Study of Parasites from Wild and Cultured *Heteropneustes* Fossilis in Selected Districts of West Bengal

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Abstract: The study was conducted to isolate and identify different parasites of Heteropneustes fossilis to determine the prevalence of parasitic infestation in terms of month, seasons and length groups of fishes. 253 numbers of H. fossilis, obtained from different districts of West Bengal between February 2016 to January 2017 were examined. The parasite infested fishes were found to suffer mainly from respiratory manifestation, bulging of belly and mortalities. The isolated parasites were Myxobolus sp., Monogeneans, Digeneans, Cestodes, Nematodes, Acanthocephalans, Ergasilus sp. Besides these, developmental stages of different parasites were also found. The most dominant parasite in H. fossilis were Nematodes mainly observed in monsoon season. Remaining most of the parasites were found to be prone in winter season. The 20-25 cm length groups of fishes were found to be more infected with parasites compared to other length groups. Three Nematode species including Procamallanus sp., Contracaecum sp. and Eustrongylides sp. were found in host fish.

Keywords: Heteropneustes fossilis, PFI, Months, Seasons, Length groups.

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I. Introduction

Heteropneustes fossilis are an important fish fauna of wetlands and they are economically important as a food source of high nutritive value. Parasites of fish constitute one of the major problems to fish health. Besides the direct losses caused by mortality, parasites have a considerable impact on growth, resistance to other stressing factors, susceptibility to predation, marketability and pave way for secondary infections [1,2,3,4]. In India parasitic diseases are most prevalent among freshwater fishes (about 78%) and encountered more frequently than microbial diseases [5]. The state of West Bengal has always attracted attention for being the highest producer of freshwater table fish and fish seed in the country. Huge amounts of hazardous substances, heavy metals, sulphide, grease, oil originated from different industries of the surroundings, domestic waste water and industrial effluents from Kolkata city pollute the aquatic environment, that can lead to stress on the fishes, which lead to many parasitic diseases [6]. The length of fish influence the parasitic infections [7,8,9]. In order to increase profitability, health care based on the knowledge of organisms, their ecology and application of the knowledge in the control of diseases is essential [10,11]. Therefore, the present study was intended to isolate and identify different parasites from *H. fossilis* and to find out Parasitic Frequency Index (PFI, %) in terms of months, seasons and length groups from selected districts of West Bengal.

II. Materials And Methods

The fish samples were collected from selected districts of West Bengal namely South 24 Parganas, North 24 Parganas and East Midnapur. The samples were collected on a regular basis once in every month. In each sampling, about 20-30 fishes were collected. The fishes were brought to the laboratory in live condition with water-filled buckets and the total lengths, body weight of fishes were taken. The vital organs like brain, skin, intestine, kidney and gills were examined for the presence of different parasites [12]. External parasites from body surface, fins and gills were removed by scraping 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 microscopes. In case of monogeneans, the gills were transfered into petridishes containing physiological saline 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 to observe parasites inside intestine. The whole gut was removed in a watch glass containing 0.8% 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 [13]. Morphometric measurements of three nematode parasites were studied during the study. Photomicrographs were taken using a Motic BA400 phase contrast microscope with in-built digital camera.

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.

Prevalence (%) =
$$\frac{\text{Total number of infected fishes}}{\text{Total no of fish hosts examined}} X 100$$

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 [14].

III. Result And Discussion:

Monthly prevalence of different parasite in Heteropneustes fossilis

Monthly distribution of parasites in *Heteropneustes fossilis* are presented in Table 1 and fig.1. Parasitic Frequency Index (PFI) of Myxobolus sp. (Fig. 4) were only found in the months of October, November and December (PFI, 20 %, 27.27% and 43.47% respectively), in rest of the months they were not found. These results were supported by [15, 16], who have recorded high prevalence of myxozoan parasites during August to January when the ambient temperature was 25°C. PFI of monogeneans was only found in the month of April (PFI, 11.76%) condition was 'occasional', in rest of the months, it was not found. Monogeneans were found mainly from January to April [17]. Poor water quality, health management, irregular feeding practices and host specificity are some of the key factors that lead to stressful conditions and subsequent parasitic infection of the host organisms. PFI of digeneans were highest in the month of December (PFI, 52.38%). After that continuous decrease of PFI from January to March and completely absent from April to July. In rest of the months October, November, December and January the prevalence of this parasite were 'common' and remaining months February, August and September the prevalence was 'occasional'. [18] observed overall higher parasitic infection in H. fossilis from July to September which agreed with present study. The parasite prevalence and intensity of variation depend on many factors like parasitic life cycle, host, feeding habits and the physical parameters of the water body where the fish inhabit. Occurrences of cestodes were found highest in the month of December with the PFI 38.09% and lowest PFI 8.69% was observed in March. From April to July it was absent and in the rest of the months the conditions were found 'common'. According to [19], the infections were more during June to September, low during February to May. It depends upon the presence of intermediate host such as piscivorous birds for the spread of cestodes infection. The hygienic conditions are also very important for the healthy environment where fishes are raised [20]. Nematodes (Fig. 5,6,&7) were observed throughout the study period. The highest PFI of Nematodes were in August (PFI, 70%) and lowest in March (PFI, 8.69%). In February, April, November and December the condition were 'occasional' and in rest of the months the occurrence were 'common'. H. fossilis are a bottom dweller carnivorous fishes mainly feeding on crustacean (copepods), worms, larvae and small fishes, which are mostly infected with nematode larvae. These copepods play major role in the spread of nematode infection in H. fossilis [21, 22]. The results of the present study satisfied all the criteria as described by [23] who reported that the month of August was observed as most suitable for proliferation of nematode parasites. PFI of acanthocephalans (Fig. 8) varied from 9.52% to 40 % throughout the year. In October, they were recorded highest (PFI, 40%) followed by a decrease up to January. It was absent from February to June and then continuously increases from July to October. [24] observed that abundance of acanthocephalans sharply increased in November then declined gradually to May. Thus it can be concluded that during winter months sudden decline in water temperature, suppress the immune system in fishes and making them more vulnerable to disease agent. PFI of Ergasilus sp. (Fig. 9) were only found in the months of October, November and December (PFI, 45%, 18.18% and 14.28% respectively), in rest of the months they were not found. [25] observed the crustacean parasites like Ergasilus sp. were found to be lower prevalence throughout the year and the prevalence of these parasites were more in the winter (November–February) season. Present study corroborates with the above findings. Developmental stages were reached peak stage in the month of January (PFI, 52.38%), lowest in the month of June (PFI, 8.69%) stated as 'common' and 'rare' respectively. These were only absent from August to December, during the study period. Statistical analysis (Table 2) revealed that there was significant difference (P<0.05, df=11) in PFI values among all the months in Heteropneustes fossilis and also significance difference (P<0.05, df=7) in PFI values among the parasites.

Prevalence of parasites in different seasons in Heteropneustes fossilis

The prevalence of parasites in different seasons is presented in Table 3, Fig.2. *Myxobolus* sp. were found 'occasionally' in winter season (PFI, 23.81%), rest of the seasons these were not found. As the water quality parameters fluctuate very quickly during winter and summer season, fish becomes affected with diseases

in these two seasons. The parasitic infection is greatly influenced by the season, which basically interferes with ecology and physiology of the fish which was strongly supported by [26, 27]. Monogeneans were found 'rarely' in summer season (PFI, 23.81%), rest of the seasons these were not found. It was reported that the reproduction rate of monogeneans were increased with temperature leading to increased parasitic abundance [28]. Digeneans were found in all seasons except summer, these parasites also found 'occasionally' in monsoon and spring seasons (PFI, 15.87% and 13.95%). Digeneans were reached peak stage in winter season (PFI, 44.05%). During winter season trematode infections were recorded maximum in case of H. fossilis as compared with other catfishes [29]. The present findings were corroborated with works of above authors. Cestodes were found in all seasons except summer, these parasites found 'occasionally' in monsoon (PFI, 22.22%) and 'rare' in spring (PFI, 9.30%). Cestodes were reached peak stage in winter season (PFI, 33.33%). During summer season, the minimum mean intensity was observed with cestodes in H. fossilis [29] which were corroborated with present study. Nematodes were found in all seasons, but it was reached peak stage during the rainy season (PFI, 55.55%) which was stated as 'common', lowest in spring (PFI, 13.95%). They were also 'common' in summer and winter season (PFI, 38.09% and 30.95%). The results of the present study corroborated with the works of [30, 31], who had reported that seasonal distribution of nematodes may be related to the fluctuation in temperature, presence of intermediate hosts and feeding habits of the hosts. Acanthocephalans were reached peak stage in winter (PFI, 30.95%), lowest in monsoon season (PFI, 22.22%). These were found 'occasionally' in monsoon season and in rest of the seasons they were not found. According to [29] in winter season, the percentage of prevalence, percentage of dominance, mean intensity and abundance for acanthocephalans were found minimum in H. fossilis. That prevalence percentage may differ due to poor water quality, health management, irregular feeding practices or stressful conditions. Ergasilus sp. were found 'occasionally' in winter season (PFI, 19.05%), rest of the seasons these were not found. During winter, the fishes lose their appetite, results in stress condition and get affected by different diseases. H. fossilis are seen to be attacked by the Ergasilus sp. which similar with the findings of [32]. The prevalence of developmental stages of parasites were highest in spring (48.84%), lowest in monsoon (PFI, 3.17%), which were stated as 'common' and 'rare' respectively. These parasites were also 'rare' in winter (PFI, 5.95%) and 'occasional' in summer (PFI, 14.28%). Statistical analysis (Table 4) revealed that there was no significant difference (P>0.05, df=3) in PFI values among seasons. However, there was significant difference (P<0.05, df=7) in PFI values among the parasites.

Prevalence of parasites in different length groups of Heteropneustes fossilis

The distribution of parasites in different length groups of *Heteropneustes fossilis* are represented in Table 5 and Fig. 3. The fishes were grouped into 10-15 cm to 25-30 cm length groups. Occurrences of Myxobolus sp. were 'occasional' in 10-15 cm and 'rare' in 15-20 cm (PFI, 16.85% and 5.81% respectively) length groups of fishes but they were absent in remaining length groups of fishes. According to [33], the degree of infection of parasites in *Heteropneustes fossilis* were higher in lower length groups which is in agreement with present study. During the study period occurrences of monogeneans were absent in all length groups except 25-30 cm length group which was stated as 'rare' (PFI, 5%). Occurrences of digeneans were 'occasional' (PFI, 18.60%) in 15-20 cm length groups of fishes. These parasites were reached peak stage (PFI, 39.47%) in 20-25 cm length groups, lowest in (PFI, 7.14%) 10-15 cm length groups. According to [34], the greater numbers of parasites were present in larger fishes due to the larger surface areas of the gut, with more volumes of food eaten, selective feeding or any change in diet might also lead to higher infestation in different length groups. This is similar to present findings. Occurrences of cestodes were 'common' in 20-25 cm and 25-30 cm length groups of fishes (PFI, 42.11% and 35% respectively) but their occurrence were 'occasional' (PFI, 12.79%) in 15-20 cm length groups of fishes. In the present study, the high incidence of infestation obtained in bigger fishes (20-25 cm) is an indicator that size of the fish is important in determining the parasitic load compared to small fishes, these data are similar to that of the previous work as reported by [35, 36]. Nematodes were found more (PFI, 71.05%) in 20-25 cm length group fishes, which was stated 'abundant'. Lowest (PFI, 12.36%) found in 10-15 cm stated as 'occasional'. This result showed that the nematode infections were more in larger size groups than the small size groups of fishes. The changes in food habits in different stage of the hosts may be the reason for fluctuation in the infestation by parasites in different groups of fishes. [37] also found a similar type of results. Occurrences of acanthocephalans were 'common' in 20-25 cm and 25-30 cm length groups of fishes (PFI, 52.63% and 30% respectively) but their occurrence were 'rare' (PFI, 9.30%) in 15-20 cm length groups of fishes. These parasites were absent in 10-15 cm length groups. These results showed that the acanthocephalans infections were more in larger size groups than the small size groups of fishes. [36] stated that the prevalence were found to be increased as the fish grow and that could be attributed to the longer time of exposure to the environment by body size. Occurrences of Ergasilus sp. were 'occasional' in 25-30 cm and 'common' in 20-25 cm (PFI, 10% and 31.58% respectively) length groups of fishes but they were absent in remaining length groups of fishes. Developmental stages were reached peak stage (PFI, 31.57%) in 20-25 cm length group, lowest in (PFI, 3.37%) 10-15 cm length group. Parasitic intensity score increased with an increase in fish size, which may be related to the period needed for colony development. High score values were found only in large fishes [38]. Statistical analysis (Table 6) revealed that there was no significant differences (P<0.05, df=3) of parasitic prevalence among the all length groups of 10-15, 15-20, 25-30 cm. However, there was significant differences (P<0.05, df=7) of parasitic prevalence among the parasites.

Identification of parasites:

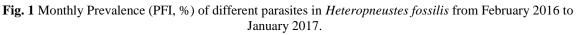
Identification of three nematode parasites collected from intestine of *Heteropneustes fossilis includes Procamallanus* sp., *Contracaecum* sp. and *Eustrongylides* sp. which were identified by morphometric measurements (Table 7), location and specificity. The morphometric measurements were given in Table 7. [39] reported that *H. fossilis* were infected with nematodes, *Procamallanus* sp. in India. In the present study the parasite, *Procamallanus* sp. resembles *Procamallanus laeviconchus* in all morphological characters except for a few variations in the measurements given by [40]. This is also prevalent in most African freshwater fishes, notably in siluroids [41]. [42] reported that *Wallago attu* were infected with nematodes, *Contracaecum* larvae in India. *Contracaecum* sp. were also reported from 19 spp. of freshwater fishes from Bangladesh [43]. In the present study the parasite, *Contracaecum* sp. larvae resembles to all morphological characters given by [40] except few variations in the measurements. [43] reported *Eustrongylides* sp. in Bangladesh. [44] also reported *Eustrongylides* sp. from *Wallago attu* in India. The occurrence of very few *Eustrongylides* larvae in *Heteropneustes fossilis* also recorded in Jaikwadi Dam [42]. The comparison with the description given by [45] showed the presence of *Eustrongylides* sp. So the present finding are in agreement with the occurrence of these parasites in *Heteropneustes fossilis* in West Bengal water bodies.

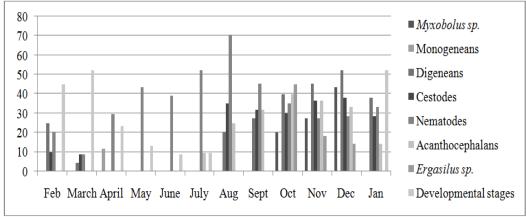
IV. Figures And Tables

TABLE 1. Monthly Prevalence (PFI, %) of different parasites in *Heteropneustes fossilis* from February 2016 to January 2017

January 2017.										
T Months	Total no. of fishes	Myxobolu s sp.	Monoge- neans	Digene ans	Cestodes	Nemat- odes	Acanth oce- phalans	Ergasilu s sp.	Develop- mental stages	
	examined	PFI	PFI	PFI	PFI	PFI	PFI	PFI	PFI	
		(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	
Feb	20	-	-	25 ^b	10 ^b	20 ^b	-	-	45 [°]	
March	23	-	-	4.34 ^a	8.69 ^a	8.69 ^a	-	-	52.17 ^c	
April	17	-	11.76 ^b	-	-	29.41 ^b	-	-	23.53 ^b	
May	23	-	-	-	-	43.48 ^c	-	-	13.04 ^b	
June	23	-	-	-	-	39.13 ^c	-	-	8.69 ^a	
July	21	-	-	-	-	52.38 ^c	9.52 ^a	-	9.52 ^a	
Aug	20	-	-	20 ^b	35°	70 ^d	25 ^b	-	-	
Sept	22	-	-	27.27 ^b	31.82 ^c	45.45 ^c	31.81 ^c	-	-	
Oct	20	20 ^b	-	40 ^c	30 ^c	35°	40 ^c	45 [°]	-	
Nov	22	27.27 ^b	-	45.45 ^c	36.36 ^c	27.27 ^b	36.36 ^c	18.18 ^b	-	
Dec	21	43.47°	-	52.38 ^c	38.09 ^c	28.57 ^b	33.33°	14.28 ^b	-	
Jan	21	-	-	38.09 ^c	28.57 ^b	33.33°	14.28 ^b		52.38 ^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%).





			2017.			
Source of Variation	SS	df	MS	F	P-value	F crit
Parasite	9825.675	7	1403.668	6.285878	7.64E-06	2.13099
Months	5206.479	11	473.3162	2.119595	0.028403	1.915305
Error	17194.49	77	223.305			
Total	32226.64	95				

TABLE 2. Two-way ANOVA of PFI (%) values for *Heteropneustes fossilis* from February 2016 to January

 2017

TABLE 3. Prevalence (PFI, %) of parasites in *Heteropneustes fossilis* in different seasons from February 2016 to January 2017.

Season	Total no. of fishes	Myxob olus sp.	Monog en- eans	Digene ans	Cestodes	Nemato- des	Acantho c- ephalans	Ergasilus sp.	Develop- mental stages
	examined	PFI (%)	PFI (%)	PFI (%)	PFI (%)	PFI (%)	PFI (%)	PFI (%)	PFI (%)
Summer (April -June)	63	-	3.17 ^a	-	-	38.09 ^c	0	-	14.28 ^b
Rainy (July-Sept)	63	-	-	15.87 ^b	22.22 ^b	55.55°	22.22 ^b	-	3.17 ^a
Winter (Oct- Jan)	84	23.81 ^b	-	44.05 ^c	33.33°	30.95°	30.95°	19.05 ^b	5.95ª
Spring (Feb- Mar)	43	-	-	13.95 ^b	9.3ª	13.95 ^b	-	-	48.84 ^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%).

Fig. 2. Prevalence (PFI, %) of parasites in *Heteropneustes fossilis* in different seasons from February 2016 to January 2017.

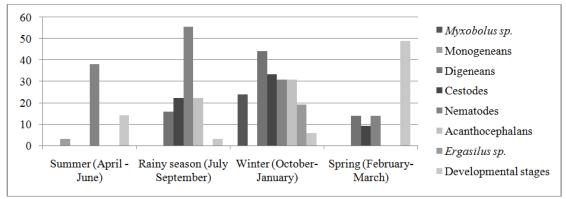


TABLE 4. Two-way ANOVA of PFI (%) values for *Heteropneustes fossilis* from February 2016 to January 2017 in different seasons.

2017 In different seasons.								
Source of Variation	SS	df	MS	F	P-value	F crit		
Season	1212.58	3	404.1933	2.059975	0.136219	3.072467		
Parasites	3168.676	7	452.668	2.307027	0.065312	2.487578		
Error	4120.467	21	196.2127					
Total	8501.723	31						

TABLE 5. Prevalence (PFI, %) of parasites in different length groups of <i>Heteropneustes fossilis</i> from February
2016 to January 2017.

Length	Total no.	Myxobol	Monog	Digenean	Cestodes	Nemato-	Acantho	Ergacillus	Developm-
(cm)	of fish	us sp.	-eneans	S		des	c-	sp.	ental stages
	examine						ephalans		
	d	PFI	PFI	PFI	PFI	PFI	PFI	PFI	PFI
		(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
10 to 15	89	16.85 ^b	-	7.14 ^a	5.62 ^a	12.36 ^b	-	-	3.37 ^a
15 to 20	86	5.81 ^a	-	18.60 ^b	12.79 ^b	34.88 ^c	9.30 ^a	-	17.44 ^b
20 to 25	38	-	-	39.47°	42.11 ^c	71.05 ^d	52.63°	31.58°	31.57 ^c
25 to 30	40	-	5	32.5°	35°	57.5°	30°	10 ^b	17.5 ^b

Fig.3 Prevalence (PFI, %) of parasites in different length groups of *Heteropneustes fossilis* from February 2016 to January 2017.

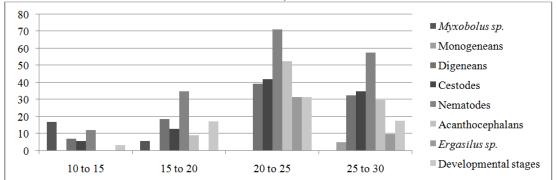


TABLE 6. Two-way ANOVA of PFI values for *Heteropneustes fossilis* from February 2016 to January 2017 in different length groups.

anterene tengan groupst									
Source of Variation	SS	df	MS	F	P-value	F crit			
length groups	3625.036	3	1208.345	8.984896	0.000503	3.072467			
Parasites	5041.162	7	720.166	5.354941	0.001249	2.487578			
Error	2824.212	21	134.4863						
Total	11490.41	31							

TABLE 7. Description of the morphological features and characteristics of three Nematode parasites observed
with their taxonomic status.

				Nematodes					
Figure no.	Name of parasite	Name of host	Attach ment Organ	Measurements with key characters					
				Measurements	Barson and Oldewage (2006)	Present study			
				Length (mm)	6.2-8.9 (7.6)	7.6			
				Maximum width(µm)	145-280 (222)	146.21			
5.	Procamall	Heteropneustes	Intestine	Buccal capsule length(µm)	60–90 (78)	59.52			
5.	anus sp.	fossilis	intestine	Buccal capsule width(µm)	58-70 (62)	56.02			
				Length of muscular part of oesophagus(µm)	380–510 (441)	313.06			
				Length of glandular part of oesophagus(µm)	960–1020 (983)	897			
				Distance of anus to tail(µm)	140-340 (227)	144			
			1						
			Intestine	Measurements	Bartlett (1996)	Present			
	Contracae	Hatanannaanataa				study			
6.	cum sp.	Heteropneustes fossilis		Total length (µm)	1600	1625			
	cum sp.			Maximum width(µm)	70	74			
				Length of oesophagus(µm)	260	240			
				Anus(µm)	70	68			
				Measurements	Melo et al. (2016)	Present study			
				Total length (mm)	8.68 mm ± 0.52 (7.46-8.46)	8			
7.	Eustrongyl	Heteropneustes	Intestine	Maximum width(mm)	$0.09 \pm 0.004 \text{ mm}$ (0.08-0.09)	0.1			
	<i>ides</i> sp.	fossilis		Length of oesophagus(mm)	$\frac{1.55 \text{ mm} \pm 0.14}{(1.22-1.73)}$	1.48			
				Width of oesophagus(mm)	$0.09 \text{ mm} \pm 0.09$ (0.05-0.07)	0.08			
				Nerve ring from the anterior extremity(mm)	$\begin{array}{c} 0.80 \text{ mm} \pm 0.10 \\ (0.54\text{-}0.91) \end{array}$	0.8			



FIGURES: (4-9)

- 4. Spore of Myxobolus sp. showing polar capsule (PC), spore (S), length of the spore(L), collected from brain of Heteropneustes fossilis.(400x) (wet mount).
- 5. Procamallanus sp. (Nematode) showing buccal capsule (BC), muscular part of oeasophagus, glandular part of oesophagus, maximum width (MW), collected from intestine of Heteropneustes fossilis (40x) (wet mount).
- 6. Contracaecum sp. (Nematode) showing oesophagus (O), anus (A), body length (L), collected from intestine of Heteropneustes fossilis (40x) (wet mount).
- 7. Eustrongylides sp. (Nematode) showing oesophagus (O), maximum body width (MW), collected from intestine of Heteropneustes fossilis (40x) (wet mount).
- 8. Acanthocephalan showing proboscis (P), maximum body width (MW), body length (L), collected from intestine of Heteropneustes fossilis. (10x) (wet mount).
- 9. Ergasilus sp. showing second antenna (SA), thoracic feet (TF), egg sac (ES), collected from gill of Heteropneustes *fossilis* (40x) (wet mount).

V. Conclusion

The present study brings about the conclusion that the *H. fossilis* were vulnerable to different parasites such as *Myxobolus* sp., Monogeneans, Digeneans, Cestodes, Nematodes, Acanthocephalans, *Ergasilus* sp. and developmental stages of parasites or eggs. The most dominant parasite in H. fossilis were Nematodes mainly observed in monsoon season. Remaining other parasites were found to be prone during winter season. Winter was the most vulnerable period to get parasitic infestation. During this period the water quality gets deteriorates and the fishes were in stressed condition which favors the parasites to infest. Larger groups fishes were found to be more vulnerable compared to smaller length groups due to widespread surface area respectively, which favors more colonization of parasites. Water quality should be maintained during winter which can prevent parasite infestation to a greater extent. Establishment of quarantine system can prevent the entry of exotic parasites.

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