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Research Article

INFESTATION OF HELMINTH PARASITES AS BIOINDICATORS OF AQUATIC POLLUTION IN *TRICHOGASTER FASCIATA* (BLOCH AND SCHNEIDER, 1801) IN TAMLUK, PURBA MEDINIPUR, WEST BENGAL, INDIA

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ABSTRACT

Trichogaster fasciata (Kholshe fish) is going to disappear day by day. Helminth parasites are the primary causes of diseases in this fish. Three infectious Helminth parasites are found that are Trematode, *Clinostomum complanatum;* Nematode, *Camallanus* sp.; and Acanthocephala, *Pallisentis ophiocephali*. Trematode is the most infectious and dangerous among the three. All invade in the specific sites, i.e. body cavity, stomach, rectum and intestine. Female Kholshe fishes are more susceptible than the males. Males are more resistant to the Helminth parasites. Abundance of diseases is highest in the summer season, followed by rainy and winter. It is due to the high temperature, pH and scarcity of dissolved oxygen in the water of the cultured fisheries. Severe Helminth infections in Kholshe fishes are one of the leading causes for their extinction in freshwater of the nature.

Keywords: *Trichogaster fasciata*, Helminth parasites, Prevalence, Seasonal variations, Aquatic Pollution.

INTRODUCTION

Trichogaster fasciata (banded gourami) is a common freshwater fish species in West Bengal. It is omnivorous and relatively sedentary, making it a suitable bioindicator species for site-specific monitoring. Global population is expected to reach 9.6 billion by 2050. The demand of animal protein is increasing every day. It is a very difficult problem to supply quality protein to the next generations. In this condition, fish culture plays a great role in supplying the required effective protein to the people. Fish culture is an important tool for the poverty alleviation of the people of developing countries. The fish contribute to around 17% of the global population's animal protein intake. Fishes are often called "Rich food for the poor people" as it supply the essential nourishment to the consumer with both macronutrients and micronutrients. Kholshe fishes, *Trichogaster fasciata* (Bloch and Schneider, 1801), are very tasty, palatable and nutritious and easily digestible. It is rich in protein (19%), vitamins and minerals (1.5%), and fat (3%). The amino acid content is very high. The medical doctors advised pregnant and milching mothers, sick and ailing patients to take the diet of small fishes like Kholshe (Mookherjee *et al*.,1946). Kholshe fishes are in the group of "Super Food" due to their high nutritional value. Kholshe fishes are available in India and in the Indian Sub-continent (Forese and Pauly,2016). They live in the natural water reservoirs, tanks, paddy fields, canals, rivers, etc. (Rainboth,1996). These fishes survive by consuming vegetable matter (85%) (Dasgupta,2004). They are omnivorous. Algae and the decaying organic matter are the favourite food of this fish (Oldalin *et al*., 2017). A full-grown Kholshe fish is 8-11 cm long,2-4 cm broad and weighs 15-20 gm per fish. A healthy, full-grown mother Kholshe fish lays 5000-13000 eggs at a time (Mitra *et al*., 2007). The laying period is from the month of April to September. This period is also their mating time; the best time is June-August (Das and Kalita,2006 & Islam *et al*., 2016; Prasad and Prasad, 1985). Kholshe fishes control the mosquitoes by eating the eggs, larvae, pupae, etc. People get relief from the invasion of Dengue, Malaria, Encephalitic diseases (Oldalin *et al*.,2017) Kholshe fishes are herbivorous cum predator. It consumes the unused feeds

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and other materials of the upper layer of the water of the fisheries and keeps the water clean (Chakraborty *et al*., 1979; Sarma *et al*., 2010). There are beautiful colour combinations on the body of the Kholshe fishes. There are three varieties which are recognised as Red, Chana and Big Kholshe. The male fishes are brighter in colour and larger in size. Females are generally whitish green and shorter in figure (Islam *et al*.,2016; Mitra *et al*.,2007). Thirty thousand fish species have been reported in the world. Out of this 800 belong to the Ornamental fish, including the Kholshe fish. This fish has a good demand in the aquarium market due to its attractive, bright colour combination. This colourful fish is called the " Living Jewels" of the environment, which are exhibited in the aquarium of various parks, temples and corporate houses (Mitra *et al*.,2007).

The health of freshwater ecosystems is increasingly threatened by anthropogenic activities such as industrial discharge, agricultural runoff, and urban wastewater contamination. These forms of pollution lead to alterations in the physicochemical properties of water, which directly affect aquatic organisms. Monitoring such ecosystems requires reliable biological indicators, and parasitological indicators have emerged as a valuable tool for such assessments. Fish parasites, especially helminths (trematodes, nematodes, and acanthocephalans), are highly sensitive to environmental conditions and can provide insight into ecological disturbances. Their complex life cycles, which often involve multiple hosts including snails, crustaceans, and aquatic birds, make them particularly responsive to environmental fluctuations. Parasite diversity, prevalence, and intensity in fish hosts can thus reflect underlying environmental changes, especially pollution stress. The use of pesticides in the paddy fields and the notorious technique of catching the fish by the use of poison and dehydration of the water bodies, fully with the help of suction pump sets, might be the leading reasons for the abolition of small fishes like Kholshe from the water areas of India. This investigation contributes to the growing body of research supporting parasitological monitoring as a valuable tool in freshwater ecosystem health assessments.

MATERIALS AND METHODS

Study area

Kholshe fishes (*T. fasciata*) were collected from the local fish market with the help of local fixed fishermen supplier of Tamluk, Purba Medinipur, West Bengal, from March 2021 to February 2022 and studied meticulously in the PG laboratory, Department of Zoology, Tamralipta Mahavidyalaya, Tamluk (22.301087°N, 87.926386° E) in an alive condition.

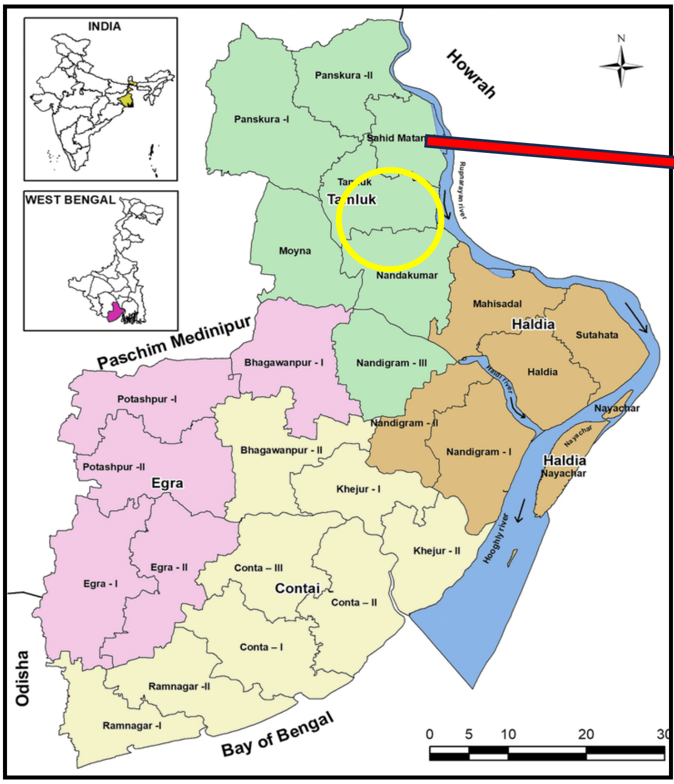

Figure 1. Map of Study area.

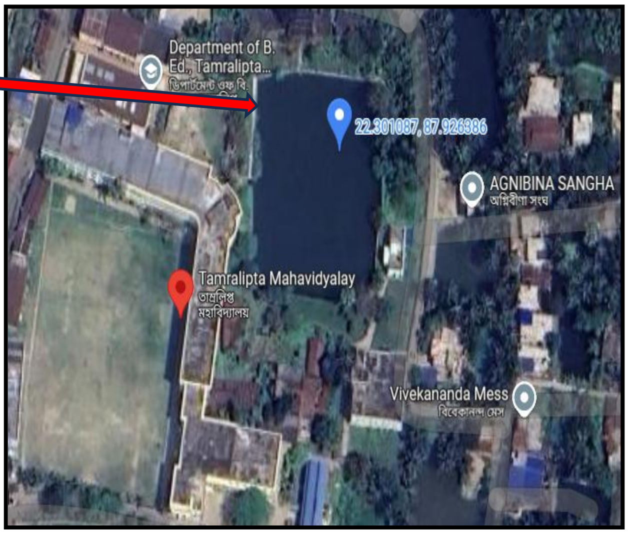

Figure 2. Satellite image of study area (22.301087°N, 87.926386° E).

Collection of fish with water samples

Water Samples along with the Kholshe fish were collected during pre-monsoon, monsoon, and post-monsoon from the pond for analysis of water temperatures as per seasons. The Konvio NEER digital water analysis kit has measured water temperatures. Collected Kholshe fishes were thoroughly examined to study the parasitic abundance and prevalence of infestation in relation to length, breadth, sex, and season. This examination was conducted individually in the PG Laboratory, Department of Zoology, Tamralipta Mahavidyalaya, Tamluk.

Figure 3. Water Sample collection pond.

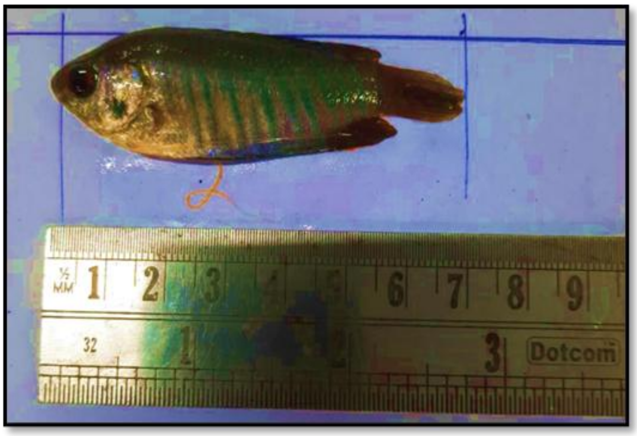

Figure 4. Length measurement of *T. fasciata.*

Figure 5. Fixed Parasites

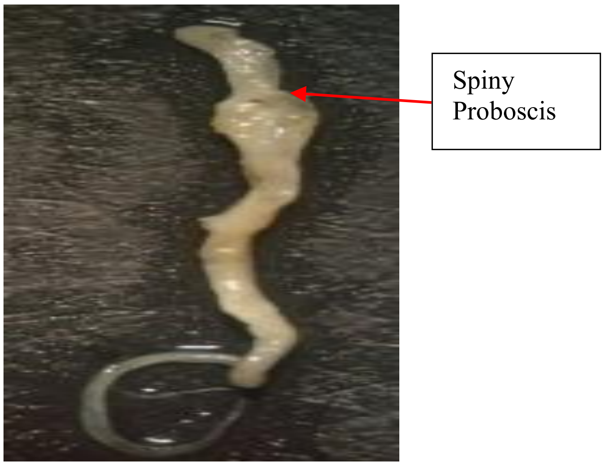

Figure 6. *Pallisentis ophiocephali*

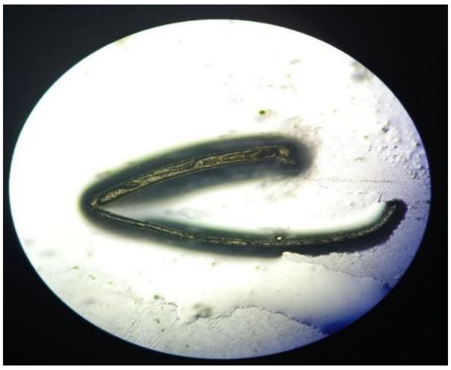

Figure 7. *Camallanus sp.*

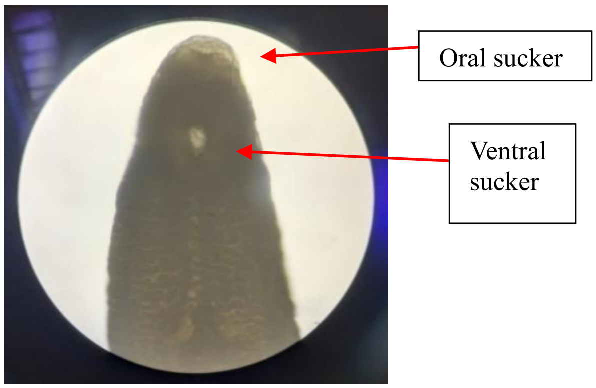

Figure 8. Microscopic view of collected Trematode (*Clinostomum complanatum*

Dissection of fishes

Kholshe fishes were dissected along the mid-ventral line by a fine, sharp knife and scissors. The gut was carefully isolated with the help of forceps and a needle, and kept in the normal saline (0.9%) solution.

Preservation of Parasites

The active living parasites were noticed and collected mainly from the intestine and cavity of the body by using forceps and a needle were placed in normal saline water (0.9%). The collected specimen parasites were fixed in AFA (Alcohol 50%: Formalin: Acetic acid (100:6:2.5), stained with acetocarmine, dehydrated in ascending grades of ethanol, cleared in Xylene, and then mounted in DPX.

Microscopic study

Fixed parasites in the slides were studied thoroughly under the microscope, Magnus MSZ-Bi. The specimen parasites were identified by following the literature of Yamaguti, S. Vol. III (1961), Gibson *et al*., 2002, Bhattacharya,2007 and Naidu, 2012.

Data analysis

The prevalence and intensity of the parasites were estimated by the following the formula suggested by Margulis *et al*.,1982. Statistical analyses like Pearson's correlation coefficient (r) and p-value were performed by using the software MS Excel 2021. The data were presented as mean ±standard error (SE), and the P-value <0.05 was considered statistically significant.

RESULT AND DISCUSSION

Results in Table 1 demonstrate that the female Kholshe fishes (*T. fasciata*) are more susceptible (80%) to the Helminth parasites than the males (53.33%), where the intensity of infection is 5.06 in females and 3.06 in males. The 24 female Kholshe fishes were infected among the 30 fishes, where the collected worms were 121. But in the case of males, only 16 Kholshe fishes were infected out of 30. A total of 40 fishes were diseased out of 60. It means 69.99 % of Kholshe fishes were infected by the parasites, which were collected from the local pond at Tamluk. In the case of female fishes, the abundance ratio is 4.03, whereas in males it is 1.93. The analysis of the results demonstrated that females are more susceptible to the Helminth parasites than the males. Fish parasite frequency index (FPFI) is also abundant in female fishes (100.04%) as compared to the male fishes (84.55%).

Table 1. Study on the intensity of infection of Helminth Parasites of *T. fasciata* in relation to sex.

Sex	No. of fish examined	No. of fish infected	No. of Parasites collected	Prevalence (%)	Intensity (±)	Abundance	FPFI (%
Male	30	16	58	53.33	3.06	1.93	84.55
Female	30	24	121	80.00	5.06	4.03	100.04

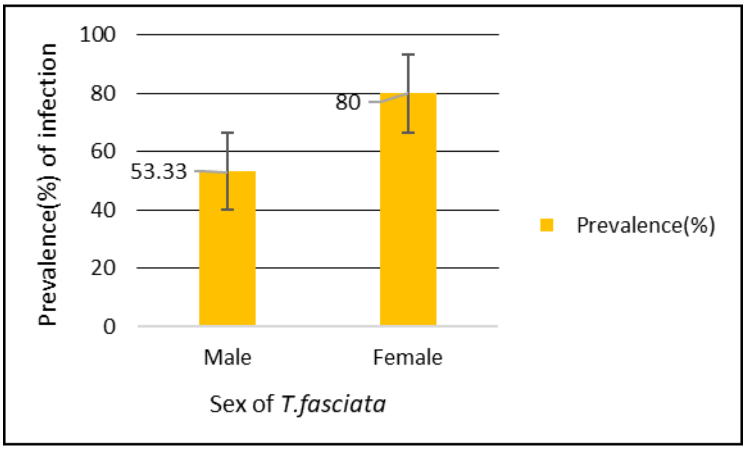

Figure 9. Intensity of Helminth Parasites infection in *T. fasciata* as per sex.

Table 2. Organ-wise detection of Helminth parasites in *T. fasciata*.

Helminth Groups	Name of the Parasites	Location	Examined Fishes	Infected Fishes	No. of worms collected	Intensity (±)	Prevalence (%)	Abundance
Trematode	*Clinostomum complanatum*	Body cavity	70	44	171	3.88	62.85	2.44
Nematode	*Camallanus sp.*	Stomach, Intestine, Rectum.		12	28	2.33	17.14	0.40
Acanthocephala	*Pallisentis ophiocephali*	Intestine		7	13	1.85	10.00	0.18

The results in Table 2 showed that the Trematode Helminth (*Clinistomum complanatum*) is the highest (62.85 %) infectious, followed by the Nematode (*Camallanus sp.*), which is 17.14 % and Acanthocephala (*Pallisentis ophiocephali*), which is only 10 %. 212 total of pathogens were collected from 63 infected out of 70 Kholshe fishes. In the case of Trematode (*C. complanatum*), 171 pathogens were collected from the 44 infected Kholshe fishes. But in Nematode (*Camallanus sp.*), it was only 28 and from 12 infected fishes. In the case of Acanthocephala *(Pallisentis ophiocephali),* 13 worms were found in 7 infected fishes. The results themselves revealed that Trematode is the highest infectious among the three. The striking site of the *C. complanatum* is the body cavity of the Kholshe fish, whereas *Pallisentis ophiocephali.* prefer to attack the intestine of the fish. But *Camallanus sp.* prefer to invade the body parts of the stomach, intestine and rectum of the digestive system of Kholshe fishes. Every pathogen chooses different favourable invasion sites in the host's body organs. There is a site specificity of the pathogens in the host body, as they choice the invading locations of the digestive system of the Kholshe fishes which are different in every parasites.

Table 3. Effects of seasonal variation on the intensity of infection of Helminth parasites in *T. fasciata*.

Seasons	No. of fishes examined	No. of fish infected	No. of Worms collected	Prevalence (%)	Intensity (±)	Abundance
Pre-monsoon (Summer)	25	24	117	96	4.87	4.68
Monsoon (Rainy)	25	22	59	88	2.68	2.36
Post-Monsoon (Winter)	25	16	23	64	1.43	0.92

Results in Table 3 revealed that the prevalence of the Helminth parasites is the maximum (96%) in the summer season, followed by the Rainy (88%) and Winter (64%). The reason is owing to the temperature and aquatic pollution in the fishery, which is much more in summer, followed by rainy and winter. The other reason might be due to the depth of the water in the fisheries. Depth of the water is the lowest in the summer season, which is one of the main reasons for aquatic pollution in the water bodies where the Kholshe fishes live. High temperature of the summer is the most favourable condition for the multiplication of the pathogens of the fishes. Due to the low depth of the water level in the fisheries, high temperature, turbidity of the clay particles, and inorganic materials, blue-green algae, brown-green algae etc. enhances the aquatic pollution in the water. As a result, dissolved oxygen decreases, which causes the fatal death of the fishes in the summer season.

Table 4. Effects of the Temperature of water on the intensity of infection of Helminth parasites in *T. fasciata*.

Seasons	Optimum range	Temperature (°C) of the examined water	Prevalence (%)	r value	Extent of correlation	p value	Significance
Pre-monsoon (Summer)	10-30°C (Pre-monsoon)	31.03±2.01	96.00	0.9988	Positively correlated	0.0060 (P ≤0.05)	Significant
Monsoon (Rainy)	25 -30 °C (Monsoon)	27.70±1.11	88.00				
Post-Monsoon (Winter)	22- 28 °C Post-monsoon (10- 20 °C)	19.71±1.94	64.00				

The positive r value indicated a proportional relationship between seasons and prevalence. As the season progresses from summer to winter, the parasitic load decreases due to the low temperature in the pond water. The value of P is 0.0060, which is less than the degree of freedom, α (0.05). Where P≤ 0.05, the correlation is statistically significant (Table 4).

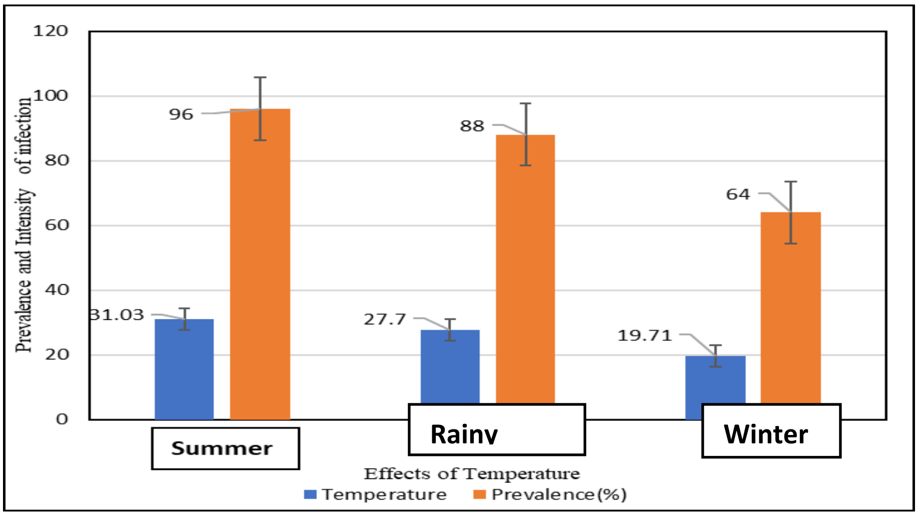

Figure 10. Graphical representation of the Prevalence of Helminth parasites in T. *fasciata* according to Temperature.

Table 5. Study on the effects of pH of the pond water on the intensity of infection of Helminth parasites in *T. fasciata*.

Seasons	Optimum pH range	pH of the examined water	Prevalence (%)	r value	Extent of correlation	p value	Significance
Pre-monsoon (Summer)		7.99±0.16	96.00				
Monsoon (Rainy)	6.5-8.5	7.54±0.09	88.00	0.9028	Positively correlated	0.0077 (P ≤0.05)	Significant
Post-Monsoon (Winter)		7.29±0.18	64.00				

The positive r value indicated a proportional relationship between seasons and prevalence. As the season progresses from summer to winter, the parasitic load decreases due to the low pH in pond water. The value of P is 0.0077, which is less than the degree of freedom, α (0.05). Where P≤ 0.05, the correlation is statistically significant (Table 5).

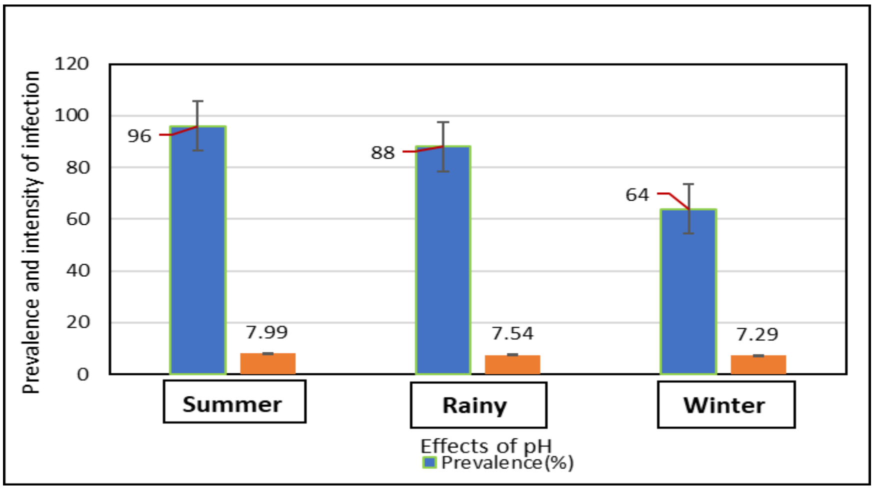

Figure 11. Graphical representation of the Prevalence of Helminth parasites in T. *fasciata* according to the pH.

Table 6. Study on the effects of Dissolved oxygen on the intensity of infection of Helminth parasites in *T. fasciata*.

Seasons	Optimum dissolved oxygen range	Dissolved oxygen	Prevalence (%)	r value	Extent of correlation	p value	Significance
Pre-monsoon (Summer)	5-10	4.99±0.32	96.00	-0.8237	Negatively correlated	0.0091 (P ≤0.05)	Significant
Monsoon (Rainy)		7.49±0.43	88.00				
Post-Monsoon (Winter)		8.16±0.37	64.00				

The negative r value indicated an inverse relationship between dissolved oxygen and prevalence. As the dissolved oxygen value is less than 5 in summer, the parasitic load increases in the pond water. It is observed from the experiment that when the value of the dissolved oxygen is in the suitable range, the parasitic load decreases gradually. The value of P is 0.0091, which is less than the degree of freedom, α (0.05). Where P≤ 0.05, the correlation is statistically significant (Table 6).

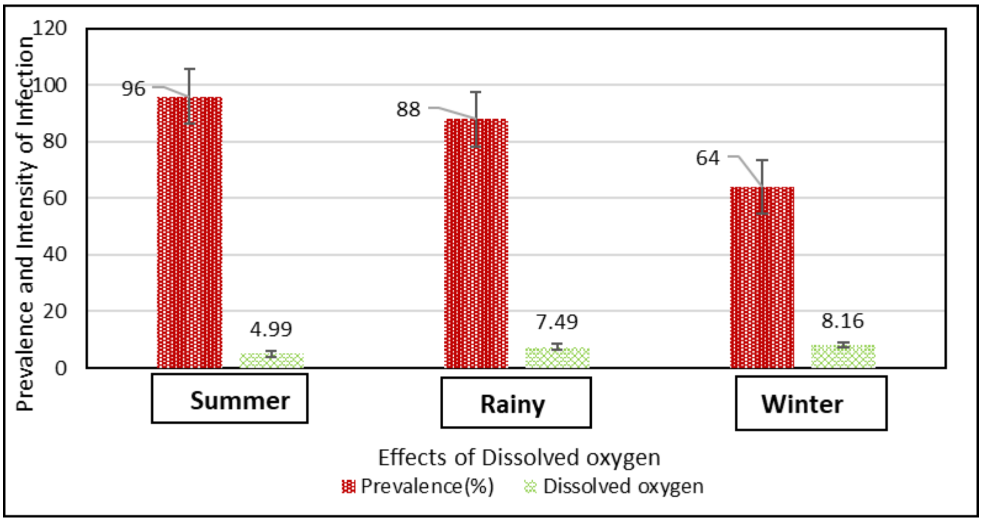

Figure 12. Graphical representation of the Prevalence of Helminth parasites in T. *fasciata* according to Dissolved oxygen.

212 Helminth pathogens are detected in the 63 infected Kholshe fishes out of 70, which are collected from the local fish supplier of Tamluk, West Bengal, which are infected with 90%. Most of the Kholshe fishes are infected with Helminth pathogens, which are very infectious to the host. Trematode Helminth parasite, *C. complanatum,* is the most dangerous to the Kholshe fishes among the three. Kholshe fishes are invaded in a particular site of the organ of the body parts of the host which they prefer. Trematode, *C. complanatum,* chooses to attack in the organ of the body cavity of the digestive system of the Kholshe fishes, whereas the Acanthocephala, *Pallisentis ophiocephali,* prefer to invade the intestine organ of the Kholshe fishes (Fielding *et al*., 2003; Lyndon & Kennedy, 2001; Taraschewski, 2000,2006). But the Nematode (*Camallanus sp*.) chooses to invade the three separate locations, likewise the stomach, intestine and rectum (Dezfuli *et al*., 2002,2003; Crompton & Joyner, 1980; Lane & Morris,2000; Jhansilakshmibai & Madhabi,1997; Levsen, 2001; Stromberg & Crites, 1974,1975). The intensity of infection of the Helminth parasites is maximum in the summer season and least in the winter season (Khanum and Parveen, 1997). Causes are due to the higher temperature, pH, and lower dissolved oxygen levels of the water where fishes are grown. Water depth of the fisheries is the lowest in the summer season. As a result, the temperature and the aquatic pollution are highest in the summer (Landsberg *et al*., 1998) along with different algae, weeds, zooplankton, phytoplankton which exhaust the dissolved oxygen in the water of the fisheries. Pollutant factors of the water of the tested tank, such as pH, dissolved oxygen, temperature, etc. are changed as per the temperature of the season. The available data from this experiment revealed the aquatic pollution is the highest in the summer followed by rainy and winter. These pollutants play a great role in the pathogenic effects of Helminth parasites. There is a direct correlation between the aquatic pollution and infectivity of the pathogens. Severe pathogenicity of these pathogens causes the death of the fishes. These factors are one of the vital reasons for the higher infection rate in summer (Austin,1998; Arizo *et al*.,2022). These factors are also one of the main causes for the extinction of the Kholshe fishes in nature.The reasons are that fish with a large length and more weight tend to carry more parasites due to increased

surface area, longer lifespan, more frequent feeding and high metabolic rate. Infection by the Helminth parasites is the most severe in the summer due to the aquatic pollution, temperature, pH and scarcity of dissolved oxygen. Infectious Helminth pathogens to this fish is one of the main causes for the diminishing of the Kholshe fishes of this planet. The findings demonstrate a strong association between helminth parasite load and water pollution. Polluted waters promote intermediate host proliferation (e.g., snails), thus facilitating the parasite life cycle. Helminth parasites in *T. fasciata* is the mirror the pollution gradient, indicating their role as reliable bioindicators.

CONCLUSION

Kholshe fishes are vanishing day by day. It is revealed that the diseases are one of the main casualties for their extinction in nature. Helminth parasites are the main culprits. Trematode Helminth parasites, *Clinostomum complanatum,* are the most notorious among the three. Trematode (*Clinostomum complanatum*) likes to attack in the fixed organ, body cavity, whereas Acanthocephala (*Pallisentis ophiocephali*) prefers the intestine, and Nematode (*Camallanus sp*.) chooses the stomach, intestine and rectum respectively of the digestive system of the Kholshe fishes. Female Kholshe fishes are more susceptible than the males. Males are more resistant to the Helminth parasites. Kholshe fishes are the highest susceptible to the Helminth pathogens in summer, followed by the rainy and winter seasons for the aquatic pollution in the water of the cultured fisheries. This study confirms that helminth parasite infestation in *Trichogaster fasciata* correlates strongly with the degree of aquatic pollution in freshwater bodies of West Bengal. The parasitological parameters such as prevalence, mean intensity, and species richness increased significantly with the worsening water quality. These findings validate the hypothesis that helminth parasites can serve as sensitive and practical bioindicators for aquatic ecosystem health. The application of parasitological indices offers a non-invasive, ecological approach to the water quality monitoring. Seasonal and site-wise variations in parasite loads also emphasize the dynamic relationship between environmental stressors and host-parasite interactions. This work demonstrates its potential as a sentinel species in freshwater ecological monitoring.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

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AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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