

Parasitic Study of Indian Major Carp, *Labeo rohita* (Hamilton, 1822) in Selected Districts of West Bengal, India

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Abstract

The work was conducted to isolate and identify different parasites from *Labeo rohita*, severity of infestation and to find out Parasitic Frequency Index (PFI, %) monthly, seasons and length wise from different selected districts of West Bengal, India. An investigation was made on Rohu (*Labeo rohita*), such way that the selected districts were had more potential fishery resources, easy to collect samples from those districts and easy transportation of collected samples to laboratory. Approximately 325 fishes were observed in between April 2012 to March 2013. The infested fishes suffered mainly from respiratory problems, blackness of the skin and mortalities. The present study revealed that the parasitic infestations were found to be the major problem in cultivable fish ponds of West Bengal. In the present study, the isolated parasites were *Myxobolus* sp., *Thelohanellus* sp., *Trichodina* sp., *Dactylogyrus* sp., *Gyrodactylus* sp., Nematodes, *Argulus* sp., *Lernea* sp., *Chilodonella* sp., *Costia* sp., *Ichthyophtherius multifiliis*, development stages and unidentified Crustaceans. Severity of (infestation) all parasites were found to be more. We were found that *Myxobolus* sp. *Thelohanellus* sp., prevalence were highest in winter (72% and 56% respectively). *Dactylogyrus* sp. and *Gyrodactylus* sp. were more in rainy season and spring respectively (72% and 38% respectively). *Chilodonella* sp., *Costia* sp. and *Ichthyophtherius multifiliis* were found high prevalence in spring season. 1 cm to 30 cm length group fishes were more infested with the parasites compare to 30.5 cm to 50 cm length groups.

1. Introduction

Freshwater aquaculture depends mainly on carp culture practices that account for around 80% of the total inland fish production and have proved sustainable at different levels of production over the years. Though the country possesses a large number of potential cultivable carp species, it is only the three Indian Major Carps viz., catla (*Catla catla*), rohu (*Labeo rohita*) and mrigal (*Cirrhinus mrigala*), that contribute a lion's share with production of over two million tonnes. Production comes from over 2.25 mha of tanks or ponds, 1.3 mha of oxbow lakes, 3 mha of reservoirs and 1.2 mha of coastal brackish water area (Ayyappan, 2007).

West Bengal being a 'rice-fish society', the State is highly significant historically, geographically and strategically since long past. The State has 37% of pond resources in India of which 70% are utilized for fish culture producing 1 to 3 mt

of fresh water fin-fish year⁻¹ of the total 0.276 mha of impounded water area, about 70% to 79% is presently under fish culture. In other water bodies the utilization is much lower except for sewage fed fisheries. (Annual report, 2007-08). The state of West Bengal has always attracted attention for being the highest producer of freshwater table fish and fish seed in the country together with the unique distinction of having the maximum water area under traditional shrimp farming.

It is important to mention here that the parasitic infestations are reportedly playing a major role in disease occurrences (78%) in Indian freshwater aquaculture (Lakra et al., 2006). India which caused an annual loss of US\$ 1 million due to disease-induced mortality and impaired growth (Vineetha and Abraham, 2009). Despite parasitic infestations, neither fish mortalities nor major disease outbreaks were recorded in the present study. Further studies on the life cycle, mode of infection, host-pathogen relationships and pathogenicity of



parasites of carps with more emphasis on parasites needed.

The objectives of the present study were to isolate and identify different parasites from Indian Major Carp (IMC) *Labeo rohita*, severity of infestation and to find out Parasitic Frequency Index (PFI, %) monthly, seasons wise and length groups wise from different selected districts of West Bengal.

2. Materials and Methods

The present study on the prevalence of parasitic infestation in freshwater carps was carried out for a period of 12 months between April 2012 to March 2013. The samples were collected from Garia, Bantala, Bamanghata, Gangajuar of South 24 Parganas District, Naihati of North 24 Parganas District, Memari of Burdwan District of West Bengal, India. The locations were selected in such a way that these units at different locations represent the concern district.

The samples were collected on a regular basis once in every month. In each sampling about 20-30 fishes were collected in live condition. The fishes were brought to the laboratory in live condition with water filled buckets and the total length, body weight of fishes were taken. The vital organs like skin, intestine, kidney and gills were examined for the presence of different parasites. The methods for collection and preservation of the samples for parasitic examination were followed as described by Soota (1980).

External parasites from body surface, fin and gills were removed by scrapping the slime with a sharp scalpel it was mixed with a drop of physiological saline and was spread on a clean dry glass slide with coverslip on top of it. The gill arches were removed and macerated on slides and examined under a compound binocular and trinocular microscope. In case of monogeneans the gills were removed into petridishes containing physiological saline water and gently scrapped to dislodge monogeneans. The monogeneans were removed on to clear slides with a fine pipette in a drop of water and covered with cover slip. For endoparasites fishes were dissected out ventrally by a sharp scalpel to observe parasites inside buccal cavity stomach and intestine. The whole gut was removed in a watch glass containing 0.9% physiological saline and was cleaned several times with tap water to free it from any unwanted materials. Small worms were searched initially with the help of magnifying glass by scrapping out mucus. (Akter et al., 2007).

Phenotypic characterization of all protozoans, monogenians, digeneans, and nematode parasites were studied as described by Soulsby (1982). Photomicrographs were taken using a Motic BA400 phase contrast microscope with in-build digital camera.

2.1. Determination of parasitic frequency index (PFI, %)

The Parasitic Frequency Index (PFI) 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.

$$(a) \text{ Prevalence (\%)} = \frac{\text{Total number of infected fishes}}{\text{Total number of fishes host examined}} \times 100$$

The frequency index were further classified into rare (0.1–9.9%), occasional (10–29.9%), common (30–69.9%) and abundant (70–100%) as per Srivastava (1980).

2.2. Determination of severity of infection/ infestation

In order to assigning numerical qualitative value to severity grade of infections surface infestation and disease syndrome severity, the generalize scheme by Lightner (1993) was followed.

Two way ANOVA was done to determine the significance of differences in Parasitic Frequency Index (PFI) of parasites among different seasons as well as different months and different length groups. It was also followed to determine the significance of differences in prevalence of parasites in different organs of the fishes as well as length groups of fishes. The critical difference (CD) was calculated to examine which of the source and month differed significantly (Snedecor and Cochran 1962).

3. Results and Discussion

3.1. Month wise prevalence of different parasites in *Labeo rohita*

Monthly distribution of parasites in *Labeo rohita* is presented in Table 1. According to (Hoffman, 1967) the incidence of fish parasites related directly to fish health. The fish parasites feed either on the digested contents of the host's intestine, or directly on host tissues. The parasites multiply rapidly under favorable conditions (Dogiel, 1956), and cause economic loss by affecting the health of fishes, often cause high mortality (Tripathi, 1959). Parasites interfere with nutrition of hosts, disrupts metabolism and secretory functions of alimentary canal and damage nervous system (Markov, 1961). Dogiel (1961) suggested 15 factors, which directly influence the parasitic fauna of fish. These factors include age, diet and abundance of fish, independence of the numbers of parasitic fauna within the fish and season.

3.1.1. Prevalence of different parasites in *Labeo rohita*

Parasitic Frequency Index (PFI) of *Myxobolus* sp. (Figures 1 and 3) were found highest during August, November and December (PFI, 74.7%, 80% and 90.47% respectively), which were stated as 'abundant' condition. The occurrence of these parasite reaches to the lowest in the month of July

(PFI, 11.53%), and the condition were 'occasional'. These results strongly supported with Bhuiyan et al., 2007, in *L. rohita*. PFI (%) of *Thelohanellus* sp. (Figures 2-6) were found throughout the year except May and July. Highest prevalence was recorded in the month of December (PFI, 85.71%) and lowest in September (PFI, 7.4%). This was probably due to the fact that decrease in water temperature caused nutritional imbalance resulting in less production of fish food organisms and reported more incidences of disease in fish during winter months these results supported by Akhter et al., (1997); Banu et al., (1993); Chandra et al., (1997); Hossain et al., (1994a, b). Disease was also 'common' in the months of November, January and March. These were 'occasional' in April, June, August and October months. PFI (%) of *Trichodina* sp. (Figure 17) varied from 9.09% to 56% throughout the year, highest in the month of January stated as 'common' and lowest in the month of October stated as 'rare'. These were found 'commonly' in April, May, June, September, November, January, March and also found 'occasionally' in the month of July. In the present study *Trichodina* sp. was extensively isolated from gills and skin, these results were similar with results of Osman 2001, Younis 2004 and El-Shahat 2004). Majumder (2011) who studies of *Trichodina* sp. on exotic carps, which were also corroborated the results of present findings and *Gyrodactylus* sp. (Figure 11) showed highest prevalence in the month of August (PFI: 81.48%), which was stated as 'abundant' and lowest prevalence in the month of November (PFI: 8%), stated as 'rare'. This was not recorded or absent in the months of March, April, May and June. It was also found commonly in the month of February (PFI: 54%). July, September, October, December and January were also found 'occasional' condition (10–29.9%).

Prevalence (PFI%) of *Dactylogyrus* sp. (Figure 12) varied from 4.16% to 94% and the occurrence of these parasite reached high in the month of December, January and February (PFI: 80.95 %, 80% and 94% respectively), which were stated as 'abundant'. PFI% reached low in the month of April and June and during this study *Dactylogyrus* sp. were not observed in the months May and March. They were found 'rarely' in the months of April and June. The result of the present study corroborates with that of Kim et al., (2001). Ogawa (1988) reported higher prevalence of *Bivagina tai* (a microcotylid, monogenean) in October to January (winter months). Ghosh et al., (1987) also observed highest infestation of *Dactylogyrus* sp. in Indian Major Carp (*Catla catla*) during winter (December and February) which was similar to this study. Nematode prevalence varied from 4.54% to 25% throughout the year, highest in the month of June i.e., 25% and lowest in the month

of October i.e., 4.54%. It was only recorded in the months of June, August and October, rest of the months they were not found. The results of the present study satisfied all the criteria as reported by Dash et al., (2008), he reported that the month of August was observed as most suitable for proliferation of nematode parasites in *Catla catla* and *Cirrihinus mrigala*. On the contrary, in *Labeo rohita* highest prevalence was observed in the month of September. This might be due to the abundance of intermediate hosts, sudden fluctuation of water quality parameters were responsible for this incidence, present finding similar with results of Dash et al. (2008); Kim et al. (2001). Present study supported with the results of Majumder (2011), who studied on exotic carps. *Argulus* sp. (Figure 10) were found during September to December and March (PFI: 14.81%, 9.09%, 8% and 4.76% respectively) which considered as 'rare' and rest of the months it was not recorded. Sometimes it was 'occasionally' (PFI: 14.81%) found in September. *Argulus* sp. the crustacean ectoparasite spent major part of the life in surface and column waters and *L. rohita* was found to be most susceptible to argulosis, present results were supported with the findings of Shella et al. (2002); Samir (2007). *Lerneae* sp. found 'occasionally' (PFI: 18.51%) in the month of September, rest of the months these were not recorded. In rainy season due to the rainfall and decrease in temperature the prevalence of diseases were more which corroborated with the findings of Moller (1978).

Chilodonella sp. (Figure 7) found 'rarely' in the month of April (PFI, 8.33%), October (PFI, 4.54%) and 'occasionally' found in December (PFI, 28.57%) and February, (PFI, 22%). Highest prevalence were recorded in the month of December (PFI, 28.57%) and lowest in October (PFI, 4.54%). PFI (%) of Digeneans (Figure 14) varies from 4.76% to 12%. These were found only in the months of August, November and December. Rest of the months they were not recorded. Present results strongly supported by Bhuiyan et al. (2007), who opinioned about Monthly fluctuation in prevalence, mean intensity and abundance of parasites in *L. rohita*. The maximum prevalence (100%) were recorded in December. Kim et al. (2001) reported the lowest prevalence of digeneans in greenlings (*Hexagrammos otakii*) during monsoon and highest during winter. The reasons for higher occurrence of digeneans in winter may be due to optimum temperature for their growth which lies in lower temperature range as in winter season. Koie (1982) found higher occurrence of *Aprocotyle simplex* in December at 6 °C. Another reason may be the availability of intermediate hosts (snail). *Costia* sp. (Figure 8) and *Ichthyophtherius multifiliis* (Figure 9) were recorded only in the month of February (PFI, 32%, 58%). The occurrence



Table 1: Prevalence (PFI %) and severity of infection of parasites in *Labeo rohita* of West Bengal during the period between April 2012 and March 2013

Month	No. of Fish investigated	Parasitic data				
		Parasites present	No. of infected fishes	PFI (%)	Site of infection	Severity of infection
April	24	<i>Myxobolus</i> sp.	6	25.00	Gill	0.5
		<i>Thelohanellus</i> sp.	5	20.83	Gill	0.5
		<i>Trichodina</i> sp.	12	50.00	Gill	1
		<i>Chilodonella</i> sp.	2	8.33	Gill	0.5
		<i>Vorticella</i> sp.	7	29.16	Gill	0.5
		<i>Dactylogyrus</i> sp.	1	4.16	Gill	0.5
May	26	<i>Trichodina</i> sp.	12	46.15	Gill	0.5
June	20	<i>Myxobolus</i> sp.	11	55.00	Gill	2
		<i>Thelohanellus</i> sp.	4	20.00	Gill	3
		<i>Trichodina</i> sp.	11	55.00	Gill	0.5
		<i>Vorticella</i> sp.	5	25.00	Gill	0.5
		<i>Dactylogyrus</i> sp.	1	5.00	Gill	0.5
		Intestinal flukes	3	15.00	Intestine	1
		Nematodes	5	25.00	Intestine	0.5
		Crustaceans	1	5.00	Body	0.5
July	26	<i>Myxobolus</i> sp.	3	11.53	Gill	0.5
		<i>Trichodina</i> sp.	7	26.92	Gill	0.5
		<i>Dactylogyrus</i> sp.	16	61.53	Gill	2
		<i>Gyrodactylus</i> sp.	4	15.38	Body	0.5
		Crustaceans	1	3.84	Fin	0.5
August	27	<i>Myxobolus</i> sp.	20	74.07	Gill	1
		<i>Thelohanellus</i> sp.	8	29.62	Gill	1
		<i>Dactylogyrus</i> sp.	22	81.48	Gill	3
		<i>Gyrodactylus</i> sp.	8	29.62	Fin	0.5
		Nematodes	2	7.40	Intestine	0.5
		Developmental stage	2	7.40	Gill	0.5
September	27	<i>Thelohanellus</i> sp.	2	7.40	Gill	0.5
		<i>Trichodina</i> sp.	14	51.85	Gill	1
		<i>Dactylogyrus</i> sp.	20	74.07	Gill	2
		<i>Gyrodactylus</i> sp.	3	11.11	Gill	0.5
		<i>Lernea</i> sp.	5	18.51	Fin	0.5
		<i>Argulus</i> sp.	4	14.81	Body	0.5
October	22	<i>Myxobolus</i> sp.	7	31.81	Gill	0.5
		<i>Thelohanellus</i> sp.	3	13.63	Gill	0.5
		<i>Trichodina</i> sp.	2	9.09	Gill	0.5
		<i>Chilodonella</i> sp.	1	4.54	Gill	0.5
		<i>Gyrodactylus</i> sp.	4	18.18	Skin	1
		<i>Dactylogyrus</i> sp.	14	63.63	Gill	2
		Nematodes	1	4.54	Intestine	0.5

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Month	No. of Fish investigated	Parasitic data				
		Parasites present	No. of Infected fishes	PFI (%)	Site of infection	Severity of infection
November	25	Digenean	2	9.09	Intestine	0.5
		<i>Argulus</i> sp.	2	9.09	Body	0.5
		<i>Myxobolus</i> sp.	20	80.00	Gill and Body	1
		<i>Thelohanellus</i> sp.	16	64.00	Gill and Fin	1
		<i>Trichodina</i> sp.	12	48.00	Gill	0.5
		<i>Gyrodactylus</i> sp.	2	8.00	Skin	0.5
		<i>Dactylogyrus</i> sp.	10	40.00	Operculum	1
		<i>Argulus</i> sp.	2	8.00	Gill	0.5
December	21	Developmental stage	2	8.00		0.5
		<i>Myxobolus</i> sp.	19	90.47	Gill	2
		<i>Thelohanellus</i> sp.	18	85.71	Gill	1
		<i>Chilodonella</i> sp.	6	28.57	Skin	0.5
		<i>Gyrodactylus</i> sp.	3	14.28	Gill	0.5
		<i>Dactylogyrus</i> sp.	17	80.95	Gill	1
		Digenean	1	4.76	Intestine	0.5
		<i>Argulus</i> sp.	1	4.76	Body	0.5
January	25	Developmental stage	1	4.76	Gill	0.5
		<i>Myxobolus</i> sp.	19	76.00	Gill	0.5
		<i>Thelohanellus</i> sp.	16	64.00	Gill	0.5
		<i>Trichodina</i> sp.	14	56.00	Gill	0.5
		<i>Gyrodactylus</i> sp.	4	16.00	Body	0.5
		<i>Dactylogyrus</i> sp.	20	80.00	Gill	1
		Digenean	3	12.00	Intestine	0.5
		<i>Myxobolus</i> sp.	20	40.00	Gill	2
February	50	<i>Thelohanellus</i> sp.	13	26.00	Gill	1
		<i>Costia</i> sp.	16	32.00	Skin	0.5
		<i>Chilodonella</i> sp.	11	22.00	Skin	1
		<i>Ichthyophtherius multifiliis</i>	29	58.00	Body and Gill	3
		<i>Gyrodactylus</i> sp.	27	54.00	Body and Gill	1
		<i>Dactylogyrus</i> sp.	47	94.00	Gill	2
		<i>Myxobolus</i> sp.	14	66.66	Gill	2
		<i>Thelohanellus</i> sp.	14	66.66	Gill & Fins	2
March	21	<i>Trichodina</i> sp.	7	33.33	Gill	1
		<i>Argulus</i> sp.	1	4.761	Body	0.5

Table 2: Two way ANOVA of PFI values for *Labeo rohita* from April-2012 to March-2013

Source of Variation	SS	df	MS	F	p-value	F crit
Parasites	47968.67	15	3197.911	10.44942	2.45E-17	1.727388
Months	5019.779	11	456.3435	1.491138	0.138994	1.847078
Error	50496.13	165	306.0371			
Total	103484.6	191				

of these parasites reached to the peak during February (PFI, 32%, 58%) and the condition was stated as 'common' and rest of the months not found.

Statistical analysis (Table 2) revealed that there was significant difference ($p < 0.05$, $df = 11$) in monthly *Labeo rohita* PFI (%) values. Similarly there was significant difference ($p < 0.05$, $df = 15$) in PFI (%) in values among the parasites.

3.2. Seasons wise prevalence of different parasites in *Labeo rohita*

The occurrence of parasites in *Labeo rohita* in different seasons is represented in Table 3. The influence of parasites in relation to the seasons has been described by many workers Bhuiyan et al. (2007); Banu et al., (1993); Chandra et al. (1997) who worked on seasonal variations. The total study period was divided into four seasons; i.e., Summer (April-June); rainy season (July-September) or Monsoon Winter (October-January) and Spring (February- March).

3.2.1. Occurrence of parasites in different seasons in *Labeo rohita*

Myxobolus sp. were found in all seasons, but it were reached peak stage during the winter season (PFI, 72%) which were 'abundant', low in summer (PFI, 24%). They were also 'occasional' in summer and monsoon season but 'common' during spring season. These results are strongly supported with Bhuiyan et al. (2007), in *L. rohita*.

Prevalence of *Thelohanellus* sp. were increased from summer to winter (PFI, 12 to 56%). These parasites occasionally found in summer and monsoon (PFI, 12%) and commonly found in spring season (PFI, 38%). This was probably due to the fact that decrease in water volume during the dry season caused nutritional imbalance resulting in less production of fish food organisms in one hand and on the other hand fall in water temperature and reduced the immune response in fish and made them more vulnerable to disease vectors. So present results supported by Akhter et al. (1997); Chandra et al. (1997) who had also reported more incidence of diseases in fish during winter months.

Trichodina sp. commonly found in summer and winter (PFI, 50% and 30% respectively), these were reached peak stage in summer (PFI, 50%), lowest in spring season (PFI, 9.8%). These were found 'occasionally' in monsoon season. As the water quality parameters fluctuate very quickly during winter and summer season, fish becomes affected with diseases in these two seasons. *Trichodina* sp. were extensively isolated from gills of tilapia and catfishes (Osman, 2001; Younis, 2004; El-Shahat, 2004) which corroborated with the present findings.

Dactylogyrus sp. were found in all seasons. Prevalence of these parasites reaches peak in monsoon (PFI, 72%), which stated as 'abundant', low in summer (PFI, 2.8%) stated as rare. These parasites were found common in winter and spring (PFI, 54% and 66% respectively). Prevalence of *Gyrodactylus* sp. were recorded highest in spring season (PFI, 38%), lowest in winter season (PFI, 13%), and these parasites found in all seasons except in summer. The results of this study are similar with that the results of Aydogdu and Altunel (2002) and Hoole et al. (2001). Aydogdu and Altunel (2002) found that *Dactylogyrus vastator* as a common parasites of carps and the infection rates were higher during summer. Hoole et al. (2001) reported that prevalence of *Gyrodactylus crysoleucas* increased in late spring and summer. The nematodes were common in the body cavity, muscle, mesenteries, alimentary canal and other visceral organs of the hosts examined. Nematodes were constantly decreased from summer to spring (PFI, 7.1% to 0%) which were stated as 'rare'. Ahmed and Ezaz (1997); Tweb and Ahmed (1981) observed nematodes from the same vital organs of *Heteropneustes fossilis*, *Channa* sp. and *Clarias batrachus*.

During the study period *Argulus* sp. were constantly decreased from monsoon to spring (PFI, 5% to 1.4%) which was stated as 'rare' and these parasites not found in summer season. *Lernea* sp. were found only in monsoon season (PFI, 6.2%), as 'rare', and not found in rest of the seasons. However, Srivastava and Mukherjee (1994); Paria and Konar (1999) reported that argulosis was not a significant problem, as only 2.39% of the total 1332 numbers freshwater fish ponds surveyed in West Bengal was affected with argulus. So the present study contradicts the earlier findings establishing the fact that the bheries are more susceptible to argulosis, because of the highly enriched organic environment. *Chilodonella* sp. were found in all seasons except in winter, these parasites also found 'rarely' in monsoon and spring seasons (PFI, 2.85% and 7.85%). *Chilodonella* sp. were reached peak stage in spring season (PFI, 15.7%). During the study period *Vorticella* sp. were found only in summer season (PFI, 17.1 and 4.2%), not found in rest of the seasons. Ectoparasitic protozoa attack the fish and cause massive destruction of the skin and gill epithelium (Sterud et al., 2003; Enayat et al., 2008) which also approved the present findings.

Prevalence of development stages of parasites were found in monsoon and winter (PFI: 2.5% and 3.2% respectively), rest of the months these were not found. Developmental stages of digenetic trematodes (*Prohemistomum* sp.) were recovered from intestine and different internal organs of *Tilapia* sp., catfish and mullets (Amer and El-Ashram, 2000). This works corroborated with the present findings. Spores or other forms

such as encysted forms of *Chilodonella hexasticha* were found in the gills (Rowland et al., 1991). Nematodes (such as the Oxyuridae and Kathlanidae) are monoxenous (single host) and occur in the intestines of detritus feeders and omnivorous fish (Khalil, 1971). Fish may become accidentally infected with free living invasive forms of parasites when feeding. For instance, spores of *Henneguya psorospermica* sedimented on the bottom (Sulman et al., 1980) are inadvertently ingested when fish (European perch) feed near the bottom which may also be the reason for higher prevalence of developmental stages in the intestine. Present findings were similar with results of Majumder (2011), who nematode parasitic studies on exotic carps. Digeneans were found only in winter season (PFI, 5.3%) which was stated as 'rare'. *Costia* sp. and *Ichthyoptherius multifilus* were found in only spring season (PFI, 22.5% and 40.8%). Not found in rest of the seasons. The digeneans was more in the intestine of *Labeo rohita* compared to gills and stomach of the fishes. This observation derives support from that of Kim et al. (2001) who observed maximum incidence of *Opecoelus sphaericus* in intestine of greenlings (*Hexagrammos otakii*). Higher incidence of digeneans in intestine may be attributed to the endoparasitic nature of this group. During the study period *Vorticella* sp. (Figure 18) and Intestinal flukes were found only in summer season (PFI, 17.1 and 4.2%), not found in rest of the seasons. Crustaceans were found 'rarely' in summer and monsoon (1.2% and 1.4%), these were not found in rest of the seasons. Prevalence of development stages (Figures 13 and 15) of parasites were found in monsoon and winter (PFI: 2.5% and 3.2% respectively), rest of the months these were not found. Digeneans were found only in winter season (PFI, 5.3%) which was stated as 'rare'. *Costia* sp. and *Ichthyoptherius multifilus* were found in only spring season (PFI, 22.5% and 40.8%). Not found in rest of the seasons.

Statistical analysis (Table 4) revealed that there was no significant differences ($p>0.05$, $df=3$) in the PFI values among the seasons. There was no significant differences ($p>0.05$, $df=15$) in the PFI values among the *Dactylogyrus* sp., *Myxobolus* sp., *Thelohanellus* sp., *Trichodina* sp., *Gyrodactylus* sp. and *Ichthyoptherius multifilus*. However the PFI values differ significantly ($p<0.05$, $df=15$) among *Dactylogyrus* sp., and *Myxobolus* sp. There was significant difference ($p<0.05$, $df=15$) in PFI values among the *Dactylogyrus* sp., *Chilodonella* sp., *Costia* sp., *Vorticella* sp., Nematodes, *Argulus* sp., *Lernea* sp., development stages of parasites, Digeneans and Intestinal flukes. However there was no significant difference in PFI values ($p>0.05$, $df=15$) among the *Myxobolus* sp., *Thelohanellus* sp., *Trichodina* sp., *Gyrodactylus* sp., *Ichthyoptherius multifilus*, *Chilodonella* sp.

and *Costia* sp. Similarly there was significant difference in PFI values among the *Myxobolus* sp., *Vorticella* sp., Nematodes, *Argulus* sp., *Lernea* sp., development stages of parasites, Digeneans and Intestinal flukes. Except *Myxobolus* sp. and *Dactylogyrus* sp. there was no significant difference ($p>0.05$, $df=15$) in PFI values among all rest parasites.

3.3. Length groups wise prevalence of different parasites in *Labeo rohita*

The influence of parasites in relation to the length of fishes has been described by many workers (Jha and Sinha, 1990; Shomorendra et al., 2005, 2007). The size of the fishes may affect prevalence and the diversity of parasitic fauna, so the Indian Major Carps examined were divided into different length groups and the results were noted and discussed made accordingly. The distribution of different parasites in different length groups of *Labeo rohita* is represented in Table 5. The fishes were grouped into 1–10 to 40.5–50 cm length groups.

3.3.1. Prevalence of parasites in different length groups of *Labeo rohita*

Prevalence of *Trichodina* sp. were more in 40.5 to 50 cm (PFI, 50%) length group fishes, which were 'common' and lowest in 20.5 to 30 cm (PFI, 9.1%), stated as 'rare'. These parasites were 'occasional' in 1–10, 10.5–20 and 30.5–40 cm length groups. Paperna and Thurston (1968) who reported that *Tilapia* sp. of all ages and sizes were susceptible to *Trichodina* sp infection throughout the year which supported the present findings also in cases of stress, several species of *Trichodina* may become pathogenic interfering with feeding and respiration of small fish (Sarig, 1968b; Ahmed, 1976). Geets and Ollevier (1996); Oniye and Aken'Ova (1999) also reported an increase in the abundance of parasites with host size. *Trichodina* are very common ectoparasites which in most cases are pathogenic to both freshwater and marine fish (Wellborn, 1967).

Gyrodactylus sp. were found more (PFI, 27.7%) in 1–10 cm length group fishes, which was 'occasional'. Lowest (PFI, 5.9%) found in 20.5 to 30 cm stated as 'rare'. These parasites were 'rare' in 20.5–30 and 30.5–40 cm length groups of fishes, 'occasional' in 1–10 and 30.5–40 cm length groups. During the study period occurrences of *Dactylogyrus* sp. were 'common' in all length groups of *Labeo rohita* except 20.5 to 30 cm length group which was 'occasional'. These parasites were reached peak (PFI, 62.1%) in 1 to 10 cm length group, lowest (PFI, 24.3%) in 20.5 to 30 cm length group. The present study revealed that overall prevalence of monogeneans were more in small size groups of hosts than the large size groups. The results of the present study corroborate with the observations of Paperna (1963a, b), Molnar (1984, 1987); Hoffman (1979); Ghosh et al. (1987), Roberts (2001); Malmberg (1970). Paperna

Table 3: Prevalence (PFI, %) of parasites in *Labeo rohita* in different seasons from April 2012 to March 2013

Period	Total no of fish examined	<i>Myxobolus</i> sp.		<i>Thelohanellus</i> sp.		<i>Trichodina</i> sp.		<i>Dactylogyrus</i> sp.		<i>Gyrodactylus</i> sp.	
		No. of infected fish	PFI (%)	No. of infected fish	PFI (%)	No. of infected fish	PFI (%)	No. of infected fish	PFI (%)	No. of infected fish	PFI (%)
Summer (April - June)	70	17	24 ^b	9	12 ^b	35	50 ^c	2	2.8 ^a	0	0
Rainy season (July-September) or Monsoon	80	23	28 ^b	10	12 ^b	21	26 ^b	58	72 ^d	15	18 ^b
Winter (October- January)	93	65	72 ^d	53	56 ^c	28	30 ^c	51	54 ^c	13	13 ^b
Spring (February- March)	71	34	47 ^c	27	38 ^c	7	9.8 ^a	47	66 ^c	27	38 ^c

Table 3 continue

Period	Total no of fish examined	<i>Chilodonella</i> sp.		<i>Vorticella</i> sp.		Intestinal flukes		Un identified crustaceans		Development stage		Digeneans	
		No. of infected fish	PFI (%)	No. of infected fish	PFI (%)	No. of infected fish	PFI (%)	No. of infected fish	PFI (%)	No. of infected fish	PFI (%)	No. of infected fish	PFI (%)
Summer (April-June)	70	2	2.85 ^a	12	17.1 ^b	3	4.2 ^a	1	1.4 ^a	0	0	0	0
Rainy season (July-September) or Monsoon	80	0	0	0	0	0	0	1	1.2 ^a	2	2.5 ^a	0	0
Winter (October-January)	93	7	7.5 ^a	0	0	0	0	0	0	3	3.2 ^a	5	5.3 ^a
Spring (February-March)	71	11	15.4 ^b	0	0	0	0	0	0	0	0	0	0

Table 3 continue

Period	Total no of fish examined	Nematodes		<i>Argulus</i> sp.		<i>Lernea</i> sp.		<i>Costia</i> sp.		<i>Ichthyophtherius multifiliis</i>	
		No. of infected fish	PFI (%)	No. of infected fish	PFI (%)	No. of infected fish	PFI (%)	No. of infected fish	PFI (%)	No. of infected fish	PFI (%)
Summer (April-June)	70	5	7.1 ^a	0	0	0	0	0	0	0	0
Rainy season (July-September) or Monsoon	80	2	2.5 ^a	4	5 ^a	5 ^a	6.2 ^a	0	0	0	0
Winter (October- January)	93	2	2.1 ^a	4	4.3 ^a	0	0	0	0	0	0
Spring (February-March)	71	0	0	1	1.4 ^a	0	0	16	22.5 ^b	29	40.8 ^c

PFI: Parasitic frequency index (%); a: rare (0.1 – 9.9%); b: Occasional (10–29.9%); c: Common (30–69.9%); d: Abundant (70–100%)

Table 4: Two way ANOVA of PFI values for *Labeo rohita* from April-2012 to March-2013 in different seasons

Source of Variation	SS	df	MS	F	p-value	F crit
Seasons	956.3282	3	318.7761	1.678918	0.184961	2.811544
Parasites	15498.65	15	1033.243	5.441847	4.63E-06	1.894875
Error	8544.149	45	189.87			
Total	24999.13	63				

tissue by *Dactylogyrus vastator*. Malmberg (1970) also reported a similar result. According to him occurrence of *Gyrodactylus anguillae* were more in young eels host in captivity. Hoffman (1979) proved that *Gyrodactylus ictaluri* was not dangerous to adult fish but may kill fry and small fingerlings in fish farms. Ghosh et al. (1987) found that monogeneans parasites were observed on fish of all size groups almost throughout the year and the tendency of higher rate of infestation in lower size groups also found. Roberts (2001) also found a similar result. He reported that *Dactylogyrus vastator* were most dangerous parasite of carp fry (2–5) cm in length and *Dactylogyrus extensus* also infect carp more in fry. In the present study prevalence of monogeneans were more in smaller size groups of hosts than larger size groups. The probable reasons behind these results may due to the low resistant power of small size fishes and host specificity of parasites.

Nematodes were ‘rare’ in 10.5–20 and 20.5–30 cm length groups, not found in rest of the length groups. This result showed that the nematodes infection were more in smaller group than the larger size group of fishes. The changes in food habits in different stage of the hosts may be the reason for fluctuation in the infestation by the nematodes in different groups of fishes. Gonzalez (1998) also found a similar result. During the study period occurrences of *Argulus* sp. were found ‘rare’ in 10–40 cm length groups and ‘common’ in 40.5 to 50 cm length group. Bigger size fishes were observed to have higher rate of protozoan parasites than the smaller ones. This might be because the bigger ones cover wider areas in search of food. There is chance of better contact of these fishes with the different stages of parasites from either surface, column or in bottom of the pond. *Argulus* is an ectoparasite, generally found in the skin and gill portion of the fish body. Large individuals preferred the skin behind the base of the pelvic and pectoral fins and lesser extends in adipose fins. Mouth, base of the dorsal fin and ventral fins of the hosts were found to be the best suited

place for the attachment of the parasite (Bazal et al., 1969; Schlute, r 1978; Shimura, 1983c; Mohan et al., 1986; Nandi et al., 1991; Chowdhury, 2002; Shella et al., 2002). The present study corroborated with the findings of earlier authors that it was found to attack mainly on external organs.

Developmental stages of parasites were rare in 1–30 cm length groups and not found in rest of the length groups. The youngers were more susceptible for developmental stages of monogenetic trematodes may be due to the poorly developed immune system and the delicate structure of gills in those young stages (Thoney and Hargis, 1991; Saleh and El-Nobi, 2003) which corroborated with the present findings. During the study period occurrence of *Chilodonella* sp. were ‘rare’ 10–30 cm length groups, ‘occasional’ in 1–10 cm length group. *Learnea* sp. were found 30.5 to 40 cm length group, which was ‘occasional’ (PFI: 16%). And not found in rest of the length groups. *Learnea* sp. is an ectoparasite, generally found in the skin portion of the fish body. Pathogenicity of lernaids largely depends on their host size and their attachment site preferences. Infection by a single or 2–3 females is very much damaging or even deadly to young or small fish (<40 mm long). Parasites may anchor in the liver, intestine or brain (Paperna and Thurston, 1968). But at present study showing more prevalence in large fishes, it may be due to large fishes (30.5 to 40 cm length group) providing of large surface area to the *Learnea* sp.

Crustaceans, Digeneans and *Vorticella* sp. were found ‘rarely’ in 10.5–30 cm length groups, rest of the length groups these were not found. *Ichthyophthirius multifiliis* and *Costia* sp. were ‘rare’ in 10.5 to 20 cm length groups; ‘occasional’ in 1 to 10 cm length group and not found in rest of the length groups. Intestinal flukes were found ‘rare’ in 10.5–20 cm length groups (PFI: 2.1%), rest of the length groups not found. The prevalence of parasitic infections correlated with fish lengths which in turn corresponds to fish age as reported by Lagler et

Table 5: Prevalence (PFI, %) of parasites in different length groups of *Labeo rohita* from April 2012 to March 2013

Parasites	Total no of fish examined	<i>Myxobolus</i> sp.		<i>Thelohanellus</i> sp.		<i>Trichodina</i> sp.		<i>Gyrodactylus</i> sp.		<i>Dactylogyrus</i> sp.	
		No. of infected fish	PFI (%)	No. of infected fish	PFI (%)	No. of infected fish	PFI (%)	No. of infected fish	PFI (%)	No. of infected fish	PFI (%)
1–10	74	25	33.7 ^c	25	33.7 ^c	13	17.5 ^b	22	29.7 ^b	46	62.1 ^c
10.5–20	140	73	52.1 ^c	56	40 ^c	38	27.1 ^b	13	9.2 ^a	73	52.1 ^c
20.5–30	185	39	21.0 ^b	23	12.4 ^b	17	9.1 ^a	11	5.9 ^a	45	24.3 ^b
30.5–40	6	1	16.6 ^b	0	0	1	16.6 ^b	1	16.6 ^b	2	33.3 ^c
40.5–50	2	1	50 ^c	0	0	1	50 ^c	0	0	1	50 ^c
Total	407										



Table 5 continue

Parasites	Total no of fish examined	<i>Chilodonella</i> sp.		<i>Learnea</i> sp.		Crustaceans		<i>Ichthyophthirius multifiliis</i>		Digenean parasites		<i>Costia</i> sp.	
		No. of infected fish	PFI (%)	No. of infected fish	PFI (%)	No. of infected fish	PFI (%)	No. of infected fish	PFI (%)	No. of infected fish	PFI (%)	No. of infected fish	PFI (%)
1-10	74	11	14.8 ^b	0	0	0	0	22	29.7 ^b	0	0	9	12.1 ^b
10.5-20	140	2	1.4 ^a	0	0	1	0.7 ^a	6	4.2 ^a	2	1.42 ^a	6	4.2 ^a
20.5-30	185	2	1.0 ^a	0	0	1	0.5 ^a	0	0	1	0.54 ^a	0	0
30.5-40	6	0	0	1	16 ^b	0	0	0	0	0	0	0	0
40.5-50	2	0	0	0	0	0	0	0	0	0	0	0	0
Total	407												

Table 5 continue

Parasites	Total no of fish examined	<i>Vorticella</i> sp.		Intestinal flukes		Nematodes		<i>Argulus</i> sp.		Development stage	
		No. of infected fish	PFI (%)	No. of infected fish	PFI (%)	No. of infected fish	PFI (%)	No. of infected fish	PFI (%)	No. of infected fish	PFI (%)
1-10	74	0	0	0	0	0	0	0	0	1	1.3 ^a
10.5-20	140	6	4.2 ^a	3	2.1 ^a	7	5 ^a	1	0.7 ^a	4	2.8 ^a
20.5-30	185	6	3.2 ^a	0	0	1	0.54 ^a	3	1.6 ^a	2	1 ^a
30.5-40	6	0	0	0	0	0	0	2	33.3 ^a	0	0
40.5-50	2	0	0	0	0	0	0	1	50 ^c	0	0
Total	407										

PFI: Parasitic frequency index (%); a: rare (0.1-9.9%); b: Occasional (10-29.9%); c: Common (30-69.9%); d: Abundant (70-100%)

Table 6: Two way ANOVA of PFI values for *Labeo rohita* from April-2012 to March-2013 in different length groups

Source of Variation	SS	df	MS	F	p-value	F crit
Length groups	982.3937	4	245.5984	2.019621	0.103086	2.525215
Parasites	13732.99	15	915.533	7.52867	5.16E-09	1.836437
Error	7296.372	60	121.6062			
Total	22011.76	79				

al. (1997). This may be attributed to probable differences in feeding habits with fish age and immunity. Oniye et al. (2004), had also indicated the importance of changing feeding habits of the fish with age on the parasite prevalence and intensity.

Statistical analysis (Table 6) revealed that there was no significant differences ($p > 0.05$, $df = 4$) in PFI values among the all length groups of 1-10, 10.5-20, 20.5-30, 30.5-40 and 40.5-50 cm. Similarly there was significant differences ($p < 0.05$, $df = 15$) in PFI values among the all parasites. However there was no significant differences ($p > 0.05$, $df = 4$) in PFI values among the *Dactylogyrus* sp., *Myxobolus* sp., *Trichodina* sp., *Thelohanellus* sp. and *Argulus* sp. but there was significant differences ($p < 0.05$, $df = 15$) in PFI

values among the *Dactylogyrus* sp., *Gyrodactylus* sp., *Ichthyophthirius multifiliis*, *Chilodonella* sp., *Learnea* sp., *Costia* sp., *Vorticella* sp., Nematodes, Development stages, Intestinal flukes and Digeneans parasites. Similarly there was no significant differences ($p > 0.05$, $df = 15$) in PFI values among the *Myxobolus* sp., *Trichodina* sp., *Thelohanellus* sp., *Argulus* sp., *Gyrodactylus* sp. and *Ichthyophthirius multifiliis* but there was significant differences ($p < 0.05$, $df = 15$) in PFI values among the *Myxobolus* sp., *Chilodonella* sp., *Learnea* sp., *Costia* sp., *Vorticella* sp., Nematodes, Development stages, Intestinal flukes and Digeneans. Similarly there was no significant differences ($p > 0.05$, $df = 15$) in PFI values among the all parasites except *Dactylogyrus* sp. and *Myxobolus* sp.



Figure 1: Cyst of *Myxobolus* sp. (arrow) present in the gills of *Labeo rohita*

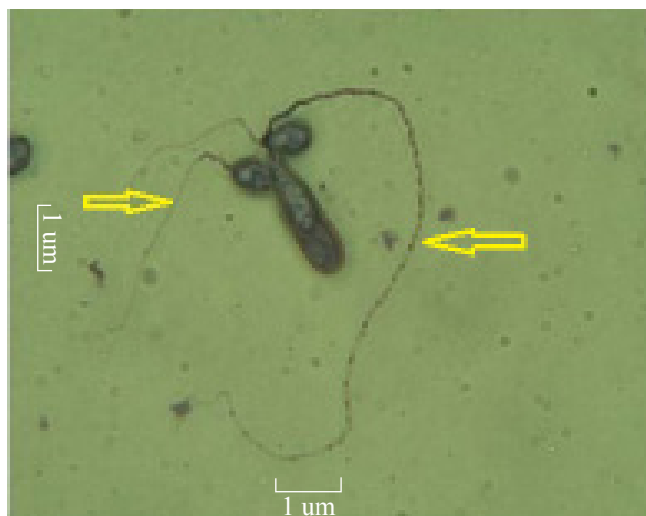


Figure 4: Two different *Thelohanellus* sp. attached to the gills of *Labeo rohita*, and with clear long polar filament (arrows) (Giemsa stained, 200x)

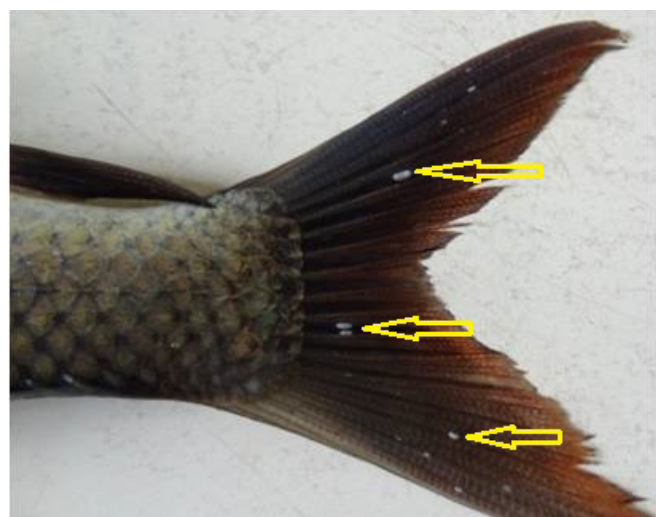


Figure 2: Cyst of *Thelohanellus* sp. (arrows) present on the caudal fin of *Labeo rohita*



Figure 5: Gills of *Labeo rohita* heavily infested with *Thelohanellus* sp. in the gills (Giemsa stained, 200x).

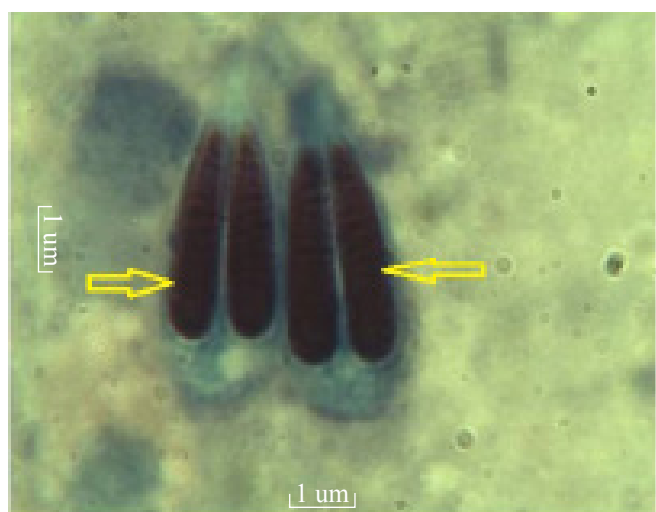


Figure 3: *Myxobolus* spp. with distinct polar filament (arrows) present in the gills of *Labeo rohita* (Giemsa stained, 1000x).



Figure 6: *Thelohanellus* sp. with distinct polar capsule (arrows) present on the dorsal fin of *Labeo rohita* (Giemsa stained, 200x).

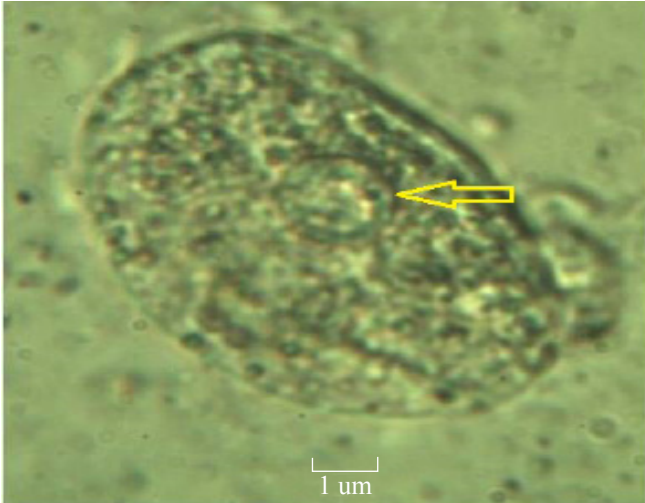


Figure 7: *Chilodenella* sp. with distinct nucleus (arrow) present on the skin of *Labeo rohita* (Wet mount, 400x).



Figure 10: *Argulus* sp. with distinct organelle isolated from the body of *Labeo rohita* (Wet mount, 40x)



Figure 8: *Ichthyoboda necatrix* (*Costia* sp.) present on the skin of *Labeo rohita* (Wet mount, 200x).

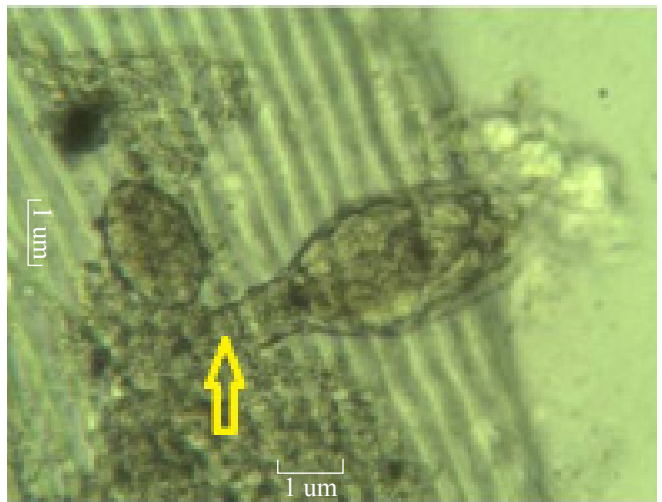


Figure 11: *Gyrodactylus* sp. attached to the skin (arrow) of *Labeo rohita* (Wet mount, 100x)

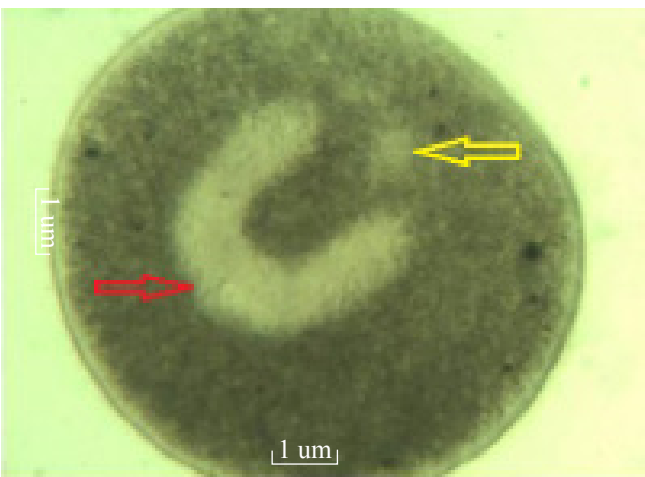


Figure 9: *Ichthyophthirius multifiliis* with distinct macro (red arrow) and micro nucleus (yellow arrow) present on the skin of *Labeo rohita* (Wet mount, 200x)



Figure 12: *Dactylogyrus* sp. present in the gills with distinct marginal hooks (circle) of *Labeo rohita* (Wet mount, 40x).

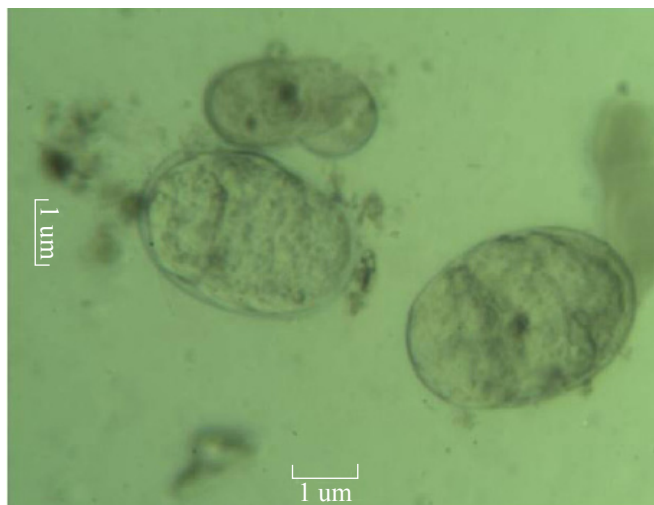


Figure 13: Developmental stages of digenean parasites present in intestine of *Labeo rohita* (Wet mount, 200x)



Figure 16: Unknown parasite found on the body surface of the *Labeo rohita* (Wet mount, 40x)



Figure 14: Magnified view of digenean parasite present in intestine of *Labeo rohita* (Wet mount, 400x)



Figure 17: *Trichodina* sp. present in the gills of *Labeo rohita* (Wet mount, 200x).



Figure 15: Developmental stages (arrow) of Digenean parasites present in intestine of *Labeo rohita* (Wet mount, 200x).



Figure 18: *Vorticella* sp. with lengthy stalk (arrow) present on the skin of *Labeo rohita* (Wet mount, 200x).

4. Conclusion

Winter was the most favorable period to get parasitic infestation. The small and medium size fishes were found to be more vulnerable due to their poor immunity power. We also recorded big size fishes were more susceptible to *Argulus* sp. and *Learnea* sp. due to wide spread surface area, which favors more colonization of parasites.

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