

## Identification and Distribution of Parasites Associated with Freshwater Fishes in Agartala, India

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**Abstract:** The parasite fauna of fishes of Tripura was poorly known. The present investigation was undertaken during September, 2004 to August, 2005 and subsequently on September 2006 to August, 2007 with an aim to study the parasites associated with different freshwater fishes in Agartala. The study was carried out in total five fish farms and four fish markets. During study period total 15 parasites were identified from different fish and prawn samples. Additional parasites were also collected and kept for further study. These parasite species were distributed in different higher taxa viz., Myxozoa (Protozoa); Ciliophora (Protozoa); Platyhelminthes-Monogenea, Cestoidae & Digenea; Nematoda; Acanthocephala; Arthropoda-Crustacea. Water samples were also analyzed from different ponds of all the selected fish farms. It was recorded that water was slightly alkaline to moderately alkaline in reaction. In most cases total alkalinity was below the desired level of fish culture and other parameters were within the range. This study reveals that parasites are abundant in this part of the country in almost all water conditions.

**Key words:** Ecology • Fresh water • Fish parasite • Myxozoa • Monogenea • *Argulus*

### INTRODUCTION

Aquaculture has been an important vector in the introduction, transfer and spread of aquatic diseases and parasites [1]. The high risk of disease transmission and parasite infestations among species has increased the level of uncertainty which farm managers have to contend with to develop the industry [2]. The majority of the disease-causing pathogens are protozoans, monogenetic trematodes and parasitic crustaceans, most of which have direct life cycles and reproduce rapidly under unfavorable pond conditions [3, 4]. Tripura is the second smallest hilly and landlocked state of the north-eastern region. But when fish is of concern Tripura stood first in fish consumption in the country, where 95% of population are fish eater. There exists a huge demand for fish, though there is a big gap between supply and demand of fish. Preliminary observation revealed that parasitic diseases are prevalent in the aquaculture system in Tripura. Till today no systematic research work on fish disease has

been carried in Tripura except some sporadic reports by different authors [5, 6, 7, 8]. There are number of important groups under metazoa viz., digenea, hirudinea (leeches), lernaeidae (copepod), argulidae (branchiura), isopods, acarina, pentastomid larvae, larvae of bivalve mollusks etc. and also protozoa to be investigated systematically in different aquaculture systems in Tripura. With this background the present research project was undertaken to make data base about parasite species of fresh water fish in Agartala for future work.

### MATERIALS AND METHODS

For the present study total five sampling stations were selected in and around Agartala. Apart from this, four fish markets were also surveyed for the fish parasites. Detailed information about all the sampling stations was noted in Table 1. To achieve the targeted objectives different procedures were adopted as demonstrated by different authors.

Table 1. Information regarding study sites.

Site No.	Name of the Site	Status	Farm bio-data	Remark
A.	Lembucherra Fish Seed Farm	Govt. Farm	Area: 6.42ha. Nursery- 5 nos. (0.40ha) Rearing- 6 nos. (0.76ha) Lake 1- 1.6 ha. Lake 2 & 3-0.95 ha.	Produce IMC & Chinese Carp seed, fry, fingerlings as well as grown table fishes in the lake.
B	Ramnagar Fish Farm	Govt. Farm	Area: 6.5 ha. No. of Ponds:12	Produce IMC & Chinese Carp fry, fingerlings as well as grown table fishes.
C	Rangamoyee Fish Breeding Farm	Private Farm	Area: 25 Acres No. of Ponds: 23 Chinese Hatchery: 4 sets	Produce IMC & Chinese Carp seed as well as grown table fishes.
D	College Tilla Fish Farm	Co- operative	Area: 3 ha. No. of Ponds: 2	Under Composite Fish Culture.
E	Central Jail Fish Farm	Govt.	Area: 1 ha. No. of Ponds: 2	Under Integrated Fish Culture.
F	Fish Markets in and around Agartala. (viz., Battatala, Golbazar, Lake Chwmohani and Durga Chowmohai)			

Mostly live or fresh fishes were collected from the above mentioned sites and brought to the laboratory of Department of Fish Health and Environment, College of Fisheries, Tripura in individual plastic buckets (containing 1-5 fishes each depending on size), where the examination were done by Whole Fish Survey and Autopsy survey [9, 10].

In whole fish survey, fish body surfaces and gills were checked with necked eye for larger ectoparasites and then examination of gills and external surfaces were done at 10X with a dissecting microscope. Scraped mucus from body surface of the fish were also examined for parasites (under the pectoral fin is a good place to look). Mucus from the margin of lesion if any were also taken and scanned through 10X, 100X, 400X. Operculum was removed with scissors carefully and gills were studied for attached parasites. Moreover, small filaments from outer gill arch were also taken out and placed in several drops of filtered pond water between a slide and cover slip and observed under microscope for attached parasites. Further, investigation of the mouth for visible parasites was also done by scrapping the roof of the mouth for a smear. Collected parasites were preserved as per standard methods [9, 10] in the sample bottles with label for later study.

In coelom / autopsy survey, coelom was cut opened by making surface cut from the anus forward to an imaginary line at the posterior portion of the operculum. Then the entire side of the coelom was cut out by cutting a rectangle of skin from behind the operculum, anterior to the anus and ventral to the backbone. After exposing the viscera, observation was done for any abnormal appearance, colour, fluid accumulation, positioning, size changes, or for the presence of cysts, worms, or other parasites. Then internal organs were removed, separated and dissected for further examination. The small and large intestines were cut out and flush out the inside material using a wash

bottle so that tapeworms and other parasites were come out. Eyes and brain were also removed and examined. Wet mounts of suspected tissues and cysts were made to examine any infestations. Collected parasites were preserved in the sample bottles and were identified following standard keys [9-13].

In water quality study, water quality parameters viz., water temperature, pH, alkalinity and dissolved oxygen (DO) were analyzed. Water and air temperatures of respective sites were measured by Hand Held Digital Thermometer (-50°C to 300°C) and also recorded. The pH of water samples of all sites were noted by the help of pH Scan WP 2 (Eutech Instruments). Alkalinities of water of all the spots were measured by following titration method [14]. DO of water samples were measured by portable DO meter 312 (Systronics).

## RESULTS AND DISCUSSION

Different water quality parameters viz., water temperature, pH, CO<sub>2</sub> Alkalinity, HCO<sub>3</sub> Alkalinity and DO of all the sampling stations (A to E) were recorded in Table 2. In each farm (site), three stations (ponds) were selected except station D and E where both are the only available stations (ponds). In each station three sampling spots were selected for monitoring the water quality parameters. 14 fish parasites were encountered during the sampling period and compiled in Table 3. From the table it was found that all the parasite species were distributed in 6 different higher taxa viz., Myxozoa (Protozoa); Ciliophora (Protozoa); Platyhelminthes- Monogenea, Cestoidae & Digenea; Nematoda; Acanthocephala; Arthropoda-Crustacea. Out of these *Argulus* sp., *Dactylogyrus* sp., *Palaegyge* sp., *Thelohanellus rohita*ae. *Camallanus anabantis*, *Spirocamellanus gubernaculus* and *Neothelohanellus catlae* were very common. Some of the parasites photographs were also presented in the Plate I - III.

Table 2: Physicochemical conditions of water of all the sites

Name of the Site	Parameter	Station 1	Station 2	Station 3
A] Lembucherra Fish Seed Farm	Water Temperature (°C)	28.6-38.6	27.8-39.3	26.8-39.0
	Air Temperature (°C)	29.4-36.2	27.2-36.7	26.6-36.8
	pH	6.4 - 9.6	7.1-9.5	7.3-9.5
	CO <sub>3</sub> Alkalinity (mgL <sup>-1</sup> )	00.00-14.7	00.00-30.38	00.00-33.32
	HCO <sub>3</sub> Alkalinity (mgL <sup>-1</sup> )	11.00-26.46	3.92-35.28	3.92-19.6
	DO (mgL <sup>-1</sup> )	7.6-11.3	7.6-10.6	4.99-11.6
B] Ramnagar Fish Farm	Water Temperature (°C)	26.8-35.0	26.5-34.9	27.2-33.6
	Air Temperature (°C)	22.2-37.0	23.4-34.5	25.5-34.6
	pH	7.2-8.5	7.1-8.4	7.1-8.1
	CO <sub>3</sub> Alkalinity (mgL <sup>-1</sup> )	00.00	00.00	00.00
	HCO <sub>3</sub> Alkalinity (mgL <sup>-1</sup> )	17.64-147.00	26.46-62.72	23.52-61.74
	DO (mgL <sup>-1</sup> )	3.8-8.4	5.1-11.1	3.8-9.1
C] Rangamayee Fish Breeding Farm	Water Temperature (°C)	26.5-36.3	27.9-35.3	28.8 - 36.1
	Air Temperature (°C)	25.2-34.8	25.6-34.7	23.4-33.0
	pH	7.7-9.2	7.7-9.6	7.4-9.5
	CO <sub>3</sub> Alkalinity (mgL <sup>-1</sup> )	00-10.78	00-9.8	00- 27.44
	HCO <sub>3</sub> Alkalinity (mgL <sup>-1</sup> )	16.6-74.48	14.7-73.5	12.74-79.38
	DO (mgL <sup>-1</sup> )	6.0-10.2	5.9-12.3	6.7-11.7
D] College Tilla Fish Farm	Water Temperature (°C)	26.5-34.7	27.0-35.3	--
	Air Temperature (°C)	26.9-36.6	27.1-38.5	--
	pH	7.8-9.0	7.4-8.6	--
	CO <sub>3</sub> Alkalinity (mgL <sup>-1</sup> )	0.0-3.92	0.0-1.96	--
	HCO <sub>3</sub> Alkalinity (mgL <sup>-1</sup> )	14.7-37.24	10.78-42.14	--
	DO (mgL <sup>-1</sup> )	6.7-10.3	6.0-11.2	--
E] Central Jail Fish Farm	Water Temperature (°C)	27.52-33.6	27.1-34.0	--
	Air Temperature (°C)	27.0-34.0	27.0-34.6	--
	pH	7.8-8.9	7.7-8.5	--
	CO <sub>3</sub> Alkalinity (mgL <sup>-1</sup> )	00.0-10.78	00.0-00.0	--
	HCO <sub>3</sub> Alkalinity (mgL <sup>-1</sup> )	15.68-63.7	43.12-112.7	--
	DO (mgL <sup>-1</sup> )	5.6-9.1	3.7-8.7	--

Table 3: List of Parasites found in different Fishes

Station No	Name of the Parasite with Major Taxa	Common Name	Host	Site
Myxozoa (Protozoa)				
1	<i>Neothelohanelius</i> sp.	Myxozoan spore	<i>Catla catla</i>	Gills
2	<i>Thelohanellus rohita</i>	Myxozoan spore	<i>Labeo rohita</i>	Gills
3	<i>Neothelohanelius catlae</i>	Myxozoan spore	<i>Catla catla</i>	Gills
Ciliophora				
4	<i>Trichodina</i>	Urceolariid ciliates	<i>Catla catla</i> , <i>Cirrhinus mrigala</i> , <i>Labeo rohita</i>	Gills
Platyhelminthes (Flat Worms)				
Monogenea				
5	<i>Dactylogyrus</i> sp.	Gill Fluke (Monogenea)	<i>Catla catla</i> , <i>Labeo rohita</i>	Gills
6	<i>Gyrodactylus</i> sp.	Monogenea	<i>Catla catla</i> , <i>Labeo rohita</i>	Gills
Digenea (Trematoda)				
7	<i>Orientocreadium batracoides</i>	Digenea Trematodes	<i>Clarias batracus</i>	Intestine
8	<i>Galactosomum anguillarum</i>	Digenea Trematodes	<i>Clarias batracus</i>	Intestine
Cestodia (Cestoda)				
9	<i>Lytocestus parvulus</i>	Cestode	<i>Clarias batracus</i>	Intestine
Nematoda (Threadworms)				
10	<i>Camallanus anabantis</i>	Threadworms	<i>Anabas testudineus</i>	Body cavity
11	<i>Spirocamellanus gubernaculus</i>	Threadworms	<i>Notopterus notopterus</i>	Intestine
Acantocephala (Thorny-headed worm)				
12	<i>Pallisentis</i> sp.	Thorny-headed worm	<i>Channa</i> sp.	Intestine
Arthropoda				
Crustacea				
13	Branchiura <i>Argulus</i> sp.	Carp Lice	<i>Labeo rohita</i>	Skin
Isopoda				
14	<i>Palaeogyge</i> sp.	Isopods	<i>Macrobrachium</i> sp.	Gills

Plate I

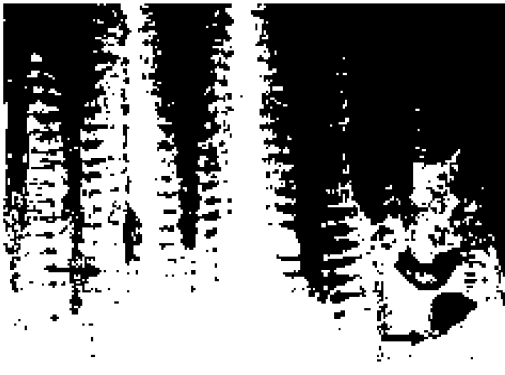


Fig.1: Wet mount of gills showing monogeneans on gills. (40x)



Fig. 2: Wet mount of gills showing *Tricodina* sp. (100x)



Fig.3: *Camallanus anabantis* (40x)

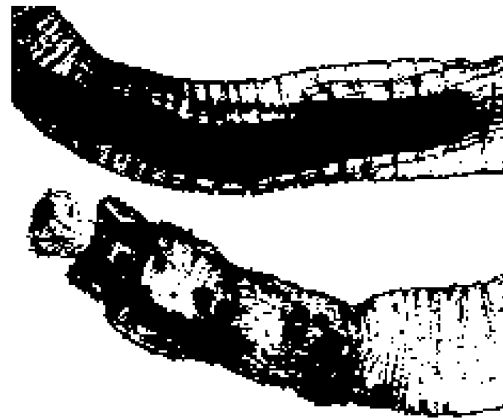


Fig. 4: *Palliserius* sp. (40x)



Fig. 5: *Neothelohanellus cathe* (Giemsa stain 200x)

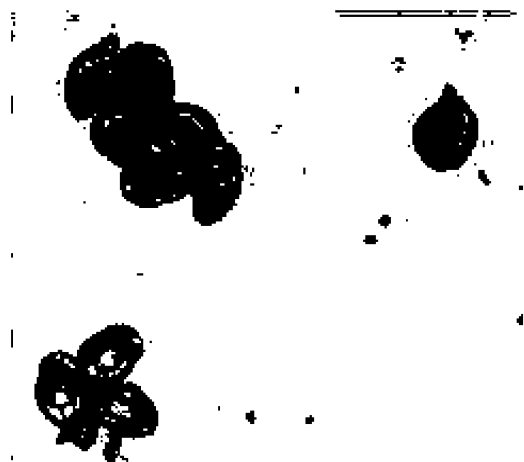


Fig 6: *Thelohanellus rohine* (Giemsa stain 200x)

Plate II



Fig. 7: *Palaegyge* sp. (Isopod infection)



Fig. 8: White gill spot disease in *Catla catla*.



Fig. 9: Collection of *Argulus* sp.



Fig. 10: *Argulus* sp.



Fig. 11: *Dactylogyrus* sp.

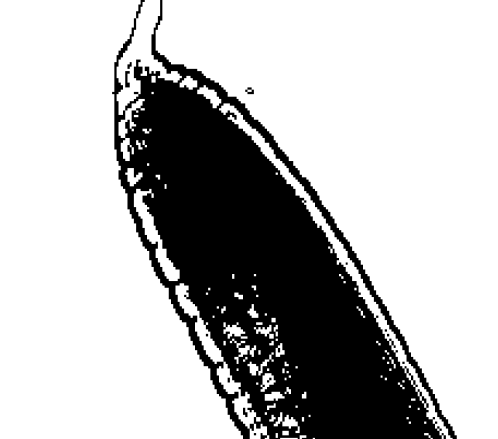


Fig. 12: *Spirocamallanus gubernaculus*

Plate III

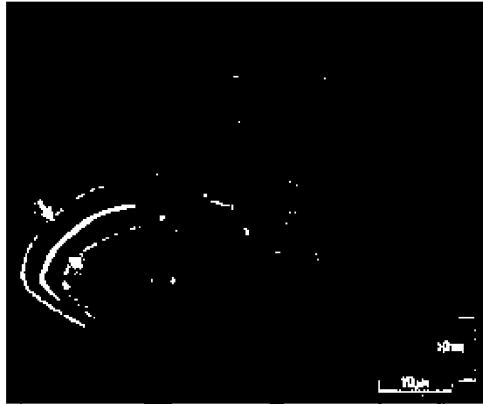


Fig. 13: Monogenean leptors (14) and anchors (Borax Carmine)



Fig. 14: Bar, anchors and 12 leptors of monogenean from gill of rohu fry (Borax Carmine)



Fig. 15: *Gyrodactylus* sp. with developing embryo (arrow) from gills of rohu (Borax Carmine)

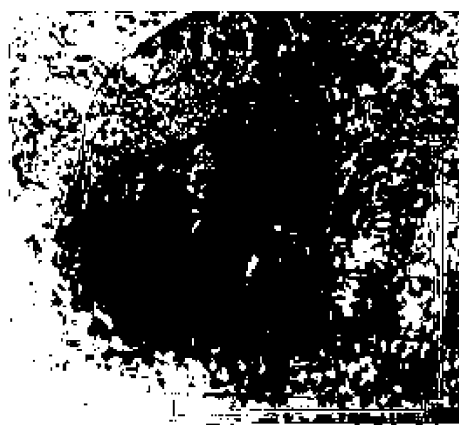


Fig. 16: Mating behaviour of *Dactylogyrus* sp. (arrow) in gills of rohu

Findings of the parasites in the 5 water bodies may indicate their wider distribution than the previously recognized in the northeastern region of India [5-8]. Moreover, the study reveals that parasites are abundant in well managed pond in spite of normal water quality (Table 2). The prevalence of different parasites in the fish farm ponds might be due to improper stocking density. The findings also in agreement of previous study [7]. Moreover, the parasite infestations in these culture systems resulted in less fish production. In fact infected brood stocks might transfer ectoparasites from farm to farm when their fry or fingerlings are used to stock un-infected

ponds [15, 16]. Among the monogenea, *Dactylogyrus* spp. was mostly observed in gills as compared to *Gyrodactylus* spp. which corroborate the earlier view [17]. Monogeneans, mainly *Dactylogyrus* spp. produce free-swimming larvae that facilitate their transmission in ponds and hence their intensities are more in gills [18]. Several classical works on monogeneans have reported that parasites are easily attracted to their specific host by means of detecting chemical substances released from the host [19]. This might confirmed the present findings in which the monogeneans were more prevalent in the gill than the other ectoparasites like Tricodinians and Myxosporeans.

The present study concludes that among the different parasites *Argulus* sp., *Dactylogyrus* sp., *Thelohanellus rohita* and *Neothelohanelus catlae* are very common even in the well managed farm ponds. However, further investigations should be carried out to clarify the epidemiology of different fish parasites in this part of India and be extended to encompass the study of genetic variations of those parasite populations in different species of definitive hosts.

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#### REFERENCES

- Klinger, R. and R.F. Floyd, 2002. Introduction to freshwater Fish Parasites, pp: 1.
- Pozio, E. and G.L. Rosa, 2005. Evaluation of the infectivity of *Trichinella papiae* and *Trichinella zimbabwensis* for equatorial fresh water fishes. *Veterinary Parasitol.*, 132: 113-114.
- Al-Rasheid, K.A.S., M.A. Ali, T. Sakran, A.A. Abdel Baki and F.A. Abdel Ghaffar, 2000. *Trichodinid ectoparasites* (Ciliophora: Peritrichida) of some River Nile fish, Egypt. *Parasitol. Int.*, 49: 131-137.
- Basson, L. and J.G. Van As, 1994. *Trichodinid ectoparasites* (Ciliophora: Peritrichida) of wild and cultured freshwater fishes in Taiwan, with notes on their origin. *Systematic Parasitology* (Historical Archive), 28: 197-222.
- Das, M.K. and R.K. Das, 1995. Fish Diseases in India a review. *Env. and Ecol.*, 13(3): 533-541.
- Saha, Ratan K. and Bagchi, Shubhashish, 2004. Occurrence of fish diseases in Tripura- a report. In: National Symposium on Disease problems in Aquaculture: Challenges, Approaches and management (6-7 Feb., 2004), CIFE, Mumbai.
- Saha, H., 2009. Occurrence, distribution and pathology of monogenetic gill parasites in *Labeo rohita* (Hamilton), M. F. Sc. Thesis, College of Fisheries, Central Agricultural University, Tripura, India.
- Saha, R.K. and D. Kamilya, 2010. Histology of monogenean infected gills of *Labeo rohita* (Ham), CAU Research New Letter, Directorate of Research, CAU, Imphal, India, 1(1): 15.
- Kabata, Z., 1985. Parasites and Diseases of Fish Cultured in the Tropics. Taylor and Francis, pp: 318.
- Lucky, Z., 1977. Methods for the Diagnosis of Fish Diseases. Ed. G. L. Hoffman. Amerind Publishing Co. Pvt. Ltd., pp: 140.
- Paperna, I., 1996. Parasites, infections and diseases of fishes in Africa- An up-to-date. CIFA Technical Paper No. 31, Rome, FAO, pp: 220.
- Roberts, R.J., 2001. Fish Pathology. W. B. Saunders, pp: 472.
- Williams H. and A. Jones, 1994. Parasitic Worms of Fish. Taylor and Francis, pp: 584.
- APHA. 1995. Standard Methods for the Examination of water and Wastewater, 20<sup>th</sup> ed., APHA-AWWA-WEF, Pub: APHA, Washington, DC 20005-2605.
- Bondad-Reantaso, M.G., R.P. Subasinghe, J.R. Arthur, K. Ogawa, S. Chinabut, R. Adlard, Z. Tan and M. Shariff, 2005. Disease and health management in Asian aquaculture. *Veterinary Parasitol.*, 132: 249-272.
- Murray, A.G. and E.J. Peeler, 2005. A framework for understanding the potential for emerging diseases in aquaculture. *Preventive Veterinary Med.*, 67: 223-235.
- Koskivaara, M. and E.T. Valtonen, 1992. *Dactylogyrus* (Monogenea) communities on the gills of roach in three lakes in Central Finland. *Parasitologia*, 104: 263-272.
- Whittington, I.D., B.W. Cribb, T.E. Hamwood and J.A. Halliday, 2000. Hostspecificity of monogenean (platyhelminth) parasites: a role for anterior adhesive areas? *International J. Parasitol.*, 30: 305-320.
- Wiegertjes, G.F. and G. Flik, (Eds), 2004. Host-Parasite Interactions. Garland Science / BIOS Scientific Publishers, The Netherlands.