Dhaka Univ. J. Biol. Sci. **32**(1): 21-32, 2023 (January) DOI: https://doi.org/10.3329/dujbs.v32i1.64187

A BRIEF ACCOUNT ON PROTOZOAN INFECTION IN ASIAN STINGING CATFISH, *HETEROPNEUSTES FOSSILIS* (BLOCH, 1794) IN DIFFERENT SEASONS OF BANGLADESH

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Keywords: Protozoan infection, Seasonal influence, *Heteropneustes fossilis,* Bangladesh

Abstract

The study was conducted to identify the protozoan parasites in a freshwater indigenous air breathing fish, *Heteropneustes fossilis* (Bloch, 1794). The host fish was collected during mid of the April 2018 to end of the March 2019 from freshwater bodies of Mymensingh, Kishoregonj, Faridpur, Jashore, Manikganj and Bogura districts of Bangladesh. Three species of phylum myxozoa namely *Henneguya singhi, Henneguya qadrii, Henneguya mystusia,* one species of phylum ciliophora namely *Trichodina siddiquae* and two species of phylum mastigophora namely *Trypanosoma singhii and Piscinoodium pillulare* were identified in *H. fossilis.* The parasites of all infected hosts were observed in gill, body slime and blood, however gills were commonly infected by parasites rather than body slime and blood. Three species of parasite *Piscinoodium pillulare, Henneguya qadrii* and *Henneguya mystusia* were first recorded in this host fish and novel locality record in Bangladesh. *H. fossilis* was found to be infected over the three major ($p<0.05$) (summer, rainy and winter) seasons and demonstrated a strong significant association (P0<05) with season during the study period. In *H. fossilis*, highest variety and highest number of parasites were found in winter season, prevalence and intensity was found 78.31% and 5.17 respectively. Lowest prevalence and intensity were found in rainy season respectively 45.87 and 4.72. Among all the parasites *Henneguya singhi* was common in all the three seasons and its rate of infection was found elevated in summer (20.69%) and rainy season (19.27%). In *H. fossilis* prevalence of female fish were highest in all the three seasons, summer (55.22%), rainy (50.75%) and winter (85.57%) than their male counterparts 38.76%, 38.09% and 68.12%, respectively.

Introduction

With the increasing interests in aquaculture, parasitic infestations are becoming threats for fish health management and aquatic crop production in Bangladesh along with the world. Although significant damage or mortality cases are less reported, equally the wild and cultured fishes infested with parasites can cause a considerable decline in the production of aquaculture. Hence, this might be an essential area for concentrating on to it for the scientists to ensure sustainable aquaculture production(1).

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A remarkable number of fish parasites are belonging to Sub-kingdom protozoa acting either as ecto-parasite or endo-parasite in cultured and wild fishes. The common pathological symptoms of protozoan infections are- lesion, inflammation, growth retardation, weight loss, in some cases mortality, due to secondary or tertiary infection by other pathogens. Thus, texture deterioration and bad appearance due to protozoan infection generally depreciate the market value of the fish resulting production forfeiture [(2)].

Heteropneustes fossilis, the 'Asian stinging catfish' is one of the most popular edible fish species in Bangladesh for its high nutritional and medicinal value. Being a species of air sac catfish group, it is equally popular to the aqua-culturists in Bangladesh, India, Pakistan, Nepal, Sri Lanka, Thailand and Myanmar. It is highly preferred in Bangladesh and locally known as 'Singhi' which is able to deliver a painful sting to human[(2)]. Among the ectoprotozoan parasites infecting *H. fossilis*, phylum ciliophorans and myxozoans, are the major important pathogens affecting fish wellbeing[(3)]. Most members of these phylum have direct life cycle enabling them to spread contamination from one individual/species to another one easily[(4)]. Generally, ciliophorans infect fish skin, fins and gills producing external ulcer and pustules [(5)] while myxozoans infect fins, skin, operculum, buccal cavity, nasal chamber, eye ball, gallbladder and wall of the alimentary canal[(6)].

Protozoan parasitic infection might have an association with the physical parameters of the surrounding environment, outstandingly temperature, tide pressure, salinity, water quality and so on. Thus, infection pattern might be influenced by season. Here, in Bangladesh we basically encounter three types of seasonal variation- summer, rainy and winter. A considerable number of studies have been done on the protozoan parasitic infestation in *H. fossilis* in relation to various parameters in our neighboring country, India[(7-16)]. However, In Bangladesh, little work has been found to describe in detail about protozoan infestation pattern in relation to their environmental/seasonal effect in *H. fossilis*[(17)]. Proposed research was an attempt to analyze the seasonal influence of protozoan infection in this host to create a base line data of protozoan infestation pattern of the wild *H. fossilis* in Bangladesh.

Materials and Methods

Host species selection: Heteropneustes fossilis (Bloch, 1794), the 'Asian stinging catfish' was selected as host species for conducting the present study. A distinctive characteristic of *H. fossilis* is that it has additional respiratory organs except gills through which it can directly breathe from air and survive prolonged period in water with less dissolved oxygen or even without water. On the other hand, protozoans are sensitive to survive without live host. So, as a host, *H. fossilis* has protracted survival capacity having accessory respiratory organs to facilitate their collection and transportation from distant sampling sites to the laboratory.

Sampling: As per the experimental design of the research, a total of 391 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 the April 2018 to end of the March 2019. Sample size of fishes collected from each area was not sharply equal.

Preparation of Sample: Fish were examined immediately after capture. Using a magnifying glass, external surface of the fish were examined, measured and recorded for any abnormalities. Their total length and weight were measured. Indications were collected from the body slime, gill slime and blood of host fish which are the best suited micro-habited for protozoan parasites to get colonized. Smears of body slime, gill slime and blood were made on glass slides on the spot and fixed them in ethanol for further observation in the laboratory.

Giemsa’s stain after acid hydrolysis: Giemsa’s stain technique was used for rapid demonstration of nuclei in ciliates and in microsporidian spores. Parasites in blood sample were identified through using this technique; slides were stained using Giemsa stain and cover slipped by DPX mount. During this process smears were fixed in Schaudinn’s fluid and rinsed well in distilled water. After that they were hydrolysed for 8 min in 1N HCL at >60°C. Again they were rinsed for several times in distilled water and stained with stocked Giemsa’s stain (diluted 1:20 with water at pH 7.0-7.2) for about 20 min and rinsed with tap water. After that they were kept dry and finally mounted with a neutral medium, Canada balsam.

Klein’s dry silver impregnation method: Klein’s dry silver impregnation method was used for staining mobiline peritrichs and other ciliates from the surface of fish. Mucus was scraped gently off gills and skin with a scalped, spread thinly on a grease-free slide, and dry rapidly.The slide was covered with a 2% aqueous solution of silver nitrate ($AgNO_3$) for 8 min. After that they were rinsed thoroughly with distilled water and were placed facing up in a dish of distilled water and expose to bright sunlight for 1-2 hours. Finally they were allowed to dry and mount with a neutral medium, Canada balsam.

Parasite count: The mounted slides were observed under microscope to note the presence of protozoans. Counts of parasites found in selective organs were recorded. Microscopic photographs were captured for the identification of species with the help of 10-megapixel digital camera. Protozoans were identified according to the description of Lom and Dyková[(18)], Sarkar[(19)], Eiras[20)], Kalavati and Nandi[(21)], Bashě and Abdullah[(22)], Kibria *et al.*[(23)]. Some parasites could not be identified up to species level because these were not got matched with any of the available published description.

Results and Discussion

A total of 391 specimen of *Heteropneustes fossilis* were collected from six districts of Bangladesh and thoroughly examined to identify protogoan parasitic infestation in all possible microhabitat such as skin, gill and fin (Table 1).

Table 1. Overall prevalence (%) and intensity of parasitic infection in different study areas.

Host	Sites	No of fish Examined	No of fish Infected	Total no of Parasites	Prevalence (%)	Intensity (±SD)
	Manikganj	61	41	187	67.21	4.56±1.36
	Faridpur	67	43	248	64.18	5.77±2.64
	Kishorganj	65	41	205	63.08	5.00±1.77
	Mymensingh	58	33	133	56.90	5.11±1.78
H.	Bogura	75	41	214	54.67	4.03±1.06
fossilis	Jashore	65	37	189	56.92	5.22±2.02

In *H. fossilis*, overall protozoan prevalence was found 60.36% and a total of six species of parasites were recorded. Among them three species belonged to phylum myxozoa (*Henneguya singhi, Henneguya qadrii and Henneguya mystusia*); one species belonged to phylum ciliophora (*Trichodina siddiquae*) and two species fitted to phylum mastigophora (*Trypanosoma singhii and Piscinoodium pillulare*) (Table 2)

Fig. 1a. *Henneguya singhi* (100x).

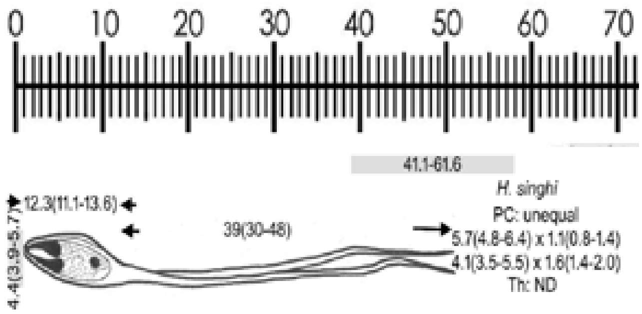

Fig. 1b. Schematic scaled diagram of *H. singhi*[41].

Fig. 2a. *Henneguya qadrii* (100x).

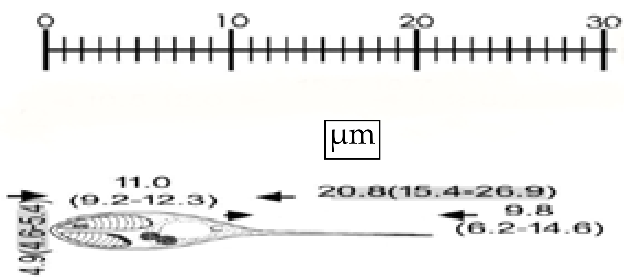

Fig. 2b. Schematic scaled diagram of *H. qadrii*[41].

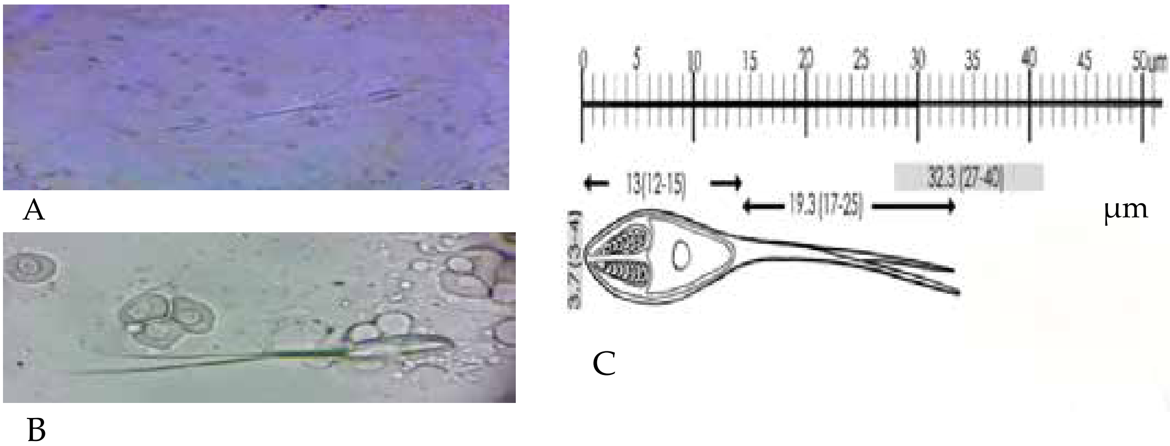

Fig. -3. *Henneguya mystusia* (A, B microscopic plate, C, Schematic scaled diagram)[41].

Fig. 4a. *Trichodina siddiquae*(100x).

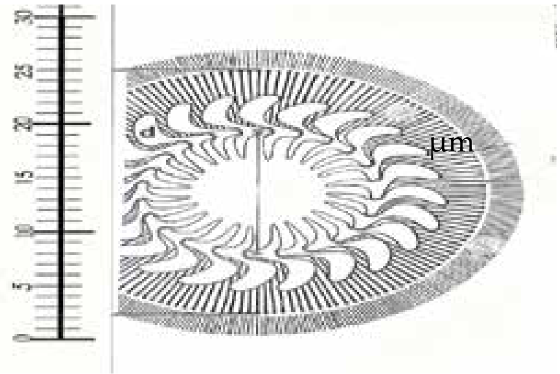

Fig. 4b. Schematic scaled diagram of *T. siddiquae*[19].

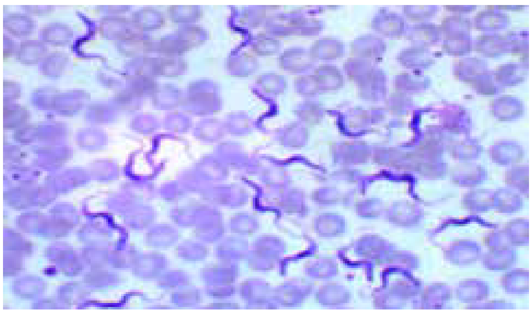

Fig. 5a. *Trypanosoma singhii* (100x).

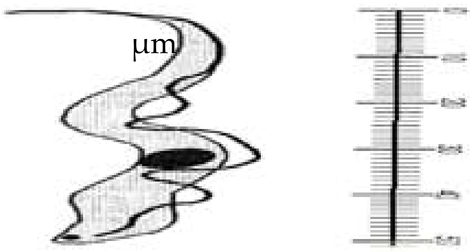

Fig. 5b. Schematic scaled diagram of *T. singhii*[19].

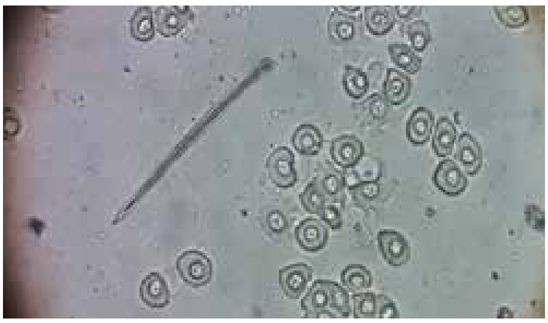

Fig. 6a. *Piscinoodium pillulare* (100x).

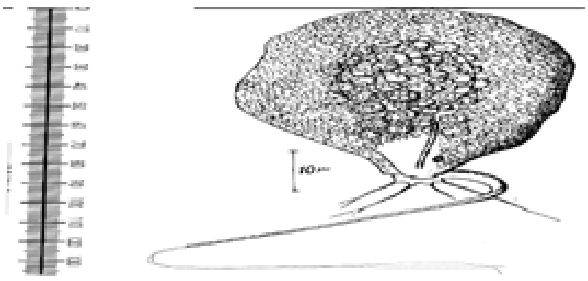

Fig. 6b. Schematic scaled diagram of *P. pillulare*[42].

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 (Table 2). Female fish were found to be more infected than their male counterparts. Female species of *H. fossilis* showed higher prevalence and intensity of infection, 66.67% and (5.20 ± 1.96) respectively (Table 2).

Table 2. Data of identified parasites in *Heteropneustes fossilis* in sampling sites.

Factors	Value (%)	P Value
Number of fishes examined	391	
Number of fishes infected	236	
Prevalence of infestation	60.36%	
Total number of parasite individuals recorded	1176	
Total number of parasite species	6	
Mean intensity ± Standard Deviation (SD)	4.98±1.92	
Total number of male hosts	*160*	
Prevalence of infestation in male host	51.25%	
Total number of female hosts	*231*	
Prevalence of infestation in female host	66.67%	
Intensity of infestation in male host	4.57±1.84	0.02**
Intensity of infestation in female host	5.20±1.96	

Group	Number of infected Hosts	Abundance	Parasites identified
Myxozoa	84	60.71%	*Henneguya singhi*
	39		*Henneguya qadrii*
	36		*Henneguya mystusia*
Ciliophora	56	25.43%	*Trichodina siddiquae*
Mastigophora	38	13.86%	*Trypanosoma singhii*
	7		*Piscinoodium pillulare*

The finding was similar to the outcomes of Ayanda[24] reporting higher parasitic infestation in female *Clarias gariepinus*. However, Ikechuk wu *et al.*[25] revealed no statistically significant difference on the infection in respect of different sexes in *C. gariepinus.* Higher infestations in male fish were however reported by several authors. Allumma and Idpwu[26] reported that in *Clarias gariepinus*, infection in host species was found to be higher in males (16.7%) than in females (6.3%).

Seasonal infection pattern:

Overall prevalence and intensity of infection was calculated in relation to the season. The associated information regarding seasonal infection of protozoa infecting *H. fossilis* showed that *H. fossilis* was found to be infected over the three major (summer, rainy and winter) seasons (Fig. 7).

In *H. fossilis*, highest variety and highest number of parasites were found in winter season, prevalence and intensity was found 78.31% and 5.17 respectively. Lowest prevalence and intensity was found in rainy season respectively 45.87% and 4.72. During summer season prevalence and intensity were found to be moderate respectively 48.28% and 4.79 (Fig. 7).

As the water quality parameters fluctuate very quickly during winter and summer season, fish become more affected with diseases in these two seasons. The parasitic infection is greatly influenced by the season, which basically interfere with ecology and physiology of the fish which was strongly supported by Ahmed *et al.* (1991)[27] and Wisheiwski (1958)[28].

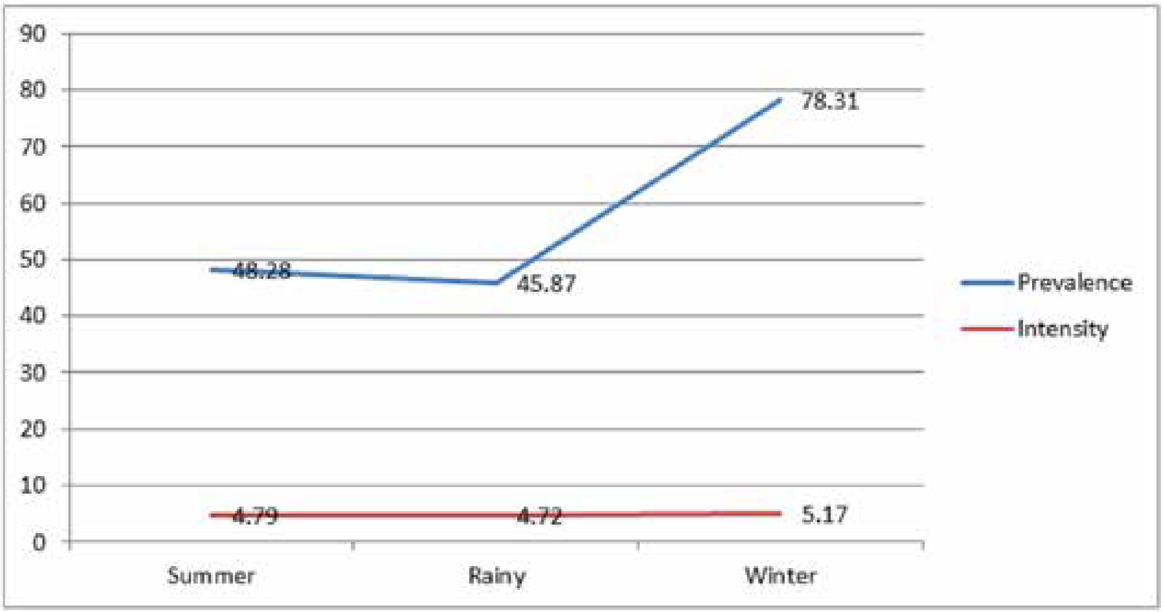

Fig. 7. Infection pattern in *Heteropneustes fossilis* in different seasons.

A total of five parasite species were collected in winter and three species in summer and rainy seasons. Among all the parasites *Henneguya singhi* was common in all the three seasons and its rate of infection was also highest in summer (20.69%) and rainy season (19.27%). Among the parasites *Henneguya mystusia* was common in both summer and rainy seasons; *Trichodina siddiquae* was common in both summer and winter and *Henneguya qadrii* was observed common in both winter and rainy season. In winter season, *Trichodina siddiquae* showed the highest (36.75%) prevalence. *Piscinoodium pillulare* (4.22%) in winter, *Trichodina siddiquae* (12.09%) in summer and *Henneguya qadrii* in rainy season (11.00%) showed the lowest prevalence (Table 3).

Table 3. Prevalence of protozoan parasites in different seasons in *H. fossilis*.

Season	Parasite	No. of infected host	Prevalence %	Intensity±SD	$S^2/\bar{x}$
Summer	*Henneguya singhi*	56	20.69	4.71 ± 0.89	0.17
	Henneguya mystusia		16.39	4.58 ±1.66	0.63
	Trichodina siddiquae		12.09	4.86 ± 0.91	0.18
Rainy	*Henneguya singhi*	50	19.27	4.14 ± 0.85	0.16
	Henneguya mystusia		15.60	5.41 ± 1.72	0.57
	Henneguya qadrii		11.00	4.75 ± 1.21	0.31
	Piscinoodium pillulare		4.22	2.57 ± 0.49	0.09
Winter	*Henneguya singhi*	130	21.08	4.43 ± 0.83	0.15
	Trypanosoma singhii		11.45	3.89 ± 0.89	0.2
	Trichodina siddiquae		36.75	4.95 ± 1.49	0.43
	Henneguya qadrii		16.27	4.56 ± 1.21	0.31

$S^2/\bar{x}$= Over dispersion

In *H. fossilis, Henneguya mystusia* showed the highest intensity (5.41 ± 1.72) in rainy season, whereas *Trichodina siddiquae* (4.95 ± 1.49) showed highest intensity in winter and *Trichodina siddiquae* (4.86 ± 0.91) in summer. Lowest intensity was shown by *Piscinoodium pillulare* (2.57 ± 0.49), *Henneguya mystusia* (4.58±1.66) and *Henneguya singhi* (4.14 ± 0.85) in winter, summer and rainy season respectively. Only a few species of parasite were frequently present in the host. When considering the parasite community from fish sample *Henneguya singhi* and *Trichodina siddiquae* were found as the component parasites and they also dominated the parasite community composition. *H. mystusia* was over dispersed in almost all the seasons and was highest (0.63) in summer.

It is difficult to explain the reasons of seasonal variation in the infection of protozoan parasites in fishes without knowing the seasonal aspects of the intermediate host-parasite system. Although changes of parasite incidence are attributed to diet and other factors such as host size and development of host immunity.[(29)] The result can be compared with the findings of Akther *et al.* (2018)[(30)] stated that the prevalence ranged from 67.86 to 81.82% in *C. punctatus*, highest prevalence was recorded in autumn and the lowest one was recorded in rainy season[(30)]. The correlation co-efficient analysis depicted that all the relationships between prevalence and abundance; abundance and mean intensity and prevalence and mean intensity were positively correlated in different seasons. Sultana (2015) also revealed that the prevalence, intensity and abundance of parasites in *Glossogobius giuris* varied with the season. It can be concluded that environmental factors directly involved with the parasitic infection of the host, such as seasonal fluctuations in water levels can cause different responses[(31)].

Table 4. Prevalence and intensity of male and female fishes in different seasons.

Host	Summer				Rainy				Winter			
	Male		Female		Male		Female		Male		Female	
H. fossilis	P	I±SD	P	I±SD	P	I±SD	P	I±SD	P	I±SD	P	I±SD
	38.76	3.89±1.44	55.22	5.24±1.43	38.09	4.56±1.39	50.75	4.79±1.38	68.12	4.85±1.42	85.57	5.35±1.36

Host	Season	No. of infected fish	No. of non-infected fish	P- value (using chi square test)
H. fossilis	Summer	56	60	$3.39x10^{-09}$**
	Rainy	50	59	
	Winter	130	36	

*P= Prevalence, I= Intensity, SD= Standard Deviation.

In *H. fossilis* prevalence of female fish were highest in all the three seasons, summer (55.22%), rainy (50.75%) and winter 85.57% than their male counterparts 38.76%, 38.09% and 68.12% respectively. The intensity of female fish was also recorded highest in all three seasons- summer (5.24 ± 1.43), rainy (4.79 ± 1.38) and winter (5.35 ± 1.36) than the male fishes 3.89 ± 1.44, 4.56 ± 1.39 and 4.85 ± 1.42 respectively (Table 4).

This could be due to certain ecological factors deriving probably from different feeding habitat in different sexes. Remarkably, Emere and Egbe[32] reported that due to the physiological state of the female, most gravid females could have reduced resistance to infection by parasites. In addition, their increased rate of food intake to meet their physiological requirements for the development of their egg might have exposed them to more contact with the parasites, which subsequently increased their chance of being infected. This was found consistent with the findings of Emere[33], who reported differences in the incidence of infestation between different sexes of host fish, which may be due to differential feeding either by quantity or quality, or as a result of different degrees of resistance to infection.

In the study, the female fish had the greatest rate of protozoan parasite infestations than their male counterparts in all seasons. It can be compared with the result stated by Chhanda and Chandra[34] that in *C. batrachus* the prevalence of female fish was 100% in both the winter and summer and mean intensity of males of *C. batrachus* was observed higher in summer [34]. Seasonal distribution of the parasites found in *C. batrachus* indicated that there were significant differences ($p<0.05$) in infection between the two sexes[34].

The results of the present study corroborated with the works of Pennycuick[35] and Ahmed[36] which reported that seasonal distribution of parasites may be related to the fluctuation in temperature, presence of intermediate hosts and feeding habits of the hosts[35-36]. However, according to Singh and Mishra[37], the prevalence, dominance, mean intensity and abundance for parasites were found minimum in *H. fossilis* in winter season[37]. That percentage may differ due to poor water quality, health management, irregular feeding practices or stressful conditions. During winter, the fishes lose their appetite, being in stressed condition and therefore are vulnerable to different parasitological infections[37].

Acknowledgement

The present work was financially supported by 'Ministry of Education' through 'Bangladesh Bureau of Educational Information and Statistics'(BANBEIS).

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(Manuscript received on 05 June, 2022; accepted on 05 September, 2022)