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Study of helminth parasites from wild and cultured *Mystus gulio* in selected districts of West Bengal, India

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Abstract
The study was conducted to collect and identify different parasites of *Mystus gulio* to determine the prevalence of helminths infestation in terms of month, seasons and length groups of fishes from different districts of West Bengal. The isolated parasites were Monogeneans, Digeneans, Cestodes, Nematodes and Acanthocephalans. The most dominant parasites in *M. gulio* were Acanthocephalans mainly observed in monsoon season. The larger length groups fishes were found to be more infected with parasites compared to smaller length groups. Three Acanthocephalans species including *Pallisentis* sp., *Raosentis podderi* and *Acathogyrus* sp. were found in host fish. Morphometric measurements of these three acanthocephalans parasites were observed during the study. Two way ANOVA was done to determine the significance of differences in Parasitic Frequency Index (PFI).

Keywords: *Mystus gulio*, Helminths, Prevalence, Seasons, Length groups

1. Introduction
Catfishes are valued fish fauna of wetlands and many of them are economically important as a food source of high nutritive value (Nimbalkar and Deolalikar) [1]. *Mystus gulio* is a carnivorous catfish commonly collected from wild area and small ponds in India, where the water quality is not good. It's food mainly consists of small fish and larvae of crustaceans (Rafique *et al*.) [2]. Due to its feeding habit, this fish can act as an intermediate or a final host for many helminth parasites. Helminths damage health of fish by inducing variable intensity of infection depending upon the quality of environmental conditions. Data regarding the parasitic infestation of helminth parasites of *M. gulio* in West Bengal is limited. Many workers like Pal and Ghosh [3], Pandey *et al.* [4], Shakir *et al.* [5] have made valuable contributions on the prevalence of helminth parasites in fish. The need to assess the parasitic infection arises because the fish suffering from parasitic infection and/or disease result into severe damage to fishery industry and also dangerous for human health. Therefore, the present study was conducted to isolate and identify different helminth parasites from *M. gulio* and to find out Parasitic Frequency Index (PFI, %) in terms of months, seasons and length groups from selected districts of West Bengal, India.

2. Materials and methods
The fish samples were collected from selected districts of West Bengal namely South 24 Parganas, North 24 Parganas and East Midnapore. 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 the fishes were taken. The vital organs like skin, intestine, kidney and gills were examined for the presence of different parasites (Soota) [6]. In case of monogeneans, the gills were removed 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 a 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 0.8% 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 (Akter *et al*.) [7].

Morphometric measurements of three acanthocephalans parasites were studied during the study. Photomicrographs were taken using a Motic BA400 phase contrast microscope with in-build digital camera.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.

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

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 Srivastava [8]. 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. It was also followed to determine the significance of differences in prevalence of parasites in different length groups of fishes (Snedecor and Cochran) [25].

3. Results and Discussions

3.1 Prevalence of different helminth parasites in *Mystus gulio*

Monthly distribution of helminth parasites in *Mystus gulio* is presented in Table 1 and Fig. 1. The Parasitic Frequency Index (PFI) of monogeneans (Fig.7) was highest in August (PFI, 72.73%) and lowest in January (PFI, 8.69%). These results supported by Pal and Ghosh [3], who have recorded monogeneans infections, begin to appear by July. Infections reach their first peaks in October after they decline and completely disappear by April to June. The occurrence of digeneans in *M. gulio* from August to January was common and highest PFI was observed in October (PFI, 60.87%). The lowest PFI of digeneans was observed in February (PFI, 10.71%) and rest of the months digeneans were absent. The prevalence and mean intensity of digenean infection was high during July to September (Madhavi) [9]. Present study corroborated with the above report. *M.gulio* were less infected by cestodes and were observed only from April to June. The PFI of cestodes varied from 8.89 to 25%. These results supported by Pandey *et al*. [4], who have recorded cestodes infections was highest in May. PFI of nematodes were highest in the month of September (PFI, 26.09%) and lowest in the month of December (PFI, 8%). In rest of the months June to November, the prevalence of this parasite were 'occasional'. Agarwal [10] mentioned that the infection was heavy during spawning of fish from March to September which concurrent to the present study. More number of fishes was infected with acanthocephalans and was found throughout the study period. PFI of acanthocephalan was highest during July and August (PFI, 75% and 77.27% respectively), which were stated as 'abundant' condition. In rest of the months, March, May, June and September to January the conditions were 'common' and remaining months February and April the prevalence was 'occasional'. The seasonal fluctuation of acanthocephalans were mainly influenced by the changes in the feeding habits of the host Awachie [11], Ahmed [12] also suggested that the prevalence of acanthocephalans increase in April to July may be due to their breeding season, as gravid female and juveniles were also available in that time. Statistical analysis (Table 2) revealed that there was significant difference ($P<0.05$, df=11) in monthly PFI values. Similarly there was significant difference ($P<0.05$, df=4) in PFI values among the parasites.

Table 1: Monthly Prevalence (PFI, %) of helminth parasites in *Mystus gulio* from February 2016 to January 2017.

Months	Total no. of fish examined	Monogeneans PFI (%)	Digeneans PFI (%)	Cestodes PFI (%)	Nematodes PFI (%)	Acanthoce -phalans PFI (%)
Feb	28	-	10.71[b]	-	-	28.57[b]
March	25	-	-	-	-	36[c]
April	23	-	-	8.89[a]	-	26.09[b]
May	24	12.5[b]	-	25[b]	8.33[a]	33.33[c]
June	24	25[b]	-	16.66[b]	20.83[b]	50[c]
July	20	35[c]	-	-	25[b]	75[d]
Aug	22	72.73[d]	36.36[c]	-	22.72[b]	77.27[d]
Sept	23	43.48[c]	43.48[c]	-	26.09[b]	52.17[c]
Oct	23	34.78[c]	60.87[c]	-	17.39[b]	65.21[c]
Nov	22	68.18[c]	45.45[c]	-	18.18[b]	68.18[c]
Dec	25	36[c]	64[c]	-	8[a]	48[c]
Jan	23	8.69[a]	30.43[c]	-	-	43.47[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 2: Two way ANOVA of PFI (%) values for *Mystus gulio* from February 2016 to January 2017.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Months	9040.914	11	821.9013	3.449801	0.001611	2.014046
Helminth Parasites	14843.52	4	3710.879	15.57583	5.19E-08	2.583667
Error	10482.82	44	238.246			
Total	34367.25	59				

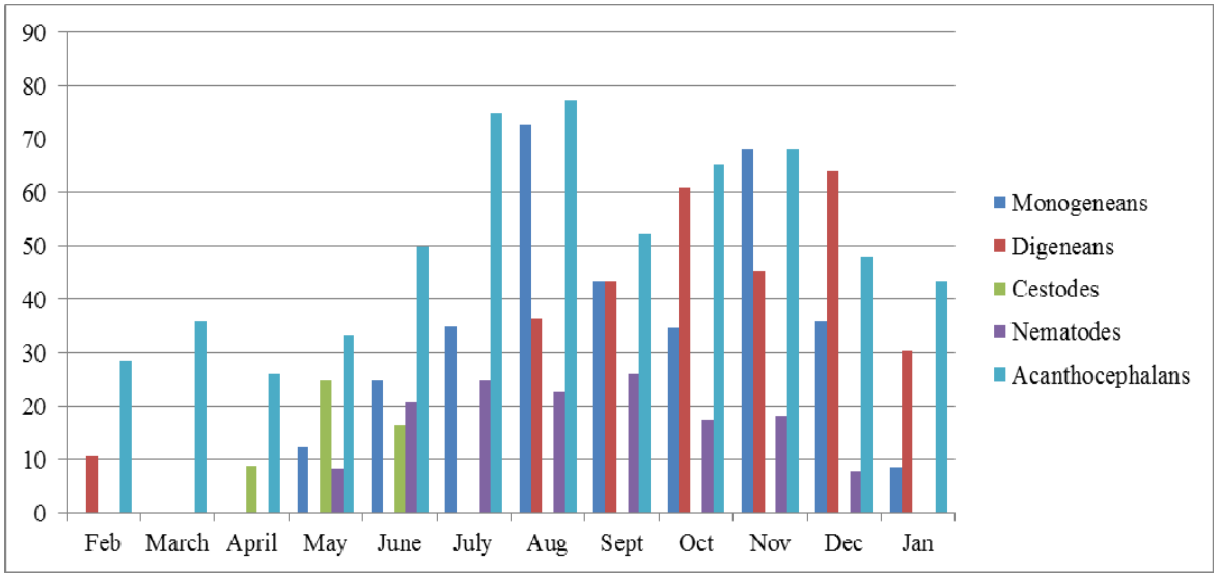

Fig 1: Monthly Prevalence (PFI, %) of helminth parasites in *Mystus gulio* from February 2016 to January 2017.

3.2 Prevalence of parasites in different seasons in *Mystus gulio*

The occurrence of parasites in different seasons is presented in Table 3, Fig 2. The infestations of monogeneans were highest during rainy season (PFI, 50.77%) and lowest in summer (PFI, 12.67%). Infestations were increased constantly from summer to monsoon season and decrease in winter and up to spring season. The results of present study are correlated with the results of Hoole *et al*. [13], Aydogdu and Altunel [21]. PFI of digeneans was found highest during winter (PFI, 50.54%) which was stated as 'common'; and gradually decreased in spring (PFI, 5.66%) and was stated as 'rare' in this season. Infestations increase continuously from rainy season to winter season and absent in summer season. Kim *et al*. [14] reported the lowest prevalence of digeneans 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. Cestodes were found only in summer season (PFI, 16.90%) stated as 'occasional'. They were not found in rest of the seasons. These results supported by Dhole *et al*. [26] who have recorded cestodes infections was highest in summer season. During summer season the manifestation of cestode parasites were highest because of temperature which helps to hatching eggs of parasites and enhances the rate of parasites while as rainy relatively shows very low infection of the parasites. Infestations of nematodes were highest during rainy season (PFI, 24.62%), followed by decreased up to spring, then increase in summer (PFI, 9.86%). The results of the present study corroborated with the works of Kim *et al*. [14], 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 found in all seasons. Infestations of these parasites were highest in monsoon season (PFI, 67.69%), followed by decreased up to spring (lowest, PFI, 30.07%), then start increased in summer (PFI, 36.62%). It was may be due to the temperature fluctuation which favors growth of these parasites. The result is strongly supported by Singh and Mishra [15], Ramudu[22]. Statistical analysis (Table 4) showed that there was significant difference ($P<0.05$, df=3) in PFI values among the seasons. Similarly there was significant difference ($P<0.05$, df=4) in PFI values among the parasites.

Table 3: Prevalence (PFI, %) of helminth parasites in *Mystus gulio* in different seasons from February 2016 to January 2017.

Period	Total no. of fishes examined	Monogeneans	Digeneans	Cestodes	Nematodes	Acanthocephalans
		PFI (%)	PFI (%)	PFI (%)	PFI (%)	PFI (%)
Summer (April - June)	71	12.67^{b}	-	16.90^{b}	9.86^{a}	36.62^{c}
Rainy season or Monsoon (July-September)	65	50.77^{c}	27.69^{b}	-	24.62^{b}	67.69^{c}
Winter (October- January)	93	36.56^{c}	50.54^{c}	-	10.75^{b}	55.91^{c}
Spring (February- March)	53	-	5.66^{a}	-	-	30.07^{c}

Table 4: Two way ANOVA of PFI (%) values for *Mystus gulio* from February 2016 to January 2017 in different seasons.

Source of Variation	SS	df	MS	F	P-value	F crit
Seasons	2454.632	3	818.2108	4.596643	0.02305	3.490295
Helminth Parasites	4376.474	4	1094.118	6.146671	0.006265	3.259167
Error	2136.022	12	178.0018			
Total	8967.128	19				

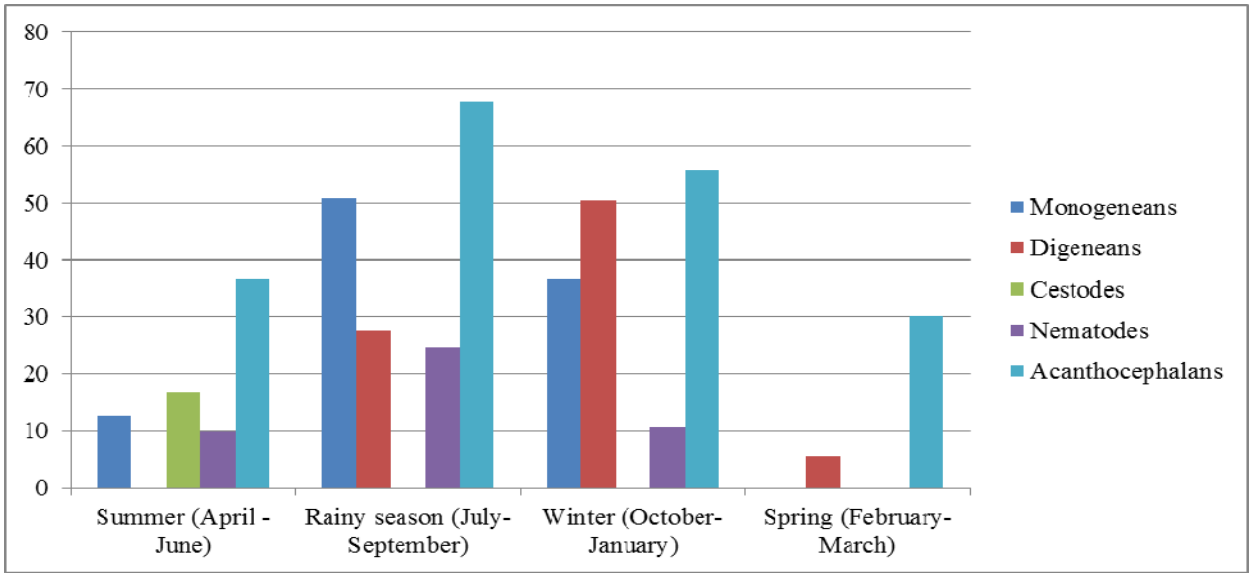

Fig 2: Prevalence (PFI, %) of helminth parasites in *Mystus gulio* in different seasons from February 2016 to January 2017.

3.3 Prevalence of helminth parasites in different length groups of *Mystus gulio*

The distribution of parasites in different length groups of *Mystus gulio* are represented in Table 5 and Fig 3. The fishes were grouped into 5-10 to 10-15 cm length groups. The occurrences of monogeneans were 'common' (PFI, 33.33%) in 10-15 cm and 'occasional' in (PFI, 17.42%) in 5-10 cm length groups of *M. gulio.* The occurrences of digeneans were 'common' (PFI, 34.67%) in 10-15 cm and 'occasional' in (PFI, 12.12%) in 5-10 cm length groups which agree with Ekanem *et al.* [16] who reported highest prevalence for all the species in larger length classes. This might be attributed to a large amount of food intake by the animals. This study also corroborated with our present findings. Cestodes were absent in 5-10 cm length group of fishes. In the 10-15 cm length groups they were 'rare' (PFI, 8%). In the larger fish the prevalence of cestode infection was found to be higher than in smaller size class (Shakir *et al.*) [5]. It is because the larger fish has spent longer time in that water, therefore has greater chances of cestode infestation with passage time. Nematodes were 'occasional' in both length groups 5-10 cm and 10-15 cm. These parasites were reached peak condition in 10-15 cm length groups (PFI, 14.66%**)**, lowest in 5-10 cm length groups (PFI, 11.36%). Larger fishes were heavily parasitized than the smaller ones. The intensity and prevalence of parasites infection increased with increasing length, size and age of the fish host (Ugwuzor [17], Geets and Ollevier[23], Oniye and Aken-Ova[24]). The reason for the higher infection rate in adult and young may be because of the longer duration of time, the older fish were exposed to the agents in the environment. This increases their chances of acquiring the parasite infection with time. The occurrences of acanthocephalans were 'common' in both length groups 5-10 cm and 10-15 cm. These parasites were reached peak condition in 10-15 cm length groups (PFI, 60%**)**, lowest in 5-10 cm length groups (PFI, 37.12%). Similar observation of infection being higher in adult was reported by Roberts [18], who noted that longer fish provide greater surface for infection than smaller fish. Statistical analysis (Table 6) revealed that there was significant differences ($P<0.05$, df=1) in PFI values among the all length groups of 5-10, 10-15 cm. Similarly there was significant differences ($P<0.05$, df=4) in PFI values among the all parasites.

Table 5: Prevalence (PFI, %) of helminth parasites in different length groups of *Mystus gulio* from February 2016 to January 2017.

Length (cm)	Total no. of fishes examined	Monogeneans	Digeneans	Cestodes	Nematodes	Acanthoce-phalans
		PFI (%)	PFI (%)	PFI (%)	PFI (%)	PFI (%)
5 to 10	132	17.42^{b}	12.12^{b}	-	11.36^{b}	37.12^{c}
10 to 15	150	33.33^{c}	34.67^{c}	8^{a}	14.66^{b}	60^{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 6: Two way ANOVA of PFI values for *Mystus gulio* from February 2016 to January 2017 in different length groups.

Source of Variation	SS	df	MS	F	P-value	F crit
Length groups	527.657	1	527.657	13.85376	0.020438	7.708647
Helminth Parasites	2239.646	4	559.9116	14.70061	0.011653	6.388233
Error	152.3505	4	38.08763			
Total	2919.654	9				

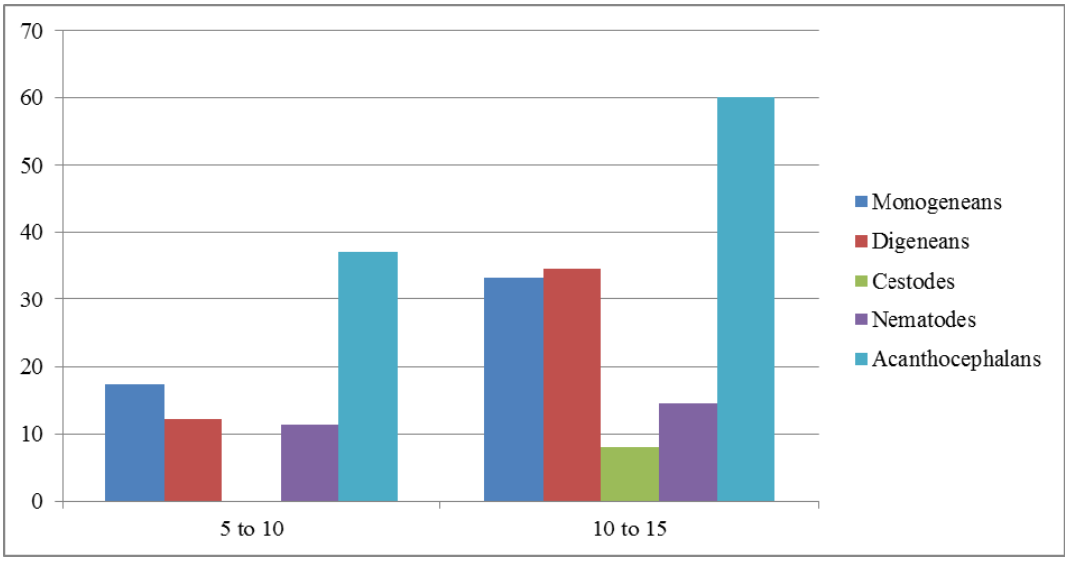

Fig 3: Prevalence (PFI, %) of helminth parasites in different length groups of *Mystus gulio* from February 2016 to January 2017.

3.4 Identification of Acanthocephalans

Two Acanthocephalans, *Raosent podderi* and *Pallisentis* sp. (Fig. 4 and 5) were recorded from *Mystus gulio* during the present investigation (Table 7). The parasites also described by Datta [19] from the intestines of small siluroid fishes, *Mystus cavasius* (Ham.) found in the Calcutta fish markets. Present parasites were collected from *M. gulio* and referred to *Acathogyrus* sp. (Fig. 6) according to the morphometry given by Rekha [20] in all characters (Table 7). These parasites were notably prevalent in siluroids.

Table 7: Description of the morphological features and characteristics of acanthocephalans observed with their taxonomic status.

Fig. no.	Name of parasite	Attachment Organ	Measurements with key characters		
4	*Pallisentis* sp.	Intestine	Measurements	Datta [19]	Present study
			Total length (mm)	6-10	7
			Maximum width(mm)	0.3	0.22
			Proboscis length × width (mm)	0.18 × 0.2	0.2 × 0.2
			Proboscis hook number in each circle	6	6
			Number of circle in Proboscis	4	4
			Anterior testis length × width	0.47 × 0.19	0.38 × 0.12
			Posterior testis length × width	0.49 × 0.18	0.46 × 0.12
5	*Raosentis podderi*	Intestine	Measurements	Datta [19]	Present study
			Total length (mm)	0.67-2.37	1.16
			Maximum width(mm)	0.27-0.53	0.33
			Proboscis length × width (mm)	0.14-0·25 × 0.12-0.18	0.16 × 0.12
			Proboscis hook number in each circle	1st and 2nd rows 6 each; 3rd and 4th rows 7 each.	1st and 2nd rows 6 each; 3rd and 4th rows 7 each.
			Number of circle in Proboscis	4	4
			Measurements of proboscis hooks (mm)	i. 0.085-0.115 ii. 0.070-0.095 iii. 0.025-0.035 iv. 0.025-0.030	i. 0.09 ii. 0.07 iii. 0.03 iv. 0.02
6	*Acathogyr-us* sp. (Female worms)	Intestine	Measurements	Rekha [20]	Present study
			Total length (mm)	4.57 – 8.6	4
			Maximum width(mm)	0.76 – 1.24	0.98
			Proboscis length x width (mm)	0.09 – 0.15 mm × 0.09 – 0.11 mm.	0.12 × 0.11
			Measurements of proboscis hooks (mm)	i. 0.071 ii. 0.059 iii. 0.048	i. 0.06 ii. 0.05 iii. 0.04

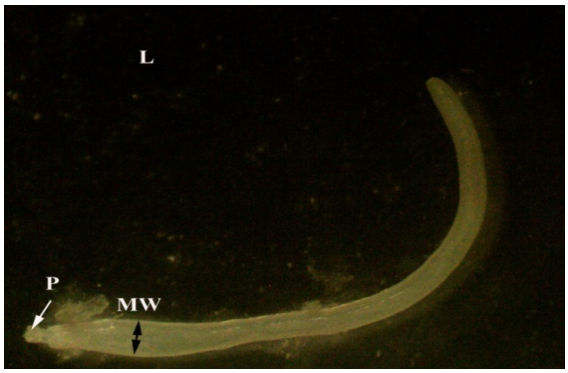

Fig 4: *Pallisentis sp.* (Acanthocephalan) showing proboscis (P), maximum body width (MW), body length (L), collected from intestine of *Mystus gulio*. (10x) (wet mount).

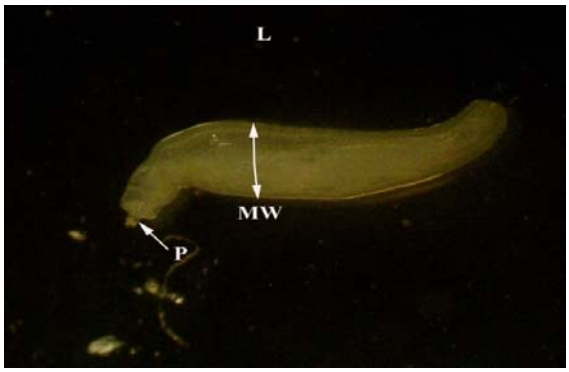

Fig 5: *Raosentis podderi* (Acanthocephalan) showing proboscis (P), maximum body width (MW), body length (L), collected from intestine of *Mystus gulio*. (10x) (wet mount).

Fig 6: *Acathogyrus* sp. (Acanthocephalan) showing proboscis (P), maximum body width (MW), collected from intestine of *Mystus gulio* (100x) (wet mount).

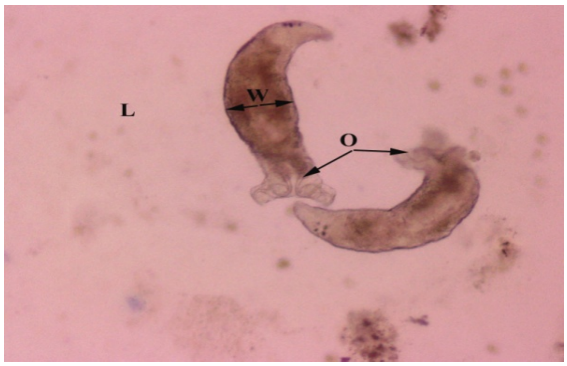

Fig 7: *Dactylogyrus* sp. (Monogeneans) showing opisthaptor (O), body length (L), body width(W), collected from gill of *Mystus gulio* (100x) (wet mount).

4. Conclusion

In the present study, recorded data shows the most dominant helminth parasite in *M. gulio* were acanthocephalans. High incidences of infections of acanthocephalans, nematodes, monogeneans were recorded in rainy seasons and cestodes in summer. Among different length groups, larger length groups were more prone to parasitic attack. The results clearly indicate that environmental factors and feeding habitat influence the seasonality of parasitic infection either directly or indirectly.

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