

Gonadal maturity assessment of butter catfish (*Ompok bimaculatus*) from major rivers and tributaries of India during spawning season

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Abstract

The present work focused on exploring reproductive biology of fish from different major rivers of India and their tributaries by comparing ovarian protein, fecundity, oocyte weight, oocyte diameter and condition factor during the spawning period. Significant correlation was found between reproductive parameters of fish in the major rivers and their tributaries. Among the parameters studied fecundity showed the highest correlation with ovarian protein level and oocyte weight in the major rivers, whereas in tributaries it was highly correlated with ovarian protein. The results from a wild population showed that the fecundity and ovarian protein level were significantly higher in the Narmada River, and the lowest in river Ganga (U.P.). Among the tributaries, maximum ovarian fecundity was observed in fish with the highest protein concentration from River Hooghly. The condition factor (K) in female *Ompok bimaculatus* were reported to be significantly high in the major River Cauveri and Sharda tributary. The oocyte weight was significantly higher in the major River Krishna and the lowest in fish from River Godavari. In fish samples collected from tributaries, those Sone River showed the highest oocyte diameter and fish from Betwa River showed the lowest oocyte diameter. It can be concluded that the aquatic atmosphere in the local area plays an important role in species specification and can affect their reproductive performance as well as their survival.

Keywords: Fecundity, Oocyte diameter and weight, Condition factor, Ovarian protein concentration, *Ompok bimaculatus*

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Introduction

Ompok bimaculatus (Bloch 1794) an indigenous freshwater catfish species belongs to the Siluridae family of the order Siluriformes. It is popularly known as butter catfish and is widely distributed along the plains and sub mountainous regions in natural water bodies, i.e. horse (large natural depressions), bars (oxbow lakes), rivers, beels (low-lying seasonal water bodies) and floodplains (Rahman, 1989; Riehl and Baensch, 1991). It also has an extensive geographical distribution covering South East- Asia (India, Pakistan, Afghanistan, Myanmar, Thailand, Java, Sumatra, Borneo and China (Talwar and Jhingran, 1991). Its feeding nature is omnivorous (Reddy, 1980; Dutta, 1994). Due to its rich lipoprotein content and soft bony structure this fish species is considered delicious and highly nutritious to the people.

O. bimaculatus population is drastically reduced because of high exploitation of natural resources and restricted breeding in captivity (Ricciardi and Rasmussen, 1999; Gibbs, 2000; Dawson *et al.*, 2003; Szollosi, 2004) due to which it has been listed as near threatened (IUCN, 2013). There is a lack of information related to reproductive aspects of near threatened fishes and their conservation and management planning except for few species (Payne, 2004; Sarkar *et al.*, 2008, 2010; Malla and Banik, 2015). Today fish diversity and associated reproductive biology are a great challenge (Dudgeon *et al.*, 2006).

In the breeding season, the gonads increase in size; somatic growth slows down and eventually stops. At this stage proteins and lipids of somatic tissue are

transferred to the reproductive organs (Aksnes *et al.*, 1986; Rao and Krishnan, 2011). Its biological aspects like condition factor and reproductive parameters like fecundity, oocyte diameter, oocyte protein content and oocyte weight are very important for the conservation of fish (Mishra *et al.*, 2013 ; Sarkar et al 2017).

The present investigation has been undertaken to correlate the variations in condition factor, ovarian protein and reproductive parameters in *O. bimaculatus* to its spawning phases in different major rivers of India and their tributaries. This attempt was made to study whether the environmental factors of rivers from different ecological regimes could be related to reproductive performance of *O. bimaculatus*. Despite the importance of the species of high conservation and commercial value, no published account of its spawning efficiency has been carried out with respect to the different riverine conditions. Therefore, this study was undertaken for the first time to generate the comprehensive and comparative account of the gonadal maturity level during the spawning period. These reference values are of vital importance in the future for comparative studies with other *Ompok* species, monitoring reproductive potential and aqua-toxicological studies.

Materials and methods

Chemicals

All the chemicals used in the sample collection and estimations were of analytical grade, and purchased locally from scientific suppliers, Lucknow.

Site of sample collection

The fish were handled in accordance with local/national guidelines for experimentation on animals and all care was taken to prevent cruelty of any kind.

The freshwater fish *O. bimaculatus* were collected from different wild populations of major rivers in India (Brahmaputra, Cauveri, Ganga: UP and WB, Godavari, Krishna, Mahanadi, Narmada, Subernrekha, Tapti) and their tributaries (Amravati, Betwa, Chambal, Ghaghra, Gomati, Hooghly, Ramganga, Sharda, Sone) during the spawning phase (2014-2015). The rivers Ramganga, Sharda, Ghaghra and Gomati, are major tributaries of the Ganga River basin in Northern India. Sone River is the largest of the southern tributaries of the Ganges. The Chambal and Betwa River are the tributaries of the Yamuna River in central and northern India, and both form a part of the greater Gangetic drainage system. The River Yamuna is the largest tributary of the Ganga in northern India, the Amaravati River is a tributary of the Cauveri River, and the Hooghly River is a long branch of the Ganges in West Bengal, India.

A minimum of ten fish from each sampling area were dissected to collect ovaries. Tissues were kept in an icebox properly to bring in the laboratory for ovarian protein estimation. A minimum of 20-25 fish from each sampling area were stored at 50% iso-propyl alcohol with their abdomen slit to be carried to the laboratory in Lucknow for the study of other reproductive parameters.

Study of reproductive parameters

Condition Factor

Condition factor (*K*) was calculated using the standard formula as follows:

$$K = \{ \text{Body weight of fish} / (\text{Total body length of fish})^3 \} \times 100$$

Ovarian protein concentration

Protein level estimations were carried out by the Lowry *et al.* (1951) method using crystalline Bovine Serum Albumen (BSA) as standard using a spectrophotometer (UV-Thermo).

Oocyte weight

The mean weight of an oocyte was determined by weighing 100 oocytes using a mini scale with hundredth gram resolution. Only those oocytes belonging to the largest size mode in the gonads were used. This was measured by Digital Balance (Smart Aqua Series) with 0.01g accuracy.

Oocyte diameter

A small portion of the ovary was taken and the diameter of the intra ovarian eggs was measured to the nearest 0.01 mm using Nikon SMZ 1500 binocular microscope loaded with NIS Elements D 4.00.00 software with image analysis devices. The maximum oocyte diameter for mature females was obtained by averaging the measurements of at least 20 of the largest oocytes. The diameter was estimated with the help of an image analysis software (NIS Element 4, Nikon SMZ1500).

Fecundity

The gonads of the individual fish were taken out carefully and preserved in isopropyl alcohol (50%). The moisture of

the ovary was removed with blotting paper. The gonadal weight was measured using a fine electronic balance. Then 0.01 g of each ovary was taken out separately from the anterior, middle and posterior regions of each ovarian lobe. The fecundity of the studied fish was observed adopting the methodology of LeCren (1951):

Fecundity = No. of eggs in the ovary sample \times Gonad Weight/Ovary Sample Weight

Statistical analysis

Data are expressed as the mean \pm S.E. Overall significance for each parameter in the major rivers and their territories was checked by one way analysis of variance (ANOVA) at $p < 0.001$, followed by multiple comparison with Newman Keul Test $p < 0.05$ in order to see the differences between the locations. To analyse interdependency of the parameters, Pearson correlations, was done by using software IBM SPSS 20. When the correlation value lies > 0.5 , the relationship is highly significant. The Pearson correlation value lies between $0.5-0.3$, the relationship is moderate significant. When the correlation value is < 0.3 , the relationship is least significant. The data were also subjected to least square regression analysis (r^2 value) and t-test at 5% significance level.

Results

The fecundity, ovarian protein level, and condition factor were significantly different in *O. bimaculatus* of same season from different major rivers and their tributaries (Figs. 1-3). The fecundity of the studied fish sampled from different the

major Indian rivers, varied from 21512.57 to 4577.23. The highest range was found for major River Narmada (21512.57 ± 5606.06) and the lowest was for River Ganga (U.P.) (4577.23 ± 2193.70). Fecundity was positively correlated with ovarian protein concentration for River Narmada (7.98 ± 0.0003) and the River Ganga (U.P.) (3.58 ± 0.0007) (Figs. 1:A, 2:A). Among the tributaries, maximum ovarian fecundity was observed in fish from the River Hooghly (20499.56 ± 6436.17) and the lowest was reported in fish from River Ramganga (8893.1 ± 55981.2) (Fig. 1:B). A similar pattern was noticed for ovarian protein concentration with the highest ovarian protein concentration (6.16 ± 0.0007) found in samples from River Hooghly and the lowest from River Ramganga (3.6 ± 0.00) (Fig. 2:B). The condition factor (K) in female *O. bimaculatus* was high in fish sampled from the major River Cauveri (0.88 ± 0.30) and Sharda tributary (1.09 ± 0.92), and the lowest in samples from the major River Ganga (WB) and its tributaries Amravati and Hooghly (Figs. 3: A, B).

The *O. bimaculatus* did not show significant differences in oocyte diameter of samples collected from the major rivers, though it was significantly different in tributary samples in the spawning season (Figs. 3:A, B). The diameter of oocytes ranged from 0.21 to 0.34 mm. It was noticed that samples collected from the Cauveri River have a higher diameter (0.34 ± 0.04 mm) and those from Mahanadi are the lowest (0.21 ± 0.02 mm) (Fig. 3:A). This pattern coordinated with condition factor. In fish samples collected from the tributaries, samples from the Sone River

(0.33 ± 0.05) showed the highest oocyte diameter and those from the Betwa River showed the lowest oocyte diameter (0.23 ± 0.02) (Fig. 3:B).

Another reproductive parameter, oocyte weight was significantly different in *O. bimaculatus* of the major rivers, but no significant difference was recorded in the samples from tributaries (Figs. 3:A, B). The oocyte weight was analysed as the highest in samples from River Krishna (0.58 ± 0.11) and lowest in those from River Godavari (0.28 ± 0.06) (Fig. 3:A). It was the highest in the tributary Chambal River (0.53 ± 0.09) and the lowest in fish from Ramganga River (0.38 ± 0.04) (Fig. 3:B).

Correlations between different reproductive parameters were seen separately in samples of the major rivers and their tributaries (Tables 1, 2; Figs. 4, 5). Equation of regression showed linear line relationship in fecundity versus ovarian protein (Figs. 4A, 5A), ovarian protein versus oocyte weight (Figs. 4B, 5B), ovarian protein versus condition factor (Figs. 4C, 5C), oocyte diameter versus condition factor (Figs. 4D, 5D) and oocyte diameter versus oocyte weight (Figs. 4E, 5E) in both major rivers and their tributaries. That was further analysed by Pearson correlation and among different major rivers, highest correlation was recorded in fecundity versus ovarian protein, oocyte diameter versus condition factor, and fecundity versus acute weight. Moderate correlation was recorded for fecundity versus oocyte diameter, ovarian protein versus oocyte weight, and oocyte diameter versus oocyte weight. The least correlation was recorded between ovarian proteins versus condition factor (Table 1).

Among different tributaries of major rivers, highest correlation was recorded for fecundity versus ovarian protein (similar to major rivers). Moderate correlation was recorded for fecundity versus oocyte diameter and oocyte weight, as well as ovarian protein versus oocyte weight (Table 2).

In the present observation, when correlation data of major river were compared with their tributaries, the value of r^2 for ovarian protein and fecundity correlation was found to be higher 0.919 in tributaries as compared to major rivers i.e. 0.721. The correlation coefficient between ovarian protein vs fecundity of major rivers and tributaries was found to be highly significant at $p < 0.01$. The ovarian protein and oocyte weight correlation ($r^2 = 0.241$; 0.22) showed a significant correlation at 0.05 level in tributaries and major rivers. The correlation coefficient ($r^2 = 0.066$; 0.015) between ovarian protein and condition factor of tributaries and major rivers was not significant at 0.05 level. Hence it was concluded from the present observation that ovarian protein appeared to increase with the increasing fecundity and oocyte weight of fish sampled from major rivers and tributaries.

Table 1: Pearson Correlations of protein concentration (mg mL⁻¹ 100mg⁻¹ tissue weight), condition factor, fecundity (100 gm BW), oocyte weight (mg) and oocyte diameter (mm) of *Ompok bimaculatus* of different major Indian rivers.

	Condition factor	Fecundity	Protein concentration	Oocyte weight	Oocyte diameter
Condition factor	1.000***	-0.046	-0.257*	-0.007	0.650**
Fecundity	-0.046	1.000***	0.958***	0.589***	0.345**
Ovarian protein concentration	-0.257*	0.958***	1.000***	0.469**	0.116
Oocyte weight	-0.007	0.589***	0.469**	1.000***	0.485**
Oocyte diameter	0.650***	0.345**	0.116	0.485**	1.000***

Values are expressed as mean ± SD. Asterisk (*) represents significance level ($p < 0.05$) in Pearson correlation (***= highest, **= moderate, *= least).

Table 2: Pearson Correlations of protein concentration (mg mL⁻¹ 100mg⁻¹ tissue weight), condition factor, fecundity (100 gm BW), oocyte weight (mg) and oocyte diameter (mm) of *Ompok bimaculatus* of different major Indian rivers tributaries.

	Condition factor	Fecundity	Protein concentration	Oocyte weight	Oocyte diameter
Condition factor	1.000***	0.017	-0.122	0.062	0.121
Fecundity	0.017	1.000***	0.849***	0.329	-0.364
Ovarian Protein concentration	-0.122	0.849***	1.000***	0.491**	-0.020
Oocyte weight	0.062	0.329**	0.491**	1.000***	0.139
Oocyte diameter	0.121	-0.364**	-0.020	0.139	1.000***

Values are expressed as mean±SD. Asterisk (*) represents significance level ($p < 0.05$) in Pearson correlation (***= highest, **= moderate, *= least).

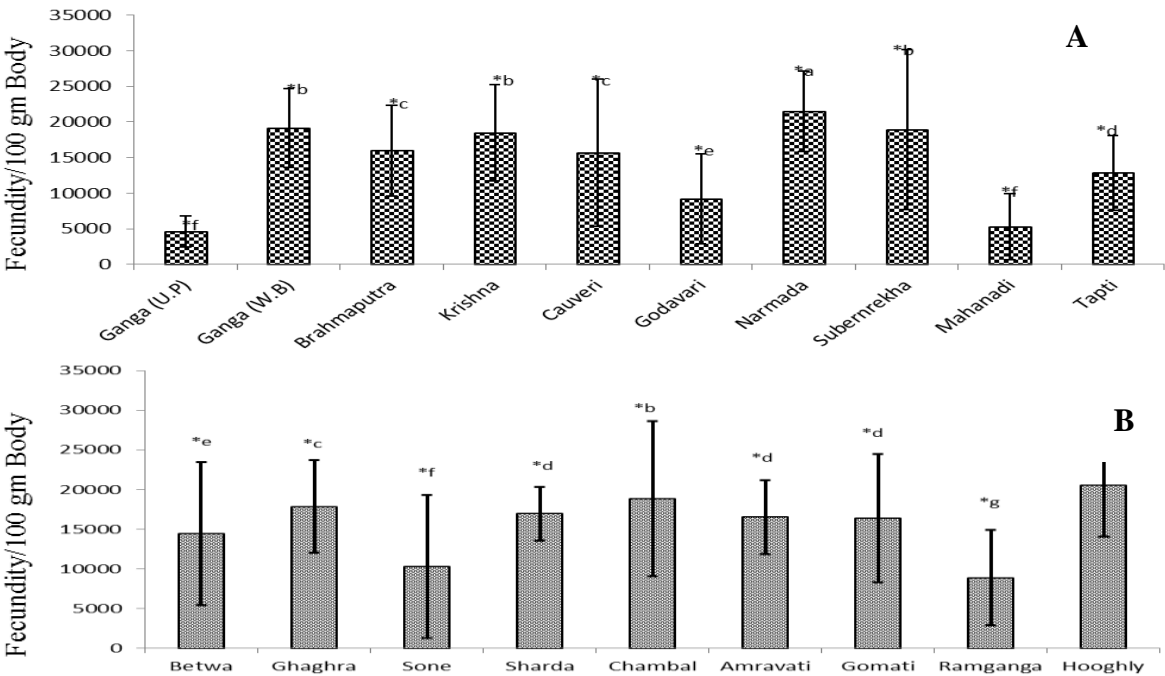


Figure1: Showing Fecundity 100 gm⁻¹ BW of *Ompok bimaculatus* Asterisk shows significant at $p < 0.001$ (one way ANOVA). River bars superscript with same letter shows non significant data (Newman Keul's, $p < 0.05$) whereas different letter shows significant difference among rivers. A: Indian major rivers, B: Indian tributaries.

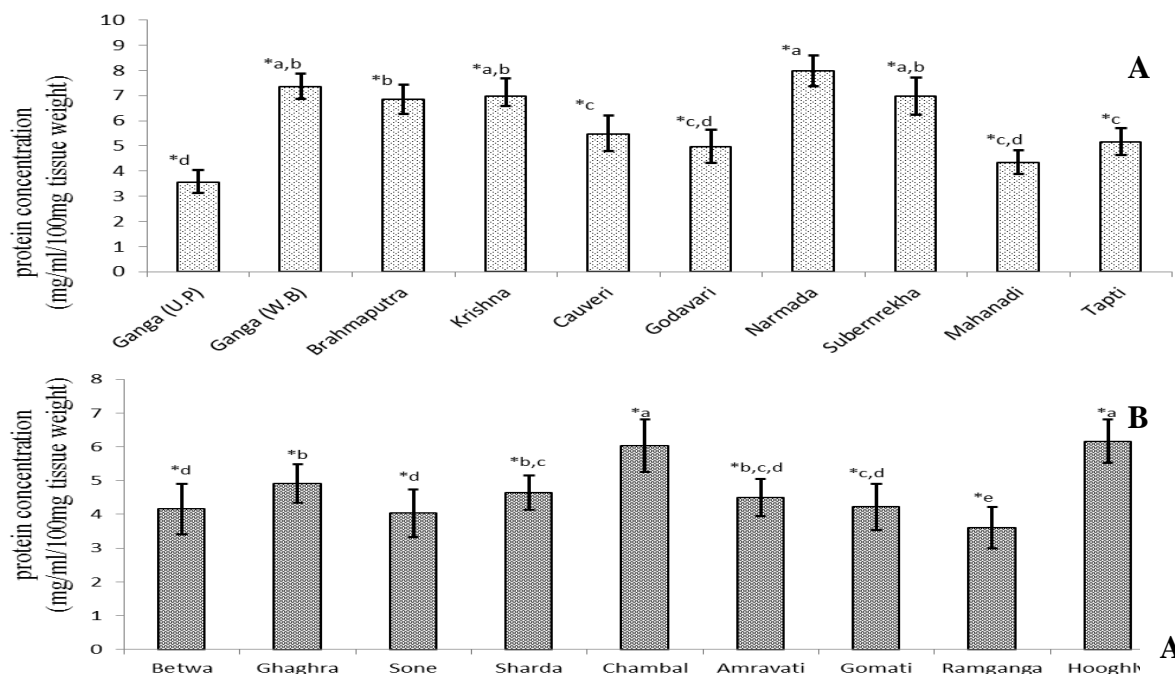


Figure 2: Showing geographical variations of *Ompok* in relation to protein concentration (mg mL^{-1} 100mg^{-1} tissue weight). Asterisk shows significant at $p < 0.001$ (one way ANOVA). River bars with same letter superscripts show no significant differences (Newman Keul's, $p < 0.05$) whereas different letters show significant differences among rivers. A: Indian major rivers, B: Indian tributaries.

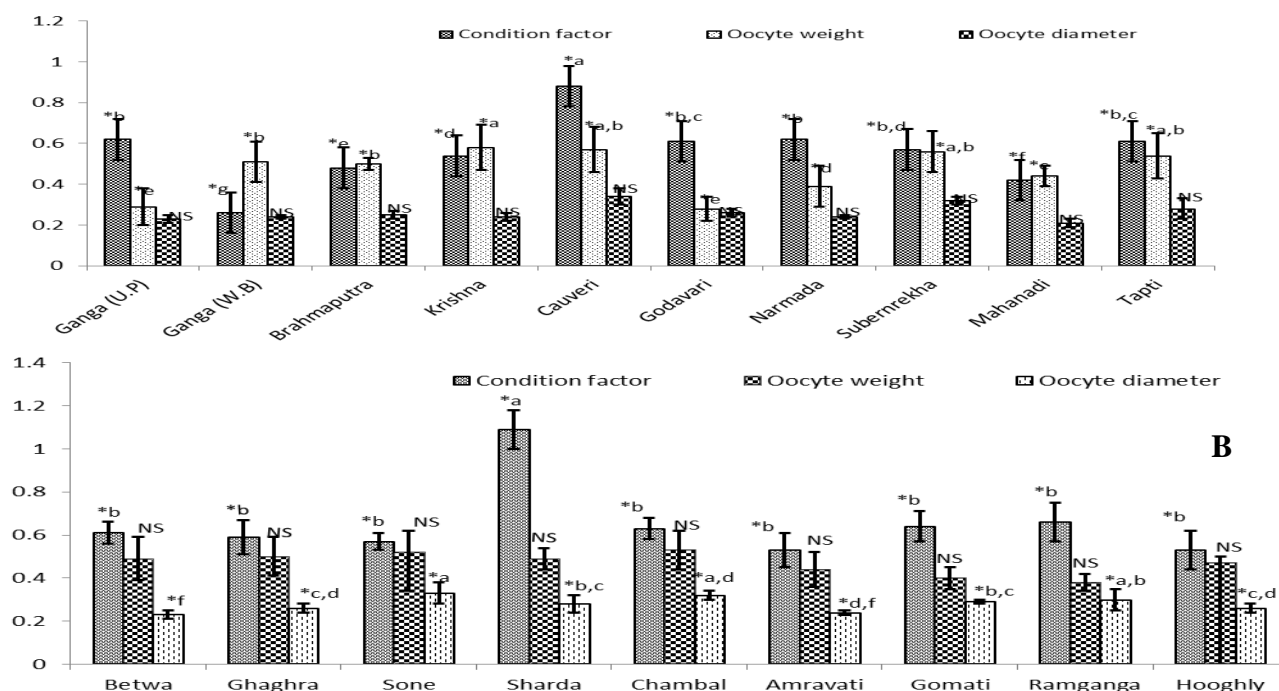


Figure 3: Showing geographical variations of condition factor, oocyte weight (mg) and oocyte diameter (mm) of *Ompok bimaculatus*. Data were significant at $p < 0.001$ (one way ANOVA). Rivers with same letter superscripts show no significant differences (Newman Keul, $p < 0.05$) whereas different letters show significant difference among rivers. A: Indian major rivers, B: Indian tributaries.

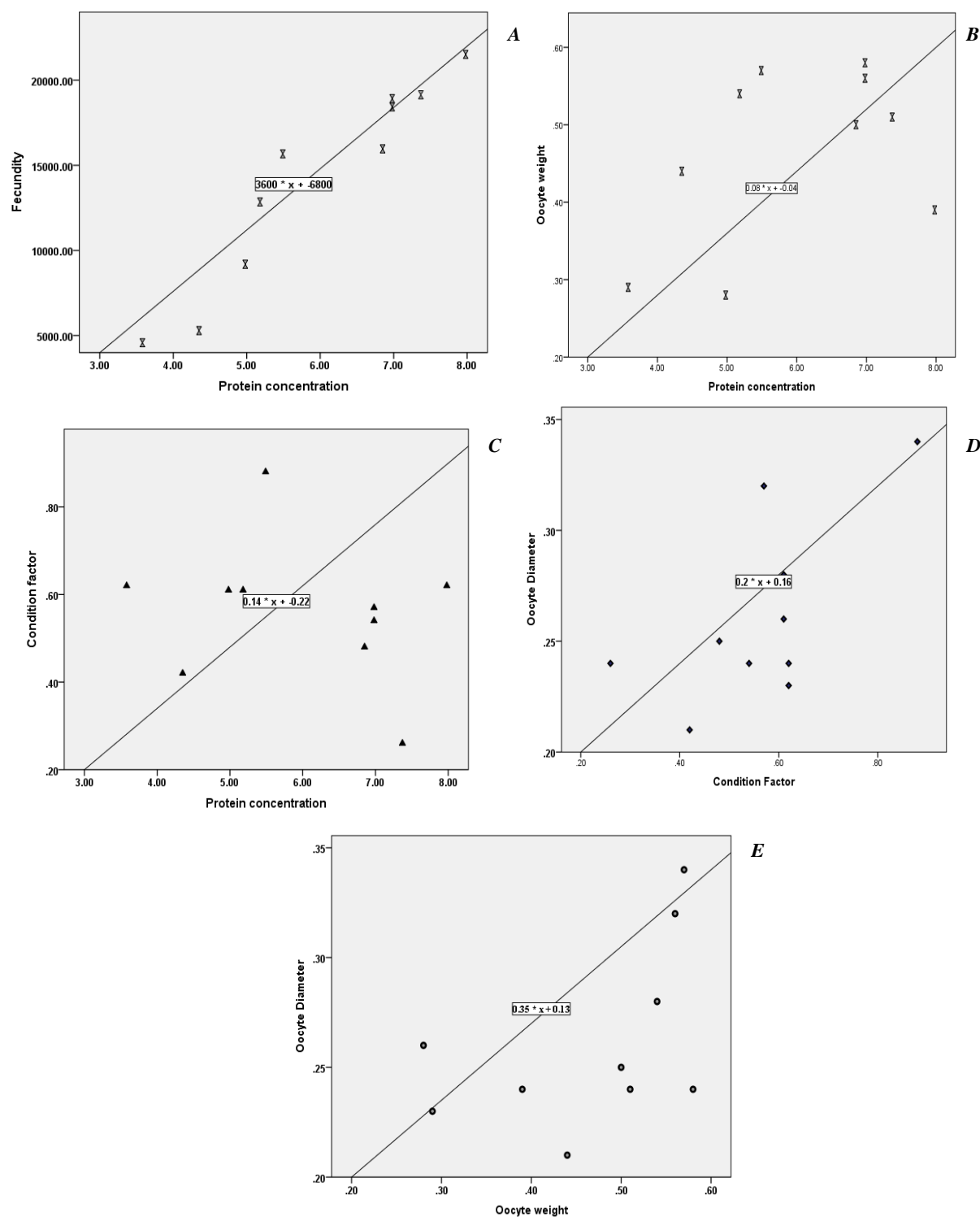


Figure 4: Showing correlations between different parameters of *Ompok bimaculatus* of different major Indian rivers: A- correlation between protein concentration and fecundity, B- correlation between protein concentration and oocyte weight, C- correlation between protein concentration and condition factor, D- correlation between condition factor and oocyte diameter, E- correlation between oocyte weight and oocyte diameter.

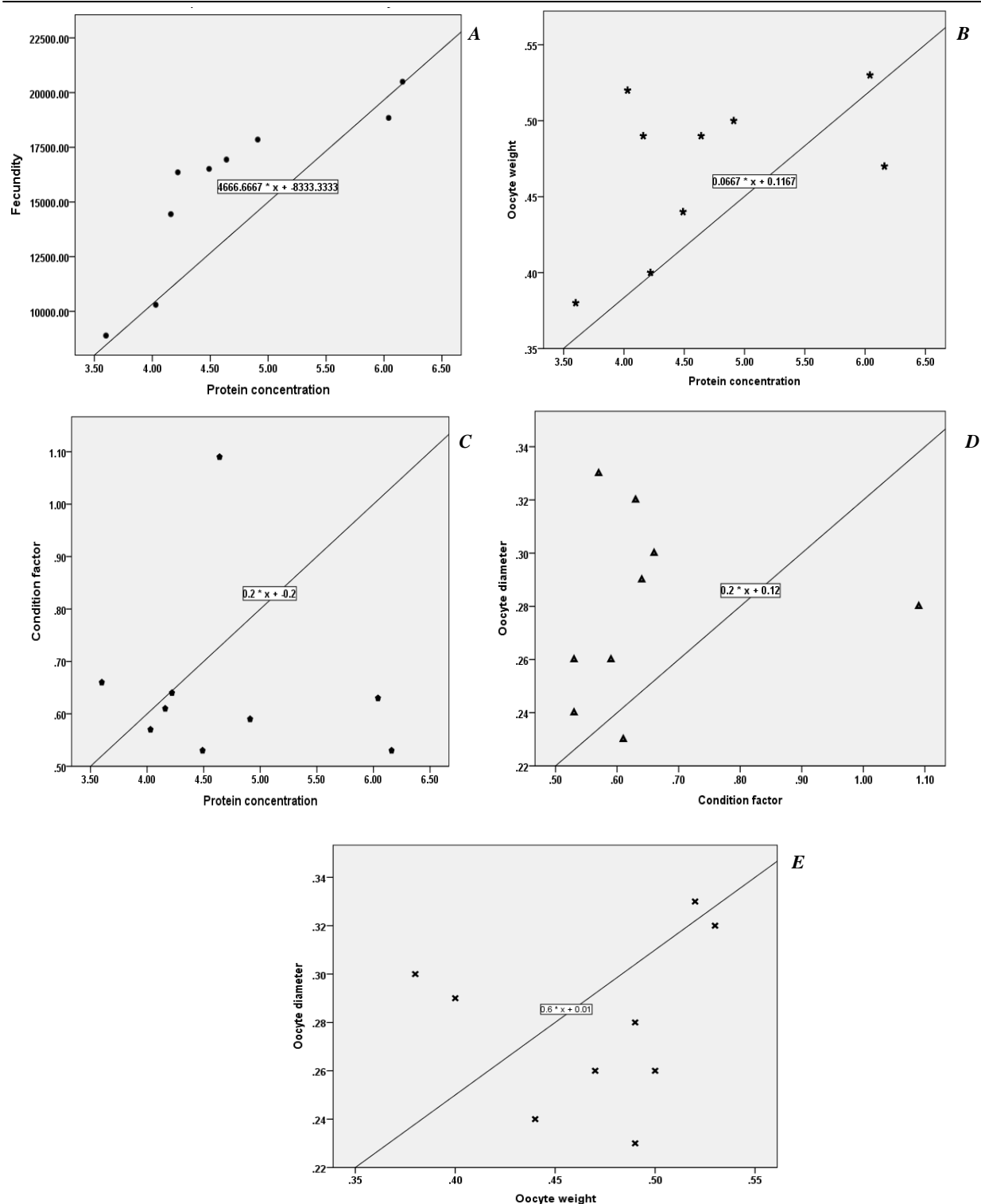


Figure 5: Showing correlations between different parameters of *Ompok bimaculatus* of different Indian tributaries of major rivers: A- correlation between protein concentration and fecundity, B- correlation between protein concentration and oocyte weight, C- correlation between protein concentration and condition factor, D- correlation between condition factor and oocyte diameter, E- correlation between oocyte weight and oocyte diameter.

Discussion

The present analysis of reproductive biology of *O. bimaculatus* assists in elucidating that different aquatic habitats and environmental conditions influence reproductive performance of fish as they affect the maturity of gonads. Due to differences in geographical conditions of water bodies this fish shows differences in breeding performance. The results showed that in different habitats a single fish species in the same season can show significant differences in fecundity, ovary protein levels, condition factor, oocyte weight and its diameter. By comparing all parameters we can submit an idea of the best strain of *O. bimaculatus* in favour of successful selective captive breeding.

Results from a correlation study reflect that value of r^2 was higher in tributaries as compared to major rivers. The major rivers follow their path from various anthropological activities therefore, they assemble more pollution in them. Among tributaries, water level is seasonal, in summer they are reduced or even dry and during the rainy season they remain flooded. Therefore, the level of pollution is variable or less in the tributaries that improves fish reproductive performance as well (Goldara and Banerjee, 2004).

In the present study it was noticed that in different wild populations of *O. bimaculatus*, the ovarian protein concentration was varied considerably in the different rivers and had a linear correlation with fecundity. The building up of the gonad is always accomplished at the expense of body protein (Love, 1970). The fecundity

was studied for capture management and potential of stock regarding the number of eggs laid by the female during the spawning season. During the spawning phase (i.e. late June to August) protein concentration was increased reaching a maximum which was attributed to lower metabolic activity. The increased protein content in the muscle was also attributed to increment with gonad maturity (Macay and Tunison, 1936; Bano, 1977). Increase in protein content of muscle with the maturation of gonads was the result of active feeding in the pre-spawning phase. Therefore, the protein cycle in fishes can be synchronized with maturity of fishes than with feeding (Shreni, 1980) as a decline in muscle protein is reported with the growth of the gonads (Srikar *et al.*, 1979; Somavanshi, 1983; Luzzana *et al.*, 1996).

The condition factor (K) is an index reflecting the interactions between biotic and abiotic factors in the physiological condition of the fishes. It shows the well-being of the population during various life cycle stages (Koops *et al.*, 2004; Oso *et al.*, 2011). It is positively related to gonadal protein. In the present study, condition factor did not show any significant correlation with fecundity in any river sample. Though value wise condition factor was highest in the Major River Cauveri and its tributary Sharda, both lay under high to moderate category for absolute fecundity and ovarian protein parameter. It suggests that a better biological condition doesn't always support reproductive performance (Oso *et al.*, 2011). This may be due to differences in the state of maturity and availability of fish food organisms or better

the nutritional condition in the riverine ecosystem.

Oocyte weight and diameter may be used as a predictor of developmental stage (Gomes *et al.*, 2011). Both have shown significant high to moderate relationship with fecundity in all collected samples. Oocyte diameter may be used on its own to measure development, but gives little information on the physiological status of the ovaries as it showed no correlation with ovarian protein (El-Sayed *et al.*, 2003). Results show that ovarian protein concentration is an important factor that can be correlated with fecundity, condition factor, oocyte weight and oocyte diameter. The factors that improve ovarian protein concentration will improve fish fecundity, condition factor as well as oocyte weight and oocyte diameter. If oocyte weight and diameter improve it will influence condition factor of fish as well.

On the basis of obtaining the results of reproductive parameters in *O. bimaculatus* from 19 rivers, fish can be divided into high absolute, moderate absolute and low absolute category though results of different parameters from varied geographical rivers, overlap each other. Fecundity and ovarian protein level also show resemblance in different rivers and their tributaries. *O. bimaculatus* from the major rivers Narmada and Ganga (W.B) and tributaries Hooghly and Chambal showed the best fecundity and ovarian protein. So these are the suggested sites for best brooders among the rivers surveyed. *O. bimaculatus* of Cauveri River and Sharda tributary was high absolute for condition factor, and oocyte diameter,

therefore suggested as best edible strain and early onset of maturity.

It can be concluded from the present study that the aquatic atmosphere of the local area plays an important role in the species specification that can affect their reproductive performance as well as their survival. Future studies should address the spatio-temporal pattern and linking biological and molecular attributes of the species to ensure adequate evaluation and conservation of this species.

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