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Assessment of the water quality of flood plain wetland (Beel) using fish protozoan parasites, west Bengal, India

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Abstract
The present experiment was undertaken in the Mathura beel of West Bengal using protozoan as bio-indicators for assessing the water quality of floodplain wetlands during May 2004 to April 2005. The fishes from different selected sites (S_1, S_2, S_3 and S_4) were collected for investigating protozoan infection in fishes the beel was stocked with the fingerlings of *Labeo rohita*, *Labeo batta*, *Catla catla*, *Cirrhinus mrigala*, *Channa punctatus* and *Tilapia mosumbica* by fisheries co-operative societies of West Bengal. The weight of fishes collected for the examination was ranging from 100-200g. The total percentage of infections were 27.50, 26.53, 24.66 and 19.37 in station S_1, S_2, S_3 and S_4 respectively. The percentage of infection recorded highest at station S_1 because of sewage received from the Military and railways. Three species of Myxozoans viz. *Myxobolus*, *Henneguya* and *Thelohanellus* and one species of *Trichodinid sciliophorana* like *Tripartiella* were recorded from the infected fishes. The maximum percentage of incidence was observed during May-September and December-February. The physico-chemical parameters like dissolved oxygen, ammonia and phosphate were positively correlated with the parasitic frequency index at the station S_1 and with phosphate and ammonia at station S_3 and S_4. The positive correlation between water quality and parasitic frequency index in S_1 indicated that protozoan could flourish in sewage contaminated water. This paper is aimed to focus that protozoan can be bio-indicator of assessing the water quality of wetland.

Keywords: Bio-indicator, fish, flood plain, protozoan parasites, water quality.

1. Introduction

Wetlands are low lying areas which are the major regulators of carbon, nitrogen and phosphorus cycles (Feierabend and Zelazny, 1987) [1]. Floodplain wetlands (Beel) exist along the backwaters of the main drainage network, get connected the main river during monsoon, become isolated during the winter (Ewel, 1990) [2]. They are important reservoirs for fresh water, excellent purifiers of the terrestrial wastes and zones for aquifer recharge that provide critical habitat for a number of fauna and flora (Duker and Borre, 2001) [3]. The Mathura Beel spread in Nadia and 24 Pargana (N) district of West Bengal, India fosters high biological diversity and has been providing hydrological and ecological services thus supporting livelihoods of a huge rural population. The beel which is spread across the highway of Kancharapa to Haringhata farm – has a total surface area of 264ha. It is a complex system of backwaters, reclaimed land and an intricate network of natural canals (Mitsch and Gosselink, 1993) [4]. Unique cultural traditions, water centered social institutions and lifestyles have evolved around the wetlands over time. The evolutionary history of this beel shows that this beel plays an important role in the ecology and economy of the fisherman of these areas. The beel is presently undergoing severe environmental crisis due to the unregulated anthropogenic activities that are disrupting the natural balance of this rich wetland system. Mathura beel has attracted most of the naturalists, limnologists and scientists and consequently has been subjected to intense biological research. The beel supports an assemblage of diversified fauna in accordance to their bio-ecological condition. Protozoans are common predators on bacteria and fungi (Hausmann *et al.*, 2003) [5], having the role of nutrient cycles (Mitchell *et al.*, 2008) [6]. Protozoans feed on and regulate the abundance of most types of aquatic microbes, and they are an integral part of all aquatic microbial food webs. They also have a long history of use as indicators. By nature, these occur in large numbers in a very limited sample volume and many biological indicators commonly used in monitoring and impact assessment studies are organisms like fishes, molluscs, polychaetes, bacteria that are logistically difficult to collect and expensive to analyze. The free-living protozoans in chief include rhizopods and ciliates that have a wider distribution in freshwater environments. Perfect characterization and monitoring of all aquatic environments typically found in freshwater settings is possible with the use of these minute organisms. Though minute in size and apparently. Insignificant, they take on a wondrous variety of form and structure. Because of their big size, they are sometimes rated as most important, but they play a useful and important role in the biosphere. But their overall role may be considered as mixed as some are useful, others harmful and few others border lined. The useful forms of the protozoans constitute important links in the food web, are employed very

well in biological and medical research, act as indicators of pollution and petroleum deposits and they are above all good natural enemies of harmful bacteria, aiding in soil fertility. Many of the protozoans are harmful as they cause dreaded diseases in man and other organisms and interfere with the production of nitrate, thereby reducing soil fertility. They are considered to be the first animals to evolve and thus have a special place in the evolutionary history of animals. Many scientists have been attracted towards protozoa during the last two decades and due to availability of fine tools and technology many facts about them have been revealed. This accelerated interest in these creatures has had a great bearing on their classification. Which has undergone important changes in the present decade. The group protozoa have now been raised to the level of sub kingdom under the kingdom Protista. So, it is very important to assess the water quality of beel through using protozoan parasites as bio-indicator of water quality of the wetlands.

2. Materials and Methods

2.1 Experiment area and its environment

The water body considered for study is a flood plain wetland locally know as Mathura Beel spreading across the district of Nadia and 24 Parganas (N), West Bengal. The water body is unique in its type having the agricultural fields and human settlements surrounding it, thus contributing varied qualities of sediment and water. It mainly receives domestic's sewage of railways and military quarters from the national highway zone. The beel receives heavy run-off of silt, chemicals and fertilizers from agricultural land which is most threatening effect on beel productivity in near future. It is a perennial water body receiving water through ground water seepage, rainwater and surface run-off from the surrounding vicinity. The water of the beel is used by residents of the surrounding for washing, bathing and household purposes as well as for irrigation of agricultural lands and mainly for fish culture practices. The varied and diverse nature of water body in terms of inputs enticed me to monitor using fish protozoan parasites.

2.2 Physiographic features of Mathura Beel

Area:	264 ha
Total length:	9km
Shape:	Crescent
Categories	Ox bow lakes
Soil type:	Sandy- clay-loam
Source of water:	Rain-fed
Type of water body:	Perennial
Average depth at monsoon:	5ft-6ft
Average depth at lean season:	3ft-4ft

2.3 Work plan of the experiment

An investigation was carried out for a period of twelve months starting from May 2004 to April 2005 for assessing the water quality beel. To get the detailed status of water bodies, four sampling stations were fixed randomly considering the whole water body into one and stations was denoted as S_1, S_2, S_3 and S_4. The station S_1 is situated near national highway. It receives domestic sewage of military and staff quarters of Kancharapara and it is highly silted and weed chocked zone. The station S_2 is locally known as Mandir of Kampa. It is used for washing and bathing of the local people. The station S_3 is known as Sardapalli. Tne water of this zone is frequently used for washing, bathing and agricultural purposes by the residents. The station S_4 is known as Raigate. The salient feature of this zone is that it is silted zone and bathing and washing activities observed during the experiment period.

2.4 Sampling Protocol

From each sampling station compound samples for water sample, sediments, macrophytes, plankton and protozoan parasites from fishes were collected randomly in labelled and clean containers once in a month and were analyzed. Fishes from each and every station were collected and brought to the laboratory in every month for protozoan parasite studies. Samples were collected in the early morning (between 7 to 8 am) at the last week of every month from each station. Monthly air temperature, rainfall and humidity data were computed from the daily data gathered from the records of the departments of Agro-meteorology and physics of the Bidhan Chandra Krishi Viswavidalaya, Nadia. West Bengal.

2.5 Sample collection technique

Compound water samples were collected monthly, from four selected stations for determining the physical-chemical parameters. The sensitive water parameters like temperature, dissolved oxygen, total alkalinity and pH were analyzed on the spot and for other parameters the sample were brought to laboratory for analysis. Live fish from each station as per the availability was brought to the labolatory for protozoan parasite identification.

2.6 Protozoan parasites

2.6.1 Collection of samples for protozoan parasite examination

The methods for collection and preservation of the samples for protozoan parasite examination were followed as described by Mandal and Nandi (1980) [7]. A monthly sample of host fish (10-30 numbers) ranged from 100-200g were brought to the laboratory in live condition.

2.6.2 Preparation of smear and fixing of protozoan parasite for identification

The scrapings were taken from gills, body surface and other body parts separately with the help of scapel and a pair of forceps. Then the scrapings on the other slides were diluted by 0.7% NaCl and thin smears were made on grease free clean glass slides. The smears were then semi dried with Bouins fluid and sometimes Schaudions fluid was used for fixation. However, to avoid specimens from becoming hard and brittle, after 15-20 minutes of fixation, slides were washed and placed in preservatives (70% ethyl alcohol). After washing the slides were dipped in coupling jars containing 70% ethyl alcohol for further preservation.

2.6.3 Staining technique

The collected smears were stained by haematoxylin and eosin and gimsa and some slide was also stained by Klein dry silver impregaration technique.

2.6.4 Microscopic Examination of sample

Prepared slides were observed under microscope to note the presence and types of protozoan parasites. Different types of

protozoan parasites were identified based on their individual characteristic features (Kudu, 1954; Nandi and Das, 1995; Rosemarie, 1998, Jayakumar and Ramasamy, 1999) [8, 9, 10, 11].

2.6.5 Determination of parasitic frequency index (PFI)

The parasitic frequency index was calculated by taking the percentage of the number of hosts infected by individual parasite species against the total number of hosts examined (Smears from skin, gills and body surface).

2.6.6 Statistical analysis

The data generated from the investigation were tested for significance of variance among the station during different months of the year through two factors (ANOVA) analysis of variance based on RCB (randomized complete block) design. The interactions between the different parameters of water and sediment and stress factors were tested through the correlation analysis. All the statistical procedures were followed after Gomez and Gomez (1984) [12] and with the help of statistical software (Microsoft Excel -2000)

3. Result

In the present experiment randomly host fishes like, *Labeo rohita*, *Labeo batta*, *Catla catla*, *Cirrhinus mrigala*, *Channa punctatus*, *Tilapia mosumbica* of weight group ranging from 100-200g were brought for examination of protozoan parasite. The total percentage of infections by the parasite in the fish in each station was 27.50, 26.53, 24.66 and 19.37 in S_1, S_2, S_3 and S_4 respectfully. Monthly data on host examined, number of hosts infected, parasitic frequency index and protozoan parasite found at S_1, S_2, S_3 and S_4 are presented in Table- 1, 2, 3 and 4 respectfully. Three species of Myxozoans viz. *Henneguya*, *Myxobolus* and *Thelahanellus* and one species of trichonids ciliophorans like *Tripertiella* were found. The maximum percentage of incidence was observed during May-September and December- February. The highest percentage of infection was observed at S_1 and lowest at the S_4. A trend of gradually decreasing in percentage of infection was found from S_1 to S_4.

The physico-chemical parameters like dissolved oxygen, ammonia and phosphate were positively correlated with the parasitic frequency index at the S_2 and with phosphate and ammonia at S_3 and S_4 but all the parameters showed positive correlation at S_1. In general, most of the physico-chemical parameters were negatively correlated with the parasitic frequency index.

Table 1: Monthly data on host examined, number of host infected and protozoan parasite found at S_1.

Month	No. of host examined	No. of host infected	Parasitic frequency index (PFI)	Name of parasite
May	12	4	33.33	*Myxobolus* sp. *Tripatiella* sp.
June	14	5	35.71	*Myxobolus* sp. *Thelohanellus* sp.
July	16	3	18.75	*Myxobolus* sp. *Thelohanellus* sp *Tripatiella* sp.
August	20	7	35.00	*Myxobolus* sp. *Thelohanellus* sp *Tripatiella* sp.
September	22	7	31.81	Myxobolus sp. *Thelohanellus* sp
October	20	5	25.00	*Myxobolus* sp. *Tripatiella* sp.
November	24	6	25.00	*Myxobolus* sp. *Thelohanellus* sp *Tripatiella* sp.
December	14	8	57.14	*Tripatiella* sp. *Henneguya* sp.
January	13	6	46.15	*Myxobolus* sp. *Tripatiella* sp. *Henneguya* sp
February	12	4	33.33	*Myxobolus* sp. *Tripatiella* sp.
March	17	4	23.52	*Myxobolus* sp. *Tripatiella* sp.
April	16	3	18.75	*Myxobolus* sp. *Tripatiella* sp. *Henneguya* sp

Table 2: Monthly data on host examined, number of host infected and protozoan parasite found at S_2.

Month	No. of host examined	No. of host infected	Parasitic frequency index (PFI)	Name of parasite
May	10	3	30.00	*Myxobolus* sp. *Tripatiella* sp.
June	14	4	28.57	*Myxobolus* sp. *Thelohanellus* sp.
July	18	6	33.33	*Thelohanellus* sp

				Tripatiella sp.
August	16	4	25.00	*Myxobolus* sp. *Thelohanellus* sp *Tripatiella* sp.
September	20	6	30.00	*Myxobolus* sp. *Thelohanellus* sp *Tripatiella* sp.
October	22	5	22.72	*Tripatiella* sp. *Myxobolus* sp
November	20	4	20.00	*Myxobolus* sp. *Tripatiella* sp. *Henneguya* sp.
December	16	4	25.00	*Myxobolus* sp. *Tripatiella* sp. *Henneguya* sp.
January	12	5	41.66	*Myxobolus* sp. *Tripatiella* sp. *Henneguya* sp
February	14	4	28.57	*Myxobolus* sp. *Tripatiella* sp.
March	16	4	25.00	*Myxobolus* sp. *Tripatiella* sp.
April	18	3	16.66	*Myxobolus* sp. *Tripatiella* sp. *Henneguya* sp

Table 3: Monthly data on host examined, number of host infected and protozoan parasite found at S_3.

Month	No. of host examined	No. of host infected	Parasitic frequency index (PFI)	Name of parasite
May	8	2	25.00	*Myxobolus* sp. *Thelohanellus* sp *Tripatiella* sp.
June	10	3	30.00	*Myxobolus* sp. Thelohanellus sp.
July	12	3	25.00	*Thelohanellus* sp *Tripatiella* sp. *Henneguya* sp.
August	18	4	22.22	*Myxobolus* sp. *Thelohanellus* sp *Henneguya* sp.
September	16	3	18.75	*Myxobolus* sp. *Thelohanellus* sp *Henneguya* sp.
October	14	4	28.57	*Myxobolus* sp. *Tripatiella* sp. *Henneguya* sp
November	16	3	18.75	*Myxobolus* sp. *Tripatiella* sp..
December	14	5	35.71	*Tripatiella* sp. *Myxobolus* sp. *Henneguya* sp
January	12	3	25.00	*Myxobolus* sp. *Tripatiella* sp.
February	8	2	25.00	*Myxobolus* sp. *Tripatiella* sp.
March	10	2	20.00	*Myxobolus* sp. *Tripatiella* sp.
April	12	3	25.00	*Myxobolus* sp. *Tripatiella* sp.

Table 4: Monthly data on host examined, number of host infected and protozoan parasite found at S_4.

Month	No. of host examined	No. of host infected	Parasitic frequency index (PFI)	Name of parasite
May	10	2	20.00	*Myxobolus* sp. *Thelohanellus* sp *Henneguya* sp.

June	12	2	16.66	*Myxobolus* sp. *Thelohanellus* sp.
July	10	2	20.00	*Thelohanellus* sp *Tripatiella* sp. *Henneguya* sp.
August	12	3	25.00	*Myxobolus* sp. *Thelohanellus* sp *Henneguya* sp.
September	10	2	20.00	*Myxobolus* sp. *Henneguya* sp. *Tripatiella* sp.
October	14	3	16.66	*Myxobolus* sp. *Tripatiella* sp. *Henneguya* sp
November	16	4	21.42	*Henneguya* sp Tripatiella sp..
December	18	4	22.22	*Tripatiella* sp. *Myxobolus* sp.
January	16	2	12.50	*Myxobolus* sp. *Tripatiella* sp.
February	12	2	16.66	*Myxobolus* sp. *Tripatiella* sp.
March	14	3	21.42	*Myxobolus* sp. *Tripatiella* sp.
April	16	2	12.50	*Myxobolus* sp. *Tripatiella* sp.

4. Discussion

This experiment was very important for assessing the water quality of floodplain wetlands through fish protozoan parasites. The protozoan parasite recorded during the investigation was *Tripartiella*, *Thelohanellus*, *Myxobolus* and *Henneguya*. Sakar and Haldar (1990) [13] suggested that the urceolariid ciliates are most prevalent in gills and body surface of various fishes in different physico-chemical factors. A peak in intensity was noticed during December and January by Saha *et al*. (1995) [14]. A similar observation was found by Pal (1975) [15]. The present observation was also compared with Saha *et al*. (1995) [14] and Pal (1975) [15].Yeomans *et al*. (1977) [16] identified Trichodinid ciliates as a potential bio-indicator, recorded from the highly polluted river in south east England. Das and Shrivastava (1984) [17] showed increased *Tricodinid* infection associated with exposure to pollutants other than oil. Voltonen and Koskinara (1989) [18] reported the skin ciliates *Trichodina* sp. from Finland lake which frequently was receiving paper and pulp mill effluent and similar observation also observed by Lentinen (1984)[19]. Das and Shrivastava (1984) [17] reported *Tricodina domergulei* on gills of *Puntius* sp. From the lake, frequently receiving industrial waste, detergent, domestic sewage, silt from soil erosion. Similar results also were observed by Dabrowka (1974) [20]. Papema and Overstreet (1981) [21] reported myxosporozoan *Myxobolus* on gills of mullet where low levels of dissolved oxygen, possibly caused by pollutants were present. Blazer (2003) [22] suggested that infection rate *Myxobolus cerabralis* also can be directly influenced by environmental factors. Acharya (2004) [23] also reported that the high percentage of *Tripartiella* sp., *Thelohanellus* sp., *Myxobolus* sp. from sewage fed fish farm compare to manage the farm. Above reference were closely related to our investigation, which confirming the water body is moderately polluted.

5. Conclusion

The fish protozoans are very effective bio-indicators for assessing the water quality of floodplain wetlands that are found in almost all freshwater bodies where they multiply in large numbers and thus are adapting to the changes in an aquatic body. With them the status of a wetlands environment can be detected in a much cost efficient manner. They can be monitored well and understanding the ecological processes with the help of these microscopic cousins is very helpful in safeguarding the diseases. Since they are efficient in heavy metal uptake, can be used as tools of bioremediation of aquatic pollutants.

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