

## Study on certain fungal diseases of indigeneous fishes of river Churni, West Bengal

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### ABSTRACT

Fishes play an important role in national productivity, socio-economic development and renewable aquatic living sources. Diseases cause economic losses in aquaculture. Fungal infections are second most prevalent diseases after bacterial diseases in economic importance. The aim of this study was to investigate the increasing aquatic fungal flora of the river Churni and the percentage of infection of various species in fishes. Investigation was carried out on fungal infected fishes from November 2014 to April 2015. A total number of 1566 fishes were examined. Out of them, about 210 fishes were found infected. The most affected species of fish were *Clarius batrachus* (26.25%) and *Heteropneustes fossilis* (22.28) followed by *Puntius sarana* (19.63). Total 15 species of fishes found infected from which 17 isolates have been found out, which belong to 8 genera and 17 species. Cultures were made on hempseeds, sesame and mustard seeds. Pure cultures were made on Sabouraud dextrose agar, glucose yeast agar and potato dextrose agar. Identification of various species was done on the basis of their vegetative and reproductive characters. During investigation period, among all the 8 genera, *Achlya* and *Saprolegnia* found more frequently and most virulent. Maximum percentage of infected fishes found in December (36.8%) followed by November (27.2%) and January (24.8%). The most prevalent form of fungus belongs to genera *Achlya* and seven species of this genera were found to cause diseases. *Aspergillus niger* and *Fuzarium sp.* were isolated first from fungal infected fishes of this region. To find out the pathogenicity of these species further investigation was needed.

**Keywords:** Churni river, Water mould, Parasitic activity and infected fishes.

### INTRODUCTION

Fish disease is a great threat limiting the economic rise in aquaculture. Both fresh water fishes and brackish water fishes were prone to fungal infection results in economic losses. Fungi that affect the fishes are considered opportunistic, attacking the fish body under stress due to unfavorable environmental conditions or attacked by any other pathogen. There are several reports of oomycetes as primary infectious agents of fishes, (Pickering and Christie, 1980). Oomycetes (zoosporic fungi) mainly spreads through swimming zoospores. Plants and animal remains in aquatic ecosystem are mainly decomposed by oomycetes. They found to be harmful to fish industry as they are parasitic to fishes. Khulbe, 1983; Aderman and Polyase, 1986; Khulbe *et al.*, 1995; Czeuczuga and Muszynska, 1999 and West, 2006 reported large number of oomycetes are parasitic to fish are mainly belong to family Saprolegniaceae. Saprolegniales are considered as opportunistic parasite, as many of them possess virulence factors which enable them to cause diseases. *Saprolegnia* and *Achlya* are most common genera in causing fish mycoses. Infections caused by *Achlya* have been reported by Chidamparam, 1942; Vishniac and Nigrelli, 1957; Scott and O' Bier, 1962; Gopal and Krishnan, 1964.

### MATERIALS AND METHODS

#### **Study Sites**

The survey was conducted in the 3 study areas (stations) of river Churni during November 2014 to April 2015. river Churni, one of the important tributaries of river

Mathabhanga, emerges at Krishnaganj, Nadia (West Bengal) flowing about at 53 kms, it finally confluences to river Bhagirathi- Hooghly near Mangaldeep, Payradanga of district Nadia. Latitude and longitude of three sites of the sampling sites are S1: Mamjoan, Nadia (23°30'E, 88°58'N), S2: Gajantala, Nadia(23°15'E, 88°53'N), S3: Kayet Para and Nadia(23°19'E, 88.56'N).

### **Mycopathological Investigation**

A total of 1566 infected individuals from each major contributing species were collected randomly from fish catching sites of the river and also from local market of the sampling sites. The infected fishes were identified by physical symptoms like decolouration of the skin, appearance of cotton wool cover of the fungus mycelia, hemorrhagic lesions on body and excess mucus secretion. Fungal tufts covered the skin, fins, gills, eyes of the fish and complete fish eggs. Infected fishes were found by naked eyes and by using hand lens.

### **Isolation of fungi from infected fishes**

Isolation of fungi from infected fishes was carried out by taking small pieces from muscles about 2 mm in diameter from different portions of body and washed thoroughly with distilled water. These tissues were then inoculated over plates containing different agar media like PDA, GY agar and SPS agar. To inhibit bacterial growth, 500 mgml<sup>-1</sup> each of penicillin and streptomycin was added to the medium. The isolates were incubated at 18<sup>0</sup>C – 22<sup>0</sup>C for 4-5 days until fungal mat developed. Isolates were then used to make pure cultures.

### **Purification of cultures**

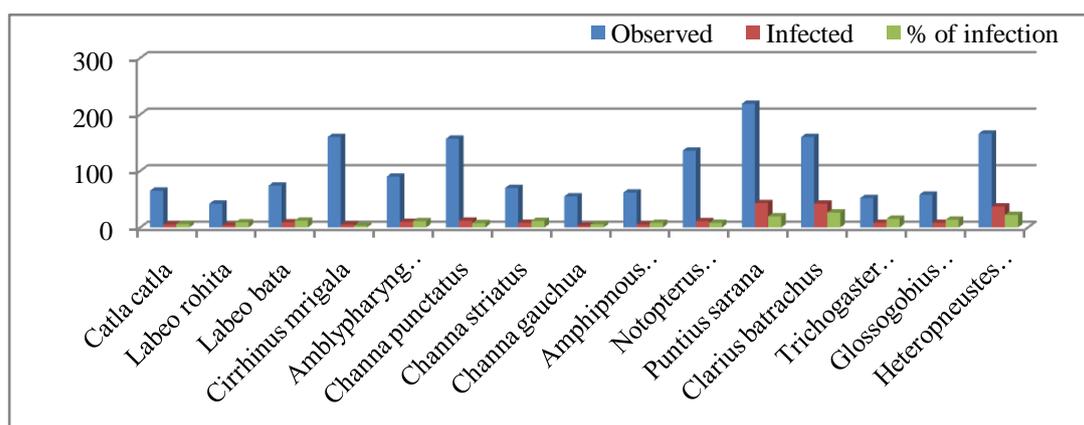
Pure cultures were prepared by taking small piece of media with fungal growth and transferred to the plates containing baits like hempseeds, sesame seeds, mustard seeds and broken pulses with sterile tap water. These cultures were incubated at 18<sup>0</sup>C-22<sup>0</sup>C for growth of colony on bait and development of sexual characters for identification. Isolated fungi were identified and the slides were observed under microscope. Identification of fungi was carried out on the basis of keys of Nelson *et al.* (1983), Raper and Fenneu, (1965), Refai *et al.* (1987), Willoughby (1994), Khulbe (2001) and Shrivastava (2009). Fishes were identified by the keys of Jhingran (1982) and Qureshi and Qureshi (1983).

## **RESULTS AND DISCUSSION**

In the present study, total 1566 fishes were examined out of which 210 were found to be infected with fungal disease. Fifteen species of fish's viz. *Catla catla*, *Labeo bata*, *Cirrhinus mrigala*, *Amblypharyngodon mola*, *Channa punctatus*, *Channa striatus*, *Channa gauchua*, *Amphipnous cuchia*, *Notopterus notopterus*, *Puntius sarana*, *Clarius batrachus*, *Trichogaster fasciatus*, *Glossogobius guiris*, *Heteropneustes fossilis* were found infected. Maximum infections were found in *C. batrachus* (24.6%) followed by *P. sarana* (19.1%), *M. cavasius* (14.7%) and *C. ranga* (14.5%). (Table 1). Here we have also succeeded to find the variation of *Achlya* species among the infected fishes. Prevalence of different fungal species in different fish species were reported. (Table 2) From the various species of fishes examined seven different species of *Achlya* were isolated namely, *A. americana*, *A. flagellata*, *A. hypogyana*, *A. klebsiana* and *A. prolifera*. *Achlya conspicua* *Achlya apiculata* (Table 3).

**Table 1**  
Total number and percentage of fungal infected fishes during sampling period

Sl No.	Host Fish	Observed	Infected	% of infection
1	<i>Catla catla</i>	65	5	5.9
2	<i>Labeo rohita</i>	42	4	9.07
3	<i>Labeo bata</i>	74	9	12.16
4	<i>Cirrhinus mrigala</i>	160	5	3.1
5	<i>Amblypharyngodon mola</i>	90	10	11.11
6	<i>Channa punctatus</i>	157	12	7.64
7	<i>Channa striatus</i>	70	8	11.42
8	<i>Channa gauchua</i>	55	3	5.45
9	<i>Amphipnous cuchia</i>	62	5	8.06
10	<i>Notopterus notopterus</i>	136	11	8.08
11	<i>Puntius sarana</i>	219	43	19.63
12	<i>Clarius batrachus</i>	160	42	26.25
13	<i>Trichogaster fasciatus</i>	52	8	15.38
14	<i>Glossogobius guiris</i>	58	8	13.79
15	<i>Heteropneustes fossilis</i>	166	37	22.28
<b>Total</b>		<b>1566</b>	<b>210</b>	<b>13.40</b>



**Fig. 1: Graphical Representation of Disease Infected Fishes**

**Table 2**  
Occurances of Different fungal species in infected fishes during sampling period

Sl No.	Host Fish	Nov.	Dec.	Jan.	Feb.	Mar.	Apr
1	<i>Catla catla</i>	<i>Asp.ni</i>	<i>A.pr</i>	<i>A.pr</i> -	-	-	-
2	<i>Labeo rohita</i>	-	<i>A.hy</i>	<i>A.pr</i>	<i>A.pr</i>	-	-
3	<i>Labeo bata</i>	-	<i>Py</i>	<i>Py</i>	-	-	-
4	<i>Cirrhinus mrigala</i>	-	<i>A.api</i>	<i>A.pr</i>	-	-	-
5	<i>Amblypharyngodon</i>	-	-	-	-	<i>A.co</i>	<i>A.con</i>
6	<i>Channa punctatus</i>	-	<i>A.api</i>	<i>A.k-</i>	<i>S.p</i>	<i>S.p</i>	<i>Aph.lev</i>
7	<i>Channa striatus</i>	-	<i>A.pr</i>	<i>All.an</i>	<i>S.h</i>	-	-
8	<i>Channa gauchua</i>	-	<i>Dy.ac</i>	<i>Dy.ac</i>	-	-	-
9	<i>Amphipnous cuchia</i>	-	-	<i>Fu.sp</i>	<i>Fu.sp</i>	-	-
10	<i>Notopterus notopterus</i>	-	<i>Py</i>	<i>Py</i>	-	-	-
11	<i>Puntius sarana</i>	<i>A.am</i>	<i>A.fl</i>	<i>A.fl</i>	<i>A.fl</i>	-	-
12	<i>Clarius batrachus</i>	<i>A.hy</i>	<i>S.f</i>	<i>S.f</i>	<i>A.pr/</i>	-	-
13	<i>Trichogaster fasciatus</i>	-	<i>A.pr</i>	<i>A.pr</i>	<i>Py.un</i>	<i>Py.u</i>	-
14	<i>Glossogobius guiris</i>	-	-	<i>Py.un</i>	<i>Py.un</i>	-	-
15	<i>Heteropneustes fossilis</i>	<i>Aph.lev</i>	-	<i>S.h</i>	<i>S.h</i>	<i>S.h</i>	-

Note: A.am.- *Achlya americana* , A.api.-*Achlya apiculata*, A.con.- *Achlya conspicua*, A.pr. *Achlya proliferata*, A.fl-*Achlya flagellata*, A.hy -*Achlya hypogyana*, A.kl –*Achlya klebsiana* , All.an.- *Allomyces anomalus*, Asp.ni.- *Aspergillus niger*, Aph.lev.-*Aphanomyces laevis*, Dy.ach.-*Dyctiuchus achlyoides*, Fu.sp.-*Fusarium sp.*, Py aph. *Pythium aphanidermatum*, Py.un.-*Pythium undulatum*, S. f.-*Saprolegnia ferax*, S.h.-*Saprolegnia hypogyana*, S.p.-*Saprolegnia parasitica*

**Table 3**  
**Seven different species of zoosporic fungi ( *Achlya*) found associated with various diseased fishes.**

Sl. No.	Isolated Fungal sp.	Various species of naturally infected fishes
1.	<i>Achlya americana</i>	<i>Channa punctatus</i> , <i>Cirrhinus mrigala</i> , <i>Clarias batrachus</i> , <i>Nandus nandus</i> , <i>Trichogaster fasciatus</i>
2.	<i>Achlya flagellata</i>	<i>Puntius sarana</i> .
3.	<i>Achlya hypogyana</i>	<i>Clarias batrachus</i> , <i>Labeo rohita</i> , <i>Nandus nandus</i> , <i>Notopterus notopterus</i>
4.	<i>Achlya klebsiana</i>	<i>Channa punctatus</i> .
5.	<i>Achlya proliferata</i>	<i>Catla catla</i> , <i>Channa striatus</i> , <i>Cirrhinus mrigala</i> , <i>Clarias batrachus</i> , <i>Trichogaster fasciatus</i> .
6.	<i>Achlya conspicua</i>	<i>Clarias batrachus</i>
7.	<i>Achlya apiculata</i>	<i>Cirrhinus mrigala</i> , <i>Channa punctatus</i>

In the month March and April the disease incidence was found to be negligible. The infection begins with the monsoon during June and was wide spread in the month of September, October, November and December. The infection was found to be at its peak in the month of December and January. The relation of some physicochemical parameters of the riverine ecosystem with disease incidence is also depicted. The peak of disease incidence was found to more when the temperature was found to be low i.e., in the months of December and January (23<sup>o</sup>c) and the incidence was found to be retarded during the months of April and May when the temperature was found to be high. During monsoon and post monsoon due to the neutral pH (7.0) and high amount of Dissolved oxygen (DO, 8.6 mg/l) and low Biological oxygen demand (BOD, 2.44 mg/l) favor the incidence. It was observed that fish of all ages got infected with fungal disease irrespective of their age and size. In the present study maximum infection was found in catfishes which get the support from the earlier findings (Robert and Jeffery, 2003).

### **CONCLUSION**

From the above depicted table (table-2) we can conclude that the *Achlya* Sp, and *Saprolegnia* were found to be the most dominant in the riverine eco system than the other fungi. It shows that maximum activity has occurred during winter when compared to the other two seasons. The infection of these fungi on the fishes causes mycosis which leads to loss of normal glaze, descaling and formation of lesions with cotton wolly outgrowth. The genus *Achlya* was found to be more virulent compared to other genus. The species *Achlya americana* were found to be more virulent, about 30% of infection was caused y these fungi. These fungi formed the surface lesions on the host with cotton wolly out growth structure than the other Species. *Saprolegnia parasitica* invaded the host tissue within three days and formed the lesion outgrowth for early infected. It shows that low temperature is conducive for parasitic activity of fungi. In the present study, we succeeded to find out 17 different species of fungi which shows fungal disease is a serious threat to aquaculture.

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