been studied after counting the total number of two sexes in the monthly collected samples. Chi-square test has been performed to investigate the differences in sex-ratio (monthly value and over-all value) from the expected ratio of 1:1.

To determine the length at first sexual maturity, after measurement of the Total Length (TL), fish specimens have been grouped in different length classes with interval of 0.5 cm. The length class in which at least 50% of the fish specimens have been observed to be mature has been regarded as length at first sexual maturity^{17,18}.

Cycle of gonadal maturation has been studied by macroscopic and microscopic observation of the different maturation stages of gonad; male and female gonads have then been grouped into different gonadal stages of development according to Nikolsky¹⁹. Additional information for differentiation of gonadal maturity stages has been gathered following the work of Azadi and Mamun¹⁵.

Spawning periodicity has been determined by monthly evaluation of the Gonado Somatic Index (GSI), condition factor and mean monthly ova diameter.

GSI and Condition Factor (K) have been measured using the following formulae²⁰:

$$\text{Gonado Somatic Index (GSI)} = \frac{\text{Gonad Weight (gm)} \times 100}{\text{Total Body Weight (gm)}}$$

$$K = \frac{(\text{TBW} - \text{GW}) \times 100}{\text{TL}^3}$$

TBW= Total Body Weight; GW= Gonad Weight; TL= Total Length

Size frequency distribution of the intra-ovarian oocytes has been studied to determine the type of oocytes development and this has been studied following the methodology of LeCren²¹.

Results and Discussion

Sex-ratio: Among the 753 specimens studied, 567 and 186 have been observed to be female and male respectively. The average ratio of males to females has been observed to be 1:3.04. Overall females have shown significant (P<0.01) dominance over males; on monthly basis except in the month of January and February, females have shown the same dominancy trend over males (table-1).

Length at first sexual maturity: The smallest males with mature gonads have been observed in the 4.5-5 cm size group. 50% of all males have been observed to be mature in the 5-5.5 cm size group and all males above 6 cm have been observed with mature testes during the spawning season. Few females in 5-5.5 cm size group have been observed with mature gonads while 50% of the females have been found with mature gonads in the size group of 5.5-6 cm. All females above 6.5 cm have been observed with mature ovaries during the spawning season (figure-1).

Cycle of gonadal maturation: Five maturity stages of ovary have been recognized as follows:

Stage I (Immature): Ovaries translucent, colorless and thread like in appearance. Ova are not visible to naked eyes, but under microscope ova are irregular in shape, with a clear nucleus and transparent as yet yolk is not formed.

Stage II (Maturing): Ovaries yellowish white in colour and still thread like in appearance; ova are visible to naked eyes; under microscope ova are spherical in shape, partly opaque in appearance due to commencement of yolk development.

Stage III (Mature): Ovaries light yellowish in colour and enlarged in size; ova are clearly visible to naked eyes; under microscope spherical in shape and opaque in appearance except the transparent periphery.

Stage IV (Ripe): Ovaries deep yellow in colour; with maximum size. Under microscope, ova are spherical in shape and opaque due to huge amount of yolk present. In this stage, ova are with their full size and start to become liberated through oviducts on putting light pressure on the abdomen.

Stage V (Spent): Ovaries pale whitish in colour; almost thread like in appearance resembling the Stage I ovary; distinguishable only with the presence of the pigmented covering of the ovary. Under microscope, ova are visible, very small in size, irregular in shape.

Four maturity stages of testes have been recognized as follows:

Stage I (Immature): Testes small in size, very fine thread like in appearance; whitish in colour.

Stage II (Mature): Testes enlarged in size and weight; creamy white in colour.

Stage III (Ripe): Testes enlarged in size and weight; yellowish white in colour. Milt comes out on putting slight pressure on abdomen.

Stage IV (Spent): Testes reduced in size and weight, transparent in appearance.

Females with immature gonads (Stage I) have been observed from January to June; highest percentage being observed in January while lowest percentage in June. Stage II (maturing

gonad) females first have been observed in February and available till August; highest percentage being observed in April and lowest percentage in August. Stage III (mature gonad) females have been observed from March to October; highest percentage being observed in September and lowest percentage in March. Ripe females (Stage IV) have been observed from April to December with highest percentage being observed in November and lowest percentage in April. Spent females (Stage V) have been observed in November and December; highest percentage being observed in December and lowest percentage in November (figure-2).

Table-1
 Monthly variation of sex ratio in *Amblypharyngodon mola*

Month	No. of Fish	Male (Observed Value)		Female (Observed value)		Ratio of male and female	P	χ^2	Remark
		No.	%	No.	%				
Sept., 2009	56	13	23.21	43	76.79	1:3.31	0.000	16.07	S**
Oct., 2009	71	14	19.72	57	80.28	1:4.07	0.000	26.04	S**
Nov., 2009	79	11	13.92	68	86.08	1:6.18	0.000	41.13	S**
Dec., 2009	66	21	31.82	45	68.18	1:2.14	0.003	8.73	S**
Jan., 2010	44	19	43.18	25	56.82	1:1.31	0.366	0.82	NS
Feb., 2010	44	20	45.45	24	54.55	1:1.20	0.546	0.36	NS
Mar., 2010	57	21	36.84	36	63.16	1:1.71	0.047	3.95	S*
Apr., 2010	50	11	22.00	39	78.00	1:3.54	0.000	15.68	S**
May, 2010	67	12	17.91	55	82.09	1:4.58	0.000	25.09	S**
June, 2010	86	12	13.95	74	86.05	1:6.17	0.000	44.70	S**
July, 2010	71	16	22.54	55	77.46	1:3.44	0.000	21.42	S**
Aug., 2010	62	16	25.81	46	74.19	1:2.87	0.000	16.52	S**

P = Probability; χ^2 = Chi-square; NS = Non Significant; S** = Significant at 1% level; S* = Significant at 5% level

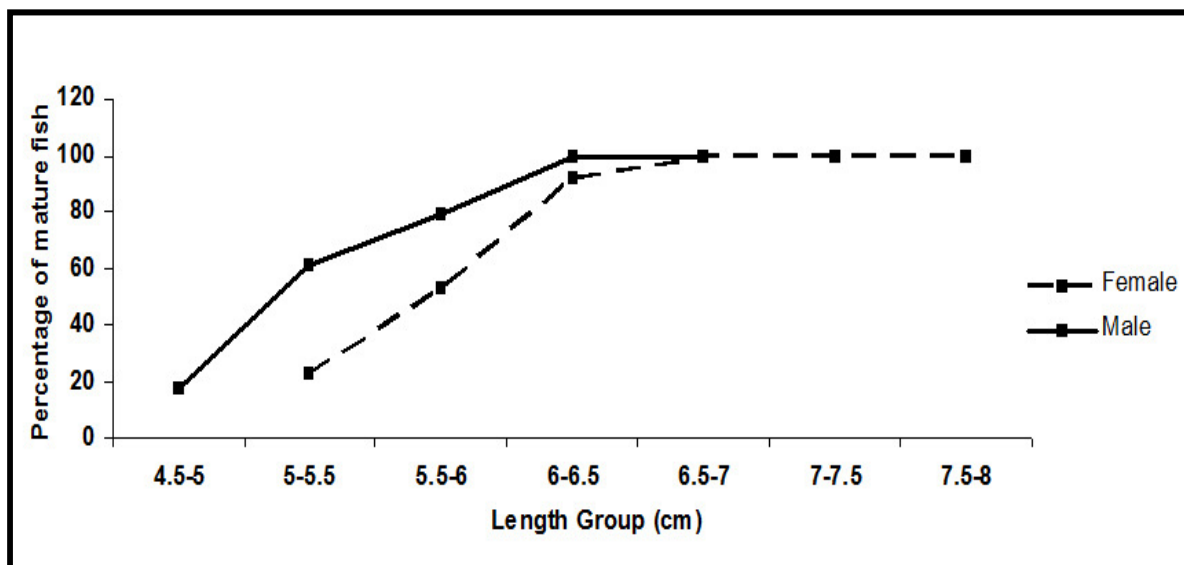


Figure-1
 Percentage of mature fish in different length groups in *Amblypharyngodon mola*

Males with immature gonads (Stage I) have been observed from January to July; highest percentage being observed in February and lowest percentage in July. Stage II (mature gonad) males first have been observed in March and available till October; highest percentage being observed in August and lowest percentage in October. Stage III (ripe gonad) males have been observed from April to December; highest percentage has been observed in November while lowest percentage in December. Spent males (Stage IV) have been observed from November to January; highest percentage being observed in December and lowest percentage in January (figure-3).

Gonado Somatic Index (GSI): In female, GSI has been observed to reach peaks twice a year, once during June and another time during November. Lowest value of GSI has been observed in the month of January; then it started to increase from February onwards and reached the first peak in June; then dropped down in July and maintained the trend till September; then again started to increase from October and reached the second peak in November; then decreased in December to reach the lowest value again in January. In male, GSI has been observed to show almost the same trend just like female fishes; reached two distinctive peaks once during June and another time during November. The lowest GSI value has been observed during February; then it started to increase from March onwards and reached the first peak in June; then started to decrease from July till August; then again started to increase from September onwards and reached the second peak in November; then again started to decrease from December to reach the lowest value again in February (figure-4).

In respect to both the sexes, GSI has shown significant ($P < 0.01$) positive relationship with Total Body Weight (TBW), Total

Length (TL), Gonad Weight (GW) and Gonad Length (GL) and the relationships are as follows:

$$\begin{aligned} \text{GSI} &= -1.19 + 2.92 \text{ TBW} \quad (r = 0.55, P < 0.01, \text{SE} = 4.80) \\ \text{GSI} &= -16.64 + 3.62 \text{ TL} \quad (r = 0.45, P < 0.01, \text{SE} = 5.15) \\ \text{GSI} &= 1.87 + 21.85 \text{ GW} \quad (r = 0.93, P < 0.01, \text{SE} = 2.11) \\ \text{GSI} &= -12.99 + 11.06 \text{ GL} \quad (r = 0.83, P < 0.01, \text{SE} = 3.22) \end{aligned}$$

In female, GSI also has shown significant ($P < 0.01$) positive relationship with Total Body Weight (TBW), Total Length (TL), Gonad Weight (GW) and Gonad Length (GL) and the relationships are as follows:

$$\begin{aligned} \text{GSI} &= 1.28 + 2.35 \text{ TBW} \quad (r = 0.46, P < 0.01, \text{SE} = 4.89) \\ \text{GSI} &= -9.31 + 2.66 \text{ TL} \quad (r = 0.33, P < 0.01, \text{SE} = 5.19) \\ \text{GSI} &= 2.46 + 20.70 \text{ GW} \quad (r = 0.92, P < 0.01, \text{SE} = 2.21) \\ \text{GSI} &= -13.55 + 11.21 \text{ GL} \quad (r = 0.79, P < 0.01, \text{SE} = 3.36) \end{aligned}$$

In male, GSI has shown significant ($P < 0.01$) positive relationship only with Gonad Weight (GW); no significant relationship has been observed with Total Body Weight (TBW), Total Length (TL) and Gonad Length (GL). The relationship between GSI and GW is as follow:

$$\text{GSI} = 0.48 + 30.05 \text{ GW} \quad (r = 0.71, P < 0.01, \text{SE} = 0.30)$$

Condition Factor: In both the sexes, condition factor has been observed to reach peaks twice a year, once during May and another time during October. Lowest value of condition factor has been observed during November; then from December it started to increase to reach the first peak in May; dropped down in June; then started to increase from July onwards to reach the second peak in October; then dropped down sharply to reach the lowest value again in November (figure-5).

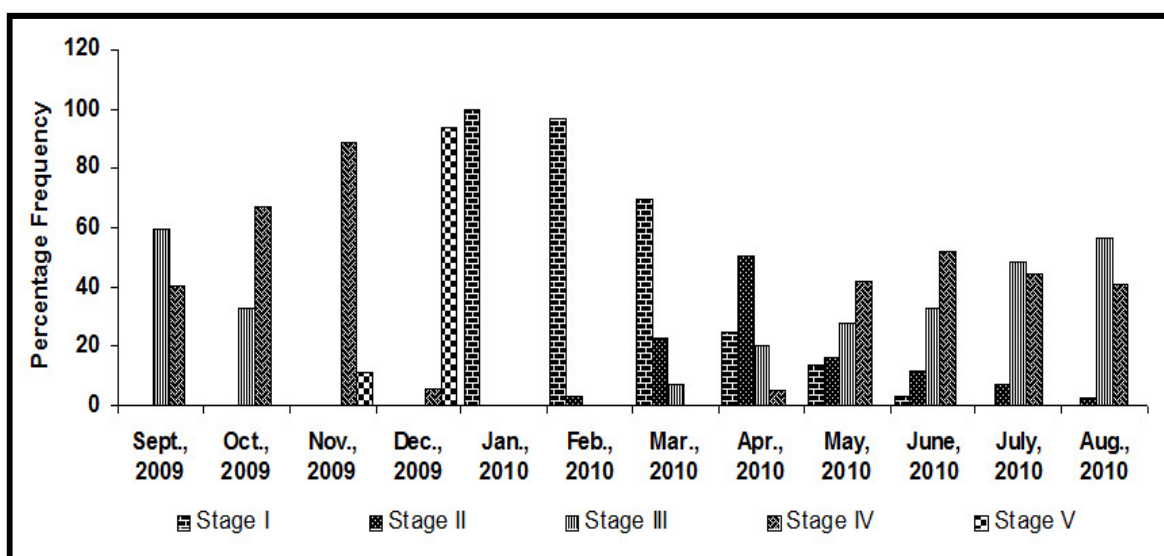


Figure-2
 Monthly percentage of different gonadal maturation stages in female *Amblypharyngodon mola*

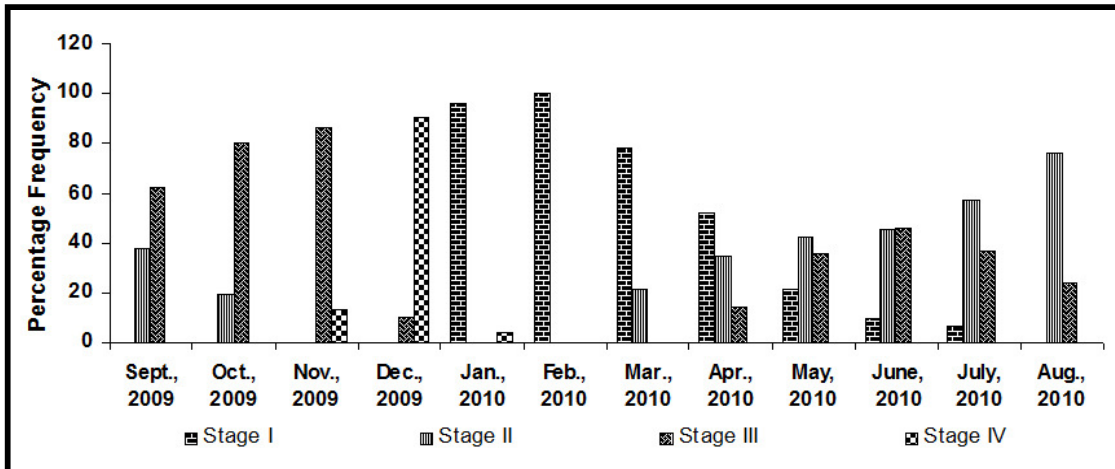


Figure-3
 Monthly percentage of different gonadal maturation stages in male *Amblypharyngodon mola*

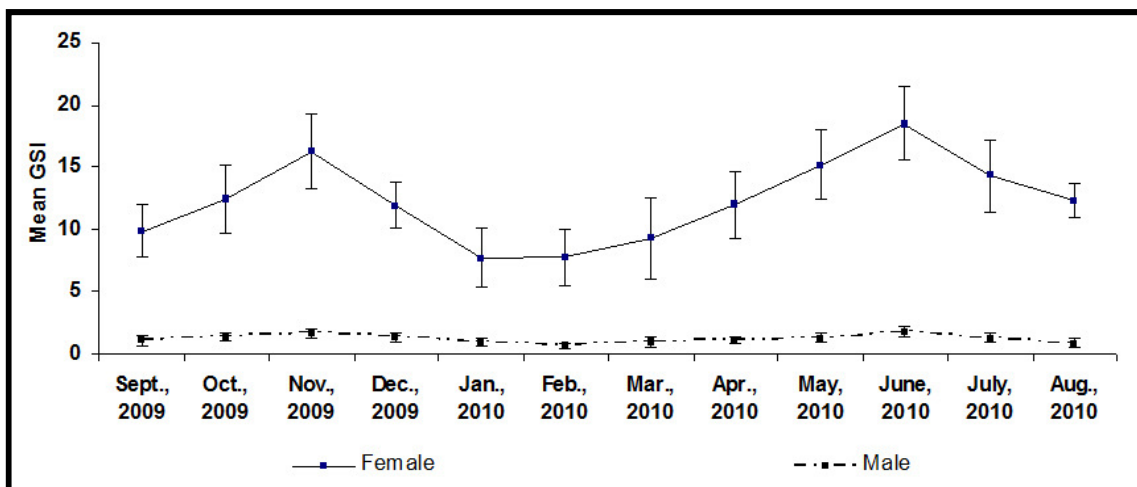


Figure-4
 Monthly variation of Gonado-Somatic-Index (GSI) of male and female *Amblypharyngodon mola*

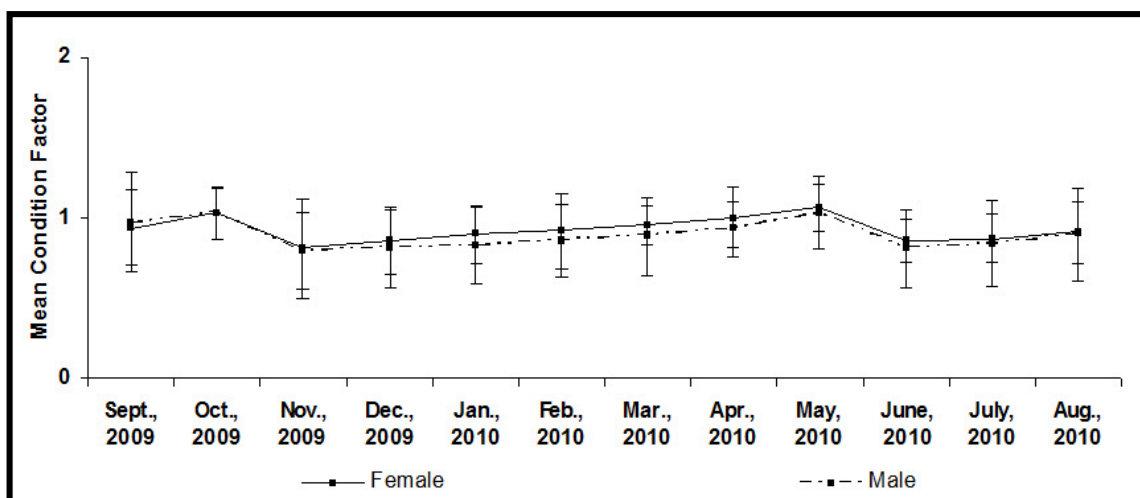


Figure-5
 Monthly variation of Condition Factor of male and female *Amblypharyngodon mola*

Size frequency distribution of intra-ovarian oocytes: Ova have been grouped in following four size classes: immature ova (0.15-0.30 mm), maturing ova (0.30-0.45 mm), mature ova (0.45-0.60 mm) and ripe ova (0.60-0.75 mm). The frequency of occurrence of ova of different diameter plotted against months have shown that immature ova (0.15-0.30 mm) have been observed from January to August and again in December; with highest percentage being observed in January and lowest percentage in August. Maturing ova (0.30-0.45 mm) have been observed from February to October except in the month of June; the highest percentage being observed in the month of April and lowest percentage in October. The mature ova (0.45-0.60 mm) have been observed from March to November; highest percentage being observed in September and lowest percentage in March. The ripe ova (0.60-0.75 mm) have been observed from April to December; highest percentage being observed in November and lowest percentage in April (figure-6).

The monthly average diameter of ova has been observed to show two peaks; once in June and another time in November. The lowest value has been observed in January; then it started to increase gradually from February onwards; made a sharp rise in April and then reached the first peak in June; then showed a fall in July till August and then started to increase from September

onwards to reach the second peak in November and then showed a sharp fall again in December to reach the lowest value again in January (figure-7).

Study of the monthly sex-ratio has depicted the female dominance over male in this population and the deviation of sex-ratio from the expected ratio of 1:1. Babu and Nair²² earlier reported female dominance in *Amblypharyngodon chakaiensis* and later other workers^{3,15,16} reported the same in *Amblypharyngodon mola*. Similar type of observation on the deviation of sex ratio from the expected value and female dominance over male in the population have been reported earlier by number of workers^{17,23-26} in other fish species which are also supporting the results of the present study. The reason behind the female dominance in this fish population is not clear; but may be it is a mechanism of population regulation as earlier described by Fagade *et al.*²⁷. On the other hand, higher metabolic strain of spawning in older males than in older females has been reported earlier^{28, 29}; thus this metabolic strain could has caused mortalities amongst males and has resulted in an excess of females specifically during the spawning period in this studied fish population.

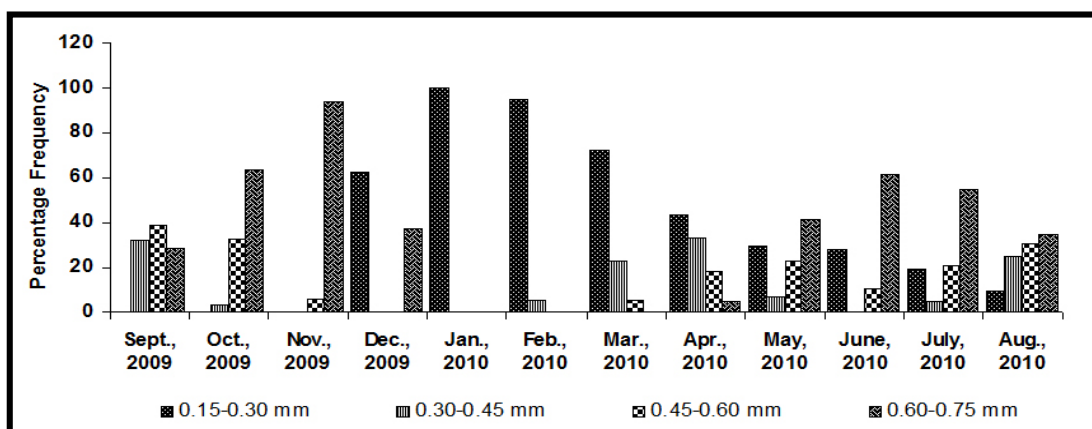


Figure-6

Monthly percentage frequency of four size groups of intra-ovarian ova in *Amblypharyngodon mola*

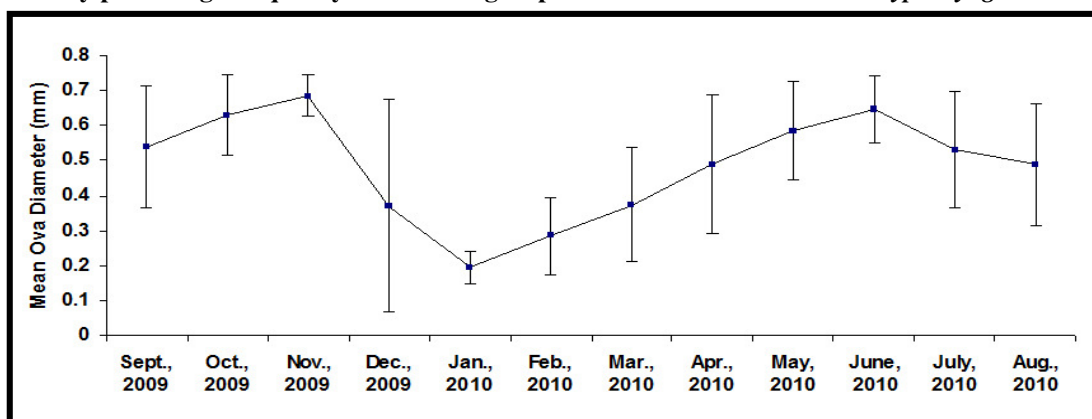


Figure-7

Monthly variation of mean intra-ovarian ova diameter in *Amblypharyngodon mola*

The result of the length at first sexual maturity study has revealed the early maturation of males over females. Early maturation of males in respect to females earlier has been reported in *Amblypharyngodon chakaiensis*²². Same trend of early maturation of males in respect to females have been reported earlier by many workers^{17, 26} in different fish species and thus supporting the observation in the present study.

Study of monthly GSI values has shown two peaks in a year; once in June and another time in November for both the sexes and high GSI values have been observed from April to December. The relationship between GSI and spawning periodicity has been stated earlier; GSI tends to increase with maturation of gonad (initiation of breeding season), becomes maximum during the period of peak maturity and declines abruptly thereafter, when the fish becomes spent after gamete extrusion and/or reabsorption^{19, 21, 24}. So, during the monthly study of GSI value in any fish species; the month(s) at which GSI value(s) reach at peak(s) depict the spawning period for that particular fish species and the months with high GSI values represent the breeding periodicity of that particular fish species. So, the two peaks of GSI value, once in June and another time in November thus depict that *Amblypharyngodon mola* spawns twice a year, once in June and then in November and high GSI values from April to December depicting this period as the breeding season for this fish species. Throughout maturation the GSI values of females have been observed to be much higher than males implying a greater proportion in body reserves has been allocated to the gonads³⁰. In respect to both sexes, GSI has shown highly significant ($P < 0.01$) positive relationship with Total Body Weight, Total Length, Gonad Weight and Gonad Length; but comparison of correlation coefficients of GSI-Total Body Weight ($r=0.55$), GSI-Total Length ($r=0.45$), GSI-Gonad Weight ($r=0.93$) and GSI-Gonad Length ($r=0.83$) have indicated that variation in GSI can be explained better in terms of Gonad Weight and Gonad Length than in terms of Total Body Weight and Total Length; in female GSI has shown the same type of relationship with Total Body Weight, Total Length, Gonad Weight and Gonad Length, and here also comparison of correlation coefficients of GSI-Total Body Weight ($r=0.46$), GSI-Total Length ($r=0.33$), GSI-Gonad Weight ($r=0.92$) and GSI-Gonad Length ($r=0.79$) have indicated that variation in GSI in female can be explained better in terms of Gonad Weight and Gonad Length than in terms of Total Body Weight and Total Length same as considering both the sexes; but in case of male, GSI has shown highly significant ($P < 0.01$) positive relationship only with Gonad Weight; so in male, variation in GSI can only be explained in terms of Gonad Weight.

Study on monthly condition factor values has shown two peaks; once in May and another time in October. The correlation between condition factor and gonad weight in fish earlier has been described; it used to increase with increasing gonad weight and reach maximum just before the spawning period and then sudden fall occur during the spawning month due to loss of gonadal products and after that again start to increase gradually^{31,32}. The two peaks of condition factor, once during

May and another one during October; and the sharp fall of the values in June and November respectively, representing June and November as the two spawning months for *Amblypharyngodon mola*. So, the condition factor values supporting the above conclusion made on spawning periodicity of *Amblypharyngodon mola* based on monthly fluctuation of GSI values.

The mean monthly ova diameter values have shown two distinct peaks, once in June and another time in November. The lowest value has been observed in January; then it started to increase gradually from February onwards; made a sharp rise in April and then reached the first peak in June; then showed a fall in July till August and then started to increase from September onwards to reach the second peak in November; then showed a sharp fall again in December to reach the lowest value in next January. Mean ova diameter value used to reach the maximum during the spawning month due to highest percentage of ripe ova available during this period in the gonad and drop down in the next month with release of ripe ova during spawning. So, the two peaks of mean monthly ova diameter values in June and November and sharp fall in July and December respectively depicting June and November as the two spawning months for this fish species. This changing trend of mean monthly ova-diameter values is in accordance to the frequency of occurrence of different size-classes of intra-ovarian oocytes; lowest value has been observed in January as during that period, only immature ova (0.15-0.30 mm) have been found in gonad; then from February onwards mean monthly ova diameter value has been observed to rise with the appearance of maturing ova (0.30-0.45 mm); in March mean monthly ova diameter value has been found to increase with increasing percentage of maturing ova and with appearance of mature ova (0.45-0.60 mm); in April the mean monthly ova diameter value has been found to rise sharply with the increasing percentage of maturing and mature ova and with appearance of ripe ova (0.60-0.75 mm); in May mean monthly ova diameter value again has been observed to make a sharp rise with increasing percentage of ripe ova and then has been found to reach the first peak in June with highest percentage of ripe ova. Then in July mean monthly ova diameter value has been observed to drop down following the release of ripe ova in the first spawning month of June; the decrement has been observed to continue till August with decreasing percentage of ripe ova; but during this time span maturing ova and mature ova percentages have been observed to rise; in September mean monthly ova diameter value has been found to increase with increased proportion of maturing and mature ova and in this month, no immature ova have been observed in the gonad; in October mean monthly ova diameter value has been found to increase sharply with increasing percentage of ripe ova and the value has been observed to reach the second peak in November with highest percentage of ripe ova in that month. In December mean monthly ova diameter value has been observed to make a sharp fall with release of ripe ova in the second spawning month of November. The study of the frequency of occurrence of different size classes of intra-

ovarian oocytes has depicted the batch-wise (asynchronous) development of oocytes in *Amblypharyngodon mola*. Oocytes have shown development in two batches; first from January to June. From January onwards immature oocytes have been observed to start their development into maturing oocytes, maturing to mature oocytes and mature to ripe oocytes and following these developmental changes, percentages of respective oocytes have been observed to change gradually and in June ripe ova percentage has been found to reach maximum; but in this month percentage of immature ova has not been found to decrease too much corresponding to the earlier month and maturing ova have been found to be completely absent in the gonad. The second batch of development of oocytes has been observed from July onwards with increasing percentage of maturing and mature ova with proportional decrement in percentage of immature ova; complete disappearance of immature ova from the gonad has been observed in September; the percentage of ripe ova also has been observed to increase from October which has been found to drop down in July and has been observed to reach the second peak in November. Development in the second batch has been observed to follow the same trend as that of the first batch. Ripe ova have been observed from April to December in the gonads and thus depicting April-December as the breeding season for *Amblypharyngodon mola*. The multiplicity of modes of ova diameter frequency in the mature ovary of this fish indicates that the fish spawns more than once in a year^{33, 34}; thus supporting the conclusion made earlier depending on monthly values of GSI, condition factor and mean monthly ova diameter. It appears that the bulk of the ova (first batch) have been spawned during June; the second batch of ova which have been observed to start their development from July onwards then have spawned in November. Absence of maturing and mature ova during December is concluding the completion of the spawning period⁸.

Conclusion

So, the present study on some aspects of reproductive biology of *Amblypharyngodon mola* has revealed the followings: i. Females dominate over males in the population. ii. Males mature earlier than females. iii. The breeding season extends from April to December with two spawning months in June and November. iv. It's a fractional spawner and oocytes development is asynchronous.

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