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Prevalence of Ectoparasitic Infestation in Indian Major Carps During Winter at Different Blocks of South 24-Parganas District, West Bengal, India

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ABSTRACT

The study was carried out to find the prevalence of ectoparasites in carp species specifically Indian Major Carps (IMC) during the post monsoon season (November'16 to March'17). Four groups of ectoparasites viz. myxozoan, ciliophoran, monogenean and crustacean were recorded from a total 500 number of carp species like, *Labeo rohita*, *Catla catla*, *Cirrhinus mrigala* and *Labeo calbasu* collected from different ponds of selected blocks of South 24-Parganas district of West Bengal. The highest prevalence (64.8%) of infestation had been recorded by Myxozoans and the lowest was by Monogeneans (4.8%). The highest and lowest ectoparasitic prevalence in carp was observed in *L. rohita* (32.9%) and *C. catla* (27.3%). Beside these, lower temperature (Average 19.3°C), low pH (Average 6.9) and marginal level of dissolved oxygen (Average 6.0ppm) were also created an unfavorable condition for parasitic infestation during this season. At the end of this experiment it was concluded that disease occurrences due to ectoparasites was high in winter with some key factors like temperature, pH and dissolved oxygen (DO).

1. Introduction

Disease is now a primary constraint for culture of many aquatic species which creates a negative effect both in economic and social development^[1]. The increase in production of culture system, increases the potentiality of disease out-break. Other than marketing concern, the biggest challenges that were faced by the fish farmers; to control many biotic and abiotic factors, which

influence fish rearing and aquaculture operations. It is well known that, the entire water area of West Bengal supports the potential fish farming compared to the other states of this country; and this high production rate in West Bengal was always lead by South 24-Parganas district till date. Freshwater aquaculture depends mainly on carp culture practices that account for around 80% of the total inland fish production according to Sanyal *et. al.* (2016)^[1]. This district was attributed as a potential source of Carp farm-

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ing. It was only with the three main Indian Major Carps viz., Catla (*Catla catla*), Rohu (*Labeo rohita*) and Mrigal (*Cirrhinus mrigala*) that contributes a lion's share in total fish production of West Bengal.

The parasitic community of fishes, show considerable variation with the environmental conditions in which fish live^[2]. Hence it is assumed that fluctuation of environment has been attributed to many disease outbreaks^[3,4]. Certain environmental conditions are more conducive to disease among which water temperature is one of the important criteria associated with disease outbreak. It was observed that the prevalence of the disease was more in the winter season^[5] than the other months of the year. The physiological and biological features of the host, affect the composition of parasite^[6]. Fish parasites cause the significant loss to wild and cultured IMCs. Large-scale mortality of IMCs often occurs in ponds and tanks due to stocking and environmental stress, followed by parasitic afflictions. Heavily ecto-parasitic or endo-parasitic infested fishes, showed interruption in normal growth and development. Mainly these parasites, feed either from the digested content of the host's intestine or the host's own tissue^[7]. Parasites cause deterioration in the food value of affected fishes and may even result in their mortality. It not only disturbed the supply of protein but also brings about a bad impact on our socio- economic condition^[8]. It was already said that there was a direct relation between disease outbreak among fishes and environmental factors. Low pH, lower temperature reduces the buffer capacity of water and that badly affects the pond ecosystem. Furthermore, the incidence of infection is accentuated by the low dissolved O₂ and relatively high CO₂ value in shallow waters, affecting the usual relationship between the invading parasites and the fish^[9], which in turn causes stress to the fish and that leads to fishes more susceptible to diseases and parasites. As, it's already mentioned that the parasitic prevalence was more in the winter season^[5] than the other seasons of the year. This study was conducted with an intention to prove this and so the field work and experiments were planned about the ectoparasitic infestation of IMC during the winter season in the selected blocks of South 24-Parganas with some key factors like temperature, pH and DO.

2. Methodology

2.1 Fish species and Study Area

Study was carried out on Catla (*Catla catla*), Rohu (*Labeo rohita*), Mrigal (*Cirrhinus mrigala*) and Kalbose (*Labeo calbasu*). The five blocks that were chosen - Joynagar-II [22°10'28.68"(N), 88°27'07.06"(E)], Budgebudge-II [22°27'54"(N), 88°10'06"(E)], Canning-I [22°20'46.05"

(N), 88°40'16.94"(E)], Bhangore-I [22°30'02"(N), 88°29'03.9"(E)] and Sonarpur [22°26'33.48"(N), 88°33'47.92"(E)] of South 24-Parganas District, West Bengal, as per the total production and IMC production rate, availability of culture area.

2.2 Sample Collection

From the selected five Blocks, 125 numbers of each species were collected from November'2016 to March'2017. A total of 500 species of Catla (*Catla catla*), Rohu (*Labeo rohita*), Mrigal (*Cirrhinus mrigala*) and Kalbose (*Labeo calbasu*) were screened for the experiment of juvenile stage (Average 250 – 400 gm weight). The samples were collected once in every month on a regular basis from every selected farm. The methods for collection and preservation of the samples for parasitic examination were followed as described by Soota, 1980^[10]. Live host or freshly dead specimen were randomly sampled and collected. The fishes were examined immediately after collection. Prior to collect the affected fish samples, its behavior and clinical signs were recorded.

2.3 Parasitic Study

The length and body weight of the fishes along with date and site of collection of specimens were recorded. The gills, fins, scales and operculum were removed with least damage and placed on separate Petri-dishes containing distilled water and examined. Each of the four gills of both sides was examined separately. The gills and body surface were checked thoroughly for any attached parasites. The dorsal, pectoral, pelvic, anal and caudal fins were placed in separate petri-dishes. Each fin was thoroughly examined; scales of each side were scrapped out along with the mucus and taken separately for examination. Microscopic examinations were done & photomicrographs of ectoparasites were taken using Olympus microscope (model no. BX51, made of Japan) with in-built digital camera (top view version 3.5). The gill, body, and tail fin smear were prepared on grease free clean slides with a drop of 0.85% NaCl solution and air dried. The India ink method^[11] were followed to identify the myxozoan spores and for permanent slide preparation; the air-dried smears were stained with Giemsa stain. The Ciliophoran parasites were subjected to silver impregnation following the method of Klein (1958)^[12]. The Monogeneans were removed on to clear slides with a fine niddle and kept in a drop of water and covered with cover slip. They were fixed in glycerol alcohol (90 parts of 70% ethyl alcohol and 10 parts of glycerol), stained in Borax carmine and finally mounted in glycerine jelly. Phenotypic characterization of all Proto-

zoans, Monogeneans, Digeneans, and Nematode parasites were studied as described by Soulsby (1982)^[13].

2.4 Prevalence Study

The Parasitic prevalence was estimated with the aid of Parasitic Frequency Index (PFI) which 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 was estimated following the formulae proposed by Margolis *et. al.* (1982)^[14].

$$\text{Prevalence (\%)} = \frac{\text{Total number of infected fishes}}{\text{Total number of fish host examined}} \times 100$$

According to Srivastava'1980^[15], the frequency index were further classified into rare (0.1-9.9%), occasional (10-29.9%), common (30-69.9%) and abundant (70-100%).

Determination the Severity of infection was characterized for assigning numerical qualitative value to severity grade of infections, surface infestations and disease syndrome severity, through the following scale by Lightner'1993^[16]:

Table 1. The Scale by Lightner'1993

Disease Syndrome Severity	Remarks
0.5	Non infective
1	Mild
2	Moderate
3	Infective
4	Excessive

2.5 Study of Water Quality

The three main water quality parameters (*viz.*, water temperature, pH and dissolved oxygen) which are related to fish health were measured as prescribed by Kumar *et. al.*'2010^[17], of each sampling ponds during the whole study period. All parameters were checked during day time, water temperature was measured by mercury thermometer, pH was measured by Pen pH meter and DO was measured by NICE Water Testing Kit (For the estimation of DO).

3. Results

Four groups of ectoparasites were identified, *viz.*, myxosporeans, ciliophorans, monogeneans and crustaceans (Fig. 1). Among the Myxozoans group, *Myxobolus* sp. scored highest as per Parasitic Frequency Index (PFI) (Table 2). A

dominating prevalence pattern was observed which represented "abundant" (Table 2) for *Myxobolus* sp. According to frequency index classification by Srivastava'1993^[15], it was found throughout the experimental season, November, 2016 to March, 2017. Prevalence of myxoboliosis were seen highest (Table 3) in *Labeo rohita* (PFI, 78.2%) among the IMCs while it has shown lowest prevalence in *Catla catla* (PFI, 68.8%). The Block Bhangore-I (Table 5) showed highest prevalence (PFI, 75.5%), while Canning-I showed lowest prevalence (PFI 67.3%).

Prevalence of *Thelohanellus* sp. (57.2 %) kept "common" trend throughout the experimental period (Table 2). This myxozoan was abundant in *Labeo rohita* (PFI 61.2%, Table 3) and lowest prevalence in *Catla catla* (55.0%). In the block wise experimental data (Table 5), *Thelohanellus* sp. showed highest prevalence in Bhangore-I (PFI, 58.8%) and lowest in Canning-I (PFI, 56.2%). The average PFI percentage of myxozoan infection throughout the experimental period was 64.8%, which was abundant according to Srivastava'1993^[15] (Table 2).

Throughout the study, only two ciliophoran specimen were found i.e. *Trichodina* sp. and *Ichthyophthirius* sp. and among these, *Trichodina* sp. were found to be more common (PFI 28.5%) while *Ichthyophthirius* sp. were rare in appearance (PFI 5.1%) throughout the experimental season (Table 2). *Trichodina* sp. were more abundant in Bhangore-I (PFI, 62.3%) and rarest in Sonarpur (PFI 7.3%, Table 5); *Ichthyophthirius* sp. were also rare in Bhangore-I (PFI 9.8%) and minimum in Canning-I, (PFI 4.1%, Table 5). As per affected fish species prevalence report (Table 3), between these two ciliophorans, *Trichodina* sp. was more common in *Labeo rohita* (PFI 30.2%) and minimum in *Labeo calbasu* (PFI 26.4%). On the other hand *Ichthyophthirius* sp. were absent in *C. catla* and it showed highest prevalence in *L. rohita* (PFI 8.1%) and lowest in *L. calbasu* (PFI, 4.6%). *Trichodina* sp. and *Ichthyophthirius* sp. were absent in Budgebudge II and Jaynagar II respectively.

In whole experimental period (November, 2016 to March, 2017), monogeneans showed rare occurrence (Table 2) according to Srivastava'1993^[15]. The average prevalence of Monogeneans was 4.8%. Among the Monogeneans only *Dactylogyrus* sp. (PFI 7.5 %) and *Gyrodactylus* sp. (PFI 2.1%) were observed and they were referred rare as per parasitic prevalence report. *Dactylogyrus* sp. showed highest prevalence (Table 5), in Bhangore-I (PFI 12.5%) and low in Jaynagar-II (PFI 7.6%). This parasite was absent in Budgebudge-II. While *Gyrodactylus* sp. was absent in both in Joynagar-II and Budgebudge-II. *Gyrodactylus* sp. showed highest prevalence in Bhangore-I (PFI 5.7%, Table 5) and lowest in Canning-I (PFI 2.2%). In our study

it was seen that both of these Monogeneans showed highest prevalence in *Labeo calbasu* (PFI for *Dactylogyrus* sp. and *Gyrodactylus* sp. were 8.7% and 3.2% respectively, Table 3) and lowest in *Catla catla*. Prevalences were 7.9% & 2.3% in *Dactylogyrus* sp, and *Gyrodactylus* sp. for *Cirrhinus mrigala* respectively (Table 3).

Beside the monogeneans, the crustaceans like *Argulus* sp., *Lernaea* sp. and *Ergasilus* sp. confirmed their prevalence (Table 2). *Ergasilus* sp. (PFI 10.2%) just reached within the boundary of occasional while *Argulus* sp. (PFI 49.9%) and *Lernaea* sp. (PFI 31.3%) were common. The average PFI value of crustacean ectoparasites was 30.4%, which denote that in winter season crustacean parasitic prevalence was common (Table 2). *Ergasilus* sp. showed highest prevalence in *L. calbasu* (18.2%, Table 3) which was occasional and *Argulus* sp. showed highest prevalence in *L.rohita* (53.8%) which was common according to frequency index by Srivastava'1993^[15]. In the surveillance report it was observed that Bhangore-I indicated highest prevalence result for *Ergasilus* sp. (PFI 18.9%, Table 5), *Lernaea* sp. (PFI 43.6%), and *Argulus* sp. (PFI 79.2%). These crustaceans parasitic diseases were completely absent in Canning-I and except argulosis, the other

two types were absent in Jaynagar-II (Table 5).

Severity grade of infections was calculated according to Lightner'1993^[16]. In myxozoans, it was observed that severity of infection was 'moderate' in both *Myxobolus* sp. and *Thelohanellus* sp. following Lightner'1993^[16]. In case of ciliophorans (*Trichodina* sp. and *Ichthyophthirius* sp.) and monogeneans (*Dactylogyrus* sp. and *Gyrodactylus* sp.), both were showed as 'non-infective'. Among crustaceans, *Ergasilus* sp., *Argulus* sp. and *Lernaea* sp. were illustrated as 'non-infective', 'moderate' and 'mild' respectively.

Statistical analysis (Table 4) by two way ANOVA revealed that there was significant differences ($P < 0.05$, $df=8$) in PFI (%) values of different fish species in relation to different parasites. Similarly there was significant differences ($P < 0.05$, $df=3$) in PFI (%) among the parasites in relation to different fish species. Statistical analysis (Table 6) by two way ANOVA also revealed that there was significant differences ($P < 0.05$, $df=8$) in PFI (%) values of parasites in relation to different places. Similarly there was significant differences ($P < 0.05$, $df=4$) in PFI (%) values of different places in relation to different parasites.

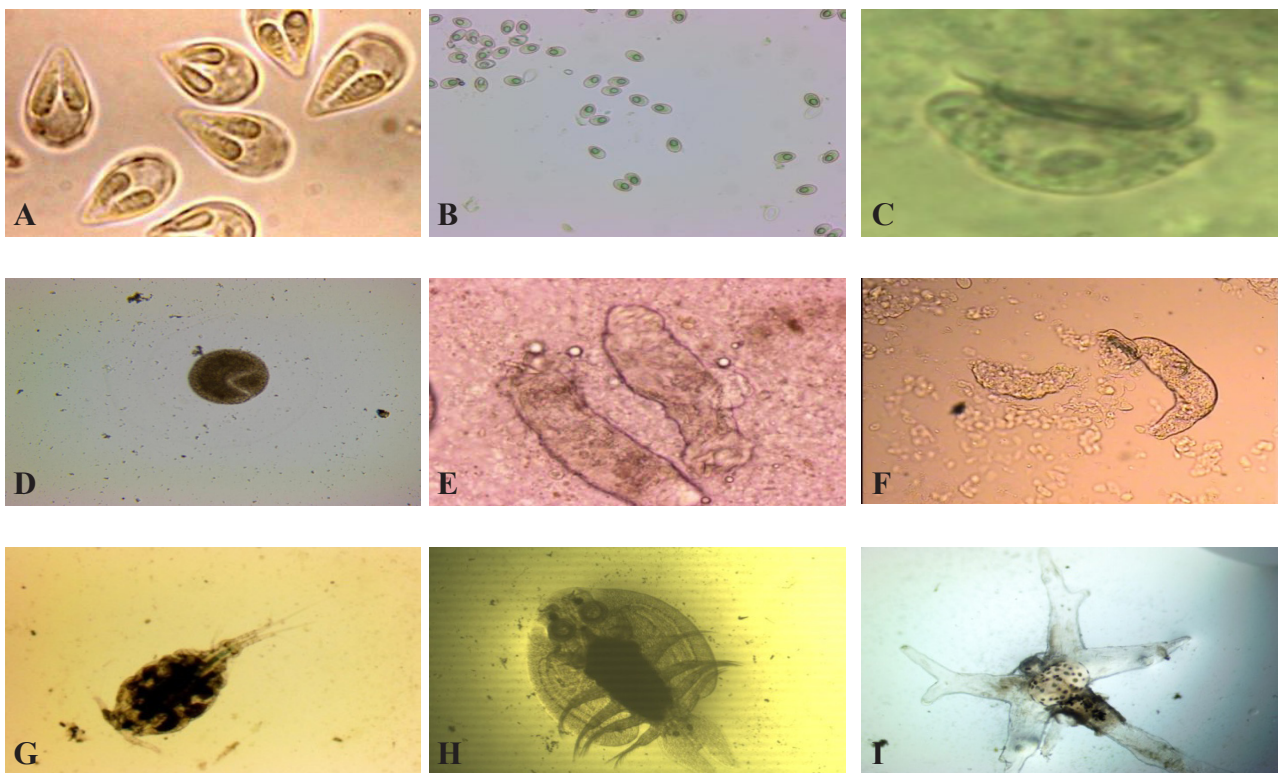


Figure 1. Identified Ectoparasites—myxosporeans, ciliophorans, monogeneans and crustaceans; A) *Myxobolus* sp., B) *Thelohanellus* sp., C) *Trichodina* sp., D) *Ichthyophthirius* sp., E) *Dactylogyrus* sp., F) *Gyrodactylus* sp., G) *Ergasilus* sp. (Copepod stage), H) *Argulus* sp., I) *Lernaea* sp.

Table 2. Prevalence & severity of infection for individual ectoparasites in IMC species from Nov.'16 to Mar'17

Myxozoans	PFI (%)	Severity of infection	Ciliophorans	PFI (%)	Severity of infection	Monogeneans	PFI (%)	Severity of infection	Crustacean	PFI (%)	Severity of infection
<i>Myxobolus</i> sp.	72.3 ± 11.3	2	<i>Trichodina</i> sp.	28.5 ± 15.4	0.5	<i>Dactylogyrus</i> sp.	7.5 ± 2.1	0.5	<i>Ergasilus</i> sp.	10.2 ± 29.9	0.5
<i>Thelohanellus</i> sp.	57.2 ± 6.1	2	<i>Ichthyophthirius</i> Sp.	5.1 ± 21.8	0.5	<i>Gyrodactylus</i> sp.	2.1 ± 2.6	0.5	<i>Argulus</i> sp.	49.9 ± 7.3	2
									<i>Lernaea</i> sp.	31.3 ± 22.6	1
Average PFI	64.8			16.8			4.8			30.4	

Table 3. The average (%) of PFI of IMC from Nov'16 to March'17

Name of the Parasitic Groups		Name of the affected Indian Major Carp							
		<i>Labeo rohita</i>		<i>Labeo calbasu</i>		<i>Catla catla</i>		<i>Cirrhilus mrigala</i>	
		PFI%	Site of infection	PFI%	Site of infection	PFI%	Site of infection	PFI%	Site of infection
Myxosporean	<i>Myxobolus</i> spp.	78.2	Gill, Fins	71.8	Gill, Fins	68.8	Fins	70.3	Gill
	<i>Thelohanellus</i> spp.	61.2	Gill, Fins	56.2	Gill	55.0	Fins	56.4	Fins
Ciliophorans	<i>Trichodina</i> spp.	30.2	Body, Gill	26.4	Body, Gill	27.5	Gill	29.7	Body, Gill
	<i>Ichthyophthirius</i> spp.	8.1	Body, Gill	4.6	Body	0.0	-	7.4	Body, Gill
Monogeneans	<i>Dactylogyrus</i> spp.	8.1	Gill	8.7	Gill	5.3	Gill	7.9	Gill
	<i>Gyrodactylus</i> spp.	2.9	Body, Fins	3.2	Body, Fins	0.0	Body	2.3	Body
Crustaceans	<i>Ergasilus</i> spp.	18.1	Gill	18.2	Gill	11.5	Gill	17.0	Gill
	<i>Argulus</i> spp.	53.8	Body, Operculum, Fins	48.9	Body	46.9	Body, base of the Pectoral and Dorsal Fins	49.8	Body, Operculum, Fins
	<i>Lernaea</i> spp.	35.4	Body, in some times Anal part of body.	31.3	Body	30.5	Body	28.0	Body, in some times base of the pectoral fins.
Avg. % PFI		32.9		29.9		27.3		29.9	Avg. % of Total PFI 30.0

Table 4. Two way ANOVA of PFI (%) values for Parasites in relation to different fishes from Nov'16 to March'17

Source of Variation	SS	df	MS	F	P-value	F crit
Parasites	20093.41222	8	2511.676528	758.6979317	6.27E-27	2.355081
Fish	141.9852778	3	47.32842593	14.29641853	1.5E-05	3.008787
Error	79.45222222	24	3.310509259			
Total	20314.84972	35				

Table 5. The average percentage of PFI, in different selected Blocks of South 24-Parganas from Nov'16 to March'17

Name of Parasite	Block Name	Joynagar-II	Budgebudge-II	Bhangore-I	Canning-I	Sonarpur
	Myxosporeans	<i>Myxobolus</i> sp.	73.8	71.8	75.5	67.3
<i>Thelohanellus</i> sp.		56.9	57.3	58.8	56.2	56.9
Ciliophorans	<i>Trichodina</i> sp.	27.9	0.0	62.3	16.7	7.3
	<i>Ichthyophthirius</i> sp.	0.0	5.2	9.8	4.1	5.4
Monogeneans	<i>Dactylogyrus</i> sp.	7.6	0.0	12.5	9.3	8.6
	<i>Gyrodactylus</i> sp.	0.0	0.0	5.7	2.2	2.8
Crustaceans	<i>Ergasilus</i> sp.	0.0	8.3	18.9	0.0	3.6
	<i>Argulus</i> sp.	16.4	31.4	79.2	0.0	72.2
	<i>Lernaea</i> sp.	0.0	16.2	43.6	0.0	33.8
Avg. % of PFI		20.3	21.1	40.7	17.3	29.3
						Avg. of Total 25.7

Table 6. Two way ANOVA of PFI (%) values for Parasites in relation to different places from Nov'16 to March'17

Source of Variation	SS	df	MS	F	P-value	F crit
Parasites	25604.4404	8	3200.55506	17.174949	1.42E-09	2.244396
Places	3226.82578	4	806.706444	4.328981	0.006536	2.668437
Error	5963.20622	32	186.350194			
Total	34794.4724	44				

The three main water quality parameters, water temperature, pH and dissolved oxygen which are related to fish health were measured of each sampling ponds during the whole study period. All parameters were checked during day time. The temperature was almost same in all the places (average 19.3°C). DO was 6.5 ppm in Bhangore-I which was highest other than four blocks; however, average was 5.96ppm which was marginal. The pH was also low (average 6.9) which also badly affects the pond ecosystem.

Table 7. Average water quality parameter of selected different Blocks from Nov'16 to March'17

Block Name Parameter	Joynagar-II	Budgebudge-II	Bhangore-I	Canning-I	Sonarpur	Avg. of Parameters
Temperature (C)	19.2	19.3	19.3	19.2	19.2	19.3
Dissolve Oxygen (ppm)	5.9	5.2	6.5	5.8	6.3	6
pH	6.9	7.1	6.5	7.3	6.6	6.9

4. Discussion

The influence of parasites in relation to the seasons had been described by many researchers. The occurrence of Myxozoans, Ciliophorans, and Crustacean were found more or less in all seasons, but it reached peak during the winter and spring season^[18,19], because in this season fishes become more susceptible to diseases due to low immunity power for the sudden change in water temperature. This study is in agreement with the reference cited above. Maximum disease prevalence was found in *L. rohita*, because of its low immunity power than other carp species. *L. rohita* made a gap with other carp species (Table 3), while *C. catla* remained in the bottom end. These results were similar with the works of others^[18,20], who worked on disease occurrence as per seasonal variations. It can be mentioned that, according to the average PFI estimation of all aggregating data of affected carp specimen (30.0%, Table 3) and selected block wise prevalence result (25.7%, Table 5), the prevalence can be attributed as “occasional” or tends to “common” in the post monsoon season. In this regard it can be said that in winter season as fish species became weak due to temperature drop, it made them more susceptible to disease.

It was observed that the highest prevalence of parasites were in December and January, this study was done following Basu and Haldar, (2004)^[21], when the ambient temperature was below 25°C^[18]. The result was supported strongly by this reference where throughout the experimental period, the average temperature, pH and DO were

19.3°C, 6.9 & 6 respectively. The suitable temperature for development throughout their life cycle and reproduction were estimated 24-28°C,^[22] which was not in agreement with the present observations. It was assumed that poor water quality, DO, low pH and low temperature were the key factors for invention of this ectoparasite.

In this study it was observed that each sampling aquatic water bodies showed average pH value 6.9 (Table 7) which is lower than the acceptable limit for aquaculture pond. Average highest & lowest pH value were 7.3 (Canning I) and 6.5 (Bhangore I). It was noted that Canning-I indicated low disease prevalence than other districts (Table 5). So, it could be concluded that this low pH value beside the low temperature were one more major factor for bringing several parasitic diseases^[18].

There were several reasons behind the low pH value in our selected aquatic water bodies; like high stocking density, algal bloom, aquatic weed; besides these biological phenomenons other important observations were connection of community drain line with aquaculture ponds, unwanted human interventions in the pond which produces decomposition, degrading animals and their parts were also present in some ponds and these were the main causes regarding aquatic pollution. These affected the average DO level which was at 6 ppm (Table 5) and that was lower than the marginal limit for aquaculture pond.

According to the surveillance report, the prevalence of the parasites reached comparatively high in the month of December and comparatively low in the month of March. It may be due to organic load in the culture ponds which

induces bio-ecological stress and made fish more susceptible to this parasitic infection. It is evident from the available literature that parasitic diseases caused significant damage not only in stocking system but also caused damage in nursery and rearing systems of carp, catfish and others^[23]. Beside these poor environmental condition, malnutrition was observed in several blocks specially Sonarpur & Canning-I. Most of the water bodies that were affected by the parasitic diseases were having high organic load as well as high stocking density and poor quality of aquatic environment.

5. Conclusion

To draw a conclusion from this study, this can be said that the post monsoon season, i.e. winter, along with the lower temperature, low pH, marginal level of DO harness to create a favorable environment for the ectoparasitic infestations, specially myxosporeans and *Argulus* sp. Due to this poor aquatic environmental temperature, fish reduces metabolic activities, which in turn also made the fishes more susceptible during the winter period towards parasitic infestations. More in depth research is needed to be carried out for studying on parasites diseases of fishes and other biotic factors.

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