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ROLE OF STOCKING DENSITY AND ABIOTIC FACTORS OF POND ON PROPAGATION AND DISTRIBUTION OF MONOGENETIC GILL PARASITES IN FARMED ROHU, *LABEO ROHITA* (HAMILTON) -A STUDY

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Abstract: In farm, monogenean infection occur by mixed species and here occurrence and distribution of monogenetic gill parasites were studied with an objective to evaluate the potentiality of monogeneans to propagate in farmed Indian major carp, rohu (*Labeo rohita*) under different stocking density (low, medium and high). Sampling of water and fish was done fortnightly and soil sample on monthly basis for about six months. Four species of monogeneans were observed. Pond with highest stocking density showed 100% prevalence throughout the period and rest showed sporadic as well as no infection pattern. Study of spatial distribution showed 1st, 2nd and 3rd gill arches had higher distribution of monogeneans than the 4th one. The posterior section of each gill arch had maximum monogenean intensity as compared to the anterior one. The mean intensity of parasites was significantly greater in higher size class of rohu. Dissolved oxygen and soil organic carbon show significant ($p>0.05$ and $p>0.01$) positive correlation with parasite intensity and prevalence respectively. The results suggest that the propagation of gill monogeneans is positively influenced by higher stocking density, organic load and dissolved oxygen and these factors don't influence their distribution pattern in gills.

Key words: Monogenean, Propagation, Stocking density, Rohu (*Labeo rohita*), Abiotic factors.

INTRODUCTION

Parasites are one of the most serious limiting factors in aquaculture. The high risk of disease transmission and parasite infestation among fish species have increased the uncertainty level which farm managers have to contend with in order to develop the fish farming industry (Pozio and Rosa, 2005). The majority of the disease-causing parasites are protozoans, monogeneans and parasitic crustaceans, most of which have direct life cycles and reproduce rapidly under unfavorable pond conditions (Basson and Van As, 1994; Al-Rasheid et al., 2000). Fish appear to co-exist with parasites, in natural habitats as well as in culture conditions, even when infestations are intense. A few parasites are, however, pathogenic to their host fish, usually to younger fish in intensive culture conditions.

Monogenean parasites have been recognized as a serious pathogen of fish in aquaculture (Ogawa, 2002; Montero et al., 2004). Among the monogenean parasites, Dactylogyrids are common which are usually numerous and have a high species density on the gills of the cyprinid fishes (Koskivaara and Valtonen, 1992). The degree of infection depends on the condition and density of host (Ergens, 1983). In wild fishes, monogeneans occurred at low intensities and in apparent equilibrium with their hosts, whereas under cultured conditions (in which fishes are concentrated more than they are in nature) these pathogens build up heavy burdens which can provoke epizootics (Obiekezie, 1991). Most species of monogeneans are restricted not only to a particular host but also to a particular part of host body. The microhabitat of gill monogeneans have been investigated in different fish species (Matejusova, 2002; Kadlec et al., 2003). Apart from stocking density, water quality parameters are also considered as the predisposing factors to infections and losses of fish due to monogeneans (Molnar, 1971).

Rohu (*Labeo rohita*), one of the important Indian major carps (IMCs) species, is commercially cultured throughout India. IMCs are susceptible to different parasitic infections and monogeneans occur mostly in the fry and fingerlings of IMCs in nursery and rearing ponds of India (Das and Das, 1995; Haldar, 1996). And in the above studies it has been observed that in natural condition monogenean infestation is not fixed to a particular species rather vast diversity exists. Moreover, information available in India is largely confined to reports of parasitic diseases and some parasite identification. Little attention has been given to the study of propagation, occurrence and distribution of parasites in different fish species in standard farm condition. Keeping in view the above, the present study was undertaken to (1) study the propagation pattern of gill monogeneans (as a whole, not species wise) under different stocking density following standard aquaculture practices, (2) investigate distribution of monogeneans in gill arches of rohu and (3) investigate the relationship between monogenean occurrences with abiotic factors.

MATERIALS AND METHODS

Study area: The present study was conducted in three rearing ponds of College of Fisheries, Tripura, India (latitude 23°16′ to 24°14′ N and longitude 91°09′ to 91°47′ E). They were designated as pond – A, B, & C, respectively. The ponds were of equal water volume (450 m^3) and the number of fry (monogenean free) stocked in pond A, B and C were 22500 (low), 34000 (medium) and 45,000 (high) respectively. Standard aquaculture management practices were followed in each pond during the study period. Each pond was inoculated with 10 fishes containing gill monogeneans two month prior to the sampling started. 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 six 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 monogeneans: 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 monogenean parasites on each section was counted using a Stereo-zoom binocular microscope (Olympus SZ51, Japan) following the method described by Hla Bu and Leong (Hla Bu and Leong, 1995). Observed parasites were collected, preserved and identified on the basis of available taxonomic characters as described by Bauer (1985), Hotenovsky (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 monogeneans was estimated as the percentage of infected fishes out of total number of fish examined.

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

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 three equal size classes including 4 – 7, 7 – 10 and 10 – 13 cm.

Spatial distribution of monogenean 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 (NH_3-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 ± 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 AND DISCUSSION

Occurrence of gill monogeneans: In pond C, monogenean occurrence was recorded continuously whereas in pond B and pond A their occurrences were noticed thrice and once, respectively within the entire sampling period. Among different monogeneans, both *Dactylogyrus* sp. and *Gyrodactylus* sp. were identified. Most of the monogeneans belonged to the Dactylogyridae family which includes *Dactylogyrus extensus, Dactylogyrus minutus, and Dactylogyrus vastator*. Monogenean infestation was as high as 572 per fish in pond C during November, 2009 and as low as 1 per fish in both pond A and B during April 2009. However, some samples showed complete absence of monogeneans.

There was no mortality observed in the infected ponds even during the peak time of monogenean intensity 572 per fish. It indicates that rohu is a hardy fish which can tolerate heavy gill parasite infestation and can manage to survive. Generally, mortality never occurred in fish longer than 3.2-3.5 cm, and can survive heavy infection with as high as 300 monogeneans per fish (Paperna, 1963).

Propagation of monogeneans (Prevalence, abundance and mean intensity): In pond A, the prevalence, abundance and mean intensity of monogeneans were zero for all the sampling days except the last sampling date (Fig. 1). In pond B, monogeneans were observed only in three sampling dates with the peak prevalence and mean intensity in the 10^{th} and 1^{st} sampling date, respectively (Fig. 2). However, in pond C, the occurrence of monogeneans was continuous with a stable prevalence of 100% throughout the investigation period (Fig. 3). The highest mean intensity of monogeneans (286 parasites per fish) was in the 9th sampling date whereas the lowest monogeneans mean intensity was 86 parasites per fish in the 3rd sampling date.

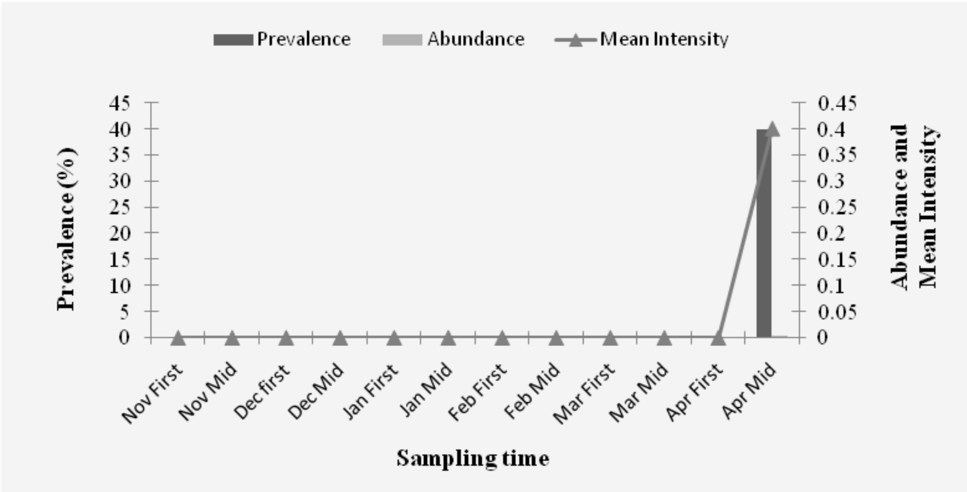

Figure 1. Prevalence, abundance and mean intensity of monogeneans on gills of rohu in pond A.

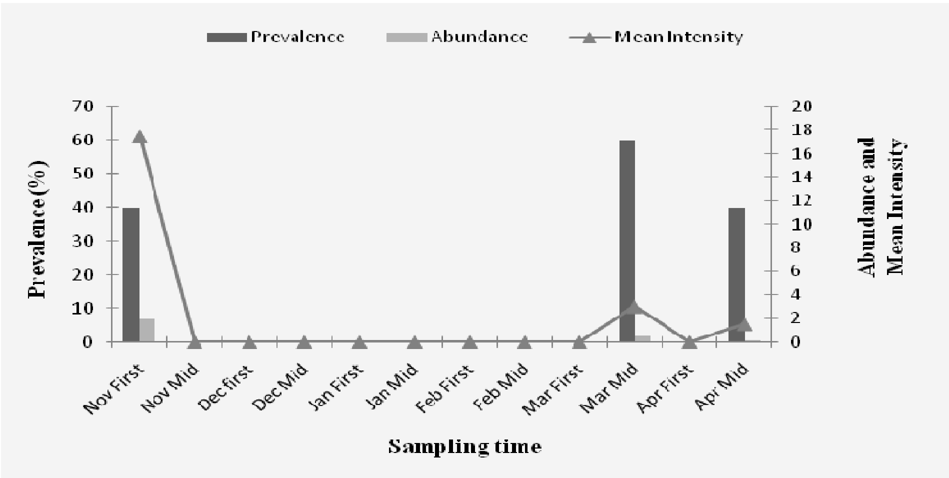

Figure 2. Prevalence, abundance and mean intensity of monogeneans on gills of rohu in pond B.

The propagation of monogeneans in terms of intensity shows increasing trend with the time, but the amplitude of the monogenean intensity seems to be regulated by the stoking density and other abiotic factors. 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 with increasing stocking density. This may be due to three reasons, one is the fact that juvenile fishes have underdeveloped immune systems, which renders the natural repellant ability of the skin and gill surface non-functional and results in increased susceptibility to ectoparasites (Lom, 1995; Barker and Cone, 2000), second the crowding stress, that cause immune suppression and higher susceptibility to pathogens (Costas et al., 2008; Di Marco et al., 2008) and third is the physiological properties of water and soil. In the study, few abiotic factors showed significant positive correlations between with the monogenean prevalence and intensity, number of researchers suggested the same (Dogiel et al., 1961; Malhotra, 1989; Lafferty and Kuris, 2005; Zargar et al., 2001).

Mean intensity of monogeneans in relation to host length: There was considerable variation of mean intensity of monogeneans with respect to different size class of fish. The highest (189 ± 43) was observed in the size class of 11 – 12 cm and lowest (94.6 ± 23) in 5 – 6 cm class (Fig. 4). Once the lengths are divided into three equal size classes including 4 – 7, 7 – 10 and 10 – 13 cm, the mean intensity of parasite was significantly higher ($p < 0.05$) in the size class of 10 – 13 cm in comparison to the lowest size class (Fig 4).

A significantly positive relationship was observed in the present study where the large size group of the fingerlings had more monogenean infestation when compared with the smaller size groups. This might be due to the availability of more space on gill for parasite attachment in large size fingerlings as compare to smaller size fishes. However, some studies reported a negative correlation between large host body size and parasite abundance (Poulin and Morand, 2005). This can be explained in term of monogenean population explosion due to available favorable conditions, as a result they occupy host's available space irrespective of host size.

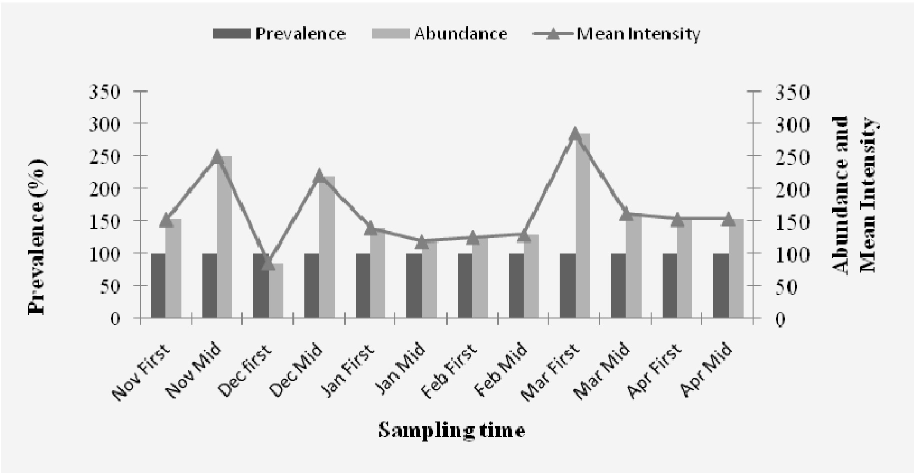

Figure 3. Prevalence, abundance and mean intensity of monogeneans on gills of rohu in pond C.

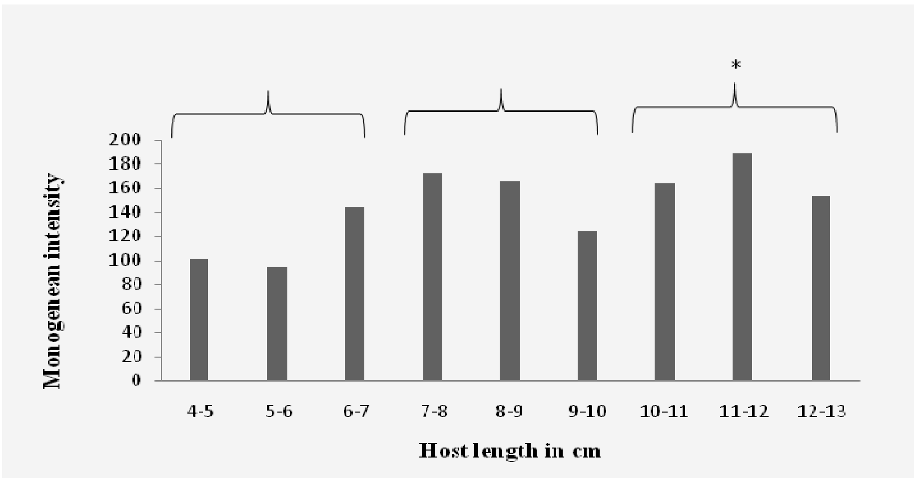

Figure 4. Mean intensity of monogeneans in relation to host length. Asterisk (*) indicates significant difference (p < 0.05) between the largest (10-13 cm) and the smallest size class (4-7 cm)

Spatial Distribution of Monogeneans on gills: Distribution of monogeneans 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 monogenean intensity of left gill pair and right gill pair of fish. The spatial distribution of monogeneans with respect to gill arch number showed highest preference of the monogeneans on the 2nd gill arch. The significance difference ($p < 0.01$) was observed between the 4th and rest of the gill arches. Also, the presence of monogeneans was more on the posterior hemibranch of each gill arch as compared to the anterior one.

Highest number of monogenean attachment to 2nd gill arch has also been reported from other studies (Chapman et al., 2000). This might be due to the higher ventilation by water currents which pass through 2nd and 3rd arches and their larger surface area (Paling, 1968). Monogeneans were also found to have significant preference to attach to posterior hemibranch than to anterior hemibrach of a gill arch. This might be explained on the basis of most suitable hiding as well as sheltered/protected place for attachment. Thus we can say that the stocking density of fishes does not influence their site preferences for attachment in host gills.

Observation on the spatial distribution of monogeneans suggested that the monogeneans had no significant preference for either the left or the right pairs of gills. Similar observation was also reported by other workers (Liang and Leong, 1991). 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 (Raymond et al., 2006).

Physico-chemical parameters and their relationship with monogenean prevalence, abundance and intensity: Values obtained from the analyses of the physico-chemical parameters are presented in Table 2. Only DO and soil OC show positive Pearson's coefficient correlation with the monogenean prevalence, abundance and intensity among other physico-chemical parameters at statistically significant level of $p < 0.05$ and $p < 0.01$ respectively (table 3 and 4). Hence, in pond C, where the stocking density was highest, highest value of soil organic matter and highest monogenean intensity were observed.

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

	Water pH Mean ±SE (Range)	Water Temp. (^{0}C) Mean ±SE (Range)	NH_3-N (mg/L) Mean ±SE (Range)	DO (mg/L) Mean ±SE (Range)	BOD_3 (mg/L) Mean ±SE (Range)	Soil pH Mean ±SE (Range)	Soil organic Carbon (%) Mean ±SE (Range)
Pond A	6.57±0.15 (5.7-7.4)	25.97± 0.94 (21.3-32.5)	0.038±0.01 (0.001-0.09)	6.91±0.38 (4.48-8.4)	15.7±1.92 (6.4-25.6)	4.90±0.15 (4.40-5.40)	0.59±0.039 (0.47-0.73)
Pond B	6.54±0.10 (6.0-7.1)	25.97±0.94 (21.3-32.6)	0.083±0.04 (0.001-0.48)	7.24± 0.69 (3.2-10.4)	14.03±2.27 (6.4-32.0)	4.03± 0.05 (3.80-4.20)	0.78±0.036 (0.66-0.91)
Pond C	6.79± 0.14 (6.0-7.6)	25.97± 0.94 (21.3-32.6)	0.093± 0.05 (0.002-0.63)	8.83± 0.42 (6.08-10.8)	13.6± 2.27 (6.4-35.2)	4.35± 0.07 (4.10-4.60)	1.025±0.114 (0.75-1.38)

Table 3. Pearson's correlation coefficient (r) between water quality of all ponds with monogenean intensity (n=36). Statistical significant correlation at 5% (*) and 1% (**) level of probability

Variables	Prevalence	Abundance	Mean intensity	pH	NH_3-N	Water temperature	BOD_3	DO
Prevelence	1							
Abundance	0.882**	1						
Mean intensity	0.884**	1.000**	1					
pH	0.275	0.301	0.304	1				
NH_3-N	0.080	0.056	0.055	0.116	1			
Water temperature	0.136	0.036	0.038	0.514**	-0.106	1		
BOD_3	-0.156	-0.061	-0.061	0.077	0.654**	-0.074	1	
DO	0.371*	0.415*	0.420*	-0.140	-0.039	-0.577(**)	0.072	1

Table 1. Spatial distribution of monogeneans on gills of rohu. * When test of mean difference (Z value) of monogeneans mean intensity among different gill arches (n= 70) was analyzed, gill arch IV showed statistical significant difference with other gill arches (p < 0.01).

Gill Arch	***MONOGENEAN ON GILL ARCH***								*Total*	***Mean***	*%*
	Left *Mean ± SE*				***Right*** *Mean ± SE*						
	72.9± 6.4				**71.2±6.1**						
Gill Section	I	II	III	IV	I	II	III	IV			
Anterior	7.9±0.8	7.9±0.7	11.4±1.1	8.2±0.8	8.3±0.8	9.3±0.9	10.0±0.9	6.8±0.6	69.9±5.8	8.73±0.5	48.6
Posterior	11.3±1.1	13.2±1.4	8.6±0.8	4.3±0.5	11.7±1.2	12.5±1.1	7.9±0.7	4.7±0.7	74.2±6.7	9.28±1.2	51.4
Total	19.2±1.8	21.1±2.1	20.0±1.9	12.5±1.3	20.0±1.9	21.8±2.0	17.9±1.6	11.5±1.3	144		
Right and left gill arch total	39.2±3.5*	42.9±3.7*	37.9±3.2*	24.1±2.3							
Mean	19.6±0.4	18.05±1.2	16.85±4.2	12.6±0.1							
%	27.2	29.8	26.3	16.7							

Table 4. Pearson's correlation coefficient (r) between soil quality of all ponds with monogenean intensity (n=18). Statistical significant correlation at 5% (*) and 1% (**) level of probability

Variables	***Prevalence***	***Abundance***	***Mean intensity***	***Organic carbon***	***Soil pH***
Prevalence	1				
Abundance	0.948**	1			
Mean intensity	0.948**	1.000**	1		
Organic carbon	0.706**	0.660**	0.663**	1	
Soil pH	-0.239	-0.127	-0.130	-0.248	1

The results from the present study showed that the monogeneans 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 monogeneans. In the study we got a significant positive correlation between Dissolved Oxygen and monogenean intensity unlike others (Raymond et al., 2006). The reason may be that the monogeneans need abundant oxygen to propagate. And further, detail studies are required to specify the reason for the positive correlation. The soil of the pond with highest stocking density (pond C) had maximum soil organic matter and monogenean intensity, and the significant correlation confirms that the crowding do enhance monogeneans infestation by providing favorable conditions like high organic load. Thus, crowding favors monogenean propagation only when condition like high level of D.O remains. Moreover, aggregation and close contacts of the fishes arises due to decreasing space per fish with higher stocking density, helps is transmission of the infection (Sasal, 2003).

In pond B, the monogenean prevalence was observed in 1st, 10th and 12th sampling occasions. There occurred a sudden crash in the monogenean population after the 1st sampling date. This observation could be related to the trend of the temperature change occurred during the period of investigation. When monogeneans were eliminated from the system the temperature of the pond water was 24°C and during reappearance it was 28.1°C. This reappearance might be due to the fact that resting eggs of monogeneans were formed during unfavorable environmental condition that helped in the reappearance of the monogeneans when the favourable condition returned (Paperna, 1963; Lom, 1995). Other studies also showed temperature dependent oviposition and hatching rate of monogeneans (Molnar, 1971; Buchmann, 1988).

There were profound variability in the prevalence, abundance and mean intensity of gill monogeneans from three ponds. During the start of the sampling monogeneans were absent in pond A. May be due to lack of favorable conditions (like high stocking density as well as organic matter or organic carbon) they could not establish themselves. They were observed only in the last sampling date where we find that organic carbon content of pond A reach near to about pond B, before organic carbon were below.

The present study conclusively demonstrates that majority of the monogenean species belonged to genus *Dactylogyrus*. Most importantly stocking density and organic load of ponds played an important role in monogenean infestation and their propagation. High D.O is also seen to enhance their propagation. Pond with more host population density had more monogenean intensity. The 1st, 2nd and 3rd gill arches showed higher distribution of monogeneans than the 4th one. Posterior hemibranch of 2nd gill arch was the most preferred site for parasite attachment. The case of factors like stocking density with other environmental parameter needs further studies to ascertain which one is the main limiting factor for the monogeneans to propagate and thus, fishes can be better managed from parasitic infection through environmental manipulation.

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