PREVALENCE OF MYXOSPOREAN PARASITES IN MULLETS OF COCHIN BACKWATERS

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Dedicated

to my

Parents and Brothers

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CERTIFICATE

Certified that the thesis entitled "**PREVALENCE OF MYXOSPOREAN PARASITES IN MULLETS OF COCHIN BACKWATERS**" is a record of independent bonafide research work carried out by **Mr. Ch. Kiran babu** during the period of study from September 1999 to August 2001 under our supervision and guidance for the degree of **Master of Fisheries Science (Mariculture)** and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or any other similar title.

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I hereby declare that the thesis entitled "**PREVALENCE OF MYXOSPOREAN PARASITES IN MULLETS OF COCHIN BACKWATERS**" is an authentic record of the work done by me and that no part thereof has been presented for the award of any degree, diploma, associateship, fellowship or any other similar title.

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सारांश

कोचीन पश्चजल की मलेट वंश की मछलियों में मिक्सोबीजाणुओं की सफलता का अध्ययन किया गया. 110 मछलियों के परीक्षण के दौरान 36.4 प्रतिशत अर्थात् 40 मछलियों में मिक्सोबीजाणुओं का संक्रमण दर्शित हुआ. मिक्सोबोलस वंश की सात प्रजातियाँ प्राप्त हुई जिनकी सफलता का प्रतिशत 1.8 से 16.4 के बीच पाया गया. मछलियों में संक्रमण को तीव्रता 1 से 100 के बीच परिसरित हुई. कुल परीक्षण के दौरान कोई आभासी विकृतिजन्य भिन्नता प्रेक्षित नहीं हुई. मलेट मछलियों के क्लोम कर्षणी व ग्रसनी क्षेत्र में मिक्सोबीजाणु परजीवी पहली बार देखे गये. परीक्षण के दौरान 110 मछलियों में से 11 में बहु आपतन दर्शित हुआ. हाँलाकि कुछ मछलियों में दीर्घ संक्रमण देखा गया, आभासी तौर पर मछलियाँ स्वस्थ पाई गई.

ABSTRACT

Prevalence of myxosporean parasites in mullets of Cochin backwaters was studied. Out of 110 fishes examined 40 (36.4%) fishes showed infection with myxosporeans. Seven species of myxosporeans, all belonging to the genus *Myxobolus* were recovered. Their prevalence varied from 1.8% to 16.4% individually. The intensity of infection in fishes ranged from 1 to 100. All the 7 species observed were found to be site/organ specific. No apparent pathological changes were observed on gross examination. Infection with myxosporean parasites was observed in gill rakers and pharyngeal region of mullets for the first time. Multiple infection was observed in 11 out of 110 (10%) fishes examined. Though heavy infections were observed in some cases, the fishes were apparently healthy.

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INTRODUCTION

Grey mullets form one of the most important fisheries of the estuaries and coastal waters of the tropical and sub-tropical regions of all seas. Their abundance in the coastal and estuarine regions may be related to their food and feeding habits. Mullets bear considerable economic importance among marine fishes, being prominently consumed along the coastal districts of several states in India. They are cultured in estuaries and ponds, and are much relished owing to their good taste.

As interest in culturing mullets increases the importance of their diseases and parasites becomes apparent. But, information regarding parasites and diseases of mullets in confined water bodies remains scanty. Though mullets enjoy a worldwide distribution, information on their parasitic fauna from the natural environment is limited to that of a few regions. Among the different groups of parasites causing significant losses in growth and production of mullets, myxosporeans with a vast number of species pose a serious threat.

Myxosporeans constitute a specific group of protozoans parasitising mostly fishes of both Marine and Fresh waters. Myxosporea include two orders, Bivalvulida and Multivalvulida. Though most myxosporeans are considered relatively non-pathogenic, some are noted pathogens and cause a variety of diseases in fish. Heavy losses have been reported because of myxosporean infections in many commercially important fishes like salmons, herring, tuna, mackerel, sole, halibut, swordfish, mullets etc., either by inflicting heavy mortalities or by rendering them unmarketable. Some of these myxosporeans are histozoic and others coelozoic. Most of them are known to exhibit high host specificity even to the organ level of fish with reported incidence in different organs like fins, scales, gills, visceral organs and even muscles.

Coelozoic species are found in body cavities such as the gallbladder, bile ducts or urinary tract and are found attached to the walls or float freely in the

fluid within the cavity. They are considered to be more primitive evolutionarily than histozoic species. Histozoic forms occur in various tissues, mostly intercellular and in some cases intracellular. The vegetative form of myxosporeans is a trophozoite represented by various forms of plasmodia, the basic form of existence in their hosts. Spores, which develop within the plasmodia, serve the purpose of transmission to a new host. The life cycle of myxosporeans is complicated and many, if not all, species require alternate development in an oligochaete worm. Histozoic plasmodia are found, ensheathed within connective tissues and appear as whitish cysts often conspicuous and enclose very large number of spores.

Spores of myxosporeans vary in shape and size depending on the genus and species. Spores are microscopic in nature and consist of three basic elements, spore valves, polar capsules and sporoplasm, their dimensions varying from 3.5 to 25 μm in the sutural plane.

Mullets form one of the most relished group of fishes in Kerala and command high market values. There are reports of myxosporean parasites inflicting mortalities in mullets. So far, no particular study has been conducted on the myxosporean parasites of mullets in Kerala. In this circumstance a study on the prevalance of myxosporean parasites in mullets of Cochin backwaters has been taken up.

REVIEW OF LITERATURE

Myxosporeans are a unique group of parasitic protozoans found exclusively in marine and fresh water fishes. More than 1330 species of myxosporean parasites have been recorded. Many are highly pathogenic causing serious diseases and massive destruction of fish in culture systems and natural reservoirs.

Myxosporeans were first discovered at the beginning of the nineteenth century and were not understood by scientists for a long time. The first mention of myxosporeans occurs in the work of Jurine (1825), who discovered their cysts in the musculature of *Coregonus fera* from a lake in Geneva. Muller (1843) termed the spores of myxosporeans as psorosperms and mistook the spores as their vegetative forms. Balbiani (1863) regarded spores as definitve structures of a vegetative nature.

Butschli (1882) was the first to correctly understand myxosporeans and in fact, gave them the name "Myxosporidia" (slime animals bearing spores). He made it clear that the polar capsule plays a definite role in the transmission of spores to a new host. Balbiani (1884) supplemented the observations of Butschli on the emergence of a sporoplasm from the spore on the opening of the valve.

Thelohan (1892,1895) was the first to classify the Order Myxosporidia, based on the characters of spore structure. Classification of this group has been subjected to numerous revisions, of which the contributions of Gurley (1893), Labb'e (1899), Doflein (1899), Auerbach (1910,1911), Poche (1913), Davis (1917), Kudo (1933), Tripathi (1948), Grass'e (1960), Meglitsch (1960), Lom and Vavra (1962) and Shul'man (1966) are notable.

Majority of the species known to date have been described on the basis of spore morphology. The similarity of spores has resulted in the misidentification of numerous species. Thus Donec and Shul'man recorded as many as 40 hosts for

some species (Masoumian, *et al.*, 1996). To enable a more accurate identification of parasites, Lom and Arthur (1989) suggested morphological characteristics which should be taken into consideration besides spore size and shape, while Molnar (1994) has called attention to the fact that host, organ and tissue specificity represent a feature, which must not be neglected when describing new species of myxosporeans.

Based on the guidelines prepared by Levine's committee (1980) and incorporating Shulman's system, Lom and Noble (1984) put forth a revised classification of myxosporea. According to this revision, the Class Myxosporea consists of 2 orders, 3 suborders, 16 families and 42 genera. Later Lom and Dykova (1992) reported that myxosporea has 52 genera and more than 1330 species.

Genus *Myxobolus* Butschli, 1882:

Butschli in 1882 established the genus *Myxobolus* with *M. mulleri* as its type species. Myxosporeans forming spores with an iodinophilous vacuole and 1 or 2 polar capsules were included under the genus *Myxobolus*. Thelohan (1892) proposed the genus *Myxosoma* to accommodate those species lacking an iodinophilous vacuole and Kudo (1933) created the genus *Thelohanellus* to include those species with a single polar capsule and an iodinophilous vacuole. Except for the presence of an iodinophilous vacuole in its spores, genus *Myxobolus* is similar to genus *Myxosoma* in all other aspects. The differentiation between genus *Myxobolus* and genus *Myxosoma* based simply on the presence or absence of an iodinophilous vacuole has been considered to be unreliable by many workers. In the absence of a unanimous opinion, Lom and Hoffman (1971) preferred to retain the generic status of *Myxosoma*. Later, Lom and Noble (1984) in their revision of class Myxosporea, suppressed the family Myxosomatidae and treated genus *Myxosoma* as a junior synonym of *Myxobolus*. Following Lom and Noble (1984), Gupta and Khera (1988b), Landsberg and Lom (1991) and Lom and Dykova (1992) treated *Myxosoma* as an invalid genus. By synonymising *Myxosoma* with *Myxobolus* many of the species, which were formerly in separate genera, became homonyms or synonyms. This led

to a state of confusion and, therefore, Landsberg and Lom (1991) revised the genus *Myxobolus* and provided a list of valid *Myxobolus* spp. reported till then.

The characters of the genus *Myxobolus* Butschli 1882 as given by Lom and Dykova (1992), are:

- Spores ellipsoidal, oval or rounded in valvular view and biconvex in sutural view, flattened parallel to the straight sutural line.
- Shell valves smooth.
- Two polar capsules mostly pyriform in shape.
- Posteriorly, the sutural ridge may extend into a crescentic ledge.
- Binucleate sporoplasm, often with an iodinophilous vacuole.
- Trophozoites as a rule large, polysporic with pansporoblast formation.
- Histozoic in fresh water fishes, some in marine (but mostly in estuarine) fishes and rarely in amphibians.
- Form as a rule large histozoic trophozoites ("cysts") with numerous spores.

A total of 467 species of *Myxobolus* have been reported from various parts of the world. Indian reports are limited to the descriptions of about 74 species by Southwell and Prashad (1918), Chakravarty (1943), Chakravarty and Basu (1948), Tripathi (1952), Qadri (1962), Bhatt and Siddiqui (1964), Lalitha kumari (1968, 1969), Chaudhuri and Chakravarty (1970), Karamchandani (1970), Narasimhamurti (1970), Mandal and Nair (1975), Narasimhamurti and Kalavati (1979, 1986), Narasimhamurti *et al.* (1980), Seenappa and Manohar (1980 a, b, 1981), Kalavati *et al.* (1981), Jayasri *et al* (1981), Jayasri (1982), Sarkar (1982,

1986, 1989, 1993, 1994, 1995), Sarkar *et al.* (1982), Kalavati and Narasimhamurti (1984b), Sarkar *et al.* (1985), Gupta and Khera (1988a,b, 1989, 1990, 1991), Dorothy and Kalavati (1992b) Kalavati *et al.* (1992) and Rajendran *et al.* (1998).

A list of *Myxobolus* spp. reported in mullets from Indian waters is given below.

List of *Myxobolus spp.* Known from Mullets in India.

No.	Parasite	Host(s)	Site	Locality	References
1	*Myxobolus anili* Sarkar, 1989	*Rhinomugil corsula* *Liza macrolepis*	Outer wall of Intestine Intestinal villi	West Bengal Andhra Pradesh	Sarkar (1989), Dorothy and Kalavati (1992b) Narasimhamurti Kalavati and Saratchandra (1980)
2	*M. episquamalis* Egusa *et al.*, 1990	*Mugil cephalus*	Scales	Andhra Pradesh	Kalavati and Anuradha (1992)
3	*M. lizae** Landsberg and Lom, 1991	*Liza macrolepis*	Gut muscles	Andhra Pradesh	Narasimhamurti and Kalavati (1979)
4	*M. macrolepi* Dorothy and Kalavati,1992	*Liza macrolepis*	Intestine	Andhra Pradesh	Dorothy and Kalavati (1992b)
5	*M. mugcephalus*** Narasimhamurti *et al.*, 1980	*Mugil cephalus*	Gills	Andhra Pradesh	Narasimamurti Kalavati and Saratchandra (1980)
6	*M. narasi**** Narasimhamurti, 1970	*Mugil waigensis*	Gut epithelium	Andhra Pradesh	Narasimhamurti (1970)

7	*M. sphaeralis* Dorothy and Kalavati, 1992	*Liza macrolepis*	Gills	Andhra Pradesh	Dorothy and Kalavati (1992)
8	*M. spinacurvatura* Maeno *et al., 1990*	*Mugil cephalus*	Mesentry	Andhra Pradesh	Kalavati and Anuradha (1992)
9	*M.* sp. Dorothy and Kalavati,1992	*Liza macrolepis*	Intestine	Andhra Pradesh	Dorothy and Kalavati (1992)

* Originally described as *Myxosoma lairdi.*

** Originally described as *Myxosoma microspora.*

*** Originally described as *Myxosoma intestinalis.*

INCIDENCE OF MYXOSPOREANS IN MULLETS:

Myxosporean parasites have been reported from mullets the world over. Species belonging to 9 genera have been recovered from mullets. Species of *Myxidium* Butschli, *Zschokkella* Auerbach, *Ceratomyxa* Thelohan, *Sphaerospora* Davis, *Davisia* Laird and *Biptera* Kovaleva, Zubchenko and Krasin occur in the gall bladder or, more rarely, in the urinary bladder or ureters of mullets and are called coelozoic forms. Species of *Myxobolus* Butschli, *Kudoa* Meglitch and *Henneguya* Thelohan on the other hand occur in various tissues and are called histozoic forms. As mentioned earlier, coelozoic forms are rarely pathogenic whereas, histozoic forms often exhibit pathogenicity.

While some myxosporeans seem to thrive in their hosts irrespective of the season, some exhibit a marked seasonal fluctuation. Some species seem to be host specific, where as others are highly polyxenous. The prevalence of myxosporean infection in a locality can sometimes be detected as being extremely high (up to 100%) although sometimes only a few fish from a given stock are infected (Lom and Dykova, 1992). Out of the total number of myxosporeans reported

from mullets world wide, majority belong to genus *Myxobolus*. A total of about 16 species of *Myxobolus* (5 species of *Myxosoma* and 11 species of *Myxobolus*) have been reported from mullets around the world (Dorothy and Kalavati, 1992). Subsequently, 5 new species of *Myxobolus* have been reported from mullets by Bahri and Marques (1996) and Fall, *et al.* (1997).

The first description of a myxozoan infecting Indian fishes came from Southwell (1915). In India, 5 genera of myxosporeans have been reported from mullets namely *Myxobolus* Butschli, *Kudoa* Meglitsch, *Biptera* Kovaleva, Zubchenko and Krasin, *Sphaerospora* Davis and *Davisia* Laird. A list of *Myxobolus* species from mullets in India has been presented in the Table 1. *Myxosoma intestinalis* n. sp. reported from gut epithelium of *Mugil waigensis* by Narasimhamurti (1970) and later renamed as *Myxobolus narasii*, was the first myxosporian parasite recorded from mullets in India. The prevalence of different species of *Myxobolus* recovered from mullets in India ranged from 1.75% to 25%.

HOST SPECIFICITY AND PATHOLOGY:

Molnar (1994) has pointed out that Myxosporeans are host, tissue and organ specific parasites. Most of them have relatively strict host specificity and show strong affinity to a certain tissue of the host fish. The majority of the known *Myxobolus* species have a well-defined site of development and there are species specific to the gills, skin, kidney, intestine etc.

Though most myxosporeans are considered harmless, histozoic myxosporeans may produce much more serious effects in marine fishes. In cases of heavy infections, they may some times turn pathogenic (Lom 1970). *Myxobolus cerebralis* infection is reported to cause huge losses in Salmon culture. Shul'man and Shul'man-Albova (1953) reported a case of serious liver damage caused by *Myxidium oviform* in *Salmo salar* (Lom, 1970). Noble (1950) and Wales and Wolf (1955) noted infection with *Ceratomyxa shasta* causing up to cent percent mortality in fingerlings of the domesticated *Salmo gairdneri* in California.

Though mortality records of wild mullet populations are scarce, a massive mullet kill (*Mugil cephalus* and *M. auratus*) that occurred in 1949 along the Atlantic and Mediterranean coast, caused by a myxosporean identified as *M. exiguus* infecting the gill filaments was reported (Paperna, 1974). Not many records are available regarding the pathogenicity of *Myxobolus* in mullets.

MATERIALS AND METHODS

COLLECTION AND TRANSPORTATION:

Mullets for the present study were collected from Cochin backwaters. Collections were made for about ten weeks from March 2001 to May 2001. Ten to 12 fishes were collected at a time. Size of the collected fishes ranged from 9 cm to 15 cm with an average of 12 cm. The collected fishes were transported to the laboratory in a plastic bin of 30-litre capacity. During transportation, proper aeration was provided using a battery powered air pump.

Maintenance:

The fishes thus brought alive to the laboratory were examined immediately or were maintained and studied at convenience. Three plastic bins of 100-lit capacity were used for maintaining the fishes for 3 to 4 days. The bins were filled with water up to $3/4^{th}$ their capacity. The salinity of the water was adjusted to 25 ppt and the fishes were stocked. Three to four fishes were stocked in each bin and maintained until examination. The water was continuously aerated with an electric aerator and replenished for every fresh collection.

Detection and study of Myxosporeans:

The fishes were killed by cervical rupture and examined under a simple dissection microscope for any externally visible myxosporean cysts. Fins, scales, gills and buccal cavity were thoroughly examined for parasites. Internal organs like heart, liver, gall bladder, alimentary canal, kidney, urinary bladder and muscles were dissected out from each fish and placed into separate petri dishes containing 0.75% saline, macerated and examined under the dissection microscope. Pieces of these tissues were smeared on glass slides with a few drops of saline, a No. 1 cover glass was placed over the smear and observed under the oil-immersion objective of a binocular light microscope. Contents of the gall bladder and urinary bladder were

observed without adding saline. Cysts of myxosporeans, if present, were carefully isolated from the tissues and placed on glass slides in a few drops of saline. A No. 1 cover glass was placed over the cyst and with a gentle tap, the cyst was ruptured and the spores spread out. The spores were then observed under the oil-immersion objective.

Smears prepared from the cysts were air-dried and fixed in acetone free methanol for one minute and stained with dilute Giemsa. Slides were then rinsed in tap water for few minutes, air-dried, cleared in Xylene and mounted with DPX.

Measurements, sketches and photomicrographs:

Measurements were taken from fresh materials with the aid of a calibrated ocular micrometer following the guidelines given by Lom and Arthur (1989). All measurements are given in micrometers (μm) unless otherwise specified. Sketches were made with the aid of a prism type camera lucida. Photomicrographs were taken using a Carl Zeiss Axiostar 1061-030 trinocular research microscope with digital camera attachment. Images were captured directly on to a PC and print outs were taken.

RESULTS

During the present study, a total of 110 fishes of *Liza* spp. were examined, of which 40 were found infected with myxosporeans. Though a total of 7 species were recorded, incidentally all of them belonged to the genus *Myxobolus*. The 7 species encountered during the present study are briefly described.

Myxobolus sp. 1:

Description:

Site of Infection: Fins.
Prevalence: 12 out of 110 fishes (10.9%) examined were infected.
Pathogenicity: Not apparent.
Host: *Liza* spp. (Mugilidae).
Life stage infected: Sub adults and adults.
Locality: Cochin backwaters.
Period: March 2001 to May 2001.

Cysts: Cysts macroscopic, appearing as opaque whitish spots, oval to elongate in shape and measured 0.2 mm to 2 mm. Usually occurred on pelvic and pectoral fins with rare occurrence on dorsal and anal fins. Number of cysts varied from 1 to 20.

Spores (Fig: 1, Plate: 1): Spores oval in valvular view with a broad anterior end and lenticular in sutural view. Polar capsules 2, unequal in shape. Intercapsular ridge and intervalvular ridge present. Sporoplasm roughly triangular in shape and found slightly detached from the spore wall. Sutural line straight and thick.

Spore dimensions: Spores measured 8.4 - 11.7 (mean, 10.75) in length and 5.88 - 7.56 (mean, 6.8) in width; polar capsules measured 2.52 - 3.36 x 1.68 (mean, 3.2 by 1.68). Details of spore measurements, with mean and range, are presented in Table 2.

Table 2. Showing details of measurements of 10 spores of *Myxobolus* sp. 1 recovered from the fins

No.	LS	BS	LPC	BPC
1	11.6	6.72	3.36	1.68
2	10.92	6.72	3.36	1.68
3	8 .4	6.72	2.52	1.68
4	10.08	6.72	3.36	1.68
5	10.08	5.88	2.52	1.68
6	11.76	7.56	3.36	1.68
7	11.76	6.72	3.36	1.68
8	10.08	7.56	3.36	1.68
9	11.76	6.72	3.36	1.68
10	10.92	6.72	3.36	1.68
Range	8.4-11.76	5.88-7.56	2.52-3.36	1.68
Mean	10.75	6.8	3.2	1.68

No. - Number;
LS – Length of spore;
BS – Breadth of spore;
LPC – Length of polar capsule;
BPC – Breadth of polar capsule.

Myxobolus sp. 2:

Description:

Site of Infection: Scales.
Prevalence: 3 out of 110 fishes (2.7%) examined were infected.
Pathogenecity: Not apparent.
Host: *Liza* spp. (Mugilidae).
Life stage infected: Adults.
Locality: Cochin backwaters.
Period: March 2001 to May 2001.

Cysts: Cysts macroscopic, appearing as whitish spots on the surface of the scales, usually at the base of the pectoral and pelvic fins, were round in shape and measured 0.5 mm to 1 mm in diameter. Number of cysts ranged from 1 to 3.

Spores (Fig: 2, Plate: 2): Spores sub spherical or spherical in valvular view and broadly lenticular in sutural view. Polar capsules 2, pear shaped and equal in size. Intercapsular and intervalvular ridges absent. Sporoplasm cup shaped with 2 small depressions below the polar capsules and a small projection at the center extending into the intercapsular space and occupies the entire extracapsular cavity. Sutural line straight and thick.

Spore dimension: Spores measured 8.4 - 10.08 (mean, 9.41) in length and 6.72 - 10.08 (mean, 8.4) in width; Polar capsules measured 3.36 - 5.04 × 2.52 - 3.36 (mean, 4.28 by 2.94). Details of spore measurements, with mean and range, are presented in Table 3.

Table 3. Showing details of measurements of 10 spores of *Myxobolus* sp. 2 recovered from the scales

No.	LS	BS	LPC	BPC
1	10.08	8.4	4.2	3.36
2	10.08	6.72	4.2	2.52
3	10.08	8.4	4.2	2.52
4	10.08	10.08	5.04	3.36
5	8.4	8.4	5.04	3.36
6	8.4	10.08	4.2	2.52
7	10.08	8.4	4.2	2.52
8	9.24	8.4	4.2	3.36
9	9.24	6.72	3.36	2.52
10	8.4	8.4	4.2	3.36
Range	8.4-10.08	6.72-10.08	3.36-5.04	2.52-3.36
Mean	9.41	8.4	4.28	2.94

No. - Number;
LS – Length of spore;
BS – Breadth of spore;
LPC – Length of polar capsule;
BPC – Breadth of polar capsule.

Myxobolus sp. 3:

Description:

Site of Infection: Gills.
Prevalance: 5 out of 110 fishes (4.5%) examined were infected.
Pathogenecity: Not apparent.
Host: *Liza* spp. (*Mugilidae*).
Life stage infected: Sub adults and adults.
Locality: Cochin backwaters.
Period: March 2001 to May 2001.

Cysts: Cysts macroscopic found attached to gill filaments and gill arch. Irregular to round in shape and measured 0.3 mm to 1 mm in size. Number of cysts ranged from 1 to 5.

Spores (Fig: 3, Plate: 3): Spores pear shaped in valvular view with a knob like protrusion at the anterior end and lenticular in sutural view. Polar capsules 2, pyriform in shape and equal in size. Sutural line straight. Intercapsular ridge present. Intervalvular ridges absent. Sporoplasm cup shaped with 2 small depressions below the polar capsules and occupied the entire extracapsular space.

Spore dimensions: Spores measured 9.6 - 11.52 (mean, 10.18) in length and 5.76 - 8.16 (mean, 6.96) in width; Polar capsules measured 3.84 - 4.8 x 1.92 - 2.88 (mean, 4.46 by 2.26). Details of spore measurements, with mean and range, are presented in Table 4.

Table 4. Showing details of measurements of 10 spores of *Myxoblus* sp. 3 recovered from the gills

No.	LS	BS	LPC	BPC
1	9.6	6.72	3.84	1.92
2	9.6	5.76	3.84	1.92
3	10.56	6.72	4.8	2.4
4	9.6	6.72	4.8	1.92
5	9.6	6.24	3.84	1.92
6	9.6	6.72	4.8	1.92
7	10.56	7.68	4.32	2.4
8	10.56	7.68	4.8	2.4
9	10.56	8.16	4.8	2.88
10	11.52	7.2	4.8	2.88
Range	9.6-11.52	6.24-8.16	3.84-4.8	1.92-2.88
Mean	10.18	6.96	4.46	2.26

No. - Number;
LS – Length of spore;
BS – Breadth of spore;
LPC – Length of polar capsule;
BPC – Breadth of polar capsule.

Myxobolus sp. 4:

Description:

Site of Infection: Gill rakers.
Prevalance: 2 out of 110 fishes (1.8%) examined were infected.
Pathogenecity: Not apparent.
Host: *Liza* spp. (Mugilidae).
Life stage infected: Adults.
Locality: Cochin backwaters.
Period: March 2001 to May 2001.

Cysts: Cysts very small, less than 0.1 mm in size, appeared as whitish, bean shaped spots under a microscope and located between the gill rakers. Number of cysts ranged from 20 to 100.

Spores (Fig: 4, Plate: 4a, b): Spores pear shaped in valvular view with a protrusion at the anterior end and lenticular in sutural view. Polar capsules 2, convergent and unequal in size. Large polar capsule is broadly pyriform in shape and small polar capsule is narrowly pyriform in shape and appeared convergent. Intercapsular and intervalvular ridges absent. Sporoplasm cup shaped with 2 small depressions below the polar capsules and a projection in the center occupying the entire extracapsular region. Sutural line straight and thick.

Spore dimensions: Spores measured 7.56 - 10.08 (mean,8.57) in length and 5.04 - 6.72 (mean,5.54) in width. Large polar capsule measured 3.36 - 5.04 (mean,4.12) × 1.68 - 2.52 (mean,2.44) and small polar capsule measured 1.68 - 3.36 (mean,2.26) × 1.68 (mean,1.68). Details of spore measurements, with mean and range, are presented in Table 5.

Table 4. Showing details of measurements of 10 spores of *Myxobolus* sp. 4 recovered from the gill rakers

No.	LS	BS	LLPC	BLPC	LSPC	LSPC
1	8.4	5.04	5.04	2.52	2.52	1.68
2	10.08	5.88	4.2	2.52	2.52	1.68
3	8.4	5.04	3.36	1.68	1.68	1.68
4	9.24	6.72	5.04	2.52	2.52	1.68
5	7.56	5.88	3.36	2.52	2.52	1.68
6	8.4	5.88	4.2	2.52	3.36	1.68
7	10.08	5.04	5.04	2.52	3.36	1.68
8	6.72	5.04	3.36	2.52	2.52	1.68
9	8.4	5.04	3.36	2.52	2.52	1.68
10	8.4	5.88	4.2	2.52	2.52	1.68
Range	6.72-10.08	5.04-6.72	3.36-5.04	1.68-2.52	1.68-3.36	1.68
Mean	8.57	5.54	4.12	2.44	2.6	1.68

No. - Number;
LS – Length of spore;
BS – Breadth of spore;
LLPC – Length of large polar capsule;
BBPC – Breadth of large polar capsule;
LSPC – Length of small polar capsule;
BSPC – Breadth of small polar capsule.

Myxobolus sp. 5:

Description:

Site of Infection: On the outer wall of pharynx.
Prevalance: 18 out of 110 fishes (16.4%) examined were infected.
Pathogenecity: Not apparent.
Host: *Liza* spp. (Mugilidae).
Life stage infected: Adults.
Locality: Cochin backwaters.
Period: March 2001 to May 2001.

Cysts: Cysts appeared as opaque whitish spots in the outer wall of pharynx. Spherical in shape and measured 0.1 mm to 0.3 mm in diameter. Numbers of cysts ranged from 1 to 17.

Spores (Fig: 5, Plate: 5): Spores slightly oval in valvular view and lenticular in sutural view. Anterior portion of the spores appeared slightly flat. Polar capsules 2, pyriform in shape and equal in size. Inter capsular and intervalvular ridges present. Sporoplasm half moon shaped with a small projection at the center and occupied the entire extracapsular region. Sutural line straight.

Spore dimensions: Spores measured 7.56 - 10.08 (mean, 8.48) in length and 5.04 - 6.72 (mean, 5.88) in width. Polar capsules measured 2.52 - 3.36 × 1.68 - 2.52 (mean, 3.28 by 1.93). Details of spore measurements, with mean and range, are presented in Table 6.

Table 6. Showing details of measurements of 10 spores of *Myxobolus* sp. 5 recovered from the outer wall of the pharynx

No.	LS	BS	LPC	BPC
1	8.4	5.88	3.36	2.52
2	8.4	5.04	3.36	1.68
3	7.56	5.04	3.36	1.68
4	8.4	6.72	3.36	2.52
5	10.08	6.72	3.36	1.68
6	8.4	5.88	3.36	2.52
7	8.4	6.72	3.36	1.68
8	8.4	5.88	2.52	1.68
9	8.4	5.88	3.36	1.68
10	8.4	5.04	3.36	1.68
Range	7.56-10.08	5.04-6.72	2.52-3.36	1.68-2.52
Mean	8.48	5.88	3.28	1.93

No. - Number;
LS – Length of spore;
BS – Breadth of spore;
LPC – Length of polar capsule;
BPC – Breadth of polar capsule.

Myxobolus sp. 6:

Description:

Site of Infection: Intestinal wall.

Prevalance: 8 out of 110 fishes (7.3%) examined were infected.

Pathogenecity: Not apparent.

Host: *Liza* spp. (Mugilidae).

Life stage infected: Sub adults and adults.

Locality: Cochin backwaters.

Period: March 2001 to May 2001.

Cysts: Cysts appeared as whitish spots, were spherical to oval in shape and measured 0.2 mm to 1.5 mm in size. Number of cysts ranged from 1 to 13.

Spores (Fig: 6, Plate: 6a, b): Spores broadly ellipsoidal with a slightly acuminate anterior end in valvular view and lenticular in sutural view. Polar capsules 2, pyriform in shape and equal in size. Intercapsular ridge present. Sporoplasm cup shaped, with 2 small depressions below the polar capsules and a small projection at the center and found delineated from the spore wall. Sutural line straight and thick.

Spore dimensions: Spores measured 6.72 - 8.4 (mean, 7.98) in length and 5.08 - 6.72 (mean, 5.46) in width. Polar capsules measured 2.52 - 3.36 × 1.68 - 2.52 (mean, 3.28 by 2.44). Details of spore measurements, with mean and range, are presented in Table 7.

Table 7. Showing details of measurements of 10 spores of *Myxobolus* sp. 6 recovered from the outer wall of the intestine

No.	LS	BS	LPC	BPC
1	8.4	5.04	3.36	2.52
2	8.4	5.04	3.36	2.52
3	7.56	5.04	3.36	2.52
4	6.72	5.04	2.52	1.68
5	8.4	6.72	3.36	2.52
6	8.4	5.88	3.36	2.52
7	8.4	5.88	3.36	2.52
8	6.72	5.04	3.36	2.52
9	8.4	5.88	3.36	2.52
10	8.4	5.04	3.36	2.52
Range	6.72-8.4	5.04-6.72	2.52-3.36	1.68-2.52
Mean	7.98	5.46	3.28	2.44

No. - Number;
LS – Length of spore;
BS – Breadth of spore;
LPC – Length of polar capsule;
BPC – Breadth of polar capsule.

Myxobolus sp. 7:

Description:

Site of Infection: Connective tissues of the peritoneum.
Prevalance: 3 out of 110 fishes (2.7%) examined were infected.
Pathogenecity: Not apparent.
Host: *Liza* spp. (Mugilidae).
Life stage infected: Sub adults and adults.
Locality: Cochin backwaters.
Period: March 2001 to May 2001.

Cysts: Cysts appeared as whitish spots in the connective tissues of the peritoneum, spherical in shape and measured 0.2 mm to 0.3 mm in size. Number of cysts ranged from 1 to 2.

Spores (Fig:7, Plate: 7): Spores spherical in valvular view and broadly lenticular in sutural view . Polar capsules 2, pear shaped, equal in size and occupy more than half the volume of the spore. Intercapsular and intervalvular ridges absent. Sporoplasm crescentic in shape, with blunt ends and a small projection at the center and filled the entire extra capsular space.

Spore dimensions: Spores measured 5.04 - 7.56 (mean, 6.38) in length and 6.72 - 7.56 (mean, 6.8) in width; Polar capsules measured 3.36 x 1.68 - 2.52 (mean, 3.36 by 2.44). Details of spore measurements, with mean and range, are presented in Table 8.

Table 8. Showing details of measurements of 10 spores of *Myxobolus* sp. 7 recovered from the connective tissues of the Peritoneum

No.	LS	BS	LPC	BPC
1	6.72	6.72	3.36	2.52
2	6.72	6.72	3.36	2.52
3	6.72	6.72	3.36	2.52
4	5.04	7.56	3.36	2.52
5	6.72	6.72	3.36	2.52
6	5.04	6.72	3.36	2.52
7	5.88	6.72	3.36	2.52
8	6.72	6.72	3.36	2.52
9	7.56	6.72	3.36	1.68
10	6.72	6.72	3.36	2.52
Range	5.04-7.56	6.72-7.56	3.36	1.68-2.52
Mean	6.38	6.8	3.36	2.44

No. - Number;
LS – Length of spore;
BS – Breadth of spore;
LPC – Length of polar capsule;
BPC – Breadth of polar capsule.

MULTIPLE INFECTIONS:

Of the 110 fishes examined, 11 fishes (10%) had infections with more than one species of *Myxobolus* , details of which are given in Table 9.

Table 9: Showing the details of multiple infections with *Myxobolus* spp.

Sl No. Of Fish	Sp. 1 (Fins)	Sp. 2 (scales)	Sp. 3 (gills)	Sp. 4 (gill rakers)	Sp. 5 (pharyngeal region)	Sp. 6 (intestinal wall)	Sp. 7 (connecti ve tissue)
1	✓	–	–	–	✓	–	–
2	–	✓	–	–	✓	–	–
3	–	–	–	✓	✓	–	–
4	✓	–	–	–	✓	✓	–
5	✓	–	–	–	–	–	✓
6	–	–	–	–	✓	✓	–
7	✓	–	–	–	✓	–	–
8	–	–	–	–	✓	✓	–
9	✓	–	✓	–	–	–	✓
10	✓	–	✓	–	–	–	–
11	–	–	–	–	✓	✓	✓

Fig 1. Spores of *Myxobolus* sp. 1 (valvular view).

Fig 2. Spores of *Myxobolus* sp. 2 (Valvular view)

Fig 3. Spores of *Myxobolus* sp. 3 (Valvular view).

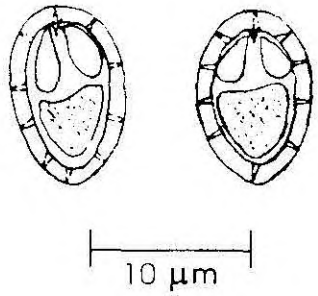


Fig. 1

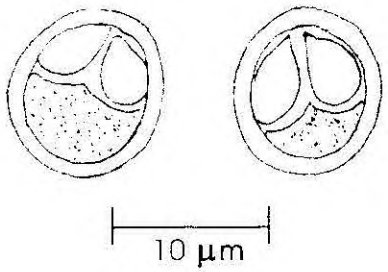


Fig. 2

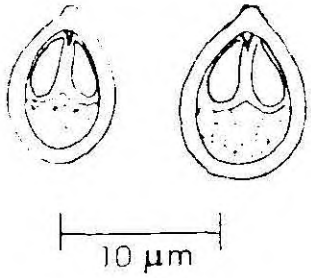


Fig. 3

Fig 4 Spores of *Myxobolus* sp. 4
a) Valvular view
b) Sutural view

Fig 5. Spores of *Myxobolus* sp. 5 (Valvular view).

Fig 6. Spores of *Myxobolus* sp. 6
a) Valvular view
b) Sutural view

Fig 7. Spores of *Myxobolus* sp. 7 (Valvular view).

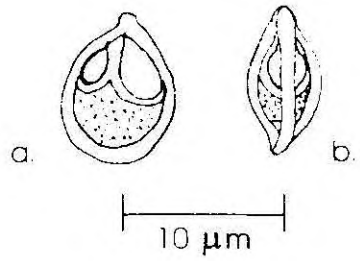


Fig. 4

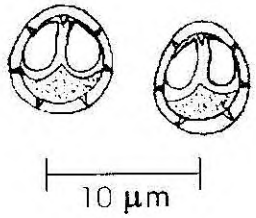


Fig. 5

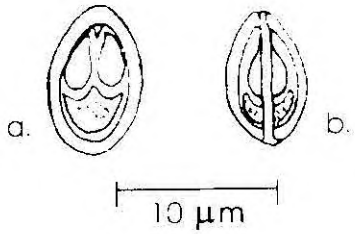


Fig. 6

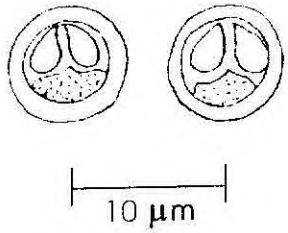
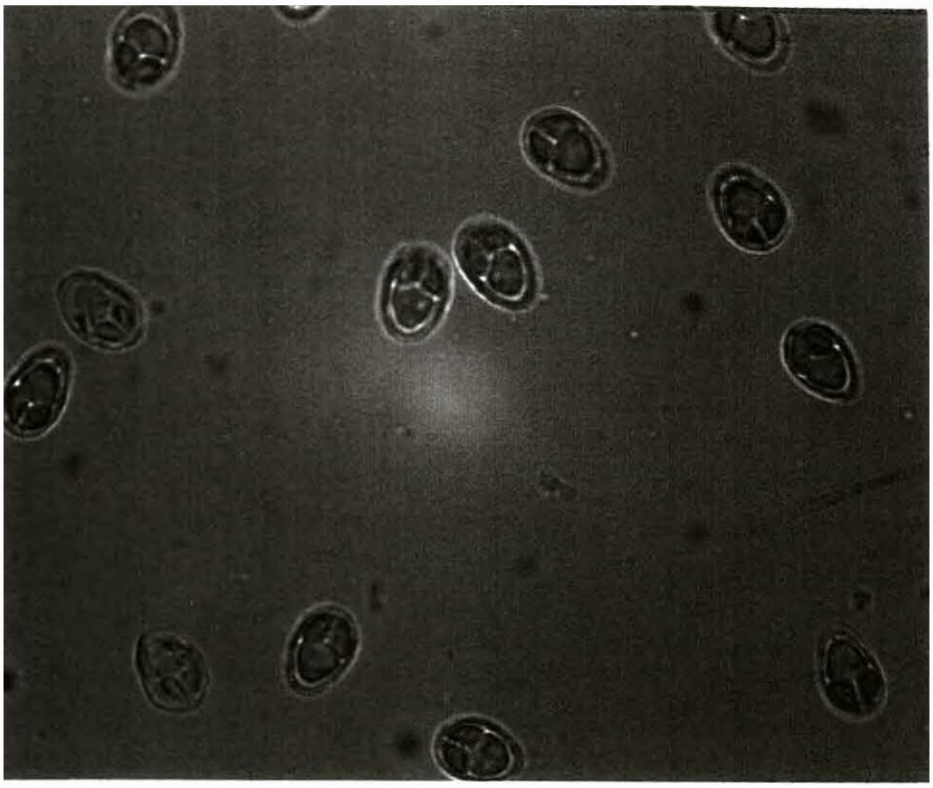


Fig. 7

Plate 1. Spores of *Myxobolus* sp. 1

Plate 2. Spores of *Myxobolus* sp. 2



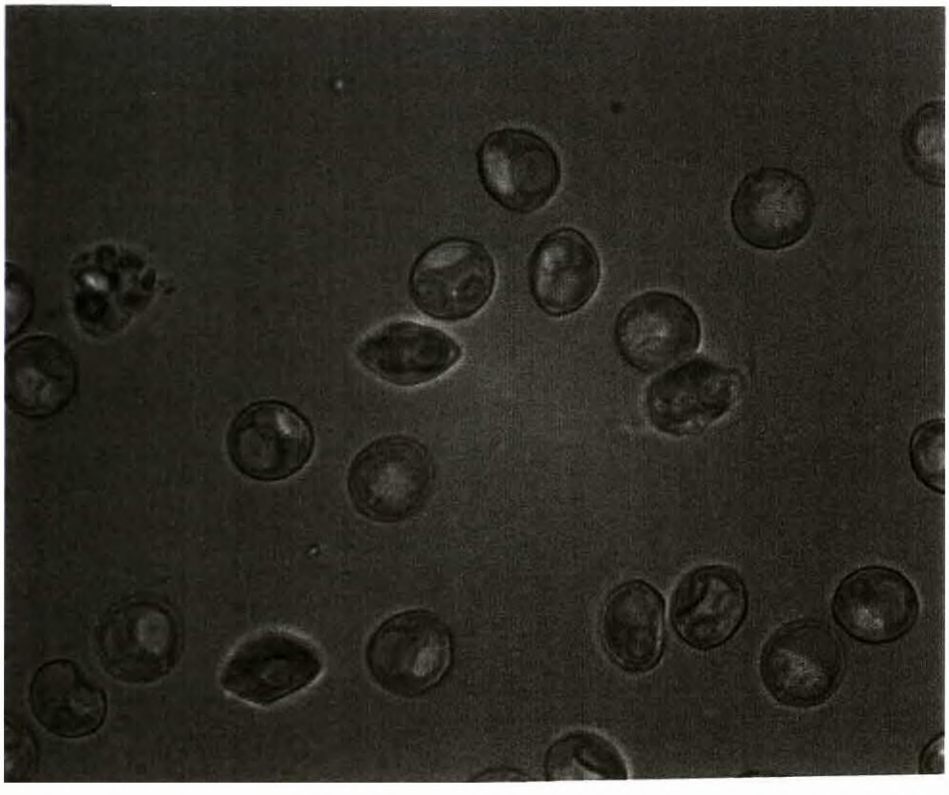
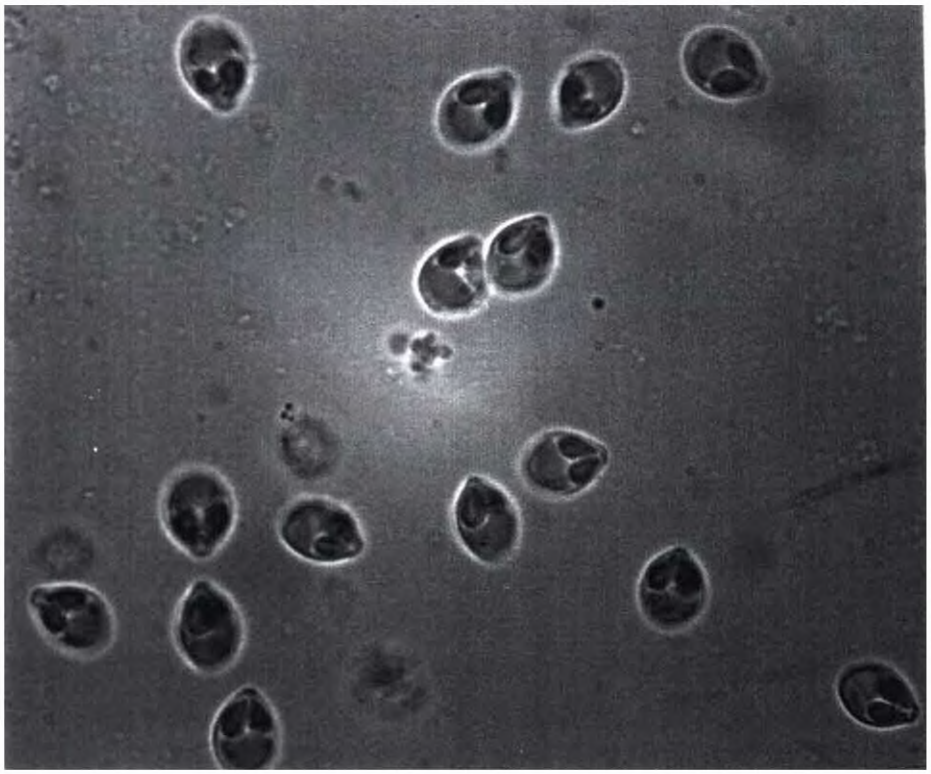


Plate 3. Spores of *Myxobolus* sp. 3

Plate 4. Spores of *Myxobolus* sp. 4



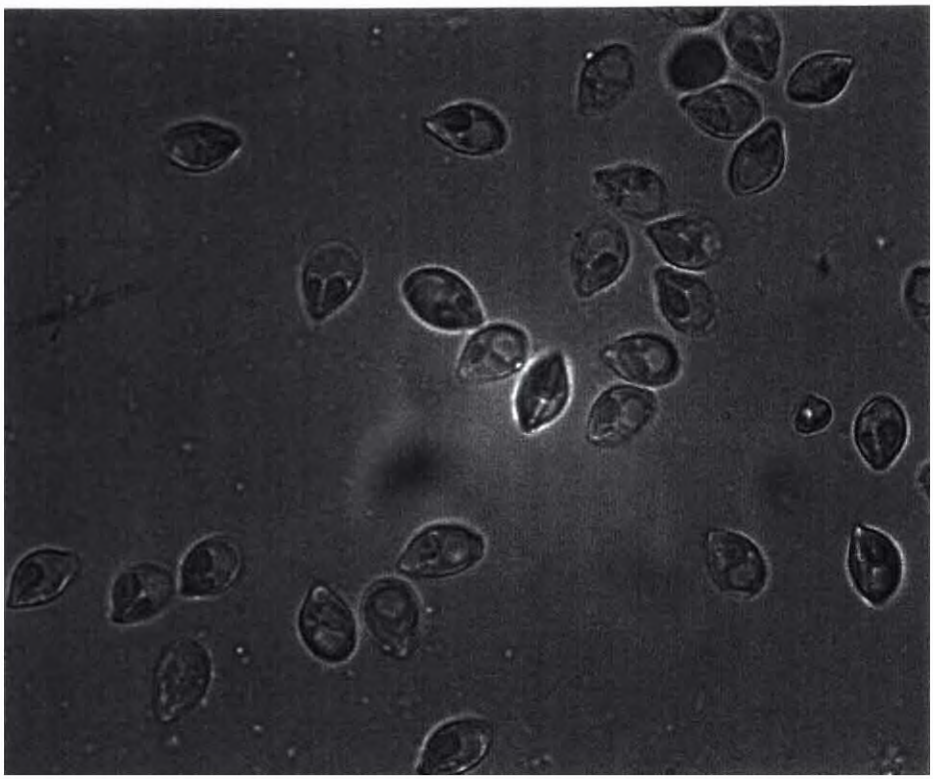
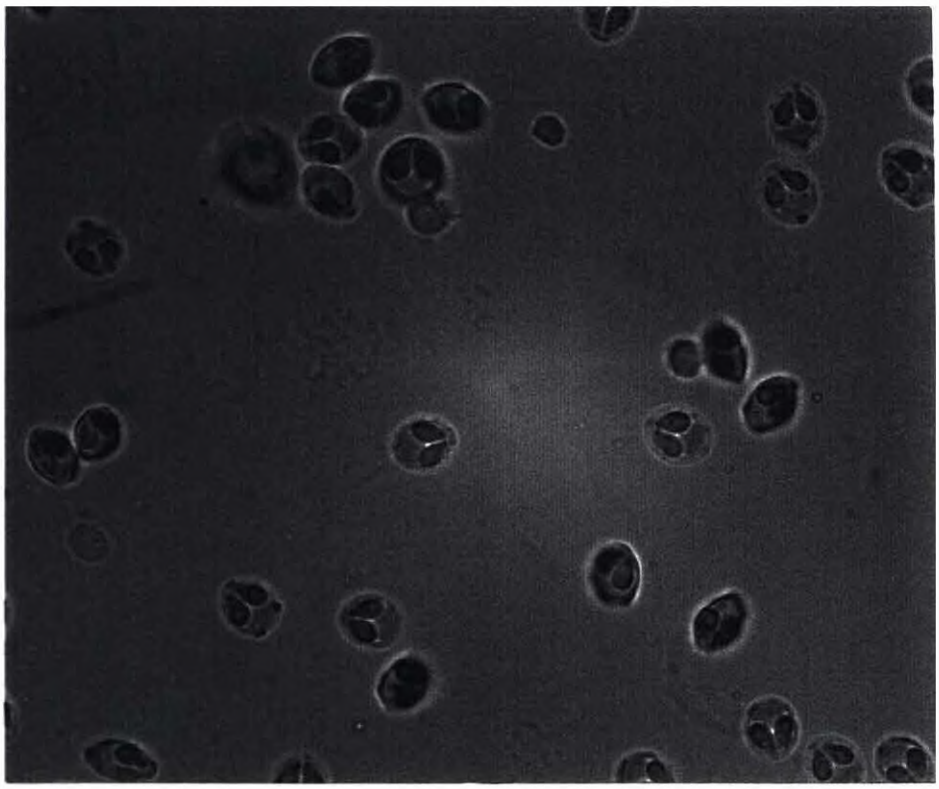


Plate 5. Spores of *Myxobolus* sp. 5

Plate 6. Spores of *Myxobolus* sp. 6



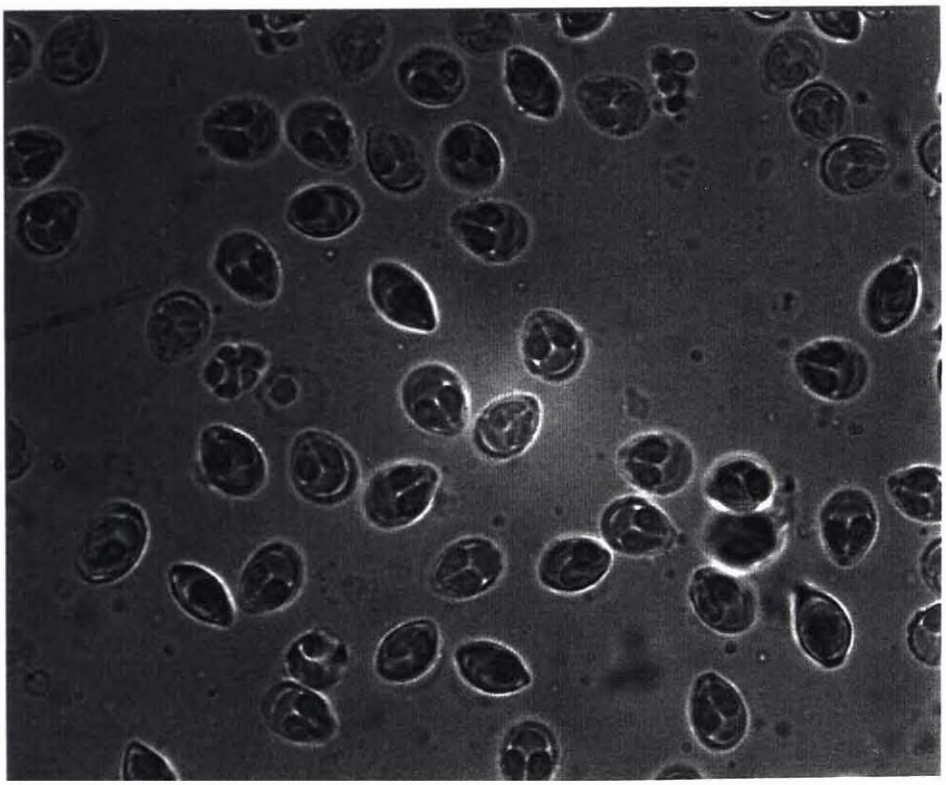
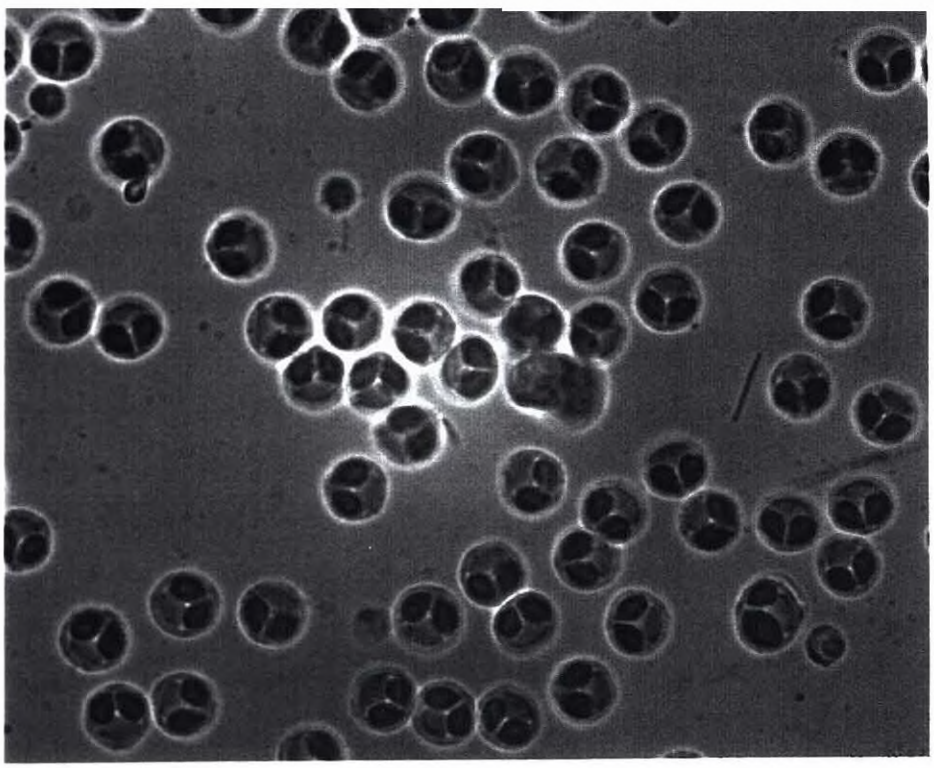


Plate 7. Spores of *Myxobolus* sp. 7



DISCUSSION

Though myxosporeans are generally considered harmless, in cases of heavy infections they are reported to inflict high mortalities and thereby cause heavy losses in Capture and Culture fisheries. Many a time these parasites may impair the growth of infected fish and reduce production. Among the 1330 species of myxosporeans reported from fishes, 467 species belong to the genus *Myxobolus*.

Mullets, which inhabit the brackish waters, are easily prone to parasitic infections. Among the protozoan parasites infecting mullets, myxosporeans are considered to be having an important role as pathogens. Till date, 9 genus of myxosporeans have been reported from mullets all over the world. Among the myxosporeans infecting mullets, species belonging to the genus *Myxobolus* are the most frequently encountered ones (Paperna and Overstreet, 1981). *Myxobolus exiguus* has been reported as the cause of mortality in mullet population that occurred in 1949 along the Atlantic and Mediterranean coast. Reports of such cases in mullets in confined water bodies are not available.

During the present study, a total of 7 species of *Myxobolus* have been recovered from mullets collected from Cochin backwaters. Of the 110 fishes examined, 40 fishes were found to be infected with myxosporean parasites. Incidentally, all the 7 species recovered were identified to be belonging to the genus *Myxobolus*. This is in agreement with the findings of Dorothy and Kalavati (1992) who have reported the prevalence of 4 species of myxosporeans, all belonging to genus *Myxobolus* from 1016 fishes of *Liza macrolepis* examined in Gosthani estuary and Visakhapatnam. Kalavati and Anuradha (1992) also reported the prevalence of 12 species of myxosporeans from 2344 *Mugil cephalus* examined from Gosthani estuary and Visakhapatnam, along the east coast of India, of which 7 species were of *Myxobolus*.

All the 7 species encountered during the present study were found in different organs namely fins, scales, gills, gill rakers, outer wall of pharynx, outer wall

of intestine and connective tissues of the peritoneum. They were characteristic in their shape and structure and differed from each other in various aspects including measurements.

The prevalence of the above 7 species individually varied from 1.8% to 16.4% with the minimum prevalence being observed in *M.* sp. 4 infecting gill rakers and maximum prevalence in *M.* sp. 5 infecting the outer wall of pharynx. The intensity of infection on the other hand varied from 1 to 2 cysts of *M.* sp. 7 observed in the peritoneal region to as high as about 80 to 100 in *M.* sp. 4 found in the gill rakers. Details regarding prevalence and intensity of infection are presented in Table 10. Prevalence of *Myxobolus* species as reported from Indian waters varied between 1.75% (Kalavati and Anuradha, 1992) and 25% (Narasimhamurti *et al.*, 1980).

All the recovered myxosporean species exhibited high levels of site/organ specificity in the host. Molnar, 1994 has pointed out that majority of the known *Myxobolus* species has a well-defined site of development and there are species specific to the gills, skin, kidney, intestine etc.

A comparison of the 7 species of *Myxobolus* recovered during the present study with the 16 species of *Myxobolus* reported from mullets so far (Dorothy and Kalavati, 1992) has been attempted to find out whether there is any similarity with the already existing species parasitising mullets. Only one species, *M.* sp. 5 that occurred on outer wall of pharynx showed some affinity to *M. exiguus* reported from the intestine of mullets from different parts of the world. Rest of the 6 species differed either in shape, structure or in measurements. Since literature pertaining to all the existing species of *Myxobolus* was not available for comparison, the taxonomic status of the parasites, as to whether they are new species or already described ones could not be ascertained. More over, owing to the limited period, a detailed study could not be conducted. So these 7 species recovered during the present study are hereby designated as *Myxobolus* sp. 1 to *M.* sp. 7.

Myxobolus sp. 4 and *Myxobolus* sp. 5 were recovered from the gill rakers and pharyngeal region respectively during the present study. Till date there has been no report on the occurrence of *Myxobolus* species in gill rakers or pharyngeal region of mullets and this is the first report of myxosporean infection from these organs of mullet. Paperna and Overstreet (1981) have stated that infections with myxosporeans differ according to site, size and geographical locality of the host, as well as by season.

Eleven out of 110 fishes (10%) examined were found to be infected with myxosporeans of more than one species. Infections with 2 species occurred in 9 cases and 3 species occurred in 2 cases. A significant observation was the occurrence of *Myxobolus* sp. 5, in 8 out of the 11 cases of multiple infections. Though no particular correlation could be drawn among the 2 or more species occurring at the same time, it has been observed that in 3 cases, *Myxobolus* sp. 3 and *M.* sp. 5 occurred simultaneously.

Myxobolus species is a histozoic form. Though some histozoic forms are believed to cause more serious damages, except for the presence of cysts of the different species, gross pathological changes were not apparent (no histopathology studies have been done) in the present study. Kent and Margolis (1995) were of the opinion that many of the myxosporean species form small, confined white pseudocysts with little associated tissue damage. However, when these pseudocysts are numerous in vital organs, such as the gills or heart, they can cause disease. During the present study, though multiple infections with 3 species were observed in 2 cases and intensity of cysts was considerably high, the fishes were healthy in external appearance.

All the 7 species observed existed as cysts deposited in different organs varying in size from less than 0.1 mm to 2 mm. *M.* sp. 1 occurred on fins especially on pectoral and pelvic fins with rare occurrence on dorsal and anal fins during heavy infestations. Cysts of *M.* sp. 2 occurred on the scales at the base of the pectoral and pelvic fins, located at the center of the scale. *M.* Sp. 3 occurred either on gill arch or attached to gill filaments. *M.* Sp. 4 was observed to be positioned

between 2 adjacent gill filaments. *M.* Sp. 5 was observed to be spread all along the outer wall of pharynx, on the outer surface with no specific location. *M.* sp. 6 occurred mostly in the anterior part of intestine with rare occurrence on the posterior part and *M.* sp. 7 occurred in the connective tissues underlying the peritoneal membrane.

Table 10: Showing details of site of infection, prevalence, cyst size and Intensity of infection

Species no.	Site of infection	Prevalence	Size of cyst	Intensity
Myxobolus sp. 1	Fins	12/110 (10.9%) fishes	0.2 – 2 mm	1 – 20
Myxobolus sp. 2	Scales	3/110 (2.7%) fishes	0.5 – 1 mm	1 – 3
Myxobolus sp. 3	Gills	5/110 (4.5%) fishes	0.3 – 1 mm	1 – 5
Myxobolus sp. 4	Gill rakers	2/110 (1.8%) fishes	< 0.1 mm	20 – 100
Myxobolus sp. 5	Pharyngeal region.	18/110 (16.4%) fishes	0.1 – 0.3 mm	1 – 17
Myxobolus sp. 6	Outer wall of intestine.	8/110 (7.3%) fishes	0.2 – 1.5 mm	1 - 13
Myxobolus sp. 7	Connective tissue underlying the peritoneal membrane	3/110 (2.7%) fishes	0.2 – 0.3 mm	1 – 2

The present study revealed that the prevalence of myxosporean parasites in mullets of Cochin backwaters is quite high, and all the 7 species recovered belonged to the genus *Myxobolus.* Though the fishes were apparently healthy on gross observation, detailed histopathological study may provide a clear picture of the damage caused at tissue levels in heavy infections.

SUMMARY

- Prevalence of myxosporean parasites in mullets of Cochin Backwaters was studied for a period of ten weeks from March 2001 to May 2001.
- 110 mullets were examined. 40 fishes were infected with myxosporean parasites. This constituted 36.4% prevalence.
- A total of seven different species of Myxosporeans were recovered from the 110 fishes examined and all the 7 species were identified to be of genus *Myxobolus*.
- Prevalence of myxosporean infection varied from 1.8% to 16.4% individually.
- The 7 species of *Myxobolus* were recovered from different site/organ namely, fins, scales, gills, gill rakers, pharyngeal region, intestine and peritoneum each species exhibiting high level of site/organ specificity.
- Occurrance of myxosporean infection in the gill rakers and pharyngeal region in mullets were reported for the first time,
- Multiple infections were observed in 11 out of 110 fishes examined.
- Fishes appeared quite healthy externally inspite of heavy incidence of myxosporean parasites.

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