Bangladesh J. Fish. Res., 4(2), 2000: 171-177

Pathology of mixed infections of saprolegniasis-myxosporidiosis in Indian major carp (*Catla catla* Ham.)

P.K. Sahoo*, S.C. Mukherjee, S.K. Nayak and S. Dey

Aquatic Animal Health Division, Central Institute of Freshwater Aquaculture
Kausalyaganga, Bhubaneswar 751 002, India
**Corresponding author*

Abstract

An outbreak of saprolegniasis in *Catla catla* in composite carp culture ponds were recorded during winter season. The typical cotton wool growths were observed on whole body surfaces of catla along with sporadic mortality. The fungal invasion was only restricted to skin and no fungal elements were visible in any internal organs after periodic acid schiff staining. On histology, periportal accumulation of mononuclear cells in liver, presence of myxosporidean cysts in antieror kidney, eosinophilic granular cells reaction in submucosa of stomach and intestine, dilated and engorged blood vessels of brain along with sloughing of epidermis and hyperplasia at gill lamellar base were pronounced changes. The possible role of release of *Saprolegnia* toxin in producing internal organs pathology has been discussed .

Key words: Saprolegniasis, Myxosporidiosis, *C. catla*

Introduction

Fungal infections of carp are serious problem in intensive system of aquaculture. Winter saprolegniasis (Winter Kill) is a serious disease affecting pond raised carps that produces clinical signs of growth of conspicuous fungal colonies growing on body surface of the fish. These cotton-wool like lesions are normally white in colour, or discoloured by the accumulation of debris between the fungal hyphae (Jeney and Jeney 1995). The fungal growths on the skin were generally thought to be secondary to primary skin/scale erosion and some predisposing factors. However, recent studies have demonstrated that winter kill of channel catfish resulted from an immunosuppression caused by a rapid decrease in water temperature in presence of zoospores of the oomycete *Saprolegnia* sp. (Bly and Clem 1991, Bly *et al*, 1992 & 1993).

The primary sequela of uncomplicated saprolegniasis is osmotic imbalance due to loss of epithelial integrity (Noga 1993). Histopathological changes described due to saprolegniasis in common carp was mostly restricted to epidermis and dermis, being characterised by degeneration of epidermal cells, focal oedema in dermis and ultimate sloughing of epidermis without any inflammatory reaction at the site of infection (Jeney and Jeney 1995). However, Alvarez *et al.* (1988) found damage to hemopoietic organs

with lymphoid cell degeneration, and vascular alterations with blood vessel enlargement and hypertrophy of sinusoidal endothelial cells of liver in brown trout due to saprolegniasis. Surprisingly, Duran *et al.* (1987) could able to detect extreme increase in liver specific enzymes with slight elevation of muscle enzymes in brown trout infected with saprolegniasis. The above inconsistent findings prompted us to study the damage caused due to saprolegniasis in internal organs of *Catla catla* obtained from pond culture systems during one winter outbreak.

Myxosporidia are also an important group of pathogens known to parasitize fish. Several species of myxosporidia have been found to infect cultivable fishes also in India (Chakravarty 1939 & 1943, Tripathi 1952, Bhatt and Siddiqui 1964, Chaudhuri and Chakravarty 1970, Karamchandani 1970, Seenappa and Manohar 1980, Mishra *et al.* 1984, Dey *et al.* 1988). Sanaullah and Ahmed (1980) from Bangladesh have reported number of cases of mass mortalities of the fingerlings of Indian major carps, *Catla catla* due to *Myxobolus* spp. Infection. They cause great problems in intensive fish culture and seed rearing leading to emaciation, retarded growth and even mass mortality (Hoshina 1952, Tripathi 1952, Dey *et al.* 1988). The enzootic nature of myxosporidiasis in Indian major carp in Orissa, India has been reported, where the pathogen produces infections ranging from sub-clinical and lethal. The fish that survive infection may become lifelong carrier and spread the infection (Mishra *et al.* 1982). Dykova and Lom (1988) reviewed the literature on myxosporeans in intensive culture of carp and concluded that the damage exerted by these species may range from almost non to serious growth impairment or direct mortality, depending on the intensity of infection, of fish condition and environmental factors. This paper describes one of the complicated field outbreaks of mixed infections of saprolegniasis and myxosporidiosis in Indian major carps from one organised freshwater farm.

Materials and methods

During one winter (November to January), it was observed that few of the ponds (0.1 to 0.4 ha area) stocked with three species of Indian major carps, were infested and infected with *Argulus* and *Saprolegnia*. It was also interesting to note that the host specificity of argulosis was restricted mostly to rohu (*Labeo rohita*), and saprolegniasis was only marked in catla. Rohu with argulosis were not included in this study. The affected ponds revealed >90% morbidity of infection during December.

Sporadic mortality was only marked in catla in the ponds few days before netting. During netting, it was observed that most of the catla (80%) were having the saprolegniasis lesions. The pH of the ponds water was varied from 6.3 to 7.7 and temperature was 18 to 24 °C. Diseased fish showing initial, advance lesions of skin ulcers and fungal growths as well as succumbed fish of about 35 numbers were collected during the period mentioned and brought to the laboratory for examination. The fungal specimens were isolated and subjected to wet mount examination with lactophenol cotton blue stain. Subsequently, the fish were anaesthetized with MS 222 (Tricane methane sulfonate, Sandoz) and necropsy was conducted. Internal organs *viz*, liver,

kidney stomach, intestine, spleen, pancreas, brain along with gills and affected skin with muscle were collected and processed for histopathology . The tissues were fixed in 10% phosphate buffered formalin and processed for haematoxylin and eosin staining. The internal organs were also stained with periodic acid- schiff (PAS; Humason 1972).

Results and discussion

The yearlings of catla (weight range, 600-1000g) were affected. Although, most of rohu of the ponds were infested with argulosis, catla were almost free from louse infestation. Rohu are more prone to argulosis in composite carp culture ponds in comparison to catla which has been reported earlier (Dey 1989).

The catla infected with saprolegniasis had cotton wool growth all over the body surface including the head region and gill surfaces. The scales were rough and loosened. Some places, fungi had invaded deep into musculature with sloughed up epidermis. Similar type of lesions were also observed in common carp due to saprolegniasis (Pickering and Willoughby 1982).

On wet mount preparation with lactophenol cotton blue staining, it was confirmed, based on their mycelia and zoo sporangia that the fungi belong to *Saprolegnia* sp. as described by earlier worker (Noga 1993).

On PAS staining, the fungal elements could not be detected in any of the internal organs. On histology, the degenerative changes in epidermis and dermis without any inflammatory reaction could be marked. Pickering and Richards (1980) also described absence or weak inflammatory response at the infected site unless secondary bacterial infections complicate the process during saprolegniasis. There was massive accumulation of mononuclear cells around the portal vessel of liver (Fig.1) with mild degenerative changes of hepatocytes. The increase in the liver-specific enzyme levels in serum of brown trout due to saprolegniasis as observed previously by Duran *et al.* (1987) might have occurred due to damage to the liver. Surprisingly, the anterior kidney revealed myxosporidian cysts in the haemopoietic areas (Fig. 2). However, the haemopoietic organs did not reveal any degenerative changes as observed in brown trout (Alvarez *et al.* 1985). The innocuous myxosporidean cysts were also observed in apparently healthy Indian major carps kidneys in many instances earlier, particularly when the fish were infected with other organisms (Kumar *et al.* 1986, Sahoo *et al.* 1998). The degree of damage to kidney and other organs due to myxosporidiosis depends on the intensity of infection in fish and environmental conditions, mostly (Dykova and Lom 1988). Thus, the presence of myxosporidean cysts in kidney only further confirmed enzootic and carrier nature of the pathogen in Indian major carp in this region as reported earlier (Mishra *et al.* 1982). The submucosa of the stomach and intestine revealed massive eosinophilic granular cell reaction (Fig. 3) which might be indicative of degree of stress in fish. The pial vessels present in periphery to the ventricles in the brain were profusely dilated and engorged (Fig. 4) . Although, Alvarez *et al.* (1988) marked enlarged blood vessels in trout saprolegniasis, the changes could be marked in blood vessels of haemopoietic tissue. The heart, spleen, pancreas and other internal

organs were devoid of any marked alteration. Other than these changes, there was hyperplasia of epithelial cells at the base of primary lamellae in the gills. However, neither the myxosporidean cyst nor the fungal elements could be marked in the gill. The cause of hyperplastic reaction could not be determined.

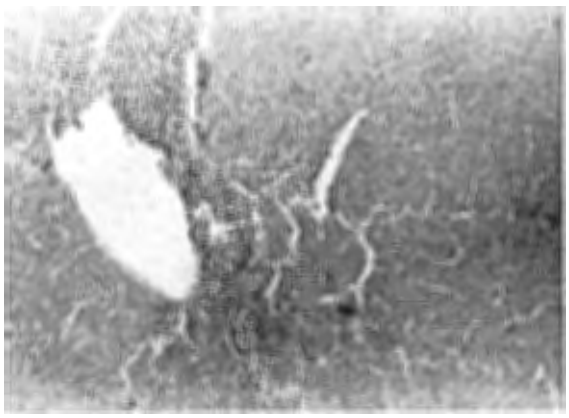

Fig.1. Liver showing periportal accumulation of mononuclear cells (H&E X200).

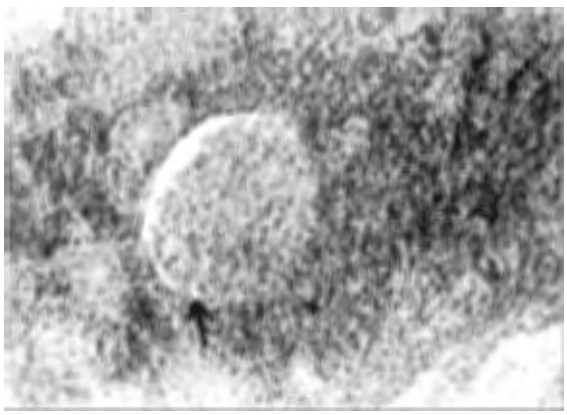

Fig.2. Myxosporidian cyst (arrow) in the anterior kidney (H & E X 400).

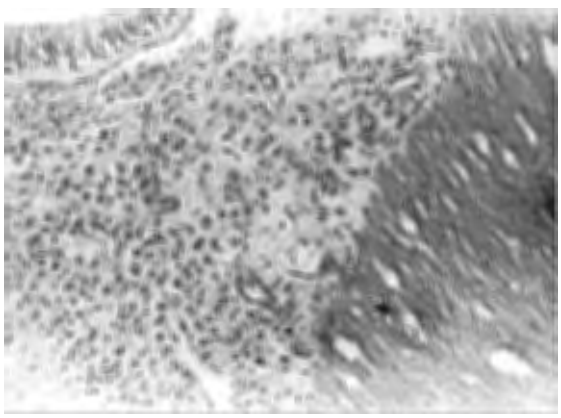

Fig. 3. Submucosa of stomach revealing massive reaction of eosinophilic granular cells (H & E X 400).

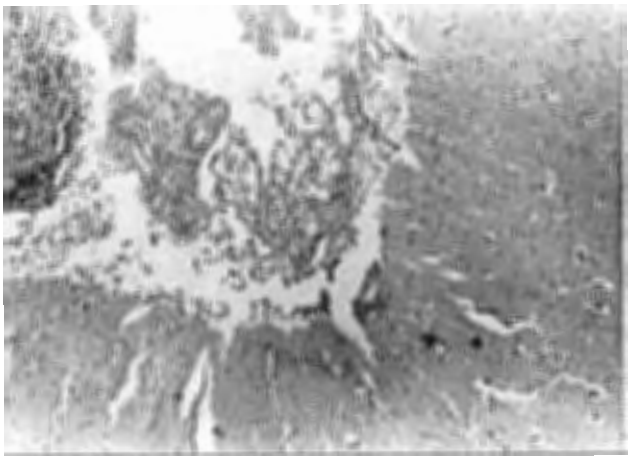

Fig.4. Dilatation and engorgement of pial blood vessels in brain (H & E X 200).

The changes observed in many of the organs might be adding the osmoregulatory problems further there by causing death of the fish ultimately. As the fungal elements could not be traced in any internal organs on PAS reaction, the observed changes may be due to release of some toxic factors by the fungus itself at the infection site, which might have reached in other organs through blood circulation. The changes in the vasculature as observed earlier and also in this experiment further added to the role of release and transport of some toxic materials by the fungi. On the contrary, according to Pickering and Willoughby (1982) there is no evidence that pathogenic saprolegnia strains produce any toxins that might be transmitted systematically. However, further studies on these aspects are warranted to confirm the cause of damage to internal organs due to saprolegniasis.

In the present study, the occurrence of myxosporidean cysts indicated the enzootic nature of this pathogen in Indian major carps in this state, and saprolegniasis could further proved to be season-specific pathogen, occurring particularly in winter due to fall of temperature leading to immunosuppression.

Acknowledgements

The authors are thankful to Director Central, Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar, India for providing necessary facilities during this study. Thanks are also due to Dr. P.V.G.K. Reddy, Dr. R. K. Jana, Dr. J. N. Saha and Mr. H.K. Muduli for their timely help in the field studies.

References

Alvarez, F., B. Razquin, A. Villena, P. Lopez Fierro and A. Zapata, 1988. Alterations in the peripheral lymphoid organs and differential leukocyte counts in *Saprolegnia*-infected brown trout, *Salmo trutta*. *Vet. Immunol. Immunopath.*, **18**: 181 -193.

Bhatt, V.S., W.A. Siddiqui, 1964. Four new species of myxosporidia from the Indian freshwater fish, *Ophiocephalus punctatus* (Bloch). *J. Protozool.*, **11**: 314-316.

Bly, J.E. and L.W. Clem, 1991. Temperature- mediated processes in teleost immunity: *In vitro* immunosuppression induced by *in vivo* low temperature in channel catfish. *Vet. Immunol. Immunopath.*, **28**: 365 - 377.

Bly, J.E., L.A. Lawson, A.J. Szalai and L.W. Dlem, 1993. Environmental factors affecting outbreaks of winter saprolegniosis in channel catfish, *Ictalurus punctatus* (Rafinesque). *J.. Fish Dis.*, **16**: 541-549.

Bly. J. E., L. A. Lawson, D.J. Dale, A.J. Szalai, R.M. Durborow and L.W. Clem, 1992. Winter saprolegniosis in channel catfish. *Dis. Aquat. Org.*, **13**: 155-164.

Chakravarty, M. M., 1939. Studies on myxosporidia from fishes of Bengal with a note on the myxosporidian infection in aquaria fishes. *Arch. Protistenk.*, **92**: 169-178.

Chakravarty, M. M., 1943. Studies on the myxosporidia from common food fishes of Bengal. *Proc. Ind..Acad. Sci.*, **18B**: 21-35.

Chaudhuri, S.R., M.M. Chakravarty, 1970. Studies on the myxosporidia (Protozoa: Sporozoa) from the food fishes of Bengal. Three new species from Ophiocephalus punctatus (Bloch). *Acta Protozool,* **8**: 167-173.

Dey, R.K., 1989. Histopathology of Argulosis in the Indian major carp, *Labeo rohita. Proc. Nat. Sem. Freshwat. Aqua.*, : 126-127.

Dey, R.K., D. Kumar and B.K. Mishra, 1988. Tissue level reaction s in the Indian major carp, *Catla catla* (Ham.), due to *Myxobolus* sp. Infections. *Asian Fish. Sci.*, **1**: 117-122.

Duran, A., L.B. Rodriguez Apracio, A. Reglero and J. Perez Diaz, 1987. Changes in Serum enzymes of saprolegnia infected brown trout, *Salmo trutta* L. *J. Fish Dis.*, **10**: 505 -507.

Dykova, I. And J. Lom, 1988. Review of pathogenic myxosporeans in intensive culture of carp (*Cyprinus carpio*) in Europe. *Folia Parasitologica*, **35**: 289-307.

Hoshina, T., 1952. Notes on some myxosporidian parasites on fishes of Japan. *J. Tokyo Uni. Fish.*, **39**: 69-89.

Humason, G. L., 1972 Animal Tissue Techniques. W.H. Freeman and Co, San Francisco. pp. 166-167.

Jeney, Zs. and G. Jeney, 1995. Recent achievements in studies on diseases of common carp (*Cyprinus carpio* L.). *Aquaculture,* **129**: 397 -420.

Karamchandani, S. J., 1970. A new species of Myxobolus from the gill cavity of Labeo bata (Hamilton). *J. Ind. Fish. Soc., India*, **2**: 170-171.

Kumar, D., B. K. Mishra and R. K. Dey, 1986. Dropsy in *Catla catla caused* by mixed infection of *Aeromonas hydrophila* and Myxosporidian sp. *Aqua. Hungarica (Szarvas),* **V**: 107-112.

Mishra, B. K., D. Kumar and R. K. Dey, 1984. Observations on the efficacy of some drugs against renal myxosporidiasis of Indian major carp. *Veterinarski Arhiv*, **54**: 211-215.

Mishra, B. K., R.K. Dey, D. Kumar, K. Suresh, 1982. Observations on renal myxosporidiosis in Indian major carp. Abstract. *Proc. Symp. Dis. Finfish Shellfish*, College of Fisheries , Mangalore,1-3 March, 1982.

Noga, E.Z, 1993. Water mold infections of freshwater fish: recent advances. *An. Rev. Fish Dis.* **3**: 291-304.

Pickering, A.D and L.G, Willoghby , 1982. *Saprolegnia* infections of salmonid fish. *In:* Microbial diseases of fish (ed. R. J. Roberts). Academic Press, London. pp. 271- 298.

Pickering, A.D. and R. H. Richards, 1980. Factors influencing the structure, function and biota of the salmonids epidermis. *Proc. Royal Soc. Edinb.*, **79 B**: 93 -104.

Sahoo, P. K., S. C. Mukherjee, A. K. Jain and Ashok Mukherjee, 1998. Light and ultrastructural changes in opisthonephros of rohu, *Labeo rohita* during acute and subchronic aflatoxin B_1

toxicity (Abstract). Fifth Asian Fisheries Forum and International Conference of Fisheries and Food Security beyond the Year 2000, Chiang Mai, Thailand

Sanaullah, M., and A. T. T. Ahmed, 1980. Gill myxoboliasis of major carp, Bangladesh. *J. Fish Dis.*, 3: 349-354.

Seenappa, D. and L. Manohar, 1980. *Myxobolus vanivilasae* n. s. parasitic in *Cirrhina mrigala* (Hamilton). *Proc. Ind. Acad. Sci.*, 89: 485-491.

Tripathi, Y. R., 1952. Studies of parasites of Indian fishes. Protozoa: Myxosporidia together with a check list of parasitic protozoa described from Indian fishes. *Rec. Ind. Mus.*, 50: 63-88.

(Manuscript received 26 December 1999)