

Comparative Analysis of Reproductive Traits in Barred Spiny Eel, *Macrognathus pancalus* (Hamilton, 1822) from Lotic and Lentic Ecosystems of Gangatic Basin, India

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Abstract: The comparative pattern of the reproductive biology dealing with sex ratio, spawning, gonadosomatic index, ova diameter and fecundity of freshwater barred spiny eel, *Macrognathus pancalus* were investigated. Fish were collected from two different geographical areas characterizing by different environmental conditions in order to elucidation of ecotypes. Male barred spiny eel was slightly dominated in the specimens collected from lentic (ponds) habitats at SRN-Bhadohi while female dominated in the population of lotic environment (Gomti River) in Lucknow region of Gangetic Basin respectively. Monthly fluctuations in gonadosomatic index were recorded with maximum value (one peak) during August in the specimens collected from ponds while two peaks were recorded during March and July in the population of river. The size at the first maturity was also determined. Ova diameter revealed the release of only one batch of mature ova in the population of ponds and two batches of ova in the population of river indicating single and double spawning periods respectively. The average relative fecundity was 82 and 72 eggs cm⁻¹ body length in the populations of lentic and lotic habitats respectively. Two predictors of fecundity: total length and body weight were evaluated and regression equations were derived. Absolute fecundity varied between 796-1618 and 375-2700 in the specimens collected from ponds and River Gomti respectively.

Key words: Gonadosomatic Index% Fecundity % *Macrognathus pancalus* % Size at First Maturity

INTRODUCTION

The freshwater spiny eel, *Macrognathus pancalus* is an inland water teleostean fish commonly known as barred or striped spiny eel found in Asia [1]. This species is distributed in India and its neighboring countries such as Nepal [2], and Pakistan, Sri Lanka, Bangladesh and Myanmar [3]. It is known to occur in estuaries and freshwater habitats such as beels, ponds, lakes and rivers [3]. The fish is commercially important and palatable as a table fish. It is the most beautiful species among the spiny eels. The demand of larger sized spiny eels always exceeds the supply in India and abroad and it fetches a good price (INR 60-80/Kg) when sold alive particularly in northern, eastern and north-eastern parts of India where people relish alive and less bony fish [1]. The smaller size of this species has an ornamental value as an indigenous aquarium fish and is being exported to America, Europe and other Asian countries [4-5]. The fish is generally caught using line and trap methods because

of its bottom dwelling habits besides cast and drag nets. The population of this species is showing the sign of dwindling because of its reckless exploitation from the natural resources in the absence of its cultivation. It is included under “near to threatened” category in India [6] and in IUCN, it is considered as least concern [7]. The investigations on various aspects of its biology are carried out by many workers such as [8] on sexual dimorphism, [9] on maturity and fecundity, [10] on unusual development of the caudal fin and [1] on biology. [11] carried out work on skin structure while [3] described the taxonomy and distribution of the species. [12-14] investigated the food and feeding habits of a closely related species, *Mastacembelus armatus*. [15] also reported the intra specific diversity of *M. armatus*. The overall pattern of reproduction in a fish species is common to all individuals in a particular region and includes a range of life history traits such as the age and size at first sexual maturity, gonadal development, fecundity and gamete size. However, the other

populations of the same fish species can develop alternative reproductive tactics which are variations with respect to the normal reproduction pattern of the species to respond to fluctuation in the environmental conditions. Both, the overall strategy and tactical variations are adaptive and aim at ensuring the survival of the species in specific environmental conditions. Fundamental to the survival of most organisms are adapted with biological rhythms with periodic (daily, monthly or annual) changes in behavior and physiology [16]. Marked reproductive seasonality in numerous vertebrate including fish ensure that reproduction and subsequent development of offspring is coordinated with optimal environmental and nutritional conditions. The studies showed that the reproductive traits such as fecundity and egg size can be influenced by food quantity [17], fishing pressure exerted on the fish and the environmental conditions [18-20]. The study on comparative aspects of reproductive cycle of fish is scanty for most tropical and sub-tropical fish species, however the some notable works carried out on this aspect are by [21] on nine spine stickleback (*Pungitius pungitius*), [22] on brown trout. The present study focused on the comparative pattern of the reproductive biology dealing with sex ratio, spawning, gonadosomatic index, ova diameter and fecundity of freshwater barred spiny eel, *Macrognathus pancalus*, from two different ecosystems.

MATERIALS AND METHODS

Monthly samples of fish were obtained for a period of 21 months from April 2009 to December 2010. Fish were caught using cast and drag nets from River Gomti, a tributary of Ganga River system at Lucknow (27°N81°E) and from ponds and canals of Sant Ravidas Nagar-Bhadohi (SRN-Bhadohi) district (25° 9' N 82°45' E) of U.P which is closely associated with Gangetic Basin in India. Body weight, total length and gonadal weight were measured and sex with gonadal stage was determined following the scheme of classification suggested by [23] for tropical and subtropical fishes. The extracted gonads were preserved in 4% paraformaldehyde solution. Intraovarian eggs were taken out for measuring the egg diameter by an ocular micrometer using 8×12.5 magnification of binocular dissection microscope. The data was pooled for further analysis due to differential availability of either male or female sex. The gonadosomatic index (GSI) was calculated using the standard formula:

$$GSI = W_G \times 100 / W_B$$

Where W_G = Weight of gonad in g, W_B = Weight of the whole fish in g. The reproductive season was determined using the GSI. Mean values of these indices were plotted monthly in order to indicate duration and peak of breeding. The linear regression of fecundity on total length, body weight were calculated based on mature (stage IV) ovaries collected during March through August 2009-2010. One ovary randomly selected from each pair was blotted dry and weighted on an electronic balance to the nearest 0.01g. The sub-samples (10-20mg) from anterior, middle and posterior regions of each ovary were taken and ova present in each sub-sample were counted and their average count was multiplied by total weight of the ovary for determining the absolute fecundity. Relative fecundities (number of ova in unit length and weight of the body) were also calculated. Average size at first maturity was defined as the size (standard length) at which 50% of individuals in the population reached sexual maturity during the reproduction period. For size at first maturity, only the third and fourth stage ovaries were taken into consideration on the basis of macroscopic examination of testes and ovaries of male and female respectively. The coefficient of correlation was estimated in order to indicate the quality of the linear regression and the strength of correlation between fecundity, body weight and body length (TL) were assessed by covariance analysis. The fecundity and body weight, body length were log transformed for the normalization. The regression analysis was done by using the following formulas.

$$\text{Log } F = a + b \log BW \text{ and } \text{Log } F = a + b \log TL$$

The mean Whiteney test was also used in order to compare the fecundity of specimens of two ecosystems. Chi square test was used to analyze the sex ratio while ova diameter was analyzed through one way analysis of variance followed by Turkey test. All the statistical analysis was done with the help of software (Graph Pad Prism 5 and Past Software). For all tests a probability of less than 5% and a confidence of 95% are considered a fiducially level of significance.

RESULTS

Correlations Between Fecundity and Body Weight: Table 1 shows the relationship between fecundity and the fish body weight. There was a significant and positive correlation between absolute fecundity and

Table 1: Relationship between total body weight and fecundity.

Relation	Ecosystem	N	a	b	r ²	F statistic	df	p
Bodyweight-Fecundity	Lotic	40	1.68±0.18	1.18±0.16	0.57	50.61	38	***
	Lentic	40	1.986±0.136	0.968±0.121	0.63	64.44	38	***

Note: N= no of observations; a and b= parameters of linear regression $y = a + bx$; r^2 = coefficient of regression; df = degree of freedom; p = probability level; significant levels= *=0.05; **=0.01; ***=0.001.

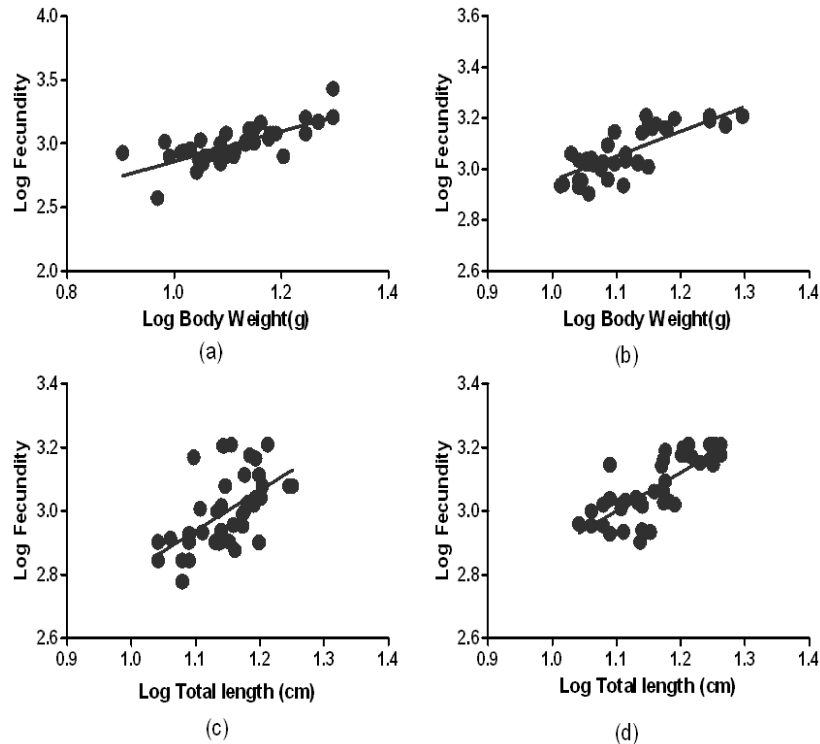


Fig. 1: Relationship between fecundity in relation to body weight and total length of barred spiny eel, *Macrognathus pancalus*: (a) & (c) Lotic ecosystem and (b) & (d) Lentic ecosystem.

fish body weight in both the ecosystems ($P < 0.0001$). The correlation was significantly stronger in the specimens of lentic ($r^2 = 0.63$; $P < 0.0001$) as compared to lotic ecosystems ($r^2 = 0.52$; $P < 0.0001$) (Fig. 1 a, b, c & d).

Reproductive Cycle and Size at First Maturity:

The spawning period of barred spiny eel in lotic and lentic habitats was different. The spawning period in barred spiny eel collected from lotic habitat extended from February to September, followed by a period of sexual rest that occurred from October to January. The peak of sexual activity was recorded in March and July in lotic environment (Fig. 2 a&b). In the lentic environment, the reproduction period of the *M. pancalus* extended from April to October with a period of sexual rest during November-January and peak of sexual activity was recorded in the month of August (Fig. 2 c&d). In each of these ecosystems, the breeding

season covered both dry and rainy seasons but the largest part of reproductive cycle occurred during rainy season. Fish sampled in dry season (October to January) was characterized by low gonadosomatic index (GSI) in both the environments and an absence of sexual stages 3, 4 and 5. There was no difference in the size at first sexual maturity of male and female barred spiny eel in both the regions (L_{50}), which was 13.1 cm during the reproductive period. The gonadosomatic indices of the individuals of both the regions were different to each other indicating that their maturation and spawning period is dependent on the location and the associated environmental conditions such as temperature, salinity, water flow, dissolved oxygen content and availability of food. The values of some physicochemical parameters recorded during experimental periods of both the environments are given in Table 2.

Table 2: Physicochemical parameters of lotic and lentic ecosystems.

S. No.	Parameters	Lotic ecosystem	Lentic ecosystem
1	Flow(m secG ^l)	0.40±0.23	0.30±0.23
2	Water temp. (°C)	29.3±0.5	30.23±0.65
3	pH	7.5±0.31	8.2±0.4
4	D.O.(ppm)	6.4±0.31	4.5±0.74
5	Total hardness (mg/l)	294.5±15.3	236±10.23
6	TDS(ppm)	324.2±7.6	410±23
7	Turbidity(NTU)	14.6±2.1	13.6±2.6
8	Alkalinity (mg/l)	232.9±30.6	74.3±4.67

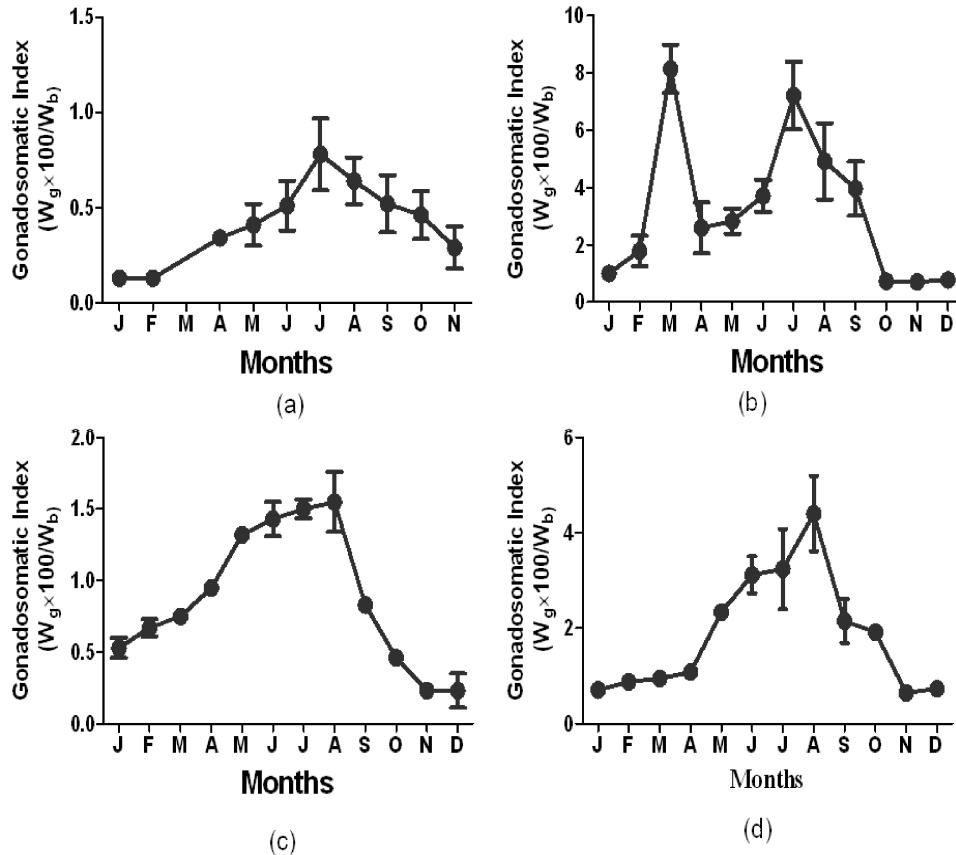


Fig. 2: Gonadosomatic index of freshwater spiny eel *M. pancalus*: (a) male of lotic ecosystem (b) female of lotic ecosystem (c) male of lentic ecosystem (d) female of lentic ecosystem.

Sex Ratio: The sex of 318 fishes of lentic environment was analyzed. The whole group consisted of 171 males and 147 females. The sex ratio (M: F) varied across the season. The minimum M: F sex ratio was observed in January (1: 0.56), while the maximum sex ratio was recorded in July (1:2.37) (Fig. 3a). The pooled sex ratio of lentic environment was male biased with 1:0.91 M: F sex ratio. The Chi square test showed that there was no significant difference from hypothetical ratio. However the sex ratio of 235 fishes of lotic ecosystem was female biased and ranged 1: 2.1 to 1: 7. In which the minimum sex ratio was recorded in the month of July and maximum in

the month of May. The overall sex ratio for the pooled observations also varied significantly from the expected sex ratio (Chi-square, $P < 0.01$), with 4.3 females for every male (Fig. 3b).

Ova Diameter: Fish sampled in lentic habitats had significantly higher Ova diameter than those sampled in the lotic habitat (Turkey test; $P < 0.05$) (Fig. 4 a&b). The ova diameter of lentic ecosystems ranged between 796-1618 μ . The size of ova ranged between 70-138 μ in the sample collected from lotic environment.

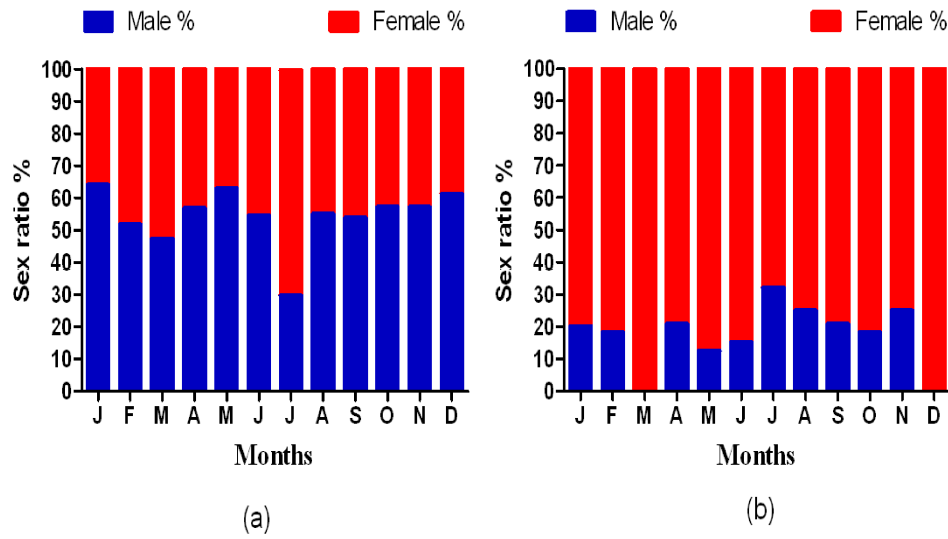


Fig. 3: Distribution of sex ratio in *M. pancalus*: (a) Lentic ecosystem, (b) Lotic ecosystem

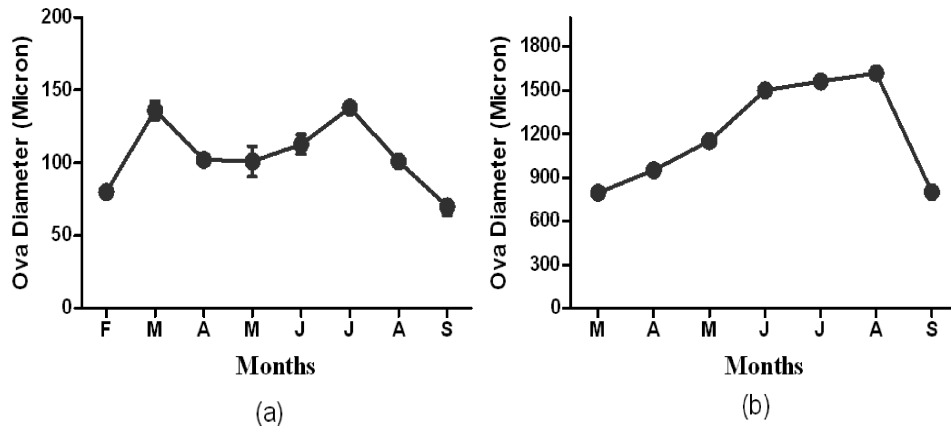


Fig. 4: Ova diameter (μ) of barred spiny eel, *M. pancalus* (a) Lentic ecosystem, (b) Lentic ecosystem

Gonad and Sexual Maturation: Paired gonads were elongate with ripening and extend towards the posterior half of the abdomen. Five maturity stages were recognized in *M. pancalus* in the samples collected from both the environments (Table 3 A&B). The gonads showed a regular seasonal development with little overlap in the different phases of maturation. The gonads developed in February and entered in the ripening stage and subsequently gonads got predominant during March and again in July in lotic ecosystem in the present study. Less active gonads were found from October through January in both the ecosystems. Mostly the ripe stage gonads were observed in month of July and August in lentic environment. The cycle of maturation and regression of gonads in the present study suggested a breeding season for barred spiny eel during August-September in lentic ecosystem and during March and July, twice in a year indicating fractional spawning in lotic habitat.

Fecundity in Barred Spiny Eel Populations:

The 'factor' of absolute fecundity of the samples of barred spiny eel collected from lotic population varied from 375 to 2700 with average value of 1048 ± 61.59 in the individuals ranging between 110-178 mm in length. The absolute fecundity varied 796-1618 with average value of 1208 ± 42.06 in the individuals collected from lentic environment ranging between 110-183 mm in length. Length wise average relative fecundity recorded was 82 eggs/cm body lengths in the individuals of lentic environment while in the lotic population the average value was 72 eggs/cm body length (Table 4). Weight wise the values were 89 and 77 eggs/g of their body weight in the specimens collected from lentic and lotic habitats respectively. The relative fecundity exhibited a different pattern between locations, being highest at the lentic population (Mean Whitney U test; $P < 0.0031$) and

Table 3A: Gonad maturation stages of male and female barred spiny eel, *M. pancalus* of lentic ecosystem

Stage	Period	Gonad condition	
		Male	Female
I(Immature Virgin)	Resting	Testes narrow, thread like and translucent. Vasa differentia not distinct. GSI : 0.22±0.025	Ovaries small, thin, thread like semi-transparent and devoid of yolk granules. GSI: 0.70±0.23
II (Maturing or Recovering Spent)	Pre-spawning	Testes slightly elongated, white in color, vasa differentia distinct. GSI: 0.42±0.026	Ovaries elongated and swollen, light yellow in color with ova small and visible to the naked eye. GSI: 1.43±0.46.
III (Ripening)	Pre-spawning	Testes creamy white in color occupying about ½ the body cavity. GSI : 0.84±0.20	Ovaries voluminous and slightly lobulated. Ovarian blood vessels visible extend almost the entire length of body cavity. Ova with distinct yolk and visible to the naked eye. GSI: 2.65±0.42
IV(Mature or Ripe)	Spawning	Testes massive creamy white. Discharge white milt under gentle pressure. GSI : 1.02±0.268	Ovaries massive occupying major portion of body cavity, ova easily ejected on slight pressure on abdomen. in diameter. GSI: 3.85±0.42
V (Spent)	Post spawning	Testes shrunken, their weight reduced. No milting. GSI: 0.86±0.23	Ovaries flabby and shrunken left with only a few residual undeveloped ova. Weight reduced. GSI: 1.35±0.48

Table 3B: Gonad maturation stages of male and female of barred spiny eel, *M. pancalus* from lotic ecosystem

Stage	Period	Gonad condition	
		Male	Female
I (Immature Virgin)	Resting	Testes narrow, thread like and translucent. Vasa differentia not distinct. GSI: 0.15±0.1	Ovaries small, thin, thread like semi-transparent and devoid of yolk granules. GSI: 0.69±0.15
II (Maturing or Recovering Spent)	Pre-spawning	Testes slightly elongated, white in colour, vasa deferentia distinct. GSI: 0.34±0.04	Ovaries elongated and swollen, light yellow in color with ova small and visible to the naked eye. GSI: 1.66±0.57
III (Ripening)	Pre-spawning	Testes creamy white in color occupying about ½ the body cavity. GSI: 0.35±0.10	Ovaries voluminous and slightly lobulated. Ovarian blood vessels visible extend almost the entire length of body cavity. Ova with distinct yolk and visible to the naked eye. GSI: 3.25±1.01
IV(Full Mature or Ripe)	Spawning	Testes massive creamy white. Discharge white milt under gentle pressure. GSI: 0.80±0.08	Ovaries massive occupying major portion of body cavity, ova easily ejected on slight pressure on abdomen. GSI: 5.67±0.94
V (Spent)	Post spawning	Testes shrunken their weight reduced. No milting. GSI: 0.32±0.09	Ovaries flabby and shrunken left with only a few residual undeveloped ova. Weight reduced. GSI: 1.63±0.31

Table 4: Relationship between total length and fecundity

Relation	Ecosystem	N	a	b	r	F statistic	df	p
Total length Fecundity	Lotic	40	1.54±0.33	1.27±0.29	0.58	19.50	38	***
	Lentic	40	1.68±0.19	1.21±0.16	0.77	54.57	38	***

Note: N = no of observations; a and b= parameters of linear regression $y = a + bx$; r^2 = coefficient of regression; df = degree of freedom; p = probability level; significant levels: = *=0.05; ** = 0.01; *** = 0.001.

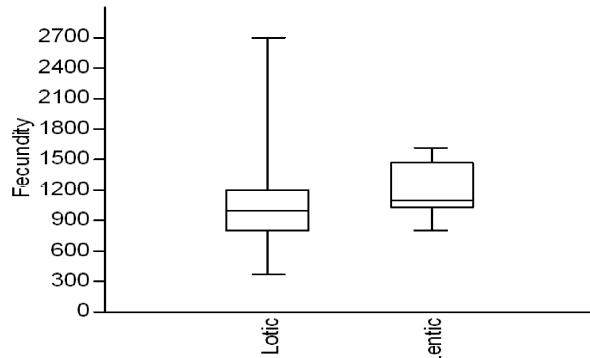


Fig. 5: Relative fecundity of barred spiny eel, *M. pancalus* from two different ecosystems. Data are illustrated in box plots that contained the median (horizontal lines) as well as 25th and 75th percentiles (bottom and top edges of the boxes).

lowest in lotic population (Fig. 5). However, statistically there was no any significant difference between the populations of lentic and lotic environments.

Correlations Between Total Length and Fecundity:

Absolute fecundity and total length were positively correlated with significant correlation in the all ecosystems ($P < 0.0001$). The correlation was significantly stronger in the specimens of lentic environment ($r = 0.77$; $P < 0.0001$) compared to the lotic environment ($r = 0.58$; $P < 0.0001$) (Table 3, Fig. 1 c & d).

DISCUSSION

Several workers reported the high population of males in fish. Working on the same species Suresh *et al.* [1] also reported the female dominance (1: 2.92) in the population of Ganga River basin at North 24 Parganas District of West Bengal. Abujam and Biswas [24] reported the male dominance in the closely related species, *Macrogathus aral* in the populations of lentic and lotic ecosystems in Dibrigarh and Tinsukhia Districts of Assam. The reasons suggested for the dominance of male in lentic and female in lotic environment in the present study may be the segregation of sexes through various periods of the year, size differences, gear selectivity related to sex difference in morphology and physiological activity and differences either by natural or artificial mortality. Sex ratio (M: F) was affected by schooling in feeding and spawning grounds. Similar results were also reported by Serajuddin *et al.* [14] in a closely related species, *Mastacembulus armatus*. However the male dominance was also reported by several workers such as

Ranagaswami [25] in *Mugil cephalus* and Gowda and Shanbhogue [26] in *Valamugil seheli*. Hoda and Qureshi [27] in *Liza klunzingeri*. On the other hand female dominance over the male was also reported by the several workers such as Effendi and Sjalpal [28], Fatima and Khan [29]. The cycle of maturation and depletion of gonads in the lentic environment as well as lotic environment is variable with the onset of monsoon season. The ripe gonads were recorded in the months of July and March and July in the lentic and lotic environments respectively indicating spawning seasons. GSI was a common metric used in fishes to investigate the reproductive investment. The monthly variation of the GSI not only indicated the different phases of reproductive cycle but also provided a fact regarding the duration of spawning season. The maximum values of both GSI (Fig. 2 a&b) and ova-diameter (Fig. 4 a) recorded in the present study during the months of March and July supported the fractional spawning i.e. twice in a year in the population of lotic environment of *M. pancalus*. In contrast to the lotic population, the maximum values of both GSI (Fig. 2 c&d) and ova-diameter (Fig. 4 b) were recorded in the month of August which indicating single spawning season during monsoon period in the population of lentic environment. Spawning in fishes is influenced by both internal as well as external factors. The hormones as internal factors are considered to be most important in order to determining the onset of maturation. The temporal synergism between growth and reproduction was regulated by several factors such as the presence of growth receptors (GH) in the fish gonad (Le Gac *et al.* [30] Gomez *et al.* [31] Gabillard *et al.* [32] establishes its role in regulation of fish reproduction. GH gene expression is also reported in the gonads of rainbow trout Li *et al.* [33] Sciarra *et al.* [34] suggesting its role as a local modulator of ovarian activity. The reproductive seasonality of fishes was regulated by the genic expression of neuroendocrine machinery such as *isotocin*, *ependemin II*, *GABA-A gamma 2*, *calmodulin* and *aromatase 2* in female Gold fish Depang *et al.* [35]. In male the up-regulation of steroid plays an important role in maintaining the reproductive investment instead of GSI in African cichlid fish Maruska *et al.* [36]. Fecundity range was found to be comparatively low in the populations of lentic habitat as compared to those of lotic habitat of *M. pancalus* due to large size of eggs recorded in the specimens of ponds (Fig. 5). Karim and Hossain [9] also reported the occurrence of low absolute fecundity (2013 eggs on an average) in the same species collected from Bangladesh. The two reproductive patterns of *M. pancalus* can be distinguished from to this study (a)

low fecundity range and large egg size in the lentic ecosystem (b) High fecundity range with small egg size in the lotic ecosystem. It has been demonstrated in fishes that depending upon the environmental conditions, females can produce either a large number of small eggs or small number of large eggs, due to their flexibility in allocating the energy reserve between these two modes of reproduction. Many studies have thus reported the relationship between fecundity and egg size in fishes Duarte *et al.* [37]. The seasonal fluctuation in the water level also influence the nutrient cycling, trophic interaction and energy cycling of aquatic environment which directly or indirectly influence the reproductive capability of fishes as well as associated biota Roderigo *et al.* [38]. In the same way temporal variations in fecundity of Arcto-Norwegian cod (*Gadus morhua*) in response to natural changes in food and temperature was reported by Kjesbu *et al.* [39]. These comparative attributes of reproductive traits provides new insights into the life history traits of *M. pancalus* in relation to environmental plasticity or genetic differentiation. However further investigations by common garden experiments on variations of life history traits in relation to environmental conditions or genetic differentiations of populations are needed to establish beyond doubt whether these parameters are plastic or genetically governed. As regards the relative fecundity the number of eggs per unit gram exceeded the number of eggs per unit body length in the specimens collected from both lotic and lentic environments. Fecundity is significantly correlated with body length and weight of the fish. A linear relationship was found to exist between fecundity and each one of these characters. Combinations of two variables, length and weight, improved predictability only slightly, therefore separate equations were derived by length on fecundity and weight on fecundity. Comparison of the quantitative variation in fecundity with length as well as weight of the ovary revealed a marked increase in egg production. Life history traits potentially vary by latitude. Fleming and Gross [40] reported in Coho salmon (*Oncorhynchus kisutch*) increased fecundity, but decreased egg size and overall egg production with increased latitude. The other Coho salmon life history studies supported positive pattern between fecundity and latitude which suggested to reproductive output maximization (Drucker, Crone and Bond, Beacham, Healey and Heard [41-44]. Life history traits latitudinal directionality provides a unique concept to determine if certain trait and life history pattern are broadly adaptive. The parrot fish (*Sparisoma cretense*) also shows the spatial pattern in the reproductive traits and associated

reproductive behavior (Pedro Afonso *et al.* [45]. Availability of food, temperature, salinity and dissolved oxygen are among the abiotic factors which are likely to impact on the fish fecundity, egg size Duponchelle [20]. Differences in food resources at the sites and or efficiency at forging and prey availability can also influence reproductive parameters and condition of fish. The high amount of dissolved oxygen, availability of more food organisms and temperature in lotic environment (Table 2) in present study may be the responsible factors for the differentiation in reproductive traits. In fishes, optimum temperature condition is a prerequisite factor for spawning observed as reported by Khan [46] and Smith [47]. Comparative analysis of reproductive traits in female tilapia in different ecosystem was also studied by Moussa *et al.* [48] in order to compare the environmental factors. The present study provides the new insights in the life history of *M. pancalus* in the different ecosystems.

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