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Do hydro-biological factors influence spawning migration: A case study of Hilsa's (*Tenualosa ilisha*) breeding habitat in Ganga River, India

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ABSTRACT

Hilsa (*Tenualosa ilisha*) is an amphihaline migratory fish and established itself as one of the most important commercial fishes of the Indo-Pacific region. It has a wide range of distribution and occurs in marine, estuarine and riverine environments. *T. ilisha* exhibits spawning migration to the freshwater environment of the river systems and thereafter nourish young ones until they return back to sea for maturity. The breeding success of this species depends on the synchronous effects of eco-environmental and biological conditions. The fish normally inhabits the lower regions of the estuaries and the foreshore areas of the sea. Hilsa prefers to reside in the lower basin region of Ganga River during their upstream migration. Several attempts have been made to explore the feeding habit of hilsa in the River system but these are inadequate. Knowledge on the feeding habitat of hilsa and seasonal variation of biotic and abiotic components are very much essential to understand the precise breeding habitat selection of hilsa in monsoon. In the present study, it was found that high turbidity, silica loads, and specific biotic components i.e. diatom population helps upcoming hilsa to spot their preferable breeding ground in the lower basin stretch of Ganga River. Management and conservation of breeding habitat improve the productivity of the fish which is directly benefiting the socio-economy of this fishery.

INTRODUCTION

Screening of a fish habitat means the analyses of all the physical, chemical and biological requirements that facilitate a conducive life for the organism in that habitat. It has been known for a long time that T. ilisha migrates from sea to fresh water. The upstream migration takes place during the southwest monsoon and consequent flooding of the rivers for spawning and breeding (Day, 1879; Rahman, 2005). Later, the juveniles return to the sea for further growth and maturation. During its upstream migration, hilsa is known to ascend 1200 km to inland river habitat (Pillay and Rao, 1963) from saline

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environment. Attempts have been made to outline environmental requirements as well as feeding habitat of hilsa (Karuppasamy and Menon, 2004; De *et al.* 2013) and food and feeding habits in different water bodies (Pillay and Rao, 1963; Halder, 1968; Ramakrishnaiah, 1972; De and Dutta, 1990; De *et al.*, 2013; Rahman *et al.*, 1992; Jafri *et al.*, 1999 and Narejo *et al.*, 2005). Attempt were also made to explain hydrological parameters influencing feeding habitat of hilsa (Hora, 1938; Gopalkrishnan, 1971; Nath, 1998; Bhaumik *et al.*, 2011). Although these studies have detailed on seasonal variation of planktonic diversity along with associated hydrological factors of different sample station in the context of T. ilisha feeding habitat, there is no explanation whether such variations in hilsa's breeding habitat have any link to its migration or not. The present study aims to feeding habits of T. ilisha, with a special emphasis to explain its migration based on available resources and upstream migration to the breeding habitat in lower basin of Ganga (the Hooghly- Bhagirathi) River system in India.

MATERIALS AND METHODS

Sample collection site and time

Water and plankton samples were collected from four different sites of which three were breeding sites (i.e. Balagarh, Belur and Godakhali) and one was non-breeding sites (i.e. Katwa) of hilsa in the upstream of hilsa in the lower basin of Ganga (Hooghly-Bhagirathi) River system in West Bengal (Fig 1, i and ii). The breeding sites were marked as per the identification made by CIFRI (Bhaumik and Sharma, 2012).

Samples were collected throughout the year (two consecutive years; 2014 and 2015). This tenure was further subdivided into three repeated seasons (i.e. premonsoon, monsoon and postmonsoon).

Water and plankton sample collection

Water samples were collected from a depth of 2.5-3.0 meters using a flexible plastic tube of 3.0 centimeters in diameter. The collected water volume was passed through plankton net with a mesh size of 15 µm into a 15ml microtube and preserved in 4% buffered formalin. Filtered water sample was stored to analyse few common hydrological parameters to characterize the different collection sites on seasonal gradient.

Analysis of water sample

Common hydrological parameters of water, like, Salinity, temperature, pH and dissolved oxygen (D.O.) were analysed in the field. The turbidity and silicates were analysed in the laboratory.

Elemental analysis of water samples were also performed using Energy dispersive X-ray spectroscopy (EDAX) at North Eastern Hill University, Meghalaya, India.

Identification and quantification of plankton

Planktons were identified up to genus level following standard manuals namely (Needham and Needham, 1962; Prescott, 1962) and counted using Sedgwick-Rafter counting cell, following the standard methods (APHA, 1992).

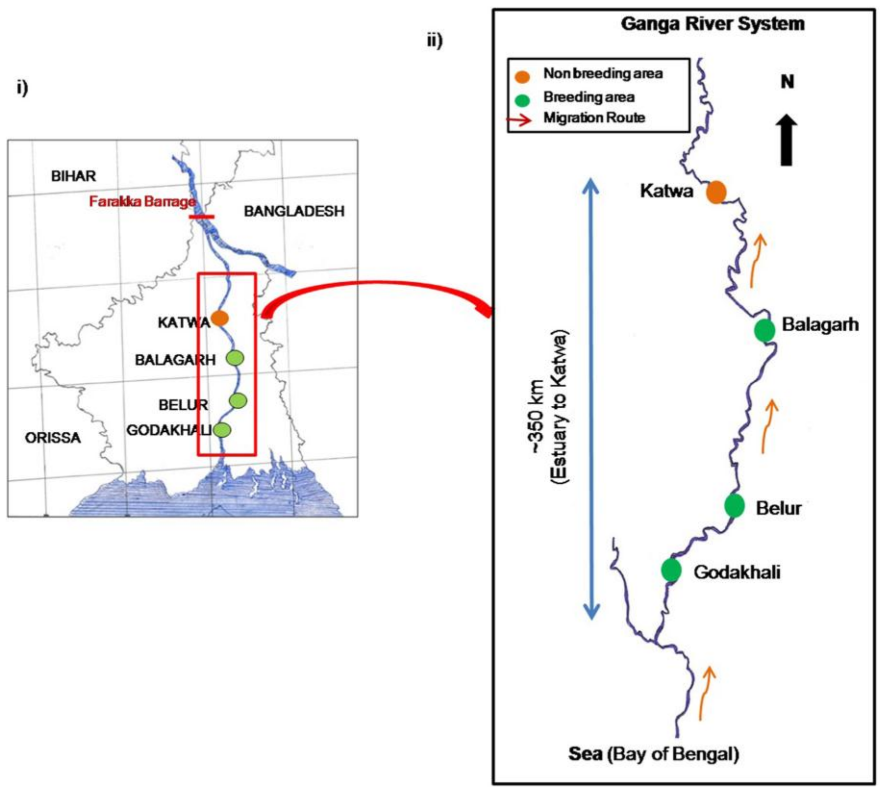

Figure 1. Overview of sample collection sites and locations: (i and ii) Route map of Lower basin of the Ganga (Hooghly-Bhagirathi River system) in West Bengal along with four sample stations [i.e. Balagarh (88.400E and 23.100N), Belur (88.360E and 22.650N) and Godakhali (88.140E and 22.390N) and one non breeding habitat i.e. Katwa (88.130E and 23.630N)].

Gut content Analysis

Gut contents were collected from adult hilsa sampled from breeding and non-breeding sites. The alimentary canal was carefully dissected out and all gut contents from the oesophagus to the anus were collected using a fine brush, and preserved them in 4 per cent buffered formalin. The gut contents from each fish were then examined under an inverted light microscope (Victory plus, Dewinter Technologies) using Sedgwick-Rafter counting cell for qualitative and quantitative analysis. The identification was done following manuals described for plankton, and quantitative analysis have been performed following the method where number of each food item is expressed as a percentage of the total number of food items found in the stomach.

Statistical analysis

Multiples means were compared through one way ANOVA followed by Tukey's post-hoc test. All values were means ± SEM. The turbidity, silicate and diatom were studied for cause-impact analysis through linear regression analysis by using SPSS 16.0 version. The 'α' level is kept at 0.05.

RESULTS

Hydrological parameters

Salinity, Temperature, pH and DO

The hydrological parameters recorded from the sampling sites throughout the study period. Out of all the parameters, salinity, temperature, pH (Eutech oakton (Multi parameter test 35) series) and DO (Lutron (DO-5510)) are shown in Fig 2. Throughout all breeding sites studied, the salinity remained below 0.5 ppt throughout the period of study (Fig 2a, i and ii), indicating that the selected sampling stations belonged to the freshwater riverine system of Ganga River. The examination of the surface temperature of sampling sites was recorded within a range between 26 -31°C. During monsoon season, temperature of all four sampling sites increased followed by a gradual fall upto 25°C during postmonsoon and premonsoon seasons (Fig 2b, i and ii). The pH of sampling sites ranged between 7.4 -8.8 indicating alkaline environment of the studied sites. Though marginal, the seasonal variation in pH was observed among all seasons and also across sampling sites. During monsoon, the pH was low (pH 7.2-8.0) in all the three breeding sites whereas level of pH remained unchanged throughout in non breeding site. Otherwise the entire stretch was under uniform alkaline condition (8.0 to 8.3) (Fig 2c, i and ii). The DO from the sampling stations showed moderate range, i.e. 6.8-9.0 ppm. The DO level decreased slightly during monsoon in the breeding sites but such change was very marginal as compared to other seasons (i.e. premonsoon and postmonsoon) (Fig 2d, i and ii).

Turbidity and Silicate

The turbidity was measured using Nephalo Turbidity Meter (Systronics). Fig 3 shows the recorded turbidity and silicate from the breeding sites. Throughout the year, there was a significant variation in turbidity across the sampling sites. Compared to non breeding ones, the turbidity of breeding sites were significantly high during monsoon. Result also showed that the turbidity in breeding sites during monsoon were high compared to pre monsoon and post monsoon seasons (Fig 3a, i and ii). In case of silicate, high silicate loads (measured through Molybdosilicate method (APHA 1998)) were observed across breeding sites during monsoon period compared to other seasons (Fig 3b, i and ii). The EDAX analysis of water samples collected from these breeding habitats during monsoon season showed high availability of silicon in water samples (Fig 3c, i, ii and iii).

After the seasonal investigations of hydrological parameters throughout two consecutive years, it was quite clear that breeding habitat of adult hilsa in Lower basin of the Ganga River was well characterized with high turbidity and silicates load containing low pH and DO level during monsoon.

Plankton analysis

Analysis of water samples from all the sampling sites recorded 27 genera of phytoplankton belong to Bacillariophyceae (13 genera), Chlorophyceae (04 genera), Cyanophyceae (08 genera), Euglenophyceae (01genera) and Chrysophyceae (01genera). There were 09 genera of zooplankton belong to Copepoda (03genera), Rotifera (04 genera), Cladocera (02 genera) (Table 1).

The quantitative analyses of planktons are shown in Fig 4. It is revealed that Chlorophyceae were dominant plankton across the sampling sites throughout all seasons. But seasonal variation has been clearly observed in case of Bacillariophyceae. Compared to other seasons, its population abundance increased remarkably during monsoon (Fig. 4b i and ii) and followed a decreasing trend during post monsoon (Fig. 4c, i and ii). Such significant changes were not observed in case of others plankton group.

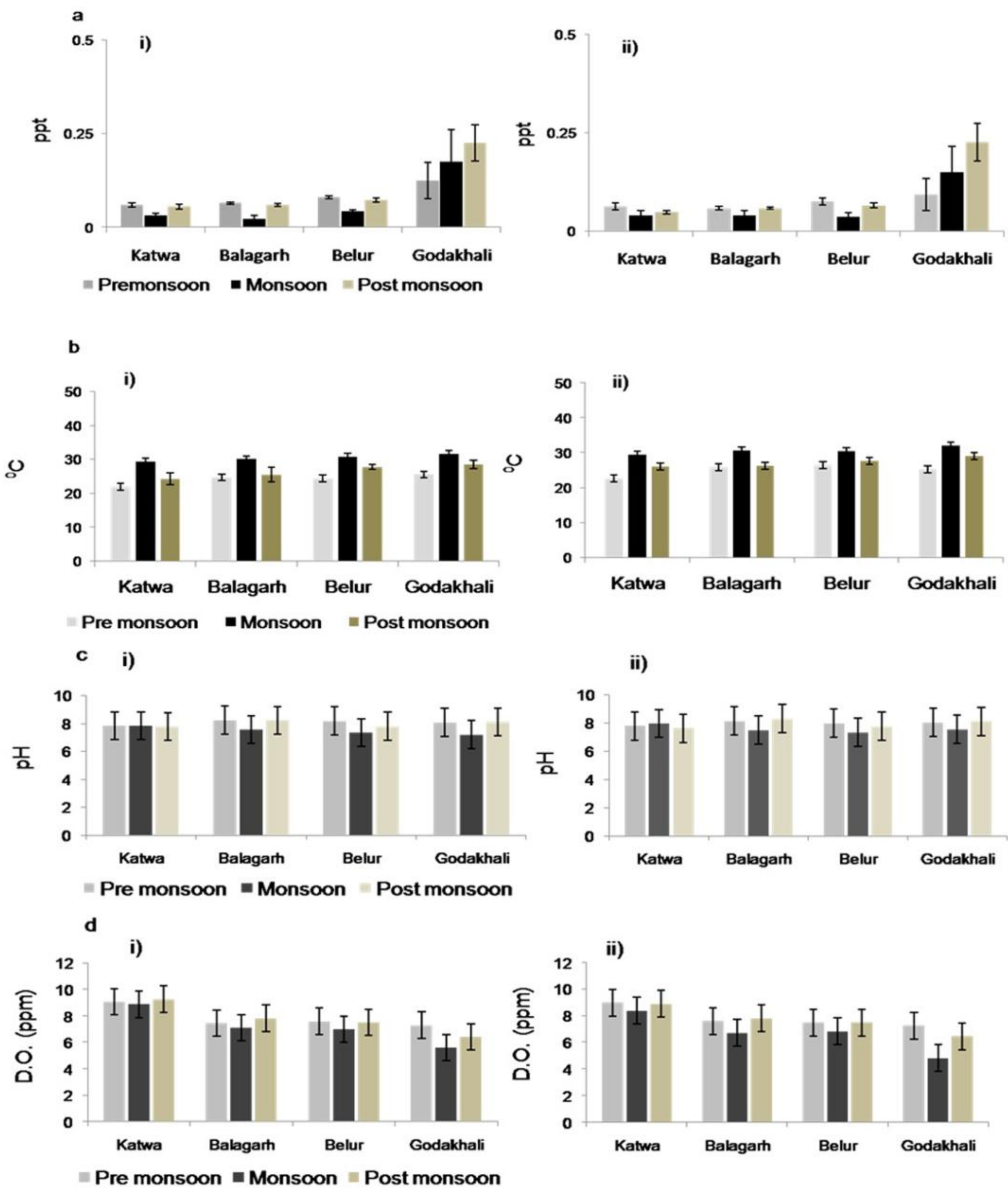

Figure 2. Seasonal variation in hydrological parameters of water bodies at different sampling stations (Breeding habitat: Balagarh, Belur and Godakhali; Non breeding habitat: Katwa) in lower basin of the Ganga River system: (a, i and ii) Salinity was in the range of freshwater (≤0.5ppt) throughout the study (b, i and ii) showing water body temperature of different sampling stations (c, i and ii) pH of different sampling stations were ranged between7.5 ± 1 (d, i and ii) Dissolved oxygen decreased in breeding habitat during monsoon. No other significant variations have been found throughout the observation period (n=12, mean ±SD; i -2014, ii - 2015).

Table1 Plankton genera (Phyto plankton and Zooplankton) identified from the sampling stations of the lower basin of Ganga river system during the study periods

PLANKTON GROUP	Genera
PHYTOPLANKTON	
Chlorophyceae	*Pediastrum* sp., *Spirogyra* sp., *Ulothrix* sp., *Volvox* sp.
Bacillariophyceae	*Ampliprora* Sp., *Bacillaria* sp. ,*Fragellaria* sp., *Tabularia* sp., *Synedra sp.*, *Biddulphia* sp., *Coscinodiscus* sp., *Ditylum* sp. ,*Diatoma* sp. ,*Nitzschia* sp. *Pinnularia* sp., *Aulacosiera* Sp., *Melosira* sp.
Cynophyceae	*Spirulina* sp., *Anabaena*sp., *Cylindrosprmum* Sp., Filamentious algae, *Nostoc* sp., *Oscillatoria* sp., *Microcystis* sp., *Microspora* sp
Chrysophyceae	*Tribonema* sp.
Euglenophyceae	*Euglena* sp.
ZOOPLANKTON	
Copepod	*Calanoida* sp., *Cyclopoida* sp., Nauplii
Rotifer	*Keratella* sp., *Trichocera* sp., *Brachionus* sp., *Lecane* sp.
Cladocera	*Daphnia* sp., *Moina* sp.

Table2 linear regression analyses of diatom versus silicate and turbidity from the breeding stations in the lower basin of Ganga river system during the course of study. The diatom populations recorded have been treated as dependent variable.

Silicate vs Diatom

	Factors	Pre monsoon	Monsoon	Post monsoon
2014	a	0.063	14.508	-1.686
	b	0.435*	1.896*	2.88*
	R^2	0.673	0.752	0.656
2015	a	15.277	17.430	24.455
	b	0.455*	1.481*	0.448
	R^2	0.601	0.717	0.214

Turbidity vs Diatom

	Factors	Pre monsoon	Monsoon	Post monsoon
2014	a	-36.570	26.141	25.966
	b	5.951*	0.074*	0.017
	R^2	0.864	0.873	0.378
2015	a	-79.151	31.634	21.825
	b	8.873*	0.034*	0.053*
	R^2	0.868	0.746	0.718

The constant (a) and regression co-efficient (b) with $p<0.05$ are indicated with asterisks.

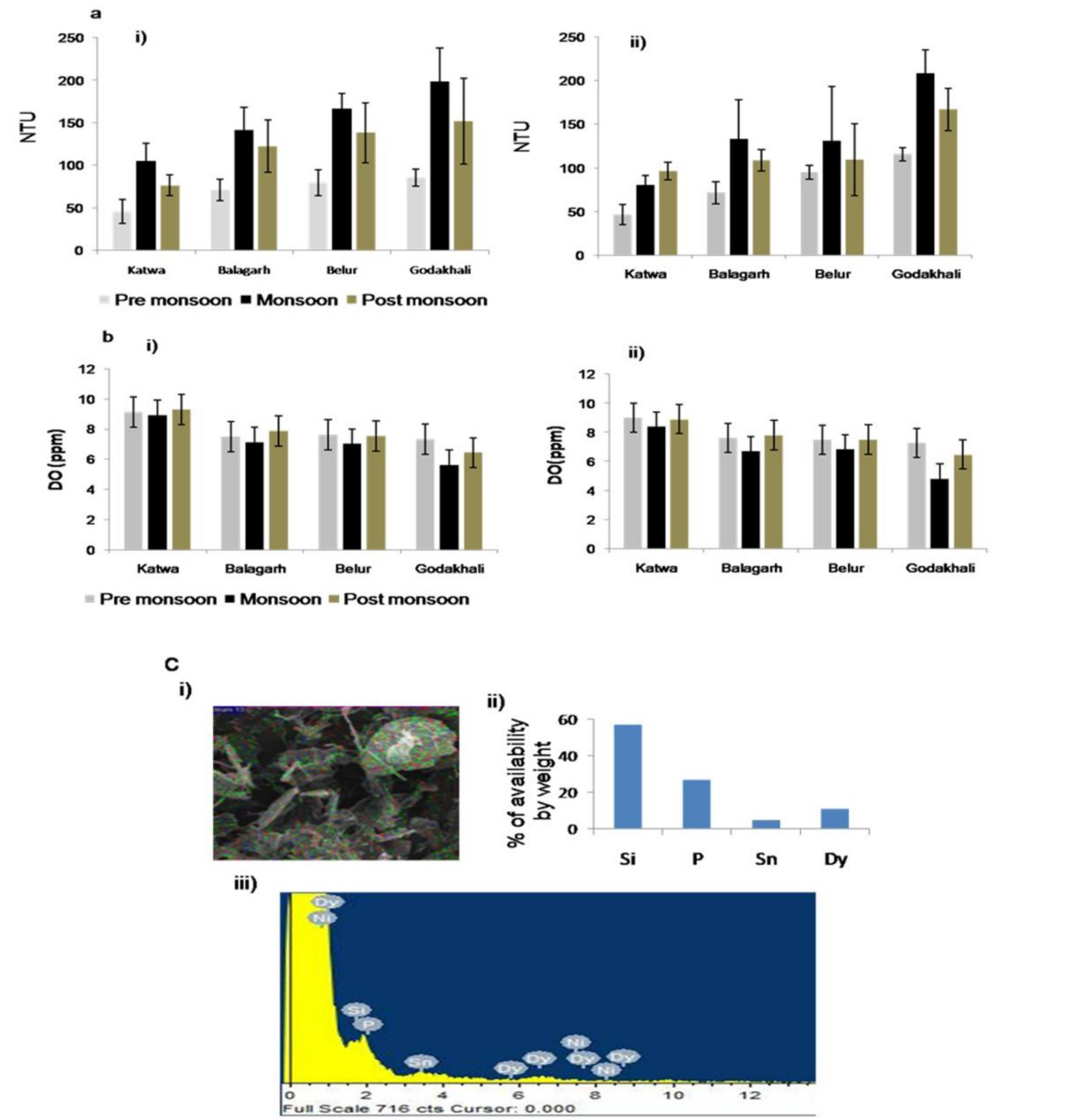

Figure 3. Seasonal variations in turbidity and silica load across the sampling stations (Breeding habitat: Balagarh, Belur and Godakhali; Non breeding habitat: Katwa) in the lower basin of Ganga River system: (a, i and ii) Turbidity levels were so high in breeding habitat during monsoon (b, i and ii) Silicates load at different sample stations in the lower basin of Ganga river system: conc. of silicates was high in hilsa's breeding habitat during the monsoon seasons (c) EDAX analysis of water sample collected from breeding habitat (i.e. Godakhali) of the lower basin of Ganga river system during monsoon season : (c, i) Scanning image of dehydrated water sample showed green dots represent Silicates as an major elements of the sample; (c, ii) Spectrum image of water sample represents the earlier detection of silicates; (c, iii) Percentage of silicates was high among other elements when plotted against % availability by weight (n=12, mean ±SD; i -2014, ii - 2015);. (c, iii) Spectrum image of water sample represents the earlier detection of silicates.

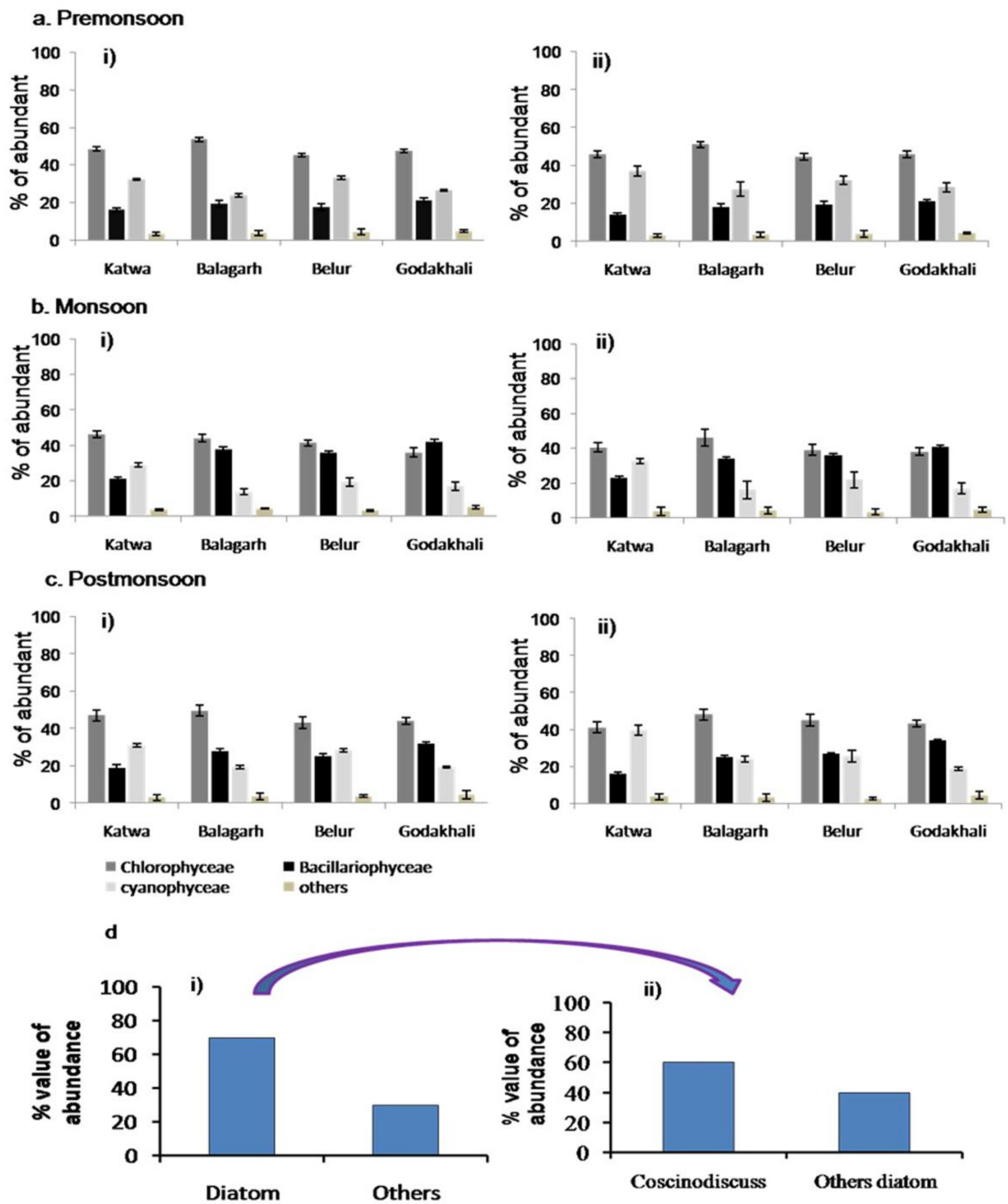

Figure 4. Quantitative analysis of plankton collected from four sampling stations (Breeding habitat: Balagarh, Belur and Godakhali; Non breeding habitat: Katwa) of the lower basin of Ganga river system: (a, i and ii) Variation in plankton genera during premonsoon season of 2014 and 2015 (b, i and ii) represents drastic increase in Bacillariophyceae group in breeding habitats during monsoon season of 2014 and 2015, whereas no changes observed in other groups of plankton (c, i and ii) Population dynamics of plankton groups during postmonsoon of 2014 and 2015 (d, i and ii) Gut content analysis of adult hilsa collected from breeding habitat (i.e. Godakhali) during monsoon season (i) Percentage abundance of diatom in the gut during monsoon season; (ii) Percentage abundance of Coscinodiscus sp. out of the total diatom genera collected from the gut of hilsa.

DISCUSSION

The breeding sites of hilsa were characterized with comparatively high temperature; high turbidity and high silicates load where pH was mildly alkaline. The DO remained low during the monsoon season. Moreover, it is also understood that the turbidity is mainly contributed by silicate loads in Ganga River System. Thus, one of the remarkable features in the breeding sites of hilsa is that it is naturally turbid with silica load. Abundance of diatom in such areas with mildly alkaline pH indicates these breeding sites as favorable 'diatom flourishing' ground. Earlier studies on the life cycle of diatom (Group: Bacillariophyceae) suggest that silicate is essential for diatoms for the production of its siliceous exoskeleton structures through a process called biomineralization (Ehrlich *et al.*, 2010; Hervé *et al.*, 2012). In general, each diatom is bounded by siliceous cell walls, composed of two mirror-image halves, the epitheca and the hypotheca. Each theca consists of a capping valve and several girdle bands, which are actually silica strips running laterally along the axis of the cell. During their vegetative reproduction, which predominates over sexual reproduction (Round *et al.*, 1990), diatoms need to build up a new silica cell wall. The silicon requirement for the silica formation is fulfilled from their surrounding aqueous habitats predominantly as silicic acid (Amo *et al.*, 1999). So the high silicate loads in hilsa breeding ground during monsoon season may be biologically correlated to growth and abundance of diatom in the breeding areas. The linear regression analysis also explained such possibility in the present case. This is evident that, of all seasons, abundance of Bacillariophyceae was comparatively high during the monsoon season in the breeding sites.

Another important observation is the presence of high diatom volume in the gut of hilsa showing its preference towards diatom as food in freshwater habitat. An explanation of such selective preference may be forwarded based on diatom biology. It is already clear that the breeding sites of hilsa have conducive aquatic environment for diatom growth and hilsa too, selectively prefers diatom. Earlier report by Hasan *et al.* (2016) affirmed that diatom stood as highly preferable planktonic food of hilsa. Hilsa being migratory and stress sensitive fish making it difficult to keep under captivity, it is really difficult to establish the linkages of breeding sites and the abundances of diatom. Eventhough, diatom biology could help to conceptualize such linkages.

In an attempt to understand diatom growth, Kroger et al. *et al.* (1999) reported that several organic cell wall components, mainly, the specific proteins called silaffin and polyamines, are associated with diatom biosilica and/ or directly participate in biosilica-nano-pattern formation. Since silica biomineralization in diatoms is much faster than abiotic silica formation; a biological flocculant is believed to assist the silica polycondensation. Long chain Polyamines (LCPAs) and silaffins have been reported to be directly involved in the molecular processes that lead to biogenesis of the elaborate patterned silica frustules (Poulsen, 2004). Both, LCPAs and silaffins are highly cationic compounds and can serve as flocculant for negatively charged silica nanoparticles whereas, at pH values 7 and above, negative charges on the surfaces of the colloidal silica particles dominate and induce electrostatic repulsion. Therefore, colloidal silica particles form a stable solid and the particles grow by the Ostwald ripening process (Ostwald, 1897; Iler, 1979). Henceforth, the mild alkalinity of hilsa's breeding sites during monsoon might help in enhancing the stability of colloidal silica particle during biomineralization of silica in Silica Deposition Vesicle of diatom cell wall. So changes in the hydrological parameters favoring biosilicification in specific breeding sites during monsoon season, provides the preferable environment for the reproduction and growth of Bacillariophyceae Data collected from the plankton analyses of lower Gangetic River system and gut content analyses of adult hilsa sampled from breeding sites during monsoon revealed prevalence of diatom in the ambiance as well as gut of hilsa, indicating probable link to such biological processes of diatom to its migration. Among the diatoms, one (*Coscinodiscus sp.*) was highly abundance in the gut of hilsa throughout all major sampling sites, particularly during monsoon. According to Sumper (2002), LCPAs appear to be the main organic component that participate in biosilicification of diatoms of the genus Coscinodiscus. These LCPAs have some specific pungent odour due to the presence of Putrescin and spermidine moiety. Interestingly, Putrescin and spermidine have chemoattraction properties for some animals (Hussain *et al.,*2016). So there seems to be a relation, found between Coscinodiscus and hilsa in their breeding sites during monsoon. It can be affirmed that there might be a relationship of silica-diatom abundance to migrations of hilsa. As of now, it may be hypothesized that the LCPA in connection with silaffin may contribute as odorant molecule in these areas to create a chemosensory signaling chain to attract fish to these areas during spawning migration. If such role exists, the mechanism behind such role could be most significant information to understand the complete biological mechanism of hilsa migration. Till today, it was reported only in goldfish that polyamine can act as stimulant of olfactory protein when applied (Rolen *et al.*, 2003). Recently, Malick *et al.* (2018) and Chatterjee *et al.* (2018a) already reported that hilsa has numerous solitary chemosensory cells on its head region which they have termed as 'sensory pad' to sense stimulant molecules in water. Chatterjee *et al.* (2018b) reported ectopic expression of odorant receptor olfactory proteins like OR and OMP traced to head epidermis of hilsa that generally bind with a ligand to activate downstream signaling of chemosensory information. It is possible that the silaffin which binds to LCPA during biosilicification may act as effective polyamine ligand to locate the breeding sites of hilsa. In insects, polyamines are reported to have a regulatory role in the expression of olfactory related genes depending on the gene, gender and mating status of diamondback moths (Zhang *et al.,*2015). In another study, drosophila was found to sense diet with high in polyamine that is beneficial for them and also increases their reproductive success (Hussain *et al.,* 2016). However, in fish, such reports are not available. Chatterjee *et al.* (2018a) forwarded a hypothesis where they assumed that the cone shaped head of hilsa has sufficiently inclined surface with creating a 'thin boundary layer', closure to the grooves on the head integument where chemosensory cells are housed. This mechanism facilitates enough time and surface to fish to maximize the process of odorant reception sensing the ligand while swimming upstream. An aquatic habitat with high silicate load is associated to silafin-LCPA ligand and hence, may guide the fish towards the habitat as favouring breeding. However, further investigations along with other aspects will enlighten such upstream migratory behaviour of fish.

CONCLUSION

Screening of biotic components and associated factors from the selected major catchment areas of hilsa throughout different seasons across the lower basin of Ganga river suggested that a specific phytoplankton (i.e. diatom or bacillariophyceae) is the predominant biotic component over others during monsoon. During monsoon season, breeding habitats of hilsa were found to have comparatively high temperature, turbidity and silicates load with mild alkaline and low DO. These hydrological factors help to increase the population of diatom during monsoon. The period when hydrological factors are non-specific to diatom pre-dominance, the migration did not appear. Based on this finding, we can propose that conservation of diatom and diatom associated ambiances should be the priority for designing and setting breeding habitat to accelerate capture fishery of this fish. This knowledge may further be extended to several other countries of indo-pacific sub continent for restoring breeding grounds and conservation of this fish.

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