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MONOGENETIC TREMATODES INFESTATIONS IN INDIAN MAJOR CARPS OF MYMENSINGH REGION

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ABSTRACT

A study was conducted to investigate the infestations of monogenetic trematodes of juvenile carps of Mymensingh region. A total of eight (8) farms, four Government (Govt.) and four Private (Pvt.) fish farms were investigated of Indian major carps - Catla (*Catla catla*), Rui (*Labeo rohita*) and Mrigal (*Cirrhinus cirrhosus*) during June, 2010 to May, 2012. Monthly samplings were carried out with 5 fishes of each species and each farm. Altogether 2880 fish host were examined of which 1424 hosts were found to be infested with monogenetic trematodes of which 626 from Govt. and 798 from Pvt. farms. The monogeneans were very common in gills of all fishes. Water quality parameters were taken and management practices were also noted. The risk of infestation by monogeneans of carp significantly ($p<0.001$) increased when the water quality parameters were deteriorated. Prevalence (%), mean intensity and abundance were found to be species specific and also varied with seasons and management systems practiced by different farmers. Prevalence (%) of monogeneans in carps was significantly ($p< 0.001$) higher in Pvt. farm than that of Govt. farms. The prevalence (%) of monogeneans were significantly ($p< 0.01$) higher in rainy season than dry season. Mrigal was more infected by monogeneans followed by Catla and Rui.

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INTRODUCTION

The groups of Monogenea are small to medium sized trematodes which complete the life cycle on one host. The chief organ of attachment is the haptor, which is posterior, mostly parasitic on the gills, some on the body and fins. Some monogenetic trematodes are serious pests in fish culture on occasion. In hatcheries and culture ponds, some dactylogyrids cause great damage to gill filaments and some caused damage to skin and fin of carps (Hoffman, 1967). They usually infect gills, skin and fins and cause damage of host tissue by their haptor, anchors and hooks during feeding. Three species of Indian major carps, Catla, Rui, and Mrigal are the principal fishes cultured throughout Bangladesh particularly in Mymensingh region. Several exotic fishes are also cultured along with our indigenous species in polyculture system for higher production (Das, 2003). Mortalities of these species accompanied by *Dactylogyrus* and *Gyrodactylus* infestations of the gills and skin has caused serious concern among fish farmers now a days. These are cosmopolitan parasites occurring highly in larval and in adult stages, produce a wide variety of effects on fishes (Chandra *et al.,* 1996).

Prevalence of fish disease has negative economic impact on aquaculture. A global estimate of disease losses to aquaculture by World Bank in 1997 was in the range of US$ 3 billion per annum (Subasinghe *et al.,* 2001). Studies of fish diseases in Bangladesh are limited to diagnosis, characterization and control of pathogens involved. Some studies have been undertaken only on socioeconomic aspects of developing pond fish culture (Rahaman and Miah, 2001; Arthur and Ahmed, 2002). Hasan and Ahmed (2002) reported economic loss due to diseases and found 7.6% loss of net profit due to disease in carp hatcheries and nurseries of two districts of Bangladesh. However, they did not assess the loss of fish due to disease in grow-out ponds and thus do not reflect the overall economic impact in fish production. There is a need to understand not only the prevalence of various diseases and pathogens but also the need to understand the economic losses resulting from disease outbreak. Production loss assessment, assessment of economic impact of disease on production and optimal investment for fish disease control are essential. Field survey is the most practical way in collecting such information directly from a large number of farmers.

However, in Bangladesh, very little is known on prevalence and extent of damage caused by monogeneans in carp fry and fingerlings (Chandra, 2006). Though there are sporadic reports of parasites and diseases in nurseries in Mymensingh area, no work was initiated on the nature of their infestation. The present work of fish monogeneans is one of the significant and priority areas. Keeping the above background in mind, the present research programme was considered to be undertaken with the study of seasonal infestations of monogeneans in different carp species, general pattern of infestation of monogeneans in different seasons and infestations of monogeneans in different farming systems of juvenile indigenous farmed carps of Mymensingh district.

MATERIALS AND METHODS

For monogenean investigation eight fish farms, 4 Government Farm (Bangladesh Fisheries Research Institute (BFRI), Fish Seed Multiplication Farm (Maskanda), Fish Seed Multiplication Farm (Shambhugonj), Fish Seed Multiplication Farm (Gouripur)) and 4 Private farms (Brahmaputra Fish Farm, Deshbondhu Fish Farm, Pankouri Fish Farm and Sornolota Fish Farm) were selected. To observe the seasonal variation three distinct seasons were used as rainy season (June-September), winter (October-January) and summer (February-May) in the experimental period. Catla, Rui and Mrigal were selected as the experimental fish and sampled regularly by visiting the experimental ponds as similar were being conducted in those pond. Experimental fish were collected for a period of 24 months from June 2010 to May 2012 for the study. Samplings were carried out from the farms at monthly intervals. During each sampling 5 fishes from each species were collected from eight ponds with the help of seine net. Fish samples were transported to the Fish Disease Laboratory of the Dept. of Aquaculture, BAU with plastic containers and bags. The young carps or juvenile were examined quickly to observe for monogeneans infestation, injury, infection and other abnormal conditions of fish body. Fishes were killed by a blow on the head. Both the opercula of the fish were removed by scissors to remove the gills and dissected gills were placed in petridish containing clean water. Prior to gill removing external examination were made by scrapping the skin and examining smear using a magnifying glass or by under microscope.

Collection of parasites from hosts gills

Most of the monogenean were recorded from the gill regions. The first and second gill arches from both sides were removed by scissors and macerated on slides and petridish. In case of too large gills only a little part of the gills were used. Gill preparations were examined at 10 × 40 magnifications. Gills containing petridish was placed under a dissecting microscope and observed the gills filaments to find out the parasites. The live monogeneans were gently rubbed to dislodge form the gill filaments by the help of a bent needle and forceps. The monogeneans were removed and picked out using a fine pipette to a small drop of water on a clean slide and covered with a cover slip.

Fixation, preservation and study of parasites

Monogenean were fixed with a drop of ammonium picrate or molmberg fixative introduced beneath the cover slip to fix and clean the worm. The corners of the cover slip were sealed with sealant to prevent it from moving and to prevent entrance of air into ammonium picrate (Chandra, 2008). Sometimes monogenean were immediately fixed in 70% alcohol for further processing. Preserved monogenetic trematodes were studied under microscope and their size shape and chitinoid structure was noted. Then the slides were marked by a marker pen according to probable monogenetic trematodes. Monogenetic trematodes were identified up to species whenever possible.

Drawing and measurement of monogenean

Ten processed specimens were selected for measurement. Actual shape of the hard parts of monogeneans such as anchors hooks bars, copulatory complex, vaginal tube were drawn with the help of a camera lucida under microscope using various magnification of eye piece and objectives. Some microscopic photographs were taken by a camera attached to the microscope. The measurements were taken with the help of oculormicrometer adjusted with stage micrometer and the microscope. All the measurements are given in millimeters (mm), ranges in parentheses by the arithmetic mean and standard deviation unless other wise stated. For statistical analysis, morphometric measurements of 10 specimens for the species were considered. Measurements were done following the recommendations of Gussev (1976) and Chandra (2008).

Identification

Identification and classification of the parasites were done following Woo (1999) and Chandra (2008). The ecological terms for prevalence (%) and intensity of infestation were used after Margolis *et al*. (1982) as-

$$\text{Prevalence} = \frac{\text{No. of hosts infected}}{\text{No. of hosts examined}} \times 100$$

$$\text{Mean intensity} = \frac{\text{No. of parasites recovered}}{\text{No. of infected fish}}$$

$$\text{Abundance} = \frac{\text{No. of parasites recovered}}{\text{No. of host examined}}$$

Statistical analysis

Prevalences of monogenean parasites were computed by the following formula (Thrusfield, 1995): Chi-square and normal tests had been performed for testing the significance of the variation in prevalences of monogenea of different species, seasons and farms (Gupta, 2005). Comparison of two prevalences were made by normal test and chi-square test was used for examining the existence of overall significant differences among more than two prevalences, but their mutiple comparisons were done by Tukey-type test (Zar, 2003). In Tukey-type test, prevalences were transformed by the following arcsin transformation formula (Zar, 2003) due to its preference by many researchers:

$$P' = \frac{1}{2}\left[\arcsin\sqrt{\frac{X}{n+1}} + \arcsin\sqrt{\frac{X+1}{n+1}}\right]$$

P' = Transformed proportion

X = No. of infested fishes

n = No. of examined fishes

Mean intensity and abundance of monogenea 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

Seasonal changes in infestations of monogenean parasites in different carp host

The prevalence of monogenean parasites were found fluctuated in irregular pattern over the study period. The highest prevalence observed in rainy season in both Govt. and Pvt. farms. The highest prevalence found in rainy season in Mrigal and the lowest is found in summer in both Catla and Rui at Govt. farms. In case of Pvt. farms, the highest mean intensity and abundance of parasitic infestations were determined as 9.30 and 6.51 respectively for Mrigal in rainy season, but they were recorded as 7.22 and 4.43 in Govt. farms. Similar infestation was observed in other fish too. The prevalence of monogenean infestation differ insignificantly ($p>0.05$) among different species in each of the seasons in Govt. and Pvt. farms (Table 1).

Table 1. Seasonal infestations of monogenean parasites for combined 2 year data recorded in different carp species during June, 2010 to May, 2012

Seasons	Species	No. of host fishies		Total load of monogenea	Prevalence (%)	χ^2 (p-value)	Mean intensity	Abundance
		Examined	Infested					
				Govt				
Rainy	Catla	160	74	436	46.25	3.620	5.89^{bc}	2.73^{bc}
	Rui	160	86	596	53.75	(0.164)	6.93^{ab}	3.73^{ab}
	Mrigal	160	98	708	61.25		7.22^{a}	4.43^{a}
Winter	Catla	160	60	296	37.5	0.450	4.93^{bc}	1.85^{ac}
	Rui	160	66	412	41.25	(0.799)	6.24^{a}	2.58^{a}
	Mrigal	160	68	390	42.5		5.74^{ab}	2.44^{ab}
Summer	Catla	160	52	236	32.5	0.757	4.54^{bc}	1.48^{ac}
	Rui	160	60	354	37.5	(0.685)	5.90^{a}	2.21^{a}
	Mrigal	160	62	348	38.75		5.61^{ab}	2.18^{ab}
Total		**1440**	**626**	**3776**	**43.47**		**6.03**	**2.62**
				Private				
Rainy	Catla	160	90	624	56.25	4.927	6.93^{bc}	3.9^{c}
	Rui	160	114	896	71.25	(0.085)	7.86^{b}	5.6^{ab}
	Mrigal	160	112	1042	70.0		9.30^{a}	6.51^{a}
Winter	Catla	160	76	456	47.5	1.604	6.0^{ab}	2.85^{ac}
	Rui	160	84	468	52.5	(0.448)	5.57^{b}	2.93^{ab}
	Mrigal	160	92	602	57.5		6.54^{a}	3.76^{a}
Summer	Catla	160	68	378	42.5	1.636	5.56^{c}	2.36^{bc}
	Rui	160	78	574	48.75	(0.441)	7.36^{ab}	3.59^{ab}
	Mrigal	160	84	690	52.5		8.21^{a}	4.31^{a}
Total		**1440**	**798**	**5730**	**55.42**		**7.18**	**3.98**

Any two values of mean intensity and abundance in each season, having no common letter are significantly different at 5% level of probability.

Seasonal changes in infestations of monogenean parasites in carp host

In the case of seasonal variation of infestations of monogenean parasites in carp fishes showed that the highest prevalence (65.83%) has been recorded for rainy season followed by 52.5% and 47.92% for winter and summer, respectively in Pvt. farm. In case of Govt. farm, the highest prevalence (53.75%) has also been observed for rainy season followed by 40.42% and 36.25% for winter and summer, respectively. The mean intensity and abundance of this parasitic disease were attained as the highest for rainy season among the Govt. and Pvt. farms (Table 2). There had been a significant ($p<0.001$) difference among the seasonal prevalences of monogenea in juvenile carp fishes of Pvt. as well as Govt. farms.

Table 2. Seasonal variation of infestations of monogenean parasites of combined 2 years in carp fishes during June, 2010 to May, 2012

Farm category	Seasons	No. of host fishes		Total load of monogenea	Prevalence (%) (Transformed prevalence, %)[1]	χ^2 (p-value)	Mean intensity	Abundance
		Examined	Infested					
Govt.	Rainy	480	258	1740	53.75 (47.14^{a})	16.323***	6.74^{a}	3.63^{a}
	Winter	480	194	1098	40.42 (39.50^{b})	(0.000)	5.66^{b}	2.29^{b}
	Summer	480	174	938	36.25 (37.05^{bc})		5.39^{bc}	1.95^{bc}
Total		**1440**	**626**	**3776**	**43.47**		**6.03**	**2.62**
Pvt.	Rainy	480	316	2562	65.83 (54.19^{a})	16.831***	8.11^{a}	5.34^{a}
	Winter	480	252	1526	52.50 (46.43^{b})	(0.000)	6.06^{c}	3.18^{bc}
	Summer	480	230	1642	47.92 (43.81^{bc})		7.14^{b}	3.42^{b}
Total		**1440**	**798**	**5730**	**55.42**		**7.18**	**3.98**
Combined	Rainy	960	574	4302	59.80 (50.63^{a})	32.676***	7.49^{a}	4.48^{a}
	Winter	960	446	2624	46.46 (42.97^{b})	(0.000)	5.87^{bc}	2.73^{b}
	Summer	960	404	2580	42.08 (40.45^{bc})		6.39^{b}	2.69^{bc}
Total		**2880**	**1424**	**9506**	**49.45**		**6.67**	**3.30**

Level of Significance : *** $p<0.001$.

1Transformed prevalences are computed and compared (pairwise) only for significant chi-square values and any two of these prevalences in each farm category having no common letter are significantly different at 1% level of probability. In each farm category, any two values of mean intensity and abundance having no common letter are significantly different at 5% level of probability.

Changes in infestations of monogenean parasites in carp in different farms

Among the different Govt. and Pvt. farms the highest prevalence found in Pankouri Pvt. farm, mean intensity and abundance were found in Deshbandhu Pvt. farm and minimum prevalence, mean intensity and abundance were found in BFRI Govt. farm. The prevalence, mean intensity and abundance were found minimum in Govt. farm (Table. 3). The farm-specific prevalences of monogenea in carp fishes differ insignificantly ($p>0.05$) both in Govt. and Pvt. farms, but the prevalence of infestation in Pvt. farm (55.42%) significantly ($p<0.001$) higher than that of Govt. farm (43.47%) whereas the highest mean intensity and abundance were 7.18 and 3.98 respectively observed in Pvt. farms.

DISCUSSION

Monogenean infestations are very common in carps in Indian farms (Gussev, 1976; Tripathi, 1957) and South East Asian countries (Chinabut and Lim, 1993). During the investigation prevalence of this fluke in Indian major carps was quite high and fishes of larger size group were more susceptible. However, several authors have noted only the correlation between outbreak of monogenean infestations and stocking densities (Johnsen and Jensen, 1986). It was also observed in our study that monogenetic infestations were higher in case of the fishes collected from Pvt. fish farm as they culture with more density.

Table 3. Infestations of monogenean parasites in combined in both 2 years carp fishes in different farms during June, 2010 to May, 2012

Type	Name of the farm	No. of the host Fishies		Total load of monogenea	Prevalence (%)	χ^2 (p-value)	Z-statistic (p-value)	Mean intensity	Abun-dance
		Examined	Infested						
GOVT	BFRI	360	142	754	39.44	1.871	4.536***	5.31^{c}	2.09^{bc}
	Maskanda	360	156	1002	43.33	(0.600)	(0.000)	6.42^{ab}	2.78^{ab}
	Sombhugonj	360	166	1084	46.11			6.53^{a}	3.01^{a}
	Gouripur	360	162	936	45.00			5.78^{ac}	2.60^{ac}
Total		**1440**	**626**	**3776**	**43.47**			**6.03**	**2.62**
PVT	Brahmaputra	360	198	1402	55.00	0.062		7.08^{ac}	3.89^{ad}
	Deshbandhu	360	198	1464	55.00	(0.996)		7.39^{a}	4.07^{a}
	Pankouri	360	202	1448	56.11			7.17^{ab}	4.02^{ab}
	Sornalota	360	200	1416	55.56			7.08^{ac}	3.93^{ac}
Total		**1440**	**798**	**5730**	**55.42**			**7.18**	**3.98**

Level of Significance : *** p<0.001. Any two values of mean intensity and abundance in each farm, having a common letter are not significantly different at 5% level of probability.

Prevalence of monogenean parasites in carp fishes was significantly (p< 0.001) higher in Pvt. farm than that of Govt. farm. The prevalence of this parasitic disease was significantly (p< 0.01) higher in rainy season than that of the others. Mrigal was more infected by this parasite than Catla and Rui. Almost identical observation were made by Barai *et al*. (2005). The food of the monogenean parasites is host mucus, epithelium, and sometimes blood. It causes mortality in hatcheries, nurseries and culture ponds, thus resulting in great economic losses (Tripathi, 1957). Margaritov (l978) described age and seasonal variations of some *Dactylogyrus* species infection which caused weight loss and sometimes severe illness and death.

Rainy and winter months were the most susceptible period of the year when fish parasites are abundant. This could be due to stocking density, water depth, temperature along with other physico-chemical parameters and management practices maintained. Banu and Khan (2004); Mohan and Bhatta (2002) reported that monogeneans are some of the very important pathogens that have had significant impact on the yield in carp hatcheries and seed production centers.

In the present study, Pvt. farms were found to be more affected by parasitic diseases compared to Govt. farm. Among private farms, Sornalata was more infected and Brahmaputr Pvt. farm was less infected. In case of Govt. farms, Sombhugonj was more infected and BFRI Govt. farm was less infected by parasitic diseases. The causes of higher/lower infestation in prevarence mean intensity or abundance were different due to different nature of management practices like pond preparation, depth of water, transparency, stocking density, feeding of fry/fingerlings etc. Chandra (1987) stated that the unfavorable environmental/ ecological conditions caused variety of fish diseases. Because water qua1ity, pond condition, stress, environmental conditions and waste product are excess in Private Farm. Where as the pond condition were comparatively better in BFRI pond. Kiskivaura *et al.* (1991) observed the prevalence of *Dactylogyrus* infection in *Rutilus rutilus* always high in eutrophic and polluted lake than oligotrophic lakes.

Mean intensity and abundance is the highest in *C. cirrhosus* and *L. rohita* collected from private Fish Farm specially in Deshbandhu and lowest in *C. catla* collected from Govt. Fish Farm of BFRI. It might be due to higher stocking density in Pvt. Fish Farm. Several authors have noted the correlation between outbreak of disease and stocking density (Akter, 2007; Johnsen and Jensen, l986). Almost similar result was reported by Barai *et al*. (2005) and Bakshi *et al*. (2006) where they have studied parasitic infestation of indigenous major carp from different areas of Mymensingh district and observed maximum infestation in *C. cirrhosus* and *L. rohita*. This may be correlated with the bottom and middle dwelling habit of *C. cirrhosus* and *L. rohita* and its comparatively slower movement in the habitat which may allow ectoparasites quick transmission from one host to another.

During the period of investigation, infestation was changed with seasonal variation. The monogeneans infection was the highest in rainy and lowest in summer season. This result agrees with (Das, 2003; Banerjee and Bandyopadhyay, 2010). Golder *et al*. (l987) observed that the average incidence of infestation during winter season was lower. Barai and Chandra (2005) reported that the highest monogeneans infection in carp fry and fingerlings were in August and September because of the highest stocking density the highest average incidence of infestation found in summers and lower in winter. It could be due to higher stocking density, lower water depth and temperature rising just after winter. Hossain (2007) mentioned that increased occurrence of disease in the rainy season might again be due to unfavorable environmental condition.

CONCLUSION

The present study highlighted the infestation and disease problems, including their identity. Monogeneans were found mainly in gills. Infestation started at the early stage of fish life, highest infestation of monogeneans was found in Mrigal. Monogeneans infestation was a common problem of fish farming system both in nursery and culture ponds in Mymensingh region. Total fish production could be significantly increased by controlling the infestations and diseases.

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