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Prevalence of Helminth Infestation in *Clarias Batrachus* (Linnaeus, 1758) with Season and Size Variation in West Bengal, India

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ABSTRACT

Parasitic infestation is a big problem in aquaculture industry. The main objective of this study was to determine the prevalence, pathology and morphometric measurements of helminth parasites of *Clarias batrachus* from two districts of West Bengal, India, during February' 2016 to January' 2017. For this purpose, 262 *Clarias batrachus*, were randomly selected and examined. The fishes were classified based on season and length. Seasonal studies demonstrate that the overall prevalence of the collected helminth parasites was highest during the rainy, followed by the winter and summer seasons. The highest infestation of helminth parasites is in length groups of 20-25 cm, compared with other length groups ($P<0.05$). Five species of helminths viz., *Orientocreadium batrachoides, Emoleptalea proteopora, Bovienia indica, Lytocestus indicus, Proteocephalus* sp. were identified during the study. Different types of histopathological changes were observed. Environmental factors and feeding habitat influence the seasonality of parasitic infection either directly or indirectly.

Key words: *Clarias batrachus*, Helminth, Length Groups, Prevalence, Season.

INTRODUCTION

Clarias batrachus (Linn.) an indigenous catfish species of India, contribute an important role in aquaculture due to their abundant occurrence, cosmopolitan distribution and high commercial value (Kumar 2010). With the growing interest in the development of aquaculture, there is also an increase in the awareness of the role of parasites and diseases as major factors affecting fish farming (Paperna 1996). *C. batrachus* (Linn.) serves as an imperative host as they carry a range of host-specific parasites (Vankara et al. 2014). Several workers have studied the helminth fauna (monogeneans, digeneans, cestodes, nematodes and acanthocephalans) of piscine hosts and have described several new species from India (Ahmed et al. 1985, Chandra et al. 1997). Therefore, the present study was conducted to isolate and identify different helminths, pathological changes of vital organ due to parasite infestation and to find out prevalence of parasites in terms of seasons and length groups from selected districts of West Bengal, India.

MATERIALS AND METHODS

A total number of 262 *C. batrachus* were collected on a regular basis between February, 2016 to January, 2017, along the four seasons, i.e. Summer (n=58), Rainy season(n=67), Winter (n=92), Spring (n=45), was analysed. Lengths of the fish were recorded and they were classified into four length groups (5-10 cm, 10-15cm, 15-20cm and 20-25cm). The fish samples were collected from two selected districts of West Bengal namely South 24 Parganas, North 24 Parganas. The fishes were brought to the laboratory in live condition 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 1980). The parasites were removed in a watch glass containing 0.8% physiological saline and were stretched over a clean slide, fixed in Neutral Buffered Formalin (NBF) and stained with borax carmine. Specimens were then stored in 70% ethanol for further study. Photomicrographs were taken using a Motic BA400 phase contrast microscope with in-build digital camera. The analysis of the helminth community was carried out considering: prevalence of parasites calculated according to Srivastava (1980) and the frequency index were further classified into rare (0.1 –9.9%), occasional (10-29.9%), common (30-69.9%) and abundant (70-100%) group. For histopathological examination, tissue samples were fixed in Bouin's fixative for 24 h, dehydrated in ascending grades of alcohol and cleared in xylene. Paraffin sections of 5 µm thickness were then stained with hematoxylin and eosin and examined under an ordinary light microscope (Roberts 2001). Two way ANOVA was done to determine the significance of differences in Parasitic Frequency Index (PFI) of parasites among different seasons and different length groups of fishes (Snedecor and Cochran 1962).

RESULTS AND DISCUSSIONS

Prevalence of Parasites in Different Seasons

The seasonal prevalence of parasites is presented in Table 1 and Figure1. Monogeneans commonly found as 'rare' in summer and rainy season (PFI, 8.62% and 4.47%). The high prevalence of Monogeneans were reported in summer season which coincides with other studies carried out (Singh and Mishra 2013). Digeneans were commonly found in rainy season and winter (PFI, 29.85% and 31.52%) and not found in rest of the seasons. Digeneans reached peak stage in winter. Kim et al. (2001) reported the highest prevalence of digeneans 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. Prevalence of cestodes was highest in rainy season (PFI, 52.24%) and lowest in spring season (PFI, 26.66%). Occurrence of Cestodes decreased from rainy season to spring season which was concurrent with other studies (Ahmed et al. 1985), where the highest rate of infection in *C. batrachus* by *L. indicus, Bovienia serialis* and *Pseudocaryophyllaeus indica* occurred in rainy season. Nematodes were found highest in rainy seasons (PFI, 23.88%) and lowest in winter season (PFI, 7.61%). Continuous development of the larval nematodes may occur with highest incidence in the rainy season which concurs with the perceptions of other investigation (Dione et al. 2014). Acanthocephalans were found highest in rainy seasons (PFI, 23.88%) and lowest in winter season (PFI, 5.43%). In rainy season, maximum helminth infections were caused by acanthocephalans in *C. batrachus* (Singh and Mishra 2013). Statistical analysis using Two way ANOVA 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) among the parasites.

Prevalence of parasites in different length groups of *Clarias batrachus*

The distribution of parasites in different length groups of *Clarias batrachus* is represented in Table 2 and Figure 2. Occurrences of monogeneans were 'rare' in 15-20 cm and 20-25 cm (PFI, 6.15% and 8% respectively) length groups of fishes. Prevalence of digeneans were highest in 20-25 cm (PFI, 38%) length group fishes and lowest in 10-15 cm length groups (PFI, 18.67%). Occurrences of monogeneans and digeneans were reached peak stage in 20-25 cm length group fishes. The present study agrees with the previous study demonstrated that the higher percentage of parasitic infection observed with the increase in size (Gaber et al. 2015). This could be due to the fact that bigger fishes cover wider areas in search of food than the smaller ones. During the study period occurrences of cestodes were reached peak in 20-25 cm length (PFI, 68%), lowest in 10-15 cm length group (PFI, 30.67%). Present result agreed with the results obtained by other researchers Ajala & Fawole (2014), who reported that the prevalence and intensity of parasitic infection is directly proportional to body length. The occurrences of nematodes were highest in 20-25cm length group (PFI, 30%) and occurrences of acanthocephalans were 'occasional' in 15-20 cm (PFI, 27.69%) length group, 'common' in 20-25 cm (PFI: 40%) length groups. Nematodes and acanthocephalans reached peak condition in 20-25cm length groups. In general, there is

Table 1. Prevalence (PFI, %) of parasites in *Clarias batrachus* in different seasons from February 2016 to January 2017.

Period	Total no. of fishes	Monogeneans PFI(%)	Digeneans PFI(%)	Cestodes PFI(%)	Nematodes PFI(%)	Acanthocephalans PFI(%)
Summer (April - June)	58	8.62[a]	-	27.59[b]	10.34[b]	17.24[b]
Rainy season (July-September)	67	4.47[a]	29.85[b]	52.24[c]	23.88[b]	23.88[b]
Winter (October- January)	92	-	31.52[c]	30.6[c]	7.61[a]	5.43[a]
Spring (February- March)	45	-	-	26.66[b]	-	-

PFI=Parasitic Frequency Index (%). a= rare (0.1 - 9.9%); b=occasional (10 - 29.9%); c = common (30 - 69.9%); d = abundant (70 -100%).

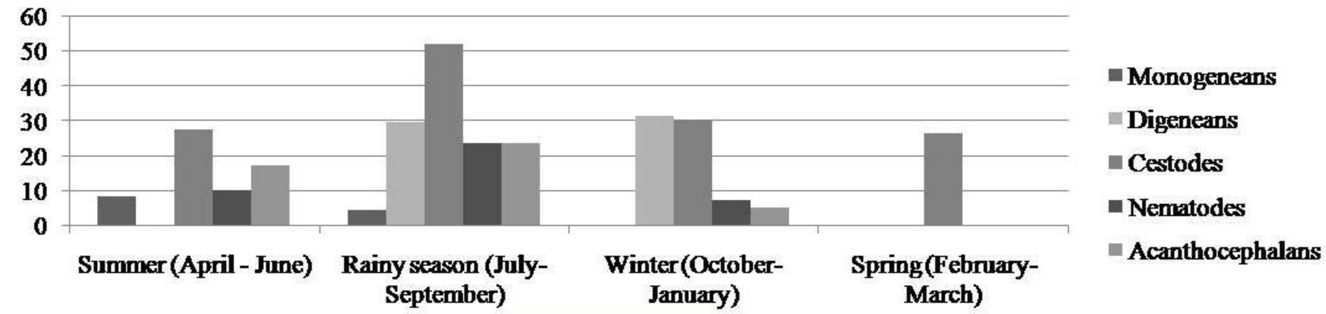

Figure 1. Prevalence (PFI, %) of parasites in *Clarias batrachus* in different seasons from February 2016 to January 2017.

Table 2. Prevalence (PFI, %) of parasites in different length groups of *Clarias batrachus* from February 2016 to January 2017.

Length (cm)	Total no. of fishes	Monogeneans PFI (%)	Digeneans PFI (%)	Cestodes PFI (%)	Nematodes PFI (%)	Acanthocephalans PFI (%)
5 to 10	72	-	-	-	-	-
10 to 15	75	-	18.67[b]	30.67[c]	4[a]	-
15 to 20	65	6.15[a]	24.61[b]	58.46[c]	16.92[b]	27.69[b]
20 to 25	50	8[a]	38[c]	68[c]	30[c]	40[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%).

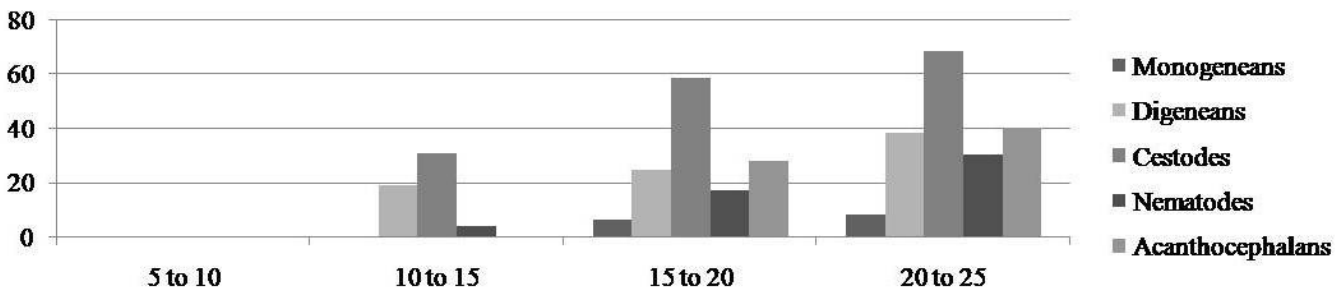

Figure 2. Prevalence (PFI, %) of parasites in different length groups of *Clarias batrachus* from February 2016 to January 2017.

a positive relationship between the level of parasitic infestation and the size and age of the host fish (Aydogdu et al. 2003) which are similar to the present observations. Statistical analysis using Two way ANOVA showed that there was significant differences (P<0.05, df=3) in PFI values among the all length groups of 5-10, 10-15, 15-20, 20-25 cm. Similarly there was significant differences (P<0.05, df=4) in PFI values among the all parasites.

Morphometric measurements

After microscopic observation, morphometric measurements were taken and represented in Table 3. During the present investigation, two caryophyllaeid cestodes, *Bovienia indica* (Figure 3.a) and *Lytocestus indicus* (Figure 3.b) were recorded from *Clarias batrachus* through morphometric analysis, comprising all measurements given by previous studies (Ash 2011). *Bovienia indica* was originally described as *Lucknowia indica* from *C. batrachus* from India (Niyogi et al. 1982) and the caryophyllid cestode, like *Lytocestus indicus, L. parvulus*, *L. Birmanicus*, *L. filiformis*, *L. fossilis,* and *L. logicollis* have been described from silurid fishes from Indian subcontinent (Chandra et al. 1997). So the present findings are the agreement of occurrence of these parasites in *C. batrachus* in West Bengal. During the study *Proteocephalus* sp. (Figure 3.c) was also identified based on the measurements given by Barson and Avenant-Oldewage (2006) and the scolex of *Proteocephalus* sp. (Figure 3c) which was armed with four cup-shaped arranged symmetrically around a protrusible rostellum. *Emoleptalea proteopora* (Figure 3d) collected from *Clarias batrachus* were morphologicaly similar to the description given by Vankara et al. (2014). Some reports of species from the genus *Emoleptalea* namely, *E. dollfusi* and *E. loossi* from *Heteropneustes fossilis*; *E. proteroptera* from *Clarias senegalensis* were already reported (Srivastava 1960). In present study, parasites collected from *Clarias batrachus* referred to *Orientocreadium batrachoides* (Figure 3e) through morphometric analysis correlated with other study (Vankara et al. 2014). There also reports of *Orientocreadium batrachoides* from *Clarias batrachus* from Philippinnes (Tubangui 1931).

Histopathological Changes

In this study, there was different significant pathological change observed in the parasitized organs as a result of helminth infections. The histopathological study in intestine showed the section of parasite (P), semi necrosed (SN) villi, completely necrosed (CN) villi, atropine necrosis (AN) of epithelial cell of mucosa (Figure 4.a). These results are very similar to those of previous surveys (Roberts 2001). In the liver of *Clarias batrachus,* as a response to the presence of the parasite, cellular necrosis (CN), congestion of blood in vessel (BC), pyknotic nucleus (PN), fibrosis (F), necrosis (N) of hepatic cell were seen (Figure 4.b). Nessa (2000) also observed similar liver pathology in *C. batrachus*. In Kidney, tubular architectural loss (TAL), glomerulo-

Table 3. Description of the morphological features and characteristics of helminth parasites observed with their taxonomic status.

Parasite	Mean size (mm)	Identified features
Bovienia indica (Figure 3.a)	17-20 x 0.69-0.8	Scolex (158-167 µm), small, unspecialized width, slightly wider than narrow; long Neck width (120-158 µm), Anterior edge of scolex blunt, almost rectangular in outline
Lytocestus indicus (Figure 3.b)	10-12 x 1.8-2	Scolex (1-1.2 × 0.8-1 mm), short, bluntly rounded, markedly narrower than body; Neck width (420-445µm), very short and distinct.
Proteocephalus sp. (Figure 3.c)	7.44-8.05 x 0.52-0.86	Scolex width (428.96-440.52 µm) with 4 cup-shaped suckers arranged symmetrically around a protrusible rostellum; suckers (0.31×0.30 mm); apical organ (0.28 ×0.28 mm).
Emoleptalea proteopora (Figure 3d)	0.70-0.79 x 0.23-0.25	Oral sucker (0.08× 0.07 mm); Testes (0.08× 0.09 mm); Pharynx (0.05 × 0.07 mm); Oesophagus (0.06× 0.07 mm).
Orientocreadium batrachoides (Figure 3.e)	2.24-2 x 0.63-0.58	Oral sucker (0.2 × 0.18 mm); Testes(0.16 × 0.24 mm); Pharynx (0.07 × 0.08 mm); Oesophagus (0.01 mm).

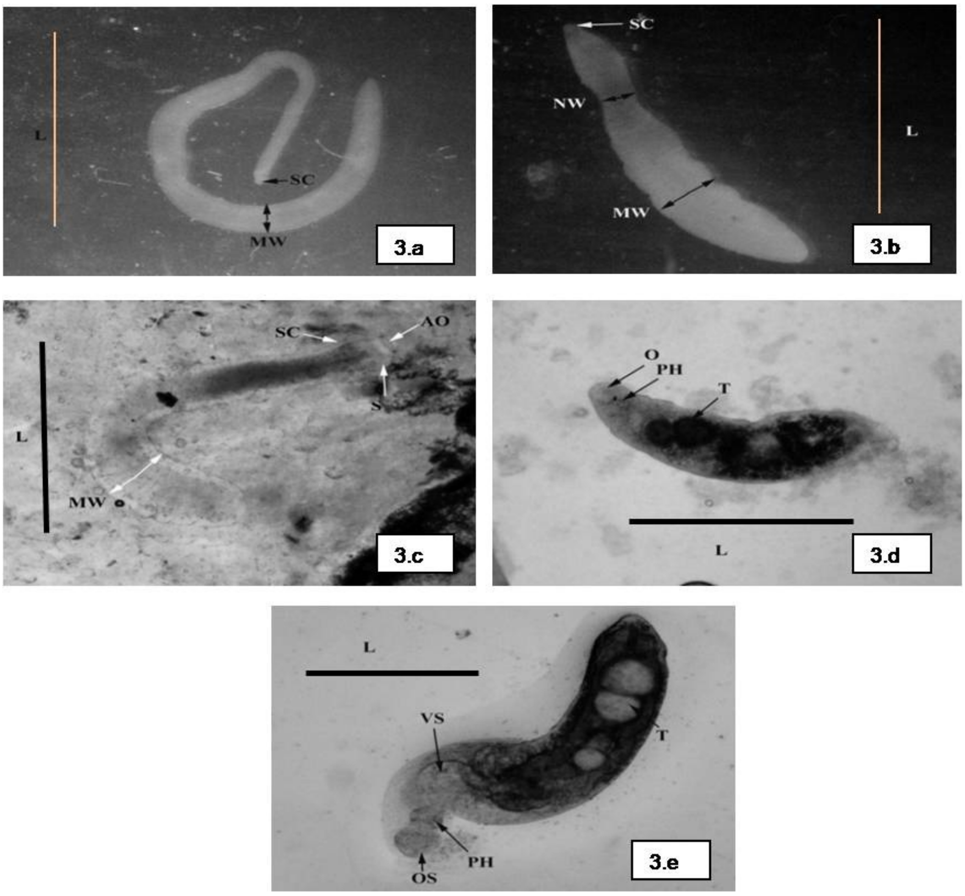

Figure 3. a-e. Helminths recovered from *Clarias batrachus* in India.
a, *Bovienia indica* (cestode) showing scolex (SC), maximum width (MW), body length (L) (100x, wet mount); b, *Lytocestus indicus* (cestode) showing scolex (SC), maximum width (MW),neck width (NW), body length (L)(100x, wet mount); c, *Proteocephalus* sp.(cestode) showing scolex (SC), maximum width (MW), body length (L), sucker (S), apical organ (AO) (400x, wet mount); d, *Emoleptalea proteopora* (digeneans) showing oral sucker (OS), pharynx (PH), testes (T) (400x, wet mount); e, *Orientocreadium batrachoides* (digeneans) showing oral sucker (OS), pharynx (PH),ventral sucker (VS) (400x, wet mount).

pathy (GP) with dilated Bowman capsules (BS), necrosis of the nephric tubule (N), vacuolization of the tubular epithelium (VTE), inflamed nephric tubules (I), deposition of granules (GR) and proteinaceous cast (PC) in the nephric tubules, degeneration of nephric tubules (DG), thickening (T) of the epithelial layer, congestion (C) of nephric tubule were noticed (Figure 4.c and Figure 4.d). From the research findings of Khatun (1999), similar pathological changes were found in kidney of *C. batrachus*. In the present study, recorded information demonstrated the most dominant helminth parasite in *C. batrachus* were cestodes. High incidences of infections of acanthocephalans, nematodes, cestodes were recorded in rainy seasons, digeneans in winter and monogeneans in summer. Among various length groups, larger length groups were more prone to parasitic attack. The outcomes of the study clearly indicate that seasons, environmental factors and feeding habitat interfere with the physiology and ecology of the fish, which in turn greatly influences the rate of parasitic infections and due to over infestation of parasite may cause high economic loss for fishery sector.

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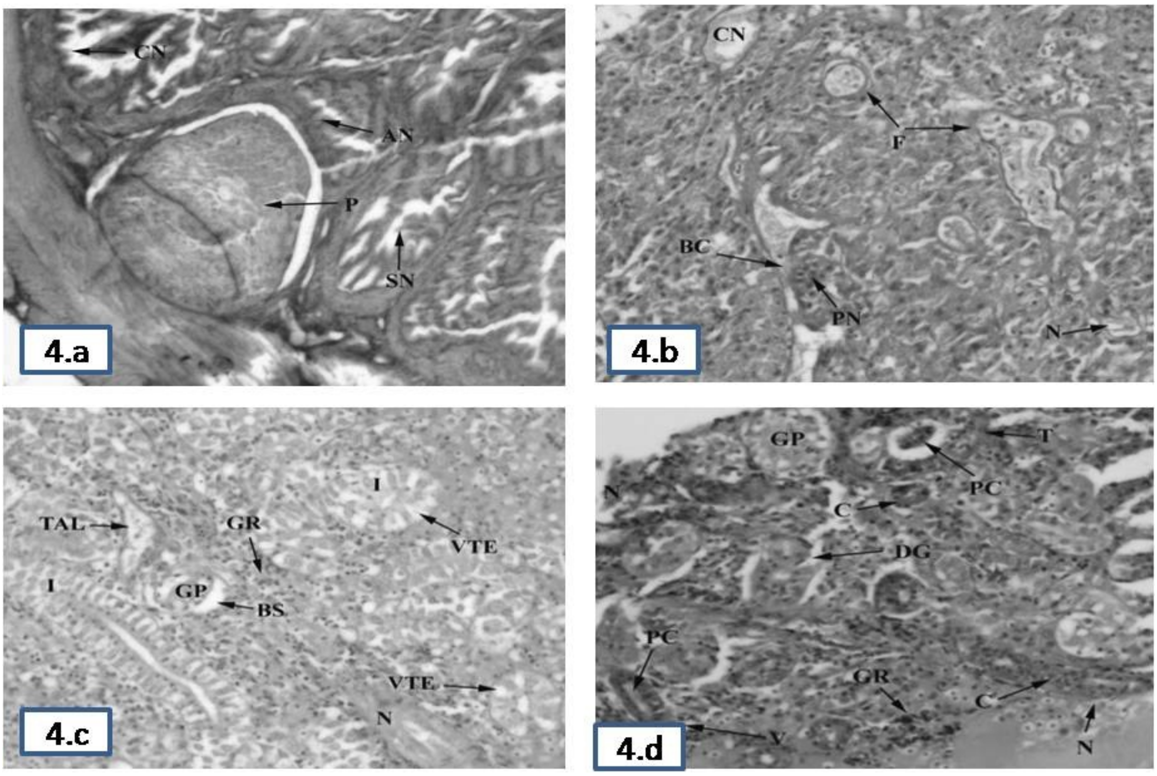

Figure 4. a-d. Histopathological section of vital organs of parasite infested *Clarias batrachus*. a, Histopathological section of intestine (H&E, 40X); b, Histopathological section of liver (H&E, 100X); c, Histopathological section of Kidney (H&E, 200X); d, Histopathological section (H&E, 200X).

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