Survey Studies for the Prevalence of Parasites from Commercially Edible Fishes in West Bengal, India

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Abstract

Indian major carps (IMCs) are one of the most economically important as well as edible food fishes all over the world. IMCs are showing different disease problems that cause harm for healthy fishes. The present study was carried out from 12 months of survey study between June 2021 to May 2022 for investigations of different causative fish parasites. In this section, our area of concerning fishes were cyprinidae family fishes the ectoparasites infect them which belong to groups of myxozoan, and protozoan fish parasites that lead to cause severe damage and finally, mass mortality has been observed. In Labeo catla (Hamilton, 1822) showing highest Myxobolus infection was observed in the rainy season (65.55%), and Thelohanellus infection was abundant in winter (65.83%). Another way, L. rohita showed a massive Myxobolus infection rate in January (56.66%), Thelohanellus and Trichodina infections highest in April (63.33% and 53.33%). In this article, the prevalence of parasitic studies has been dealt with. As per seasonal diversity, host age, size of the hosts and sex-wise prevalence of parasitic infection over the fish sectors. Here, parasitic infections were shown at different time scales which are stated in this research article. As per the sex wise study, the highest infection was observed in females as compared to male fishes. The large sized fishes were highly prone to parasitic diseases as subjected to small young fishes.

Keywords: Economical; Survey study; Ectoparasite; and Prevalence.

INTRODUCTION

quaculture is a very highly produced sector around the world. It is a vast way

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for economical as well as eco-friendly medium. This helps every fish farmer for their wealth being purposed. Fishes are food products which contain several minerals, which have good nutrient values and several people intakes around the world. Nowadays, fish are infected with several deadly causative pathogens like parasites, bacteria, fungi and viruses. These are caused by major diseases on fish farms. The limitations in are parasitic illnesses in aquaculture because of increased fish density in the lentic areas of water where the fish diseases can spread quickly from one host to another (Sinha, 2018). They interfere with fish growth and decrease immune systems *i.e.*, they damage fish immune profile and as a result, fishes become died.

Parasites are abundant and various, which helps

to contribute to the natural communities (Kuris et al., 2008). Parasites intake their feed from hosts and due to some extension, they give a potentially negative impression on the infected organisms, it creates dynamicity in the population chain, biodiversity, community structure and food web connectivity (Marcogliese, 2003; Wood et al., 2007; Johnson et al., 2008). Parasites play a key role in ecosystems by interfering with the abundance and density of fish populations and food web establishment (Acosta et al., 2020). So, the population diversity of parasites is often used as an indicator to study species richness and biodiversity, as well as the dynamics of fish populations (Levy et al., 2019; Marcogliese, 2002). Several fish parasites having complex life cycles may contain the definitive fish host and one or more intermediate invertebrate hosts (Juntaban et al, 2021). Myxozoans are diverse and abundant cnidarian parasites, they are widely studied for development of the infections (Abdel-Ghaffar et al., 2005; Székely and Molnár, 1999). Myxosporeans are common parasites of fishes around the world (Lom and Dyková, 1994), which creates major damage to the economically important fresh and marine water fish species (Kaur and Attri, 2015). The genera Myxobolus (Bütschli, 1882), and Thelohanellus (Kudoa, 1933) are among the most studied myxozoan fish parasites. The myxozoan parasite samples are very important, they contain 67 genera and more than 2600 species (Morris, 2010).

This study observed the primary infection in freshwater fishes, which contain parasites. Parasitic infection is the entry point of other secondary infections (e.g., bacteria and fungi). Parasitic infections are predisposed season wise and are generally affected by the physiology and ecology of the host fishes. Therefore, appropriate health status is useful for controlling actions elicited in aquaculture invention. West Bengal is a "rice fish culture". This state is enormously important historically as well as geographically for a long past.

The state of West Bengal has been played centre of attraction for a high amount of freshwater fish production and also has the distinction of a wide range of water areas under traditional shrimp farming. The disease is the only factor that creates constraints in the aquaculture industry, inhibiting economic success in this sector. The objectives of this study were to isolate, and identify diverse parasites from IMCs, and to find out the "Parasitic Frequency Index" (PFI) in the status of months and seasons from selected several water bodies districts of West Bengal. In this study, we aim to

identify their prevalence in different time scales. In future prospects, we will focus on the prevention measures pathway.

MATERIALS AND METHOD

2.1 Study location

The study was performed in the district of North 24 Parganas (Monirampore, Barrackpore, Icchapur and Titagarh) area of West Bengal, India, which has several export farms of IMCs. Among them, several of these exporters partially rely on small-scale farms in nearby rural areas to maintain the supply of IMCs, which helps to reduce costs. Around 4500-5000, fish were produced monthly in freshwater farms.

2.2 Experimental setup

A total of 320 fish were collected from four differently situated IMCs farms in the district of North 24 Parganas (Monirampore, Barrackpore, Icchapur and Titagarh) of West Bengal, India was visited during the period from June 2021 to May 2022. At each farm, the water quality parameters were measured. 320 fishes were collected in diverse fish farms, and average body weight (26 \pm 2.4 g) and length (15 \pm 0.5 cm) were respectively collected for the study. In polyethene bags filled with oxygenated pond water, all fish were transported to the lab for additional examination. After reaching the working station, specimens were stocked in a 5000 litre cultured tank with a submersible water filter system (SOBO, Aquarium Internal Filter WP 1000F) for pumping, aeration, and filtering water continuously and commercially available fed was given 2% of their body weight.

2.3 Collection and processing of specimens

In this current study, we observed the prevalence of parasitic infestation in freshwater IMCs, and it was carried out over 12 months of study between June 2021 to May 2022. The live fish specimens were collected from different waterbodies of Monirampore (n=240; 80 no. each in the month of June, among them male 36, and female 44; 80 no. each in the month of October, among them male 38, and female 42; 80 no. each in the month of February, among them male 40, and female 40), Barrackpore (n=240; 80 no. each in the month of July, among them male 34, and female 46; 80 no. each in the month of November, among them male 38, and female 42; 80 no. each in the month of March, among

them male 36, and female 44), Icchapur (n=240; 80 no. each in the month of August, among them male 38, and female 42; 80 no. each in the month of December, among them male 40, and female 40; 80 no. each in the month of April, among them male 34, and female 46), and titagarh (n=240, 80 no. each in the month of the September, among them male 40, and female 40; 80 no. each in the month of January, among them male 34, and female 46; and 80 no. each in the month of May, among them male 38, and female 42) districts of North 24 Parganas, West Bengal, India. These different sampling sites were selected for concerning purposes. The live specimens were collected regularly once every month and they were collected in live conditions. The fish were brought to the laboratory in live condition with water filled containers and the length and weight of the fish were measured. A total of 44 fish farms were facing problems of fish being lethargic and showing excessive mortality. Fish are intake 1% body weight commercial fed every day, which is available in the market. Each pond's size is around 1.25 bighas.

2.4 Sampling for parasitic study

First, the live fish specimens were put to anaesthetize with a dose of sodium bicarbonate buffered MS-222 (NaHCO3) (Tricaine methanesulfonate, Argent laboratories; 30 mg/l). The skins and gills were thoroughly examined for the presence of different parasites. The collection and preservation methods were opined by Soota (1980). The site of collection and date were noted for future prospects. The live fishes were screened for the presence of parasites within 12 hours. An external sign and health status were well documented. With special interest skin and gills were gently removed at least damaged and kept on separate petri plates containing physiological saline (0.65%) water and thoroughly examined. Each pair of gills were separated and checked thoroughly for the presence of any kind of infection. Morphological characters were well described by Soulsby (1982). Photographs were taken using Magnüs MLX PLUS (India) with an in-built digital camera (Fig. 1).

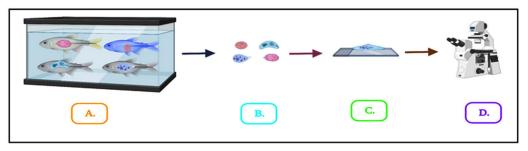


Fig. 1: In this schematic diagram, fishes were infected with different parasites in an aquaculture tank (A), isolation the parasites were gently dissected (B), the fresh smear was made grease free slide with the help of water (C) and finally observed under microscope at different magnifications (D).

2.5 Sex identification of fishes

Fish were tested physically for sex, and the urogenital papillae were used as a test. In addition, the ovaries in females and the gonads in men are visible.

2.6 Determination of Parasite Frequency Index (PFI) and Prevalence Study

The Parasite Frequency Index (PFI) was determined by using 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 (Vijaysundardeva *et al.*, 2018).

The prevalence rate of parasite infection was measured using the model (Amos *et al.*, 2018):

Prevalence (%) =
$$\frac{\text{No. of fish host infected}}{\text{Total no. of host examined}} \times 100$$

The prevalence of parasites infection based on the sex of the fish (Amos *et al.*, 2018):

$$Prevalence (\%) = \frac{No. of a particular sex of fish infected}{Total no. of a particular sex of fish examined} \times 100$$

The model was used to estimate the parasite intensity (Amos *et al.*, 2018):

Intensity =
$$\frac{\text{Total no. of parasite species in a sample of fish examined}}{\text{No. of fish host infected}}$$

The frequency index was clarified into rare (0.1-9.9%), occasional (10-29.9%), common (30-69.9%) and abundant (70-100%) (Vijaysundardeva *et al.*, 2018).

2.7 Statistical analysis

It was statistically established that the infection was sex dependent using chi-square analysis.

RESULTS

3.1 Infection of host fishes

Screening of *Labeo rohita* showed infections (e.g., *Myxobolus*, *Thelohanellus* and *Gyrodactylus*) and *L. catla* (e.g., *Myxobolus* and *Thelohanellus*) were observed (Table. 1).

3.3 Month wise parasitic prevalence in Labeo catla (Hamilton, 1822)

The month wise diversity of parasites in *Labeo catla* is given below. The occurrence of parasitic fauna like *Myxobolus* sp. (Fig. 2A) and *Thelohanellus* sp. (Fig. 2B) were obtained from *L. catla* in this current study. The Parasitic Frequency Index (PFI) of *Myxobolus* sp. was found highest in "July" (73.33%) opined as "Abundant" and the lowest prevalence was found in February (3.33%) indicated as "Rare". These findings are fully satisfied with the previous study (Das *et al.*, 1989; Seenappa and Manohar,

Table 1: Cyprinidae family fishes showing parasitic infection

Sl. No.	Name of fishes	Myxobolus infection	Thelohanellus infection	Gyrodactylus infection
1.	Labeo rohita (Hamilton, 1822)	+	+	+
2.	L. catla (Hamilton, 1822)	+	+	-
3.	Cirrhinus mrigala (Hamilton, 1822)	-	-	-
4.	L. bata (Hamilton, 1822)	-	-	-
5.	L. calbasu (Hamilton, 1822)	-	-	-

[&]quot;+" = infected and "-" = non-infected

3.2 Identification of Parasites

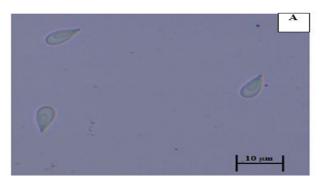
In this current study, different fish skin and gill lesions, such as erosions, ulcerations, haemorrhages, profuse mucus, dullness of the coloring and whitish discoloured areas were documented depending on the strength and method of the parasite attachment, and immunological response of the host. The macroscopic inspection of infected fish also revealed various behavioural abnormalities, including weakness, despair, anorexia, flashing, and an increase in opercular movements.

The microscopic observations of fish that were infected by protozoan *Trichodina* revealed lesions on the skin including increased opercular movement, dull colour, pale spots, and excessive mucus production. *Trichodina mutabilis* which was found in *Poecilia reticulata* (Peter, 1859), was identified using the sticky disk's diameter (60 to 65 m), the denticle ring's diameter (40 to 45 m), and the number of denticles (28 to 30) (Kazubski and Migala 1968). As per microscopical evidence, the presence of polar capsules, and spores indicates that the observed specimen belongs to the class Myxosporean parasites (Lom and Arthur, 1989). Due to this parasitic infection, the gill of fish shows whitish colouration and behavioral abnormalities.

1980; Narasimhamurti and Kalavati, 1984; Basu and Halder, 2003) who have documented that myxozoan parasites were highly prevalent in August to January when the ambient temperature was below 25°C and the lowest prevalence in February (3.33%). The PFI range of *Thelohanellus* sp. suggests that the January (76.66%) month was found to be the "Abundant" condition, and the rare condition was found to be in May, August, September, February and March but a previous study (Kaur et al., 2012) showed that 100% prevalence of infection in May against complete 0% prevalence in this study, and the probable reason will be due to the diverse geographical attributes. The earlier report stated that Thelohanellus sp. highest prevalence was found in February (31.66%) it opined "Common" distribution and the lowest in August (11.66%), and it stated "Occasional" type of distribution. This will be possible in case of high stocking density, depth of the water, temperature fluctuations and several physicochemical parameters which co-relate with the available literature (Banu and Khan, 2004).

3.4 Season-wise occurrence of parasites in Labeo catla (Hamilton, 1822)

The seasons' change is highly influenced by the occurrence of parasites which has been available



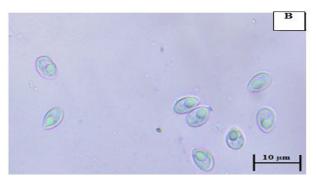


Fig. 2: In these figs., Myxobolus sp. infection was observed in Labeo catla, scale bar 10 micrometre (μm) 2A; and Thelohanellus sp. infestation was observed in Labeo catla, scale bar 10 micrometre (μm) 2B.

in several research literatures (Bhuiyan *et al.*, 2007; Banu *et al.*, 1993; Chandra *et al.*, 1997). The total study period was separated into four seasons, they were-summer (April-June), rainy (July-September), winter (October-January) and spring (February-March).

The infection cycle of *Myxobolus* sp. was remarkably shown as a seasonally constant increase from summer to winter with the highest during rainy (65.55%), and it shows "common" the lowest observation in the season of spring (6.66), and it shows "rare" distribution. The fluctuations of the parasitic prevalence due to seasonal, ecological and physiological conditions play a vital role in the fish (Ahmed *et al.*, 1991; Wisheiwski, 1958). The variety of parasitic fauna determination is involved in the diet and lifespan of the host, large hosts need more habitats appropriate for parasites than do small ones (Polanski, 1961).

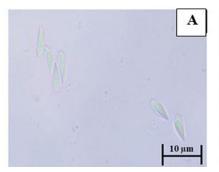
Thelohanellus sp. highly increased its observation in the winter (65.83%) season and it opined "common" type of distribution and in the spring (4.99%) season it shows a "rare" kind of distribution. The presence of the parasite hosts showed parasitic specific nature.

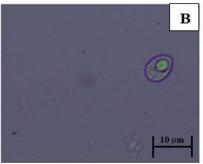
The result suggested that infection site specificity

of the hosts for parasite attachment. The direction of the water force moves towards the respiratory current over the gills.

3.5 Parasitic prevalence in Labeo rohita (Hamilton, 1822)

The majority of parasitic taxa well reported in L. rohita during this current study period are Myxobolus sp. (Fig. 3A), Thelohanellus sp. (Fig. 3B), and Trichodina sp. (Fig. 3C) The PFI values of the isolated parasites showed the abundance values in the host specification and gradually common, occasional and finally rare status is also given as per PFI values. Infection with Myxobolu 6s sp. was documented as a "common" situation in the L. rohita as the PFI values reach their maximum in the month of January (56.66%). The occasion and occurrence of the Thelohanellus sp. were documented lowest in the month of April (63.33%), which was present throughout the year except for September. The establishment of Thelohanellus sp. infection "Common" was observed in the month of April (63.33%). Our findings, corroborated with the available researcher articles (Akther et al., 1997; Hossain et al., 1994; Bhuiyan et al., 2007; Banu et al., 1993; and Chandra et al., 1997) in L. rohita. PFI of Trichodina sp. highest in the month of April (53.33%), which was established as a "Common" condition.





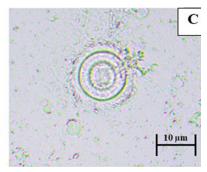


Fig. 3: Here, Myxobolus sp. infection was observed in $Labeo\ rohita$, scale bar 10 micrometre (μm) (3A); and Thelohanellus sp. infestation was observed in $Labeo\ rohita$, scale bar 10 micrometre (μm) (3B); and Trichodina sp. infected was observed in $Labeo\ rohita$ scale bar 10 micrometres (μm) (3C).

3.6 Season-wise occurrence of parasites in Labeo rohita (Hamilton, 1822)

The seasonal prevalence of parasites in *L. rohita* was not diverse. In the case of Myxobolus sp. it shows baseline levels distribution in the all over seasons. The winter and spring seasons show peaks for Myxobolus sp. infection in fish. These results are strongly supported by another research article (Bhuiyan et al., 2007) in L. rohita. The prevalence of Thelohanellus sp. infection in fishes shows peaks. The decreases in water volume led to nutritional disruption which declines food fish production, in another hand fall in water temperature and declines in the immune response caused by more disease-prone fishes, then mortality will be rise. The prevalence peak of Trichodina sp. showed moderate peaks in the spring and summer seasons. This protozoan parasite is observed in the skin of L. rohita because the ectoparasitic in nature and the only protozoan parasite which was found throughout the study times.

In the consideration of sex-wise study, the

compared to males (Figs. 8, 9, 10, and 11), which is fully satisfied with the previous study (Wahab *et al.*, 2021).

3.7 Age-wise study of parasitic variations

In this current study, it is shown that parasite infections are highly correlated with the age of fish. The prevalence of parasites was lower in young fish than in adult fish greater than six months (Fig. 12). These findings were fully satisfied with the previous study (Saha *et al.*, 2015).

3.8 Length-wise infections of parasites

The length of the fish is highly related to parasitic infections. It is revealed that lengthy fishes are highly susceptible to infections as compared to small fishes. Here, we concluded that the smallest fishes are significantly less susceptible to the infection than the other length groups and the length of the fishes are highly important for the study of parasitic study (Saha *et al.*, 2015) (Fig. 13,14).

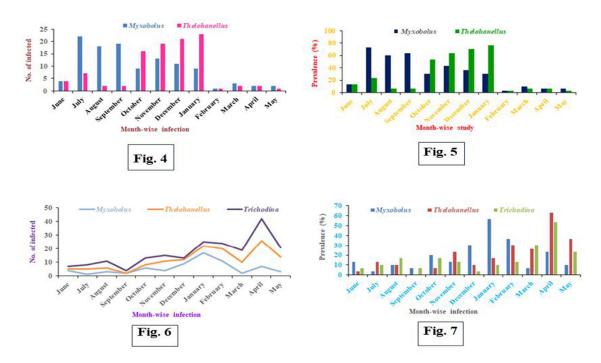
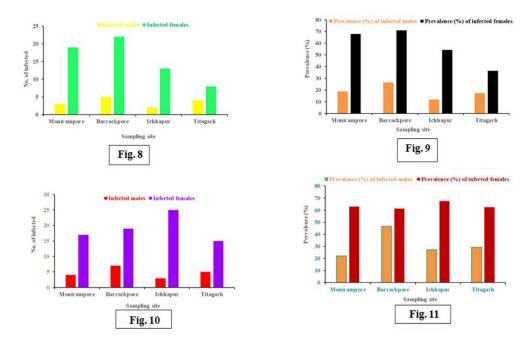


Fig. 4: Shows month-wise infected individuals at different time scales in *Labeo catla*. Here, it showing a number of infections in fish of different months. In comparison with fig. 3, it shows that month-wise parasite infection is highest in the months of April and May. In fig. 2 months of July to January showing the highest number of parasitic observations but in another way **Fig. 5** Showing number of parasitic incidence highest in April and May as compared to other months. **Fig. 6**, Month-wise prevalence (%) rates is shown in *Labeo catla*. Here, the highest peak is observed in the month of July for *Myxobolus* infection. In the month of January showed the highest abundance of *Thelohanellus* infection as compared to other months in *Labeo rohita*. In **Fig. 7** *Myxobolus* infection is highest in January. The month of April shows highest rate of *Thelohanellus* infection and also *Trichodina* showing highest peak in April as compared to other months in *Labeo rohita*.

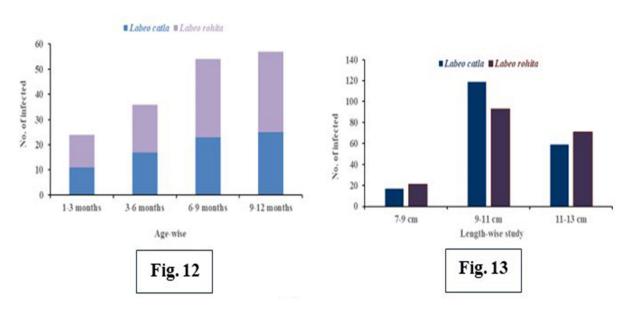


In **Fig. 8**, diseased fish farms of the Barrackpore were the highest female (22) infections observed and lowest female infections were found in Titagarh (8). Highest male fishes' infection is observed in Barrackpore (5) and lowest infection is observed in Ichhapur (2) of *Labeo catla*. sampling site-wise female fishes of *Labeo catla* is showing highest number of parasitic incidences as compared to the male sex.

In **Fig. 9**, female fishes of Ichhapur (25) were observed highest number of infections and lowest number of infections is observed in Titagarh (15). Highest number of infected male fishes is found in Barrackpore (7) and lowest number of male infections is found in Ichhapur (3) of *Labeo rohita*.

In **Fig. 10**, highest prevalence rate of females is observed in Barrackpore (>70%) as compared to other sampling sites and lowest in Titagarh (>30%). In another way, highest prevalence rate of males is observed in Barrackpore (>20) as compared to other sampling sites and lowest is observed in Ichhapur (>11%) of *Labeo catla*.

In **Fig. 11**, highest prevalence rate of female fishes is found in Ichhapur (>65%) and lowest prevalence rate of female fishes 61.29%. Highest prevalence of male fishes is found in Barrackpore (>40%) and lowest observation is found in Monirampore (22.22%) of *L. rohita*.



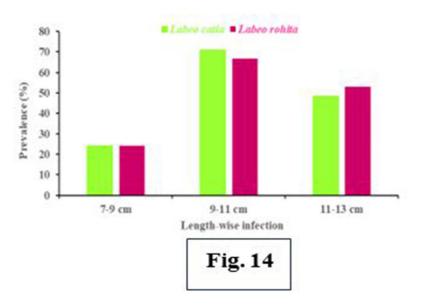


Fig. 12 In this graph comparison study between age-wise in *L. catla* and *L. rohita*. As per this study, the age group between 9-12 months showed the highest number of parasitic infections in both fishes

Fig. 13 In this pictorial presentation, a contrasting study between *L. catla* and *L. rohita* regarding length-wise revealed abundance level of parasitic infections showed 9-11 cm fishes.

Fig. 14 In this figure showed the highest parasitic prevalence is observed between *L. catla* and *L. rohita* in the case of length-wise evaluation, 9-11 cm length fishes showed the highest peaks of prevalence in both fishes.

3.9 Water quality investigation

An overview of the water quality investigations were obtained in the sampled disease fish farms

(Table 2) situated in different districts of West Bengal, India. These findings suggested that the presence of parasites can disturbs water quality.

Table 2. Water quality parameters of different diseased fish farms located in West Bengal, India

Parameter	Monirampore	Barrackpore	Icchapur	Titagarh
рН	8.10	8.08	8.23	8.01
Conductivitya	5.33	5.17	7.91	6.25
TDS	3.44	3.18	5.32	4.67
Alkalinityb	211	177	226	182
Hardnessb	232	169	252	195
Dissolved oxygenb	3.9	4.1	5.2	4.9
Ammoniac	0.446	0.592	0.645	0.539
Total phosphorusc	0.101	0.148	0.145	0.123
Temperature (°C)	27.7	28.1	29.7	29.2

^aunit μs/cm; ^bppm (Parts per million); ^cmg/l.

DISCUSSION

Myxozoan infection is a common parasitic disease of freshwater fishes that belongs to different

taxa (Kaur and Attri, 2015). These infected fishes are unmarketable because of the presence of visible large cysts in the gill lamellae. As per guidelines of food hygiene regulations, those fishes unable for consumption (Betke *et al.*, 2001). Myxosporean

parasites show strong host-specific interactions (Molnár, 1994). Accumulation of toxic chemicals and water eutrophication with algal blooms contribute to poor water quality that creates stress factors and increases fish susceptibility to parasite stimulation (Coutant, 1998). The myxozoan and protozoan fish parasites are found most abundant from January to April when the temperature diverges between 19 to 27 °C. But, changes in months vary in high temperature reducing fish infections. In high temperatures, the life cycle of these parasites becomes hampered and remains in the dormant stage. In this current study, the parasitic prevalence is carried out in a temperature dependent manner and it occurs mainly during the winter season. While in the rainy season parasitic infection was observed in moderate amounts (Hossain et al., 2008), and also environmental queues play a major role in their living purposes. The high prevalence of protozoan parasites can indicate such factors as environmental parameters like seasonality, fish behaviour and handling management in farmed fish (Florindo et al., 2017). Stress conditions are caused by variations in the aquatic ecosystems that create parasitic infections along with host susceptibility and social status correlated with the fish specimens (Alves et al., 20010; Gómez and Morgan, 2003). In the case of protozoan infection where concentrations of ammonia in the water were at higher levels (Florindo et al., 2017). Due to the use of higher crude protein which increased nitrogenous substances in the water bodies (Florindo et al., 2017). The highly diverse nutrients in the water can elicit the proliferation of protozoan parasites (Smallbone et al., 2016).

Another approach the length and age of the fish play an important factor in varying the prevalence of parasitic infection in IMCs. Here, elevated levels of parasitic prevalence graphs are indicated in adults and large fishes as compared to the younger and smaller ones (Saha et al., 2015) (Fig. 12). In this study, adult fishes are highly infested with parasitic infections as compared to young fishes. The age and length of the fishes are provided significant attributes for parasitic infections. Adult fish are having increased metabolic activity due to a high amount of food intake. These findings correlated with other finds (Bashirullah, 1973; Dogiel, 1961). Their findings also supported that, parasitism is associated with the age and food habits of the fish specimens.

The sex-wise study reveals that female fishes are highly infected by parasitic infestations as compared to males. Due to the strong immune systems in males which protect them from parasitic infections as compared to female fishes (Smith, 1969; Satpute and Agarwal, 1974; Sinha and Chakrabarti, 1984; Rajaiah, 1997). The host's sex has an important bearing on the regulation and recurrence of the parasites. The biochemical differences in the quantity and quality of the steroid hormone presumably present in male and female hosts may account for the differences in myxozoan and protozoan parasite infections in male and female hosts. Thus, it can be concluded that the influence of the seasons, host age, sizes, and sex play a significant role in the prevalence of *Myxobolus*, *Thelohanellus* and *Trichodina* sp. parasitic infections in *L. catla* and *L. rohita*.

CONCLUSION

In this current study, the winter season with the low water temperature, high level of dissolved oxygen, moderate pH and low hardness provides favourable environmental queues for ectoparasitic infections like Myxobolus, Thelohanellus and Trichodina sp. Therefore, water quality plays a major role in the increasing rate of pathogens and their capability to survive on the host. So, the concentration of fish seeds and water quality paradigm will be kept appropriately controlled to avoid parasitic infections in the fish hatcheries or ponds (Sinha, 2018). In this study, the addition of diverse key points which are majorly involved in the prevalence study of parasites. This study will help for future enthusiastic researchers who work in that field.

Declaration of Competing Interest

The authors have declared no conflict of interest.

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