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Incidence of protozoan infestations in juvenile farmed fishes with relation to water quality parameters

David Rintu Das

Bangladesh Fisheries Research Institute, Floodplain Sub station, Santaher, Bogura, Bangladesh

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***Corresponding Author**

DR Das
drd4272@yahoo.com

ABSTRACT

An investigation on protozoan parasitic infestations in juvenile farmed fishes was made in relation to water quality parameters and management practice. A total eight (8) different fish farms (four Government and four Private) of Bogura region were selected and investigated during June 2016 to May 2018. All together 2880 fish host samples of *Catla catla* (960), *Labeo rohita* (960) and *Cirrhinus cirrhosus* (960) were collected and examined, out of which 1350 fishes (Catla- 346, Rui –478 and Mrigal– 526) were infested by protozoan parasites. Most of the farms were managed carefully and have more or less similar type of practices. Monthly samplings were carried out with 5 fishes for each species from each farms. Mrigal (67.08%) was more susceptible than Rui (65%) and Catla (38.75%) to protozoan parasites. The risk of being infested by protozoa of juvenile carp in private farm is about 5.82 times more than that of government farms. Most of the variables regarding water qualities except p^H, alkalinity and water depth exhibit significant ($p<0.001$)) negative association with the occurrence of the parasitic diseases. The risk of developing protozoa in juvenile carps was significantly ($p<0.05$ or $p<0.001$) reduced with increasing temperature, dissolved oxygen, hardness and transparency of water.

INTRODUCTION

One major problem in proper fish culture is the parasitic infestation in early stages of their life cycle. In fish culture system, production depends on the availability of adequate healthy juveniles. It has often been a difficult task to obtain healthy fry and fingerlings for successful fish culture system. Fish culture also depends on the quality of management, which depends on the understanding of the biology of fish and the aquatic environment in which they live (Das et al., 2018).

Nursery ponds for rearing fry and fingerlings are limited in number. Management practices in the nursery pond of both Private and Government sector are yet to be at a standard level. Fish farmers often raise questions about the quality of fry obtained from nursery ponds and they observed reduced growth of juvenile carps may be due to many factors. Protozoan parasites are common in juvenile carps and other cultured species in nurseries. Recent cause of heavy mortalities of these species accompanied by myxosporidean infestations of the gills has caused serious concern among fish farmers. The myxosporia is a group of cosmopolitan parasites occurring highly in larval stages and also in adult stages. They produce a variety of effects (Bhuiyan et al., 2010) in the major carps and their hybrids (Kalavati and Nandi 2007). Akter et al. (2007) reported that 61 % of carp fry in nurseries of the greater Mymensingh districts were infected with protozoan parasites. The highest mortalities of carp fingerlings were due to the infection by *Trichodina*, *Myxobolus* and *Ichthyophthirius*. As such juvenile carp fishes remain susceptible to both ecto and endoparasites. But very little information are available about the infestation of protozoan parasites in juvenile carps in Bangladesh. For this reason the present work was, therefore, undertaken to know the incidence of protozoan parasitic infestations in juvenile farmed fishes in their relation to water quality parameters.

MATERIALS AND METHODS

The study was conducted for a period of 24 months from June, 2016 to May, 2018. The investigation were recorded from eight (8) (four

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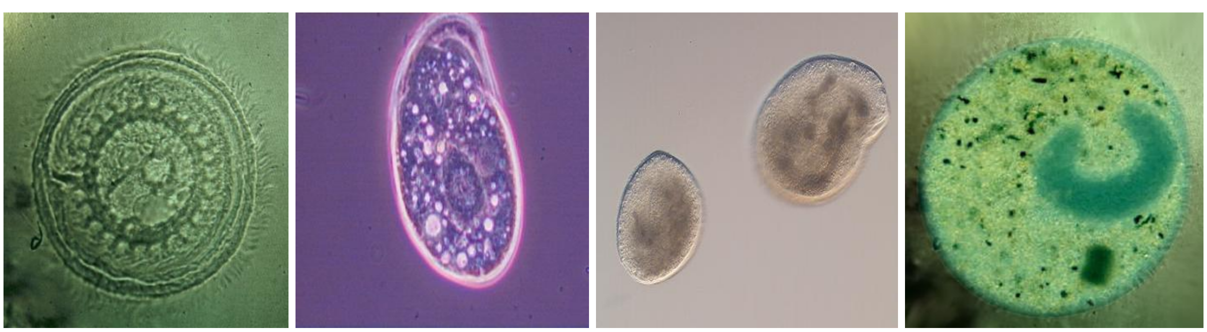
Government and four Private) ponds in different months and seasons. Water samples were taken by clean black colored bottle from the selected pond. Water quality parameters like temperature, dissolved oxygen and pH was determined by using thermometer, DO meter and pH meter. Transparency of the ponds was measured by a Secchi-disc of 30 cm in diameter and depth of water was measured by a ribbon of cm. Ammonia, alkalinity and hardness were measured by Bromophenol/blue indicator and HI: 3811-0 Solution by Titrimetric method (HI: 3811-0 Alkalinity test kit) respectively. The experimental fishes were sampled regularly by visiting the experimental ponds. Samplings were carried out from the farms at monthly intervals. During each sampling five fishes for each species were collected from every pond with the help of seine net. Most of the protozoan parasites were recorded from the différent régions of the fish body like pelvic, pectoral, anal, caudal regions, skin, gill regions, body surface, muscle and mouth cavity. In the laboratory fishes were killed by a blow on the head and only a little portion of the infested part of different regions were taken into petridish containing water and gently scrapped to dislodge live parasites. The specimens were kept over clear slides with a fine pipette in a drop of water and covered with cover slip. Ammonium picrate solution was added beneath the cover slip to fix and clear the parasites. Four corners of the cover slips were then sealed with sealant to prevent it from moving. Then the slides were marked for tracking and recording. To observe the seasonal infestation, only distinct seasons were included. The whole year was divided into three seasons – summer (March - June); rainy season (July-October) and winter (November - February). Multivariable logistic regression models were fitted for detecting the risk factor (s) that significantly influences the infestation of juvenile carps by protozoan parasites. Infestation of protozoan parasites in different types of juvenile carp fishes corresponding to the different seasons as well as farms were compared by Tukey tests (Zar, 2003). All the statistical analyses were done by SPSS (Statistical Package for Social Science) and MS Excel.

RESULTS

Water quality parameters in carp culture

Seasonally, fluctuation of water quality parameters

Temperature, dissolved oxygen (DO), pH, ammonia, alkalinity, hardness, tansparency and depth of water were recorded in different months and seasons. In the present investigation, these parameters were exhibited significant variations during summer, rainy and winter season. Significantly increased value of ammonia, alkalinity, hardness and decreased value of temperature and dissolved oxygen (DO) were observed during these seasons which were within the unfavorable range of fish culture over the study period as indicated in table 1.



Trichodina sp *Chilodonella* sp. *Myxobolus* sp. *Ichthyophthyrius* sp.

Figure 1
Different protozoa were identified based on morphology according to Lom and Dyková (1992).

Table 1
Seasonal fluctuations of water quality parameters of culture fish farms in Bogura during the period from June, 2016 to May, 2018

Seasons	Water quality parameters (Mean ± SD)							
	Water temp (^{0}C)	DO (mg/l)	pH	Ammonia (ppm)	Alkalinity (ppm)	Hardness (ppm)	Transparency (cm)	Depth of water (cm)
Rainy	32.97 ±1.27	5.87 ±0.40	7.30 ±0.35	1.70 ±0.10	105.33 ±13.01	93.67 ±11.72	35.00 ±2.00	173.00 ±2.65
Winter	19.43 ±4.68	4.57 ±0.70	7.07 ±0.15	1.87 ±0.47	154.33 ±12.06	125.00 ±26.96	36.67 ±4.04	141.00 ±13.23
Summer	27.78 ±3.46	5.53 ±0.60	7.40 ±0.26	1.73 ±0.40	158.00 ±8.89	142.00 ±9.87	34.67 ±4.04	157.33 ±9.81
Mean ±SD	26.3 ±2.02	5.3 ±0.22	7. 2 ±0.08	1.7 ±0.09	138.0 ±8.61	118.8 ±8.75	36.3 ±1.15	155.5 ±4.80

Nature of infestation of protozoan parasites

The prevalence (%), mean intensity and abundance of protozoans were found in irregular patterns over the 24 months. Monthly average prevalence (%), mean intensity and abundance were shown in Figures 1, 2 and 3.

Host wise monthly infestations of protozoan parasites

During the study period infestations of protozoan parasites in Catla, Rui and Mrigal were recorded from two sectors (Govt. and Pvt. carp culture ponds) in different months. The different parasites were found in different species. The results are given below host wise in Figures 1, 2 and 3.

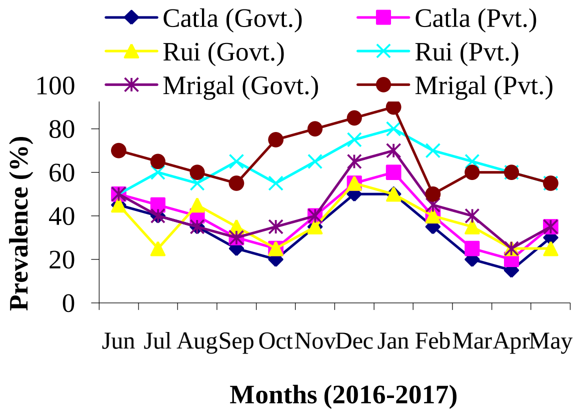
Catla: The monthly prevalence of parasite in *C. catla* collected from Govt. farm was higher during December - January (50%) and only lower in April (15%), mean intensity was higher in January (6.30) and lower in May (3.16), abundance was higher in January (3.15) and lower in April (0.55). In combined prevalence in two year was 33.34%, mean intensity 4.58 and abundance 1.53 (Figures 1- 3).

Similarly, the higher infestations was recorded (60 %) in January in Pvt. farms, lower in April (20 %), mean intensity in January (6.50) and in August (4.37), abundance in January (3.9) and in April (0.95). During the two year combined prevalence 38.75%, mean intensity 5.28 and abundance was 2.04 (Figures 1- 3) were recorded respectively.

Rui: The monthly prevalence in Govt. farm was found higher only in December (55%), but lower during in April, May, July and October (25%), whereas mean intensity and abundance was higher in January (7.50) and December (3.90) and lower in May. The combind prevalence for two year was 36.67%, mean intensity 5.57 and abundance 2.04 (Figures 1- 3).

The Pvt. farms had also higher prevalence (80%) in January and reduced in June (50%). Both mean intensity and abundance maintained similar nature of infestations like prevalence. Similarly combined infestations for two year data revealed comparative higher prevalence (65%), mean intensity (7.46) and abundance (4.85) (Figures 1-3).

Mrigal: In Govt. farm prevalence recorded higher in January (70%) and lower in April (25%), mean intensity and abundance showed higher in January and lower in May and April. Overall prevalence during two year investigation was 42.50 %, mean intensity 5.97 and abundance 2.54 (Figures 1- 3). Similar pattern of higher prevalence revealed in Pvt. farms showed 90%, lower 50% in February, both mean intensity and abundance found higher in January but lower in July and February. Similarly overall prevalence during two year survey showed 67.08%, mean intensity 9.56 and abundance 6.41 (Figures 1- 3).



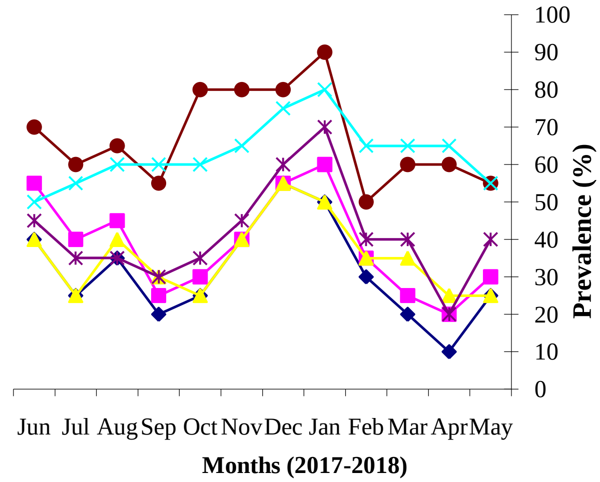
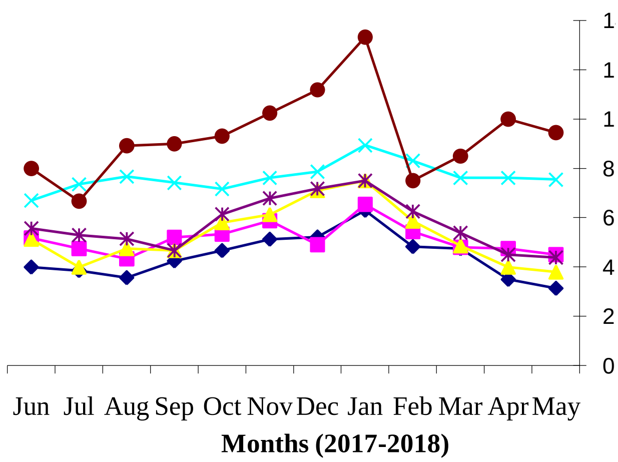


Figure 1
Monthly variation of prevalence (%) of protozoan parasites in different juvenile carp species (June, 2016 to May, 2018).



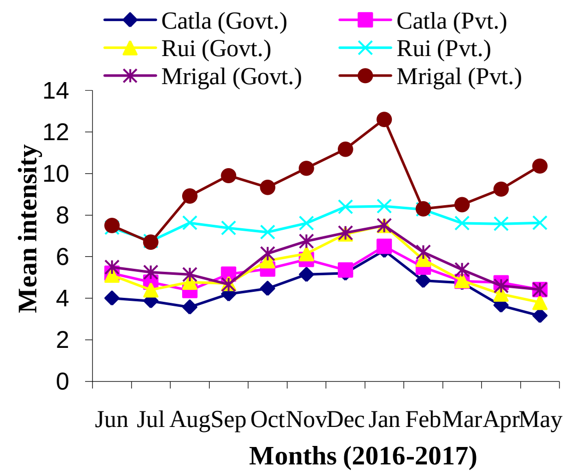
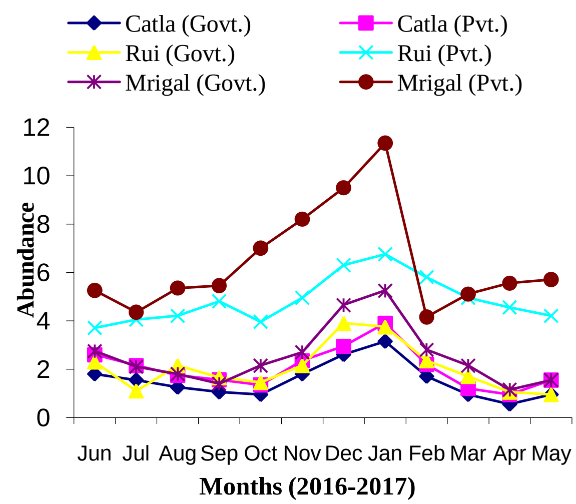


Figure 2
Monthly mean intensity of protozoan parasites in different juvenile carp species (June, 2016 to May, 2018).



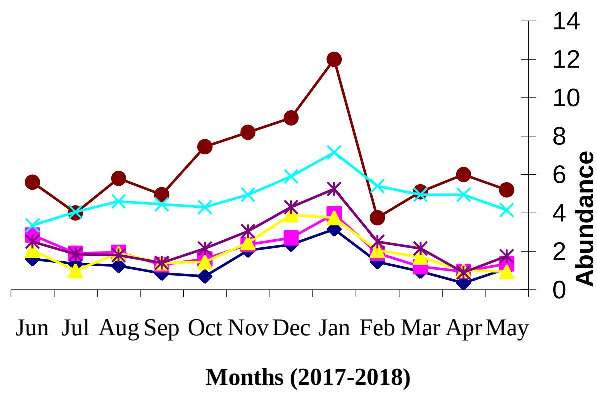


Figure 3
Monthly abundance of protozoan parasites in different juvenile carp species (June, 2016 to May, 2018).

Multivariable logistic regression model for protozoan parasites

By fitting multivariable logistic regression model for protozoan parasites it is evident (Table 2) that each of the categories of the variables like species and organization exert a significant ($p<0.001$) positive association with the infestation of protozoa in juvenile carps comparing with their respective reference (minimum level of infestations) categories. But most of the variables regarding water qualities except p^H, alkalinity and water depth exhibit significant ($p<0.05$ or $p<0.001$)) negative association with the occurrence of this parasitic disease. The effect of p^H and alkalinity are significantly ($p<0.05$ or $p<0.01$) positive on the infestation of this parasite. Odd ratios indicate that Rui and Mrigal are 2.09 and 2.72 times more likely to be infested by protozoa than that of Catla. The risk of being infested by protozoa of a juvenile carp in private farm is about 5.82 times more than that of one of a government farms. The risk of developing protozoa in juvenile carps significantly ($p<0.05$ or $p<0.001$) reduces with the increment of temperature, dissolved oxygen, ammonia, hardness and transparency of water. The highest average reduction (68.9%) is found for one unit increase in ammonia followed by 44.6%, 7.3%, 3.8% and 2.7% for increasing one unit in dissolved oxygen, transparency, temperature and hardness of water respectively. The likelihood of developing protozoa significantly ($p<0.05$ or $p<0.01$) goes up in juvenile carps on an average by 40.5% and 1.2% with one unit increase in p^H and alkalinity of water respectively when other variables remain constant.

Table 2
Multivariable logistic regression for detecting risk factor (s) to be infested juvenile carps by protozoan parasites

Variables	Category	Coefficient (b)	SE (b)	Wald's P	OR	95% CI
Species	Catla (Ref.)	-	-	-	-	-
	Rui	0.737***	0.151	0.000	2.090	1.55 - 2.81
	Mrigal	0.999***	0.153	0.000	2.715	2.01 - 3.66
Organization	Govt (Ref.)	-	-	-	-	-
	Pvt.	1.762***	0.222	0.000	5.824	3.77 - 8.99
Season	Summer (Ref)	-	-	-	-	-
	Rainy	0.341	0.196	0.082	1.406	0.96 - 2.06
	Winter	0.211	0.196	0.283	1.235	0.84 - 1.81
Temperature		-0.039*	0.018	0.029	0.962	0.93 - 0.99
DO		-0.590***	0.097	0.000	0.554	0.46 - 0.67
pH		0.340*	0.140	0.015	1.405	1.07 - 1.85
Ammonia		- 1.168***	0.146	0.000	0.311	0.23 - 0.41
Alkalinity		0.012**	0.004	0.003	1.012	1.00 - 1.02
Hardness		-0.027***	0.005	0.000	0.973	0.96 – 0.98
Transparency		-0.075***	0.012	0.000	0.927	0.91 – 0.95
Water depth		-0.005	0.004	0.259	0.995	0.99 – 1.00

Level of Significance: * $p<0.05$, ** $p<0.01$ and *** $p<0.001$

DISCUSSION

Physico-chemical features such as water temperature, total alkalinity, DO, pH, total hardness transparency and depth of water of the monthly record showed fluctuations in different months. The water quality parameters of pond for management practices might be affected and in many times can cause several problems in culture systems. Most of the farmers have no clear idea about the prevention and control of disease and parasites of their commercial fish. In some cases, it was found that the farmers maintained higher stocking density and supplied minimum amount of feed. So, the fish did not get appropriate nutrition required and ultimately productions get lowered. Also, it was known that where stocking density was high occurrence of disease problem was also higher.

Water quality parameters are related to disease infestation as they fluctuate more rapidly. Protozoan prevalence was positively correlated with diseases with the increasing temperature of water (Das *et al*., 2018; Banerjee and Bandyopadhyay, 2010). The total alkalinity and hardness showed more or less direct relationships with pH and NH_3. More or less inverse relationship was found between DO and ammonia; which had also been observed by Hossain *et al*. (2008). Optimum range of unionized ammonia for fish is 0.0 to 0.025 (Jhingran, 1988). The amount of NH_3 always fluctuated in the ponds. A close relationship between pH and carbonate content of water was also observed. The monthly prevalence data revealed that ectoparasitic infection was heavy unionized ammonia was toxic to fish, while the ammonium ion (NH_4+) was nontoxic. The concentration of unionized ammonia should not exceed more than 0.025 ppm (Das *et al*., 2016).
Fish releases CO_2 through its skin and gills. This activity is hampered if the amount of this gas is more in the water. As a result fish suffers from suffocation leading to death (Hossain *et al*., 2007). According to Barai *et al*. (2005) free CO_2 at a concentration of more than 15 ppm was detrimental for pond fishes. Prolonged exposure to low concentrations of DO can be harmful to fish life because they would die at a level of 1 mg/l. Growth and feeding decreases at 1-5 mg/l of DO and growth and production is optimum at more than 5mg/l (Asmat *et al*., 2006).

Lower concentration of pH increases the toxicities of hydrogen sulphide (H_2S), copper and other heavy metals to fish. Fishes are prone to attack of parasites and diseases in acidic waters. When pH rises over 11, the gills and lens and cornea of fish eyes were destroyed (Bhuiyan *et al.*, 2010). As a result the fishes become weak and infected by parasites. The present data on pH, free CO_2 and hardness are similar to those reported by Ahmed *et al.* (2009). Water with less than 5ppm $CaCO_3$ equivalent causes slow growth, distress and eventual death of fishes. Primary production of pond decreases if hardness rises over 300 ppm (Akter *et al*., 2009). Bhuiyan and Musa (2008) reported that the highest concentration of free CO_2 was recorded in August and in winter months. Das and Chandra (2017) also observed high levels of free CO in June and lower levels in January.

Epizootics were reported in China as early as Tenth century. It is the most pathogenic protozoan parasite of fishes. The first major outbreak in North America was described in 1898. From 1940 it has become a serious disease of carp in Russia. From Bangladesh and India this has been regularly reported and became a major problem in carp farming (Chandra, 2006; Banerjee and Bandyopadhyay, 2010). As the disease was basically of cold weather of European countries, rising of temperature in aquaculture system would be able to control its infection. Now it has been a problem for temperate countries, with slow adaptation of this parasite with the movement of indigenous and exotic fishes for aquaculture production (Das et al., 2018).

Protozoan parasite was first described in Europe (Kiernik, 1909). From Asia it was found on skin and gills of *Aristichthys nobilis* and *Puntius gonionotus* in Viet Nam (Ha, 1999). Mitra and Haldar (2004) reported its presence in a freshwater wild fish, *Nandus nandus*. *P*resent report recorded *L. rohita* and *C. cirrhosus* in Mymensingh, Bangladesh. Introduction of exotic fish might be the source of foreign parasites in local host. Their slow adaption to our indigenous species in the new environment is another host cause of dispersal. The species is parasitic on many freshwater fishes including the carp, *L. rohita* and *C. cirrhosus.* It infests on the skin, fins and the gill epithelium of the host fish. The parasite causes serious damage to the gill of the host (Das, 2003).

Protozoan is a widely distributed parasites and is an obligatory ectoparasites. It forms large plasmodia in gills and sometimes in other organs of several fish especially cyprinids. It caused massive mortality in Mrigal in the culture ponds. It remains attached to the gills of the host fish. It is a common parasite of the economically important fish, Mrigal and affects the growth and development of the host. However, the intensity rate of infestation are variable in different farm management system (Pvt. and Govt.), depending upon species of the carps and seasons. This parasite causes severe damage to the economically important fishes like, carp fry and fingerling throughout the country. The present results on the incidence of protozoan parasitic infestations in juvenile carp fishes are agreement with (Banu and Khan, 2004).

Prevalence of protozoan parasites in carps was significantly (p< 0.001) higher in Pvt. farm than that of Govt. farm. The prevalence of this parasites in winter season was significantly (p< 0.01) higher than that of summer and rainy season. Mrigal was more infected than Catla and Rui. But its prevalence varified significantly (p< 0.01) only with Catla. This results are in agreement with Das *et al*. (2018). (Chandra, 1987) stated that the unfavorable environment and ecological conditions caused variety of fish diseases. So, it can be mentioned that susceptibility of disease occurring is depended on many factors like, season, stocking density, water depth, dissolved oxygen etc.

CONCLUSION

However, for producing healthy juvenile fish farmers should maintain proper density of juvenile carp population and have to check regularly the occurrence of disease, dissolved oxygen concentration, temperature, ammonia, hardness or any kinds of abnormalities for immediate taking measures for sustainable seed production.

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