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Certain Aspects of Reproductive Biology of *Mystus dibrugarensis* From Dihing River, Assam

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ABSTRACT

Mystus dibrugarensis (Chaudhuri 1913) has high demand on market due to its good taste as well as ornamental value in indigenous market. According to IUCN status it has been categorized as least concern but it occurs in less amount. The present paper describes morphological changes in gonads during its entire reproductive cycle, gonado-somatic ratio (GSR) and maturity period for proper breeding and fisheries guidelines for rearing of this species. This species was observed as isochronal species as their breeding restricted from late April to July. Early maturation of male specimen was observed during the study period. Five stages of both testicular and ovarian cycle were explained namely immature, maturing, matured, ripe and spent. Highest gonado-somatic ratio was recorded in pre-monsoon (March-May) and that of lowest in post-monsoon (Sep-Nov) for both male and female specimen.

Keywords Gonad, GSI, Maturity, Ornamental.

INTRODUCTION

Fishes exhibits a particular seasonal phage of egg or milt production. The knowledge of maturity cycle and its functional mechanism provides necessary inputs on successful breeding programs of the species. The most suitable method of determining the reproductive cycle in fishes is to observe seasonal development changes in the gonads. *Mystus dibrugarensis* which is locally known as *singora* ; is found in the rivers of upper Brahmaputra basin, Assam. It is bottom dweller, mostly inhibiting the river (Das and Biswas 2008) but occasionally also found in the *beels*, especially during monsoon periods. In NE India it is preferred as food fish, moreover it also popular for its ornamental value. Individuals of 6–9 cm size have high ornamental value (Das and Biswas 2008). Reproductive biology of fish provides the basic information essential for evaluating the commercial potentialities of its stock, life history, culture practices and actual management (Lagler 1956, Doha and Hye 1970). Knowledge of yearly maturity cycle of culturable fishes make success in fish culture (Stoumboudi *et al.* 1993).

Many workers have worked on the different aspects of reproductive cycle of different *Mystus* species viz., *M. vittatus* (Rao and Sharma 1984, Hoque and Hossain 1993, Rao *et al.* 1999, Islam *et al.* 2011), *M. gulio* (Sarkar *et al.* 2002, Islam *et al.* 2008), *M. cavasius* (Roy and Hossain 2006), *M. bleekeri* (Musa and Bhuiyan 2007), *M. tengara* (Gupta and Banerjee 2013). But till date there is no report on maturity cycle of *M. dibrugarensis*. Considering the importance of this species and in view of the paucity of knowledge, the present work was aimed to study the maturity cycle of this selected species.

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MATERIALS AND METHODS

Collection of sample : Maximum number of specimens were caught at Nitaimukhghat (station 1) and Dihingmukh (station 2) of Dihing River of Assam between July 2014 and August 2016. The total length (TL) of the fishes were taken from the tip of the snout to the extended tip of the caudal fin by using slide caliper and body weight (BW) of each fish specimen was taken to the nearest of 0.1 cm and 0.01 g, respectively using a electronic balance after blot-drying excess water from the body. Sex was identified on the basis of presence or absence of genital papillae. Fishes were dissected to remove the gonads and placed in blotting paper and some were stored in 5% formalin. After dissection, the weight of gonads were recorded to nearest 0.01 g.

Sexual dimorphism and sex-ratio : Sex differentiation of *M. dibrugarensis* was done by careful visual inspection of the external genital character. Later dissection was also done to confirm the visual inspection.

Sex-ratio was expressed as the proportion of males to the total number of the fish sampled. It was done by separating the two sexes into two groups by following Mahmood *et al*. (2011). Chi-square test (χ^2) was done to test the ratio difference was significant or not, assuming that the ratio of male to female in the population to be 1 : 1.

Maturity cycle : Routine assessment of gonadal development was normally done by assessing individuals to stages by characters which can be differentiated with the naked eye. Depending on the morphology of gonads and portion of abdominal cavity occupied by gonads, both testicular and ovarian cycle of male and female respectively were done by following Nikolsky (1963). Gonad weight gives an easily measured quantitative record of changes in gonad condition.

Gonado Somatic Ratio (*GSR*) : For estimation of GSR fresh specimen were weighed and the gonads were removed and weighed separately. GSR was calculated sex-wise in relation with the different and maturity stages. Routine assessment of gonad mass as proportion of the total body weight was done on monthly basis by following Biswas (2002).

Occurrence of fish at their different maturity stages : Study of various maturity stages was done on the basis of morphology of gonads, gonad size, space occupied by the gonads and classified into 5 stages (Nikolsky 1963). In each month numbers of specimen were enlisted in the different maturity stage column. After grouping in each month, their percentage of occurrence was calculated (Agarwal 1996).

Ova-diameter and fecundity : Ovaries were dissected out from the specimen and kept in 8% formalin. Sub-samples were taken randomly from anterior, middle and posterior part of both the ovaries and mixed randomly and to ova-diameter measurement with the help of stereo microscope and Leica software.

Absolute fecundity and relative fecundity was studied by following Bagenal (1957) and Hardisty (1964) respectively.

RESULTS

Sexual dimorphism and sex ratio : Sex differentiation in *M. dibrugarensis* is relatively simple. Male species have a conical projection termed as genital papilla in the ventral side of the body in front of the anal fin. It was a soft, elongated structure, broad at the base and gradually tapering towards the end and this structure hang freely from the body. The tip of the papilla is beyond the base of the first anal fin. In female this genital papilla was absent whereas they possess a round genital opening and swelling abdomen when they were matured.

The sex ratio (M : F) taken from monthly sample showed a wide variation from 0.36 : 1 to 1.75:1. Overall sex-ratio was 0.932:1, significantly tilted towards female ($\chi^2 = 5.87$, at p 0.01). Monthly sampled data indicated that distribution of sexes fluctuated significantly in favor of female in April, May, June, July ($\chi^2 = 5.23$, 4.59, 4.33, 4.26 at p 0.05) while in February, it showed the preponderance of

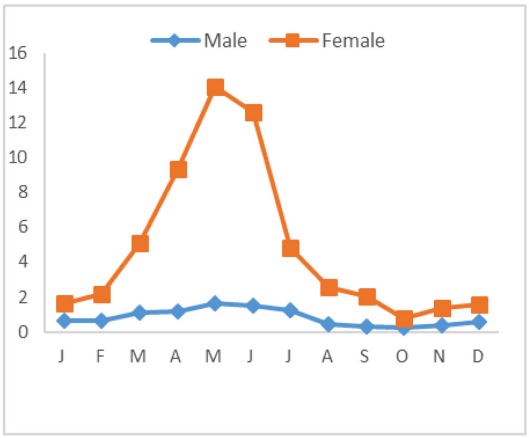

Fig. 1. Monthly variations of GSR.

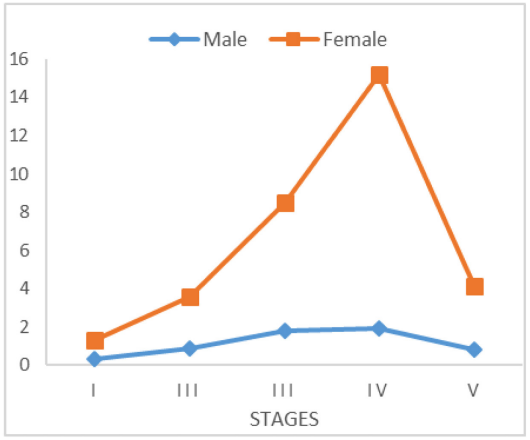

Fig. 2. Variations of GSR in different maturity stage.

male (χ^2= 7.22 at p 0.01). In the remaining months they follow almost 1:1 sex ratio (Table 1).

Morphology of gonads : Depending on the morphology of testis and ovary, portion of abdominal cavity occupied by gonads, at different developmental period and size of the intra-ovarian oocytes, five maturity stages of gonad have been identified for *M. dibrugarensis.* The gonads in immature stage (Stage I) is highly rudimentary and pinkish in color. In maturing stage, gonads occupied about half of the body cavity and blood vessels started to develop. Gonads occupied 3/4th of the body cavity with well-developed blood vessels in mature stage. As the maturity progressed, the gonads became thicker in size, changed to orange/reddish in color due to development of blood vessels and ultimately it occupied the entire ventral cavity of the fish. Gonads found highly reduced and shrank in spent stage (Stage V). Details of testicular and ovarian cycle were summarized in Table 2.

Occurrence of specimen during different stages: Females with immature gonads (Stage I) was encountered from August to February, mostly in November. Stage II (maturing females) first have been observed in October to April ; highest being observed in February. Stage III (mature females) first appeared in October but was available till April, highest being

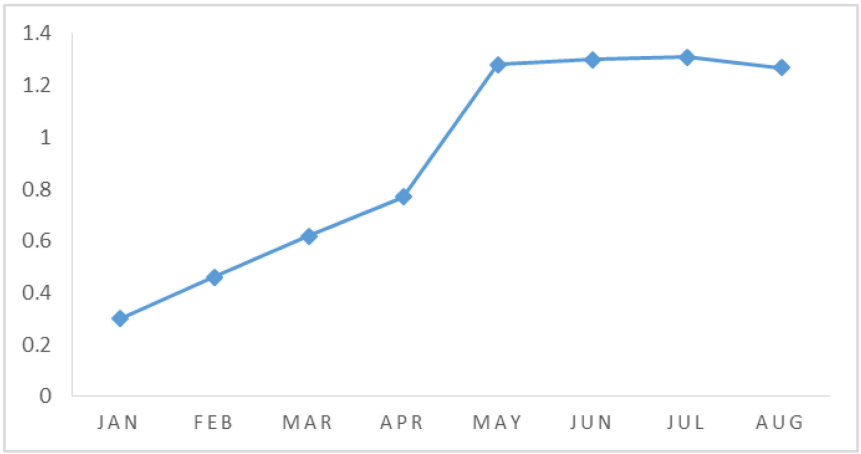

Fig. 3. Progression of ova diameter in different month.

Fig.4. Test species.

Fig.5. Ovary (immature stage).

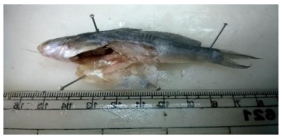

Fig.6. Ovary (maturing stage).

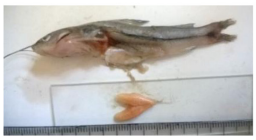

Fig.7. Ovary (matured stage).

Fig.8. Ovary (ripe stage).

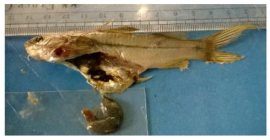

Fig.9. Ovary (spent stage).

Fig.10. Testes (maturing stage).

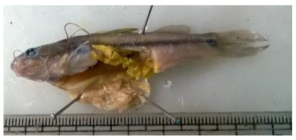

Fig.11. Testes (matured stage).

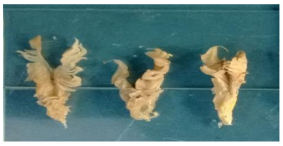

Fig.12. Testes (ripe stage).

Fig. 4. Test species. **Fig. 5.** Ovary (immature stage). **Fig. 6.** Ovary (maturing stage). **Fig. 7.** Ovary (matured stage). **Fig. 8.** Ovary (ripe stage). **Fig. 9.** Ovary (spent stage). **Fig. 10.** Testes (maturing stage). **Fig. 11.** Testes (matured stage). **Fig. 12.** Testes (ripe stage)

observed in March and lowest in June. Ripe females (Stage IV) were found from April to August with a peak in June. Spent female (Stage V) were recorded from June to October, highest being observed during August and lowest in June.

On the other hand males with immature gonads (Stage I) have been observed from September to February, the peak being in October. Maturing males (Stage II) were first encountered in November and were available till April ; highest in February. Stage III (mature males) were observed from January to June ; highest being observed in March and that of lowest in June. Ripe males (Stage IV) were observed from March to August with the highest percentage encountered in May and lowest in March. Spent male (Stage V) were available from May and lasted till October, but mostly in September. Development of finger like projection (testicular lobule) was observed in testes from maturing stage which was not seen in immature stage. These projections were also seen to be increased in size and volume. Early maturation of male in respect to females was observed in this species. It has also been reported by

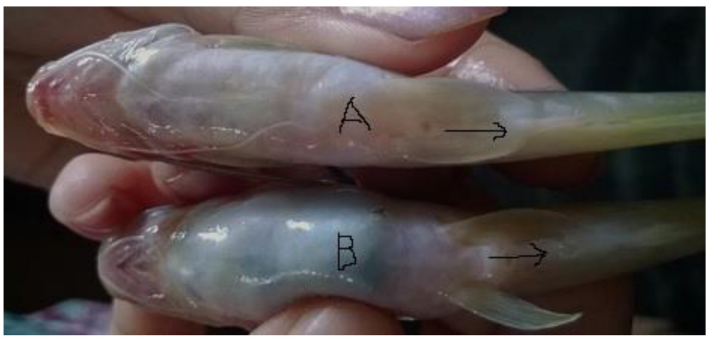

Fig. 13. Sexual dimorphism (A male & B female).

Table 1. Morphological description of gonad at different stages of maturity.

Stages	Male	Female
Stage I (Immature)	Testes hair like, flat, colorless or grey ; closed to vertebral column. It occupies 1/4 of ventral cavity	(Figs. 4, 5). Ovary opaque and pinkish in color. It occupies 1/4 of ventral cavity
Stage II (Maturing)	At this stage (Fig.10) testes 'V' shaped, whitish structure and have finger like projections (testicular lobule). It occupies ½ of ventral cavity	Maturing ovary (Fig.6) ½ of ventral cavity; blood capillaries developed; few oocytes can be seen with magnifying glass
Stage III (Mature)	Testes becomes creamy, occupies about ¾ of ventral cavity (Fig.11). Testicular lobules increased but sperm cannot be extruded	Ovary becomes transparent, vascularized and deep yellowish; eggs opaque and visible without any aid; occupies ¾ of the ventral cavity (Fig.7)
Stage IV (Ripe)	Stage IV (Ripe)	Ovary highly enlarged, covers the entire ventral cavity and consists of yellowish or creamy round eggs ; vagina become pinkish in color (Fig. 8)
Stage V (Spent)	Testes become flat, flaccid and whitish; testicular lobule starts to disappear. Gonad appears empty (Figs. 13A - 13B)	It decreased to ½ of body cavity walls of gonad are found loose and transparent ; remnant ripe eggs visible from outside (Fig. 9)

various workers in other fish species (Parameswarn *et al.* 1974, Rahman 2005, Suresh *et al.* 2006, Banik *et al.* 2012).

Monthly variations of GSR : The development of gonad can be represented by an index called gonadosomatic ratio. Monthly average GSR was found lowest in October and highest in May in both sexes (Table 2). It was ranged from 0.12 to 3.63 in male and 0.26 to 19.1 in female. The highest GSR value in male was 1.66 (± 0.41) and 12.4 (±3.8) in female whereas lowest GSR in male 0.28 (± 0.03) and 0.49 (± 0.17) in female. Highest GSR value was recorded in pre-monsoon (March-May) and that of lowest in post-monsoon (Sep-Nov) for both male and female. A gradual increase GSR was recorded from pre-spawning to spawning period and thereafter, there was a sharp decline during post-spawning period (Fig. 1).

Ova diameter and fecundity : The ova diameter increased from January (0.30 ± 0.05 mm) on wards up to July (1.31 ± 0.17 mm). The occurrence of ripe ova was decreased from August that indicated the cessation of spawning.

Both absolute fecundity (7026±2200) and relative fecundity (830.2 ±167.8) was found maximum in April. Likewise minimum absolute fecundity (411±154.7) and relative fecundity (44 ± 22.7) was observed in August. Monthly variation of ova diameter and fecundity was presented in the Table 3.

Spawning season : *M. dibrugarensis* spawn once in a year that occurs from late April to early August. Highest GSR value in pre-monsoon (March-May), occurrence of ripe ova from April to July and unavailability of ripe specimen after August indicating the spawning period was started from April and ends in August.

DISCUSSION

Sex differentiation in *M. dibrugarensis* was found distinct. Presence of genital papilla in male and genital pore in female helps to distinguish their sex easily. This observation was also recorded by various workers in other *Mystus* species (Bhatt 1971 a and b, Ng 2001, Musa and Bhuiyan 2007, Darshan *et al.* 2011 and 2013, Gupta 2015).

The study on sex-ratio provides information on the proportion of male and female fish in the population means the dominance of sex in a given population. It also provides the basic information necessary for fish reproduction and stock size assessment (Vicentini and Araujo 2003). The overall sex-ratio of *M. dibrugarensis* was significantly in favor of

Table 2. Sex ratio and gonado somatic ratio of *M. dibrugarensis.* * Significant at $p^{0.05}$ and ** significant at p 0.01.

Month	Sex ratio (M:F)	χ^2	GSR	
			Male	Female
Jan	1.16:1	0.526	0.62 ± 0.25	1.03 ± 0.22
Feb	1.53:1	7.23**	0.66 ± 0.32	1.52 ± 0.63
Mar	0.36:1	13.0**	1.09 ± 0.28	4.03 ± 0.99
Apr	0.37:1	10.3**	1.21 ± 0.73	8.15 ± 3.27
May	0.53:1	4.59 *	1.66 ± 0.41	12.4 ± 3.8
Jun	0.40:1	8.02**	1.49 ± 0.45	11.1 ± 4.06
Jul	0.57:1	4.26*	1.27 ± 0.44	3.58 ± 0.68
Aug	0.51:1	6.45*	0.47 ± 0.21	2.13 ± 0.63
Sep	0.72:1	1.42	0.31 ± 0.11	1.71 ± 0.22
Oct	1.21:1	0.49	0.28 ± 0.03	0.49 ± 0.17
Nov	1.75:1	3.27	0.41 ± 0.19	0.99 ± 0.46
Dec	0.932:1	5.87*	0.55 ± 0.22	1.01 ± 0.44
Total	0.932:1	5.87*	--- ---	

female (χ^2 = 5.87, at p 0.01) which is in conformity with the finding of Mahmood *et al.* (2011). However, the occurrence of more female in certain months is related to gonadal development and due to their heavier weight they can't escape from fishing gears (Biswas 2002). Female dominance in some specific months was also reported by several workers (Bhatt 1971a, Rao and Sharma 1984, Roy and Hossain 2006) in different *Mystus* species. Variation in sex ratio is probably due to various reasons like age difference, difference in size at first maturity and also due to differences in natural and fishing mortality between the two sexes.

Fish biologists described maturity stages of *Mystus* species into 5-7 stages. Five, six and seven maturity stages were reported by Gupta and Banerjee (2013), Mithu *et al.* (2014), Rastogi and Saxsena (1968) in *Mystus tengara*. Basu *et al.* (2015) mentioned five maturity stages viz., immature, mature, early ripening, ripe and spent in *Mystus vittatus*. In the present study, maturity stages of *M. dibrugarensis* was categorized into five stages based on their progression of gonad. Testicular lobule development in the test species was observed from maturing stage which was also reported by Gupta and Banerjee (2013) in *M. tengara.*

Monsoon rain influence the spawning activity of various *Mystus*. As in the north east India monsoon generally started from June and last till July. The test species were said to be pre-monsoon and monsoon breeder as its spawning occurs from late April to early August. Spawning of *M. tengara* attributed to monsoon rain i.e. from April to early September (Rastogi and Saxsena 1968, Guraya *et al.* 1975 and 1976). Ovarian maturation of *M. vittatus* peaked during April to July when the monsoon started (Arockiaraj *et al.* 2004, Basu *et al.* 2015) where as in *M. montanus* it peaked during October-December. It has been reported that a particular fish species may have different spawning periodicity depending on environmental condition of the habitat (Biswas *et al.* 1984). As for instance, single peak in *M. gulio* was observed which is comparable to the observations made in other estuaries (Pantulu 1961, David 1963) that indicated it spawns once in a breeding season (Kaliyamurthy 1981). The test fish was found to be spawn once in a year that was similar with the above mentioned findings.

In the present experiment, GSR for the females was always higher than those of the males that was supported by Paswan (2014). GSR increased with the progression of gonads, found maximum during the period of peak maturity as also observed in other fish species (Biswas 2002). In the pre-spawning stage, there was a gradual increase in GSR reaching a peak during spawning period ; thereafter, there was a declining phase during post-spawning period. This result was similar with the finding of Basu *et al.* (2015), Sarker *et al.* (2002). Increase in GSR values

Table 3. Monthly variation of ova diameter and fecundity.

Month	Ova diameter (mm)	Absolute fecundity	Relative fecundity
Jan	0.30 ± 0.05	904 ± 116	87 ± 26.3
Feb	0.46 ± 0.04	3898.9 ± 1873.4	358 ± 246
Mar	0.62 ± 0.07	5507 ± 1230	646 ± 174.2
Apr	0.77 ± 0.12	7026 ± 2200	830.2 ± 167.8
May	1.28 ± 0.24	6007.1 ± 2611.7	536.2 ± 246
Jun	1.30 ± 0.31	3196 ± 459.9	380.5 ± 28.8
Jul	1.31 ± 0.17	506.2 ± 290.2	49.5 ± 18.6
Aug	1.27 ± 0.21	411 ± 154.7	44 ± 22.7

indicated development of the gonads during February to May which was found almost steady in June and a drop from August till October which indicated spent stage of the fish (Fig.1). GSR was peaked during November-December (Arockiaraj *et al.* 2004) in *M. montanus.*

Occurrence of maximum sized intra-ovarian egg was observed in May-July in the test fish was similar to the finding of Chakraborty *et al.* (2007). Progression of ova diameter in different month was presents in Figs. 2 and 3. The peak season was coinciding with the peak breeding and indicating commencement of active breeding from April to July. Declination in the GSR value from August indicated that the spawning is over by August *M. dibrugarensis*. The highest GSR was recorded in ripe stage whereas the lowest was in immature stage for both sexes.

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