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Seasonal Incidence of Myxozoan Parasitic Infection in *Catla catla* of Sewage Fed Ponds

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A B S T R A C T

Disease outbreaks in the fisheries sector are the paramount threats and strictures that affects the production at about 50% loss and marketability of fishes in the developing countries. Various parasites use the fishes as their hosts as they are the chief sources of proteins and especially poor management of the sewage fed fisheries sector creates physiological stresses within them which makes them more susceptible to parasitic infestations like myxozoan infection. Studies on the myxozoan parasites are not much available from sewage fed ponds of India. Keeping this in mind, fishes mainly the Indian Major Carps were randomly sampled and collected from the three sewage fed ponds in the East Kolkata wetlands. Myxozoan parasites were identified and parasitic prevalence was estimated along with the water quality parameters of the ponds. Among the IMCs the percentage of infection was highest in *Catla catla* (23.72%) and mainly two genera of Myxozoans had been found in the Catla fishes examined from the sewage fed ponds, where both the genus (*Myxobolus* & *Thelohannelus*) contributed almost equally (49.88 & 50.12%) in the spore formation within the gill lamella. Seasonal pattern of Myxozoan infection was studied and found to be highest during winter (42.73%) followed by monsoon (23.35%) and summer (5.08%). Water attributes like free CO_2 showed highly significant positive correlation with myxozoan disease in *Catla catla.* Whereas pH, total alkalinity showed highly significant negative correlation with Myxozoan infestation. So, for developing proper myxozoan disease management strategies in the sewage fed ponds and to monitor the reclamation and restoration of these sewage fed wetlands, the study can be helpful.

***Key words*:** *Catla catla*, Indian major carps, Myxozoan parasite, Sewage fed ponds

Fisheries and aquaculture sector in India contributes about 5-7% share of global fish production and trade, attaining second position among the top aquaculture producing nations in the World trailing China [1-2]. From the stand point of economics, the fisheries and aquaculture sector faces serious constrains or damage from periodical disease outbreaks [3-6]. These outbreaks may occur due to environmental stress or may be due to the encounter of different pathogens such as virus, bacteria, fungus, protozoan and monogenean parasites, which may be associated with intensification and diversification of the aquaculture practices [7-8]. Among the pathogens, parasitic diseases are reported to cause significant loss in aquaculture sector in India by interfering with metabolism and nervous control of their host fishes [9-11]. In West Bengal, fish farmers who adopt fresh and sewage-fed water fish farming methods, experience fish mortality and/or retardation in growth due to parasite infection like myxozoan infection. These parasites are most common in aquaculture of Indian subcontinent and considered as the emerging parasites of economically important aquaculture species [12]. Among the 1350 species of myxozoans described some have been found to affect both marine and freshwater fishes in natural and simulated condition [13-18]. Some studies on myxozoan parasites from freshwater fishes of Manipur, Kerala and Punjab [19-21] were reported in recent years, but the information from other parts of India is very few.

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MATERIALS AND METHODS

Fish collection

On an average 75 numbers each of live host or freshly dead specimen of *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* were randomly sampled and collected from the three sewage fed ponds in the East Kolkata wetland during September 2018 to August 2020 viz. for two years in monthly interval. The fishes were carefully examined for ectoparasites immediately after collection.

Examination of host fishes and collection of parasites

For observation and identification of parasites, the external surface such as scales, fins, skin and fin base of host

fishes were examined under a magnifying glass for ectoparasites or any kind of lesions. Then scrapping of the skin was done by a scalpel to collect the mucus in a petri dish for microscopic examination. Protozoan parasites were collected from the mucus or body surface by pipette, dropper and needle in a slide.

For permanent slide preparation the smears were prepared by removing an individual from the infected fish and pressing it on a clean grease-free slide with a drop of distilled water or 0.5% normal saline solution. This was teased apart and smeared uniformly, dried in air, fixed in acetone-free absolute methyl alcohol for 5–10 minutes and stained with the Giemsa stain (the stock solution was diluted at the ratio of 1:20 drops of buffered distilled water at pH 7.0) for 30–35 minutes. Finally, the smears were washed in buffered water (pH 7.0) and dried in air.

Identification of parasites

Parasites were identified under a compound microscope by following the description and figures of Lucky [22]; Kabata [23], Mukherjee *et al*. [24].

Prevalence study

The parasitic prevalence was estimated with the help of parasitic frequency index (PFI) which was calculated by taking the percentage of the number of hosts infected by an individual parasite species against the total number of hosts examined in a particular area under investigation.

Prevalence frequency index (PFI) was estimated following the formula given by Margolis *et al*. [25].

$$\text{Prevalence (\%)} = \frac{\text{Total No. of infected fishes}}{\text{Total No. of fish host examined}} \times 100$$

Study of water quality

The water quality parameters viz, water temperature, pH, DO, salinity, unionised ammonia, total alkalinity, free carbon dioxide and hardness were measured per month from the three sewage fed ponds following the protocols described in APHA (2012).

Statistical analysis was performed among various parameters like parasitic prevalence, seasonal and month wise variation of prevalence, physicochemical attributes by using SPSS Ver. 18. Differences were considered as statistically significant at a probability value of $P < 0.05$ [26].

RESULTS AND DISCUSSION

Among the fishes examined from the sewage fed ponds of East Kolkata wetlands, Indian Major Carps were found to be more disease prone in comparison to the exotic fishes in the context of Myxozoan infection like the observation of Das *et al*. [27]. Sadguru and Verma [28] reported highest prevalence of Myxozoan parasite as 19.91% for freshwater ponds, whereas in sewage fed ponds the prevalence was reported to be 23.72% throughout the year owing to significantly greater (P>0.05) mean of parasites in sewage than from a normal pond.

Table 1 Pond wise Mean ± SD and range of parasitic infestation in three Indian major carps from sewage-fed ponds

	Myxozoans	Trichodinids	Argulids	Dactylogyrus	Fungal	Bacterial
			Catla catla			
Pond-1						
Mean ± SD	23.60±21.30	29.35±28.63	1.93±3.76	18.00±16.88	0.95±2.30	1.11±2.36
Range	0-70.37	0-81.48	0-11.53	0-50	0-7.69	0-7.69
Pond-2						
Mean ± SD	24.68±24.82	30.73±26.11	2.02±4.49	19.72±20.56	0±0	0±0
Range	0-80.76	0-80.76	0-16.66	0-69.23	0-0	0-0
Pond-3						
Mean ± SD	22.88±19.26	29.50±24.25	1.56±3.56	13.93±14.18	0±0	2.22±4.02
Range	0-76	0-80	0-14.28	0-38.46	0-0	0-13.63
			Labeo rohita			
Pond-1						
Mean ± SD	21.04±17.46	26.33±26.28	5.78±11.79	15.86±14.78	1.46±2.73	0.32±1.09
Range	0-53.57	0-81.48	0-11.53	0-50	0-7.69	0-7.69
Pond-2						
Mean ± SD	26.29±27.40	32.11±30.02	4.60±10.37	19.18±22.87	1.32±4.02	0±0
Range	0-88	0-80.76	0-16.66	0-69.23	0-16	0-0
Pond-3						
Mean ± SD	13.30±17.61	17.36±20.38	2.40±5.63	14.39±14.68	0.00±0	1.74±3.23
Range	0-73.91	0-80	0-14.28	0-38.46	0-0	0-13.63
			Cirrhinus mrigala			
Pond-1						
Mean ± SD	22.13±21.70	30.7±24.99	1.3±3.33	19.40±18.41	0±0	0±0
Range	0-74.07	0-84.61	0-12	0-61.53	0-0	0-0
Pond-2						
Mean ± SD	14.10±17.87	32.16±25.98	0±0	18.11±18.30	0±0	0±0
Range	0-62.96	0-81.48	0-0	0-55.55	0-0	0-0
Pond-3						
Mean ± SD	14.32±16.61	15.65±15.99	0±0	14.68±15.73	0±0	2.47±4.00
Range	0-58.33	0-58.33	0-0	0-58.33	0-0	0-12.5

The data on the incidence of myxozoan infection in the three Indian major carps examined showed that the percentage of infection was highest in *Catla catla* (23.72%), followed by *Labeo rohita* (20.21%) and *C. mrigala* (16.86%) among the Indian major carps (Table 1). This observation was almost similar to the study of Khatri *et al*. [29] where also Catla was

reported as mostly affected genera among the major carps. Das *et al*. [30] gave the clue behind this more susceptibility of catla towards Myxozoan infection that the fishes have wide opercular cavity and mouth through which spores of myxozoan gain easier entry into the host fish.

Myxozoan protists were mostly encountered in the gills, fins of fishes in the present study as observed by Molnar [31. Basu *et al*. [32] reported that the intensity of infection of myxozoan was higher in hybrid than pure carps. Reimer *et al*. [33] stressed upon the host specificity of Myxozoan protists which corroborates this study where Indian major carps were the main target of these parasites. Cruze *et al*. [34] did not find any significant difference in the prevalence of infection of *Kudoa* sp. spores between seasons, but in the present study the prevalence of infection was significantly (P>0.05) different between months and seasons.

Mainly two genera of Myxozoans had been found in the Catla fishes examined from the sewage fed ponds, where both the genera (*Myxobolus* & *Thelohannelus*) contributed almost equally (49.88 & 50.12%) in the spore formation within the gill lamella in contrast to the observation of Kalavati and Nandi [35] where *Myxobolus* was found to be the dominant genus.

In the present study it was observed that the infection due to myxozoans was more prevalent in the size group of 70-290mm in Indian major carps, i.e., mostly the young one. This was in agreement with the observation of other workers [36-37]. In all probability these young ones receive the infection from brood fishes during the fry and fingerlings stages.

Myxozoan infection was found to be highest during winter (42.73%) in *Catla catla* followed by monsoon (23.35%) and summer (5.08%) (Fig 1). Winter season was found to be more disastrous from the point of view of disease outbreak in carp aquaculture sector in India [38-39]. This may be due to higher stocking density of fishes in the composite fish culture ponds. The high density and the consequent stress developed and low level of water during winter months probably facilitated the greater availability of spore to infect the fishes. In an investigation by Mukhopadhyay and Halder [40], two myxozoan parasites were obtained from Indian major carps of sewage fed pond in monsoon. They opined that in sewage fed pond, there was a greater accumulation of waste in monsoon months that favours the parasites to flourish. Rainfall, flooding or inadequate land disposal or manure may contaminate surface water with parasite from the adjacent agricultural areas [41]. Sadguru and Verma [42] in their study on myxozoan parasites on carps showed lowest rate of infection among the fishes in monsoon which differs with the present examination.

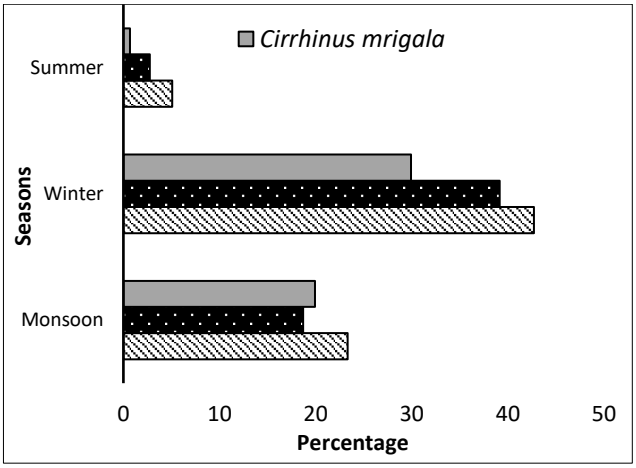

Fig 1 Seasonal variation in percentage of occurrence of myxozoan infection from various carps in sewage fed ponds studied

Table 2 Pond wise Range and Mean±SD of Physico chemical Parameters of the studied Sewage fed ponds

Water quality parameters		Pond-1	Pond-2	Pond-3
Water temperature (°C)	Mean ± SD	20.3-28	20.5-28.5	21-29.5
	Range	24.47±2.51	24.38±2.73	24.85±2.80
Dissolved oxygen (mgl^{-1})	Mean ± SD	0-10.8	2-8.5	2-8.3
	Range	5.65±2.61	5.78±1.52	6.34±1.56
pH	Mean ± SD	6.5-8.2	7-8.4	7.1-8.5
Salinity (ppt)	Range	0.1-0.4	0-0.4	0-0.3
	Mean ± SD	0.19±0.09	0.18±0.08	0.16±0.08
Total alkalinity (mgl^{-1})	Range	112-182	143-341	112-198
	Mean ± SD	133.33±21.30	207.75±57.16	155.88±28.98
Hardness (mgl^{-1})	Range	120-224	169-400	125-210
	Mean ± SD	181.83±33.97	252.71±71.35	161.83±30.16
Unionised ammonia (mgl^{-1})	Range	0.4-2.2	0.5-2.5	0.3-2.6
	Mean ± SD	1.53±0.55	1.72±0.69	1.00±0.55
Carbon-di-oxide (mgl^{-1})	Range	0-9.2	0-5.3	0-6.2
	Mean ± SD	2.03±3.60	1.75±1.79	0.44±1.36

The physicochemical attributes

In the present study free CO_2 ranged from 0 - 6.07mgl^{-1} (Table 2). Though the optimum free CO_2 level in a water body is 5 to 10 mgl^{-1}, but for sewage fed ponds the value can be more i.e., up to 20 mgl^{-1} [43]. During the investigation free CO_2 was nil during February to May which was similar to the observation of Saha and Mondal [44]. The absence of free CO_2 may be due to high phytoplankton blooms. Free CO_2 showed highly significant positive correlation (P<0.05) with myxozoan disease in *Catla catla*. Total alkalinity showed highly significant (P<0.05) negative correlation with Myxozoan infestation. Presence of high organic matter in the studied ponds and consequent decrease of pH was observed. However, waste water fish ponds, generally contain high amount of bases from different detergents and hence do not permit the pH of water to be very low and hence a productive range of pH is usually maintained in a wastewater fish pond [45]. The pH was negatively correlated (P<0.05) with all the diseases in the fishes of the sewage fed ponds. The pollutants encountered in the sewage fed ponds may weaken the fish, resulting in more colonization by myxozoan parasites and development of clinical diseases associated with these myxozoans. Multiple pollutants in these water bodies might have a synergistic effect

on disease susceptibility and exposure time may profoundly instigate the disease proliferation in carps [46-49.

CONCLUSION

Phylum Myxozoa- a paradoxical group of organisms can cause is a wide range of diseases in fishes in the semi-intensive culture system. But the knowledge of disease occurrence, the pathogen involved, the extent of damage it causes, the role of environmental factors in disease promotion is very much inadequate and unsystematic from various parts of India having reported highest parasite virulence in *Catla catla* with mortality up to 80-90%. Strategies are to be developed to improve fish production by combating the diseases caused by the myxozoan parasites through proper identification, characterization and treatment.

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