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Original Article

Studies on reproductive biology of *Mystus tengara* (Ham.-Buch., 1822), a freshwater catfish of West Bengal, India

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Abstract: Studies on reproductive biology are essential to assess culture potential of a fish species. *Mystus tengara* is a popular food fish as well as preferred as an ornamental fish in West Bengal. Till date detailed report on reproductive biology of this fish species in the agro-climatic context of West Bengal is lacking. Therefore, the present work was aimed to study the detailed reproductive biology of *Mystus tengara* with an emphasis on sex ratio, length at first sexual maturity, cycle of gonadal maturation and spawning periodicity using standard methods. Results of the study revealed female dominance of the species over male in the population. However, the males showed earlier maturation than females. Five gonadal maturity stages namely immature, maturing, mature, ripe and spent were identified both for female and male fishes. Monthly study of gonadosomatic index (GSI), condition factor and mean ova diameter revealed that the breeding season for this fish species extended from May to September with a single spawning month in July. Total spawning behaviour along with synchronous oocytes development was also observed in this fish species.

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Introduction

Mystus tengara is commonly known as Tengara Mystus which is a freshwater species, inhabits both flowing and standing waters. The species is distributed in India, Nepal, Bangladesh and Pakistan (Talwar and Jhingran, 1991). In West Bengal it is locally known as tengara and is a preferred food fish due to its good taste, high nutrient profile; and in recent times it has also got its importance as ornamental fish too (Gupta and Banerjee, 2012).

Studies on reproductive biology of any fish species are essential for assessing commercial potentialities of its stock, life history, culture practice and actual management of its fishery (Doha and Hye, 1970). Reproductive potential of a population is one of the basic exigencies to designate the individuals of that population in respect to their gonadal conditions (Jhingran and Verma, 1972). In order to make success in fish culture, it is important to assess the yearly breeding cycle of culturable fishes (Stoumboudi et al., 1993). Spawning of fish occurs during a particular phase of reproductive cycle; some of them breed once annually while others at regular intervals throughout the year. Knowledge of gonadal development and spawning season of a species allow subsequent studies on spawning frequency of its population, which is important for its management (Chakraborty et al., 2007). Study of sex-ratio, length at first sexual maturity, cycle of maturation and spawning periodicity etc. are essential part of reproductive biology investigation of fishes (Reddy, 1979; Vazzoler, 1996).

A number of workers (Qasim and Qayyum, 1961; Bhatt, 1971a, b; Rao and Sharma, 1984; Roy and

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Hossain, 2006; Musa and Bhuiyan, 2007) have studied different aspects of reproductive biology of different species of *Mystus*. Rastogi and Sexena (1968) and Guraya et al. (1975) have studied seasonal changes in morphology and activity of *Mystus tengara* ovary. However, till date there is no report on reproductive biology of *Mystus tengara* in the agro-climatic context of West Bengal. The objective of the present study was to study the reproductive biology of *Mystus tengara* collected from a selected wetland in West Bengal.

Materials and Methods

Monthly samples of *Mystus tengara* were collected from an undisturbed wetland Baruipur, South-24-Paraganas district of West Bengal (Latitude N 22°34', Longitude E 88°43') for a period of 12 months starting from September, 2008. In total, 721 specimens (never less than 50 in a month) were collected during the entire study period for evaluation of reproductive biology of the fish.

Sampled fish were brought to the laboratory, total length (to the nearest of 0.1 cm) was measured by a measuring scale, washed thoroughly with clean water, soaked by a blotting paper and total body weight was measured (to the nearest of 0.01gm) by an electronic balance (Sartorius, Model No. BT 223S).

Fish specimens were dissected out ventrally to remove gonads carefully. Surface moisture of gonads was removed using blotting paper and the weight and length of the gonads were measured to the nearest of 0.01 gm and 0.1 cm, respectively.

Sexes of the sampled fish specimens were determined after examination of the gonads. Monthly variation in sex ratio was determined from the total number of two sexes in monthly collected samples. Chi-square test (Zar, 1999) was performed to investigate the differences in sex-ratio (monthly value and over-all value) from the expected ratio of 1:1.

Fish specimens were grouped into different length classes with interval of 0.5 cm and the length class in which at least 50% of the fish specimens were observed to be mature was regarded as length at first sexual maturity (Rao and Sharma, 1984; Suresh et al., 2006; Mitra et al., 2007).

Cycle of maturation was studied by macroscopic and microscopic observation of the different maturation stages of gonad; male and female gonads were grouped into different gonadal stages of development according to Nikolsky (1963). Additional information for differentiation of gonadal maturity stages was gathered following the work of Bhatt (Bhatt, 1970, 1971b) who worked on two other species of *Mystus*.

Spawning periodicity was determined by monthly evaluation of the Gonadosomatic Index (GSI), condition factor and mean ova diameter. GSI and Condition Factor (K) were measured using the following formulae (Htun-Han, 1978):

$$\text{GSI} = \frac{\text{Gonad Weight (gm) x 100}}{\text{Total Body Weight (gm)}}$$

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

Where TBW is total body weight, GW is gonad weight and TL is total length.

Size frequency distribution of the intra-ovarian oocytes was studied on monthly basis to determine the type of oocytes development. For measuring the size frequency distribution of the intraovarian oocytes, a small representative part from the middle portion of the right or left ovary was taken out separately and put into physiological saline solution (0.85% NaCl) in a petridish. The ova present in the ovary samples were separated and spread on a glass slide to measure the diameter under a microscope fitted with micrometer following the method of LeCren (1951). The ocular micrometer reading was standardized with that of the stage micrometer for measurement of ova diameter in micrometer (μm) and then the values were transformed to mm unit. Ova were then grouped into four size classes; immature ova (0.10-0.30 mm), maturing ova (0.30-0.45 mm), mature ova (0.45-0.60 mm) and ripe ova (0.60-0.85 mm) depending on the maturity status of the ova and then monthly percentage frequency of

Table 1. Monthly variation of sex ratio in *Mystus tengara*.

		Male (Observed Value)		Female (Observed value)		Ratio of male and female	P	χ2	Remark
Month	**No. of Fish**	**No.**	**%**	**No.**	**%**				
Sept., 2008	52	24	46.15	28	53.85	1:1.17	0.579	0.31	NS
Oct., 2008	60	22	36.67	38	63.33	1:1.73	0.039	4.27	S*
Nov., 2008	62	22	35.48	40	64.52	1:1.82	0.022	5.23	S*
Dec., 2008	45	18	40.00	27	60.00	1:1.50	0.180	1.80	NS
Jan., 2009	56	22	39.29	34	60.71	1:1.54	0.109	2.57	NS
Feb., 2009	62	25	40.32	37	59.68	1:1.48	0.128	2.32	NS
Mar., 2009	75	31	41.33	44	58.67	1:1.42	0.133	2.25	NS
Apr., 2009	62	22	35.48	40	64.52	1:1.82	0.022	5.23	S*
May, 2009	66	22	33.33	44	66.67	1:2.00	0.007	7.33	S**
June, 2009	65	21	32.31	44	67.69	1:2.09	0.004	8.14	S**
July, 2009	62	19	30.65	43	69.35	1:2.26	0.002	9.29	S**
Aug., 2009	54	22	40.74	32	59.26	1:1.45	0.174	1.85	NS

P = Probability; $\chi 2$ = Chi-square; NS = Non Significant; S** = Significant at 1% level; S* = Significant at 5% level

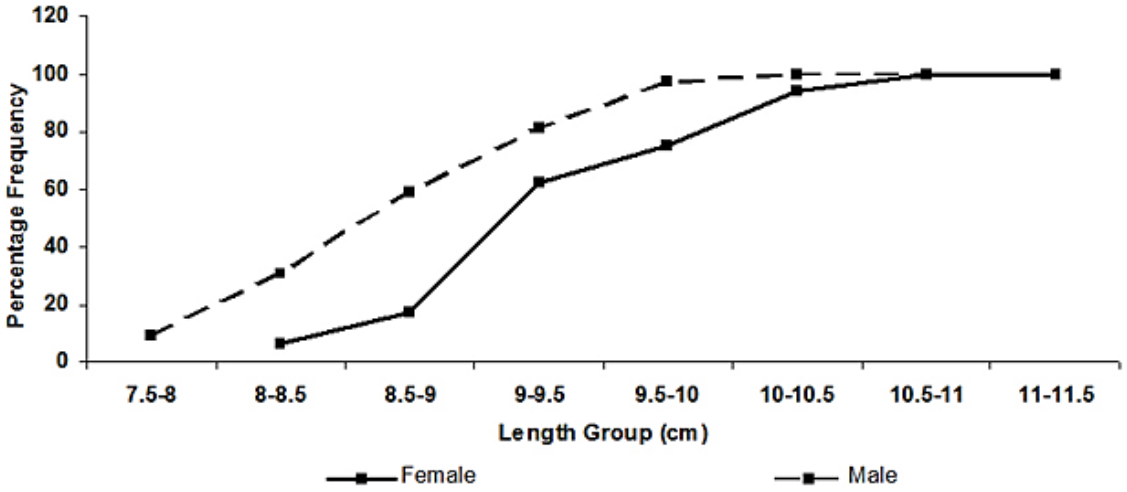

Figure 1. Percentage of mature fish in different length groups in *Mystus tengara*.

four size classes was calculated to get the information on the spawning type.

Results

Sex-ratio: Among the 721 specimens studied, 451 and 270 were observed to be female and male, respectively (Table 1). The average ratio of males to females was observed to be 1:1.67. Overall females showed significant (P<0.01) dominance over males; though on monthly basis only from May to July (P<0.01) and also in April, October and November (P<0.05) significant dominance of females over males was observed.

Length at first sexual maturity: The smallest males with mature gonads were observed to appear in 7.5-8 cm size group. 50% of all males were observed to be mature in the 8.5-9 cm size group and all males above 10 cm were observed with mature testes during the spawning season. Few females in 8-8.5 cm size group were observed with mature gonads while 50% of the females were found with mature gonads in the size group of 9-9.5 cm. All females

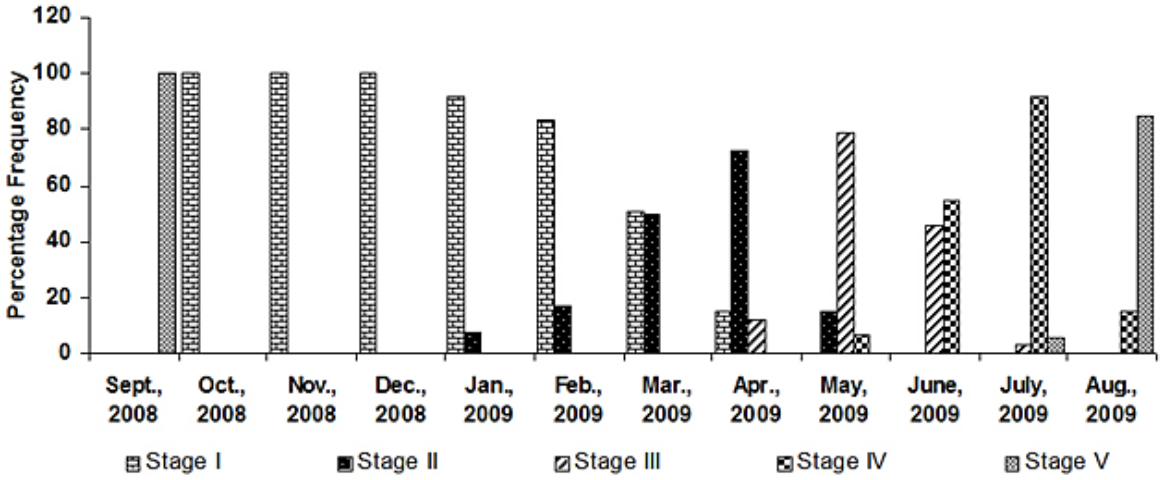

Figure 2. Monthly percentage of different gonadal maturation stages in female *Mystus tengara*.

above 10.5 cm were observed with mature ovaries during the spawning season (Fig. 1).

Cycle of gonadal maturation: Five stages of maturity of ovary and testes were recognized as follows:

Stage I (Immature): Ovaries translucent and colorless; ova not visible to naked eyes, but under microscope ova were irregular in shape, transparent, yolk not formed. Testes whitish in color, very narrow, thread like, no testicular lobules were visible.

Stage II (Maturing): Ovaries yellowish white in color; ova visible to naked eyes but not prominent; under microscope ova were spherical in shape, slightly opaque due to deposition of yolk at the central position. Testes milky white in color, thread like, appearance of little testicular lobules.

Stage III (Mature): Ovaries deep yellowish in color and enlarged in size; ova clearly visible to naked eyes; under microscope spherical in shape and completely opaque (except the periphery) in appearance due to presence of yolk. Testes light yellowish in color, testis lobes increased in size and length; increased number of testicular lobules.

Stage IV (Ripe): Ovaries reddish yellow in color; with maximum size; ova clearly visible to naked eyes; under microscope were spherical in shape and opaque due to presence of huge amount of yolk. In this stage, ova were with their full size, came out on putting light pressure on the abdomen. Testes yellowish white in color, testes lobes extended much and two lobes were nearly touching each other. Testicular lobules increased in number and length. Milt came out while putting slight pressure on abdomen.

Stage V (Spent): Ovaries very much reduced in size, shrunken and reddish in appearance. Under microscope, few ripe ova along with irregular shaped small translucent ova were visible. Testes translucent, thread like and flaccid in appearance; no testicular lobules were visible.

Females with immature gonads were observed from October to April; highest percentage being observed from October to December while lowest percentage was observed in April. Maturing females first were observed in January and available till May; highest and lowest percentage being observed in April and January, respectively. Mature females were observed from April to July; highest percentage was observed in May while lowest percentage in July. Ripe females were observed from May to August; highest percentage being observed in July and lowest percentage in May. Spent females were observed from July to September; highest percentage being observed in September and lowest percentage in July (Fig. 2).

Males with immature gonads were observed from October to May; highest percentage being observed in November and December while lowest percentage was observed during May. Maturing males first were observed in January and available till June; highest

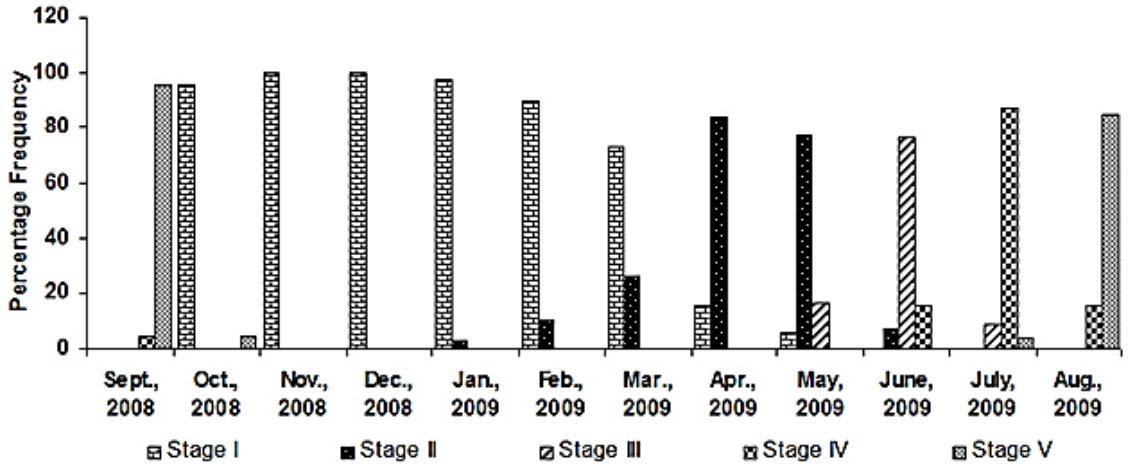

Figure 3. Monthly percentage of different gonadal maturation stages in male *Mystus tengara*.

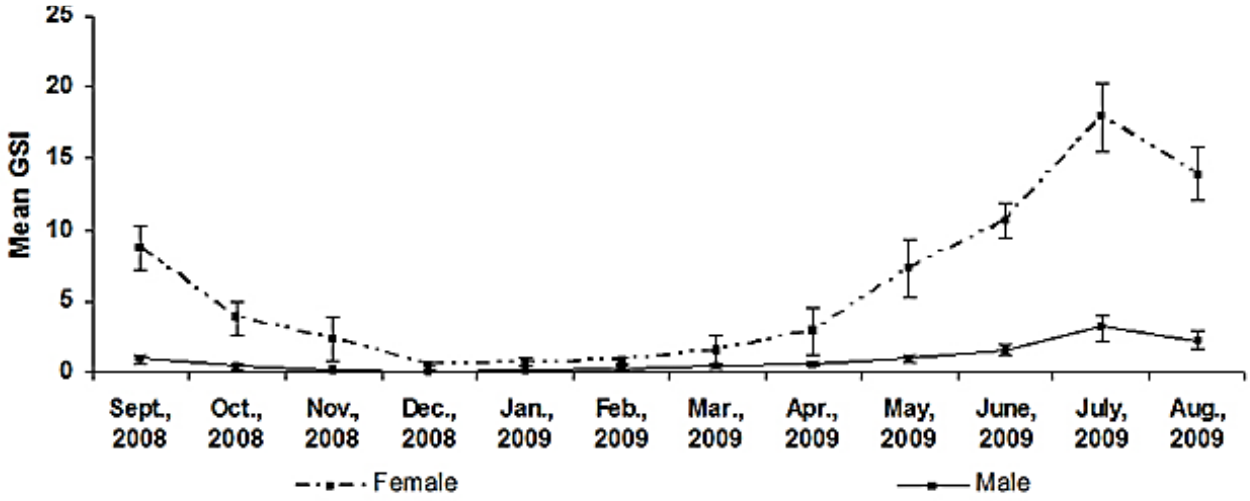

Figure 4. Monthly variation of Gonado-Somatic-Index (GSI) of male and female *Mystus tengara*.

percentage being observed in April and lowest percentage in January. Mature males were observed from May to July; highest percentage was observed in June while lowest percentage in July. Ripe males were observed from June to September with highest percentage being observed in July and lowest percentage in September. Spent males were observed from July to October; highest percentage being observed in September and lowest percentage in July (Fig. 3).

Gonado Somatic Index (GSI): In both female and male, GSI was observed to reach peak once a year during the month of July. The lowest value of GSI was observed in the month of December; then it started to increase from January onwards and reached the peak in July; then dropped down in August to reach the lowest value again in December (Fig. 4).

In respect to both sexes, GSI showed significant (P<0.01) positive relationship with Total Body Weight (TBW), Total Length (TL), Gonad Weight (GW) and Gonad Length (GL) as follows:

GSI = -3.38 + 0.73 TBW (r = 0.39, P<0.01, SE = 4.37)
GSI = -7.59 + 1.06 TL (r = 0.21, P<0.01, SE = 4.65)
GSI = 0.31 + 8.85 GW (r = 0.95, P<0.01, SE = 1.55)
GSI = -8.87 + 6.42 GL (r = 0.63, P<0.01, SE = 3.68)

Condition Factor: In both female and male, condition factor was observed to reach peak once a year during June. The lowest value of condition factor was observed in the month of December; then it started to increase from January onwards and reached the peak in June; then dropped down in July to reach the lowest value again in December (Fig. 5).

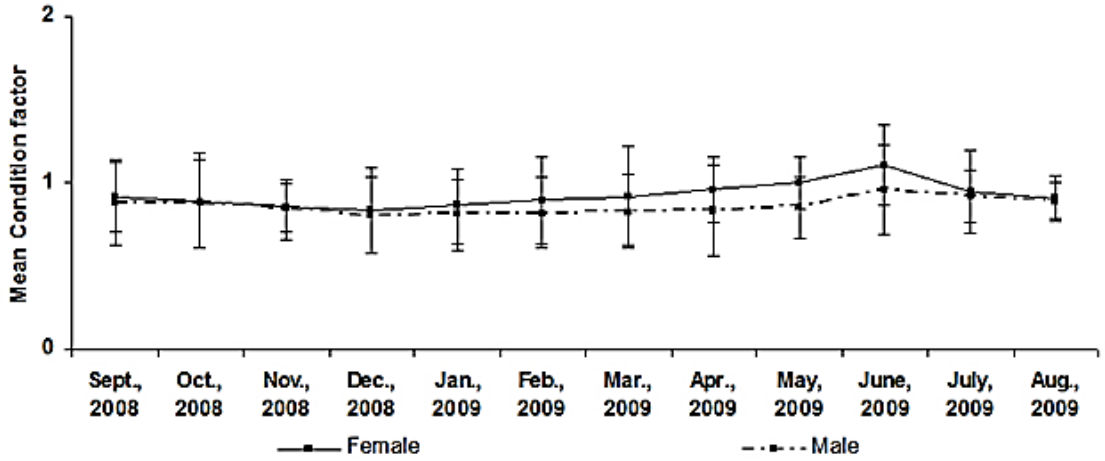

Figure 5. Monthly variation of Condition Factor of male and female *Mystus tengara*.

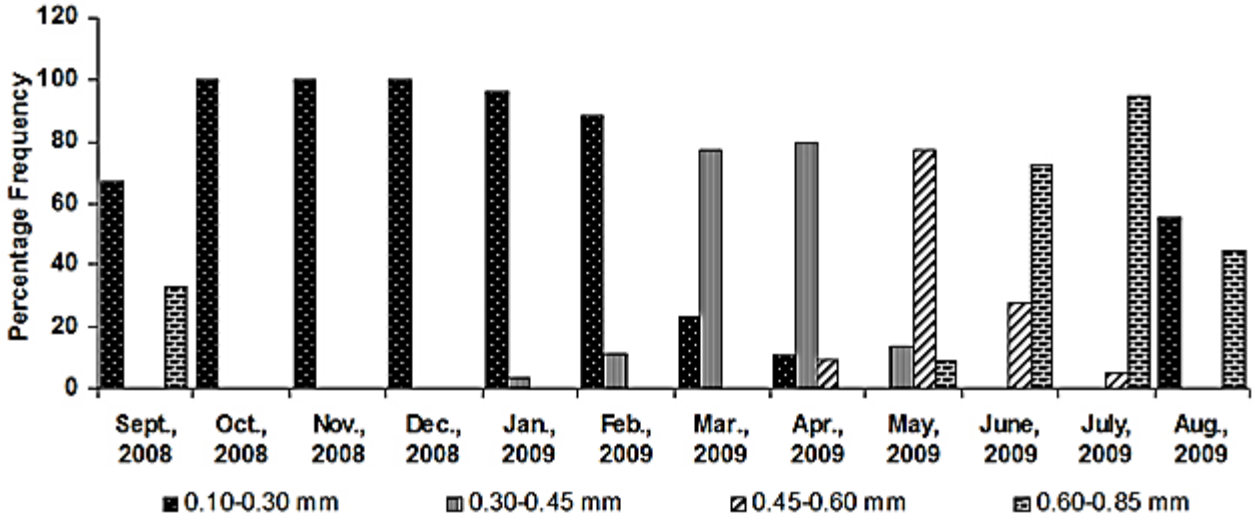

Figure 6. Monthly percentage frequency of four size groups of intra-ovarian ova in *Mystus tengara*.

Size frequency distribution of intra-ovarian oocytes: The frequency of occurrence of ova of different diameter plotted against months showed that immature ova were observed from August to April; the percentage occurrence of immature ova was observed to be highest from October to December and lowest percentage being observed in April. Maturing ova were observed from January to May; highest percentage being observed in April and lowest percentage in January. Mature ova were observed from April to July; highest percentage being observed in May and lowest percentage in July. Ripe ova were observed from May to September; highest percentage was observed in July while lowest percentage in May (Fig. 6).

The mean monthly ova diameter was observed to reach peak once in a year, in the month of July. The lowest value of mean monthly ova diameter was observed in October; then it started to increase gradually from November onwards to reach the peak in July; then dropped down in August to reach the lowest value again in October (Fig. 7).

Discussion

Monthly record of sex-ratio of *M. tengara* indicated a female dominance over male in the population and deviation from the expected ratio of 1:1. Bhatt (1971b), Rao and Sharma (1984), Roy and Hossain (2006), Musa and Bhuiyan (2007) etc. also reported female dominance over male in different species of *Mystus*. Similar observations on the deviation of sex ratio from the expected value and female dominance over male in the population of other fish species have been reported earlier by number of workers (Suresh

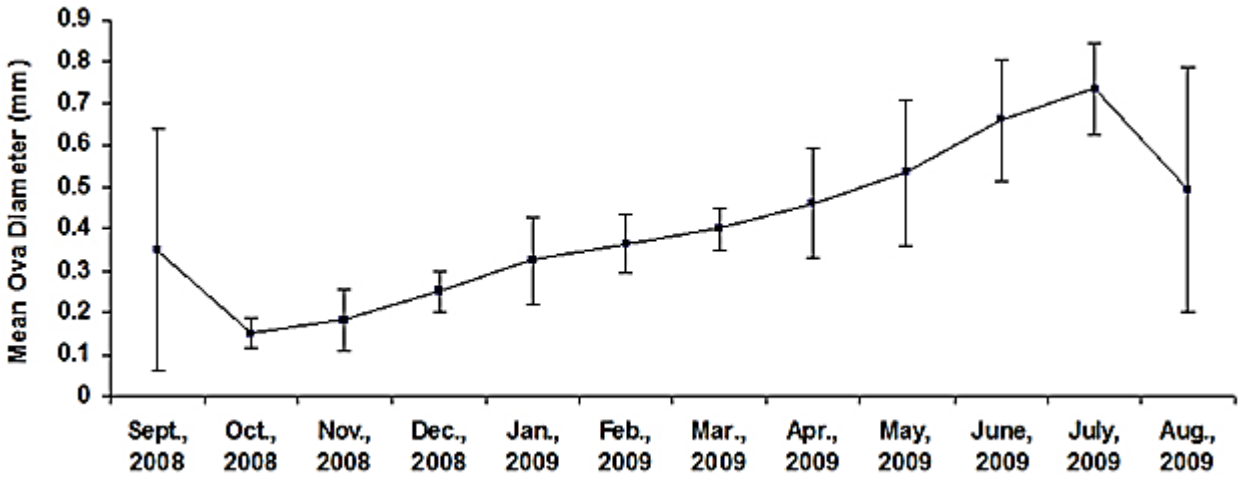

Figure 7. Monthly variation of mean intra-ovarian ova diameter in *Mystus tengara*.

et al., 2006; Mondal and Kaviraj, 2010; Olurin and Savage, 2011; Parvin et al., 2011; Banik et al., 2012) 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. (1984). On the other hand, some authors (Ursin, 1963; Cooper, 1983) concluded that the metabolic strain of spawning was generally greater in older males than in older females rendering more mortality amongst males than females specifically during the spawning period in this studied fish population. Early maturation of males over females also could account for greater strain in males. Bhatt (1971b) and Rao and Sharma (1984) also reported earlier maturation of males than females in different species of *Mystus*. Same trend of early maturation of males in respect to females have also been reported by many workers (Suresh et al., 2006; Banik et al., 2012; Rahman and Tachihara, 2005) for other fish species and thus supporting the observation of the present study.

Study of monthly GSI values showed only one peak in the month of July for both the sexes and high GSI values were observed from May to September. The relationship between GSI and spawning periodicity has been stated earlier; GSI tend to increase with maturation of gonad (initiation of breeding season), become maximum during the period of peak maturity and decline abruptly thereafter, when the fish becomes spent after gamete extrusion and/or reabsorption (Le Cren, 1951; Nikolsky, 1963; Olurin and Savage, 2011). Therefore, 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. Results of the present study indicate that *Mystus tengara* is a single-spawner with July as the spawning month and the breeding season expanding from May to September. Throughout maturation, the GSI values of females were observed to be much higher than males implying that a greater proportion in body reserves was allocated to the gonads in females (Chatzifotis, 2004). GSI showed 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=0.39), GSI-Total Length (r=0.21), GSI-Gonad Weight (r=0.95) and GSI-Gonad Length (r=0.63) have indicated that variation in GSI can be explained better in terms of Gonad Weight than in terms of Total Body Weight, Total Length and Gonad Length for this fish species.

Monthly condition factor (K) values showed only one peak during June and dropped down in July. The correlation between condition factor and gonad weight indicated that K value increased with

increasing gonad weight and reached maximum just before the spawning period and then dropped during the spawning month due to loss of gonadal products (Marammazei et al., 2000; Hernandez et al., 2003; Kiran and Puttaiah, 2003) confirming July as the spawning month for this fish species. Guraya et al. (1975) also reported July as its spawning month, though Rastogi and Saxena (1968) reported June as the spawning month. The small duration of breeding season is very common in *Mystus*; Qasim and Qayyum (1961) reported June-September as the breeding season for *Mystus vittatus*; while Bhatt (1971a, b) found *Mystus vittatus* and *Mystus cavasius* to breed during August-September in Aligarh.

Mean monthly ova also showed maximum value during July, the spawning month due to highest percentage of ripe ova available during this period in the gonad further confirming July as the spawning month for *Mystus tengara*. The changing trend of mean monthly ova-diameter corresponded with the frequency of occurrence of different size-classes of intra-ovarian oocytes depicting a synchronous development of oocytes in *Mystus tengara* (Qasim and Qayyum, 1961; Bhatt, 1971b; Rao and Sharma, 1984). Ripe ova were present from May to September in the gonads indicating this period as the breeding season for *Mystus tengara*. Absence of maturing and mature ova during August is an indication of the completion of the spawning period for this fish species (Chakraborty et al., 2007).

Till now, fishery of *Mystus tengara* is capture-based. It is now essential to start its captive culture. Since *Mystus tengara* is available with a sex ratio of almost 1:2 (male: female) two females for one male can be stocked for captive breeding of *Mystus tengara* to get success. The present study reveals that the right time to collect the brooders from nature is May-June for *Mystus tengara*. However, proper strategies to conserve the fish species in its natural habitat are required. In this respect length at first sexual maturity is of special interest in fisheries management and is widely used as an indicator of minimum-permissible capture size (Lucifora et al., 1999). The data of the present study have revealed that the minimum capture size for *Mystus tengara* in nature is 10-10.5 cm.

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