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COMMUNITY STRUCTURE OF PROTOZOAN PARASITES IN *HETEROPNEUSTES FOSSILIS* (BLOCH 1794) IN BANGLADESH

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Key words: Protozoan parasite, Community structure, Prevalence, Intensity, Distribution, *Heteropneustes fossilis*

Abstract

The study was conducted to prepare a database for the infection status of protozoan parasites on an important host fish species of Bangladesh, *Heretropneustes fossilis.* Host samples were collected from the freshwater habitats of six different districts of Bangladesh- Manikganj, Faridpur, Mymensingh, Kishoreganj, Bogura, and Jashore. *H. fossilis* was noted to be infected by 6 parasite species, of which 3 belonged to myxozoa (*Henneguya singhi*, *Henneguya qadrii* and *Henneguya mystusia*); 1 belonged to ciliophora (*Trichodina siddiquae*) and 2 (*Trypanosoma singhii* and *Piscinoodium pillulare*) belonged to mastigophora.The parasites, *Trypanosoma singhii* and *Henneguya singhi* were recorded as new locality record in *H. fossilis*. The three parasites *Piscinoodium pillulare, Henneguya qadrii* and *Henneguya mystusia* were the first recorded parasites in this fish and the first locality record in Bangladesh. The parasites were observed to occupy gill, body slime, and blood. Gill parasites were abundantly found compared to body slime and blood parasites. The highest prevalence (67.21%) of infection of *H. fossilis* was observed in Manikganj and the lowest prevalence (54.67%) of infection was observed in Bogura. Parasites of *H. fossilis* showed the highest diversity in fishes of Faridpur (2.63). Species richness of parasites was highest in Manikganj (0.38) and species evenness was relatively low (0.13-0.19) in almost all the study sites.

Introduction

The 'Asian stinging catfish', *Heteropneustes fossilis* is a species of air sac catfish group found in Bangladesh, India, Pakistan, Nepal, Sri Lanka, Thailand, and Myanmar. It has great demand in Bangladesh due to its medicinal value. The stinging catfish can deliver a painful sting to humans. Poison from a gland on its pectoral fin spine has been known to be extremely painful[(1)]. It has a pair of accessory respiratory organ that extends backward from the gill-chamber or either side of the ventral column. It can receive oxygen directly from the air and can survive a long time in the water with less oxygen even without water. It is fairly common in Bangladesh[(2,3)].

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Protozoan parasites are one of the important disease-causing agents of wild and cultured *H. fossilis* in Bangladesh. Among the ectoprotozoan parasites infecting *H. fossilis* phylum ciliophora and myxozoa, are the major important agents affecting fish wellbeing[4]. These groups have a natural life cycle that easily spreads from one fish to another[5]. Ciliophorans infect fish skin, fins, and gills[6], producing external ulcers and pustules[7], while myxozoans infect fins, skin, operculum, buccal cavity, nasal chamber, eyeball, gall bladder, and alimentary canal wall [8]. Along with the destruction of host's skin and gill epithelium, some other protozoa impede various biological activities such as respiratory system obstruction and growth retardation[9]. Even moderate infection of these organisms may cause a fatal disease since the infected fish lose their appetite and stop feeding[10]. In many instances, individuals of protozoan parasites provoke the secondary infection[11] of other pathogens like viruses, fungi, and bacteria. They are the most dangerous parasitic group that probably cause, more diseases in fish cultures than any other type of animal parasites[10,11], including mortality leading to constraints in national aquaculture production. In this way, parasitic protozoan diseases are responsible not only for significant losses to the commercial fishing industry but also for a negative social impact in developing countries where aquaculture activities contribute to food production of high nutritional value to the needy population[10,11].

However, the pathological effects exerted by these parasites depend on factors related to parasite species, factors related to host species and environmental factors[5,12]. A little work has been found to describe in detail about incidence of the protozoan infestation in *H. fossilis* in Bangladesh[12]. The present work determining the community structure of parasites aims to be served as valuable baseline data for the protozoan parasitic infestation on *H. fossilis* in Bangladesh. Further studies towards finding the remedies for protecting these valuable fish resources will boost the country's economy and help mitigate the protein requirements.

Materials and Methods

Selection of species: Heteropneustes fossilis (Bloch, 1794), the 'Asian stinging catfish' is a fairly common air sac catfish species in Bangladesh selected as host species for conducting the present study. A special characteristic of the fish is that it has additional respiratory organs other than gills. It can receive oxygen directly from the air and can survive a long time in the water with less oxygen or even without water. Simultaneously, our concerned 'protozoan' parasites are very sensitive and cannot survive so prolonged except live fishes. This is the reason to be selected as host fish with protracted survival capacity having accessory respiratory organs those facilitated collections and transportation from distant sampling places.

Collection of host sample: According to the experimental design of the research, a total of 391 individuals of host fish species, *H. fossilis* were collected alive from the freshwater bodies of Kishoreganj (Kuliar char- 24°10'40" N, 90°50'57" E and Pakundia- 24°30'07"N, 90°67'71"E), Mymensingh (Ishawrganj-24°41'16" N, 90°35'58" E and Trishal- 24°57'18"N, 90°43'84"E), Faridpur (Modhukhali- 23°32'50"N, 89°31'22"E and Boalmari- 23°44'04"N, 89°66'84"E), Jashore (Purondorpur, Jhikorgacha Upazila- 23°5'51" N, 89°5'53" E and Monirampur-22°59'32"N, 89°11'53"E), Manikganj (Singair- 23°81'45"N, 90°12'47"E and Ghior- 23°93'74"N, 89°86'05"E) and Bogura (Sherpur- 24°68'21"N, 89°41'47"E and Sadar- 24°87'45"N, 89°38'34"E) with the help of fishermen during mid of April 2018 to end of March 2019. The sample size of fishes collected from each area was not sharply equal.

Sample preparation: The hosts were examined as soon as possible after capture. Immediately after collection, the external surfaces of the fish were observed using a magnifying glass. The external surface of the fish was examined and recorded for any abnormalities. After collecting the samples, their total length and weight were measured. Evidence was collected from the body slime, gill slime and blood of host fishes which are the best suited micro-habitat for protozoan parasites to get harbor. Smears of body slime, gill slime and blood were made on glass slides on the spot and fixed in ethanol for further observation in the laboratory. Giemsa's stain technique was used for rapid demonstration of nuclei in ciliates and in microsporidian spores and Klein's dry silver impregnation method was used for staining mobilie peritrichs and other ciliates from the surface of fish. The slides were observed under a microscope to note the presence or absence of protozoan parasites. Counts of parasites found in selective organs were recorded. Microscopic photographs were captured to identify species with the help of a 10-megapixel digital camera, measurement of parasites was done by an ocular micrometre aided by Euromax software. Protozoans were identified according to the description of Lom and Dyková (1992)(13), Sarkar (1985)(14), Eiras (2002)(15), Kalavati and Nandi (2007)(16), Bashĕ and Abdullah (2010)(17) and Kibria *et al.* (2010)(18). Some parasites could not be identified up to species level because these were not get matched with any of the available published descriptions.

Calculation: Measurement of prevalence, mean intensity and abundance of infection were calculated according to Margoles *et al.* (1982)(19). Simpson's Diversity Index(20) evaluated both species richness and abundance of parasites found in the samples. Shannon's Diversity index(21) was used to measure the diversity. The most commonly used index of evenness based on the Shannon- Wiener index(22) was used. Margalef Index of Species Richness(23) evaluated the richness of parasites within the samples. Statistical analyses were carried out using Microsoft Excel 2010. Significance levels were set at $p \leq 0.05$.

Results and Discussion

A total of 391 species of *Heteropneustes fossilis* were collected from several districts of Bangladesh and thoroughly examined to identify parasitic infestation in all possible microhabitat such as skin, gills, and fins. In *H. fossilis*, the overall protozoan prevalence was found at 60.36% and six species of parasites were recorded. Among them three species belonged to sub phylum myxozoa (*Henneguya singhi, Henneguya qadrii* and *Henneguya mystusia*); one species belonged to sub-phylum ciliophora (*Trichodina siddiquae*) and two species fitted to phylum mastigophora (*Trypanosoma singhii* and *Piscinoodium pillulare*). A total of 1176 (n) individuals of protozoan parasites were collected from 236 infected fish (out of 391 fish examined). Of them 60.71% were under phylum myxozoa, 25.43% of parasites were under phylum ciliophora and 13.86% of parasites were under phylum mastigophora (Fig. 1).

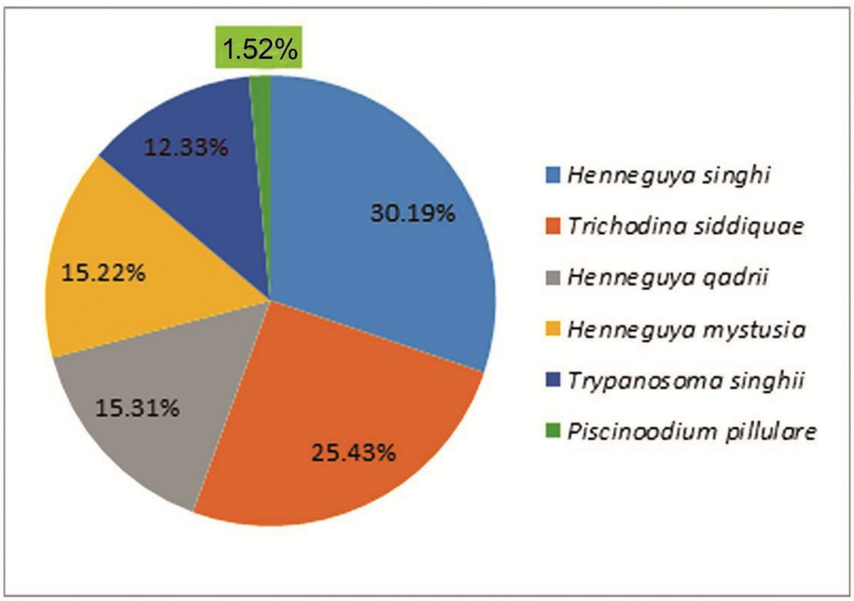

Fig. 1. Abundance of protozoan parasites in *H. fossilis*.

Among the parasites found in *H. fossilis*, *Henneguya singhi* was the most abundant 355 (30.19%), followed by *Trichodina siddiquae* 299 (25.43%), *Henneguya qadrii* 180 (15.31%), *Henneguya mystusia* 179 (15.22%), *Trypanosoma singhii* 145 (12.33%) and *Piscinoodium pillulare* 18 (1.53%) (Fig. 1).

The protozoan parasite *Trichodina siddiquae* from *H. fossilis* was previously recorded in Rangamati hill district in Bangladesh[24]. The parasite *Trypanosoma singhii* was previously recorded in India[25,26] and *Henneguya singhi*[27] was also previously recorded in India in the same host, but in this study, these were recorded as new locality records in Bangladesh for this host. The rest three parasites (*Piscinoodium pillulare, Henneguya qadrii* and *Henneguya mystusia*) were first recorded in *H. fossilis,* as well as the first locality record in Bangladesh (Table 1).

Table 1. Status of identified parasites record in Bangladesh.

Parasites	Sampling area	Record status
Trichodina siddiquae	Faridpur, Kishoreganj, Bogura	Previously recorded in Bangladesh
Piscinoodium pillulare	Manikganj	New host record New locality record in Bangladesh
Trypanosoma singhii	Manikganj, Faridpur, Mymensingh	New locality record in Bangladesh
Henneguya qadrii	Kishoreganj, Jashore	New host record New locality record in Bangladesh
Henneguya mystusia	Faridpur, Mymensingh	New host record New locality record in Bangladesh
Henneguya singhi	Manikganj, Bogura, Jashore	New locality record in Bangladesh

Host fishes were collected from different districts of Bangladesh. The prevalence and intensity of host fishes vary in respect of prevailing in various sites. Prevalence and intensity of parasitic infestation according to host's macrohabitat have been shown in Table 2. In *H. fossilis* highest prevalence (%) of parasitic infection was observed in Manikganj (67.21%), which was followed by Faridpur (64.18%), Kishoreganj (63.08%), Jashore (56.92), Mymensingh (56.90%), and Bogura (54.67%), respectively. The highest mean intensity was recorded in Faridpur (5.77 ± 2.64) followed by Bogura (5.22 ± 2.02), Jashore (5.11 ± 1.78), Kishoreganj (5.00 ± 1.77), Manikganj (4.56 ± 1.36) and Mymensingh (4.03 ± 1.06).

According to the evidence recorded in Table 2, it is undoubtedly demonstrated that parasites of *H. fossilis* had a higher index of species richness in Manikganj (highest value-0.38). The lowest value was observed in Kishoreganj, Bogura, and Jashore (0.19) compared with the number of component parasites to the number of parasite species found in each site, Manikganj and Faridpur were the richest among all the sites. The number of component parasites at these sites was recorded as three (3). *Henneguya singhi* was recorded as the most dominant parasite species in these two sites. At the same time, *Trichodina siddiquae* was found as the predominant parasite species in Bogura, whereas, *Henneguya qadrii* was observed as a dominant parasite in Kishoreganj, and *Trypanosoma singhii* was found as the dominant parasite in Mymensingh. Species Diversity Index was measured highest in Faridpur (2.63) and lowest in Manikganj (1.75), whereas low diversity was observed in Jashore. The parasite communities of the sampling sites exhibited higher diversity (2.00) in Kishoreganj, Mymensingh, and Bogura. Species evenness was moderately low (0.13-0.19) in almost all the sites (Table 2).

Table 2. Community structure of protozoan parasites in *H. fossilis* in various districts in Bangladesh.

Characteristics	Manikganj	Faridpur	Kishoreganj	Mymensingh	Bogura	Jashore
Number of fish examined	61	67	65	58	75	65
Number of fish Infected	41	43	41	33	41	37
Prevalence	67.21%	64.18%	63.08%	56.90%	54.67%	56.92%
Mean intensity (±SD)	4.56±1.36	5.77±2.64	5.00±1.77	5.11±1.78	4.03±1.06	5.22±2.02
No. of parasites collected	187	248	205	133	214	189
No. of parasite sp.	3	3	2	2	2	2
Identification of dominant sp.	*Henneguya-singhi*	*Henneguya mystusia*	*Henneguya-qadrii*	*Trypanosoma -singhii*	*Trichodina-siddiquae*	*Henneguya-singhi*
Proportion of dominant parasite	73	47	54	53	54	63
Species evenness	0.15	0.19	0.13	0.14	0.13	0.13
Species Richness 'R'	0.38	0.36	0.19	0.20	0.19	0.19
Simpson's Diversity Index	1.75	2.63	2.00	2.00	2.00	1.89

Parasites of *H. fossilis* commonly infected gill, blood and body slime. A total of 1176 parasites of 6 genera were collected from these three organs of *H. fossilis*. During the study period, a total of 676 parasites of 4 species were collected from the gill. The ciliophoran parasite, *Trichodina siddiquae*; the myxozoan parasite, *Henneguya qadrii, Henneguya mystusia,* and the mastigophoran parasite, *Piscinoodium pillulare* were recorded from gills of host fish. The body slime parasites were recorded infected by the only myxozoan parasite *Henneguya singhi*. Around 145 parasites of a single species were collected from the blood of the host fish. The parasite *Henneguya singhi* presented the highest prevalence (21.48%) and *Trichodina siddiquae* showed the highest intensity (5.34 ± 1.50) in gill. The parasite *Trypanosoma singhii* exhibited the lowest prevalence (1.79%) in blood and *Piscinoodium pillulare* displayed the lowest intensity (2.57 ± 0.49) in the gill of *H. fossilis* (Table 3).

Parasites in different investigated organ from *H. fossilis* revealed that fishes of district Faridpur were highly infected, followed by Bogura, Kishoreganj, Jashore, Manikganj, and Mymensingh. Organ specificity of parasitic manifestations in district Manikganj was gill>body slime>blood, whereas in Faridpur was gill>blood and in Mymensingh was blood > gill. In Bogura, it was gill > body slime and in Jashore was body slime > gill. It was the only gill in Mymensingh (Fig. 2).

Table 3. Micro-distribution of protozoan parasites in different organs in *H. fossilis*.

Group of parasite	Name of parasite	No. of fish infected	Preva-lence	No. of parasite	Intensity ±SD	Organ infected
Ciliophora	*Trichodina siddiquae*	56	14.32	299	5.34±1.50	Gill
	Henneguya qadrii	39	9.97	180	4.62±1.12	-
Myxozoa	*Henneguya mystusia*	36	9.21	179	4.97±1.66	-
Mastigophora	*Piscinoodium pillulare*	7	9.72	18	2.57 ±0.49	-
Mastigophora	*Trypanosoma singhii*	38	1.79	145	3.82 ±0.88	Blood
Myxozoa	*Henneguya singhi*	84	21.48	355	4.23±0.92	Body slime

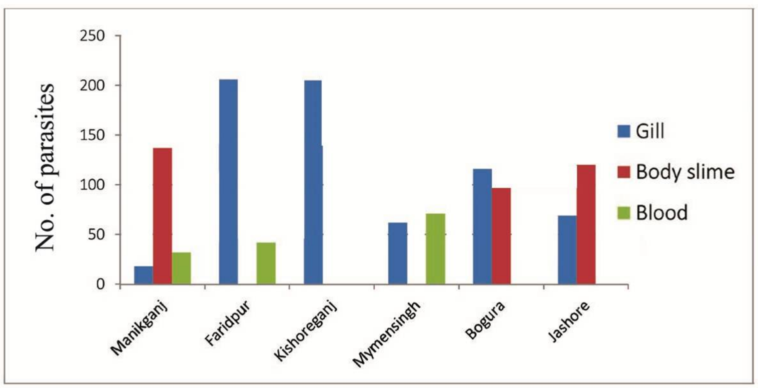

Fig. 2. Micro-distribution of parasites of *H. fossilis* in different districts in Bangladesh.

Gills were observed to harbour the highest number of protozoan parasites. This could be because the gills are the centre of filter feeding and are the sites of gaseous exchange. This observation agrees with the report of Emere and Egbe (2006)[28], who reported the highest load of protozoan parasites in the gill of *Synodontis clarias*. The report of Nyaku *et al.* (2007)[29] also revealed the highest load of protozoan parasites in the gills of *Auchenoglanis ocidentalis, Oreochromis niloticus,* and *Bagrus bayad* in River Benue. Roger and Gainer (1975)[30]and Chakroff (1976)[31] had shown different protozoan parasites in gills. The sieving ability of the gill rakers may help to trap some organisms, and this could be attributed to the presence of the protozoan parasites there[32]. Due to the presence of spores within the entire length of the gill filament, the blood vessels were dilated, and bounding endothelial cells were highly compressed[33]. Rukyani (1990)[34], Azevedo *et al.* (2010)[35] and Campos *et al.* (2011)[36] reported the alterations in the capillary network, hyperplasia of gill epithelium and structural disorganization of secondary lamellae. These alterations may partially compromise gill functions and diminish respiratory capacity and ionic exchange[37]. Earlier, similar observation had also been made by McCraren *et al.* (1975)[38] in gill infections in American catfish with *Henneguyaexilis*, in *C. Punctatus* with *Henneguya waltairensis,* and in carp with *Myxobolus koi.*

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