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INVESTIGATION OF MINYTREMA MELANOPS MORTALITY IN WOODS RESERVOIR NEAR THE CFFF

Topical Report

By
Judy A. Cooper

July 1980

Work Performed Under Contract No. AC02-79ET10815

The University of Tennessee Space Institute
Energy Conversion Division
Tullahoma, Tennessee

U. S. DEPARTMENT OF ENERGY

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ACKNOWLEDGEMENTS

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SUMMARY

The Department of Energy MHD Coal-Fired Flow Facility is located on Woods Reservoir at the University of Tennessee Space Institute. Part of the role of UTSI as participants in the DOE program is to document environmental aspects of coal-fired MHD. This document reports the findings of UTSI in investigating a fish kill on Woods Reservoir. The results show the fish mortality to be of natural causes, in no way attributable to CFFF operations.

Occasionally seasonal fish kills have occurred in Woods Reservoir during the past several years with minimal documentation of causative factors. During the early spring (April 1980) approximately one hundred fish of a single species *Minytrema melanops* (spotted sucker) died near the embayment of Rollins Creek. An investigation was conducted to determine the cause of death. Inasmuch as only a single species was involved it was assumed that the kill resulted from an infectious or parasitic disease rather than from pollution. This view was substantiated by an examination of the water quality data during the period. Specimens were examined macroscopically and microscopically for external and internal pathogens. Heavy infestation of the myxosporidian spore *Myxosoma sp.* was found localized in the gills of the fish. Scanning electron photomicrographs revealed a heavy infection by the spores and this coupled with the stress of spring spawning was concluded to be a contributing factor in the death of *M. melanops*.

I INTRODUCTION

A. HISTORICAL BACKGROUND

The environmental program of the magnetohydrodynamics (MHD) Coal-Fired Flow Facility (CFFF) includes water quality monitoring of Woods Reservoir adjacent to the facility. Expansion of the existing MHD program at the University of Tennessee Space Institute (UTSI) initiated the construction of the CFFF which will have a point source discharge to Rollins Creek, a tributary of Woods Reservoir. Moreover, several point source discharges of domestic and industrial wastes are known to impact Rollins Creek (TDPH 1978). In early 1979 prior to operation of the CFFF, a water quality program was developed to establish baseline conditions for the stream.

Stream use classification of Rollins Creek includes domestic and industrial water supply, recreation, irrigation, fish and aquatic life, livestock watering and wildlife use. An overall objective of the MHD aquatic environmental monitoring program has been to maintain existing stream conditions and assure water quality standards applicable to stream usage.

During April 1980 monitoring effort detected a fish kill in the embayment of Rollins Creek near the CFFF. Seasonal fish kills, but without documentation, in Woods Reservoir have been reported in the past. Preliminary investigation of this recent fish kill by the Chemistry and Environmental Science Section revealed a heavy infestation of protozoan parasites among the gills of the fish. Thus the major objective of the present study was to determine causative factors contributing to the mortality of the fish.

B. REVIEW OF THE LITERATURE

Species from every class of Protozoa are known to parasitize fish inhabiting natural waters. Some protozoans harmful to fresh water fish include the flagellates (Costia nectrix and Cryptobia salmositica), the ciliates (Ichthyophthirius and Trichodina), sarcodina (Schizamoeba), the coccidians (Eimeria and Haemogregarina) and the cnidosporidians (Myxobolus and Myxosoma).

Myxosoma has been found in several species of fishes (Dogiel, et al. 1958). Myxosoma cerebralis, the causative agent of whirling disease, is reported in trout by Amlacher (1970) and in salmon by Wedemeyer, et al. (1976). Symptoms of the disease include rotatory movements of the fish and a black coloration in the caudal region of the body. Progressive stages of the disease show defective opercula, mandibular and vertebral deformities, and depressions in the cranium (Dogiel, et al. 1958 and

Hoffman and Meyer 1974). Myxosporidians are also known to cause disease of the gills. Gills become inflamed and swollen, and in some instances of heavy infection, the myxosporidian cyst ruptures resulting in damage to the tissue, causing hemorrhaging of the gill filaments. Myxosporidiosis of other organs with minimal pathological changes has also been reported (Dogiel, et al. 1958).

Species of Myxosoma are known to parasitize at least nine families of fish (Table I); the families Centrarchidae, Cyprinidae, Catastomidae and Salmonidae appear to be the most common hosts.

II METHODS AND MATERIALS

Approximately 50% of the fish, Minytrema melanops, were examined externally for evidence of ectoparastic infection of helminths, leeches, protozoans, and crustaceans. The fish were dissected and examined internally for monogenetic trematodes, cercariae of digentic trematodes, plerocercoids of cestodes, and acanthocephalans. Subsequently tissue samples of the fins, gills integument, muscles and viscera were prepared for microscopic examination. Wet mount preparation of macerated tissues and scrapings from the integument and fins were examined using a Nikon light microscope at a magnification of 400X. Observations were made using bright-field, dark-field, and phase microscopy. Selected smears were stained with Lugol's solution as a test for an iodinophilic vacuole in the spores observed.

Additional samples were preserved in a 5% formalin solution for further study. Samples for electron microscopy were freeze-dried in liquid nitrogen and gold coated in a mini-coater by Commonwealth Scientific for observation in the Amray 1000Å scanning electron microscope.

A control group (seemingly unaffected specimens) was also examined, in the same manner as the experimental group, to determine if the spore was commensal or a pathogen. Ten spotted suckers were obtained to serve as the control group by means of electrofishing with assistance from fishery biologists of the Tennessee Wildlife Resource Agency.

III DISCUSSION OF RESULTS

Since Minytrema melanops was the only species affected in the mortality in Woods Reservoir, the death was assumed to be a result of an infectious or parasitic disease rather than from pollution. This was further confirmed by water quality analysis of Rollins Creek, a tributary of Woods Reservoir. The data shows that the dissolved oxygen, pH, and temperature of this limnological station were within the standard for fresh water aquatic life during this period (Table II). Comparison of the data with water quality criteria conducive for fish growth suggested by Wedemeyer, et al. (1976) indicates that water chemistry for Rollins Creek was within these proposed limits (Tables II & III). However, there was an

TABLE I

Members of Selected Families of Fish Susceptible to Infection by Species of Myxosoma

Family	Host Species	Pathogen Species
Catostomidae (Suckers)	Carpiodes cyprinus (quillback)	Myxosoma rotundum
	Catostomus commersoni (white sucker)	M. bibullatum
		M. catostomi
		M. commersoni
		M. ellipticoides
	Ictiobus bubalus (small mouth buffalo)	M. endovasa
		M. multiplicatum
		M. okobojiensis
		M. ovalis
	Minytrema melanops (spotted sucker)	M. microthecum
Centrarchidae (Sunfishes)	Lepomis cyanellus (green sunfish)	M. cartilaginis
	L. macrorchirus (bluegill)	M. cartilaginis
	Micropterus salmoides (large mouth bass)	M. cartilaginis
	Pomoxis nigromaculatus (black crappie)	Myxosoma sp.
Cyprinidae (Minnows and Carp)	Carassius auratus (goldfish)	M. acuta
		M. sachalinensis
		M. sphaerica
	Chrosomus neogaeus (fine scale dace)	M. parellipticoides
	Cyprinus carpio (carp)	M. brachialis
		M. dujardini
		M. encephalica
	Ericymba buccata (silverjaw minnow)	M. grandis
	Notropis cornutus (common shiner)	M. media
		M. notropis
		M. orbitalis
		M. robustum
	N. hudsonius (spottail shiner)	M. grandis
	Pimephales promelas (fat head minnow)	M. hoffmanni

TABLE I (Continued)

Family	Host Species	Pathogen Species
	Rhinichthys atratulus (black nose dace)	*M. grandis*
Cyprinodontidae (Killifish)	*Fundulus diaphanus* (banded killifish)	*M. funduli*
	F. heteroclitus (mummichog)	*M. funduli* *M. hudsonis* *M. subtecalis*
	F. majalis (striped killifish)	*M. funduli*
Esocidae (Pikes)	*Esox lucius* (northern pike)	*M. anurus*
	E. masquinongy (muskellunge)	*M. cuneata* *M. muelleri*
Gasterosteidae (Stickleback)	*Eucalia inconstans* (brook stickleback)	*M. eucaliaii*
Percidae (Perches)	*Perca flavescens* (yellow perch)	*M. neurophila* *M. scleroperca*
	Percina caprodes (log perch)	*M. scleroperca*
Percopsidae (Trout Perches)	*Percopsis omiscomaycus* (trout perch)	*M. procerum*
Salmonidae (Salmon)	*Oncorhynchus gorbuscha* (pink salmon)	*Myxosoma cerebralis*
	O. keeta (chum)	*M. cerebralis* *M. dermatobia* *M. squamalis*
	O. kisutch (coho salmon)	*M. dermatobia* *M. squamalis*
	Salmo gairdneri (rainbow trout)	*M. cerebralis* *M. squalmalis*
	S. salar (Atlantic salmon)	*M. cerebralis*
	S. trutta (brown trout)	*M. cerebralis*
	Salvelinus fontinalis	*M. cerebralis*

TABLE II

WATER QUALITY DATA FOR ROLLINS CREEK DURING APRIL

PH	PH	EPA STD >6.5	<9.0
CONDUCTIVITY	MICROMHO	NONE	
HARDNESS	PPM	TENN STD	<150 PPM
ALKALINITY (TOTAL)	PPM	EPA STD >20. PPM	
ALKALINITY (MO)	PPM	NONE	
CALCIUM	PPM	NONE	
IRON	PPM	TENN STD	<.3 PPM
POTASSIUM	PPM	NONE	
MAGNESIUM	PPM	NONE	
SODIUM	PPM	NONE	
SILICON	PPM	NONE	
CHLORIDE	PPM	TENN STD	<250 PPM
PHOSPHATE (TOTAL)	PPM	NONE	
SULFATE	PPM	TENN STD	<250 PPM
TOTAL SOLIDS	PPM	TENN STD	<500 PPM
DISSOLVED O2	PPM	EPA STD >5. PPM	
AMMONIA	PPM	STD *	
TEMPERATURE	DEG	TENN STD	<30.5 C

DATA TAKEN AT STATION 2

	PH	CONDUCTIVITY	HARDNESS	ALKALINITY	ALKALINITY (M.O.)	CALCIUM	IRON	POTASSIUM	MAGNESIUM
April 1, 1980	7.2	87	46	90	90	18.13	.88	.79	2.89
April 7, 1980	7.3	119	54	102	102	16.46	.82	.97	2.69
April 16, 1980	8.0	108	65	61	61	17.08	.71	1.10	2.89
April 21, 1980	8.9	130	55	68	61	17.29	.53	.90	2.71

	SODIUM	SILICON	CHLORIDE	PHOSPHATE	SULFATE	TOTAL SOLIDS	DISSOLVED O2	AMMONIA	TEMPERATURE
April 1, 1980	1.76	3.21	6.41	.03	5.77	93	10.2	.05	15.2
April 7, 1980	1.55	3.74	5.21	.02	6.79	103	8.8	.05	16.4
April 16, 1980	1.76	5.29	5.84	.04	4.91	58	11.6	.05	15.5
April 21, 1980	1.55	4.24	6.83	.04	4.13	136	13.5	.05	24.2

TABLE III

SUGGESTED WATER QUALITY CRITERIA CONDUCIVE TO OPTIMUM HEALTH OF WARMWATER AND COLDWATER FISHES*

Water Chemistry	Upper Limits for Continuous Exposure
Acidity	pH 6-9
Alkalinity	At least 20 ppm (as $CaCO_3$)
Ammonia (NH_3)	0.02 ppm
Cadmium	0.0004 ppm in soft water (<100 ppm alkalinity)
Cadmium	0.003 ppm in hard water (>100 ppm)
Chlorine	0.003 ppm
Chromium	0.03 ppm
Copper	0.006 ppm in soft water; 0.03 ppm in hard water
Hydrogen sulfide	0.002 ppm
Lead	0.03 ppm
Mercury (organic or inorganic)	0.2 ppb maximum, 0.05 ppb average
Nitrogen	Maximum total gas pressure 110%
Nitrite (NO_2^-)	100 ppb in soft water, 200 ppb in hard water
Polychlorinated biphenyls (PCB's)	0.002 ppm
Total suspended and settleable solids	80 ppm or less

* Taken from Wedemeyer, et al. 1976

increase in the mean temperature for April over the previous month. It is believed the suckers had entered the tributary for spring spawning, environmentally induced by the rise in temperature.

After reviewing the physicochemical data, an investigation was made for possible biological or physiological factors contributing to the death of the fish. Gills of the infected fish were noticably swollen and inflamed; fish which came to the surface clearly exhibited anoxic stress symptoms. External examination and autopsies of approximately fifty per cent of the affected fish yielded no evidence of infection of helminths, leeches, protozoans, or crustaceans, organisms which commonly parasitize fish. However, separate microscopic examinations of the fins, gills, muscles, and integument revealed an infection of myxosporidian spores (Figure 1) localized in the gills. The genera Myxosoma and Myxobolus are morphologically indistinguishable myxosporidian spores. However, the genera can be separated on the basis of a Lugal solution stain (Appendix A). Myxobolus contains a glycogen vacuole which stains mahagony red with iodine stain while Myxosoma does not absorb the stain (Kudo 1971 and Hoffman 1967). Spores were assigned to the genus Myxosoma, since the test for the idinophilic vacuole was negative.

Additional observations of the spores were made on the electron microscope (Figures 2-5). Electron photomicrographs of the gills revealed a heavy spore infection (Figures 2 and 3). According to W. A. Rogers, fish parasitologist of Auburn University, the gills were as heavily infected as any he had ever observed (personal communication of May 28, 1980).

When the control group was examined, numerous spores were found among only thirty per cent of the suckers obtained by electrofishing. Seventy per cent of the control group had few to no spores sparsely distributed throughout the gills.

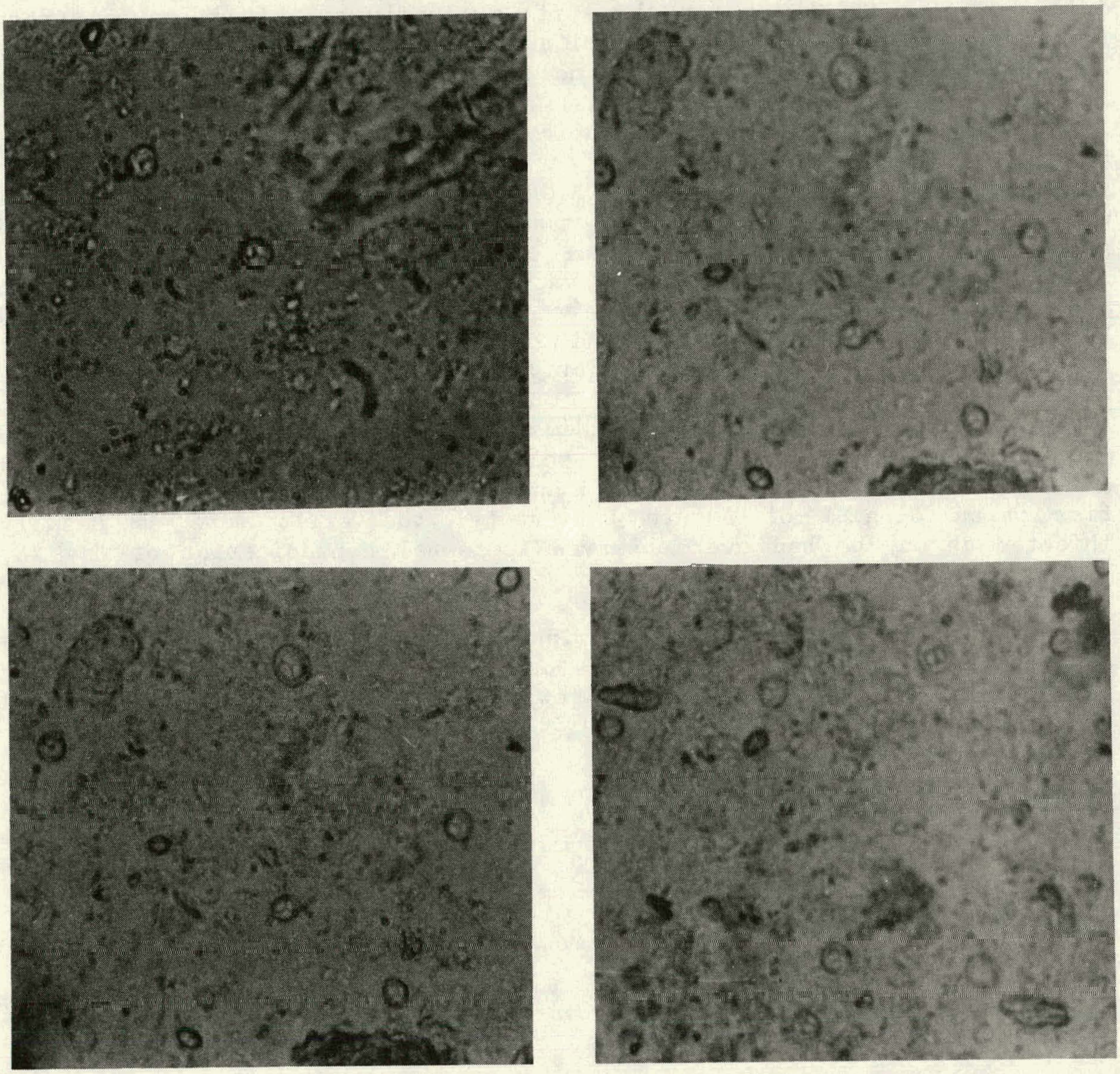

Figure 1. Spores of Myxosoma sp. from the Gills of Minytrema melanops X400

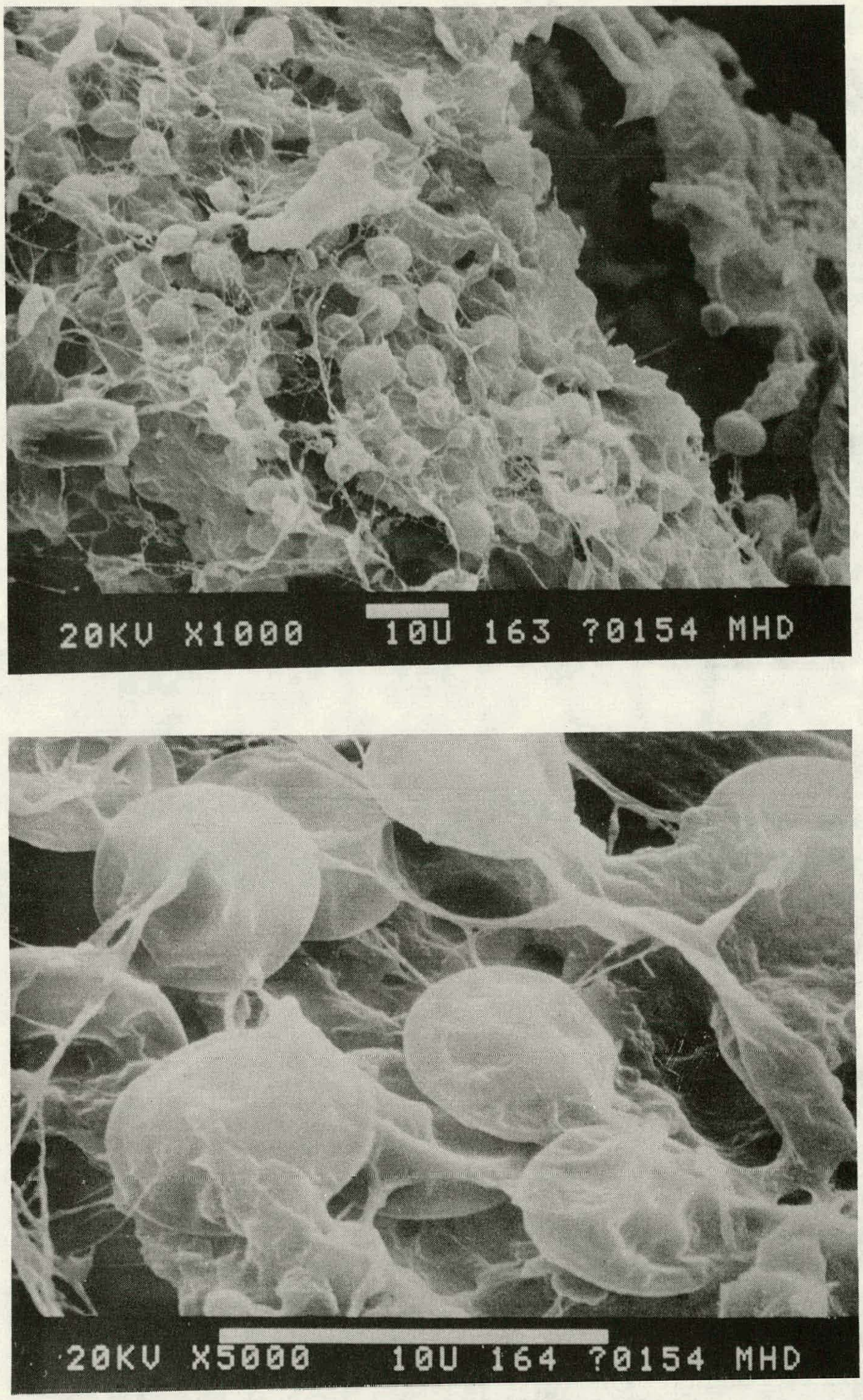

Figure 2. Spores of Myxosoma sp. Heavy Infestation of Gills from Minytrema melanops (Scanning Electron Photomicrograph)

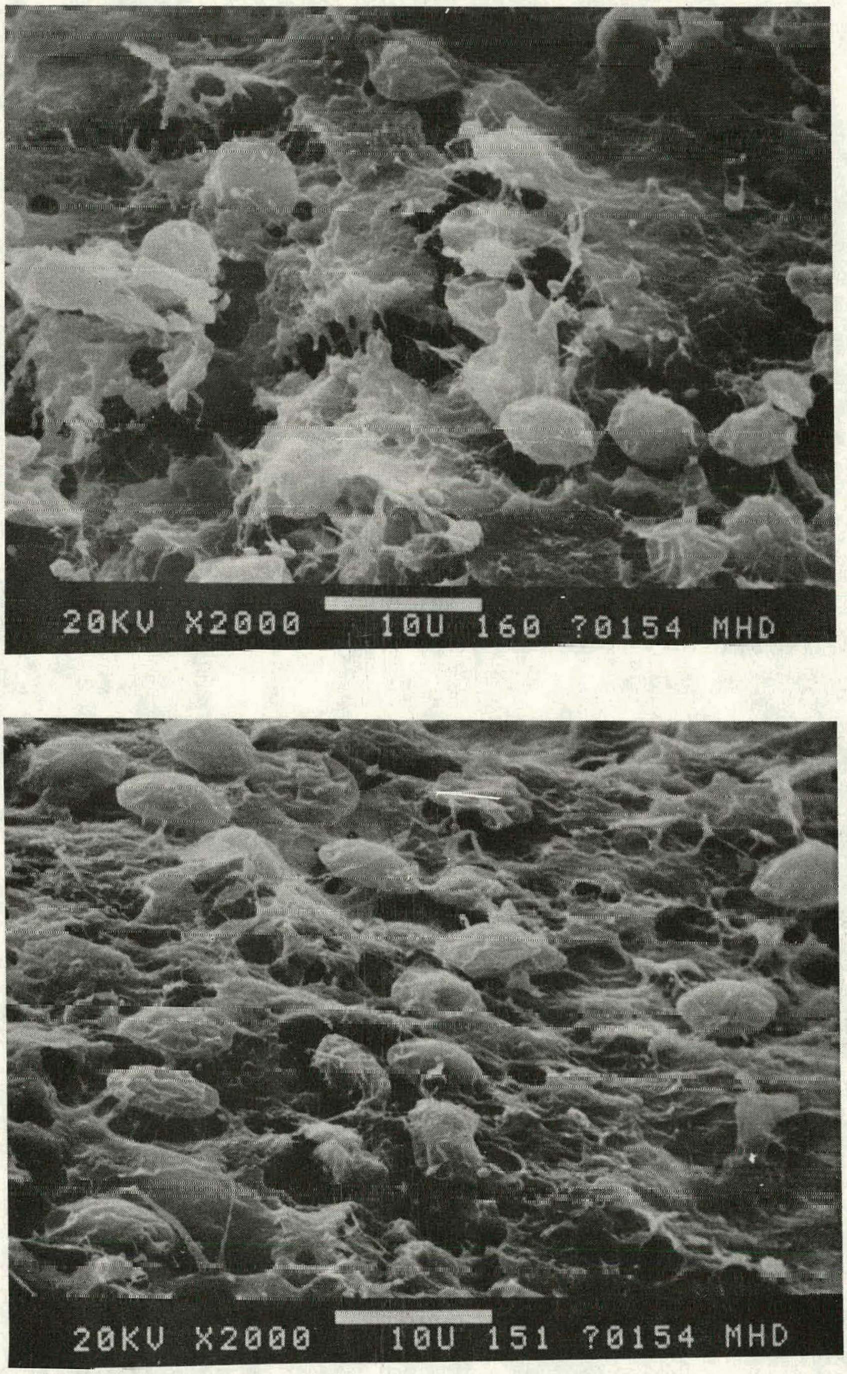

Figure 3. Scanning Electron Photomicrographs of Spores of Myxosoma sp. X2000 Note: Heavy infestation

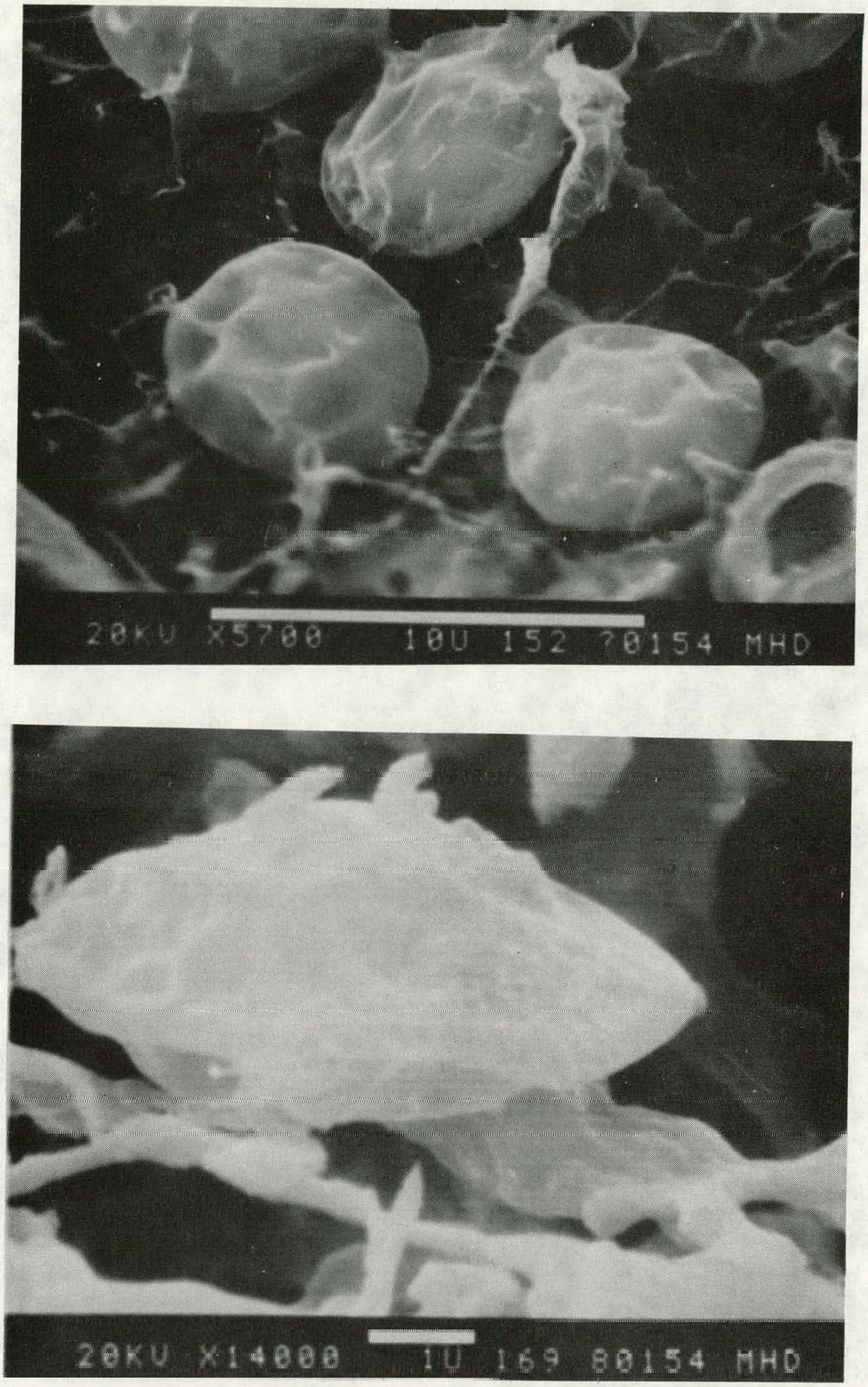

Figure 4. Scanning Electron Photomicrographs of the Spore *Myxosoma sp.* from the Gills of Spotted Suckers

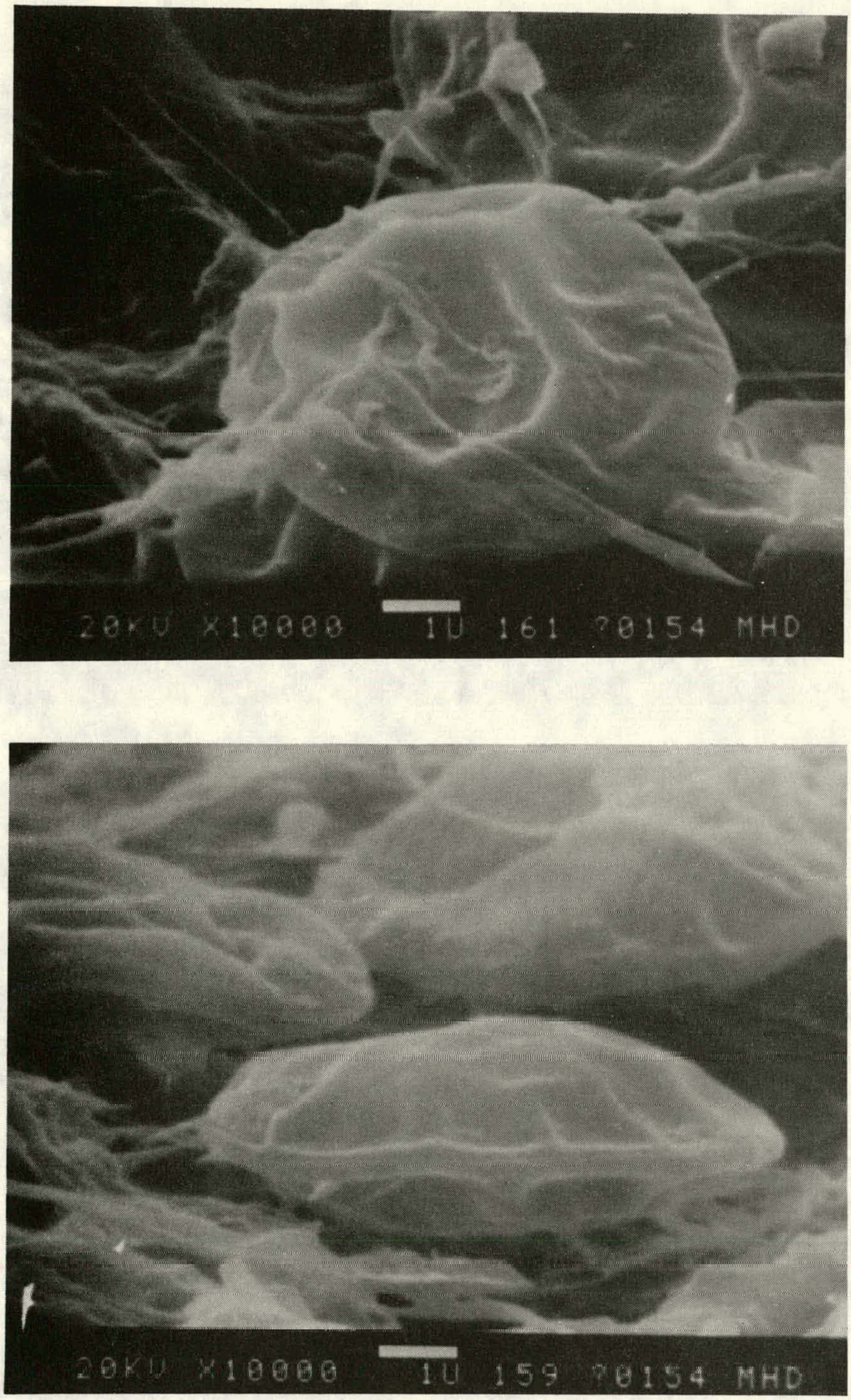

Figure 5. Spore of Myxosoma sp. x 10,000 Note Groove in Lower Photo

IV CONCLUSIONS

Environmental changes are stressful and lower the resistance of fresh water fish. The role of stress as a predisposing factor in the host-parasite-environment interaction has been discussed by Wedemeyer, et al. (1976) who includes diseases produced by protozoan parasites as a stress-mediated infectious disease. It is believed that *Myxosoma* sp. is a facultative fish pathogen of *M. melanops* and under favorable environmental conditions epizootics can occur during the spawning season when there are seasonal fluctuations in the temperature.

During most of the year the host, *M. melanops*, inhabits deep waters in which dense populations are not formed, but in the spring the host migrates into tributaries and assembles in large shoals, thus providing favorable conditions for the dissemination of the parasite. During this period the host is highly susceptible to infestations. It is concluded that *Myxosoma* sp. is harbored as a potential pathogen by normal, healthy *M. melanops* but can overtake the host under proper environmental conditions especially when the host's resistance is low. The spores remain within the host until its death and subsequent decomposition releases them into the water to become the source of further infection.

On the basis of those findings presented in this paper, it is concluded that *Myxosoma* sp. coupled with the stress of spring spawning was a contributing factor in the death of the fish in Rollins Creek. It is recommended as a preventive measure that upon confirmation of the disease, that dead fish be removed from the water and destroyed to interrupt the direct life cycle of the microbe.

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APPENDIX A

PREPARATION OF LUGOL'S SOLUTION

LUGOL'S SOLUTION

Dissolve 10 g iodine crystals and 20 g potassium iodide in 200 ml distilled water. Add 20 ml glacial acetic acid a few days prior to using; store in amber glass bottles. (Millipore Corporation, 1975).

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