Aspects of reproductive biology of the lesser spiny eel *Macrognathus aculeatus* (Bloch, 1786) from river Ganga, Uttar Pradesh, India

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ABSTRACT

A total of 315 samples of *Macrognathus aculeatus* (Bloch, 1786) were collected for this study from the river Ganga at Sant Ravidas Nagar, Bhadohi, Uttar Pradesh during June 2016 to May 2017. Chi-square test (χ^2) confirmed that the male to female sex ratio was 1:0.93 which was not significantly different from hypothetical ratio of 1:1. The maximum numbers of stage IV gonads were encountered during the month of June indicated its spawning period. Mass and length ratio showed a negative allometric growth ($b < 3$) in males (2.08102), females (2.50212) and for the sexes pooled (2.25371). The maximum and minimum values of condition factor for both the sexes of *M. aculeatus* were recorded during non-breeding and breeding seasons respectively. The ova diameter of ripe eggs of *M. aculeatus* ranged between 1.25-1.40 mm during the month of June while the single mean peak value (male = 1.40 ± 0.091 ; female = 4.49 ± 0.059) of the gonado-somatic index pointed out that the fish has only one breeding season during monsoon. Absolute fecundity varied from 800 to 1510, with an average value of 1247 eggs. Fecundity was found to be highly correlated with the total length ($r = 0.9041$), body weight ($r = 0.8901$), ovary length ($r = 0.8721$) and ovary weight ($r = 0.9210$) which were found to be significant ($p < 0.001$).

Keywords: Fecundity, Ganga River, *Macrognathus aculeatus*, Reproduction

Introduction

Studies on reproductive biology of fish is considered to be important for their management under controlled conditions and also from the evolutionary point of view (Kashyap *et al.*, 2016). The knowledge of reproductive potential of fish can be used in formulating the degree of rearing facilities needed and to assess the aquacultural potential. Reproductive biology studies in fish include the assessment of sex ratio, maturity stages, duration of reproductive season, gonadosomatic index (GSI) and quantification of reproductive capacity (Maurua *et al.*, 2003; De Carvalho *et al.*, 2009; Fontoura *et al.*, 2009; Cardoso *et al.*, 2019). Reproductive biology is one of the most important biological aspects which is a prerequisite for evaluation of the commercial potentialities of a fish stock and its preservation, conservation and management prospects (Jakobsen *et al.*, 2009; Hliwa *et al.*, 2017; Cardoso *et al.*, 2019). GSI has been used as an indicator of reproductive activity of fish and for determination of the breeding season (Sley *et al.*, 2015; Almukhtar *et al.*, 2016). The condition factor is affected by food availability, environmental conditions, population density, physical factors and the physiology of fish including development of gonad maturity (Parrish and Mallicoate, 1995; Uthayakumar *et al.*, 2013).

The spiny eel, *M. aculeatus* is one of the common species of Mastacembeliformes and widely distributed in different freshwater habitats of Asian and South-east Asian countries such as Bangladesh, China, India, Indonesia, Korea, Laos, Malaysia, Nepal, Singapore, Taiwan, Thailand and Vietnam (Jayaram, 1981; Das and Kalita, 2003; Rahman *et al.*, 2012). The spiny eels inhabit mainly in medium to large sized rivers and are abundantly distributed in low lying wetlands, freshwater streams, ponds, canals, beels and tanks. They are also available in freshwater and brackishwater within tidal influence and are benthopelagic and potamodromous in nature (Bhuiyan, 1964; Taki, 1978; Riede, 2004). They are often found in small puddles in which mud abounds and popularly known as the 'ditch eels' (Hora, 1935) and are also known as the 'lesser spiny eels' (Munro, 1955). *M. aculeatus* attain upto 15 inches in length and are considered as commercially important species, palatable as an excellent food fish with high nutritive value (Day, 1878; Sahoo *et al.*, 2009) and also used as an aquarium species (Vidthayanon, 2002). Hossain *et al.* (1999) pointed out the high nutritional value of *M. aculeatus* as it contains 15.3% carcass protein, 22.1% muscle protein, 4.12% lipid, 78.1% moisture, 2.3% ash and 5.3 KJ g⁻¹ energy. Prasad *et al.* (1983) also reported the seasonal variation in fat and water content of this fish and the serum cholesterol level was minimal (155.0 ± 29.5 mg ml⁻¹) as recorded by Chandra (1982).

M. aculeatus occupies the third level in the food chain in waters and also helps to control water pollution by consuming detritus (Rahman *et al.*, 2009). The demand of the species always exceeds the supply and the supply mostly depends on the capture resources (Sahoo *et al.*, 2009). *M. aculeatus* is under the category of 'least concern' according to the International Union of Conservation of Nature (IUCN, 2018). In spite of its preference and palatability, very less information is available on the biology of this fish species (Rahman *et al.*, 2012). A survey of literature showed that very little work has been carried out on different aspects of the biology of *M. aculeatus* inspite of its economic importance. The studies include the estimation of fecundity of *M. aculeatus* in India (Prasad *et al.*, 1979); length-weight and length-length relationship (Lazarus and Reddy, 1986; Hossain *et al.*, 2006), reproductive biology (Nabi and Hossain, 1996), sex ratio, length-frequency distributions and morphometric relationships of length-length and length-weight (Rahman *et al.*, 2012). Qasim and Qayyum (1961) studied the spawning frequencies and breeding season of *Rhynchobdella aculeata* (*M. aculeatus*).

Keeping in mind the paucity of information on the biology of this species, the present study was planned to determine the length-weight relationship, condition factor, sex ratio, gonadosomatic index (GSI) and fecundity of *M. aculeatus*.

Materials and methods

Collection of fish samples

A total of 315 specimens of the spiny eel, *M. aculeatus* were collected from the Ganga River passing from Sant Ravidas Nagar, Bhadohi (25°9' N - 25°32' N; 82° 14' E - 82°45' E) using cast nets and gillnets (mesh size = 3 to 4.5 cm) during June 2016 to May 2017. The individuals were primarily cleaned with distilled water and two body parameters *viz.*, total length (TL) and body weight (BW) were measured.

Weight-length ratio and condition factor

Weight and length relation was established using the equation $BW = a \cdot TL^b$ (Le Cren 1951), where, BW is the total weight and TL is the total length. Parameter 'a' and 'b' were calculated by means of a linear regression on transformed data ($\log BW = \log a + b \log TL$), where 'a' is the intercept and 'b' represents the slope of the regression line. Covariance analysis (ANCOVA) was performed to determine possible differences between the parameters for males and females.

Monthly variations in the body condition of fish were obtained using Fluton's condition factor (K), using the formula: $K = BW \times 100 / TL^3$ which indicates the type of growth (King, 2013).

Reproductive biology

To determine the sex, an abdominal incision was made and the gonads were removed and cleaned with distilled water. Weight and length of ovary were taken and then preserved in 4% formaldehyde solution in labelled vials for further studies. Stages of maturity were determined according to the method suggested by Qayyum and Qasim (1964) for the tropical and subtropical fishes. Identification of gonadal conditions was done on the basis of their colour, appearance and their extent in the body cavity.

Gonadosomatic index (GSI)

GSI for each fish was computed by expressing the gonad weight as percentage of the total body weight. GSI was calculated using the standard formula:

$$GSI = (OW/BW) \times 100$$

where, OW = weight of gonad in g, BW = weight of the fish in g.

Ovarian diameter

Intraovarian eggs were measured and their diameters were taken with the help of an ocular micrometer using 8 x 12.5 magnification of binocular dissecting microscope.

Fecundity

Fecundity evaluation was carried out by taking 10 mg subsamples of eggs from the anterior, middle and posterior regions of each ovary. Ova present in the subsamples were counted using binocular dissecting microscope at 1 x 12.5 magnification and their average count was multiplied by the total weight of the ovary for the determination of absolute fecundity.

$$\text{Fecundity (F)} = N \times \text{Weight of gonad (OW)}$$

where, N = No. of eggs in sample

Relationships of fecundity with various parameters (TL, BW, OL and OW) were also worked out. The probability level was set as less than 0.05.

Results

Sex ratio

The male:female sex ratio ($\sigma:\phi$) recorded in the present study was 1:0.93. However, the chi-square test (χ^2) showed no significant departure from the hypothetical ratio (1:1). Male *M. aculeatus* maintained numerical superiority over female (Table 1) in all months except during February, August, September and December. The sex ratio also varied with size which was found to be non-significant ($p > 0.05$) from the hypothetical ratio (1:1) in all size groups (Table 2).

Table 1. Sex ratio of *M. aculeatus* in different months sampled from river Ganga

Months	Male abundance	Female abundance	Sexes combined	Sex ratio ($\sigma^7:\text{f}$)	Chi-square value (χ^2)	Significance
Jan	24	20	44	1:0.83	0.366	NS
Feb	13	14	27	1:1.07	0.036	NS
Mar	14	10	24	1:0.71	0.685	NS
Apr	16	14	30	1:0.87	0.133	NS
May	6	6	12	1:1	0.0	NS
Jun	17	13	30	1:0.76	0.542	NS
Jul	12	8	20	1:0.66	0.833	NS
Aug	20	22	42	1:1.09	0.095	NS
Sep	8	12	20	1:1.5	0.183	NS
Oct	11	5	16	1:0.43	2.618	NS
Nov	11	7	18	1:0.63	0.934	NS
Dec	12	20	32	1:1.66	2.13	NS
Total	164	151	315			
Mean	13.66	12.58		1:0.93		NS

σ^7 =Males, f =Females, NS= Non-significant

Table 2. Sex ratio of *M. aculeatus* in different size groups sampled from river Ganga

Size range (mm)	Male abundance	Female abundance	Sexes combined	Sex ratio ($\sigma^7:\text{f}$)	Chi-square value (χ^2)	Significance
7-10	18	12	30	1:0.66	1.25	NS
11-14	27	11	38	1:0.40	4.18	NS
15-18	19	22	41	1:1.15	0.22	NS
19-22	22	18	40	1:1.22	0.40	NS
23-26	23	24	47	1:1.04	0.02	NS
27-30	24	26	50	1:1.08	0.27	NS
31-34	22	28	50	1:1.27	0.73	NS
35-38	09	10	19	1:1.24	0.05	NS
Total	164	151				NS

σ^7 =Males, f =Females, NS= Non-significant

Gonads and maturity stages

Testes of *M. aculeatus* are paired, slender and creamy white in colour, located dorsally in the abdominal cavity which elongate with ripening and extend towards the posterior half of the abdomen. The testes are suspended by a delicate and thin mesentery called mesorchia. From the anterior inner margin of each testis arises a vas efferens which runs along the whole length of the testis. The two vas efferens of the two sides join posteriorly to form a common duct, the vas deferens that communicates with the exterior.

Ovaries of *M. aculeatus* are paired and elongated, situated in the posterior half of the body cavity and are suspended medially by mesovaria. Ripe ovaries are light yellow in colour. Anteriorly, the two lobes representing the two ovaries are free, but posteriorly they bend downwards and inwards to form a short single oviduct, which leads to the genital aperture.

In the present study, five maturity stages were recognised in both males and females of *M. aculeatus*

(Table 3; Fig. 1). In both sexes, gonads show a regular seasonal development with little overlap in different phases of maturation. Spawning season of *M. aculeatus* was estimated on the basis of the occurrence of adults in different stages of maturity in each month. Both sexes were completely matured in the month of June with ripe gonads (stage IV).

GSI

Remarkable changes were observed in the weight of gonads during development. Low GSI indicated the inactive phase of gonads (January-February and October-December). As gonadal activity picked up, the GSI also increased, attaining the peak value in the ripe stage. GSI increased markedly from May and peaked in the month of June in both the sexes and declined rapidly at the onset of spawning in August as it was indicated by the appearance of spent specimens (Fig. 2). Therefore, it was concluded that the fish spawned once in a year with one spawning peak. In the present study, comparatively high values of GSI in both male (1.40 ± 0.091) and female (4.49 ± 0.059) were recorded in the breeding season during May to June.

Table 3. Maturity stages of gonads in *M. aculeatus*

Stage	Testes	Ovary
Stage I (Immature virgin)	Testes are small, paired, slender, thread like, distinguished microscopically from the ovaries. Vasa deferentia not very distinct and difficult to locate	Structures extending one third of the body cavity. Eggs of circular shape, semitransparent
Stage II (Maturing virgin or Recovering spent)	Testes slightly elongated, opaque and white in colour. Vasa deferentia distinct and easy to locate	Ovaries thin, slightly elongated. Ova spherical opaque
Stage III (Ripening)	Testes ivory in colour, more prominent than Stage II. Viscous fluid oozes out with slight pressure on the abdomen	Ovaries yellowish, elongated, slightly lobulated, ovarian blood vessels visible extending almost the entire length of the body cavity. Eggs visible to naked eye
Stage IV (Ripe or Fully mature)	Testes flabby, massive, creamy white in colour, grown to maximum in size, occupying substantial part of the body cavity and discharge white milt on gentle pressure.	Ovaries yellow white in colour, ovarian membrane very thin. Eggs opaque and very distinct, almost round and easily ejected if slight pressure is applied on the abdomen
Stage V (Spent)	Testes shrunken, their weight drastically reduced in this stage. No milting with pressure on abdomen	Ovaries flabby, shrunken and left with only a few residual ova in recently spawned fish. Majority of ova small, transparent, invisible to naked eye.

Fig. 1. Maturity stages of *M. aculeatus* in different months sampled from river Ganga. (a) Males and (b) Females Fig. 2. Mean values of the gonadosomatic index (GSI) and condition factor (K) in males and females of *M. aculeatus*

Weight-length ratio and condition factor

The weight-length ratio showed significant difference between genders (ANCOVA, $p < 0.05$). The growth coefficient was lower than three ($b < 3$), indicating that *M. aculeatus* shows a negative allometric growth for males (2.08102), females (2.50212) and for combined sexes

(2.25371). The rate of change in weight relative to length obtained in size range of 7-38 cm yielded the following equations: Males: $\text{Log BW} = -4.73810 + 2.08102 \text{ Log TL}$; Females: $\text{Log BW} = -6.10542 + 2.50212 \text{ Log TL}$; Sexes combined (Males and Females): $\text{Log BW} = -5.31351 + 2.25371 \text{ Log TL}$. The average values of condition factor (K) of *M. aculeatus* ranged from 0.32151 to 0.5060 (males) and 0.3251 to 0.5487 (females) with maximum and minimum value during January (non-breeding time) and June (peak breeding time) respectively. The details are given in Fig. 2.

Ova diameter

In the present study, the size of ova ranged between 0.35-1.70 mm while the ripe eggs of *M. aculeatus* were spherical and diameter ranged between 1.25-1.40 mm during the month of June (Fig. 3). The diameter of ova collected from anterior, middle and posterior regions of the ripe ovary were almost of the same size. Likewise,

Fig. 3. Size frequency distribution of intraovarian oocytes of *M. aculeatus* sampled from river Ganga. (a) March, (b) April, (c) May, (d) June and (e) July

the ova from the two lobes of gonads were also of similar diameter.

Fecundity

In the present study, absolute fecundity varied from 800 to 1510, with an average value of 1247 in the specimens of *M. aculeatus*. Fecundity was also found to be highly correlated with the TL and BW with values of correlation coefficient 'r' 0.9041 and 0.8901 respectively and significant at $p < 0.001$. Also, the values of correlation coefficient were 0.8721 and 0.9210 for fecundity-OL and fecundity-OW relationships respectively (Table 4).

Discussion

In the present study, males outnumbered females of *M. aculeatus*. Male dominance over female may be due to accidental or natural mortality such as spawning stress of females in the population. Similar results were

reported by Abujam and Biswas (2011) and Kashyap *et al.* (2016). However, it is worth mentioning here that several other workers (Qayyum and Qasim, 1964; Cardoso *et al.* 2019) recorded the dominance of females over males. In the present study, male and female sex ratio was 1:0.93 which did not differ significantly from the hypothetical sex ratio of 1:1. Rahman *et al.* (2012) reported that male and female sex ratio in *M. aculeatus* was 1:1.35 which differed significantly from the expected value of 1:1. The minor differences in the sex ratio could be attributed to the differences in the growth between sexes, sexual dimorphism and mortality rates.

In *M. aculeatus*, the cycle of maturation and depletion of gonads occurs only once in a year and synchronised in both the sexes. It was observed during the present study that both male and female *M. aculeatus* had higher GSI values during May to June, which indicated that the fish

Table 4. Logarithmic regression equations of various relationships in *M. aculeatus*

Relationship	Regression Equations	Correlation coefficient (r^2)	p value
Fecundity-TL	$\log F = 3.10 + 0.83 \log TL$	0.9041	0.001
Fecundity-BW	$\log F = 3.71 + 0.52 \log BW$	0.8901	0.001
Fecundity-OL	$\log F = 2.08 + 1.57 \log OL$	0.8721	0.001
Fecundity-OW	$\log F = 3.12 + 0.17 \log OW$	0.9210	0.001

has only one breeding season and spawns during this time with a peak value of 1.40 ± 0.091 for male and 4.49 ± 0.0590 for female. Similar observations were perceived by Nabi and Hossain (1996) in the spiny eel, *M. aculeatus*. Ali *et al.* (2003) reported that the GSI in *M. aculeatus* ranged from 0.44 ± 0.06 to 14.40 ± 1.48 while Sultana *et al.* (2017) reported the mean GSI of all samples of *M. aculeatus* as 12.50 ± 1.38 .

In the present study, most of the stage IV ripe gonads were recorded from May to July and spawning was found to be at its peak in core monsoon period. An increase in GSI value means the advancement of maturity. High value of GSI in both males and females during the period from May to July indicated full development of gonads. Recovering gonads of stage II remain quiescent from October through January, because during this period both temperature and photoperiod remained minimum. During February/March an increase in development of gonads takes place with the increase in temperature. Peak condition of development of gonads was recorded during June when both temperature and photoperiod were maximum.

According to Le Cren's (1951) concept of 'cube law' the value of 'b' for an ideal fish must be 3.0, but this value may vary significantly from one species to another. In the present study, it was found that both adult males and females follow negative allometric growth pattern ($b < 3$) and the species during its development exhibited a more increase in length than in weight (Le Cren, 1951; Froese, 2006). The rate of increase in weight in relation to length (regression coefficient, b) was found to be higher in females than males, which could be attributed to the enormous growth of ovaries in females.

In the current study, maximum condition factor (K) was observed in the month of January (non-breeding time) and minimum in June (peak breeding time) in both the sexes. The condition factor decreased with growth and corresponding increase in weight with maximum value during non-breeding seasons while it reduced at peak breeding season in the closely related spiny eel, *Mastacembelus armatus* (Uthayakumar, 2013). The high values of GSI and low condition factor were recorded in the month of June which indicated that there exists no relation between GSI and condition factor. The present finding is in corroboration with Cardoso *et al.* (2019) who reported the reverse pattern for mean condition factor and GSI in *Prochilodus lacustris*. The condition factor relatively depended upon a number of factors such as environmental conditions and ecological parameters, seasons, food availability, gonadal development, fishing pressures and stress conditions (Zargar *et al.*, 2012; Ahmad, 2013; Ali *et al.*, 2014; Komsari *et al.*, 2015).

According to Qasim and Qayyum (1961), breeding has direct relation with monsoon cycle and freshwater teleosts found in the plains of Uttar Pradesh have their breeding season from July to September end. The breeding behaviour of *M. aculeatus* seems to follow the same pattern and have relatively short spawning seasons from late May and gradually progresses to the middle of June and late July when the rivers are flooded with rain water. Present finding is also in accordance with the findings of Narejo *et al.* (2003) and Rahman *et al.* (2006) who observed that *M. armatus* has only one breeding season during May to July with a peak in July as well as with that of Nabi and Hussain (1996) in spiny eel, *M. aculeatus*.

Fully developed and ripe eggs were spherical with diameter ranging between 1.25-1.40 mm. Similar to the present findings, Nabi and Hussain (1996) also reported that the eggs were spherical and uniform in diameter (1.00 mm) in ripe gonads of *M. aculeatus*.

In the current study, fecundity varied from 800 to 1510, with an average value of 1247 in the specimens of *M. aculeatus* ranging between 12-19 cm in size. Prasad *et al.* (1979) estimated the fecundity (210-1828) of *M. aculeatus* in India. In the present study, highly significant ($p < 0.001$) correlation co-efficient (r) was observed between fecundity-TL (0.9041), fecundity-BW (0.8901), fecundity-OL (0.8721) and fecundity-OW (0.9210) for *M. aculeatus*. Taslim *et al.* (2002) pointed out that the co-efficient of correlation between fecundity versus TL and BW were found to range from 0.34-0.45 and 0.33-0.59 respectively in *M. aculeatus*. Similar observations were reported by many authors such as Das *et al.* (1989) in *Heteropneustes fossilis*, Khan *et al.* (2002) in *Plotosus canius*, Rheman *et al.* (2002) in *Liza parsia* and by Kashyap *et al.* (2016) in *Channa punctatus*. The finding of this study could be useful for management of the *M. aculeatus* stocks in the wild populations as well as under captive culture conditions.

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