ISSN: 2347-5129
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.352
IJFAS 2016; 4(3): 449-457

www.fisheriesjournal.com
Received: 23-03-2016
Accepted: 24-04-2016

Abha Mishra
Department of Applied Animal Sciences, Babasaheb Bhimaro Ambedkar University, Lucknow, U.P., India.

Anurag Rawat
Department of Applied Animal Sciences, Babasaheb Bhimaro Ambedkar University, Lucknow, U.P., India.

Sneha Verma
Department of Applied Animal Sciences, Babasaheb Bhimaro Ambedkar University, Lucknow, U.P., India

Uttam Kumar Sarkar
CIFRI, Barrackpore, Kolkata, India.

Ravindra Kumar
National Bureau of Fish Genetic Resources, Canal Ring Road, Lucknow, U.P. India

Correspondence
Abha Mishra
Department of Applied Animal Sciences, Babasaheb Bhimaro Ambedkar University, Lucknow, U.P., India.

Spawning dynamics of female freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794) in different major rivers of India

Abha Mishra, Anurag Rawat, Sneha Verma, Uttam Kumar Sarkar and Ravindra Kumar

Abstract
The pattern of reproductive responses depends on unevenness in the thermal regimes of different Indian River. The objective of this study was to investigate the reproductive strategies of butter catfish *Ompok bimaculatus* in wild populations of Indian major rivers (Brahmaputra, Cauvery, Ganga, Godavari, Krishna, Mahanadi, Narmada, Subarnarekha, and Tapti). Changes in gonado-somatic index (GSI), ovarian protein, fecundity and ovarian histology were observed in preparatory, pre-spawning and spawning phase of reproductive cycle. The fecundity was ranged minimum to maximum in Godavari to Narmada. GSI, fecundity and ovarian protein concentration distributions were correlated significantly ($P < 0.05$) by linear regression analysis. The histological assessment showed that ovaries exhibited seven stages of oocyte development, which were oogonia, chromatin nucleolar, early perinucleolar, yolk granules, late perinucleolar, vitellogenesis and vitellogenic oocyte stages in all the three phases of reproduction. The observation of fish sampled from different rivers showed that Narmada River has better adaptive condition for growth and breeding of *O. bimaculatus* in comparison of others rivers.

Keywords: *Ompok bimaculatus*, GSI, ovarian protein, fecundity, histology.

1. Introduction
The *Ompok bimaculatus* (Siluriformes: Siluridae) is an indigenous freshwater fish species commonly known as the "butter catfish". It is widely distributed throughout the Indian sub-continent and South East Asia including India, Pakistan, Afghanistan, Myanmar, Thailand, Java, Sumatra, Borneo and China [35]. The butter catfish inhabits plains and sub mountain regions, and is also found in rivers, lakes, tanks and ponds. The population of this species has seriously declined due to over-exploitation of natural resources and competition from alien species, wide use of pesticide and insecticides from agricultural area and restricted breeding in captivity [14, 17, 31, 34].

The major river leads to a progressive ecosystem degradation due to uncontrolled anthropological activities [11]. These toxic compounds are transferred to the biota through the food chain. In order to correlate the environmental health of the ecosystems of major rivers, the female *Ompok bimaculatus* fish species was monitored for different reproductive parameters. Among the main aspect that comprises the reproductive strategy of fish species are GSI, ovarian protein concentration, fecundity and ovarian morphology. Understanding of these aspects can be considered the first step in establishing the principal life-history patterns of fish species [26]. This will help in observing the environmental stress and long term negative effects on population. The gonado-somatic index is particularly useful since it is extremely influenced by generic stressors due to a number of environmental variations. GSI expresses either gonadal recrudescence or gonad growth from the regressed state to full maturity. Abnormal index values could in fact be associated to ecological disturbance or environmental changes [8]. GSI is an important indicator of reproductive periodicity and is also used for gonadal maturation determination [19]. Fecundity plays a key role in assessment of fish stock production, management, conservation and provides a complete picture of population dynamics [32]. Fecundity, GSI and ovarian protein level are important to estimate the reproductive potential of a species in relation to their size and environmental conditions in which they live [3, 8, 16].

The ovarian morphology show signs of interaction between pollution and physiological processes [2, 15, 18, 36]. The ovarian developmental study is very vital for understanding the fish culture and conservation. It occurs in three phases which are the primary growth phase, secondary growth phase and maturation phase [9, 12]. Very few studies have been made on the ecology in relation to the fisheries of the river system [6, 7, 33].

Information on the ovarian development of *O. bimaculatus* is scarce. Thus the objective of this study was to evaluate whether biological differences of reproductive responses in the observed fish species between the different Indian major rivers could be useful in defining habitat, geographical and ecological situations and conservation strategies of freshwater ecosystems. This study would provide useful information on its spawning pattern and strategy, which is very important for sustaining the aquaculture, management and conservation of *O. bimaculatus* in the country.

2. Materials and methods

2.1 Chemicals

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

2.2 Study Area

Nine major rivers viz. Brahmaputra, Cauvery, Ganga, Godavari, Krishna, Mahanadi, Narmada, Subarnarekha, and Tapti were selected to trace the reproductive pattern of butter cat fish, *Ompok bimaculatus* (Fig.1). These rivers are distributed in four climatic zones based on the rainfall viz., heavy rainfall with more or less moderate temperature, heavy seasonal rainfall with persistent high temperature, indiscrete rainfall with fluctuating temperature in summer and winter, very low rainfall with high temperature fluctuation in summer [13].

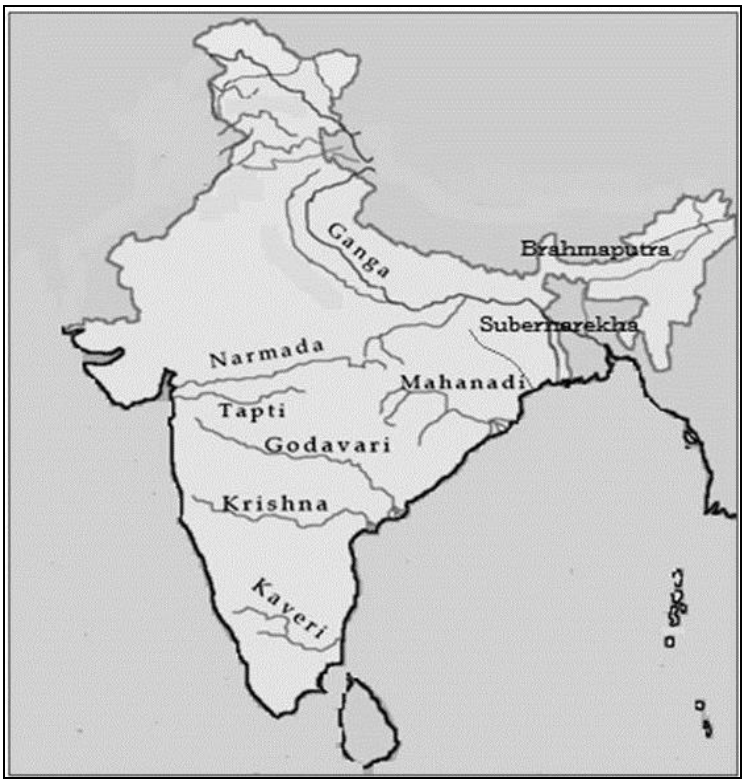


Fig 1: Geographical location of Indian Major rivers, Brahmaputra, Cauveri, Ganga, Godavari, Krishna, Mahanadi, Narmada, Subemrekha and Tapti taken under survey in present Study.

Table 1: Different collection sites and GPS co-ordinates of different Indian Major rivers

Rivers	Collection Sites	GPS Coordinates
Brahmaputra	Guwahati	N 25º13'24" E 89º41'41"
Cauveri	Mysore, Menakshipuram	N 11°21 ′40″ E79°49′46″
Ganga	Kanpur, Varanasi, Farakka	N 26°30′28″ E 80°19′01″ N 24°48'58" E 87°55'55"
Godavari	Nirmal	N 19º55'48" E 73º31'39"
Krishna	Vijaywada, Rajamundri	N 17°55′28″ E 73°39′36″
Mahanadi	Chattisgarh	N 9°11′50″ E 99°22′57″
Narmada	Hosangabad, Jabalpur	N 21º39'3.77" E 72º48'42.8"
Subarnarekha	Ranchi	N 21°33′18″ E 87°23′31″
Tapti	Surat	N21º14'53.67"E 73º35'21.87"

2.3 Animal 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.

Minimum 10 number of the freshwater fish *O. bimaculatus* were collected from different sites of Indian major rivers (Brahmaputra, Cauveri, Ganga, Godavari, Krishna, Mahanadi, Narmada, Subernrekha, Tapti) in three reproductive phases

viz. preparatory (March-April), pre-spawning (May June) and spawning (July august) phases during year 2012-2013 (Table 1, Fig. 1). The weight and length were recorded in the sampling area. The ovary weight was also recorded at the sampling site. For the estimation of fecundity, the ovary of the five matured female fish from different sites were taken out and preserved in isopropyl alcohol (50%) to bring in the laboratory. From the remaining five fish, part of ovary tissue was fixed in Bouin's solution for histological purpose and rest of ovary tissue was kept in a chill-box (0 ^{0}C) properly to bring in the laboratory. In the laboratory tissue were kept in deep fridge (-20 ^{0}C) till ovarian protein estimation.

2.4 GSI determination
The Gonadosomatic index (GSI) were determined by simply dissection of the gonad and weighed using the digital balance (Shimadzu-AY220). The GSI was calculated as:
GSI= weight of gonad x 100/ body weight

2.5 Estimation of ovarian protein concentration
Ovarian protein concentration was estimated by the method of (1951) [24] method using crystalline Bovine Serum Albumen (BSA) as standard with a spectrophotometer (UV-Thermo).

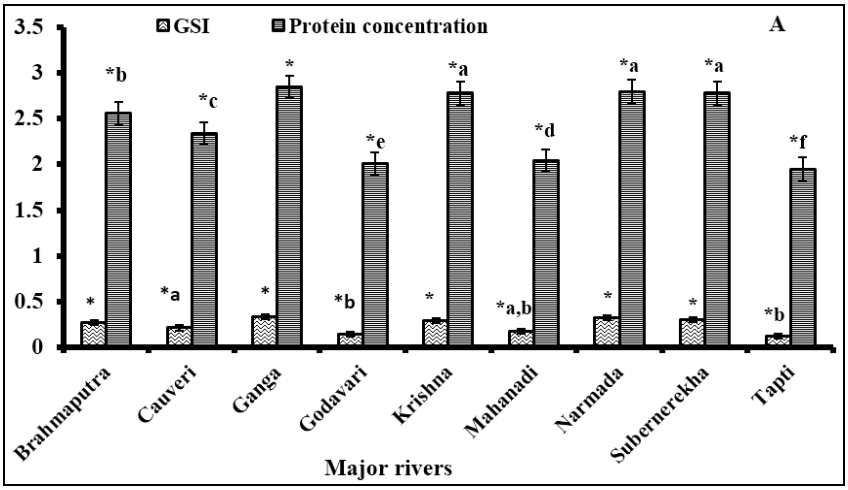
2.6 Estimation of fecundity
In laboratory ovary of the matured female fish were taken out from isopropyl alcohol (50%) and with the help of blotting paper, the moisture content of ovary was removed. The gonadal weight was recorded in the fine digital balance. Then 0.01 g of each ovary was taken out separately from anterior, middle and posterior regions of each ovarian lobe. The fecundity was estimated by the methodology of [22]:
Fecundity = No. of Eggs in the ovary Sample x Gonad Weight / Ovary Sample Weight.

2.7 Ovarian histological processing
Bouin's fixed ovaries were embedded in paraffin. The sections were cut at 7µm and staining was done using Harris' haematoxylin and eosin. Microphotographs were taken with bright field microscope (Olympus CX41, Japan). The microscopic developmental stages of oocyte were categorised according to Janseen *et al.* (1995) [28]. The oocyte diameter of each developmental stage of different Indian major rivers from each reproductive phase was measured at 40x magnification with the micrometer of image analysis software (Magnus Pro).

2.8 Statistical analysis
Data were expressed as the mean ± S.E. The significance for each parameter was analysed by one way analysis of variance (ANOVA) at $P < 0.001$. Newman keul Test at $P < 0.05$ were used to determine significance deference among sampling sites. To analyze interdependency of the parameters, Pearson correlations, was done by using software IBM SPSS 20. When the correlation value is > 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.

3. Results
Sampling were done on three different reproductive phase, preparatory, prespawning and spawning in nine major rivers of India. Results showed that *O. bimaculatus* more or less registered same type of developmental stages in different wild population from major rivers. Then also due to difference in their local habitat i.e. river conditions wild population of *O. bimaculatus* registered difference in protein level and their fecundity as well. This study supported the importance of local environment in the reproductive performance of same species.

3.1 GSI, ovarian protein and fecundity among different Wild River
The GSI ranged from 0.12 to 0.33 in preparatory phase, 0.34 to 0.48 in prespawning phase and 2.35 to 14.79 in spawning phase of *O. bimaculatus* in all major rivers. The GSI showed a significant difference in different reproductive phases and sampling areas of same season for females ($P < 0.001$; preparatory phase: F = 125, prespawning phase: F = 25 and spawning phase: F = 89616.67). The results showed that the samples collected from Ganga River in preparatory season and Narmada River in prespawning and spawning phase registered a higher value of GSI (Fig. 2A, B; 3A).
The ovarian protein level of studied fish sampled from different major rivers varied in respect to rivers and with seasons as well (Fig. 2A, B; 3A; from 1.05 to 2.85 mg/ml/100mg tissue weight in preparatory phase, 3.1 to 3.96 mg/ml/100mg tissue weight in pre-spawning phase and 4.35 to 7.98 mg/ml/100mg tissue weight in spawning phase). Highest protein concentration was observed from Ganga (in preparatory phase) and from Narmada (in prespawning and spawning phase). Protein concentrations were low in case of Mahanadi and Godawari as in case of GSI. It showed a significant difference among reproductive phases and sampling areas (Fig. 2A, B; 3A).



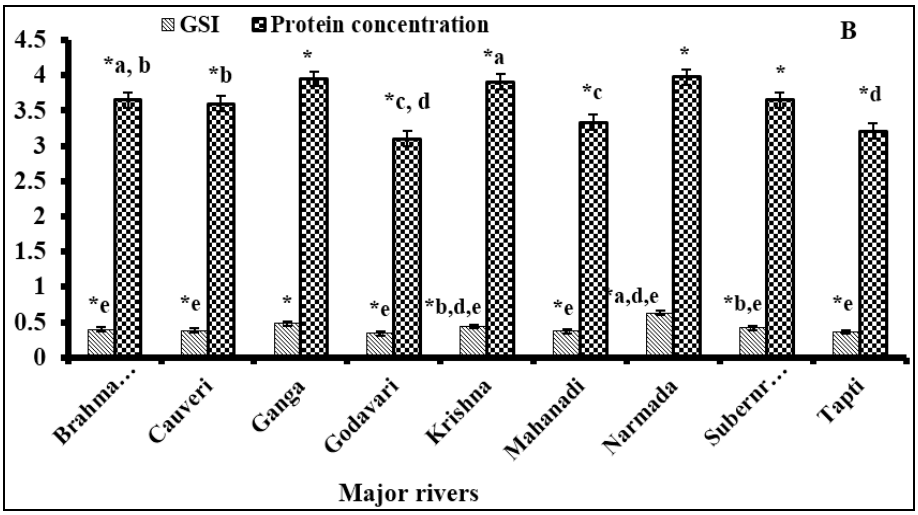


Fig 2: Showing geographical variations of *Ompok bimaculatus* of different Indian major rivers in relation to ovarian protein concentration *(*mg/ml/100mg tissue weight), GSI in preparatory season (A) and prespawning season (B). Data expressed in mean ± SEM. Asterisk shows significant at $P < 0.001$ (one way ANOVA). The bars superscripted with same letter show no significant data (Newman Keul's, $P < 0.05$) whereas different letter shows significant difference among major rivers.

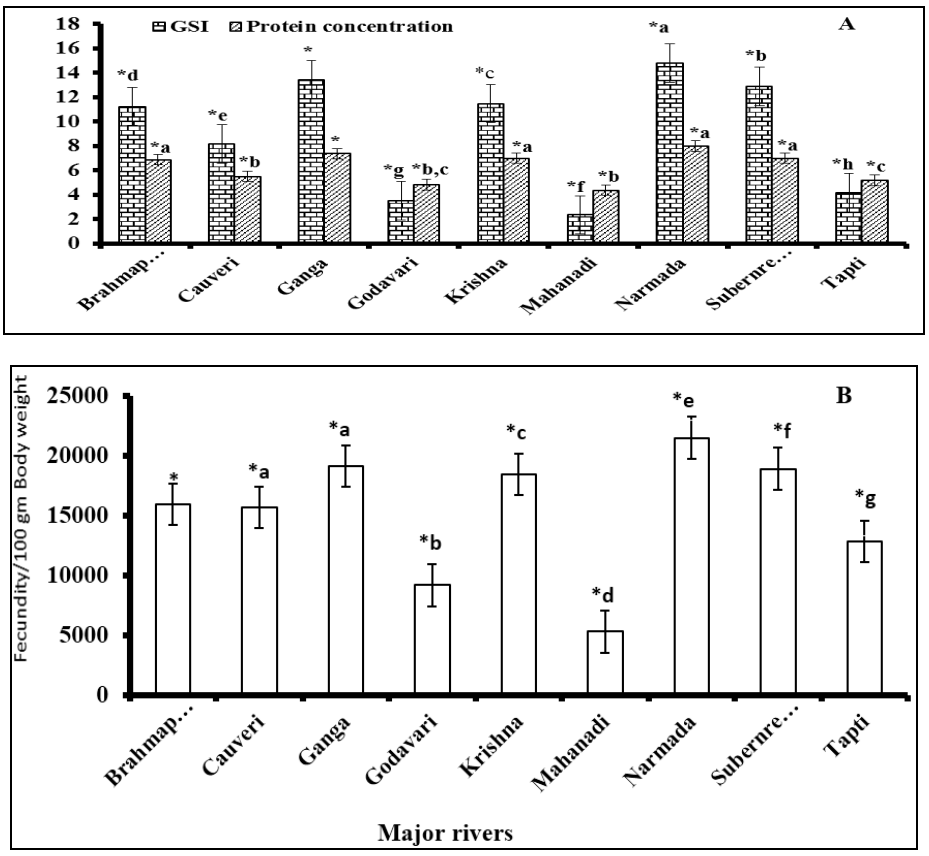


Fig 3: Showing geographical variations of *Ompok bimaculatus* of different Indian major rivers in relation to ovarian protein concentration *(*mg/ml/100mg tissue weight), GSI and protein concentration (A) and fecundity (B) in spawning season. Data expressed in mean ± SEM. Asterisk shows significance at P <0.001 (one way ANOVA). The bars superscript with same letter shows no significant data (Newman Keul's, $P < 0.05$) whereas different letter shows significant difference among major rivers.

The fecundity of female *O. bimaculatus* was highest recorded from Narmada river (21512.57/100gm body wt) and lowered from Mahanadi river (5293.23/100gm body wt) among the sampled *O. bimaculatus* (Fig. 3B).

3.2. Relationship between GSI vs Protein concentration vs Fecundity

The Pearson's correlation relationship between GSI and protein concentration in preparatory, prespawning and spawning phase was found to be linear. The linear relationship reflects that on increase in GSI, ovarian protein content was increasing so as the fecundity in their respective phase. The correlation coefficient (r) between these parameters in preparatory, prespawning and spawning phase was 0.84, 0.391 and 0.965 respectively ($P < 0.001$) (Table 2).

The pearson's correlation showed a significant correlation between fecundity and GSI ($r = 0.903$; $P < 0.001$). Fecundity vs ovarian protein concentration was also showed a highly

significant correlation (r = 0.886; $P < 0.001$ level). Hence it was concluded from the present observation that fecundity appeared to increase with increasing GSI and ovarian protein concentration in spawning season (Table 2).

Table 2: Correlations of GSI, protein concentration and fecundity of *O. bimaculatus* of different Indian major rivers. (*) represents significance level in Pearson correlation (***=highest, **= moderate, *= least)

Season	Correlations		Protein concentration
Preparatory phase	GSI	Pearson Correlation	.917***
		Sig. (2-tailed)	.001
Prespawning phase	GSI	Pearson Correlation	.625***
		Sig. (2-tailed)	.072
Spawning phase	GSI	Pearson Correlation	.982***
		Sig. (2-tailed)	.000
	Fecundity	Pearson Correlation	. 941***
		Sig. (2-tailed)	.000
			GSI
	Fecundity	Pearson Correlation	.950***
		Sig. (2-tailed)	.000

3.3. Comparison of follicle stages among different Rivers

According to maturity stages, ovaries change in size, color, and turgidity. Macroscopic analysis showed that the resting ovaries presented small sizes and a gelatinous-semitransparent appearance, while advanced maturing and mature ovaries had a vascularised dark brownish one (Table 3).

The ovary of preparatory phase was grossly characterized as two lobed, small sizes, slightly pinkish and transparent. Histologically, the ovary showed that it largely contained the oogonium and peri-nucleus stages in this period. But some of the oocytes were also contained small amount of yolk vesicles during this phase. These early oocytes contained a larger central nucleus. The histological sections of ovary represented that the most of the oocytes were in early peri-nucleus follicle stage, whereas the late peri-nucleus follicle stages were also distinguishably observed in few of the Rivers viz., Narmada, Tapti and Mahanadi river (Table 3; Fig. 4, 5A). At the maturing stage or prespawning phase, an increase in ovarian volume observed. It occupied one-fourth of the ventral cavity and increasingly vascularised. The oogonia were rounded and prominent nucleus was present. Ovigerous folds and yolk vesicle were also seen. Germinal layer was thin, the previtline cells were seen and oocyte was in oil drop stage. Plate shows large number of VI stage follicles that are in their late perinucleolus stage (Table 3; Fig. 5B, 6). The histological sections of spawning phase revealed that oocyte had full of lipid yolk and protein yolk in centre. It pushed lipid yolk to the periphery. Dark blue color rounded or hexagonal previtellogenic oocytes were present. Nucleus deformed, cytoplasm had abundant protein yolk globules. The ovarian wall was much vascular and thin in the spawning phase of reproduction. Large mature oocyte with full of lipid yolk was noticed. Some of the oocyte showed germinal vesicle. Ovary was filled with large number of germinal vesicle migratory oocyte (Table 3; Fig. 5C, 7).

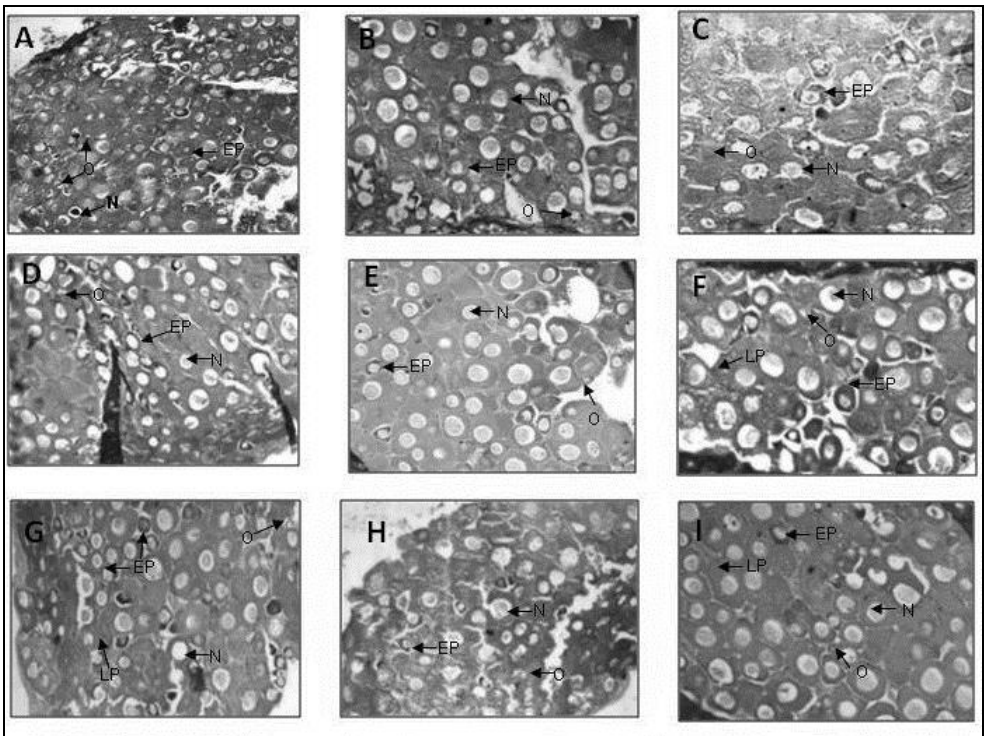


Figure 4: Histological presentation of preparatory season of different Indian major rivers: A: Brahmaputra, B: Cauveri, C: Ganga, D: Godavari, E: Krishna, F: Mahanadi , G: Narmada , H: Subernrekha , I: Tapti. Photomicrograph of ovary represents: EP- early perinucleolus, N- nucleus, O- oogonia, LP- late perinucleolus. Images were acquired at 40x magnification.

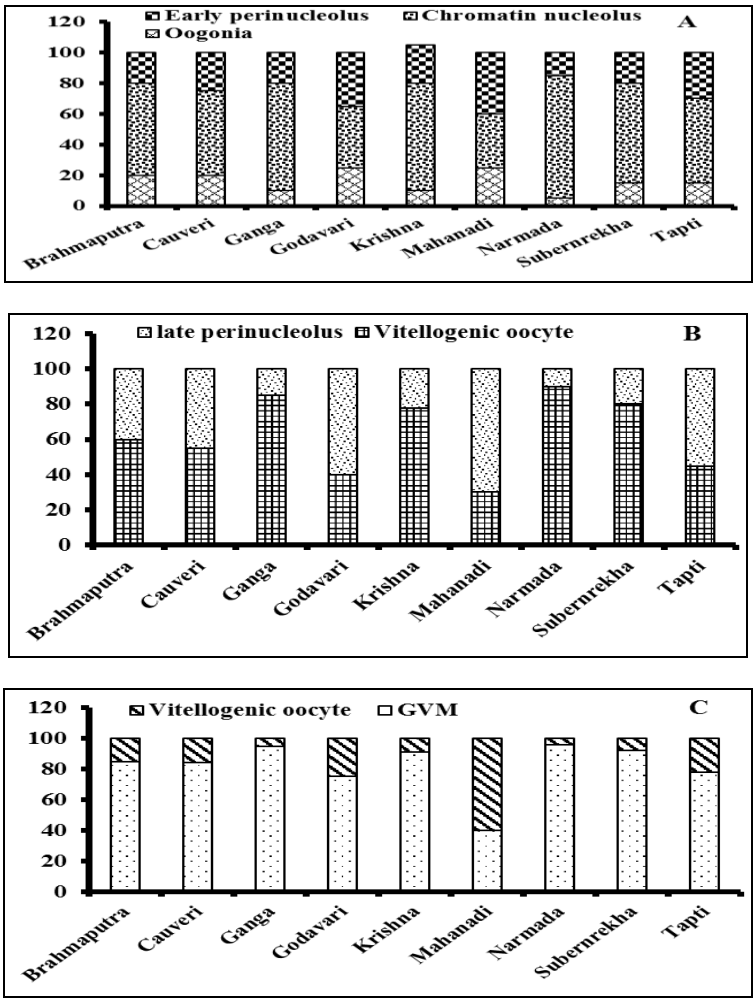


Fig 5: Showing variations of oocyte developmental stages in histological sections in preparatory phase (A), prespawning phase (B) and spawning phase (C) *of Ompok bimaculatus* with different Indian major rivers. GVM: Germinal Vesicle Migratory oocyte.

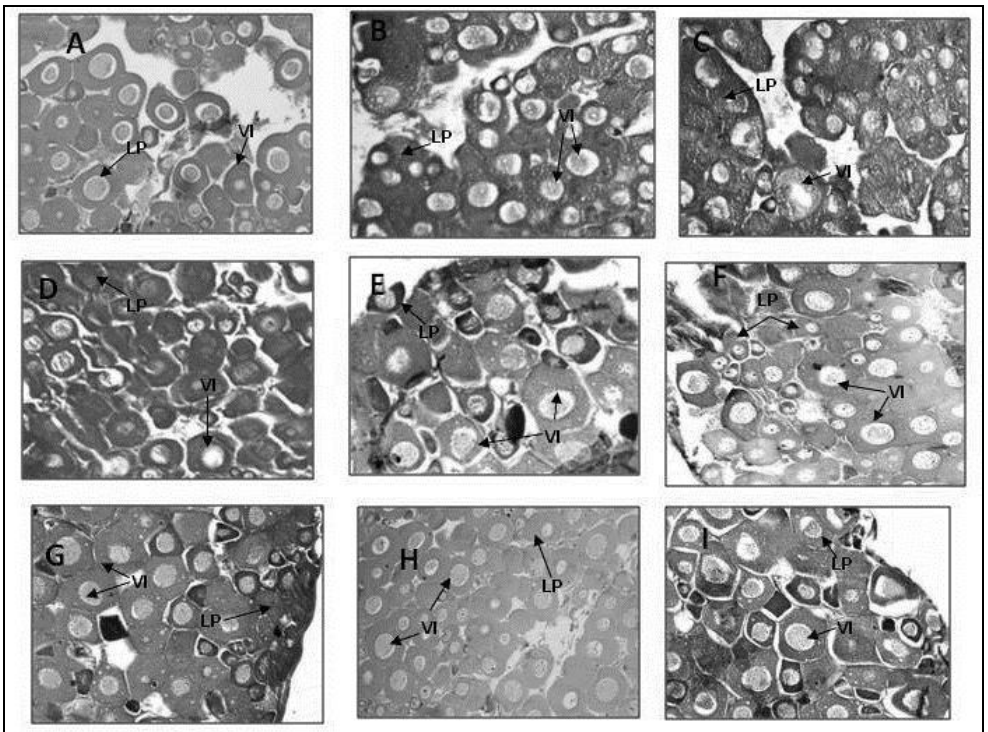


Figure 6: Histological presentation of pre-spawning season of different Indian major rivers: A: Brahmaputra, B: Cauveri, C: Ganga , D: Godavari, E: Krishna, F: Mahanadi, G: Narmada, H: Subernrekha , I: Tapti. Photomicrograph of Ovary represents: LP- late perinucleolus, VI- vitellogenic oocyte. Images were acquired at 40x magnification.

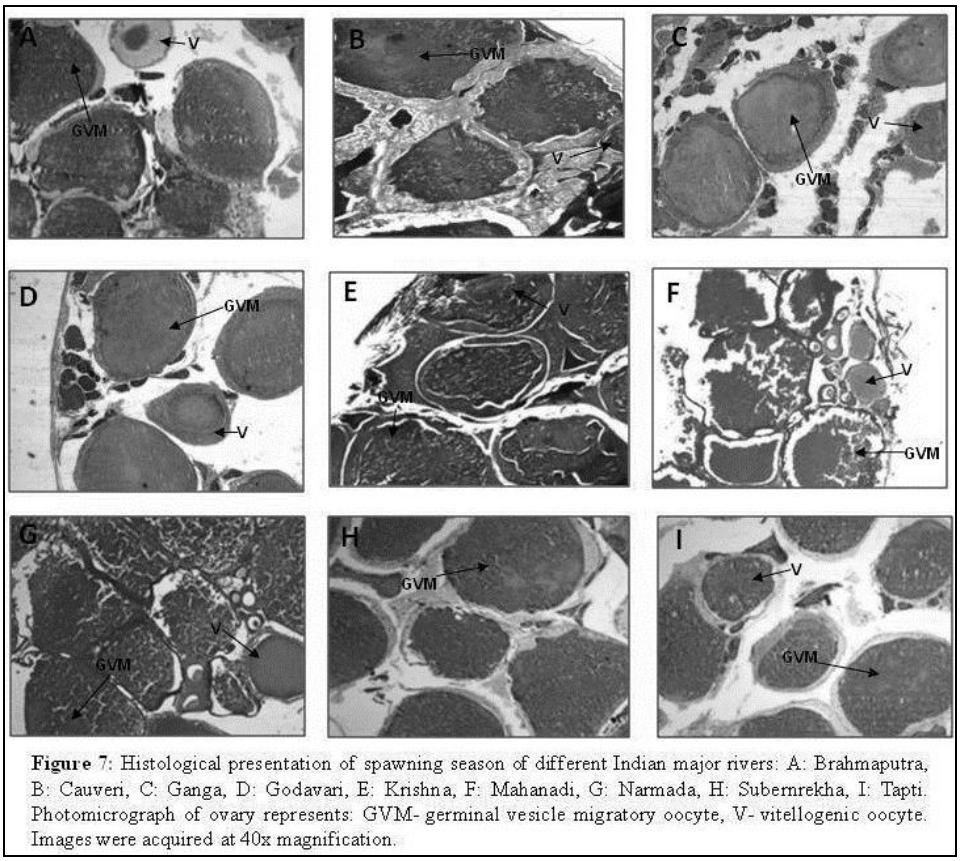


Figure 7: Histological presentation of spawning season of different Indian major rivers: A: Brahmaputra, B: Cauveri, C: Ganga, D: Godavari, E: Krishna, F: Mahanadi, G: Narmada, H: Subernrekha, I: Tapti. Photomicrograph of ovary represents: GVM- germinal vesicle migratory oocyte, V- vitellogenic oocyte. Images were acquired at 40x magnification.

Table 3: Macroscopic and microscopic examination of oocytes and its developmental stages.

Ovarian phase	Macroscopic appearance	Histological observation
Preparatory	Ovary small in size, two lobed, slightly pinkish and transparent	Oogonia, early perinucleolus and chromatin nucleolus stage were seen.
Prespawning	Ovaries occupy one-fourth of the ventral cavity, reddish in color and increasingly vascularised	Oocytes filled with yolk granule, vitellogenic and late perinucleolus oocytes.
Spawning	Highly vascularised, dark brown in color, occupy most of the ventral cavity, large and lobular in appearance, eggs excruted with slight abdominal pressure	Predominance of germinal vesicle migratory oocyte and vitellogenic oocytes.

4. Discussion

The reproductive parameters observed in this study can be attributed to the differences in abiotic factors such as photoperiod, temperature and rainfall as well as biotic factors like food availability and physiological characteristics which are generally considered as determining factors that trigger the reproductive cycle [23]. The present research was undertaken to investigate the fluctuations in gonadosomatic index, ovarian protein level and fecundity of freshwater catfish *Ompok bimaculatus* associated with ovarian histology in preparatory, pre-spawning and spawning season of different Indian major rivers, respectively.

Gonadosomatic index was the major determinant of reproductive development. However its use in examination of reproductive biology is more suitable when it associated with the other markers of reproduction viz., fecundity, ovarian protein, macroscopic and histological observations. A significant relationship was found between different reproductive phases. During the preparatory phase in which the ovary showed reduced size, the GSI was about 0.12 to 0.33. In the prespawning phase, a wide GSI variation was shown (0.36 to 0.63). Such variation is probably due to the oocyte growth and development. In the ripe or spawning stage, GSI reaches the highest values (2.35 to 14.79) due to increased weight of ovary which occupy most of the ventral cavity. GSI also showed higher variations in rainy season than the other season thus further confirming that the breeding or spawning take place during the rainy season.

The ovarian protein level was varied considerably in the different rivers in different reproductive phases and has a linear correlation with fecundity and GSI. The gonad development is always accomplished at the disbursement of body protein [24]. The protein concentration was increased and reaching maximum in the spawning season as compared to other phases which was attributed to lower metabolic activity and increased muscle protein content which is attributed in gonad maturity increment [4, 25].

Fecundity is a life-history trait that can be estimated by the number of oocytes that complete their development and are released in each reproductive period, i.e., reproductive investment. It is a measure of the reproductive potential of fish [10, 28]. The relationship between fecundity and the variables of body weight and gonad weight were linearly positive for the *O. bimaculatus* sampled from different Indian major rivers.

Fecundity exhibited adaptive fluctuations with different habitats which reflect changes in the environment. These variations may be due to food supply, temperature and habitat [27, 30]. The highest value of fecundity and GSI, and correlation between fecundity vs GSI was found in Narmada River, a central Indian major river and the fifth longest river in the Indian subcontinent which indicate that this river has more healthy fish as compared to others. The histological analysis of oocytes in different developmental stages was also similar to those of other teleost reported in the literature [1, 9, 12, 21, 29].

5. Conclusion

To sum up, the obtained results of reproductive parameters in *O. bimaculatus* from 9 major rivers, fish collected from Narmada River had shown best for GSI, fecundity and ovarian protein. So this is suggestive best brooders site among other rivers. Therefore it can be concluded that local environment plays important role in the reproductive success. This study provides important information for brood stock management and conservation measures of *O. bimaculatus* in the freshwater ecosystem.

6. Acknowledgement

Authors are thankful to DBT, New Delhi for financial assistance (BT/PR14628/AAQ/03/537/2010).

7. References

1. Abascal FJ, Medina A. Ultrastructure of oogenesis in the bluefin tuna, *Thunnus thynnus*. Journal of Morphology. 2005; 264:149-160.
2. Adams SM, Bevelhimer MS, Greeley Jr. MS, Levine DA. The SJ. Ecological risk assessment in a large river-reservoir: 6. Bioindicators of fish population health. Environmental Toxicology and Chemistry 1999; 18:628-640.
3. Bagenal TB, Braum E. Eggs and early life history. In: Methods of assessment of fish production in fresh waters. T.B. Bagenal (ED). IBP Handbook 3, Blackwell Scientific, Oxford, 1978.
4. Bano Y. Seasonal variation in the biochemical composition of *Clarius batrachus.* L. Proceeding of the Indian Academy of Sciences 1977; 85(3):147-155.
5. Biswas SP, Borbora SH, Dutta BC. Present status of capture fisheries in the upper stretches of the Brahmaputra. Bulletin of Life Sciences 1995; 5:31-40.
6. Biswas SP, Boruah S. Ecology of the River Dolphin (*Platanista gangetica*) in the Upper Brahmaputra. Hydrobiologia 2000a; 430:97-111.
7. Biswas SP, Boruah S. Fisheries ecology of the north-eastern himalayas with special reference to the Brahmaputra River. Ecological Engineering 2000b; 16:39-50.
8. Brown-Peterson NJ, Peterson MS, Nieland DL, Murphy MD, Taylor, RG, Warren JR. Reproductive biology of female spotted sea trout, *Cynoscion nebulosus*, in the Gulf of Mexico: differences among estuaries? Environmental Biology of Fishes 2002; 63:405-415.
9. Cek S, Bromage N, Randall C, Rana K. Oogenesis, hepatosomatic and gonadosomatic indexes and sex ratio in rosy barb (*Punctius conchonius*). Turkish Journal of Fisheries and Aquatic Sciences. 2001; 1:33-41.
10. Chech Jr. JJ, Moyle PB. Reproduction. In: Fishes-An introduction to Ichthyology. Upper Saddle River, Prentice Hall, 2004, 726.
11. Cognetti G, Maltagliati F. Biodiversity and adaptive mechanisms in brackish water fauna. Marine Pollution Bulletin 2000; 40:14.
12. Coward K, Bromage NR. Histological classification of ovarian growth and the dynamics of ovarian recrudescence in Tilapia zilli (Gervais). Journal of Fish Biology. 1998; 53:285-302.
13. Das MK, Naskar M, Mondal ML, Srivastava PK, Dey S, Rej A. Influence of ecological factors on the patterns of fish species Richness in tropical Indian rivers. Acta Ichthyologica et Piscatoria 2012; 42(1):47-58.
14. Dawson TP, Berry PM, Kampa E. Climate change impacts on freshwater wetland habitat. Journal for Nature Conservation. 2003; 11:25-30.
15. Depledge MH, Fossi MC. The role of biomarkers in environmental assessment (2). Invertebrates. Ecotoxicology 1994; 3:161-172.
16. Fawole OO, Arawomo GAO. Fecundity of *Sarotherodon galilaeus* (Pisces: Cichlidae) in the Opa reservoir, Ile-Ife, Nigeria. Revista de Biologia Tropical 2000; 48:201-204.
17. Gibbs JP. Wetland loss and biodiversity conservation. Conservation Biology 2000; 14(1):314-317.
18. Ham KD, Adams SM, Peterson MJ. Application of multiple bioindicators to differentiate spatial and temporal variability from the effects of contaminant exposure on fish. Ecotoxicology and Environmental Safety 1997; 37:53-61.
19. Hojo RES, Santos GB, Bazzoli N. Reproductive biology of *moenkhausia intermedia* (Eigenmann) (Pisces, Characiformes) in Itumbiara reservoir, Goias, Brazil. Revista Brasileira de Zoolgia 2004; 21:519-524.
20. Janseen PAH, Lambert, Goos HJTH. The annual ovarian cycle and the influence of pollution on vitellogenesis in the flounder, *Pleuronectes flesus*. Journal of Fish Biology. 1995; 47:509-523.
21. Knight JT, Butler GL, Smith PS, Wager RNE. Reproductive biology of the endangered oxyleyan pygmy perch *Nannoperca oxleyana*. Whitley. Journal of Fish Biology. 2007; 71:1494-1511.
22. Le Cren ED. The length weight relationship and seasonal cycle in gonad-weight relationship and condition in the perch (*Perca fluviatilis*). Journal of Animal Ecology. 1951; 20:201-209.
23. Love RM. The chemical Biology of fishes. Academic press, Landon, 1970.
24. Lowry OH, Rosebrough N, Farr AL, Randall RJ. Protein measurement with Folin Phenol reagent. Journal of Biological Chemistry. 1951; 193:265-285.
25. Macay M, Tunison AV. Cortland Hatchery Report No. 5. N.Y. State Cons. Deptt. Us Bur of fish and Cornell Univ. Matthews, W.J., 1998 Patterns in freshwater fish ecology. New York, Chapman and Hall, 1936, 756.
26. Mazzoni R, Silva APF. Aspectos da história de vida de Bryconamericus microcephalus (*Miranda Ribeiro*) (Characiformes, Characidae) de um riacho costeiro de Mata Atlântica, Ilha Grande, Rio de Janeiro, Brasil. Revista Brasileira de Zoologia 2006; 23(1):228-233.
27. Mekkawy IAA, Hassan AA. Some reproductive parameters of *Synodontis Schall* (Bloch and Scchneider, 1801) from the river Nile, Egypt. Journal of fisheries and aquatic sciences. Aquatic Sciences 2011; 6:456-471.
28. Menezes NA, Vazzoler AEAM. Síntese de conhecimento sobre o comportamento reprodutivo dos Characiformes da América do Sul (Teleostei, Ostariophysi). Revista

Brasileira de Biologia. 1992; 52(4):627-640.
29. Morris JA, Sullivan CV, Govoni JJ. Oogenesis and spawn information in the invasive lionfish, Pterois miles and Pterois volitans. Scientia Marina 2011: 75:147-154.
30. Niklosky GV. Theory of fish population dynamics as the biological background for rational exploitation and management of fishery resources. Edinburg, Oliver and Boyd Ltda. 1969; b:323.
31. Ricciardi A, Rasmussen JB. Extinction rates of North American freshwater fauna. Conservational Biology 1999; 13:1220-1222.
32. Sato Y, Fenerich-Verani N Nuner APO, Godinho HP, Verani JR. Padroes reprodutivos de peixes da bacia do Sao Francisco. In: Aguas, peixe e pesca no Sao Francisco das Minas Gerais. H.P. Godinho and A.L. Godinho (Eds). Pontifi´cia Universidade Cato´lica de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil, 2003, 229-274.
33. Singh SP, Mehrotra SN, Yadava YS, Choudhury M, Biswas BK, Biswas DK. Investigation on factors relating to decline of fishery in the River Brahmaputra and its tributaries. Annual Report, CICFRI, Barrackpore, 1988.
34. Szollosi-Nagy. In Proceedings of the United Nations seminar, Delft, Netherlands, 2004, 25-26.
35. Talwar PK, Jhingran A. Inland fishes of India and adjacent countries, Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi 1991; 2(16):1158.
36. Walker CH. The use of biomarkers to measure the interactive effects of chemicals. Ecotoxicology and Environmental Safety 1998; 40:65-70.