Low pH, dissolved oxygen and high temperature induces *Thelohanellus rohita* (myxozoan) infestation in tropical fish, *Labeo rohita* (Hamilton)

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Abstract In *Labeo rohita*, myxozoan infection is very common and here occurrence and distribution of myxozoan gill parasites were studied with an objective to evaluate the relationship between myxozoan infections with different abiotic factors. All ponds were infected with myxozoan infection. Sampling of water and fish was done fortnightly and soil sample on monthly basis for about 6 months. *L. rohita* is infected with the one myxozoan species *Thelohanellus rohita*. The first, second and third gill arches showed higher distribution of myxozoans than the fourth one. Posterior hemibranch of second gill arch was the most preferred site for parasite attachment. The mean intensity of parasite was significantly higher ($p < 0.05$) in the size class of 8–9 cm in comparison to the other size classes. There were profound variability in the prevalence, abundance and mean intensity of gill myxosporidians from three ponds. During the start of the sampling, myxozoans were present in all the ponds but their intensity varied in different ponds. These may be due to the variability in the abiotic factors of individual ponds. Most importantly acidic pH, lower DO and higher temperature promote myxozoan infestation and their propagation. Low soil pH is also seen to enhance their propagation. Our data clearly highlighted that prevalence, intensity and abundance of *T. rohita* strongly influenced by the above environmental parameters and suggested that its life cycle is probably effected by

their change; our hypothesis must be regarded as speculative as long as further detail study is not carried out.

Keywords Myxozoan (*Thelohanellus rohita*) · Gill · Rohu (*Labeo rohita*) · pH · Dissolved oxygen · Temperature

Introduction

One of the biotope mostly exploited by different fish ectoparasites are the gills of fishes (Fernando and Hanek 1976). In most cases, these pathogens showed a preference for specific sites of the gill apparatus of their host (Rohde 1979). For example, Myxosporidian trophozoites of the species of *Myxobolus* from *Notemigonus crysoleucas* develop in the distal half of a primary lamella (Cone and Wiles 1985). On the same line, some *Myxobolus*, *Henneguya* and *Thelohanellus* species are characterized by strict tissue specificity and species showing affinity to the epithelium, connective tissue, cartilage or vascular tissue usually occur in a strictly defined location within the gill apparatus (Molnár 2002). Such restriction of microhabitat is interpreted in many ways: it could be due to variation of the relative volumes of water current passing over the different gills (Wiles 1968; Paling 1968; Smith 1969; Suydam 1971), or due to interspecific competition (Holmes 1973). According to Kristan et al. (2006), the factors responsible for narrow microhabitat specificity are not clear. For the past three decades the spatial structure of parasites communities has been in the center of many ecological studies (Bashirullah and Rodriguez 1992; Dzika 1999; Nie 2000; Simkova et al. 2002; Matejusová et al. 2003; Kristan et al. 2006; Turgut et al. 2006).

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The study of the distribution of core and secondary species of this component community in the host population in relation to sex, age, and fish size was done by many researchers (Tombi and Bilong Bilong 2004; Jeannette et al. 2010); but no study is reported about the distribution pattern with respect to the environmental factors. The aim of this work is to evaluate the relationship between occurrence and distribution pattern of myxozoans with different environmental factors.

Materials and methods

Study area

The present study was conducted in three rearing ponds of College of Fisheries, Tripura, India (latitude 23°54.334'–23°54.354' N and longitude 91°18.406'–91°18.437' E). They were designated as pond—A, B, and C, respectively. The ponds were of equal water volume (450 m³) and containing fry of rohu fishes and their stocking density was same for all the ponds (22,500 rohu fry). Standard aquaculture management practices were followed in each pond during the study period. Each ponds were found be infected with gill myxozoans. Ponds were under close observation and measures like bird nets and net enclosures were installed to prevent invasions of predators.

Sampling

Sampling was done for about 6 months from 1st November 2008 to 15th April 2009 at fortnightly interval. Water samples were collected from the surface up to the depth of 15 cm from four sampling spots. They were brought to the laboratory and were analyzed immediately upon arrival. Soil samples from each pond were collected in marked polythene bags, brought to the laboratory and dried by exposing to air in dry place. Five fish of 4–13 cm size and 0.5–20 g body weight classes were caught randomly from each pond with a cast net at fortnightly interval. They were kept in several clean empty containers filled with pond water to acclimatize the fish prior to laboratory analyses.

Examination of fish

A total of 180 fish were examined during the entire study period (60 fish from each pond). Fish were killed by insertion of a pointed needle into the brain via the upper part of the eye. The total body weight and length of each fish were recorded before examining the fish for parasites.

Propagation and occurrence of gill myxozoans

In the laboratory, the operculum was removed to expose the gill. Each gill arch was separated in an order and was placed separately in Petri dish containing filtered pond water/tap water. The number of myxozoan parasites on each section was counted using a Stereo-zoom binocular microscope (Olympus SZ51, Japan) following the method described by Hla Bu and Leong (1995). Observed parasites were collected, preserved and identified on the basis of available taxonomic characters as described by Lucky (1977), Kabata (1985), and Hoffman (1999).

After counting total number of parasites from the entire gill section they were expressed in terms of prevalence, abundance and mean intensity following the formula proposed by Margolis et al. (1982).

The prevalence of myxozoans was estimated as the percentage of infected fishes out of total number of fish examined.

Prevalence (%)

$$= \frac{\text{Total number of infected fish}}{\text{Total number of fish hosts examined}} \times 100$$

The abundance was estimated as the ratio between the total number of parasites in a sample and the total number of fish examined.

$$\text{Abundance} = \frac{\text{Total number of parasites recovered}}{\text{Total number of fish hosts examined}}$$

The mean intensity was determined as the ratio between the total number of parasites in a sample and the number of infected fish in a sample (parasite host-1).

$$\text{Mean intensity} = \frac{\text{Total number of parasites recovered}}{\text{Total number of infected fish}}$$

Mean intensity of monogeneans was also recorded based on different size class of fish. They were recorded after dividing the lengths into seven equal size classes.

Spatial distribution of myxozoans on gill

For studying spatial distribution of monogeneans, gills were excised and each arch was placed in a separate Petri dish containing filtered pond water and observed under a Stereo-zoom binocular microscope (Olympus SZ51, Japan). Gill arches from each side (both left and right) of the fish were numbered I–IV from the anterior portion of the gill arch below the operculum to the posterior. Each gill arch number was again divided into two hemibranch, anterior and posterior. From each gill portion, numbers of monogeneans were recorded.

Physico-chemical parameters

Different water and soil quality parameters including temperature, water pH, dissolved oxygen (DO), ammonia nitrogen ($\text{NH}_3\text{-N}$), biochemical oxygen demand (BOD_3), soil pH and soil organic carbon (OC) were analyzed following standard procedure (APHA 2005; Carter 1993).

Statistics

Statistical analysis of data was performed using SPSS-15.0 (SPSS Inc., Chicago, IL, USA) software. Correlation and regression analysis was used to find out relationship between different parameters. Results are presented as mean $\pm$ standard error. Comparisons of mean values were determined by Z and F test. Probability levels of 0.01 and 0.05 were used to find out the significance in all cases.

Results

Occurrence of gill myxozoans

In pond C, myxozoan occurrence was recorded continuously whereas in pond B and pond A their occurrences were nil once and five times, respectively within the entire sampling period. The rohu fishes were found to be infected with *Thelohanellus rohita*. Their infestation was as high as 645 per fish in pond A and as low as one per fish in all the ponds during various months of sampling period. However, many samples showed complete absence of myxozoans.

There was no mortality observed in the infected ponds even during the peak time of myxozoan intensity 645 per fish. It indicates that rohu is a hardy fish which can tolerate heavy gill parasite infestation and can manage to survive.

Prevalence, abundance, and mean intensity

In pond A, the prevalence, abundance and mean intensity of myxozoans were zero for most of the sampling days (five sampling days out of 12 sampling days) (Fig. 1). During the last sampling day's occurrence were continuous. In pond B, myxozoans were observed in most of the sampling dates except during the January, 2009. However, the peak abundance and mean intensity was observed in the 11th sampling date (Fig. 2). In pond C, the occurrence of myxozoans was continuous with almost a stable prevalence of 100% throughout the investigation period except fourth and tenth sampling dates (Fig. 3). The highest mean intensity of myxozoans (99.6 parasites per fish) was in the sixth sampling date whereas the lowest myxozoans mean intensity was 28.5 and 25.4 parasites per fish in the fourth and ninth sampling date respectively. In all the ponds, the

Fig. 1 Prevalence, abundance and mean intensity of myxozoans on gills of rohu in pond A

Fig. 2 Prevalence, abundance and mean intensity of myxozoans on gills of rohu in pond B

Fig. 3 Prevalence, abundance and mean intensity of myxozoans on gills of rohu in pond C

trend of variation in their intensity was decreasing up to the month of January and then slowly their abundance get peaked till last sampling, but the amplitude of the myxozoan intensity seems to be regulated by different abiotic factors. In pond C, the prevalence and intensities were highest and stable as compared to other two ponds.

Mean intensity of myxozoans in relation to host length

There was considerable variation of mean intensity of myxozoans with respect to different size class of fish.

Fig. 4 Mean intensity of monogeneans in relation to host length. Star indicates significant difference ($p < 0.05$) between the different size groups

The highest (78.42 ± 18.1) was observed in the size class of 8–9 cm and lowest (4.63 ± 0.18) in 4–5 cm class (Fig. 4). The mean intensity of parasite was significantly higher ($p < 0.05$) in the size class of 8–9 cm in comparison to the other size classes (Fig. 4).

Spatial distribution of myxozoans on gills

Distribution of myxozoans in the left and right side of gill pairs of infected fish as well as in the anterior and posterior hemibranch of each gill arch are shown in the Table 1. There was no significant difference ($p > 0.05$) between the myxozoan intensity of left gill pair and right gill pair of fish. The spatial distribution of myxozoans with respect to gill arch number showed highest preference of the myxozoans on the first gill arch. The significance difference ($p < 0.01$) was observed between the fourth and rest of the gill arches. Also, the presence of myxozoans was more on the posterior hemibranch of each gill arch as compared to the anterior one.

Physico-chemical parameters and their relationship with myxozoan prevalence, abundance, and intensity

Values obtained from the analyses of the physico-chemical parameters are presented in Table 2. Only DO and water temperature show significant Pearson's correlation coefficient ($p < 0.01$ and $p < 0.05$ respectively) with the myxozoan prevalence whereas the pH shows correlation with both abundance and intensity of myxozoans at statistically significant level of $p < 0.05$ and $p < 0.01$ respectively (Table 3) whereas among soil parameters only soil pH showed significant relation with myxozoans prevalence and abundance ($p < 0.05$ and $p < 0.01$ respectively) (Table 4).

Discussion

The results from the present study showed that the myxozoans were not found continuously in all the ponds throughout the investigation period. In fact, the infected ponds exhibited low as well as high prevalence, abundance and intensities of gill myxozoans. There was no mortality observed in the infected ponds even during the peak time of myxozoan intensity 645 per fish. It indicates that rohu is a hardy fish which can tolerate heavy gill parasite infestation and can manage to survive. In pond C, the prevalence and intensities were highest and stable as compared to other two ponds. Hence, these parasites are very efficient in establishing itself in the studied host if certain unknown factors remain optimum for the parasite. The variability in their abundance may be due to physiological properties of water and soil of individual pond. In the study, few abiotic factors showed significant positive correlations with the myxozoan prevalence and intensity, number of researchers suggested the same (Pampoulie et al. 2001; Gbankoto et al. 2001).

A significantly relationship was observed in the present study where the large size group of the fingerlings had more infestation when compared with the smaller size groups but on the other side larger groups of length more than 9 cm has less infestation. This might be due to the well developed immunity among the fishes, further study need to be undertaken to elucidate the observation. However, some studies reported a negative correlation between large host body size and parasite abundance (Poulin and Morand 2000). Highest number of myxozoan attachment to second gill arch has also been reported from other studies (Jeannette et al. 2010). This might be due to the higher ventilation by water currents which pass through second and third arches and their larger surface area (Paling 1968). Myxozoans were also found to have significant preference to attach to posterior hemibranch than to anterior hemibranch of a gill arch. This might be explained on the basis of most suitable hiding as well as sheltered/protected place for attachment.

Observation on the spatial distribution of myxozoans suggested that they had no significant preference for either the left or the right pairs of gills. Similar observation was also reported by other workers (Jeannette et al. 2010). This could be due to the fact that similar volumes of water flowing through the left and right side of the gill might have brought equal amount of infective larval stages to the gill (Paling 1968). The results from the present study also showed that the myxozoan occurrence is strongly correlated with the pH of both water and soil, therefore pH is an important factor for the regulation of their propagation. In the study, we found pH and DO to have negative influence on the propagation of myxozoans. Thus, low pH and low DO will promote their propagation and vice versa.

Table 1 Spatial distribution of monogeneans on gills of rohu

Gill Section	Gill Arch									
	Monogenean on gill arch									
	Left Mean $\pm$ SE					Right Mean $\pm$ SE				
	6.40 $\pm$ 0.86					6.98 $\pm$ 1.09				
	I	II	III	IV		I	II	III	IV	
Anterior	2 $\pm$ 0.33	3.16 $\pm$ 0.39	3.40 $\pm$ 0.46	2.66 $\pm$ 0.35		3.03 $\pm$ 0.49	3.92 $\pm$ 0.49	3.78 $\pm$ 0.57	2.63 $\pm$ 0.38	24.58 $\pm$ 3.66
Posterior	5.92 $\pm$ 1.32	3.97 $\pm$ 0.49	3.22 $\pm$ 0.40	1.29 $\pm$ 0.19		5.29 $\pm$ 0.94	4.87 $\pm$ 0.99	3.10 $\pm$ 0.59	1.31 $\pm$ 0.19	28.97 $\pm$ 5.11
Total	7.92 $\pm$ 1.65	7.13 $\pm$ 0.88	6.62 $\pm$ 0.86	3.95 $\pm$ 0.54		8.32 $\pm$ 1.43	8.79 $\pm$ 1.68	6.88 $\pm$ 1.6	3.94 $\pm$ 0.57	53.55
Right and left gill arch total	16.24 $\pm$ 3.08	15.92 $\pm$ 2.56	13.5 $\pm$ 2.46	7.89 $\pm$ 1.11						
Mean	8.12 $\pm$ 0.2*	7.96 $\pm$ 0.83*	6.75 $\pm$ 0.13*	3.95 $\pm$ 0.01						
%	30.32	29.72	25.25	14.73						

* When test of mean difference (Z value) of myxozoans mean intensity among different gill arches ($n = 120$) was analyzed, gill arch IV showed statistical significant difference with other gill arches ($p < 0.01$)

Table 2 Average physico-chemical parameters of waters and soil in three ponds

	Water pH Mean $\pm$ SE (range)	Water Temp. ($^{\circ}$ C) Mean $\pm$ SE (range)	NH ₃ -N (mg/L) Mean $\pm$ SE (range)	DO (mg/L) Mean $\pm$ SE (range)	BOD ₅ (mg/L) Mean $\pm$ SE (range)	Soil pH Mean $\pm$ SE (range)	Soil organic carbon (%) Mean $\pm$ SE (range)
Pond A	6.79 $\pm$ 0.1 (6.0–7.6)	25.97 $\pm$ 0.94 (21.3–32.6)	0.093 $\pm$ 0.05 (0.002–0.63)	8.83 $\pm$ 0.42 (6.08–10.8)	13.6 $\pm$ 2.27 (6.4–35.2)	4.35 $\pm$ 0.07 (4.10–4.60)	1.025 $\pm$ 0.114 (0.75–1.38)
Pond B	6.54 $\pm$ 0.10 (6.0–7.1)	25.97 $\pm$ 0.94 (21.3–32.6)	0.083 $\pm$ 0.04 (0.001–0.48)	7.24 $\pm$ 0.69 (3.2–10.4)	14.03 $\pm$ 2.27 (6.4–32.0)	4.03 $\pm$ 0.05 (3.80–4.20)	0.78 $\pm$ 0.036 (0.66–0.91)
Pond C	6.57 $\pm$ 0.15 (5.7–7.4)	25.97 $\pm$ 0.94 (21.3–32.5)	0.038 $\pm$ 0.01 (0.001–0.09)	6.91 $\pm$ 0.38 (4.48–8.4)	15.7 $\pm$ 1.92 (6.4–25.6)	4.90 $\pm$ 0.15 (4.40–5.40)	0.59 $\pm$ 0.039 (0.47–0.73)

The data procured for different parameters at various study sites of a water body for the whole period of study were pooled and the average values determined

Table 3 Pearson's correlation coefficient (*r*) between water quality of all ponds with myxozoan intensity (*n* = 36)

Variables	Prevalence	Abundance	Mean intensity	pH	NH ₃ -N	Water temperature	BOD ₃	DO
Prevalence	1							
Abundance	.483**	1						
Mean intensity	.484**	.997**	1					
pH	-.074	-.416*	-.431**	1				
NH ₃ -N	-.201	-.205	-.201	.116	1			
Water temperature	.417*	.086	.066	.514**	-.106	1		
BOD ₃	.024	-.098	-.098	.077	.654**	-.074	1	
DO	-.521**	-.097	-.088	-.140	-.039	-.577**	.072	1

Statistical significant correlation at 5 % (*) and 1 % (**) level of probability

Table 4 Pearson's correlation coefficient (*r*) between soil quality of all ponds with monogenean intensity (*n* = 18)

Variables	Prevalence	Abundance	Mean intensity	Soil pH	Organic carbon
Prevalence	1				
Abundance	.420	1			
Mean intensity	.414	.997**	1		
Soil pH	.502*	.594**	.587*	1	
Organic carbon	-.029	.255	.248	.003	1

Statistical significant correlation at 5 % (*) and 1 % (**) level of probability

The present study conclusively demonstrates that *Labeo rohita* is infected with the myxozoan parasite belonged to genus *Thelohanellus* sp. The first, second and third gill arches showed higher distribution of myxozoans than the fourth one. Posterior hemibranch of second gill arch was the most preferred site for parasite attachment. There were profound variability in the prevalence, abundance and mean intensity of gill myxosporidians from three ponds. During the start of the sampling, myxozoans were present in all the ponds but their intensity varied in different ponds. These may be due to the variability in the abiotic factors of individual ponds. Most importantly acidic pH, lower DO and higher temperature promote myxozoan infestation and their propagation. Low soil pH is also seen to enhance their propagation.

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