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Filamentous Fungi Present in the External Mucus of Gizzard Shad (Dorosoma cepedianum)

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Filamentous Fungi Present in the External Mucus

of Gizzard Shad (*Dorosoma cepedianum*)

(TITLE)

BY

Kenneth C. Stetina

THESIS

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ABSTRACT

The mucus layers of 30 gizzard shad (*Dorosoma cepedianum*) from the Lake Charleston side channel reservoir were sampled to identify what fungi or fungal spores were present. The fish sampled had a mean length of 171 mm, a mean weight of 41 g, and a mean age of 2.1 years. Hemp seed, fish scale, and polycell-gel substrates were examined, and four genera of fungi were identified. *Fusarium* was most frequently encountered, followed by *Pythium*, *Allomyces*, and *Saprolegnia*, respectively. Older fish (2.5 and 3.5 yrs) had a greater diversity of associated fungi than did younger fish (1.5 yrs), probably due to their benthic feeding habits and greater reproductive stress.

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INTRODUCTION

Fungi which parasitize fish have been documented for a number of years (Agersborg, 1933; Kanouse, 1932; Scott, 1964). Fungal infections are quite evident in still, man-made impoundments with high fish densities. Examples of suitable aquatic ecosystems include farm fish-ponds, fish hatcheries, and sport fishing recreation areas.

Aquatic fungi can have adverse effects on fish populations. For example, fish eggs are susceptible to fungal attack which results in a small percentage of fry hatching from the eggs. The fry which survive have little defense against fungal infection as they are weak and have not accumulated a sufficient coat of protective mucus. Although the mucus coating generally acts as a barrier to prevent infection, it can be removed when a fish is roughly handled or slightly bruised. When this occurs, fungal infection and a high rate of mortality are likely to follow (Scott, 1964). Even if the fry mature into fingerlings, and then yearlings, they often fall prey to fungi under environmental stress. Among the conditions which preclude fish to fungal infection are environmental changes, parasites which act as primary invaders, piscivorous predators which disrupt the mucus layer, mechanical scraping off of the mucus layer on various substrates, and open wounds caused by fighting for territories and mates or

fishermen who extensively handle the fish and then throw them back.

This study was designed to sample the mucus layer of 30 gizzard shad (Dorosoma cepedianum Lesneur) from the side channel reservoir of Lake Charleston and determine what fungi or fungal spores are present. This was accomplished by culturing the mucus and identifying the fungi which grew out from the mucus. Lake Charleston is located a mile and a half south of the city of Charleston, Illinois (T12N, R10E, Sect.30). Lake Charleston is a highly eutrophic, man-made, side channel reservoir which dates to 1947 and the construction of the Riverview dam. The dam blocked off the Embarrass River with the flooded area behind the dam being used as the water supply for Charleston. In 1978, as a result of heavy siltation, the lake had an average depth of 0.46-0.61 m and provided a 74-day supply of water for the city of Charleston. In the event of a drought, however, the water supply would have been severely threatened. With this in mind a new dike and side channel were constructed in 1981. The depth of Lake Charleston was subsequently increased to 2.44 m and the city's water supply increased to 174 days. The lake depth is kept more or less constant by a station which pumps filtered water from the side channel into the reservoir (Figure 1). The lake can be successfully stocked with fish as the fish population cannot move from the lake.

The fish being sampled, the gizzard shad, does not occur naturally in Lake Charleston. It was stocked in the lake as a forage fish for piscivores, especially the large mouth bass (Micropterous salmoides Lacepede). There is evidence that large mouth bass only eat young gizzard shad and do not bother older fish (Smith, 1979).

LITERATURE REVIEW

GIZZARD SHAD NATURAL HISTORY

The gizzard shad (Dorosoma cepedianum) is a member of the class Osteichthyes, order Clupeiformes, family Clupeidae. Originally, its range extended from Minnesota eastward to the St. Lawrence River and from New Jersey southward to the Gulf of Mexico. Today it is found in the southwestern states where it has been introduced as a forage fish. Although the gizzard shad is generally considered to be a fresh water fish, it has also been recovered in estuaries and brackish waters. The gizzard shad is recognized by its white or silvery body, dorsal fin in which the last ray extends into a long filament, and an inferior or subterminal mouth. In addition, it has a very acute sense of hearing because its swimbladder is connected to the inner ear by a narrow diverticulum of the bladder (Moyle and Cech, 1988). Although similar in appearance to the threadfin shad (Dorosoma petenense Gunther), the gizzard shad differs

in its mouth position, smaller scales, and higher anal fin ray count (Smith, 1979).

The gizzard shad is found in greatest abundance in oxbow lakes and reservoirs where it may comprise the greatest percentage of the fish biomass. As a result of its economic importance, research to date on the gizzard shad has been centered on natural history and physiology. No previous studies have been published on fungal spores present in the mucus of the gizzard shad.

Spawning occurs in April, May and June. Females lay several thousand adhesive eggs which attach to almost any available substrate. The eggs hatch in about three days at 25° C (Smith, 1979). The shad larvae are cylindrical in shape and exhibit a terminal mouth that contains teeth on both jaws. The growth rate in the first growing season is quite rapid with an average length of 100 mm attained by the end of this period. Maturity is reached in the second year of life with the average total life expectancy being 6-7 years (Smith, 1979).

Young gizzard shad feed on surface and subsurface zooplankton until they reach 25 mm in length. As they age, their digestive system undergoes developmental changes to allow benthic feeding (Heinrichs, 1982). First, the mouth changes from a supraterminal position to a subterminal position to facilitate benthic feeding. The pharynx is then modified for straining microscopic particles. Next,

pharyngeal organs with goblet cells and taste buds develop, the esophageal and gastric glands appear, and the stomach becomes muscular to function as a crushing organ. Intestinal length increases, most likely to accommodate the shift in diet from zooplankton to a herbivorous diet on benthic material (Heinrichs, 1982).

MUCUS LAYER

In fresh and salt water fish, the mucus layer is the outermost covering. It covers the scales and epidermis and provides an extra barrier which potential parasites must penetrate. Since it is slick, the mucus layer may also aid the fish in evading predators. Research completed by Fletcher and Grant (1969) suggests that teleost fish possess the ability to produce antibodies in the external mucus, although the fish must be alive for this inhibitory morphogen to be present. If the mucus is removed from the fish or if a dead fish is exposed to pathogenic spores, the external mucus is readily colonized (Wood et al., 1988). In healthy fish, the mucus is constantly being replaced through continuous secretions by goblet cells in the dermis. The old mucus is sloughed off and spores are lost with it.

FUNGI ASSOCIATED WITH FISH

Many types of aquatic fungi can attack fish. In a study performed by Pickering and Willoughby (1977), five genera

of fungi were found in epidermal lesions of perch (Perca fluviatilis Linnaeus). They (Pickering & Willoughby, 1977) postulated that Argulus foliaceus Linnaeus, a fish louse, broke the mucus layer and allowed for secondary infection by fungi. The five genera of fungi observed in the epidermal legions were Leptomitus Agardh, Achlya C.G.Nees, Saprolegnia C.G.Nees, Aphanomyces deBary, and Pythiopsis deBary. In salmonid fish inhabiting the British Isles, Saprolegnia parasitica Coker (Coker, 1923; Kanouse, 1932) has invariably been isolated from fungal infections. Saprolegnia parasitica has also been observed on trout (Salmo trutta Linnaeus), char (Salvelinus alpinus Linnaeus), and salmon (Salmo salar Linnaeus) (Willoughby and Pickering, 1977). Saprolegnia parasitica has also been observed on eels (Anguilla japonica Temmincke et Schlegels) in Japan (Hoshina et al., 1960). Scott (1964) concluded that Saprolegnia parasitica, S. ferax (Gruith.) Thuet., S. delica Coker, S. monoica Pringsheim, Achlya bisexualis Coker & Couch, as well as other isolates of Saprolegnia parasitize wounded platyfish (Xiphophorus maculatus Gunther) under controlled laboratory conditions.

FUSARIUM NATURAL HISTORY

Fusarium is a member of the form class Hyphomycetes, form order Moniliniales, form family Moniliniaceae. The life cycle of Fusarium, an imperfect genus, includes no

known sexual phase. The mycelium is extensive and cottony in culture, often with some tinge of pink, purple or yellow in the mycelium or medium. The conidiophores are variable, slender and simple or stout, short, branched irregularly or bearing a whorl of phialides, single or grouped into sporodochia. The conidia (phialospores) are hyaline, variable, principally of two kinds, often held in small moist heads: 1) macroconidia: several-celled, slightly curved or bent at the pointed ends, typically canoe-shaped: 2) microconidia: 1-celled, ovoid or oblong, borne singly or in chains. In addition, some conidia are intermediate to these two types and are two or three-celled, oblong or slightly curved. Fusarium is typically parasitic on higher plants or saprobic on decaying plant material (Barnett and Hunter, 1972).

PYTHIUM NATURAL HISTORY

Pythium is a member of the class Oomycetes, order Peronosporales, family Pythiaceae (Bold et al., 1980). Pythium has a well-developed mycelium consisting of highly-branched hyphae. The zoosporangia are either filamentous and undifferentiated or well-defined spheroidal structures, each with an emission tube of variable length. In addition, the zoosporangia may exhibit internal proliferation. Biflagellate, reniform zoospores are expelled into a vesicle at the tip of the discharge tube where cleavage and

maturation occur. Pythium thalli are monoecious. Oogonia are spherical or ellipsoidal and may be either terminal or intercalary. Each oogonium produces a single egg which becomes an oospore after fertilization. Antheridia are of various shapes and may be either stalked or sessile. One to several antheridia are associated with each oogonium and form distinct fertilization tubes. Members of the genus Pythium are saprobic or parasitic on both plant and animal matter in water and soil (Sparrow, 1960).

SAPROLEGNIA FERAX NATURAL HISTORY

Saprolegnia ferax (Gruith.) Thuet. is a member of the class Oomycetes, order Saprolegniales, family Saprolegniaceae (Bold et al., 1980). The mycelium of S. ferax consists of stout, irregularly branched hyphae. Sporangia, which frequently exhibit lateral proliferation from the base, are cylindrical or slightly tapered in appearance. Gemmae when present, are pyriform and jointed. The thallus of S. ferax is monoecious, producing numerous oogonia and antheridia. Spherical or oval oogonia ar either terminal or intercalary, but are never arranged in chains. Each oogonium contains a single row of up to 20 eggs. Short, tuberous antheridia are associated with each oogonium and fertilization tubes are suppressed. Saprolegnia ferax is found in fresh water and soil (Sparrow, 1960) and is often a fish pathogen of economic importance (Scott, 1964).

ALLOMYCES ARBUSCULA NATURAL HISTORY

Allomyces arbuscula Butler is a member of the class Chytridiomycetes, order Blastocladiales, family Blastocladiaceae (Bold et al., 1980). The vegetative thallus of Allomyces arbuscula closely resembles that of Allomyces macrogynus. The two species can be separated using gametangial size. The female gametangia of Allomyces macrogynus are at least twice the size of the male gametangia, while the male and female gametangia of A. arbuscula are equal in size. Allomyces arbuscula produces two or rarely more gametangia terminally on its branches.

The resting spores of Allomyces are able to survive in the dry state for months or years. Species included in this genus are more often found in the warmer parts of the world, such as the Southern United States, Mexico, Central and South America, the West Indies, Southern Asia, the East Indies, Africa and Southern Europe (Bessey, 1965). Members of the genus Allomyces are found in both soil and water where they are saprobic on plant and animal remains (Sparrow, 1960).

MATERIALS AND METHODS

Thirty gizzard shad were netted from the side channel reservoir of Lake Charleston using a cast net. After draining off superficial water, each fish was placed in a pan and sacrificed by severing the spinal cord. The mucus was then scraped off the fish using a new, single-edged razor blade. The mucus of each fish, including a small number of fish scales, was divided equally and placed into two petri dishes. The petri dishes were previously used but were cleaned with alcohol. To each dish was added 10 ml of filtered, autoclaved Lake Charleston water to prevent the mucus from desiccating. The fish were then placed in a plastic bag and returned to the laboratory to record length, weight and age. The age of each fish was determined by examining the otolith (Cailliet et al., 1986). Once in the laboratory, 20 ml of filtered, autoclaved Lake Charleston water was added to each dish to bring the total volume to 30 ml. One dish received two split, boiled, hemp seeds, while the other received polycell-gel. Polycell-gel is a glucose-peptone medium with trace micronutrients. Streptomycin sulfate was added to prevent bacterial growth. Dry vinyl wallpaper paste granules were added until a gel-like consistency was reached (Willoughby et al., 1984). The cultures were incubated at 20° C, with observations made every other day to record fungal growth. Once the fungal

hyphae were conspicuous, the culture was transferred to a second petri dish with the same medium for further growth. Each colony was identified to genus, and, if the appropriate sexual structures developed, species. Frequency data were used to determine which species is most commonly associated with gizzard shad.

RESULTS

Thirty gizzard shad were sampled from the side channel reservoir. Fish length ranged from 147 mm to 206 mm, with a mean of 171 mm. Fish weight after removal of the mucus layer ranged from 24 g to 68 g, with a mean of 41 g. The youngest fish was 1.5 yrs and the oldest was 3.5 years, with a mean age of 2.1 yrs. Length, weight, and age data for each fish are presented in Table I.

Four genera of fungi were found in the mucus layers of the 30 gizzard shad sampled: Allomyces (Appendix I, Figs. 1-3), Pythium (Appendix I, Fig. 4), Saprolegnia (Appendix I, Figs. 5-7), and Fusarium (Appendix I, Figs. 8-10). The plates baited with split hemp seed produced more fungal cultures with greater diversity than the plates using polycell-gel.

In the plates baited with hemp seed, Fusarium was the most prevalent, occurring in 22 of the 30 cultures. Fifteen of the Fusarium colonies were noted growing on fish scales

that had been removed with the mucus. Pythium colonies were found in 11 cultures, occurring on both hemp seed and fish scales. Allomyces arbuscula was noted on hemp seeds in 2 cultures. Saprolegnia ferax was observed only once, growing on hemp seed (Table II).

The plates using the polycell-gel technique showed fungal growth in 7 of the 30 cultures. Fusarium was the most prevalent, occurring in all 7 cultures in polycell-gel and on fish scales. Pythium was observed in one culture, growing in the polycell-gel (Table III).

Fusarium and Pythium were associated with fish in the 1.5 yr age class. Fusarium, Pythium, Allomyces arbuscula, and Saprolegnia ferax were associated with fish in the 2.5 yr age class. Fusarium, Pythium, and Allomyces arbuscula were associated with fish in the 3.5 yr age class (Table IV).

DISCUSSION

Fusarium was found most commonly in the mucus layer of the gizzard shad sampled, regardless of fish age. In 17 cultures, Fusarium was found growing on fish scales, rather than on hemp seed or in polycell-gel. Fish scales contain between 41 and 84% organic matter, mainly collagen and icthylepidin, providing a nutrient-rich substrate which allows fungal growth. In addition to the organic

components, fish scales may contain up to 59% inorganic salts, primarily $CaCO_3$ and $Ca_3(PO_4)_2$. Small amounts of $Mg_3(PO_4)_2$ are also found in fish scales (Brown, 1957). Though inorganic salts are added to the polycell-gel medium, the concentration and relative proportions of each may differ enough to inhibit the growth of Fusarium as compared to the fish scales.

Pythium was second only to Fusarium in frequency, occurring in the mucus of 11 of the 30 gizzard shad sampled. Pythium was found in association with all age classes of fish sampled. Though 5 pure colonies of Pythium were noted, this genus often occurred in mixed colonies with Fusarium, Allomyces arbuscula, and Saprolegnia ferax. When Pythium was found in association with another fungus, its growth is typically less vigorous. Pythium may function as a saprobe which decomposes dead hyphae of other fungi.

Saprolegnia ferax was isolated from a single fish belonging to the 2.5 yr age class. Allomyces arbuscula was isolated from 2 fish, one in the 2.5 yr age class and one in the 3.5 yr age class. Since Allomyces is not common in this area, it probably was a contaminant that was introduced into the cultures from previously used petri dishes. Both of these species were associated exclusively with older fish. Two sets of conditions may explain why mature fish have a more diverse fungal compliment. Older fish are benthic feeders and are constantly exposed to both bacteria and

fungi on the lake bottom. The chance of encountering fungal spores is thereby increased. Secondly, older fish have reached reproductive age and are subjected to reproductive stress, which may temporarily weaken their resistance to fungal infection. When the fish were collected from the side channel reservoir, spawning was in progress. Energy typically allocated to life processes, such as continual replacement of the mucus layer and the production of associated antibodies, was used instead for reproductive activities.

Table I. Length, weight, and age of 30 gizzard shad (Dorosoma cepedianum) collected from the side channel reservoir at Lake Charleston.

FISH NUMBER	LENGTH (mm)	WEIGHT (g)	AGE (yrs)
1	178	51	2.5
2	183	54	2.5
3	183	49	2.5
4	160	38	1.5
5	147	32	1.5
6	152	32	1.5
7	168	36	2.5
8	163	34	1.5
9	173	30	1.5
10	165	36	2.5
11	170	44	1.5
12	152	30	1.5
13	168	40	2.5
14	183	48	2.5
15	163	32	1.5
16	173	44	2.5
17	163	53	1.5
18	150	24	1.5
19	178	50	1.5
20	160	28	1.5
21	160	24	1.5
22	191	56	2.5
23	155	26	1.5
24	196	53	2.5
25	163	37	2.5
26	206	68	3.5
27	188	52	3.5
28	199	28	3.5
29	155	32	1.5
30	196	58	2.5

Table II. Fungi associated with the mucus layer of gizzard shad, identified using hemp seed bait.

FISH NUMBER	FUNGUS	SUBSTRATE
1	No growth	none
2	Fusarium	hemp seed
	Pythium	hemp seed
3	Fusarium	hemp seed
	Allomyces arbuscula	hemp seed
4	Fusarium	fish scale
5	Fusarium	fish scale
6	Fusarium	fish scale
7	Pythium	hemp seed
8	Fusarium	hemp seed
	Pythium	hemp seed
9	Fusarium	fish scale
10	Fusarium	fish scale
	Pythium	fish scale
11	Fusarium	fish scale
12	Fusarium	fish scale
13	Fusarium	hemp seed
14	Fusarium	fish scale
15	Fusarium	hemp seed
16	Pythium	hemp seed
17	Fusarium	fish scale
	Pythium	fish scale
18	Fusarium	hemp seed
19	Pythium	hemp seed
20	Fusarium	fish scale
21	Fusarium	fish scale
22	Pythium	hemp seed
	Saprolegnia ferax	hemp seed
23	Fusarium	fish scale
24	Pythium	hemp seed
25	Fusarium	fish scale
26	Fusarium	fish scale
27	Fusarium	hemp seed
28	Allomyces arbuscula	hemp seed
	Pythium	hemp seed
29	Fusarium	fish scale
30	Pythium	hemp seed

Table III. Fungi associated with the mucus layer of gizzard shad, identified using polycell-gel.

FISH NUMBER	FUNGUS	SUBSTRATE
1	No growth	none
2	No growth	none
3	No growth	none
4	No growth	none
5	No growth	none
6	No growth	none
7	No growth	none
8	No growth	none
9	No growth	none
10	No growth	none
11	No growth	none
12	No growth	none
13	No growth	none
14	Fusarium	polycell-gel
15	No growth	none
16	No growth	none
17	No growth	none
18	No growth	none
19	No growth	none
20	Fusarium	polycell-gel
21	Fusarium	polycell-gel
22	Fusarium	polycell-gel
	Pythium	polycell-gel
23	No growth	none
24	No growth	none
25	Fusarium	fish scale
26	No growth	none
27	Fusarium	fish scale
28	Fusarium	fish scale
29	No growth	none
30	No growth	none

Table IV. Genera of fungi isolated from gizzard shad mucus associated with fish age and substrate.

AGE CLASS	SUBSTRATE		
	HEMP SEED	POLYCELL-GEL	FISH SCALE
1.5 year	Fusarium Pythium	Fusarium	Fusarium Pythium
2.5 year	Allomyces Fusarium Pythium Saprolegnia	Fusarium Pythium	Fusarium Pythium
3.5 year	Allomyces Fusarium Pythium		Fusarium

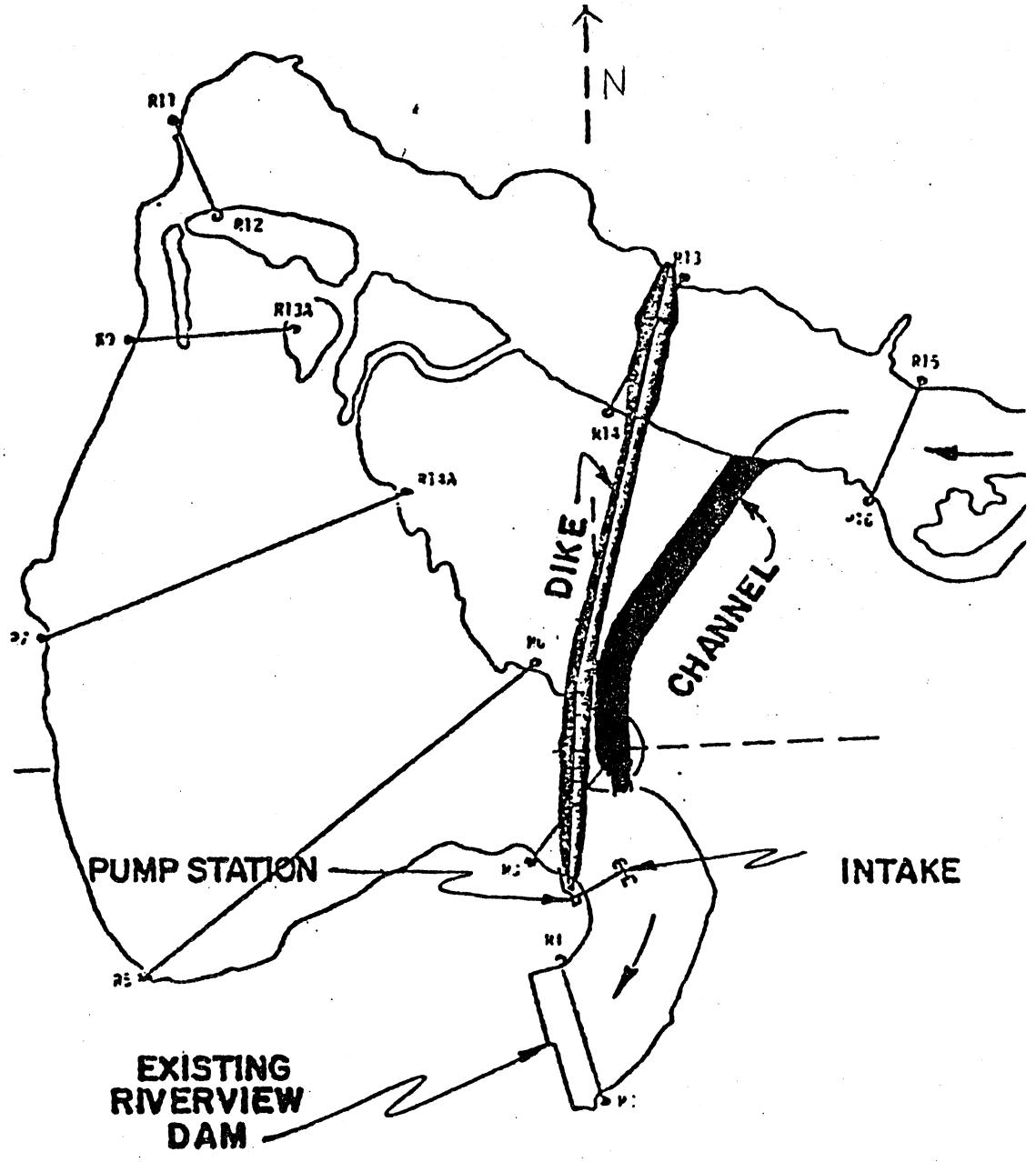

Figure 1. Map showing Lake Charleston and the side channel reservoir (T12N, R10E, Sect.30).

APPENDIX I: PHOTOGRAPHS OF FUNGI IDENTIFIED

Figure 1. *Allomyces arbuscula* showing meiosporangia and vegetative filaments (200X).

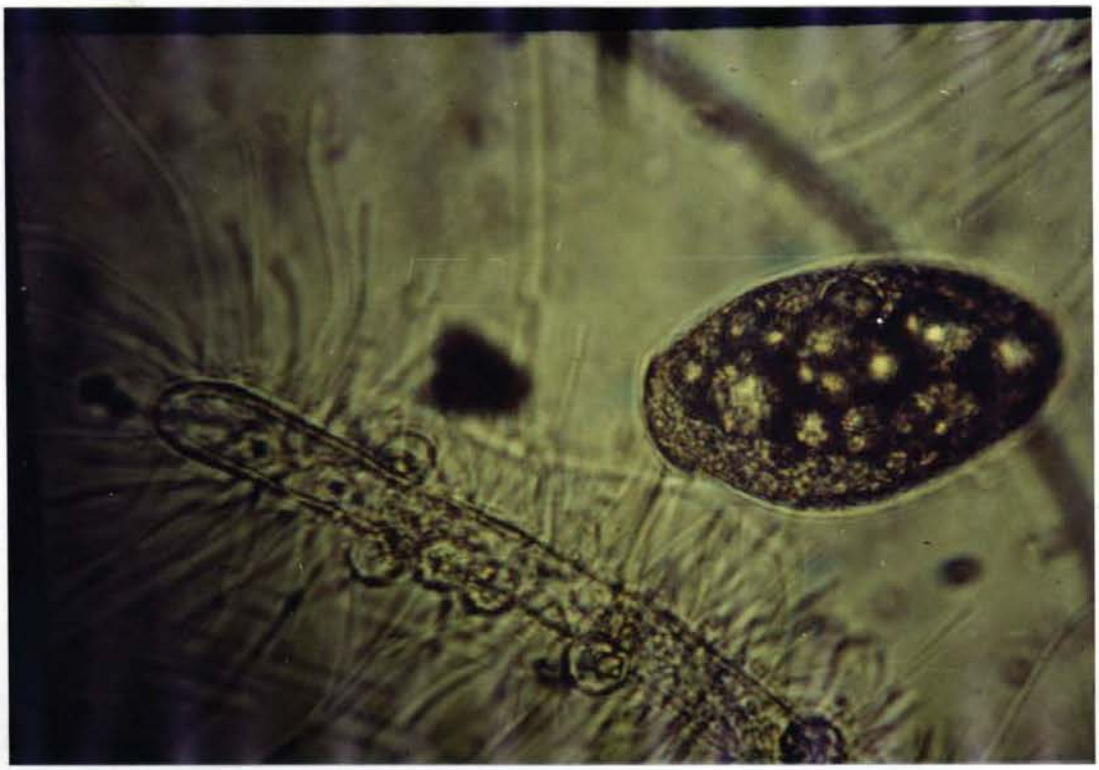

Figure 2. Deciduous meiosporangia of *Allomyces arbuscula* (400X).

Figure 3. Zoosporangia of Allomyces arbuscula (400X).

Figure 4. Vegetative filaments of Pythium (400X).

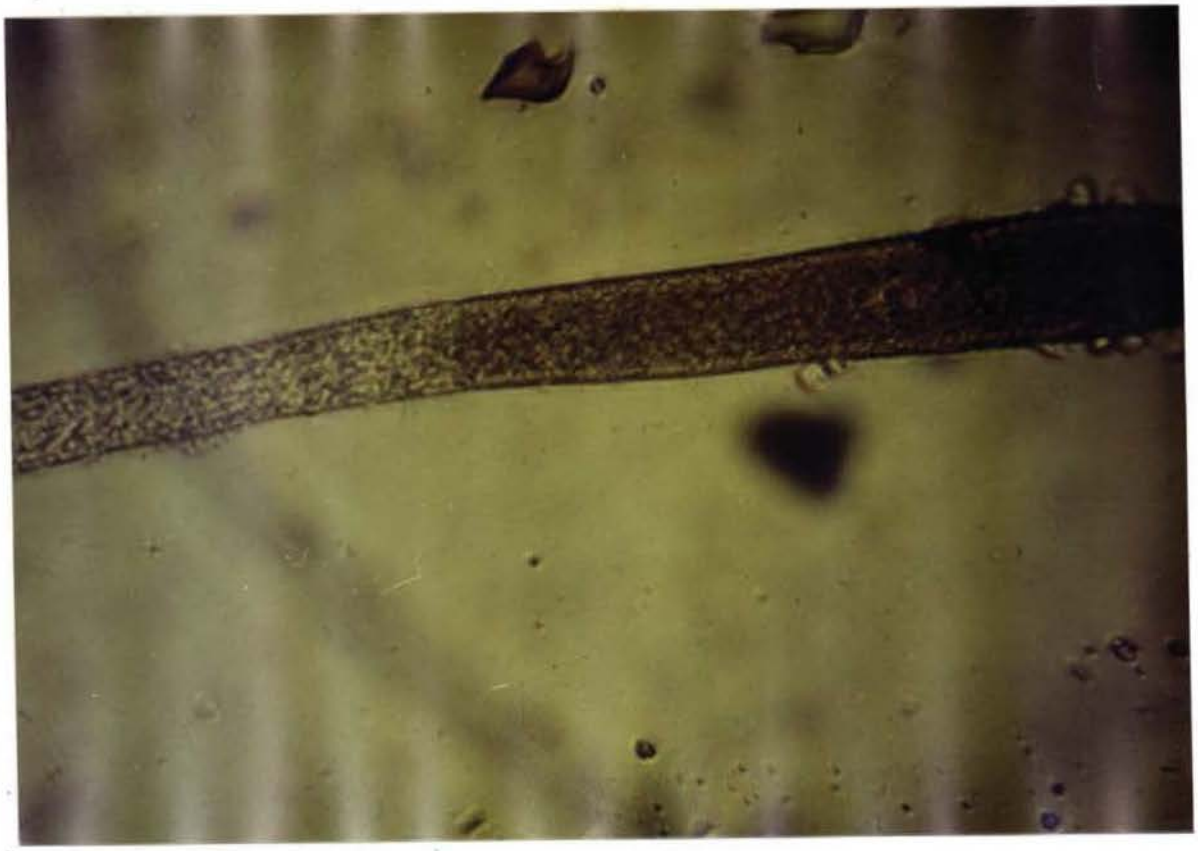

Figure 5. Zoosporangia of Saprolegnia ferax (200X).

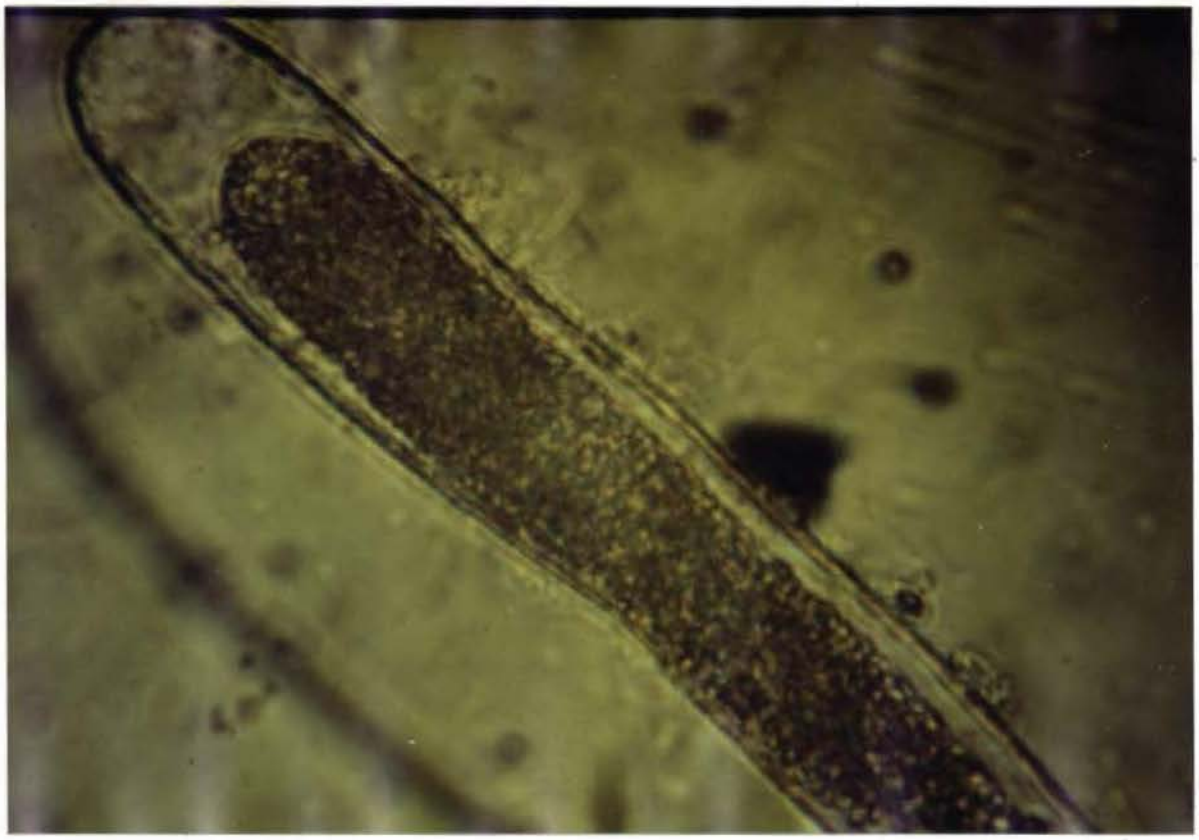

Figure 6. Zoosporangia of Saprolegnia ferax showing internal proliferation (400X).

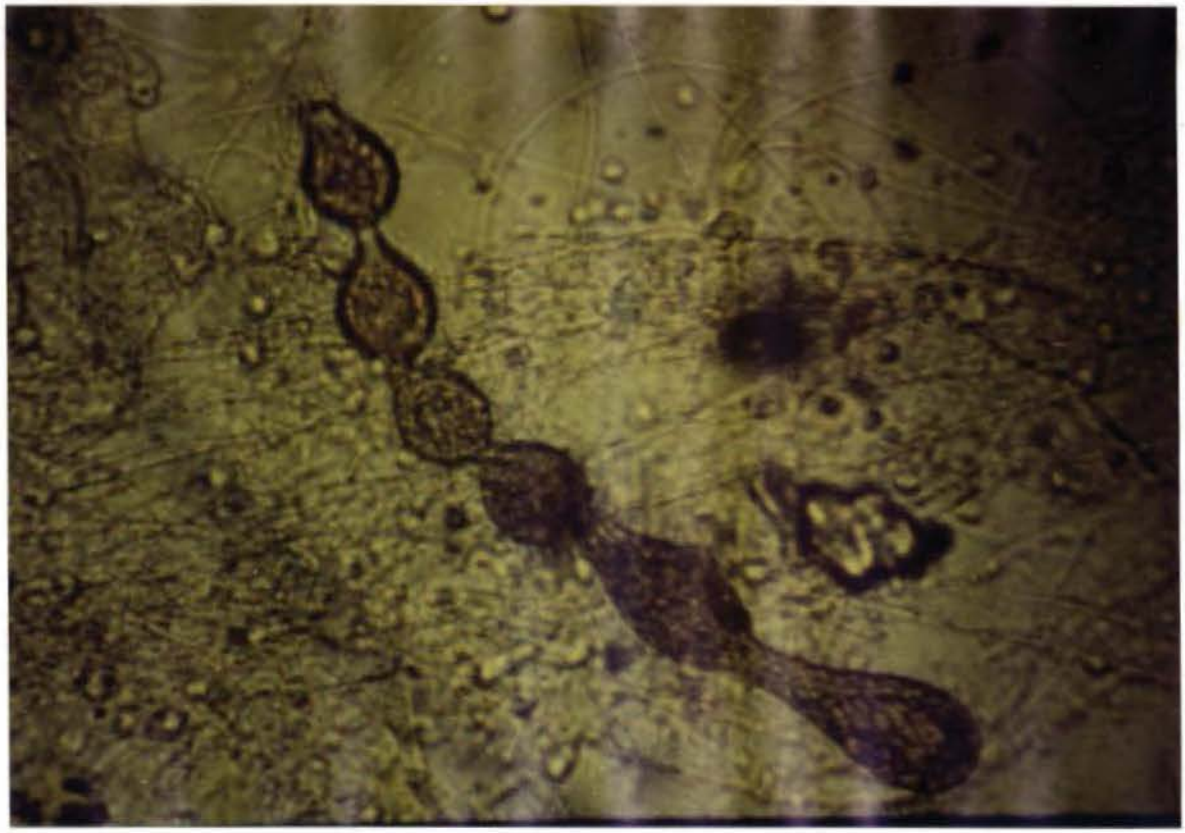

Figure 7. Chain of gemmae from Saprolegnia ferax (200X).

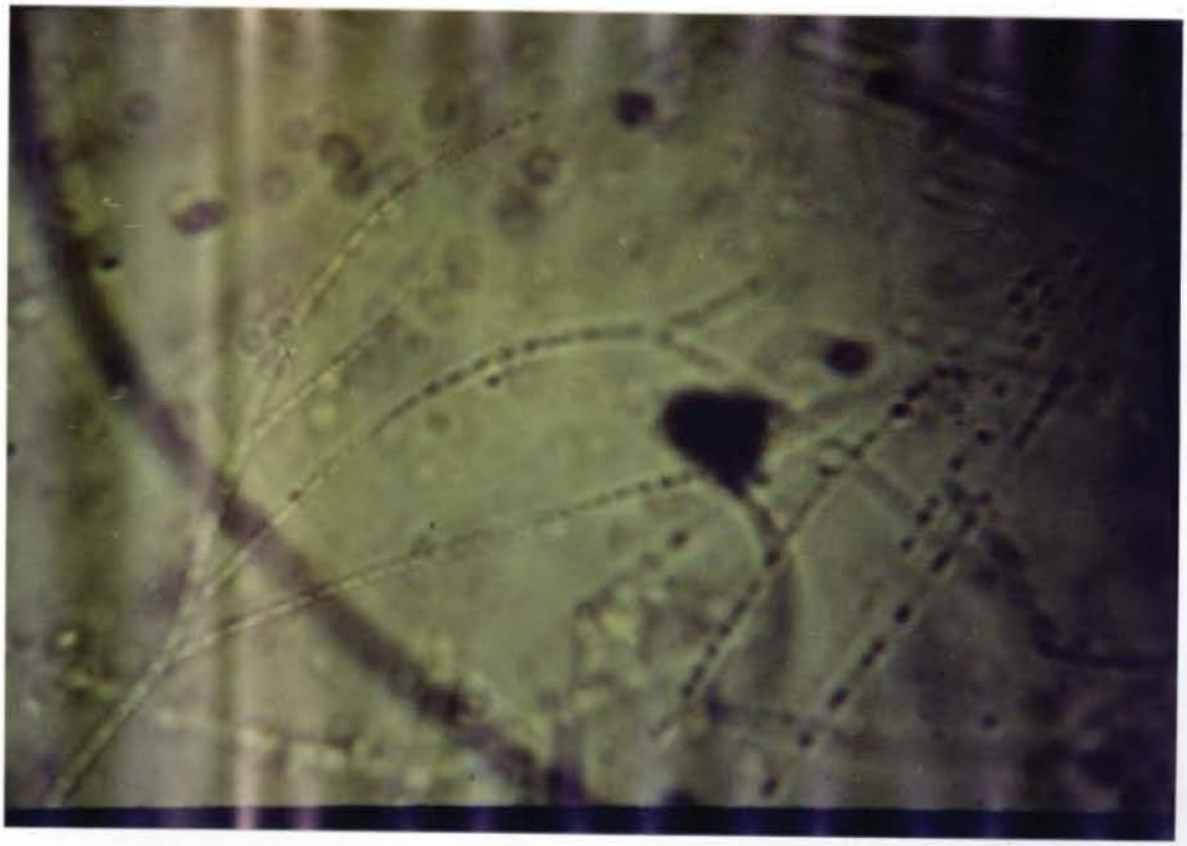

Figure 8. Vegetative filaments of Fusarium showing septae (400X).

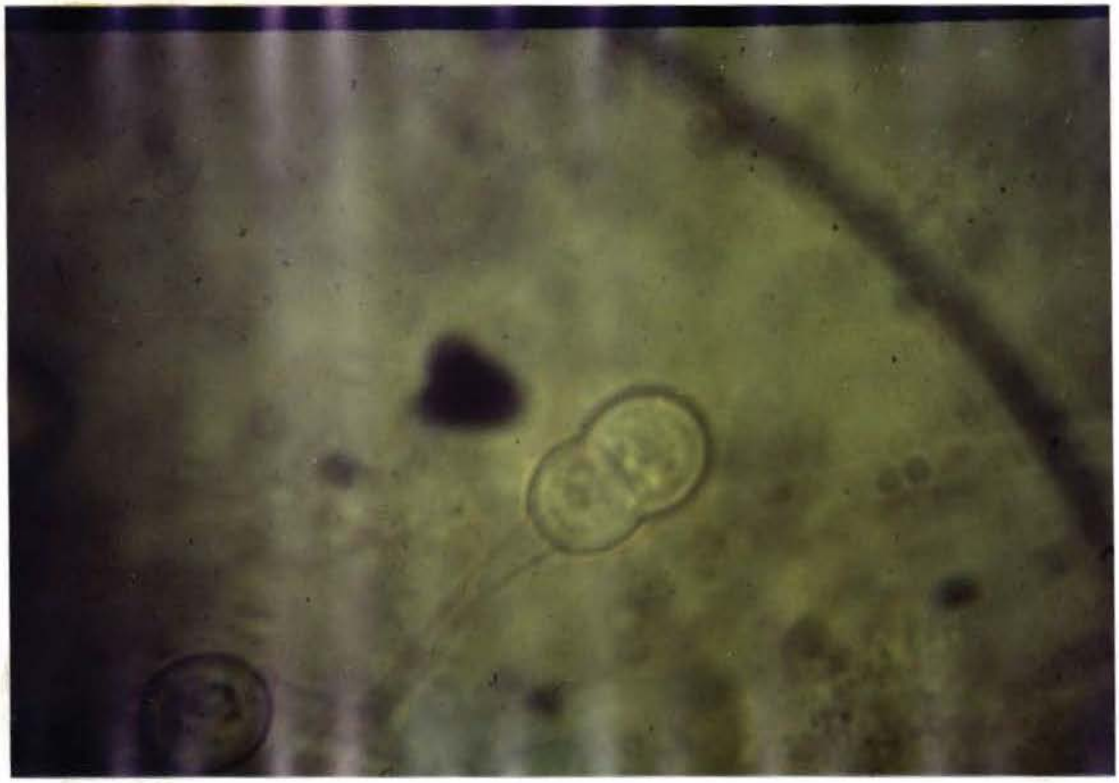

Figure 9. Developing conidia of Fusarium (1000X).

Figure 10. Older conidia of Fusarium with three septae and canoe tipped ends (400X).

REFERENCES

Agersborg, H.P.K. 1933. Salient problems in the artificial rearing of salmonoid fisheries, with special reference to intestinal fungistosis and the cause of white-spot disease. Trans. Amer. Fish. Soc. 63: 240-250.

Barnett, H.L. and Hunter, B.B. 1972. Illustrated Genera of Imperfect Fungi. Burgess Publishing Company. Minneapolis. pp 126-127.

Bessey, E.A. 1950. Morphology and Taxonomy of Fungi. Hafner Publishing Co., Inc., New York. pp 84-86.

Bold, H.C., C.J. Alexopoulos, and T. Delevoryas. 1980. Morphology of Plants and Fungi. Harper & Row, Publishers, Inc., New York. 819 p.

Brown, M.E. 1957. The Physiology of Fishes. Academic Press, Inc., New York. 447 p.

Cailliet, G.M., M.S. Love, and A.W. Ebeling. 1986. Fishes: A Field and Laboratory Manual on Their Structure, Identification, and Natural History. Wadsworth Publishing Company, Belmont. 146 p.

Coker, W.C. 1923. The Saprolegniaceae, With Notes on Other Watermolds. University of North Carolina Press, Chapel Hill.

Fletcher, T.C. and P.T.Grant. 1969. Immunoglobulins in the serum and mucus of the plaice (Pleuronectes platessa). Biochem. J. 115(5): 65.

Heinrichs, S.M. 1982. Ontogenic changes in the digestive tract of larval gizzard shad, Dorosoma cepedianum. Trans. Amer. Microsc. Soc. 101(3): 262-275.

Hoshina, T., Sano, T. and M. Sunayama. 1960. Studies on the saprolegniasis of eel. J. Tokyo Univ. Fish. 47: 59-79.

Kanouse, B.B. 1932. A physiological and morphological study of Saprolegnia parasitica. Mycologia 24: 431-452.

Moyle, P.B. and J.J. Cech. 1988. Fishes: An Introduction to Ichthyology. Prentice-Hall, Inc., Englewood Cliffs. 221 p.

Pickering, A.D. and L.G. Willoughby. 1977. Epidermal lesions and fungal infection on the perch, Perca fluviatilis L., in Windermere. J. Fish Bio. 11: 349-354.

Scott, W.W. 1964. Fungi associated with fish diseases. Devel. Indus. Microbio. 5: 109-123.

Smith, P.W. 1979. The Fishes of Illinois. University of Illinois Press, Urbana, pp 31-33.

Sparrow, F.K., Jr. 1960. Aquatic Phycomycetes. The University of Michigan Press, Ann Arbor. 1187 p.

Willoughby, L.G. and A.D. Pickering. 1977. Viable saprolegniaceae spores on the epidermis of the salmonid fish Salmo trutta and Salvelinus alpinus. Trans. Brit. Myc. Soc. 68(1): 91-95.

Willoughby, L.G., Pickering, A.D. and H.G. Johnson. 1984. Polycell-gel assay of water for spores of saprolegniaceae (fungi), especially those of the Saprolegnia pathogen of fish. Hydrobiologia. 114: 237-248.

Wood, S.E., Willoughby, L.G. and G.W. Beakes. 1988. Experimental studies on uptake and interaction of spores of the Saprolegnia diclina-parasitica complex with external mucus of brown trout (Salmo trutta). Trans. Brit. Myc. Soc. 90(1): 63-73.