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Description and Seasonal Variation in Incidence of a New Species of Myxosporidian Parasite (Class Myxosporidea) of the Bluegill Sunfish, Lepomis Macrochirus Rafineque, in Kentucky

Stephen Bayes Crider

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Stephen Bayes Crider

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My wife, Norma Ann, typed the manuscript, and it is to her and to my son, Stephen James, that this paper is dedicated.

ABSTRACT

A new histozoic myxosporidian parasite (Class Myxosporidea), Myxobolus meglitschi sp. nov., infecting young-of-the-year and yearling bluegill sunfish, Lepomis macrochirus Raf., from Shanty Hollow Lake, Warren County, Kentucky, was described. Phenology was investigated from January 20 to December 20, 1969.

The magnitude of infection varied seasonally in bluegill. Incidence was highest in July and August (43.9%) in host populations, declining, but not disappearing from September to December 20, 1969. The infection exhibited a yearly mean incidence of 22.31%.

The pattern of distribution of cysts on hosts varied seasonally. During periods of low incidence cysts were confined primarily to a postanal area below the lateral line. During July and August cysts were widespread on hosts.

Initial infection of hosts may occur accidentally through contact with spores, incidental to feeding upon protozoan intermediate transfer hosts or other benthic organisms; or, during spawning, by spore contact with eggs or

larvae. Development of the parasite from initial spore contact to the production of detectable size cysts appeared to require 80-90 days. The parasite may spread on the host by means of autoinfection.

Pathology was limited to scale erosion at the point of contact by cysts.

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INTRODUCTION

A general parasitological survey of fish taken from Shanty Hollow Lake, Warren County, Kentucky, in October, 1968, disclosed the presence of a previously undescribed, histozoic species of myxosporidian parasite (Class Myxosporidea, Genus Myxobolus) infecting young-of-the-year and yearling bluegill sunfish, Lepomis macrochirus Raf.

This study was undertaken to establish the identity of this parasite and determine its seasonal variation in incidence in Shanty Hollow Lake from January 20 to December 20, 1969.

While there have been numerous contributions to the taxonomy of the genus Myxobolus, studies dealing with seasonal incidence are relatively few in number. Bond (1938a) described and investigated seasonal occurrence of Myxobolus bilineatum Bond and Myxosoma subtecalis Bond in the mummichog, Fundulus heteroclitus (Linnaeus). Myxobolus bilineatum infected 4 to 25% of host populations, while Myxosoma subtecalis infected up to 91%. The highest percentage of infected fish was observed in spring, summer, and fall. Fish (1938) concluded that six to eight months were

required for Myxobolus inornatus Fish to complete its life cycle. He believed peak occurrence of this parasite in the black bass, Huro floridana (LeSueur) (= Micropterus salmoides Lacépède), was influenced by environmental factors since occurrence varied seasonally.

In a second report on seasonal relationships of myxosporidian parasites, Bond (1939) noted that other workers reported seasonal cycles for certain species. Cycles reached a peak in summer and declined or disappeared in infected populations during other periods. Based on these reports and his own observations of infection cycles of three separate parasites of F. heteroclitus, Bond concluded that changes in infection cycles were related to: (1) seasonal temperatures and their effects on host fish; (2) natural habitat of the parasites, either coelozoic (organ infecting) or histozoic (tissue infecting); (3) exposure of cysts and method of spore liberation; and (4) species of the parasite.

Iverson (1954) examined 1,687 silver salmon, Onycorhynchys kisutchi (Walbaum), from two canneries in Anacortes, Washington. He noted that 5.1% were infected with Myxosoma squamalis Iverson and speculated that the life cycle of this parasite required approximately seven months. Iverson did not concern himself with seasonal relationships and reported no maximal or minimal level of infection. He theorized, however, that young fish could receive the parasite from spawning adults. Guilford (1963)

investigated the occurrence of Myxosoma scleroperca Guilford in the yellow perch, Perca flavescens (Mitchill), and the log perch, Perca caprodes (Raf.). He believed this parasite developed throughout the summer, reaching a peak in the fall of the year. At this time of the year large inflammatory cysts were observed in the eyes of host fish, whereas cysts were either absent or quite small during other seasons. In October, 1952, Guilford found that 11.2% of those fish checked were parasitized. Subsequent investigations by Guilford in the fall of 1961 and the summer of 1962 revealed infection levels of 19% and 6.6%, respectively.

An eight-month study of Myxosoma cartiliginis Hoffman, Putz and Dunbar (Hoffman et al. 1965) disclosed that spores developed throughout the summer causing the greatest production of cysts in the head cartilage of young-of-the-year bluegill to occur in late summer.

Davies (1968) found that Myxobolus muelleri Bütschli showed no seasonal variation in occurrence, spore shape, or size on the dace, Leuciscus leuciscus (L.), from the River Lugg, England. Davies noted this same parasite harbored in the roach Rutilus rutilus (L.). The parasite exhibited only slight variation in occurrence, spore shape and spore size. The highest percentage of infected R. rutilus was detected in June, slowly declining in severity thereafter.

Lewis (1968) reported that the percentage of golden shiner, Notemigonus crysoleucas (Mitch.), infected by

Myxobolus argenteus Lewis, was highest in late summer and declined in the fall. Detectable cysts were observed four to five months after hatching of host fish.

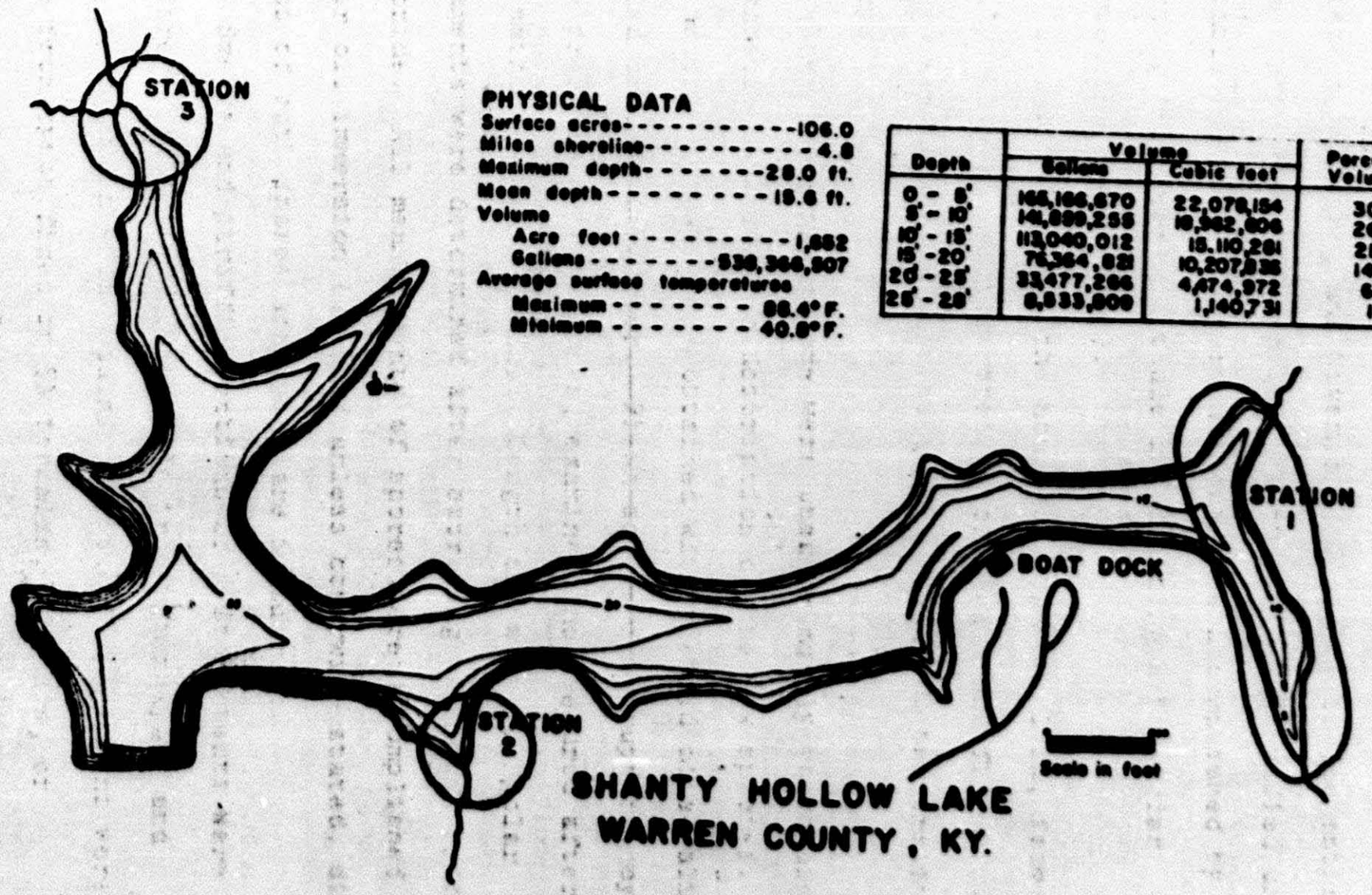
METHODS AND MATERIALS

From January 20 through December 20, 1969, monthly samples of bluegill were collected from three stations on Shanty Hollow Lake, Warren County, Kentucky (Figure 1). Concurrently, surface water temperature at each station was recorded using a Wexler Celsius thermometer. Sampling began at 8 AM and terminated at 12 Noon each sampling period. Fish were taken using 3 x 3 ft., 4 x 10 ft., or 5 x 50 ft. cotton or nylon seines of 1/4 in. and smaller mesh. A monthly sample of 75 bluegill, selected at random from seine collections, was transported to the laboratory for examination. Supplemental specimens were obtained during population studies in the lake, from fishermen's creels, and from federal hatchery stocks used for population replenishment at Shanty Hollow.

Fish from all collections were measured to the nearest millimeter for total body length, width, and depth (Lagler 1956) with a vernier caliper.

The number and patterns of distribution of cysts on host fish were determined by examination of specimens with the use of a dissecting microscope at 200X magnification. Each cyst observed was plotted on a generalized diagram of a bluegill. Fish were examined both externally and

FIGURE 1. Bathymetric map and physical and hydrographic data for Shanty Hollow Lake, Warren County, Kentucky (Pfeiffer 1967). Sampling stations are indicated.



PHYSICAL DATA

Surface acres - - - - - - - - - - - - 106.0
Miles shoreline - - - - - - - - - - 4.8
Maximum depth - - - - - - - - 28.0 ft.
Mean depth - - - - - - - - - - 15.6 ft.
Volume
- Acre feet - - - - - - - - - - 1,652
- Gallons - - - - - - - - 538,366,807

Average surface temperatures
- Maximum - - - - - - - - 88.4° F.
- Minimum - - - - - - - - 40.8° F.

Depth	Volume		Percent Volume
	Gallons	Cubic feet	
0 - 5'	166,186,670	22,078,154	30.7
5' - 10'	141,899,258	18,962,606	26.3
10' - 15'	113,040,012	15,110,281	21.0
15' - 20'	76,364,821	10,207,838	14.2
20' - 25'	33,477,266	4,474,972	6.2
25' - 28'	8,833,809	1,140,731	1.6

SHANTY HOLLOW LAKE
WARREN COUNTY, KY.

internally.

Stomach analyses were made on fish taken in March, September, October, and November. Contents of the entire alimentary canal were removed, sorted, and identified using suitable keys. Age determinations were accomplished by counting the number of scale annuli using a binocular microscope (Lagler 1956).

Biweekly, between the hours of 9 AM and 1 PM, from April to August, various species of legal size fishes caught by fishermen were identified and examined for the presence of cysts.

The following methods were used primarily to obtain diagnostic data for the description of the new species. Dimensions of cysts were obtained with the aid of a calibrated ocular micrometer. Fresh spores from ruptured cysts were prepared as wet mounts, examined and measured after the method of Kudo (1921a). Length and width of polar filaments were determined after extruding them with hydrogen peroxide. All measurements of spores were accomplished under oil immersion (1000X); unless otherwise stated, dimensions of both spores and cysts are in microns.

Smears and paraffin sections of cyst contents were differentiated with Heidenhain's iron hematoxylin and Mallory's aniline-blue stains. Tissue preparations were made from cysts fixed in 5% formalin, Gilson's, or Kaformacet (Romeis 1948) solution. Feulgen's nuclear stain was used to test for the presence of nuclear material and

fresh Lugol's solution was used to test for presence of an iodinophilous vacuole.

DESCRIPTION OF THE STUDY AREA

Shanty Hollow Lake

Shanty Hollow Lake is a 106 acre recreational impoundment located in northwestern Warren County, Kentucky, approximately 16 miles northeast of the city of Bowling Green. The lake was formed when private interests dammed a series of small tributaries of the Green River in 1951. In 1953, the lake was purchased by the Kentucky Department of Fish and Wildlife Resources and added to the system of state owned fishing lakes.

Pfeiffer (1967) studied the physical, chemical, and biological aspects of Shanty Hollow Lake. According to his data, Shanty Hollow, at normal pool, has a maximum depth of 28.0 feet, a mean depth of 15.6 feet, 4.8 miles of shore line, and a total volume of 1652 acre feet. A bathymetric map and physical and hydrographic data prepared from Pfeiffer's survey are presented in Figure 1.

Shanty Hollow Lake exhibited considerable fluctuation in water level throughout 1969 because of a leak in the dam. Periodic soundings taken at the dam during the study showed that the lake rose from 14 feet in January to 28 feet in July, then fell to a low of 12 feet in early December. The percentage loss in volume represented by the decrease in

depth between July and December was 1380 acre feet or 80.8% of the total volume.

Because Shanty Hollow is a recreational lake, State fishery personnel have attempted to maintain high fishing potential by fertilization and restocking programs. Applications of inorganic fertilizers were made by them between April and September each year from 1960 to 1965, inclusive. Pfeiffer (1967) concluded that fertilization caused a substantial increase in the quantity and quality of the fishing during those years. The program of fertilization terminated in 1965 but was reinitiated in 1969 in the hope that the declining bluegill standing crop could be strengthened. Concomitantly, 6,000 two-inch bluegill were introduced in November, 1968, and 150,000 one-inch fish stocked on March 29, 1969 (Pfeiffer, personal communication, 1969).

Species diversity of ichthyofauna (Table 1), in addition to bluegill, was determined from seining operations, creel surveys, population studies and stocking records of the Kentucky Department of Fish and Wildlife Resources.

Sampling Stations

Station 1, located at the southern end of the lake, encompassed two large coves. Inflow was provided by runoff and two intermittent limnokrenes. The substrate at this station was mud. On June 21, Polycystis aeruginosa Kutzing formed a bloom in several areas within the station.

TABLE 1. Species diversity of ichthyofauna determined from seining operations, population studies, creel surveys, and stocking records of the Kentucky Department of Fish and Wildlife Resources, January through December, 1969.

Centrarchidae		
	Lepomis macrochirus Rafinesque	(Bluegill Sunfish)
	Lepomis cyanellus Rafinesque	(Green Sunfish)
	Lepomis megalotis (Rafinesque)	(Longear Sunfish)
	Lepomis gibbosus (Linnaeus)	(Pumpkinseed Sunfish)
	Pomoxis nigromaculatus LeSueur	(Black Crappie)
	Pomoxis annularis Rafinesque	(White Crappie)
	Chaenobryttus gulosus (Cuvier)	(Warmouth Sunfish)
	Micropterus salmoides (Lacépède)	(Largemouth Black Bass)
Percidae		
	Stizostedion vitreum (Mitchill)	(Yellow Walleye)
Ictaluridae		
	Ictalurus punctatus (Rafinesque)	(Channel Catfish)
	Ictalurus natalis (LeSueur)	(Yellow Bullhead)
Atherinidae		
	Labidesthes sicculus (Cope)	(Brook Silverside)

Wind action concentrated the bloom into viscous mats on the water's surface. All traces of the bloom disappeared by mid-July.

Station 2 consisted of two small coves and was located on the western shore of the lake, 1500 feet south of the dam. Inflow into this area was from runoff and the substrate consisted of mud and gravel. By August 23, a bloom of Anabaena circinalis (Kutz.) Rabenhorst formed, persisting until late September. As was the case at Station 1, wind caused the bloom to accumulate in mats on the water's surface.

Station 3, a large embayment in the northernmost arm of the lake, was fed by two intermittent rheokrenes and by runoff. Stream flow ceased in late July and commenced in December. The substrate for most of the area was mud mixed with sand, gravel, and rubble. The banks and bottom of the creek channels, which cut across the station, were composed of masses of compacted leaves, sticks, and mud. During July, P. aeruginosa formed a bloom, followed by a bloom of A. circinalis in August and September. Neither bloom accumulated at this station to the extent observed at the other stations.

THE HOST, LEPOMIS MACROCHIRUS RAF.

The bluegill sunfish was first described by Rafinesque (1819) during his survey of the fishes of the Ohio River drainage system. Trautman (1957) and Whitaker (1968)

indicate that its known range extends over much of central and eastern United States. In his survey of the fishes of Kentucky, Clay (1962) briefly reviewed the biology and distribution of bluegill within the state.

Bluegill apparently prefer lentic habitats which contain small amounts of suspended, clayey silts, and substrates consisting of organic debris with aquatic vegetation in shallow water. This species is extremely prolific in that a single female is capable of producing thousands of eggs during a breeding season. If individuals become too numerous within a restricted area, they will stunt quite dramatically. This species hybridizes with the warmouth, longear, green, orangespotted, redear, and pumpkinseed sunfishes (Trautman 1957, Clay 1962).

Studies of types of food organisms utilized by bluegill have been made by Ricker (1949), Hayne and Ball (1956), Minckley (1963), and Gerking (1962, 1964). These authors noted that the diet of the bluegill consisted primarily of benthic organisms such as bloodworms, dragonfly naiads, mayfly nymphs, oligochaetes, ostracods, and helgrammites. Fry consumed planktonic forms such as protozoans, algae, and microcrustaceans during early phases of their growth period. Benthos are added to their diet as they grow older.

Apparently, low water temperature reduces the activity of bluegill and affects food uptake. Wohlschlag and Juliano (1959) observed decreases in bluegill metabolism during winter and increases during the spring and summer months.

They noted that fish grew rapidly during warm water periods when food was plentiful but decreased their uptake of food, stopped growing and, in fact, lost weight with the onset of cooler weather. These authors attributed cessation of growth and loss of weight to a decrease in the supply of available food. Lagler, Bardach, and Miller (1962) noted a sharp increase in food uptake by bluegill beginning in late April and early May. This increase continued through August, then declined abruptly in September and remained at a low level during the rest of the year. From examination of their data, it was apparent that once water temperature exceeded 4° C, both activity and food uptake increased, then declined in accordance with a decrease in water temperature in the fall and winter.

Seasonal factors apparently regulate depth distribution of bluegill. Larimore (1957) and Wohlschlag and Juliano (1959) observed large schools of bluegill over shallow banks in early spring and summer. Subsequent observations revealed such schools engaging in feeding and spawning activity; with the onset of cooler water temperature the schools retreated to deeper water.

RESULTS

Myxobolus meglitschi sp. nov.

Identification

Identification and differentiation of different genera and species of Myxosporidia is almost entirely dependent upon morphological features including spore size, shape, polar capsule size and shape, and the presence or absence of specialized structures. The unique diagnostic morphological features of *M. meglitschi*, determined from measurements of characteristics of fresh spores, are summarized in Table 2. Terminology follows that of Noble (1944).

Cyst

The dull white to cream colored, smoothly ovoid, easily ruptured cysts appeared only on external surfaces of the host. Cyst were noted along the sides of the body and at the bases of pectoral, pelvic, and anal fins as well as on the abdomen and caudal peduncle (Figure 2). On heavily infected fish, cysts sometimes were observed filling the pores of scales of the lateral line. They had a distinct tendency to appear as isolates rather than as closely compacted masses. The undersurface of scales was the most frequently noted point of attachment for cysts; however, not infrequently, cysts were anchored in the

TABLE 2. Characteristics of M. meglitschi sp. nov. compared with those of its closest relative, M. kostiri Herrick (1936).

Character	M. meglitschi sp. nov. Range	Mean	No. Obs.	M. kostiri Herrick Range	Mean	No. Obs.
Cyst						
Length	200 - 800µ	600µ	50	750 - 1500µ	N/G*	N/G
Width	75 - 350µ	220µ	50	N/G	N/G	N/G
Thick.	65 - 150µ	105µ	50	N/G	N/G	N/G
Spore						
Length	16.0 - 18.1µ	17.33µ	250	8.8 - 11.2µ	9.66µ	100
Width	14.0 - 17.0µ	15.31µ	250	6.4 - 8.0µ	7.4µ	100
Thick.	10.0 - 12.0µ	11.0µ	250	4.9 - 5.8µ	5.4µ	57
Capsule, Ant.						
Length	7.0 - 9.0µ	8.21µ	250	3.3 - 4.9µ	4.35µ	25
Width	5.0 - 7.4µ	5.74µ	250	1.6 - 2.8µ	2.46µ	25

TABLE 2. Continued.

Character	*M. meglitschi* sp. nov. Range	Mean	No. Obs.	*M. kostiri* Herrick Range	Mean	No. Obs.
Capsule, Post.						
Length	7.0 - 9.0μ	8.21μ	250	4.1 - 4.9μ	4.7μ	25
Width	5.0 - 7.4μ	5.74μ	250	2.4 - 3.3μ	2.5μ	25
Polar Filament						
Length	30.0 - 32.0μ	31.0μ	10	N/G	30.0μ	2
Width	0.7 - 0.8μ	0.75μ	10	N/G	N/G	N/G
# Coils	5 - 7	N/G	250	N/G	13	N/G

* Indicates information not given.

FIGURE 2. Examples of cyst locations on hosts. CY, cyst

epidermis. Non-scaled surfaces were devoid of cysts. In many instances, scales which served as points of anchorage for cysts were eroded or recessed (Figure 3).

Cyst dimensions averaged 600 x 220 x 105μ, with the longest axis most often oriented parallel to the long axis of the host.

A membrane of reticulated cells, which stained deep blue in Heidenhain's iron hematoxylin and Mallory's aniline-blue, surrounded the spores (Figure 4). This membrane was 4.6 to 7.0μ thick. Although the membrane was present, no inflammation or erosion of tissue other than scales was observed on bluegill as has been described by Kudo (1926) and Nigrelli and Smith (1938) for other species on different hosts. Mature spores occupied the central area of immature cysts while developmental stages of spores were generally located on the periphery. Older, more mature cysts contained only mature spores.

Sporogony

Globose, uninucleated sporonts (stage which develops into one or more spores), averaging 3.0μ in diameter, were observed on the periphery of cysts. The nucleus of this stage was dense and centrally located (Figure 5a). Oblong, binucleated sporonts were approximately twice as large as uninucleated forms, and both stages had relatively uniform cytoplasm that displayed no granules or vacuoles.

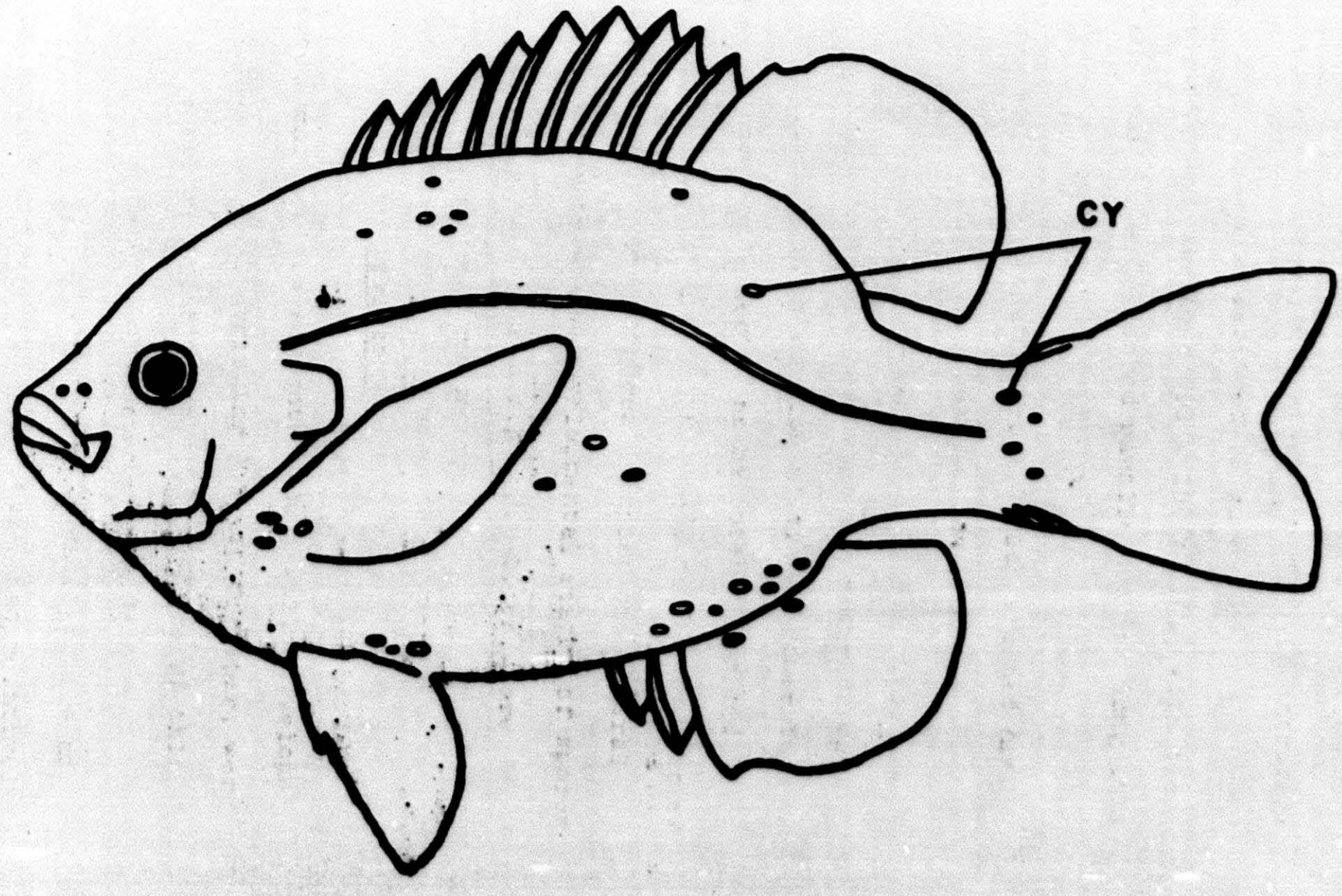

CY

FIGURE 3. Scale erosion at the point of attachment of a cyst of M. meglitschi. (400X)

FIGURE 4. Mallory's aniline-blue stained, sectioned cyst of M. meglitschi. Spores are surrounded by a membrane of reticulated cells. Membrane averages 4.6 - 7.0μ thick. CM, cyst membrane. (200X)

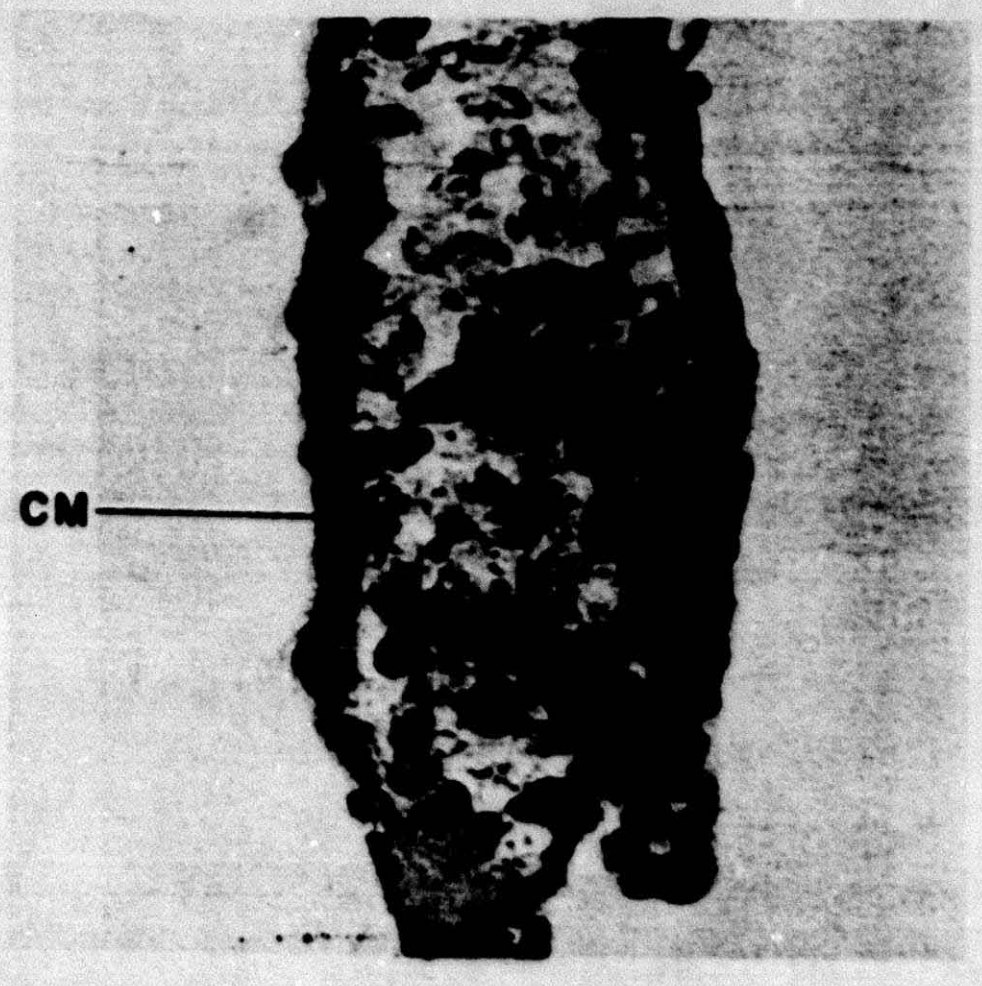

CM

FIGURE 5. Some stages of sporogony, M. meglitschi:

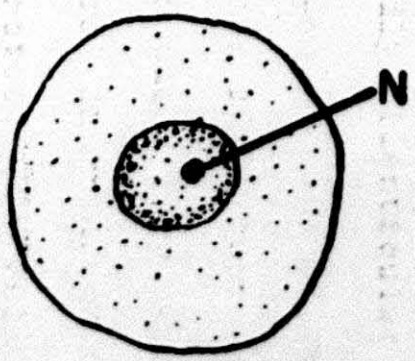
a. uninucleated sporont; N, nucleus;

b. pansporoblast; P, pansporoblast;

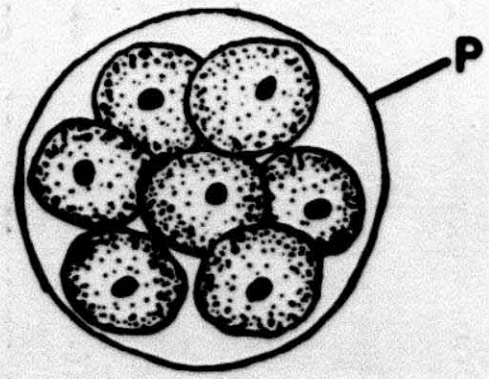
c. monosporous stage; S, sporont; H, hyaline membrane;

d. disporous stage;

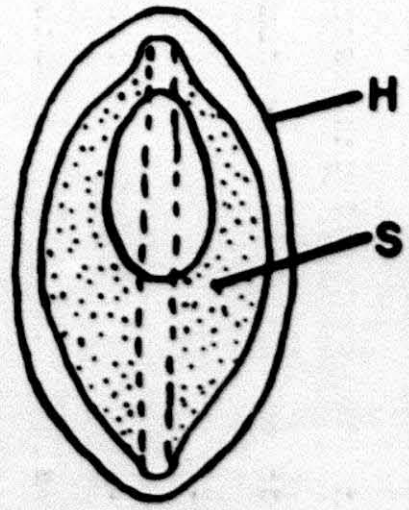
e. polysporous stage; IS, immature spore.



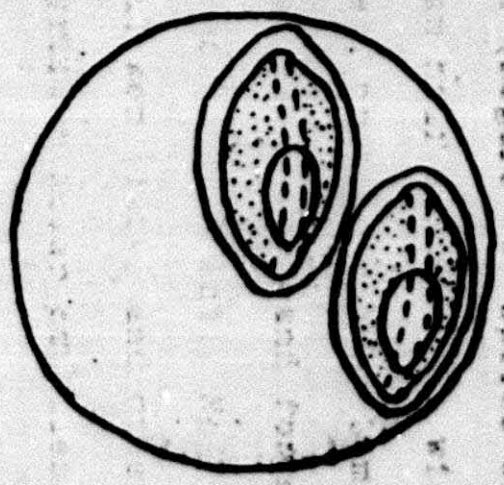
a.



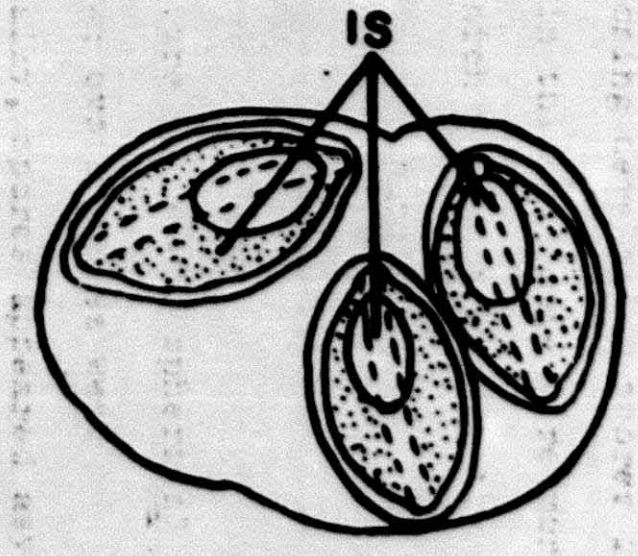
b.



c.



d.



e.

Pansporoblasts (sporont producing two or more spores) containing two to eight nuclei were frequently found in immature cysts (Figure 5b). It was assumed that these stages gave rise to mono-, di-, and polysporous stages found in mature cysts (Figure 5c,d,e). Maturing individual sporonts and pansporoblasts were surrounded by a thin hyaline membrane, apparently enclosing developing spores in a fluid-like medium. Stages within this membrane were more refractile than mature spores which were not apparently surrounded by this structure (Figure 6a,b).

Spore

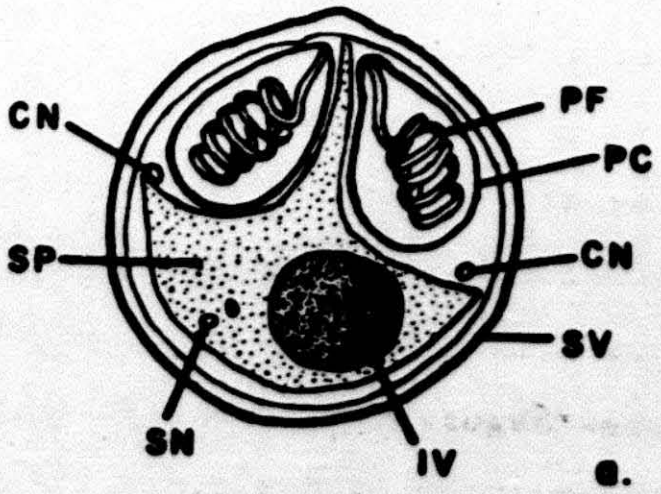
Externally, the typical mature spore was spherical or ovoid (Figure 6a,b) in face view; ovoid spores were attenuated posteriorly. Internally, spores appeared asymmetrical in face view with the polar capsules and inclusion bodies in the sporoplasm consistently lying off-center of an imaginary line drawn from pole to pole (Figure 6a,b). From the lateral and apical views (Figure 6c) spores were broadly bilenticular and more than half as thick as wide (Table 2). The paired, unstriated, proteinaceous (Lom and Corliss 1967) spore valves were equal in size and formed a conspicuous ridge where they joined (Figure 6c). No sutural line, as described by Walliker (1969) and others, was noted. Two well separated pyriform polar capsules, equal in size, and attached side by side, occupied the anterior portion of the spores (Figure 6a,b). In many

FIGURE 6. Spores of M. meglitschi; external and internal morphology:

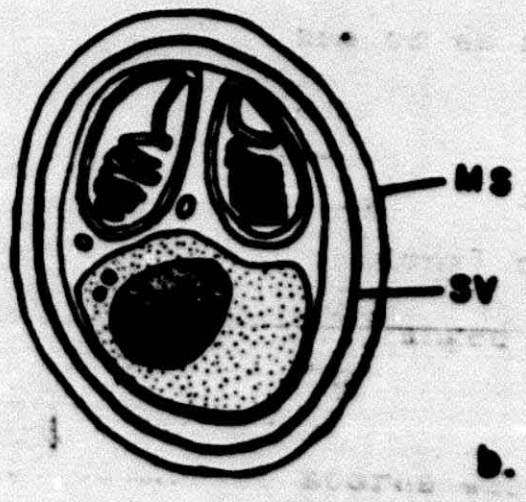
a. mature, spherical spore, face view; PC, polar capsule; PF, polar filament; SN, sporoplasm nuclei; CN, capsular nucleus; IV, iodinophilous vacuole; SP, sporoplasm;

b. mature ovoid spore, face view; MS, mucous sheath; SV, spore valve;

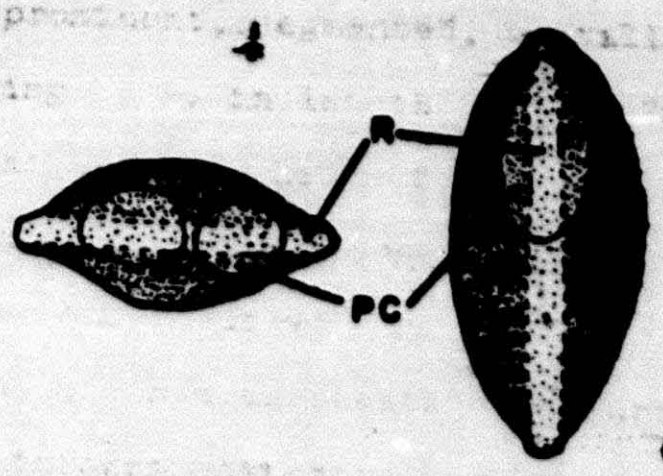
c. apical and lateral views of spores; R, ridge; PC, polar capsule.



a.



b.



c.

instances one or both capsular nuclei were in evidence when spores were treated with Feulgen's nuclear stain (Figure 6a). Each capsule contained a distinct polar filament with five to seven coils (Figure 6a,b), averaging 31.0 x 0.75μ in length when extruded. Posteriorly, spores contained a prominent sporoplasm (vital, living portion containing the sporoplasm nuclei which are the gametes), with a conspicuously eccentric, iodinophilous glycogen vacuole. The vacuole averaged 4.0 x 3.0 x 0.4μ in diameter (Figure 6a,b). Two nuclei were usually evident within the sporoplasm; however, on occasion, as few as one or as many as four were present.

A mucous sheath, first demonstrated by Lom and Varva (1963) and described by Hoffman (personal communication, 1969), surrounded mature spores and ranged in thickness from 1.0 to 2.0μ (Figure 6a,b).

Atypical variants of mature spores were observed in cysts during the summer and early fall. The variants demonstrated a prominent, segmented, distally knobbed caudal process, averaging 11.2μ in length (Figure 7a). Variants have also been reported by Lewis (1968) and others. Development of a caudal process was observed on July 26 (Figure 7b,c,d,e) and required 2.5 hours for formation. During formation, the mucous sheath that surrounded the spore tended to thicken posteriorly. There, the spore valves opened and a portion of the sporoplasm streamed from the spore and into the thickened sheath carrying with it

FIGURE 7. Caudal process of atypical variant and sequence of formation, M. meglitschi:

a. Caudal process of atypical variant, M. meglitschi; MS, mucous sheath; SV, spore valve; CP, caudal process.

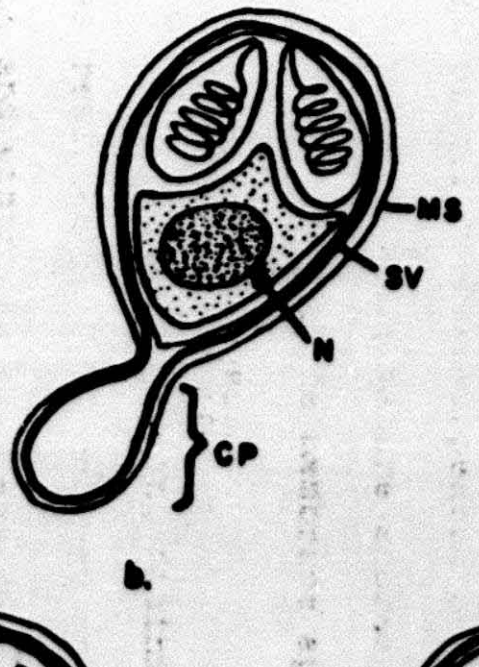
b. Caudal process; sequence of formation, 15 minutes after initiation; MS, mucous sheath; CP, caudal process; N, nucleus; SV, spore valve.

c. 45 minutes after initiation; MS, mucous sheath; CP, caudal process; N, nucleus; SV, spore valve.

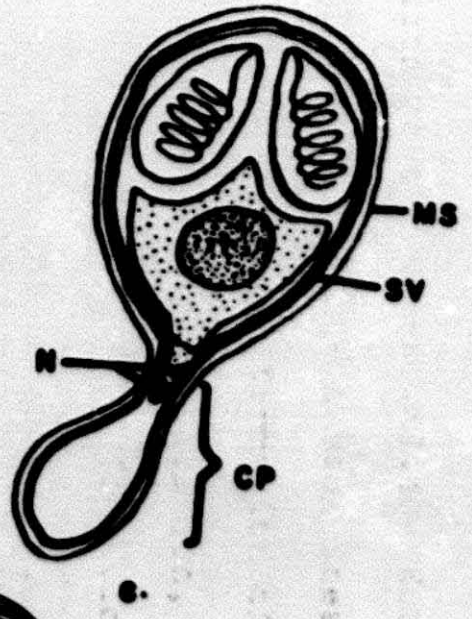
d. 75 minutes after initiation; MS, mucous sheath; CP, caudal process; N, nucleus; SV, spore valve.

e. 150 minutes after initiation; MS, mucous sheath; CP, caudal process; SV, spore valve.

a.

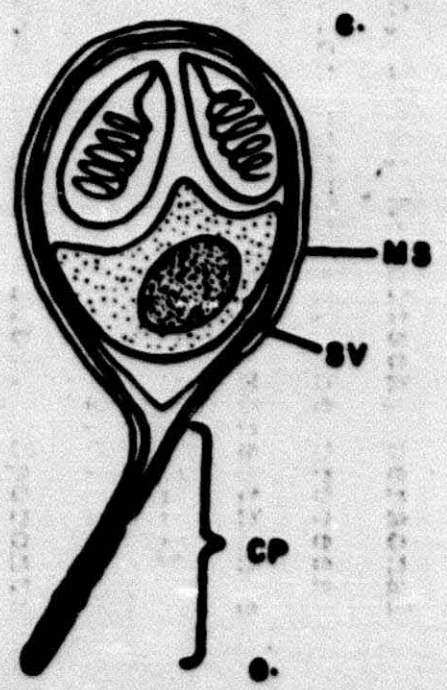


b.



c.

d.



e.

two bodies, apparently nuclei. If the bodies were nuclei, they probably were sporoplasmic or valvular nuclei. The two valvular nuclei have been reported to degenerate after formation of the spore valves (Noble 1944), while sporoplasmic nuclei and the mucous sheath have been surmised to be concerned with the formation of caudal processes (Hoffman, personal communication, 1969; Meglitsch, personal communication, 1969). As formation continued, the process gradually decreased in size until it was little more than a narrow protuberance (Figure 7a,e). Variants with a fully formed caudal process appeared pyriform (Figure 7a,e) in contrast to typical, mature spores (Figure 6a,b). Sporonts enclosed by the hyaline membrane apparently did not form or display a caudal process.

Techniques of fixation, staining, and dehydration of spores resulted in a pronounced degree of shrinkage in spore length and width, as well as capsule length and width (Table 3). Most spore dimensions were reduced by more than twice the amount noted by Kudo (1921b) for other species. The standard error of the means included ± one standard deviation.

Relationships

Hoffman (1967) listed 56 species for the genus Myxobolus, and Lewis (1968) described one, totaling 57 known North American forms. Numerous reports of infection of integument and scales by Myxobolus spp. have been made;

TABLE 3. Measurements of: 1. fresh, 2. fixed, and 3. fixed, stained, and dehydrated spores of *M. meglitschi* sp. nov.

Character	Range	Mean	Std. Error*	No. Obs.	% Chg. Fm. Fresh Spores
		1. **Fresh**			
Spore					
Length	16.0 - 18.1μ	17.33μ	0.95	250	
Width	14.0 - 17.0μ	15.31μ	0.83	250	
Capsule					
Length	7.0 - 9.0μ	8.21μ	0.33	250	
Width	5.0 - 7.4μ	5.74μ	0.57	250	
		2. **Fixed (Formalin)**			
Spore					
Length	11.5 - 14.0μ	12.83μ	0.48	100	26.0
Width	10.0 - 12.0μ	10.50μ	0.54	100	31.0
Capsule					
Length	5.0 - 5.5μ	5.18μ	0.69	100	37.0
Width	3.0 - 4.0μ	3.46μ	0.29	100	40.0
		3. **Fixed, Stained, and Dehydrated**			
Spore					
Length	10.0 - 12.0μ	11.33μ	0.62	100	35.0
Width	8.0 - 10.2μ	8.87μ	0.56	100	42.0
Capsule					
Length	4.0 - 4.8μ	4.21μ	0.30	100	49.0
Width	2.5 - 3.0μ	2.78μ	0.20	100	52.0

* Standard error of the mean includes ± 1 standard deviation.

Kudo (1920) noted 10 species; Bond (1938a), Herrick (1936, 1941), Kudo (1929, 1933, 1934), Lewis (1968), Lewis and Summerfelt (1964), Meglitsch (1937), Nigrelli (1948), Otto and Jahn (1943) and Yasutake and Wood (1957) account for 13 more. However, none of these forms are known to parasitize integument or scales of bluegill. There are only two accounts of parasitism of this host by any member of the Class Myxosporidea. Hoffman, et al. (1965) observed M. cartilaginis in the cartilage of the head, gill arches and bases of large fin rays, and Otto and Jahn (1943) found Myxobolus osborni Herrick (1936) in the gall bladder.

M. meglitschi most closely resembles Myxobolus kosteri Herrick in spore symmetry and contents, as well as in color and in cyst location on hosts. Differences in spore characteristics and number of coils in the polar filaments between the two forms are presented in Table 2.

Etymology

This species is named in honor of Dr. Paul A. Meglitsch for his numerous contributions to the field of Parasitology.

Establishment of M. meglitschi in Hosts

During internal examination of recently sacrificed host specimens, sections of gut tissue and the contents of alimentary canals (Table 4) were carefully examined for the presence of spores, amoeboid stages (migrating diploid sporoplasm) or multinucleated trophozoites (the growing,

TABLE 4. Percentage frequency by total number of items found in alimentary canals of 75 bluegill, Shanty Hollow Lake, Warren County, Kentucky, in March, September, October, and November, 1969.

Item	Percentage Frequency
Cladocera	37.0
Diptera	35.0
Copepoda	7.0
Odonata	4.0
Megaloptera	4.0
Algae	4.0
Ostracoda	3.0
Detritus, sand grains, pebbles	3.0
Vegetation other than algae	1.0
Unidentified material	1.0
Fishes	.5
Terrestrial insects	.5
Total %	100.0

vegetative individual). There was no evidence that M. meglitschi was present internally even though the fish examined were obviously parasitized externally. It was generally agreed by Debaisieux (1925), Kudo (1926, 1929), Noble (1944), Cheng (1964), and Lom and Corliss (1967) that spores entered the gut and anchored themselves to the gut epithelium by use of the polar filaments. Once this occurred, the sporoplasm, released as an amoeboid stage, migrated through the gut wall and established itself in tissues or organs, there undergoing successive stages of development which culminated in spore formation.

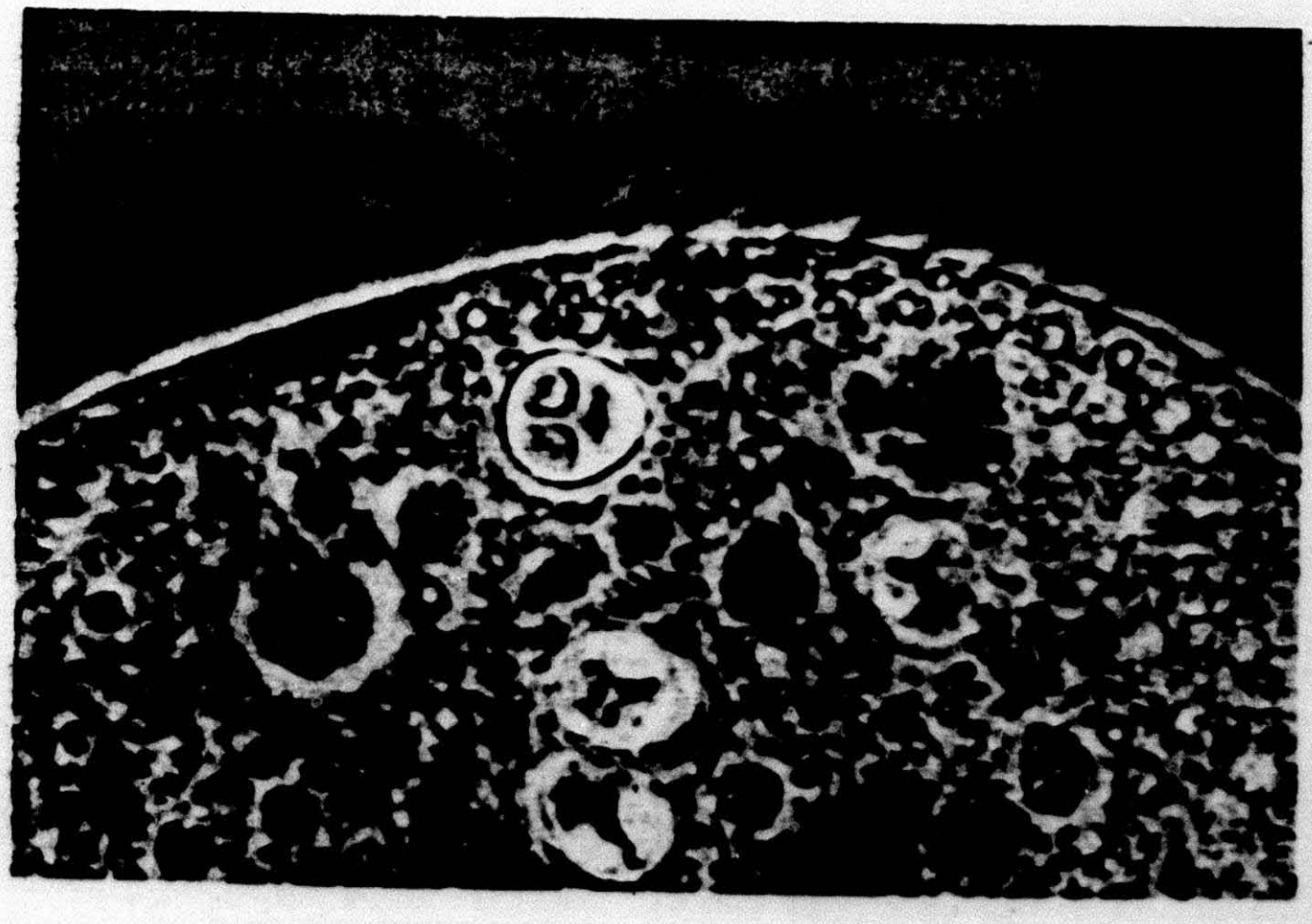
In order to test the establishment pattern, two separate attempts were made to induce infection in bluegill through the use of feeding experiments similar to those described by Hoffman et al. (1965), Walliker (1968), and Weissenberg (1968). The initial attempt was made in February by placing infected tissue and a suspension of spores in a small aquarium with 10 noninfected fish. The experiment failed because the fish became heavily parasitized by the protozoan Ichthyophthirius spp. and died soon after exposure to spores.

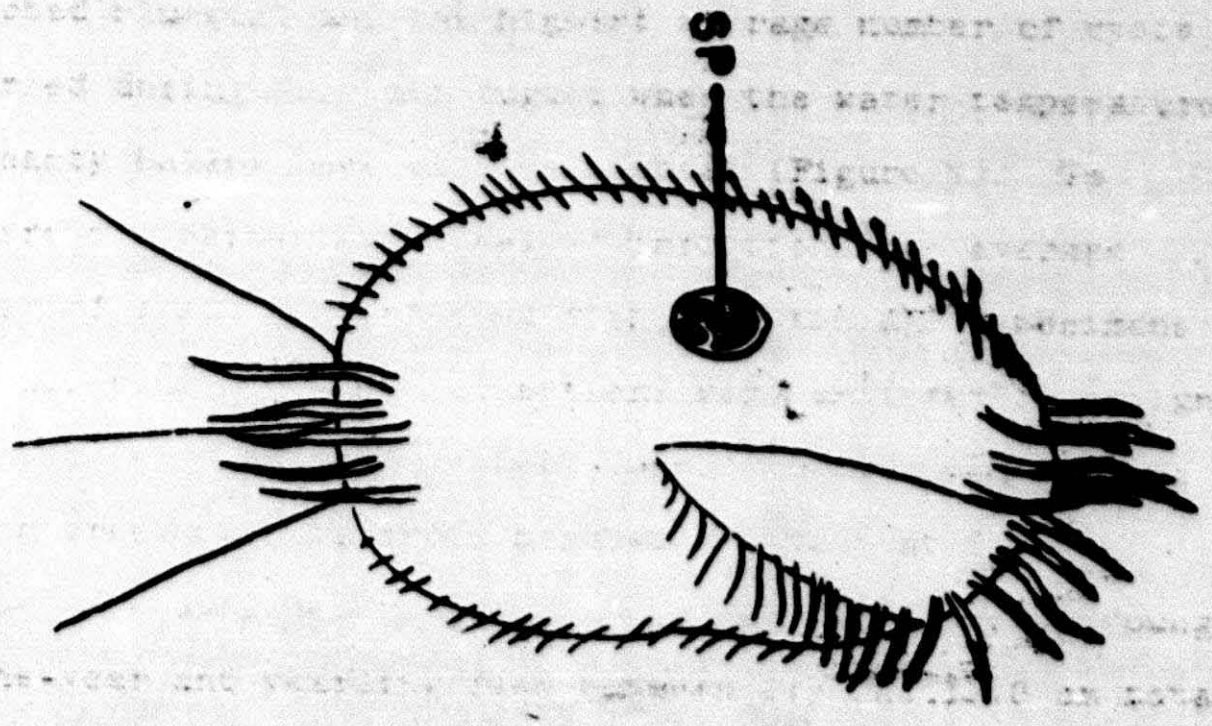
On March 21, during a routine examination of a wet mount of ruptured cysts, prepared with a drop of water from Shanty Hollow Lake as a mounting medium, numerous ciliated protozoans (Stylonychia sp.) were observed in the process of ingesting spores of M. meglitschi, apparently as a food source. Continued observation of this activity disclosed .

that virtually all observed Stylonychia sp. had ingested numerous spores (Figure 8). Subsequent investigations between March 21 and early July revealed a total of five kinds of ciliated protozoans which ingested spores: Stylonychia sp., Paramecium sp., Lacrymaria sp., Dileptus sp., and Euplotes sp. The mode of acquisition of spores varied from deliberate, aggressive feeding by Stylonychia sp. to apparently incidental ingestion by Lacrymaria sp. Spores were retained as long as 4.5 hours after ingestion. Spores noted in these forms were counted; 30 vacuoles from 21 individual protozoans contained from 1 to 12 spores each, depending upon the protozoan species being checked. Examination of spores released either by excretion or death and subsequent rupture of the protozoan's pellicle showed no signs of disintegration or digestion. Extrusion of polar filaments by action of digestive juices, which would have impaired spore viability (Hoffman et al. 1965), was not observed prior to, during, or immediately after ingestion or release of spores.

Transmission of myxosporidian parasites has been surmised to occur incidental to feeding or by ingestion of small invertebrates carrying spores; these invertebrates acted as intermediate transfer hosts (Hoffman et al. 1965; Hoffman, personal communication, 1969; Weissenberg 1968; Weissenberg, personal communication, 1969). In an effort to support this hypothesis, a second attempt to induce infection utilizing spore-carrying protozoans was undertaken

FIGURE 8. Spores of M. meglitschi in food vacuoles of Stylonychia sp. 240 minutes after ingestion. Digestion of spores is not apparent and polar filaments have not been extruded. SP, spore. (400X)




SPS

in July and August. The experiment failed to produce satisfactory results because of the inability to keep fish alive under laboratory conditions.

Phenology

Variation in Incidence

From January 20 to December 20, the mean incidence of infected bluegill per monthly sample varied from 1.38% in April and May to 53.3% in August (Table 5). The mean annual infection level was 22.31%. Based on combined monthly samples, 27.8% of the bluegill were infected during January and February, 12.69% in May and June, 43.9% in July and August, and 8.95% in November and December. The greatest number of cysts (115) and the highest average number of cysts per infected fish (18.0 per infected fish) occurred during July. Both the highest mean incidence of infected bluegill and the highest average number of cysts occurred during July and August when the water temperature at Shanty Hollow Lake was the highest (Figure 9). To compare the percentage level of infection, the average number of cysts per infected fish, and the age, specimens from all monthly seine collections were arbitrarily assigned to three length classes. Legal size, adult fish examined during creel surveys and a population study at Shanty Hollow were assigned to a fourth length class. Only young-of-the-year and yearling fish between 2.5 and 11.0 cm total

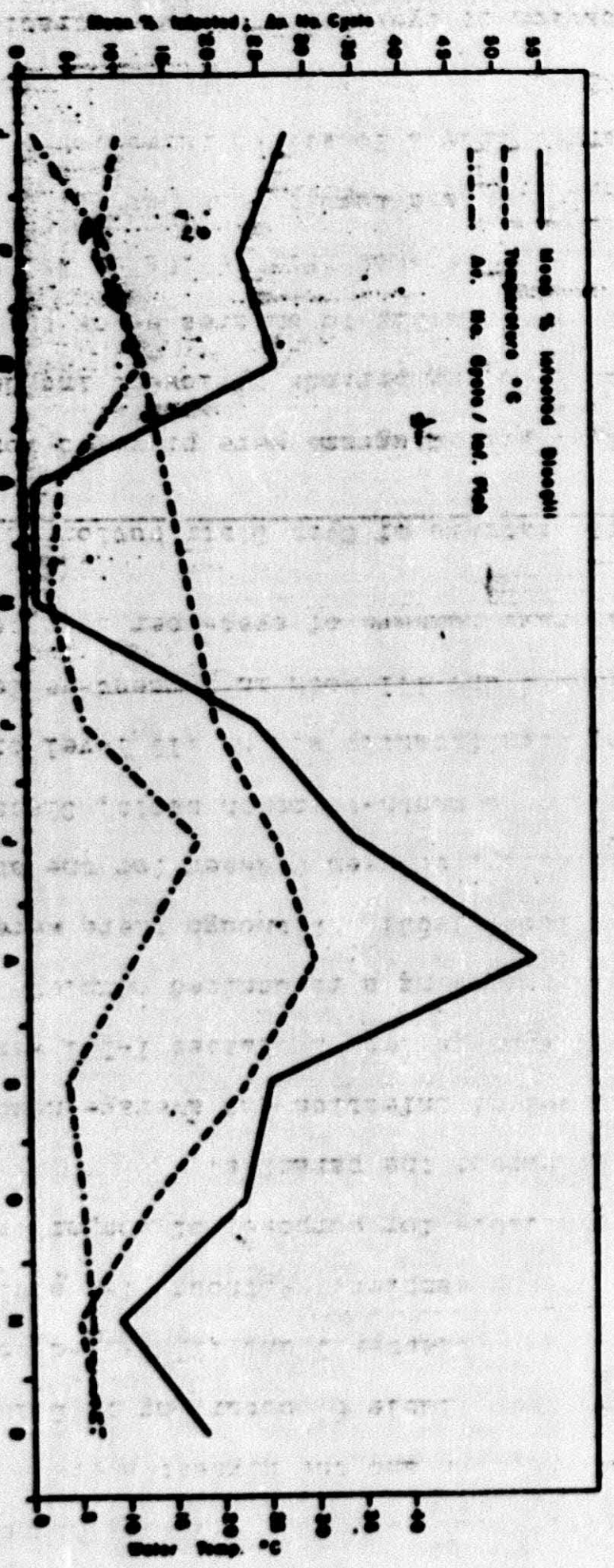
TABLE 5. Percentage infection by M. meglitschi sp. nov. in three length classes of bluegill. Numbers in parentheses below length class represent size ranges (total body length).

Date	Length Class I (2.5–4.9 cm)			Length Class II (5.0–7.9 cm)		
	No. Ckd.	No. Inf.	% Inf.	No. Ckd.	No. Inf.	% Inf.
Jan. 20	10	0	0	60	21	35.0
Feb. 7	18	4	21.2	52	12	23.1
Mar. 22	27	3	14.8	45	16	35.6
Apr. 26	12	0	0	55	1	1.8
May 10	18	0	0	51	1	1.9
Jun. 21	24	8	33.2	51	10	19.7
Jul. 19	13	5	38.4	58	18	31.0
Aug. 29	30	19	63.5	40	21	52.5
Sep. 19	2	0	0	28	10	35.8
Oct. 25	2	0	0	65	15	23.0
Nov. 15	5	0	0	68	7	10.7
Dec. 20	19	2	10.5	54	10	18.4

TABLE 5. Continued.

Date	Length Class III (8.0-11.0 cm)			Total		
	No. Ckd.	No. Inf.	% Inf.	No. Ckd.	No. Inf.	% Inf.
Jan. 20	5	0	0	75	21	28.0
Feb. 7	5	1	20.0	75	17	22.6
Mar. 22	3	1	33.3	75	20	26.6
Apr. 26	8	0	0	75	1	1.38
May 10	6	0	0	75	1	1.38
Jun. 21	0	0	0	75	18	24.0
Jul. 19	4	3	75.0	75	26	34.6
Aug. 29	5	0	0	75	40	53.3
Sep. 19	45	9	20.0	75	19	25.3
Oct. 25	8	2	25.0	75	17	22.7
Nov. 15	2	0	0	75	7	9.4
Dec. 20	2	1	50.0	75	13	18.5

FIGURE 9. Variation in percentage of bluegill infected by M. meglitschi sp. nov., average number of cysts per infected fish, and water temperature, Shanty Hollow Lake, Warren County, Kentucky, January through December, 1969.



body length were infected (Table 5), with the highest percentage level of infection and the highest average number of cysts per infected fish (Table 6) occurring in Class II (5.0-7.9 cm) bluegill. Classes I and III failed to exhibit infection in 50% of the samples. Although few adult fish (Class IV) were available for purposes of comparison, none were ever found to harbor the parasite.

Percentage level of infection and average numbers of cysts per infected fish in length Classes I-III were statistically analyzed using a randomized complete block design (Steel and Torie 1960). Although there were no differences, statistically, between Classes for the above mentioned criteria on a month-to-month basis, Class II bluegill differed significantly at the .10 level of probability from Classes I and III both in percentage level of infections and average numbers of cysts per infected fish.

Variation in the Pattern of Cyst Distribution on Hosts

Composite cyst plot diagrams were prepared for each month of the study; during periods of lowest incidence, cysts were confined primarily to an area below the lateral line and posterior to the anus (Figure 10a). At the time of highest incidence, July and August, the pattern of cyst distribution changed from a localized infection to one which was widespread over the body (Figure 10b). The pattern of the shift in location of cysts and the concentration of cysts, postanally, below the lateral line throughout the

TABLE 6. Average number of cysts of M. meglitschi sp. nov. in three length classes of bluegill. Numbers in parentheses next to length class represent size ranges (total body length).

Date	Length Class I (2.5-4.9 cm)			
	No. Ckd.	No. Inf.	Range	Avg. No. Cysts per Inf. Fish
Jan. 20	10	0	0	0
Feb. 7	18	4	1 - 2	1.2
Mar. 22	27	3	2 - 6	4.3
Apr. 26	12	0	0	0
May 10	18	0	0	0
Jun. 21	24	8	1 - 26	10.7
Jul. 19	13	5	2 - 36	16.8
Aug. 29	30	19	2 - 66	20.8
Sep. 19	2	0	0	0
Oct. 25	2	0	0	0
Nov. 15	5	0	0	0
Dec. 20	19	2	1 - 6	3.5

TABLE 6. Continued.

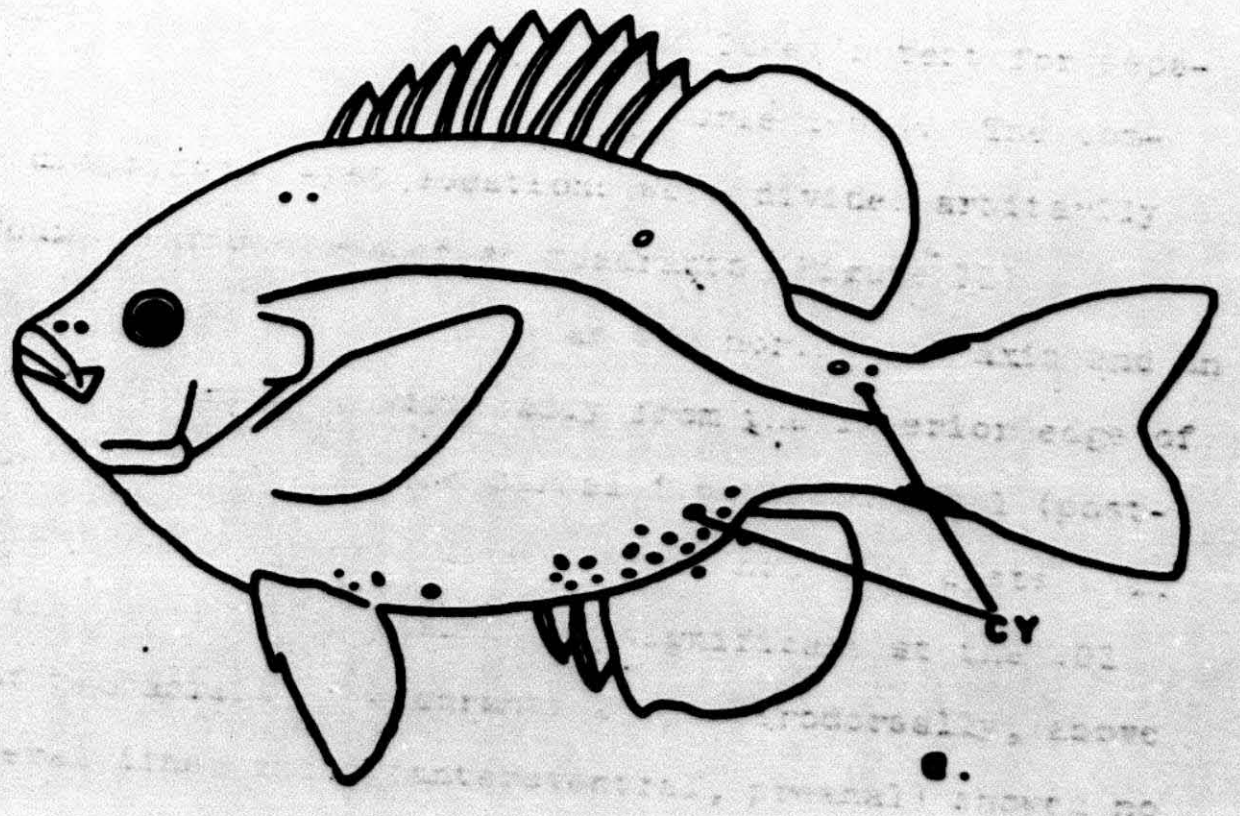
Date	Length Class II (5.0-7.9 cm)			
	No. Ckd.	No. Inf.	Range	Avg. No. Cysts per Inf. Fish
Jan. 20	60	21	1 - 16	3.8
Feb. 7	52	12	1 - 22	10.0
Mar. 22	45	16	1 - 27	8.8
Apr. 26	55	1	12 - 12	12.0
May 10	51	1	9 - 9	9.0
Jun. 21	51	10	1 - 20	7.7
Jul. 19	58	18	1 - 115	24.9
Aug. 29	40	21	1 - 51	13.2
Sep. 19	28	10	1 - 15	5.3
Oct. 25	65	15	1 - 42	11.4
Nov. 15	68	7	1 - 88	19.9
Dec. 20	54	10	1 - 32	7.3

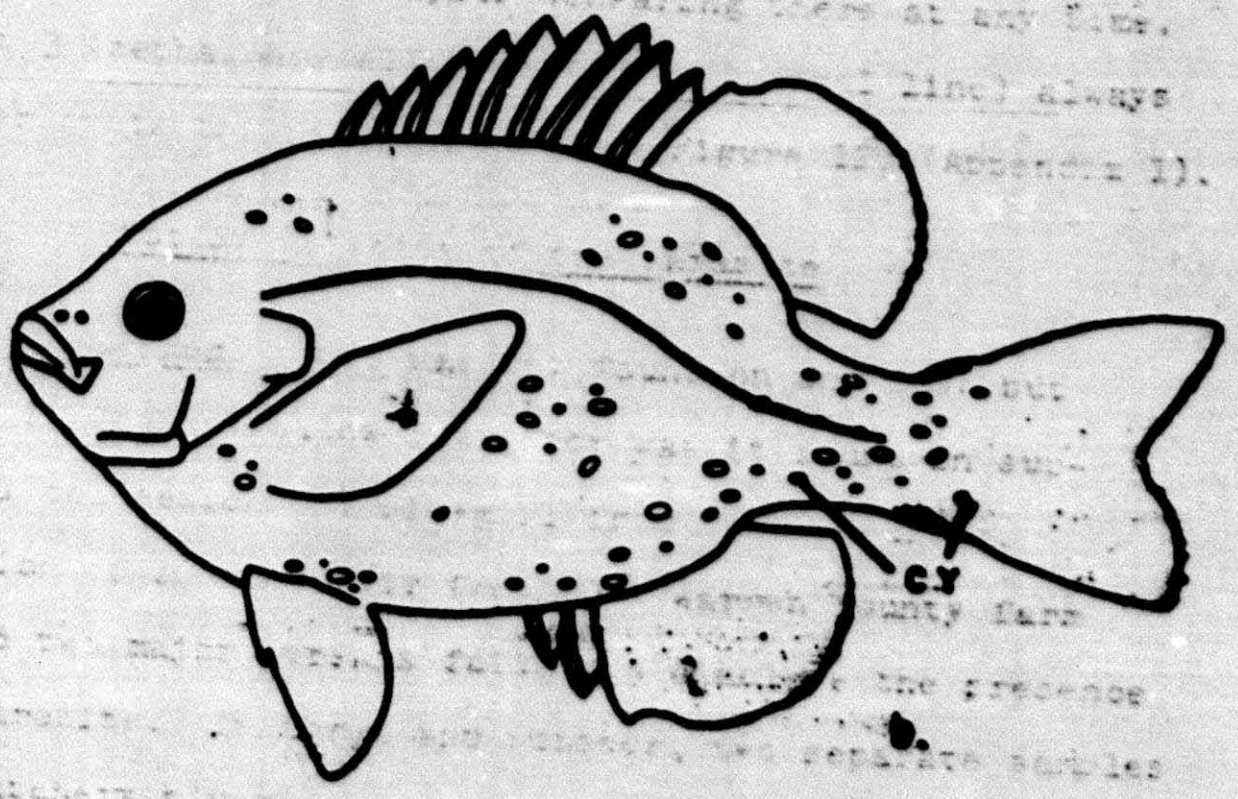
TABLE 6. Continued.

Date	Length Class III (8.0-11.0 cm)				Weighted Avg. No. Cysts per Inf. Fish
	No. Ckd.	No. Inf.	Range	Avg. No. Cysts per Inf. Fish	
Jan. 20	5	0	0	0	1.2
Feb. 7	5	1	17 - 17	17	9.4
Mar. 22	3	1	24 - 24	24	12.3
Apr. 26	8	0	0	0	4.0
May 10	6	0	0	0	3.0
Jun. 21	0	0	0	0	6.1
Jul. 19	4	3	2 - 25	12.3	18.0
Aug. 29	5	0	0	0	11.3
Sep. 19	45	9	1 - 22	7.1	4.1
Oct. 25	8	2	1 - 8	4.5	5.3
Nov. 15	2	0	0	0	6.6
Dec. 20	2	1	8 - 8	8	6.2

FIGURE 10. a. Pattern of cyst distribution April, May, 1969. CY, cyst

b. Pattern of cyst distribution July, August, 1969.


CY


CY

year appeared unique. Significance of this pattern on the bodies of bluegill was analyzed statistically using a randomized complete block design and Tukey's test for separation of means derived (Steel and Torie 1960). The composite diagrams of cyst locations were divided arbitarily into four areas designated as quadrants (Figure 11) delineated by the lateral line as the horizontal axis and an imaginary line running vertically from the anterior edge of the anus to the base of the dorsal fin. Quadrant 1 (postanally, below the lateral line) always had more cysts than any of the other three quadrants, significant at the .01 level of probability. Quadrants 2 (posterodorsally, above the lateral line) and 4 (anteroventral, preanal) showed no difference in numbers of cysts appearing there at any time. Quadrant 3 (cephalodorsally, above the lateral line) always had fewer cysts than any other area (Figure 12) (Appendix 1).

Host Specificity of the Parasite

Myxobolus meglitschi was not found on any host but bluegill in Shanty Hollow Lake, nor was it found in supplemental collections of bluegill from other sources. Examination of 100 bluegill from five Warren County farm ponds and two major streams failed to disclose the presence of the parasite. In March and October, two separate samples of 100 hatchery bluegill were examined with negative results. A sample of 50 bluegill taken from Nolin and Barren River Reservoirs in October was similarily unparasitized.

FIGURE 11. Quadrant designations for cyst distribution.

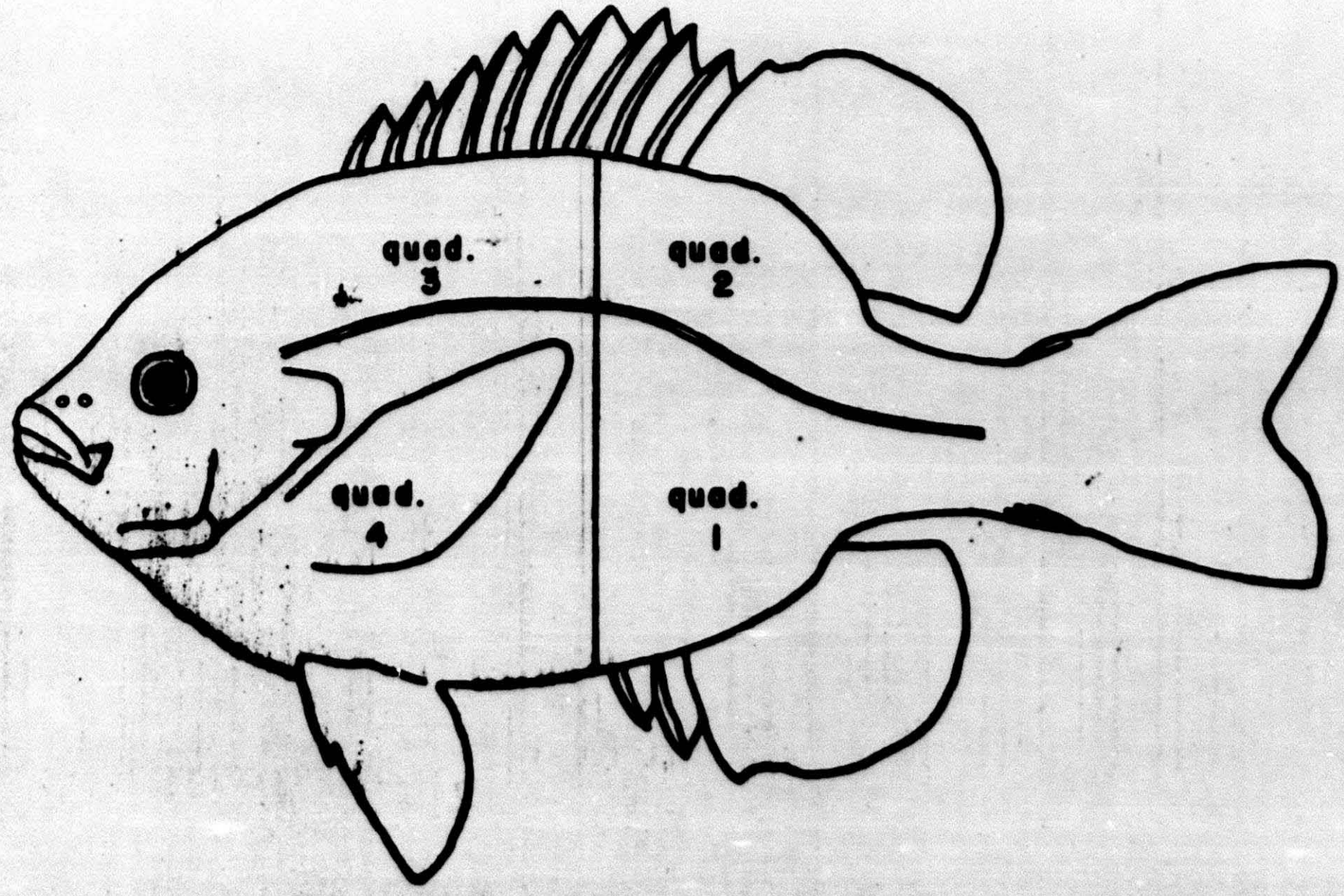
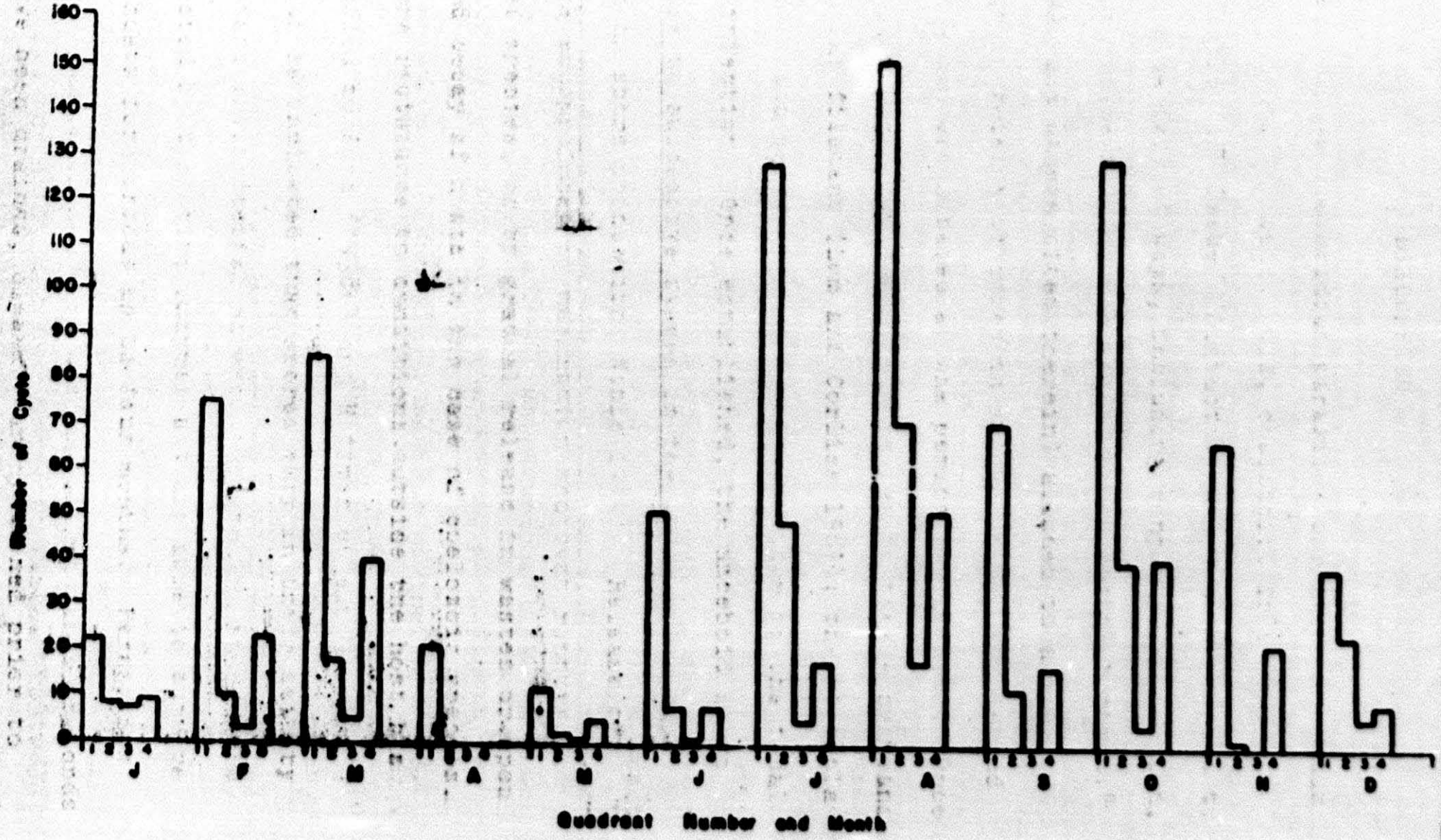

quad. 3
quad. 2
quad. 4
quad. 1

FIGURE 12. Number of cysts of M. meglitschi appearing in four quadrants (see Figure 11) on host fish, January through December, 1969.


160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
Number of Cycle
J
F
M
A
M
J
J
A
S
O
N
D
Quadrant Number and Month

DISCUSSION

Presently, myxosporidian parasites are known to occur in two locations in hosts: viz, coelozoic, in cavities; and histozoic, in tissue. Contact with the host appears to occur in a variety of ways, including congenital infection, ingestion incidental to feeding, and "accidental contact." Kudo (1926 and others cited therein) alluded to auto-infection by release of amoeboid stages from uninucleated sporonts which are located at the periphery of cysts. Once on or in hosts, spores anchor themselves to tissue by means of the polar filament (Lom and Corliss 1967); the sporoplasm is released and migrates as an amoeboid stage to the final site of infection. Once established, the amoeboid stage gives rise to successive stages in the life cycle, culminating in spore production (Noble 1944). Release of spores is accomplished by maturation and cyst rupture in histozoic species, or by gradual release in wastes or upon death and decay of hosts in the case of coelozoic species.

Spore resistance to conditions outside the host has been studied by Bond (1938b) and Hoffman et al. (1969). These authors indicated that spores vary in their ability to withstand environmental stress. Generally, spores are not affected by digestive enzymes and are able to survive outside hosts for periods up to four months. Parasitism by myxosporidians of hatchery fish placed in tanks or ponds which have been drained, cleaned, and poisoned prior to

stocking has been reported by several workers.

Finally, although little work has been done with regard to the seasonal nature of myxosporidian parasites, it is generally agreed that some species exhibit seasonal variation, showing increased incidence in summer, with decline or disappearance in infected populations during fall, winter, and early spring.

Occurrence of M. meglitschi on bluegill varied with the season of the year as has been reported for other myxosporidians. Analysis of Figure 9 showed the following trends: (1) a 3-month period in late winter (January through March) in which the level of infection was 25.7% of the fish sampled; (2) an 82-day period in early spring between March 29 and June 21 when incidence dropped to 1.38%; (3) an abrupt rise in incidence throughout the summer, from June 21 through August, peaking at 53.3% of the fish sampled harboring the parasite; and (4) an abrupt decline, but not disappearance of infection in the fall from September through December 20. The summer maximum of incidence occurred at the same time water temperature was at its zenith. The decline in incidence from September through December 20 and the decline in water temperature were coincident.

The highest number of cysts and the greatest average number of cysts per infected fish occurred during July, declining during other months (Figure 9, Table 6). Spore sizes varied, but exhibited the same size ranges (Table 2) throughout the year.

Inability to locate spores in gut contents or amoeboid stages or trophozoites in tissue, and failure of laboratory experiments to yield conclusive data on the developmental period of this parasite, leaves the method of establishment open to speculation.

The histozoic nature of M. meglitschi and the manner in which spores are liberated appears to provide the environment with a continuous supply of spores. That these spores contacted hosts was evidenced by (1) the presence of parasitized fish at every sampling station at some time during the year, (2) observed incidence during each of the twelve months in which sampling occurred, with a yearly mean incidence of 22.31%, and (3) infection of young-of-the-year fish after they were placed in the lake by stocking and/or appeared after spawning. Contact with spores could have occurred within the spawning beds during the egg or larval stage if spores were present as a result of previous liberation into the environment or if cysts on spawning adults ruptured over the nest.

A second, more likely possibility of contact with spores of M. meglitschi was through ingestion of spores incidental to feeding. Since protozoans occurring in samples of lake water have been found to ingest spores, fry which might utilize these intermediate transfer hosts as a source of food could have become parasitized. Likewise, spores could have been picked up with detritus during benthic feeding; however, no spores were observed in alimentary canals of

hosts.

The effect of seasonal changes in water temperature on the activity of hosts with respect to regulation of spawning and feeding was documented by Lagler (1956), Lagler et al. (1962), Wohlschlag and Juliano (1959), and Gerking (1962, 1964). Bluegill in Shanty Hollow spawned in April with fry apparently susceptible to parasitism at the onset. Feeding by young-of-the-year and yearling fish proceeded at an increased level beginning in April and continued throughout the summer. Informal checks of stomachs during this period revealed that 100% of stomachs examined contained some food items. However, during late winter, late fall, and early winter of 1969, over 50% of stomachs checked were found empty. The seasonal rise in percentage level of infection from June through August appeared to be reflected, at least in part in the young-of-the-year and yearling fish, by an exhibited increase in feeding activity.

The period of time required for development of *N. meglitschi*, from establishment and release of amoeboid stages on hosts to production of detectable size cysts, cannot be stated with any degree of certainty. Nevertheless, inferences can be made from Figure 9. Incidence of infection was lowest for the 82-day period between March 29 and June 21; on March 29, 150,000 non-parasitized bluegill were stocked in the lake. Additionally, spawning occurred in the natural population during this period adding a second, presumably initially non-infected, host source. Both hatchery fish and

naturally spawned fish subsequently were presumably exposed and susceptible at the time of introduction. The rise in incidence from June through August in young-of-the-year fish may have reflected an 80- to 90-day period of development. Thus, fish exposed in late March exhibited cysts in July, and fish spawned in May were demonstrably parasitized by August.

A possible explanation for the periodic decline (Figure 9) in the number of cysts per infected fish can be related to this same 80- to 90-day period of development. From January 20 to March 22 (Table 6), a steady increase in the number of cysts per infected fish was evident, followed by a decline, then an increase between June and August. A third period of decline then an increase was observed during the period September through November. Periods of alternating rise and fall in numbers of detectable size cysts indicated that maturation and rupture of mature cysts occurred during this 80- to 90-day period. Additionally, fish from the May and September seine collections exhibited eroded scales with remanents of what appeared to be the membrane which had surrounded cysts.

The pattern of cyst distribution and presence of cysts of various sizes containing mature spores and developmental stages, particularly uninucleated sporonts on the periphery of cysts, may indicate a spread of the parasite on hosts by autoinfection as described by Kudo (1926) and other workers. However, the significance of the formation of the caudal

process as a mechanism of autoinfection cannot be ignored. Taxonomic studies of the various species of myxosporidia are replete with figures showing variants with processes which are entire or forked. The time of the year when variants appeared has been largely ignored by most workers, although the parasites themselves were most often first observed during the warmer periods of the year. Lewis (1968), in describing M. argenteus, first observed by her in early spring, noted the presence of variants in most cysts with one cyst containing large numbers of variants having both entire and forked caudal processes. Parker (personal communication, 1970) has also observed variants in cysts and the formation of caudal processes in the fall of the year.

Variants in cysts of M. meglitschi comprised approximately 2% of the contents of cysts during the summer and fall. If it is assumed that the nuclei and accompanying cytoplasmic material observed entering the caudal process of the variant of M. meglitschi are sporoplasmic and therefore gametic (Noble 1944) in nature, the rupture of the distal end of the caudal process could free an amoeboid stage in the cyst and account for the presence of the forked process observed and reported for many forms. The caudal process of M. meglitschi was never observed to be forked. Upon rupture of mature cysts these amoeboid stages could infect the same host, or parasitize eggs or larval stages in spawning beds, becoming a means of perpetuation of the infection within host populations.

The sequence of caudal process formation, release of amoeboid stages within cysts, and subsequent rupture of cysts releasing amoeboid stages which may have resulted in the spread of the parasite on hosts might account, in part, for the postanal concentration of cysts. Since the dorsal surface of the body immediately behind the head exhibited the fewest numbers of cysts, it may be that amoeboid stages were unable to migrate to this region in appreciable numbers.

Although some myxosporidian parasites have been reported to be disfiguring or lethal to hosts (Nigrelli and Smith 1938, Nigrelli 1948, Hoffman et al: 1965), M. meglitschi apparently does not injure or incapacitate its host. The only observed effect was scale erosion by cysts. Infected fish swam and fed in the same manner as non-infected fish when both were placed in aquaria and observed.

SUMMARY AND CONCLUSIONS

A new histozoic myxosporidian parasite, Myxobolus meglitschi sp. nov., infecting the scales and epidermis of bluegill sunfish in Shanty Hollow Lake, Warren County, Kentucky, was described. The parasite was endemic to Shanty Hollow Lake, host specific for bluegill, and appeared to infect fish one year old and younger. While older fish were examined during the study, none were observed to harbor this parasite. Infected fish ranged in size from 2.5 to 11.0 cm total body length. Various stages in the life cycle and the formation of a caudal process was observed during the course

of the study.

Phenology was studied from January 20 through December 20, 1969. The magnitude of infection in the bluegill varied seasonally, with a yearly mean percentage level of infection of 22.31%. The highest percentage of infected fish occurred during July and August (43.9%), abruptly declining but not disappearing from September to December. The lowest percentage of incidence occurred from March 29 to June 21 when only 1.38% of fish in samples were found to be parasitized. The highest average number of cysts (18.0 per infected fish) occurred in July. Both the peak period of incidence (July and August) and the highest average numbers of cysts per infected fish (July) were coincident with the period in which the water temperature at Shanty Hollow Lake was the warmest.

Neither the method of initial establishment nor the period of time required for completion of the life cycle after establishment of M. meglitschi in bluegill was determined with any degree of certainty. Contact by hosts with spores may occur incidental to feeding upon intermediate transfer hosts carrying spores. Five different species of protozoans were observed to utilize spores of M. meglitschi, apparently as a source of food. Spores could also have been ingested with detritus as bluegill fed upon benthic organisms. A third possible means of contact with the parasite may have occurred during spawning, if spores or amoeboid stages came into contact with eggs or larva in

spawning beds. The time required for completion of the life cycle appeared to be 80 to 90 days, as indicated by the periodic rise and fall in the average numbers of cysts of detectable size per infected fish, and a period from March 29 to June 21 when the percentage of infected fish dropped to 1.38%, then rose to 24% between those dates. It was during this period that 150,000 young-of-the-year hatchery fish were stocked in the lake and spawning in the natural population occurred. Both host sources were, presumably, initially non-parasitized.

The pattern of distribution of cysts on the body of the host varied seasonally. During periods of lowest infection within the population, cysts were confined primarily below the lateral line in a postanal area, whereas during the period of highest infection, cysts were found not only postanally below the lateral line, but widespread over the host's body. Cyst concentration and the presence of cysts of various sizes containing mature spores and developmental stages, on individual hosts, may indicate spread of the parasite by autoinfection. Kudo (1926) and other workers noted the presence of uninucleated sporonts on the periphery of cysts which released what appeared to be amoeboid stages. Uninucleated sporonts were in abundance in cysts of M. meglitschi. A second possibility concerns the observed formation of a caudal process with migration of nuclei and cytoplasmic material into the distal end of the process. If it is assumed that these nuclei were sporoplasmic and

therefore gametic in nature, release of the amoeboid stage from the caudal process and a subsequently from the cyst upon its rupture, would allow the parasite to be spread on individual hosts.

Observed pathological effects of M. meglitschi on hosts were limited to scale erosion at the point of contact by cysts.

APPENDIX 1. Statistical comparison of numbers of cysts of M. meglitschi appearing in four quadrants on the bluegill sunfish January - December, 1969.

Month	Quadrant 1	2	3	4	$\bar{x}$
1	22	8	7	9	11.5
2	75	10	3	23	27.8
3	85	18	5	40	37.0
4	21	0	0	0	5.3
5	12	2	1	5	5.0
6	51	8	1	8	17.0
7	128	49	5	38	55.0
8	151	71	18	52	73.0
9	71	12	0	17	20.0
10	130	40	4	41	53.8
11	67	1	0	22	22.5
12	39	24	6	9	19.6
Σ	852	243	50	264	
$\bar{x}$	71	20.3	4.2	22	

ANOVA

Source	df	ss	ms	F
Total	47	62,173		
Treat.	3	30,210	10,070	179.3 **
Rep.	11	30,110	2737.3	48.8 **
Error	34	1.853	56.15	

APPENDIX 1. Continued.

Tukey's Test

Quadrant (Treatment)

1	4	2	3
71.0	22.0	20.3	4.2

Month (Replication)

8	7	10	3	2	11	9	12	6	1	4	5
73.0	55.0	53.8	37.0	27.8	22.5	20.0	19.5	17.0	11.5	5.3	5.0

LITERATURE CITED

Bond, F. F. 1938a. Cnidosporidia from Fundulus heteroclitus (Linnaeus). Trans. Amer. Microscop. Soc., 57: 107-122.

_____. 1938b. Resistance of myxosporidian spores to conditions outside the host. J. Parasitol., 24: 470-471.

_____. 1939. The seasonal incidence of myxosporidian parasites infecting Fundulus heteroclitus (Linnaeus). Trans. Amer. Microscop. Soc., 58:156-163.

Clay, W. M. 1962. A Field Manual of Kentucky Fishes. Kentucky Dept. of Fish and Wildlife Res. Frankfort, Ky. 147 p.

Cheng, T. C. 1964. The Biology of Animal Parasites. W. B. Saunders Co. Philadelphia. 727 p.

Davies, E. H. 1968. Myxosporidian parasites from the fish of the River Lugg. J. Protozool., 15:471-480.

Debaisieux, P. 1925. Protistologica II: Etudes sur les myxosporidies III: Myxobolus notatus Mayor. Arch. De Zool. Exp. et Gens., 64:353-385.

Fish, F. F. 1938. Notes on Myxobolus inornatus n. sp., a myxosporidian, parasitic in the black bass (Huro floridana LeSueur). Trans. Amer. Fisheries Soc., 69:173-177.

Gerking, S. D. 1962. Production and food utilization in a population of bluegill sunfish. Ecol. Monogr., 32: 31-78.

_____. 1964. Timing and magnitude of the production of a bluegill sunfish population and its food supply. Verh. Internat. Verein. Limnol., 15:496-503.

Guilford, H. G. 1963. New species of myxosporodia found in percid fishes from Green Bay (Lake Michigan). J. Parasitol., 49:474-478.

Hayne, D. W. and R. C. Ball. 1956. Benthic productivity as influenced by fish predation. Limnol. and Oceanog. 1:162-175.

Herrick, J. A. 1936. Two new species of Myxobolus from the fishes of Lake Erie. Trans. Amer. Microscop. Soc., 55:194-198.

_____. 1941. Some myxosporidian parasites of Lake Erie fishes. Trans. Amer. Microscop. Soc., 60:163-170.

Hoffman, G. L. 1967. Parasites of North American Freshwater Fishes. University of California Press. Berkeley. 486 p.

_____, R. E. Putz, and C. E. Dunbar. 1965. Studies on Myxosoma cartilaginis n. sp. (Protozoa: Myxosporidea) of centrarchid fish and a synopsis of the Myxosoma of North American freshwater fishes. J. Protozool., 12:319-332.

Iverson, E. S. 1954. A new myxosporidian, Myxosoma squamalis, parasite of some salmonid fishes. J. Parasitol., 40:397-404.

Kudo, R. R. 1920. Studies on myxosporidia; a synopsis of genera and species. Illinois Biol. Monogr., 5:1-265.

_____. 1921a. On the nature of structures characteristic of Cnidosporidian spores. Trans. Amer. Microscop. Soc., 40:59-74.

_____. 1921b. On the effect of some fixatives upon myxosporidian spores. Trans. Amer. Microscop. Soc., 40:161-168.

_____. 1926. On Myxosoma catostomi Kudo 1923, a myxosporidian parasite of the sucker, Catostomus commersoni. Arch. Protistenk., 56:90-115.

_____. 1929. Histozoic myxosporidia found in freshwater fishes of Illinois, USA. Arch. Protistenk., 65:364-378.

_____. 1933. A taxonomic consideration of myxosporidia. Trans. Amer. Microscop. Soc., 52:195-216.

_____. 1934. Studies on some protozoan parasites of fishes of Illinois. Illinois Biol. Monogr., 13:7-44.

Lagler, K. F. 1956. Freshwater Fishery Biology. W. C. Brown, Co., Dubuque, Iowa. 421 p.

_____, J. E. Bardach, and R. R. Miller. 1962. Ichthylogy. John Wiley and Sons, Inc., New York. 545 p.

Larimore, R. W. 1957. Ecological life history of the warmouth (Centrarchidae). Illinois Nat. Hist. Survey Bull., 27:1-83.

Lewis, S. D. 1968. Myxobolus argenteus sp. n. (Protozoa: Myxosporidea), a parasite of the golden shiner. J. Parasitol., 54:1034-1037.

Lewis, W. M. and R. D. Summerfelt. 1964. A myxosporidian, Myxobolus notemigoni sp. n., parasite of the golden shiner. J. Parasitol., 50:386-389.

Lom, J. and J. O. Corliss. 1967. Ultrastructural observations on the development of the microsporidian protozoan Plistophora hyphessobryconis Schaperclaus. J. Protozool., 14:141-152.

_____ and J. Varva. 1963. Mucous envelopes of spores of the subphylum Cnidospora (Doflein, 1901) Acta Soc. Zool. Bohemoslov, 27:4-6.

Meglitsch, P. A. 1937. On some new and known myxosporidia of the fishes of Illinois. J. Parasitol., 23:467-477.

Minckley, W. L. 1963. Studies of the ecology of a spring stream: Doe Run, Meade County, Kentucky. Wildlife Monogr. No. 11. 124 p.

Nigrelli, R. F. 1948. Epickle cell hyperplasia in the cells of the redhorse sucker (Moxostoma aureolum) associated with an infection by the myxosporidian, Myxobolus moxostomi sp. nov. Zoologica [New York], 33:43-46.

_____ and G. M. Smith. 1938. Tissue responses of Cyprinodon variegatus to the myxosporidian parasite Myxobolus lintoni. Zoologica [New York], 23:195-201.

Noble, E. R. 1944. Life cycles in the myxosporidia. Quart. Rev. Biology, 19:213-235.

Otto, G. R. and T. A. Jahn. 1943. Internal myxosporidian infections of some fishes of the Okoboji region. Iowa Acad. Sci., 50:323-335.

Pfeiffer, P. W. 1967. Some physical, chemical, and biological characteristics of Shanty Hollow Lake. Kentucky Fish. Bull., No. 31. 1-31.

Rafinesque, C. S. 1819. Prodrome de 70 neuveaux genres d'animaiux decouvertes dans l'interieur des Etats - Unis d'Amerique durant l'annee 1818. Jour. de physique, de chimie, d'Historie naturelle et des arts. 88:417-429.

Ricker, W. E. 1949. Utilization of food by bluegills. Invest. Indiana Lakes and Streams. 3:311-318.

Romeis, B. 1948. Mikroskopische Technik. 15th ed. Leibnuz Verlag. Munich. 228 p.

Steel, R. G. D. and J. H. Torie. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Co. Inc., New York. 481 p.

Trautman, M. B. 1957. The Fishes of Ohio. Ohio St. Univ. Press, Columbus. 683 p.

Walliker, D. 1968. Studies on Myxidium oviforme, a myxosporidian parasite of Irish salmon, Salmo salar. Parasitol., 58:839-844.

_____. 1969. Myxosporidea of some Brazilian fresh water fishes. J. Parasitol., 55:942-948.

Weissenberg, R. 1968. Intracellular development of the microsporidian Glugea anomala Moniez in hypertrophying migratory cells of the fish Gasterosteus aculeatus (L.), an example of the formation of xenoma tumors. J. Protozool., 15:44-57.

Whitaker, J. O. 1968. Keys to the Vertebrates of the Eastern United States, Excluding Birds. Burgess. Pub. Co., Minneapolis. 256 p.

Wohlschlag, D. E. and R. O. Juliano. 1959. Seasonal changes in bluegill metabolism. Limnol. and Oceanog., 4:195-209.

Yasutake, W. T. and E. M. Wood. 1957. Some myxosporidia found in Pacific Northwest salmonids. J. Parasitol., 43:633-642.