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Mycotic Involvement in Epizootic Ulcerative Syndrome of Freshwater Fishes of Bangladesh: A Histopathological Study

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Abstract

Five species of freshwater fishes (*Cirrhinus mrigala, Catla catla, Puntius sophore, Glossogobius giuris* and *Labeo rohita*) were affected by the epizootic ulcerative syndrome (EUS) in the Bangladesh Agricultural University campus. A monthly sampling was carried out from October 1997 to March 1998.

Clinically and histologically *C. mrigala* was severely affected by EUS, followed by *C. catla, P. sophore* and *G. giuris*. *L. rohita* was the least affected species. Large, deep and whitish ulcers were recorded in the lateral region (near the dorsal fin) and caudal region where parts of fins, scales and muscle were lost in most of the EUS affected fish. Massive necrosis of the epithelium and muscle tissue was observed in the affected tissue surrounding the granuloma. The granuloma due to fungal infection consisted of mononuclear epithelloid cells and fibrillar structures. Fungal granulomas were also found in the liver of *C. mrigala* and *C. catla* and in the kidney of *C. mrigala* only. However, the liver and kidney of other species had necrotic hepatocytes, renal tubules and glomeruli associated with many inflammatory cells.

Water temperature, alkalinity and hardness were reduced in the months of December, January and February in comparison with other months. Clinically and histologically, in the colder months (December, January and February), all fish specimens were more affected by EUS in comparison with other months. The clinical signs, infection mode and histopathology of the disease observed in the present study resemble mycotic granulomatosis. Thus, the consistent involvement of fungus and its similarity in pathology to EUS in other countries, indicate that fungus is one of the pathological agents of EUS.

Introduction

The epizootic ulcerative syndrome (EUS) is an ulcerative condition of wild and cultured freshwater fishes in Asia and the Indo Pacific region (Tonguthai

1985; Roberts et. al., 1986; Lilley et. al., 1992). Bangladesh is probably the 12th country in the Indo Pacific region affected by EUS which was first reported in February 1988 from the Meghna Dhanagoda Project of Chandpur District. The second outbreak showed marked seasonality between October 1989 and March 1990 and in the subsequent years, the disease recurred during the same season (Barua 1994). The lowering of water temperatures together with heavy rainfall, low alkalinity and pH fluctuations were recognized as predisposing factors (FAO 1986; Lilley et. al., 1992). A wide range of biological etiologies including viruses, fungi and bacteria have been associated with outbreaks (Roberts 1994).

Clinically, the disease starts as red spots on the body surface of affected fishes. The lesions may develop as necrotizing dermatitis and become dermal ulcers (Callinan et. al., 1989). Clinically and histopathologically, the lesions of EUS in *Puntius* sp. and Indian major carps closely resemble the fungal condition of pond cultured ayu (Egusa 1971).

In Bangladesh EUS affected at least 31 species of fishes among which snakeheads(*Channa* sp.) and *Puntius* sp. were most severely affected. In November,1988 the first outbreak of EUS occurred among Indian major carps (Barua 1994). By 1995 the incidence of EUS was high in many places of Bangladesh. In 1996 outbreaks were less severe whereas in1997 and 1998 the incidence again increased. Barua (1994) has reported on the status of EUS and environmental influences of EUS in Bangladesh. Rahman and Chowdhury (1996) experimented on the bacterial status of EUS in Indian major carps. Roberts et. al., (1993) also worked on the mycotic aspects of EUS from snakeheads and *L. rohita* of Bangladesh. However, no detailed study on the histopathology of EUS in Bangladesh was reported. Thus the present study was designed to characterize the histopathology associated with five species of EUS affected fish: *Cirrhinus mrigala, Catla catla, Puntius sophore, Glossogobius giuris,* and *Labeo rohita.*

Materials and Methods

The EUS affected fishes were collected from four culture ponds in and around the Bangladesh Agricultural University campus, Mymensingh, Bangladesh. Sizes of ponds varied between 0.16 to 0.65 hectares using similar culture methods in all sampling ponds. Monthly samples of *Cirrhinus mrigala, Catla catla, Puntius sophore, Glossogobius giuris* and *Labeo rohita* were collected from October 1997 to March 1998. Twelve fishes per species were examined initially from which four fish were observed randomly for the presence of any injury, infection and other abnormalities. Fish size varied between 10-35 g and 10-19cm. Histopathological observations were conducted on the skin, muscle, liver and kidney fixed in 10% neutral buffered formalin. The samples were processed by an automatic tissue processor, embedded, sectioned at 5 μm, then stained with haematoxylin and eosin. Prevalence of infection was recorded specieswise during the investigation period. Temperature, alkalinity, and hardness of the ponds were also measured monthly.

Result

Five species of freshwater fishes were found to be affected by EUS in ponds around Bangladesh Agricultural University, Mymensingh, during this investigation. Clinically, *C. mrigala* was severely affected by EUS exhibiting large, deep, whitish ulcers in the mid-lateral and caudal regions where scales, skin and muscle tissue had been eroded. *C. catla* had grey-whitish necrotic areas extending deep into the muscle of the dorsoventral region. In *P. sophore*, necrotic areas of the dorsoventral region appeared whitish. Greyish necrotic areas were also visible in the lateral and caudal regions of *G. giuris*. However in *L. rohita*, necrotic haemmorhagic areas were relatively mild, occurring in the dorso-lateral regions. Clinical signs of EUS were more severe in the months of December, January and February whereas in October and March most of the fish looked healthy (Table 1).

Table 1. Clinical signs of experimental fishes in various months.

Months Species	October	November	December	January	February	March
C. mrigala	Scale loss & mild 'H' in 'dv' region	Scale & skin loss; 'H' deepens up to muscle in 'dv' region	Tissue necrosis extends' in 'dv & 'C' regions	Necrosis in size & depth from 'D' to 'A' region. 'cf' loss & 'H' in 'C' parts	Marked 'N' in 'C' & 'D' regions; fin loss	Healing of 'N' organ & mild scale loss
C. catla	Normal appearance	Scale loss; 'H' in 'dv' & 'C' regions	Dark-brown 'N' areas in 'dv' regions	Gray-white 'N' areas reached up to deep muscle in 'dv' region	Brownish-white 'N' area in 'dv' region & loss of 'df'	Mild 'H' lesions present
P. sophore	Healthy appearance	Scale protrusion & rough body surface	Dark-brown 'H' lesions from 'D' base to ventral region	'N' area at 'df' base & lateral regions	White 'N' areas extended to deep muscle	Light brown 'N' areas present
G. giuris	Healthy appearance	Thin body; no lesions	Scale loss in lateral sides & light brown 'H' lesions	'N' in lateral sides & 'C' regions	Gray-white layer over 'N' areas	Normal appearance
L. rohita	Normal appearance	Weak body & rough skin surface	Scale & skin loss; dark 'H' lesions in lateral region	'N' area at 'df' base & lateral region	Gray-white layer over 'N' areas	Healthy fish

H = heamorrhagic
N = necrotic
dv = dorsoventral
A = abdominal
C = caudal
D = dorsal
cf = caudal fin
df = dorsal fin

The highest prevalence of infection was recorded in January while the lowest were in October and March. Among the species, the highest prevalence was recorded in *C. mrigala* and the lowest were in *L. rohita* and *G. giuris* (Table 4).

Histologically, *C. mrigala* was found to be the most severely affected fish species. Massive necrosis of the epithelium and muscle tissue associated with numerous fungal granulomas, epithelioid cells and several coiled fibroblasts were seen (Fig. 1A). *C. catla, P. sophore* and *G. giuris* showed myofibrillar necrosis charactrised by the presence of mycotic granuloma, epithelioid cells and other pyknotic cells (Figs. 1B, 1C and 1D). Pathologically, the skin and muscles of *C. mrigala* were more affected followed by *C. catla, P. sophore, G. giuris. L. rohita* was the least affected (Table 2 and Fig. 1E).

Fungal granulomas were also recorded in the liver and kidney of EUS affected *C. mrigala,* associated with necrotic hepatocytes, renal tubules and glomeruli (Figs. 2A and 3A and Table 2) . Numerous pyknotic nuclei, epithelioid cells and inflammatory cells were scattered in the affected liver and kidneys of *C. mrigala* specimens. In the liver of *C. catla,* early stage mycotic granulomas were observed with some hepatocytic necrosis (Fig. 2B). The kidney of *C. catla* and the liver and kidney of *P. sophore, G. giuris* and *L. rohita* showed necrotic hepatocytes, renal tubules, with inflammatory cell infiltration,

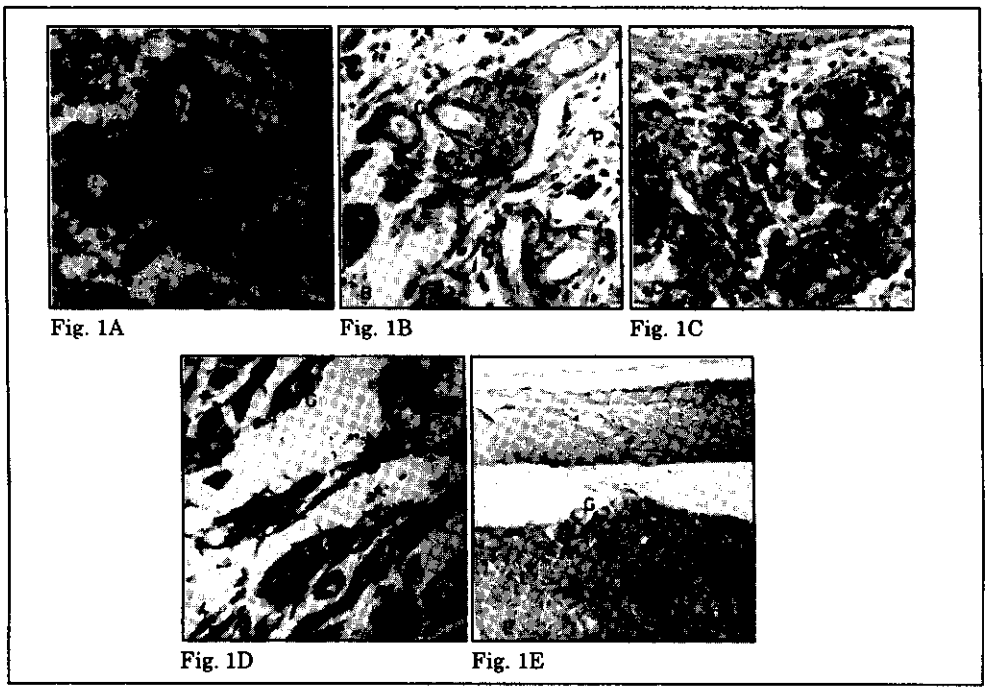

Fig. 1. Photomicrographs of the cross section of the skin and muscle of naturally affected EUS fishes in Bangldesh. A. Well-developed fungal granuloma consisting of epithelioid cells (e) with a rim of fibroblasts in the muscles of *C. mrigala* (arrow). (H&E, X 430). B. Many fungal granuloma (G) in the necrotic muscles of *C. catla* showing pyknotic nuclei (P). H&E, X 430). C. Fungal hyphae in the host inflammatory tissues (arrow) of the muscles of *P. sophore.* (H&E, X 220). D. Early to mature granulomas (G) in necrotic muscles of *G. giuris* with inflammatory cells (arrow). (H&E, X 220). E. Early to mature granulomas (G) in the muscle-hypodermal border of *L. rohita.* Melanocytes (m) are seen underneath the dermal layer. (H&E, X 110).

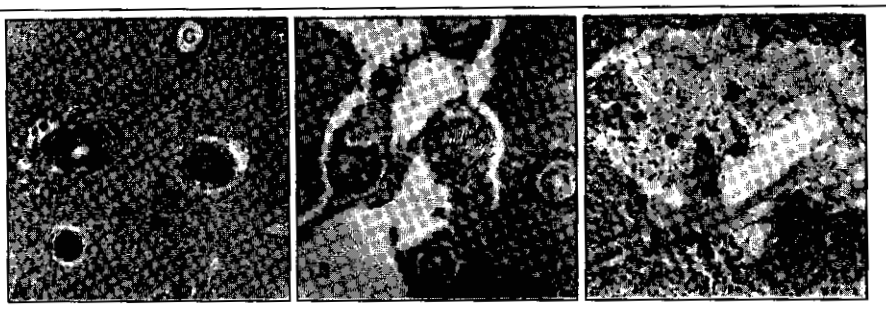

Fig. 2A Fig. 2B Fig. 2C

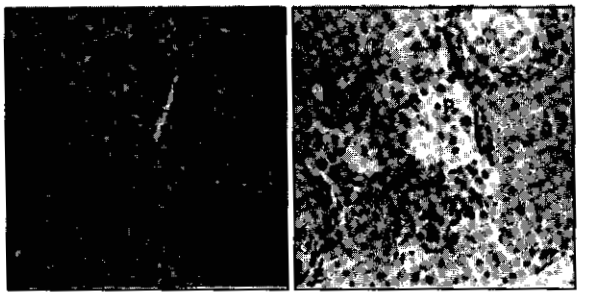

Fig. 2D Fig. 2E

Fig. 2. Photomicrographs of the liver of various EUS affected specimens as observed in the present experiment. A. Mycotic granuloma (G) in *C. mrigala*. (H&E, X 100). B. Early to mature granulomas (G) and necrotic hepatocytes in *C. catla*. (H&E, X 260). C. Congestion and severe necrosis of hepatocytes (arrow) in *P. sophore*. (H&E, X 220). D. Mild necrosis and accumulation of inflammatory cells (arrow) in *G. giuris*. (H&E, X 220). E. Necrotic hepatocytes with many pyknotic cells (P) in *L. rohita*. (H&E, X 430).

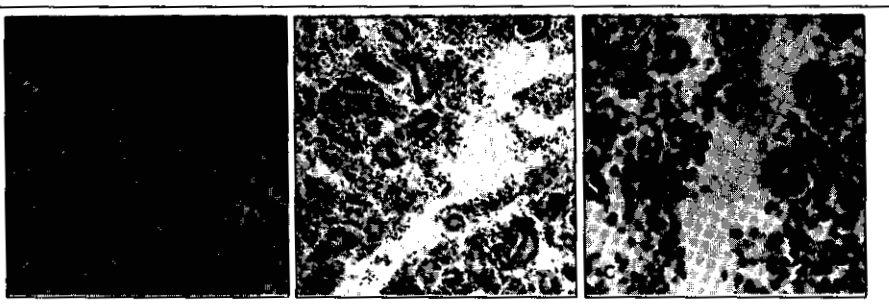

Fig. 3A Fig. 3B Fig. 3C

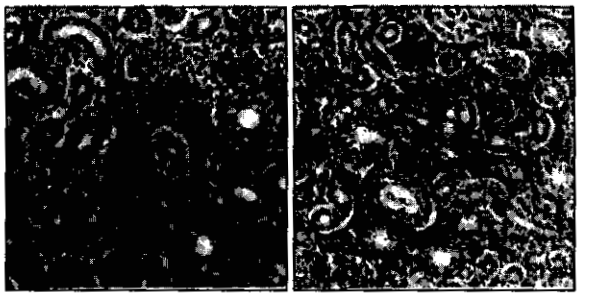

Fig. 3D Fig. 3E

Fig. 3. Cross section of the kidneys of various EUS affected specimens as observed in the present experiment. A. Epithelioid cells (e) displacing renal tubules and formation of granulomas (G) in *C. mrigala*. (H&E, X 430). B. Necrosis of renal tubules (R) and haemopoietic tissue (arrow) in *C. catla*. (H&E, X 220). C. Epithelioid cells (e) displacing most of the renal tubules (R) in the necrotic kidney of *P. sophore*. (H&E, X 430). D. Almost normal kidney of *G. giuris* with some inflammatory cells are observed in between the renal tubules. (H&E, X 220). E. Normal renal tubules (R) and haematopoietic cells in *L. rohita*. (H&E, X 220).

Table 2. Monthwise pathological changes in the organs of various fishes during experimental period.

Organs	Months / Species	October	November	December	January	February	March
Skin-Muscle	*C. mrigala*	Lost skin cells; normal muscle	Skin cell lost; necrotic muscle	Skin totally lost; Muscle necrosis & 'FG' appeared	Lost skin, 'FG' with 'e' cell in necrotic muscle layer	Same as January	Lost 'e' cells, 'd' muscle more or less normal
	C. catla	Lost 'e'; and muscle normal	Skin lost partly; mild necrosis in muscle	Lost skin and part of muscle necrosis and 'FG' appeared	Lost skin and muscle; 'FG' severe in necrotic muscle layer	Increase no. of 'FG' in necrotic muscle layer; 'P' cells present	Skin lost partly; muscle normal
	P. sophore	More or less normal skin and muscle cells	'H' epidermis & 'd' myofibrils arranged normally	Lost skin and part of muscle; necrotic muscle tissue	Necrotic muscle, 'P' cell, many 'FG' with 'E' cell in muscle	Same as January	Lost skin cells & myotomes normal
	G. giuris	Mild 'H' skin and normal 'M'	Lost skin and 'M' cells	Necrotic muscle, 'E' cell appeared	Dense 'FG' in necrotic muscle layer; 'P' nuclei	Necrotic muscle with mild 'FG'	Lost cells of 'e' & 'd'; mild 'H' in 'M' cells.
	L. rohita	Normal skin and muscle cells	Lost skin and 'M' normal	Lost skin & part of muscle	Muscle more or less normal few 'FG' near dermis	Lost cells of skin; normal 'M' cells	Skin and muscle cells more or less normal in fashion
Liver	*C. mrigala*	Normal	Mild necrotic hepatocyte	Necrotic hepatocyte and 'bc' appeared	Many 'FG' in necrotic hepatocyte	Same as January	Mild necrosis in hepatocyte
	C. catla	Normal	Normal	Mild necrotic hepatocyte	'FG' in necrotic hepatocyte, 'P' cell present	No. of 'FG' reduced, 'P' cell present	Mild necrosis in hepatocyte
	P. sophore	Normal	Normal	Necrotic hepatocyte with 'P' nuclei	Necrotic hepatocyte; 'P' nuclei & 'bc' appeared	Reduced necrotic cell & 'bc' appeared	More or less normal hepatocyte
	G. giuris	Normal	Normal	Mild necrosis in hepatocyte	Necrotic hepatocyte & 'bc' appeared	Same as January	Normal
	L. rohita	Normal	Normal	Normal	Necrotic hepatocyte & 'FH' present, 'P' nuclei	Necrotic hepatocyte; 'P' nuclei present	More or less normal cell
Kidney	*C. mrigala*	More or less normal	Rupture 'KT' and many 'P' cell & 'bc' present	'H' kidney, many 'bc' and 'P' cell present	Necrotic 'KT' & glomeruli; 'FG' present	Same as January	Rupture 'KT' and 'bc' present
	C. catla	Normal	Rupture 'KT'	Rupture KT & glomeruli; 'P' cell present	Lost 'KT', glomeruli in many places	Necrotic 'KT' & glomeruli	Normal
	P. sophore	Normal	Normal	Mild necrosis in 'KT' and glomeruli	Same as December	Necrosis and lost cells in many places	Mild necrosis; 'bc' present
	G. giuris	Normal	Normal	Normal	Necrosis of 'KT' and glomeruli	Same as January	More or less normal
	L. rohita	Normal	Normal	Normal	'bc' and 'P', nuclei in between 'KT'	Mild 'H' kidney cells	Normal

FG = Fungal granuloma; E = Epithelioid; H = Haemorrhagic ; P = Pyknotic ; KT = Kidney tubule; bc= blood cell; FH = Fungal hyphae; e = epidermal, d = dermal; M = myotome

but no mycotic granulomas in all specimens sampled in December, January and February. The liver and kidney of all fish sampled in the months of October, November and March, were almost normal (Table 2). Thus, pathologically all the fish specimens were more affected in the months of December, January and February. *C. mrigala* was the most severely affected species, followed by *C. catla, P. sophore, G. giuris* and *L. rohita* (Tables 1 and 2).

Water temperature, alkalinity and hardness were higher in the months of October, November and March and lower towards December, January and February (Table 3).

Discussion

Clinically, *C. mrigala* was the most severely affected among the five fish species investigated. Except for the affected areas of the body and degree of severity, clinically there are some similarities among the various species. Signs of EUS in *C. mrigala* appeared as large deep whitish ulcers in the mid lateral and caudal regions where scales, skin and muscle tissues had been eroded. In *C. catla, P. sophore* and *G. giuris,* grey-whitish necrotic areas were found in various parts of the body, whereas in *L. rohita* the signs appeared as mild haemorrhagic lesions. Hatai (1994) described red spots in the body of mycotic granulomatosis affected ayu and in extreme cases the lesions became swollen, eroded and ulcerous. Callinan et. al., (1989) also described red spots on the body surface of early EUS affected mullet, lesions later progressing to necrotizing determatitis and dermal ulcers.

Histologically, *C. mrigala* was also found to be the most severely affected and *L. rohita* as the least affected species. In *C. mrigala,* chronic necrosis of muscle tissue with numerous mycotic granulomas were observed in the affected muscles. The fungal granulomas were associated with epithelioid cells and several coiled fibroblasts. Similarly Mohan and Shankar (1994) also described numerous granulomas forming as a result of chronic inflammatory response with fungal hyphae in EUS affected fresh and brackishwater fishes of India. The present histological findings also agree with the observations on EUS of freshwater fishes of South and Southeast Asia (Roberts et. al., 1992; Roberts et. al., 1993) and on red spot disease of brackishwater fishes of Australia (Callinan et. al., 1989). Hatai et. al., (1994) also reported fungal hyphae and many granulomas in the internal organs and musculature of *Colisa lalia* suffering from an invasive mycosis in Japan. In this case, the granuloma consisted of mononuclear cells including neutrophils, macrophages, epithelioid cells and fibrillar structures. Similar mycotic granulomatosis were also reported in cultured ayu in Japan (Miazaki and Egusa 1973; Hatai 1980). Noga et. al., (1988) also described similar inflammatory responses in ulcerative mycosis of Atlantic menhaden. In the present experiment fungal granulomas were also observed in the liver of *C. mrigala* and *C. catla* and in the kidney of *C. mrigala.* The presence of typical mycotic granulomas in the internal organs including pancreas, kidney, liver, gonad, spleen, heart and alimentary tract were also described by Hatai et. al.,(1994). Kumar et. al., (1989) also

Table 3. Mean and range of water quality parameters from sampled ponds in Bangladesh from October, 1997 to March, 1998.

Parameters	October	November	December	January	February	March
Temperature (°C)	29 (25-31)	24 (20-28)	20 (15-25)	16 (12-21)	22 (16-26)	28 (25-31)
Alkalinity (ppm)	55.5 (48-60)	51.0 (44-57)	37.5 (33-42)	48.5 (23-34)	43.0 (38-49)	50.0 (45-56)
Hardness (ppm)	65.5 (60-70)	55.5 (49-58)	43.0 (38-47)	30.0 (24-35)	40.5 (35-44)	61.0 (55-66)

Figures in the parentheses indicate range.

Table 4. Prevalence (%) of infection in various species during the experimental period.

Species	October	November	December	January	February	March
L. rohita	8	25	58	50	50	8
C. catla	17	33	67	75	58	17
C. mrigala	33	50	75	83	67	33
P. sophore	17	33	58	67	42	17
G. giuris	8	25	42	58	25	8

reported similar pathological changes in the skin, muscle, kidney, liver and heart of *Puntius*, *Mastacembelus* and *Channa* in India.

Clinically and histologically, it was observed that in all the fish species studied, EUS infections were most severe during the colder months of the year (December, January, February) when temperature, alkalinity and hardness reached their minimum levels. Similar observations were made by Palisoc (1990), Barua (1994) and Chinabut (1994) on EUS outbreaks in the Philippines, Bangladesh and Thailand. The reduction of water temperature together with periods of heavy rainfall, low alkalinity, and pH fluctuations were recognized as pre-disposing factors for the occurrence of EUS (FAO 1986; Lilley et. al., 1992). In addition, Barua (1994) reported that fish stressed from environmental changes usually ate less, became weak and were more susceptible to pathogens. In the present investigation, the highest prevalence of infection was recorded in January while the lowest were in October and March. Therefore it could be assumed that the prevalence of infection might be related to the seasonal variations.

Histopathological examination showed that many granulomas and fungal hyphae were present in the musculature and other internal organs. However, identification of the fungus was not carried out in the present study. The epizootic ulcerative syndrome which is now a serious disease in Southeast Asian countries, was associated to a particular species of *Aphanomyces* (Lilley et. al., 1992; Roberts et. al., 1993). The histopathology of the disease, as observed in the present study closely resembles that of mycotic granulomatosis observed in cultured ayu in Japan (Hatai et. al., 1994). Present observations of EUS affected muscle, liver and kidney tissues indicate that the fungus plays the greatest role in the production of granuloma. It is therefore likely that the

fungus which was responsible for the production of these granulomas might be *Aphanomyces* sp. Other pathogens may also play a role in the occurrence of EUS, but these could not be determined in the present investigation. Thus, identification of a specific fungus and other pathogens associated with EUS of fish need to be carried out in future experiments.

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