AN ABSTRACT OF THE THESIS OF

ROWAN WAYNE GOULD (Name) for the MASTER OF SCIENCE (Degree)

in MICROBIOLOGY (Major) presented on Nov 6, 1969 (Date)

Title: OCCURRENCE OF THE PROTOZOAN FISH PARASITE CERATOMYXA SHASTA IN THE DESCHUTES RIVER BASIN AND THE ULTRASTRUCTURE OF THE SPORE

Redacted for Privacy

Abstract approved: Dr. J. L. Fryer

The purpose of this paper was twofold, (1) to determine the distribution of the infective stage of Ceratomyxa shasta (Noble) in the Deschutes River basin, and (2) to study the ultrastructure of the spore stage of the protozoan parasite.

Liveboxes containing juvenile rainbow trout were placed at specific test points in the Deschutes River system. After an exposure period, the fish were examined for the presence of the parasite. C. shasta was detected in this manner in each of the three main tributaries to the Deschutes River. In the Metolius River the source of infection was Suttle Lake. In the upper Deschutes River the source was Crescent Lake. Crooked River was thought to contain the infective stage of C. shasta in the lower reaches because of contamination with Deschutes River water entering by irrigation canals. Odell and Davis Lakes (not directly connected to the Deschutes River) contain

the infective stage, but are thought to constitute a closed system.

The ultrastructure of the spore of C. shasta was examined by electron microscopy. Penetration of embedding plastic into the spore was possible only when the specimens were placed in 10% formalin for an extended period of time.

The valves of the spore form knobs at the suture line similar to those of other myxosporidians. A septate desmosome similar to that described in Hydra (Wood, 1959) was identified between the two valves. Within the sporoplasm are two vesicular nuclei with nucleoli, endoplasmic reticulum, electron dense circular bodies, mitochondria and ribosome packets. The polar capsule and filament of C. shasta are morphologically similar to those of other myxosporidians.

Occurrence of the Protozoan Fish Parasite Ceratomyxa shasta in the Deschutes River Basin and the Ultrastructure of the Spore

by

Rowan Wayne Gould

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

June 1970

APPROVED:

Redacted for Privacy

Associate Professor of Microbiology
in charge of major

Redacted for Privacy

Chairman of the Department of Microbiology

Redacted for Privacy

Dean of Graduate School

Date thesis is presented Nov. 6, 1969

Typed by Gwendolyn Hansen for Rowan Wayne Gould

ACKNOWLEDGEMENTS

I would like to express my appreciation to the following people:

To my major professor, Dr. J. L. Fryer, associate professor of Microbiology, Oregon State University for his constant support and advice.

This project was funded by Oregon Fish Commission project 815 from July 1, 1968 to July 1, 1969, and by the Oregon Game Commission project 908 from July 1, 1969 to November 1, 1969.

To Mr. James E. Sanders for his invaluable advice and assistance.

To the personnel at Wizard Falls Hatchery, Oregon Game Commission and to Game Commission biologists Jim Griggs and Don King for their assistance in the distribution survey.

To Mr. A. H. Soldner for his technical assistance in the electron microscopy of Ceratomyxa shasta.

To Mr. Tom Kuzma for his help in the placement and collection of liveboxed fish and his technical assistance in spore purification.

To my wife Cheryll and my parents for their continual patience and understanding.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

OCCURRENCE OF THE PROTOZOAN FISH PARASITE *CERATOMYXA SHASTA* IN THE DESCHUTES RIVER BASIN AND THE ULTRASTRUCTURE OF THE SPORE

INTRODUCTION

Ceratomyxa shasta (Noble), a protozoan parasite of fish, has received increased attention since it was first described (Noble, 1950). The organism has been found to be responsible for losses of juvenile hatchery reared rainbow and steelhead trout (*Salmo gairdneri* Richardson), coho salmon (*Oncorhynchus kisutch* Walbaum), and chinook salmon (*Oncorhynchus tshawytscha* Walbaum). Wild juvenile and adult fish have also been infected by this protozoan disease. Other species of trout and salmon appear to be susceptible, but to a lesser degree (Schafer, 1968). These infections have led to extensive examinations of selected waters for the occurrence of the disease, mode of transmission and methods for control. One of the studies, the distribution of *C. shasta* among salmonid fishes in the Columbia River basin of Oregon (Sanders, Fryer and Gould, 1969), has provided the background for one of the topics discussed in this paper.

This thesis is divided into two sections. The first is concerned with the distribution of *C. shasta* in the Deschutes River basin. The second is a description of the spore ultrastructure.

Previous work (Sanders *et al.*, 1969) indicated that the infective stage of *C. shasta* could only be found in the Columbia River as far

inland as the mouth of the Deschutes River. In addition they demonstrated that the lower portion of the Deschutes River also contained the infective stage. The purpose of this study was to identify waters containing the infective stage of C. shasta in the Deschutes system and certain high mountain lakes, thus expanding the previously collected distribution data.

The electron microscope was used to describe the spore ultrastructure of C. shasta in the second section of this paper. Relatively few studies can be found in the literature concerning myxosporidian fine structure. This facet of the study was undertaken to provide some information on the spore organelles. Such knowledge may be useful in understanding the transmission and life cycle of the parasite.

SURVEY OF THE LITERATURE

History of Ceratomyxa shasta

Ceratomyxa shasta was first identified by Noble in 1950 when a myxosporidian parasite caused considerable loss in fingerling rainbow trout (S. gairdneri Richardson) from Crystal Lake Hatchery, Shasta County, California (Noble, 1950). Noble described the trophozoite stage as variable in shape. The sporoblast averaged 12.7 by 19 microns and contained two spores. A small percent of sporoblasts contained four spores. The spore dimensions were found to be six by 14 microns with no striations on the shell valve. A distinct raised suture line was present as well as polar capsules which averaged 1.8 microns in diameter. Sporoplasm appeared to fill the spore and apparently contained two nuclei. Noble found that the parasite was widely dispersed within the various organs of infected fish and presumably caused death.

Wales and Wolf (1955) described three protozoan diseases of trout in California; one of which was C. shasta. They found that fish experienced extensive mortality accompanied by the presence of the protozoan when raised in Crystal Lake water. The disease ceased to be a serious problem when Rock Creek water was utilized in the hatchery.

Wales and Wolf also noted that infected fish were sluggish and tended to "seek quiet, shallow areas of the ponds or raceways." The fish exhibited a much distended visceral cavity. Attempts to transmit the disease from fish to fish proved to be unsuccessful.

After 1955 only isolated reports of C. shasta were found in the literature. Wood and Wallis (1955) reported the occurrence of the disease in adult spring chinook salmon returning to Dexter holding ponds on the middle fork of the Willamette River. This was the first report of infection in that species. Wood (1960) also reported an incidence of the parasite in adult coho salmon. The fish were being held for maturation in the Yakima River. He suggested that the disease may be present only in the Columbia River system in Washington because infections were not observed in the Puget Sound and other coastal areas. Fryer and Conrad (1965) mentioned C. shasta as being one of six infections isolated from one spring chinook salmon at the Willamette River Salmon Hatchery in Oregon.

Conrad and Decew (1966) reported an incidence of C. shasta in juvenile salmonids in Oregon at Bonneville Hatchery on the Columbia River, and at Pelton Hatchery on the Deschutes River. The species affected included spring chinook salmon, coho salmon, and steelhead trout. The spring chinook were found to be the least affected by the epizootic. A description of pathology in the spring chinook pointed out that emaciation was the only recognizable symptom early in the season.

Later, in the fall, fluid filled blebs, necrotic tissue and kidney pustules became apparent. In the infected coho salmon, raised vents and emaciation were very common. The fish contained large amounts of visceral fat, however. In juvenile steelhead (most severely diseased) caudal peduncles were often dark and abdomens distended, fluid filled, and lined with caseous material. Hemorrhagic areas were noted from the vent to the pelvic fins.

In Conrad and Decew's report on C. shasta, it was mentioned that the epizootic was reduced considerably by eliminating water carrying the infective stage and substituting a disease free source. This observation is similar to that described by Wales and Wolf (1955) in Crystal Lake.

W. E. Schafer (1968) published an extensive report on the epizootiology of C. shasta in the area around Crystal Lake Trout Hatchery. He cited some new susceptible species which included brook trout (Salvelinus fontinalis Mitchill) and brown trout (Salmo trutta Linnaeus). Schafer's observations indicated the parasite was infective when the water exceeded 50 ^{o}F; however, temperatures below 50^{o}F did not eliminate an established infection. He also noted that lower temperatures tended to depress the development of the epizootic.

Additional information on the pathology was also included in the paper. At a temperature range of 52-56^{o}F first symptoms (lack of appetite, listlessness, and redistribution to slack water) appeared at

about 24 days. Internal changes occurred at about 20 days which included small whitish, opaque areas on the large intestine. Average time from infection to mortality was 41 days.

Transmission studies showed that infection could occur when fish were exposed for as little as 15 minutes in water carrying the infection. Another experiment pointed out that the infective agent of the disease could pass through an opening of about 44 microns. Schafer found that he could not pass the disease from fish to fish, but could transfer the disease by inoculating schizonts, trophozoites, and spores (collectively) into the visceral cavity of non-infected rainbow trout. It was found that this mode of transmission resulted only in spore production within the fish.

A recent article deals with the occurrence of C. shasta in fish from the Columbia River basin and Oregon coastal streams (Sanders, Fryer, and Gould, 1969). The paper cites three susceptible species to the parasite which have not been previously mentioned: Atlantic salmon (Salmo salar Linnaeus), cutthroat trout (Salmo clarki clarki Richardson), and sockeye salmon (Oncorhynchus nerka Walbaum). Sanders et al. noted other tributaries to the Columbia River that contain the infective stage of C. shasta. These are LaCamas Lake, the Willamette and Cowlitz Rivers. The paper also mentions that the presence of a spore does not necessarily indicate that a water source contains the infective stage. Both Schafer (1968) and Sanders et al. (1969) point out that the infectious stage of the disease might possibly be some stage of the life cycle other than the spore itself.

Ultrastructure of the Myxosporidians

The amount of information published on the structure of C. shasta is limited to light microscope examinations by Noble (1950). Studies employing the use of the electron microscope to probe the ultrastructure are nonexistent. It was found, moreover, that the ultrastructure of the myxosporidians in general has been more or less neglected until the present decade. P. P. Grassé (1960) published his work on the trophozoite of Sphaeromyxa sabrazesi, a myxosporidian which parasitizes the Hippocampus. He described the wall of the trophozoite as being composed of microvillosites and the contents of the trophozoite as being composed of enormous vacuoles. Within the thin streams of cytoplasm between the vacuoles, he found a somatic nucleus, numerous mitochondria, and lipid inclusions. He also described the generative cell within the cytoplasm and suggested that gametes were not formed within the myxosporidian, thus eliminating the possibility that the parasite might be a metazoan. He did, however, consider the parasite more advanced on the evolutionary ladder than the other protozoans.

Cheissen, Schulman, and Vinnitchenko (1961) published the next work on myxosporidian ultrastructure. They used the spores of Myxobolus uniporus and Myxobolus carassii. The valves, polar capsules, and sporoplasm were studied in some detail. Within the sporoplasm were large corpuscles which were devoid of interior

structure. The authors have suggested that these may be mitochondria, but indicated more work was necessary to establish this hypothesis.

Cheissen et al. (1961) worked primarily with morphology and capsule content. They showed it to have a wall consisting of a two-layered osmiphilic membrane on the outside, and a weaker osmiphilic layer underneath. They also pointed out a cap or stopper apparatus on the polar capsule. Within the polar capsule was found the polar filament. They suggested that the filament consisted of two parts, each tightly coiled around the other. Lom and Vávra (1963), in a paper published on the fine morphology of the spores of Microsporidia, pointed out that in preliminary observations with Henneguya psorospermica (a myxosporidian), the polar filament consisted of a single strand twisted into spiral folds within the polar capsule. This observation is generally held to be correct.

Lom and Vávra (1965) have described the development of polar capsules. They compared the polar capsule to the nematocyst of Hydra. The developmental similarities led them to believe that the myxosporidians were really metazoans, as suggested by Grell (1956) and Gottschalk (1958). That same year, Lom again published on the extrusion of the polar filaments and other features of myxosporidian spores (Lom, 1964). The ultrastructure presented in the paper was predominately extruded filaments and spore valves of Myxobolus. Lom

and de Puytorac (1965a) presented some of their observations on the ultrastructure of myxosporidian trophozoites. They used plasmodes of Henneguya psorospermica, Myxobolus sp., Zschokella nova, and Myxidium lieberkuhi. Their results showed only slight variations in trophozoite ultrastructure among the myxosporidians. Lom and Vávra (1965) also published a paper on the development of the polar filament in Henneguya.

The developmental stages of myxosporidians have been the main topic of study with the electron microscope and have led to most of our knowledge of their ultrastructure. Lom and de Puytorac (1965b) presented a collection of data on myxosporidian ultrastructure and polar capsule development. In their explanation of the detailed life cycle of the parasite, they indicated several interesting structures. Among those found in different myxosporidian trophozoites were smooth, thin, limiting membranes or bristled membranes with villosites. Others were pinocytotic vacuoles, mitochondria of usual structure, dictyosomes, endoplasmic reticulum, poorly defined vacuoles, various inclusions, and myelinic formations. They also show by electron micrographs that the development of the spores is by valvogenic cells, capsulogenic cells, and sporoplasmogenic cells of sporoblasts, an observation based on light microscope studies by previous authors.

Lom and de Puytorac also mention that the mature spores of myxosporidians are generally so ill preserved by fixation that detailed

study is difficult. It was noted by this author that work has been done predominantly on trophozoites and sporoblasts, but very little on the mature spore itself.

Schubert (1968) expanded the work done by Lom and his co-workers on Henneguya using Henneguya pinnae. He followed the development very closely from the trophozoite stage through the mature spore. Detailed micrographs and descriptions indicated the position of the plasmode in the host and the position of germative cells in the plasmode. The germative cells appear to divide to form a two-cell stage. One cell then surrounds the other. This enclosed cell divides repeatedly to form a multicellular stage. These cells then differentiate to form the components of the spore.

MATERIALS AND METHODS

Distribution Studies in the Deschutes Basin

Before and during this study, attempts to transmit C. shasta within the laboratory by previous investigators (including those in our group) proved to be unsuccessful. As yet, the mode of transmission is unknown. For this reason, the choice of experimental method was limited. The only procedure for inducing the disease was to expose a susceptible animal directly to a water supply carrying the disease. On-site field testing was therefore necessary to determine the infectability of a particular point in the Deschutes River basin.

Testing Procedures

Juvenile rainbow trout (S. gairdneri Richardson) from Wizard Falls and Fall River Hatcheries (OGC)[1] were used to determine the distribution of the parasite. The two hatcheries were chosen because of their location within the test area. The average size of the fish used in the study ranged between 75 and 200 per pound during the exposure periods.

To hold the fish during experimentation, liveboxes were constructed. The cylindrical shaped liveboxes measured 16 inches in

[1] Oregon Game Commission.

diameter and 24 inches in length. They consisted of a stainless steel frame covered with one-fourth inch mesh aluminum screen. Riveted to half of one end of the livebox was a round stainless steel plate hinged in the middle and used as a door. This door was held fast to the frame by a removable thumb screw.

The liveboxes were placed at selected sites within the test area. At certain intervals, the fish were examined to observe their progress and to remove mortalities. The animals were not fed upon being transferred to the test site but did obtain natural food to some extent. Cannibalism of mortalities was often present. At the end of an exposure period, usually around 30 days, the liveboxes were removed and the fish examined. The exact length of exposure was determined by the condition of the fish when they were examined at the site. If the number of mortalities was low and the fish did not appear debilitated, the livebox was usually left for a longer interval of time. The minimum exposure period was 14 days--the earliest length of time that spores have been diagnosed in diseased fish (Sanders et al., 1969).

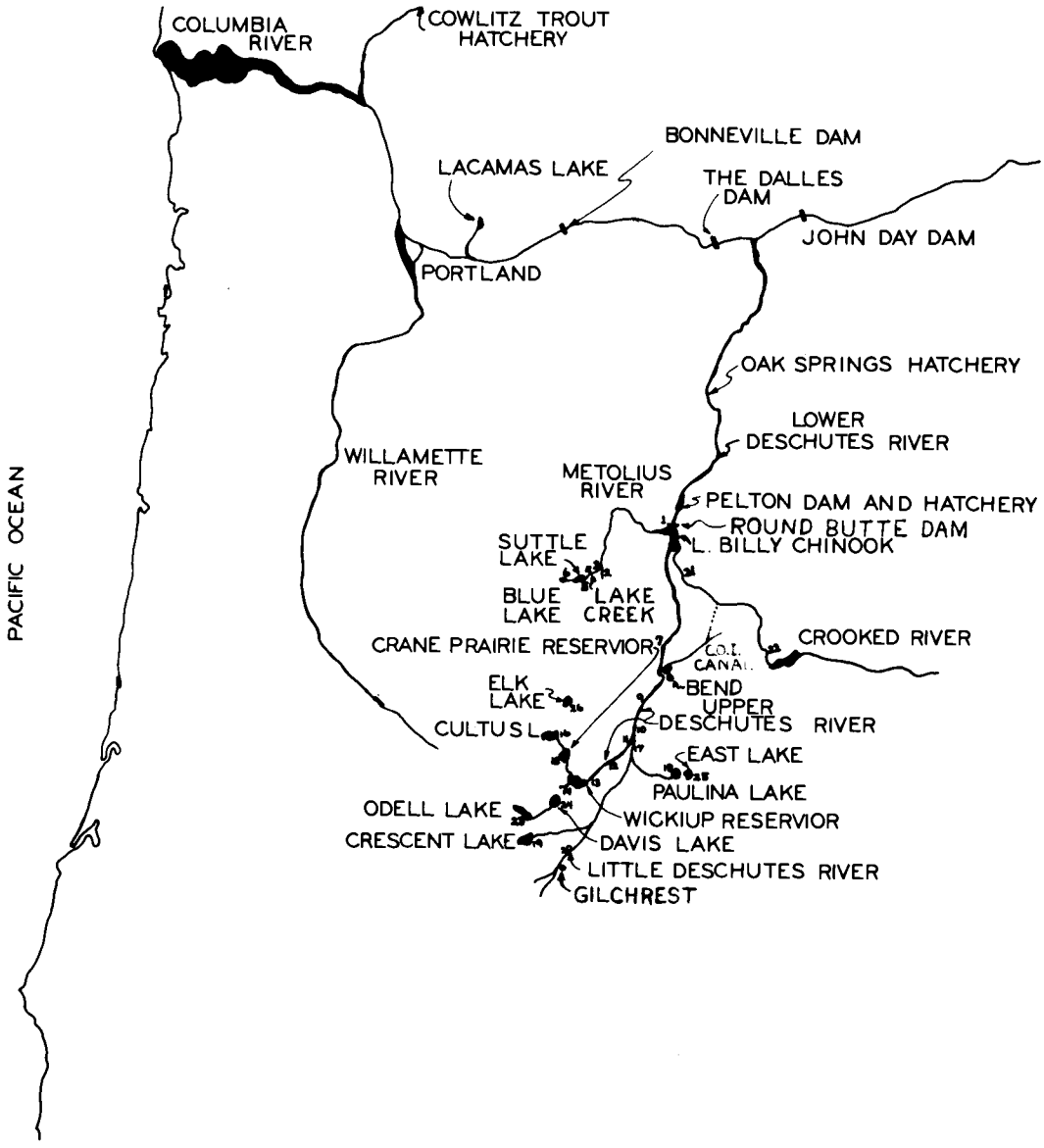
Test Area

Exposure sites for the distribution experiment (Figure 1) were limited to two areas. The first included the Deschutes River and most of the major tributaries which make up the Deschutes basin. The

Figure 1. Map of sites where fish were held in liveboxes to determine the occurrence of the infective stage of Ceratomyxa shasta.

Numbered Locations of Livebox Sites

1. Round Butte Dam
2. Metolius above Lake Creek
3. Lake Creek at the mouth
4. Lake Creek at Lake Creek Lodge
5. Suttle Lake
6. Blue Lake
7. Tumalo
8. North Canal Dam (Mirror Pond)
9. Lava Island Falls
10. Harper Bridge
11. Above the confluence of the Little Deschutes River
12. Pringle Falls
13. Below Wickiup Dam
14. Davis Creek
15. Crane Prairie
16. Big Cultus
17. Lower Little Deschutes River
18. Paulina Lake
19. Crescent Lake
20. Little Deschutes at Gilchrest
21. Lower Crooked River
22. Upper Crooked River below Prineville Dam
23. Odell Lake
24. Davis Lake
25. East Lake
26. Elk Lake


COLUMBIA RIVER
COWLITZ TROUT HATCHERY
BONNEVILLE DAM
LACAMAS LAKE
THE DALLES DAM
JOHN DAY DAM
PORTLAND
OAK SPRINGS HATCHERY
LOWER DESCHUTES RIVER
WILLAMETTE RIVER
METOLIUS RIVER
PELTON DAM AND HATCHERY
ROUND BUTTE DAM
L. BILLY CHINOOK
SUTTLE LAKE
BLUE LAKE
LAKE CREEK
CRANE PRAIRIE RESERVIOR
C.O.I. CANAL
CROOKED RIVER
ELK LAKE
BEND
UPPER DESCHUTES RIVER
CULTUS L.
EAST LAKE
PAULINA LAKE
ODELL LAKE
WICKIUP RESERVIOR
CRESCENT LAKE
DAVIS LAKE
LITTLE DESCHUTES RIVER
GILCHREST
PACIFIC OCEAN

second included some of the larger high mountain lakes in the central Oregon region which do not flow directly into the Deschutes River.

The lower Deschutes is composed of water from three major tributaries: the Metolius River, the upper Deschutes River, and the Crooked River. All three meet at Lake Billy Chinook which is formed by Round Butte Dam (Figure 1).

The Metolius River

The Metolius River arises from subterranian springs one mile above Camp Sherman, Oregon and flows directly to Lake Billy Chinook. One of the major tributaries of the Metolius, Lake Creek, originates from Suttle Lake. Suttle Lake, in turn, is fed by Link Creek which flows from Blue Lake (Figure 1).

The Upper Deschutes River

The upper Deschutes River has its origin at Little Lava Lake and flows to Crane Prairie Reservoir. From there, the river flows to Wickiup Reservoir and then to Lake Billy Chinook. Major tributaries of the upper Deschutes River include the Cultus River, which flows from Cultus Lake to Crane Prairie Reservoir, Davis Creek which enters Wickiup Reservoir from a lava flow separating Wickiup Reservoir and Davis Lake (Figure 2), and the Little Deschutes River, which enters below Wickiup Reservoir. The Little Deschutes River

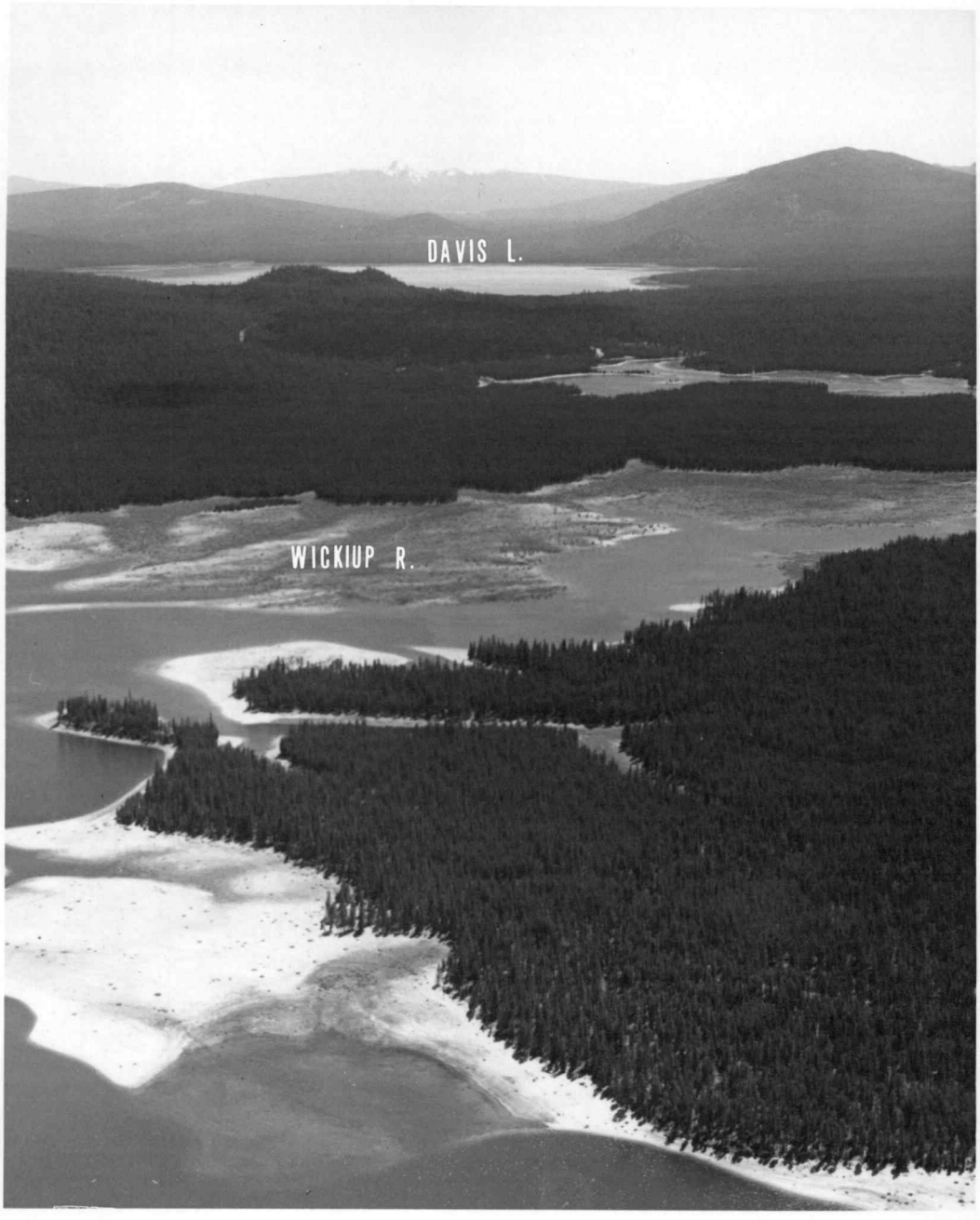


Figure 2. Aerial photograph showing the lava flow which separates Davis Lake and Wickiup Reservoir.

has tributaries which include Crescent Creek, from Crescent Lake and Paulina Creek, from Paulina Lake.

The Crooked River

The Crooked River flows from the Ochoco Mountains to Prineville Reservoir. From there it enters into Lake Billy Chinook and the Deschutes River.

Bodies of Water not Connected to the Deschutes River

Bodies of water tested but not part of the Deschutes watershed were East, Davis, Odell, and Elk Lakes. Odell and Davis Lakes are connected by Odell Creek and may constitute a closed system. The large lava flow separating Davis Lake and Wickiup Reservoir (Figure 2), allows no direct water exchange as shown by flourescent tracer methods (O. S. G. C., 1961, 184-185). This finding is subject to question, however.

Examination of Fish for Ceratomyxa shasta

The method for examination of liveboxed fish was similar to that used by Sanders in his work concerning the occurrence of C. shasta among salmonids in Oregon waters (Sanders, 1967). Smears from the intestine and occasionally the gall bladder were air dried and stained by the Ziehl-Neelsen method for acid-fast bacteria (Soc. American

Bacteriologists, 1957). The first step in the staining procedure was slightly modified by omitting the heating of the carbol fuchsin.

Examination of the smears with a light microscope revealed blue, kidney shaped spores with red polar capsules (440X). As in Sander's work, the blue sporoplasm of the spore was sometimes difficult to see, depending on the thickness of the smear. The presence of the polar capsules, however, was enough criteria to diagnose the presence of a spore (440X and 970X).

Ultrastructure of the Spore

Purification of Spore Samples

The spores used to determine the ultrastructure of C. shasta were obtained from adult coho salmon from the Bonneville Salmon Hatchery. The intestinal tracts were removed during spawning operations and stored at 4°C.

The intestinal contents were removed and examined. Those which contained a large number of spores were pooled for processing.

The sample was initially centrifuged at 300 rpm to remove the larger debris. The supernatant was filtered through a Swinney filter (Gelman Hypodermic Adapter) which contained only the wire pad (70 micron pore diameter). This suspension was diluted to 200 ml with distilled water and centrifuged at 1000 rpm for five minutes. The

washing process was repeated four times yielding a pellet[2] with sufficient spores for electron microscope examination.

Fixation and Embedding[3]

The purified samples were placed in 10% formalin for one month. The formalin was decanted and the sample washed three times by suspension and centrifugation in cold monobasic-dibasic sodium phosphate buffer (0.125M, pH 7.1). The material was left in suspension approximately five minutes between centrifugations. The spore pellet was then resuspended in a 2% osmium tetroxide solution in phosphate buffer for 24 hours at 4°C. This sample was centrifuged to a pellet, resuspended for five minutes in the cold buffer, and again centrifuged.

The pellet was embedded in 2% Ionagar, diced to small chunks, and dehydrated as follows:

30% ETOH	1 change for 10 minutes
50% ETOH	1 change for 10 minutes
70% ETOH	1 change for 10 minutes
90% ETOH	1 change for 10 minutes
100% ETOH	2 changes for 10 minutes each
100% ETOH	2 changes for 10 minutes each

The pellet was infiltrated with plastic mixture by adding the required volume of benzyl dimethylamine (BDMA) catalyst to a third portion

---

[2]The spores of C. shasta are of much greater density than the intestinal debris. They can therefore be separated quite easily.

[3]A contracted study with A. H. Soldner, Electron Microscopy Service, Oregon State University.

of anhydrous propylene oxide and adding liquified plastic a few milliliters at a time. This process took place over a 24 hour period. The sample was then transferred to embedding capsules filled with properly catalized plastic mixture and cured for 72 hours at 60°C. The plastic mixture consisted of:

Araldite 6005	28%
Epon 812	8%
Dodecenyl succinic anhydride (DDSA)-hardner	64%
Benzyl dimethylamine (BDMA)-catalyst	1 drop/ml plastic

Ultramicrotomy and Microscopy

Silver-grey sections were cut with a diamond knife on a Porter-Blum MT-2 ultramicrotome and placed on Formvar coated copper grids. Sections were stained with 1% aqueous uranyl acetate for 20 minutes. The sample was again stained with 1% aqueous neutralized phosphotungstic acid for 20 minutes. Completed sections were examined with a Phillips E. M. 300 electron microscope at 80 KV.

RESULTS

Distribution Studies in the Deschutes Basin

Sanders et al. (1969) demonstrated that the lower Deschutes River contained the infective stage of C. shasta. In addition, the infective stage was shown to be present in Pelton Reservoir just below the Pelton Regulating Dam by Conrad and Decew (1966). There was no evidence of the disease in the livebox placed above Pelton Reservoir in Lake Billy Chinook. Scuba diving operations, however, did reveal the presence of rainbow trout mortalities containing spores of C. shasta on the bottom of the Metolius and Upper Deschutes arms of the reservoir.

The Metolius River

Fish were held in the Metolius branch of the Deschutes River (Figure 1). Fish placed just above the mouth of Lake Creek in Metolius water and fish placed in the mouth of Lake Creek yielded no spores on microscopic examination (Table 1). Water temperatures ranged between 48 and 52°F. A livebox placed further upstream on Lake Creek at Lake Creek Lodge also contained uninfected fish after 31 days exposure (Table 1). The livebox was located in backwater that approached temperatures of 65°F. Mortalities occurred in fish

Table 1. Incidence of the infective stage of Ceratomyxa shasta in Lake Billy Chinook and the Metolius branch of the Deschutes River.

Location of livebox (Figure 1)	Number of fish exposed	Date livebox was set	Temp. °F	Days exposed	Fish Recovered		No. infected	Percent Infected[2]
					Live	Mort		
Round Butte Dam(1)[1]	35	8- 9-68	60°F	35	18	0	0	0
Metolius above Lake Creek(2)	35	6-18-68	53°F	62	34	0	0	0
Lake Creek at the mouth(3)	35	6-18-68	52°F	62	34	1	0	0
Lake Creek at Lake Creek Lodge(4)	40	6-19-69	63°F	35	23	1	0	0
Suttle Lake(5)	35	7-24-68	68°F	25	1	20	20	95
Blue Lake(6)	35	6-18-69	59°F	62	2	4	0	0
Blue Lake	35	8-22-68	57°F	33	30	0	0	0

[1] Numbers correspond to Figure 1.

[2] Percent of recovered fish (live + mortalities) infected with C. shasta.

held in Suttle Lake, the source of Lake Creek (Table 1). Spores of C. shasta were found in 95% of the fish. In Blue Lake, located just above Suttle Lake, two livebox settings yielded no evidence of the disease (Table 1). Water temperatures during both experiments were above the 50 to 52°F which approaches the lower temperature limit for the occurrence of the disease (Schafer, 1968).

The Upper Deschutes River

Liveboxes placed above the confluence of the Little Deschutes River yielded uninfected fish (Figure 1, Table 2). Below the confluence of the Little Deschutes River, fish placed at Harper Bridge and Lava Island Falls were uninfected (Table 2). Ten miles below Lava Island Falls, however, at Mirror Pond near Bend infected fish were diagnosed. Two test periods, June of 1968 and July of 1969, produced incidences of 16% and 30%, respectively (Table 2). Fish placed in Mirror Pond in August of 1968 were uninfected. Below Mirror Pond at Tumalo, an incidence of 64% was found (Table 2). Temperatures in the above exposures ranged from 58 to 60°F.

Fish recovered from the lower portion of the Little Deschutes River (Figure 1) in July of 1968 and 1969 were not infected with C. shasta. Water temperatures were well above 50°F in both cases. In August of 1968 a 35% incidence was found (Table 3). The water temperature was approximately 61°F during the exposure period. A

Table 2. Incidence of the infective stage of Ceratomyxa shasta in the Upper Deschutes branch of the Deschutes River

Location of livebox (Figure 1)	Number of fish exposed	Date livebox was set	Temp. °F	Days exposed	Fish Recovered		No. infected	Percent infected[2]
					Live	Mort		
Tumalo(7)[1]	35	5-18-68	65°F	33	4	10	9	64
North Canal Dam(8)	35	5-18-68	55°F	33	19	12	5	16
North Canal Dam	35	8-22-68	57°F	33	0	0	0	0
North Canal Dam	40	6-19-69	63°F	35	13	0	4	30
Lava Island Falls(9)	35	8-22-68	--	33	26	7	0	0
Harper Bridge(10)	38	8-23-68	59°F	32	30	6	0	0
Above the confluence of the Little Deschutes(11)	35	7-26-68	60°F	14	19	0	0	0
Above the confluence of the Little Deschutes	38	8-23-68	63°F	32	37	0	0	0
Pringle Falls(12)	35	8-23-68	58°F	32	33	0	0	0
Below Wickiup Dam(13)	38	8-23-68	48°F	32	26	10	0	0
Davis Creek(14)	35	7-20-68	55°F	30	35	0	0	0
Crane Prairie(15)	35	8- 8-68	75°F	24	13	0	0	0
Big Cultus(16)	35	6-15-68	--	71	14	1	0	0

[1] Numbers correspond to Figure 1.
[2] Percent of recovered fish (live + mortalities) infected with C. shasta.

Table 3. Incidence of the infective stage of Ceratomyxa shasta in the Little Deschutes River and some of its tributaries.

Location of livebox (Figure 1)	Number of fish exposed	Date livebox was set	Temp°F	Days exposed	Fish Recovered		No. Infected	Percent Infected[4]
					Live	Mort		
Lower Little Deschutes(17)[1]	35	7-14-68	72°F	23	15	5	0	0
Lower Little Deschutes	35	8-23-68	61°F	32	16	9	9	36
Lower Little Deschutes	10[2]	6-30-68	66°F	25	0	4	0	0
Paulina Lake(18)	200[2]	June, 1968	62°F	90	25[3]	0	0	0
Crescent Lake(19)	35	7- 7-68	68°F	29	15	6	1	5
Crescent Lake	75	7-19-69	65°F	25	3	24	13	48
Little Deschutes at Gilchrest(20)	10[2]	6-30-69	60°F	25	0	5	0	0

[1] Numbers correspond to Figure 1.

[2] These sites were tested by personnel of the Oregon Game Commission. The fish were examined in our laboratory.

[3] A sample portion of the surviving fish.

[4] Percent of recovered fish (live + mortalities) infected with C. shasta.

livebox placed further upriver on the Little Deschutes in the Gilchrest area revealed no infected fish (Table 3). Tributaries to the Little Deschutes River, Crescent Creek and Paulina Creek, produced opposite results (Table 3). Fish placed in Crescent Lake contracted the disease with a 5% incidence in July of 1968. A second group exposed in June of 1969 had a 48% incidence. Water temperature was between 62 and 68°F during both tests. Fish exposed in Paulina Lake were uninfected with C. shasta.

The Crooked River

Results obtained from the Crooked River differed within a short distance along its route with no apparent change in river environment. Fish held for 21 days in the upper Crooked River above Prineville in June of 1969 were found to be uninfected (Table 4). Water temperature was about 60°F. In the lower Crooked River at Opal Springs, fish did not seem to contract the disease within a 30 day period. Subsequently, the livebox was left for an additional ten days and yielded a 13% incidence (Table 4).

Bodies of Water not Connected to the Deschutes River

Odell and Davis Lakes were found to contain the infective stage of C. shasta (Figure 1, Table 4). In August of 1968 an incidence of 85% was diagnosed in fish held in Odell Lake for 24 days. The water

Table 4. Incidence of the infective stage of Ceratomyxa shasta in the Crooked River branch of the Deschutes River and in some lakes in the Deschutes basin not flowing directly to the Deschutes River.

Location of livebox (Figure 1)	Number of fish exposed	Date livebox was set	Temp. °F	Days exposed	Fish Recovered		No. Infected	Percent Infected[2]
					Live	Mort		
Lower Crooked River(21)[1]	35	8-10-68	--	40	24	8	4	13
Upper Crooked River below Prineville Dam(22)	40	6-20-68	64°F	20	9	0	0	0
Odell Lake(23)	35	8- 7-68	67°F	24	10	16	22	85
Davis Lake(24)	35	8-24-68	61°F	31	3	6	1	11
East Lake(25)	200	June, 1968	62°F	90	9	1	0	0
Elk Lake(26)	35	8-24-68	61°F	31	20	0	0	0

[1] Numbers correspond to Figure 1.

[2] Percent of recovered fish (live + mortalities) infected with C. shasta.

temperature averaged about 67°F. Odell Creek, which feeds Davis Lake, had been shown to contain the infective stage of the disease by our research group in 1967. At that time Davis Lake was also shown to contain the protozoan. An 11% incidence of C. shasta was found in fish from one livebox during this study (Table 4). Holding fish was difficult because water temperatures in Davis Lake are usually around 75°F during the summer; but when these fish were recovered in August of 1968, the temperature was 61°F.

East and Elk Lakes were the last two sites tested for the infective stage of C. shasta. None of the fish recovered from these waters were infected (Table 4).

Ultrastructure of the Spore

Noble (1950) described light microscopic structure of C. shasta when he first identified the organism. Sanders (1967) found that spores from Oregon fish were almost identical to those described by Noble. An unstained spore is pictured in Figure 3.

Spore Valves

Figure 4 is a transverse section through the middle of a C. shasta spore, below the polar capsules. As in other myxosporidians, the two valves form knobs where they come together at the suture line. These knobs range in diameter between 0.3 to 0.4 μ. The remainder

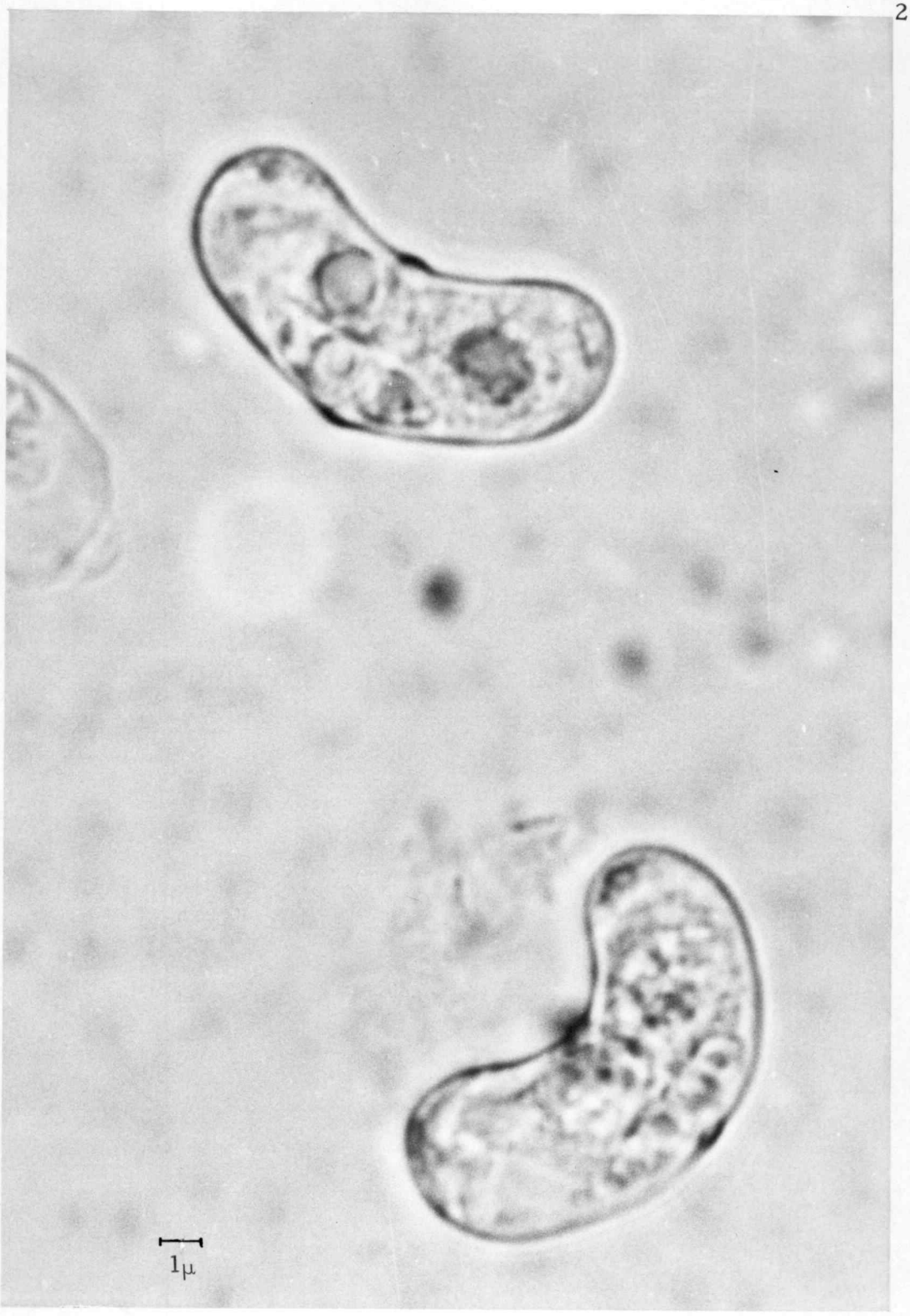
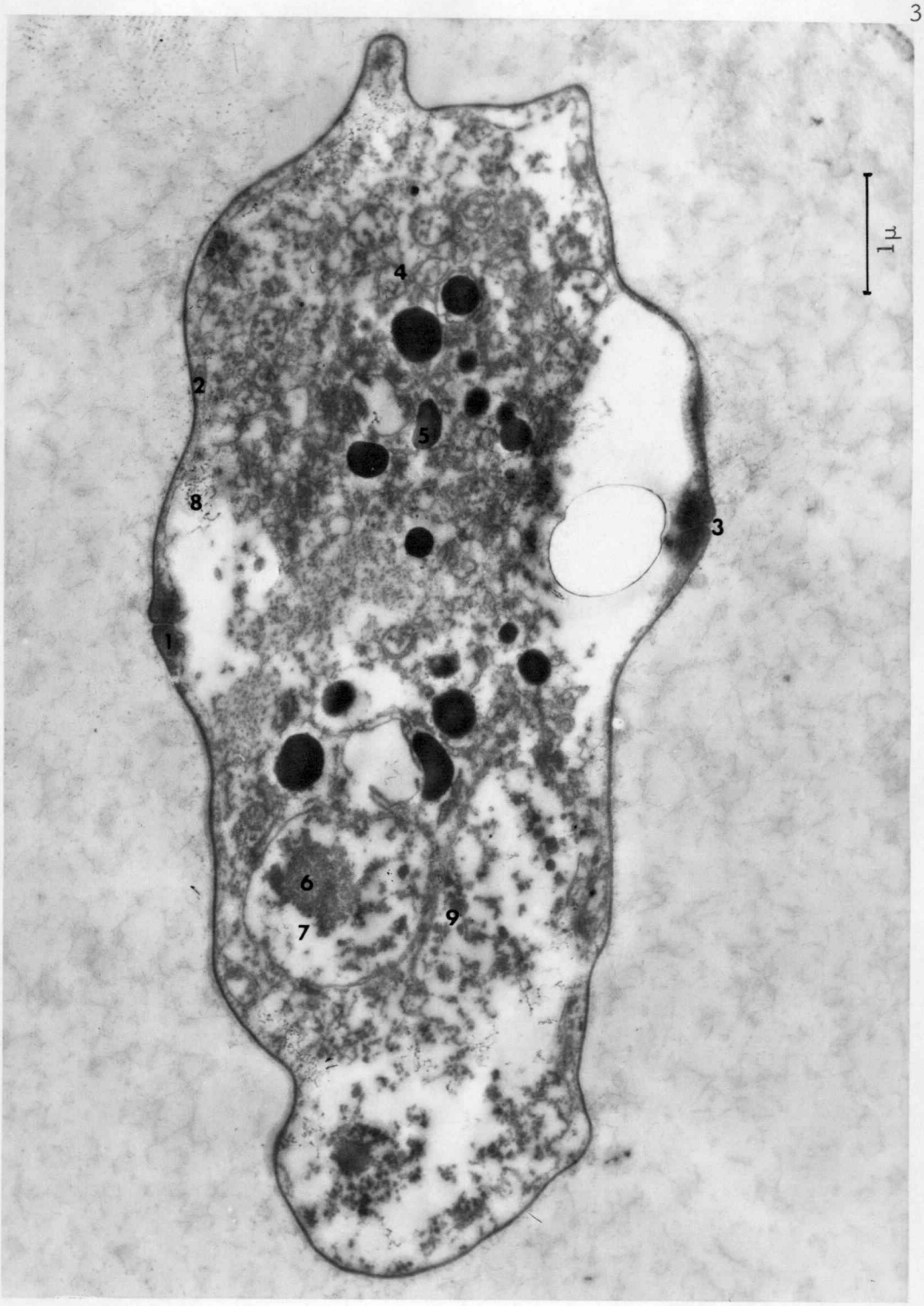


Figure 3. Photograph of an unstained spore of Ceratomyxa shasta (6,500X).

Figure 4. Transverse section of Ceratomyxa shasta below the polar capsules (17,700X).

1. Knob of the valve
2. Valve
3. Suture line
4. Endoplasmic reticulum
5. Electron dense body
6. Nucleolus
7. Nucleus
8. Ribosome packet
9. Mitochondria


1μ
1
2
3
4
5
6
7
8
9

of the valve varies in width with the widest portion of the end of the spore. The valve has a granular appearance with evidence of some vacuolation and indistinguishable structure. The irregular valve shape is probably an artifact of fixation. The valves consist of three layers (Figures 4 and 7), the outer layer is more electron dense than the inner, even though they both appear to consist of the valvogenic cell wall. The outer layer is about 20 mμ thick while the inner layer is slightly thinner. These dimensions could have been altered by the process of fixation. Between the inner and outer layer of the valve is a granular matrix.

The two valves at the suture line are connected by a septate desmosome (Figure 8). The separation of the two valves is about 400 Å. The septate lamellae are about 80 Å apart. This particular structure was seen in two electron micrographs which clearly showed the suture line. It is interesting to note that in both cases there were exactly 15 lamellae present.

Sporoplasm of Ceratomyxa shasta

In transverse and cross sections, the endoplasmic reticulum (endoplasm) surrounded by ectoplasm can be recognized (Figures 4, 5, and 6). Although vesicles were evident, the endoplasmic reticulum appeared very granular. The endoplasm may be separated into two separate areas (Figure 4) divided on each side of the suture line by a uniformly granular area.

Figure 5. Cross section of spore of Ceratomyxa shasta through the two nuclei in the sporoplasm (30,000X).

1. Vesicular nucleus
2. Nucleolus
3. Double membrane of nuclei
4. Mitochondria
5. Valve
6. Inner layer of valve
7. Matrix of valve
8. Outer layer of valve
9. Endoplasmic reticulum
10. Electron dense body
11. Nucleopore

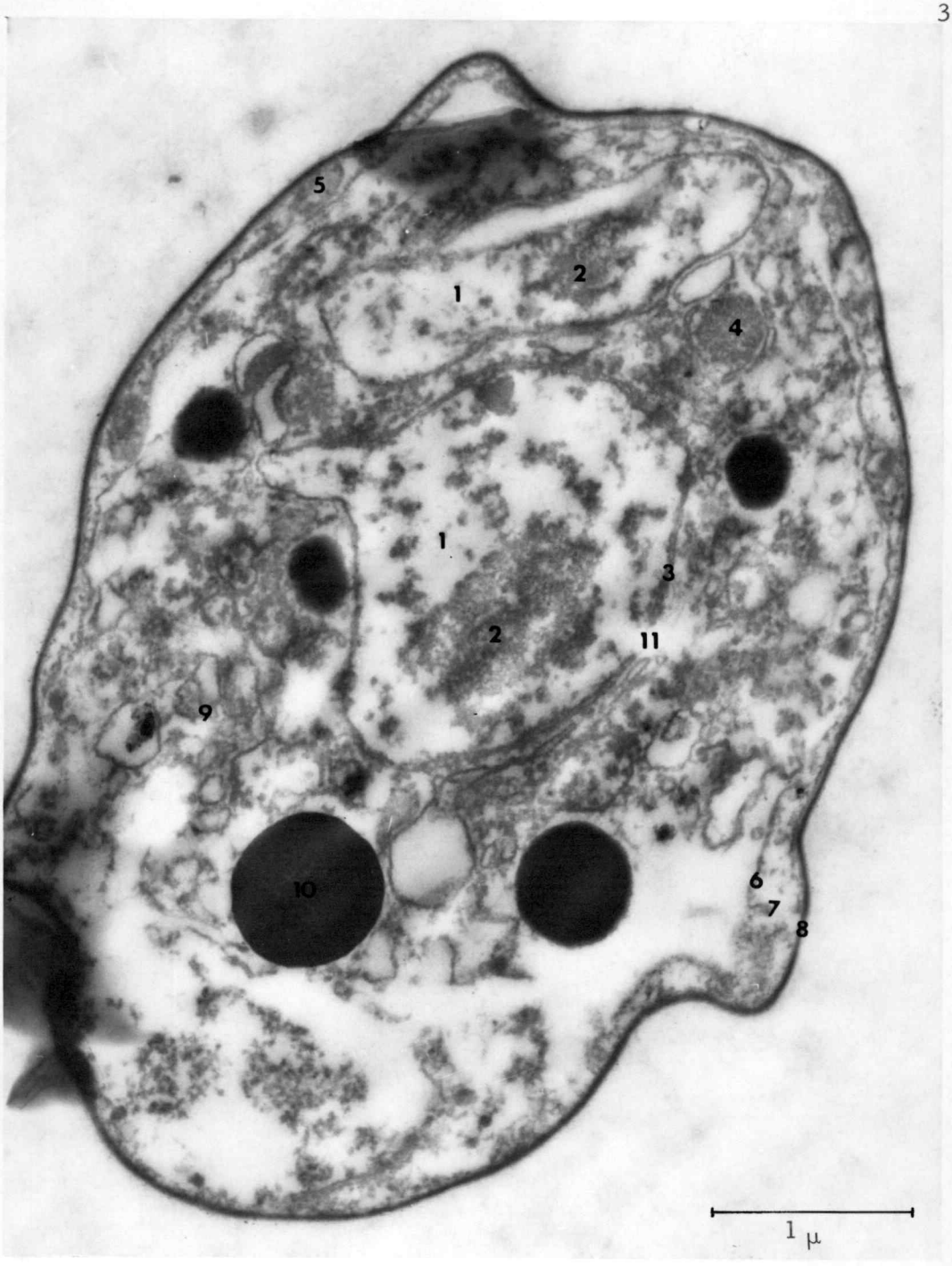

5
1
2
4
1
3
2
11
9
10
6
7
8
1 μ

Figure 6. Transverse oblique section of spore of Ceratomyxa shasta through one end of the spore and the polar capsules (23,600X).

1. Valve
2. Ribosome packet
3. Mylin-like structure
4. Electron dense body[1]
5. Suture line
6. Knob of valve
7. Degenerated capsulogenic cell
8. Compressed cellular material
9. Granular layer surrounding the polar capsule
10. Polar capsule wall
11. Granular band surrounding the polar filament
12. Polar filament
13. Stopper or cap

[1] Chatter marks are probably from insufficient penetration of the hardner or plastic mixture.

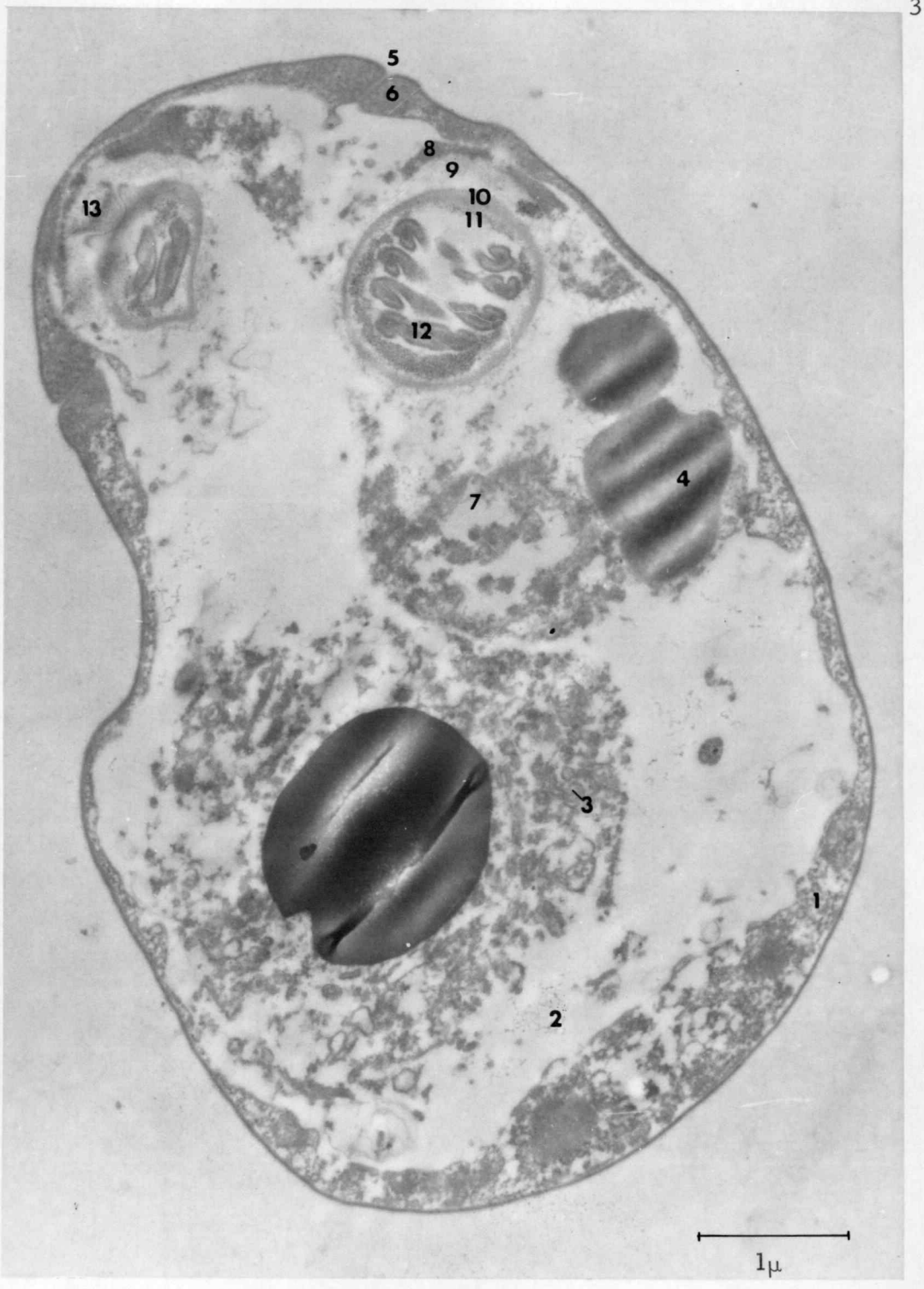
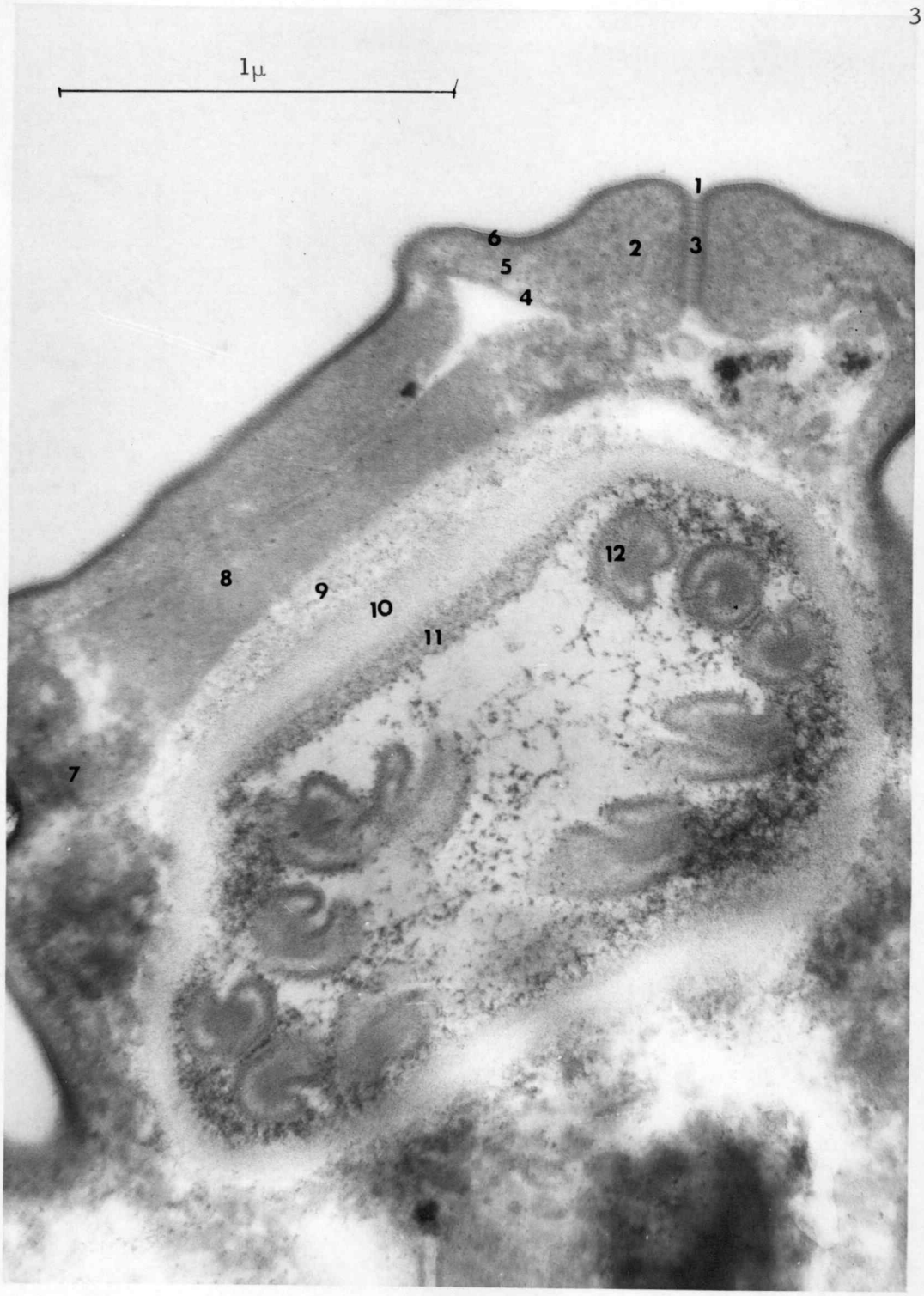

5
6
8
9
10
11
13
12
4
7
3
1
2
1μ

Figure 7. Polar capsule and suture line of spore of Ceratomyxa shasta (61,000X)

1. Suture line
2. Knob of valve
3. Septate desmosome
4. Inner layer of valve
5. Matrix of valve
6. Outer layer of valve
7. Cellular material of capsulogenic cell
8. Compressed cellular material
9. Granular layer surrounding the polar capsule
10. Polar capsule wall
11. Granular band surrounding the polar filament
12. Polar filament


1μ
1
2
3
4
5
6
7
8
9
10
11
12

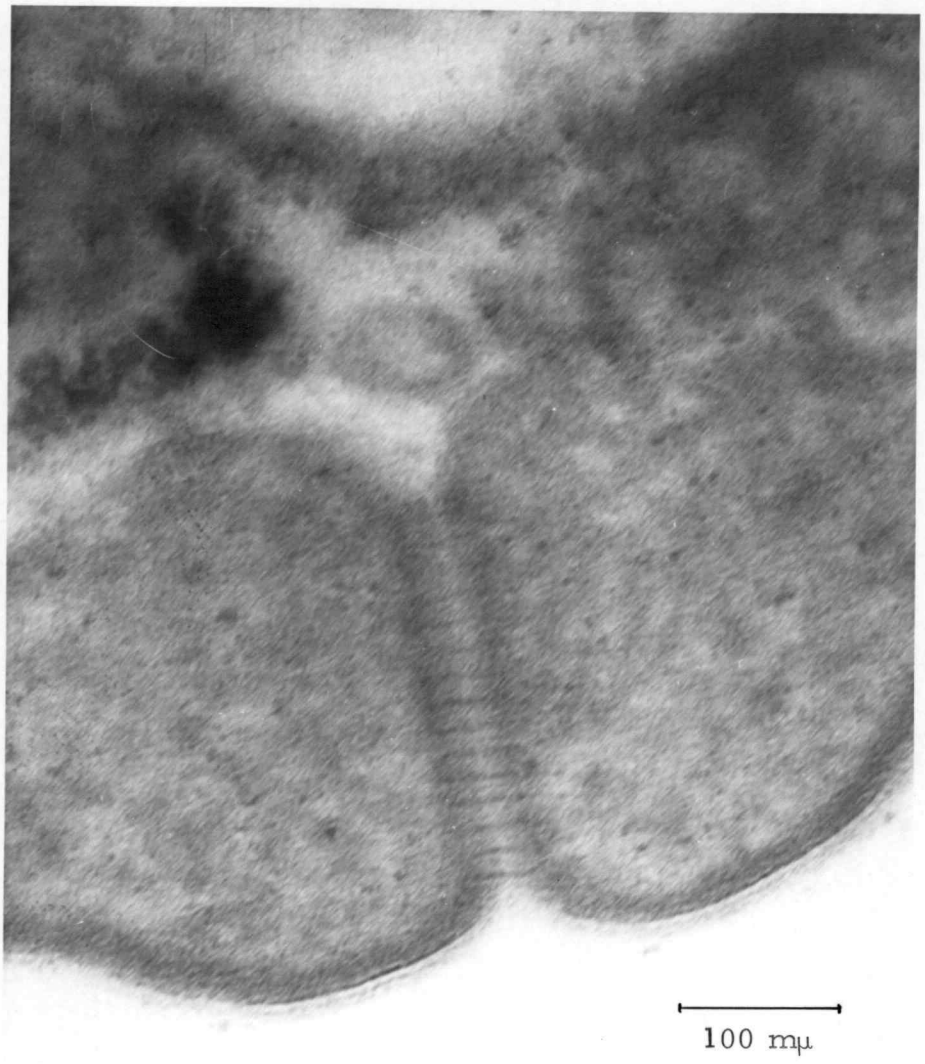


Figure 8. High mag of septate desmosome at the suture line (213,500X).

At different sites within the sporoplasm electron dense bodies of various shapes and sizes can be seen. These osmiphilic structures appear throughout the spore, but are most concentrated in the nuclear regions. Also within the sporoplasm are small corpuscular bodies appearing as part of the granular endoplasmic reticulum (Figures 4 and 5). They may be Golgi bodies. A peculiar structure which may be a mitochondrion can be seen in Figure 4 (no. 4). They are not numerous within the sporoplasm. Spiraled structures (Figure 6) which may be part of the vesicular endoplasmic reticulum, could also be myelin figures.

Very prominent within the sporoplasm is the large vesicular nucleus (Figures 3 and 4). The nuclear membrane appears to be double with large nucleopores (Figure 5). The nuclear membrane is about 20 mμ thick. The cross section through the sporoplasm indicates that the sporoplasm may contain two nuclei (Figure 5). Both appear to be bounded by a double membrane. A nucleolus surrounded by chromatin was found within each nucleus (Figure 5).

The Polar Capsule

The polar capsule of C. shasta lies within an area which is probably a degenerating capsulogenic cell (Figures 6 and 7). The capsule appears to compress the degenerating contents of the capsulogenic cell where it lies proximal to the suture line (Figure 7).

This compression might occur as the polar capsule develops to full size and maturity. Between the degenerated capsulogenic cell contents and the polar capsule wall lies a slightly granular zone (Figure 7) which is more pronounced next to the compressed area. This granulation completely surrounds the polar capsule.

The wall of the polar capsule varies in thickness from 50 to 125 mμ. At one point a stopper or cap (Cheissen et al., 1961 and Lom, 1964) can be seen lying within the wall itself (Figure 6). At this stage of spore maturation, the wall of the capsule is unbroken beneath this stopper-like structure. The polar capsule wall is composed of finer, more compact granules than the surrounding layers.

A band of electron dense granules which surround the polar filament is found within the polar capsule (Figures 6 and 7). The polar filaments appear to be situated within the polar capsule as a twisted band wound within a ball as suggested by Lom and Vávra (1963) in Henneguya psorospermica. The outer layer of the band is more electron dense than the center.

DISCUSSION

Distribution Studies in the Deschutes Basin

Ceratomyxa shasta has been extensively studied with respect to occurrence of diseased fish, especially returning adult salmon. Sanders (1967) demonstrated that infected fish could be found as far inland as Oxbow Hatchery on the Snake River (Idaho Fish and Game). Fish exposed in the Columbia River above the Deschutes River, however, were not infected by the parasite. The Deschutes, therefore, could be the most inland source of infection for returning anadromous and resident salmonids. Tests in the lower Deschutes indicated the existence of the infective stage of C. shasta (Sanders et al., 1969).

C. shasta is present in each of the three main tributaries to the lower Deschutes River. Suttle Lake on the Metolius branch is a principle source of water containing the infectious agent. It is likely that the infective stage is carried down Lake Creek into the Metolius, and from the Metolius into Lake Billy Chinook. The source of infection in the upper Deschutes River appears to be Crescent Lake. The infective stage is carried down Crescent Creek to the Little Deschutes River. The Little Deschutes then enters the upper Deschutes River above Harper Bridge. The upper Deschutes appears to be free of the infective stage of the disease above the mouth of the Little Deschutes River. Infected fish were recovered from the

Crooked River at Opal Springs which enters one mile above Lake Billy Chinook. The source of this infection is probably overflow irrigation canals from the upper Deschutes River. These canals enter the Crooked River about five miles above Opal Springs. The last source of the infection may be the Odell Lake-Davis Lake complex. They appear to form a closed system with subterranian outflow. The drainage area has remained undetected even though tests have been made with flourescent dye techniques. It appears that some water drains into the lava fields separating Davis Lake and Wickiup Reservoir, but none appears to enter the Davis Creek arm of Wickiup Reservoir at any detectable level. If water does enter Wickiup Reservoir and the flourescent dye was filtered out, it is probable that the infective stage of C. shasta is also removed.

To evaluate the data from this experiment, it must be understood that constructive, valid results are obtained only when a site yields infected fish. The necessary inexactness of the testing procedure makes it possible to draw only the broadest of conclusions. Along the proposed route of infective water, some groups of fish did not become infected. The uninfected fish removed from the mouth of Lake Creek and the Metolius River can probably be best explained by the low water temperatures (50 to 52°F). Below the mouth of the Little Deschutes, dilution of the infective stage may have been instrumental in surpressing the appearance of the disease. Seasonal

occurrence of the infective state (seen at Mirror Pond and in the Little Deschutes), water depth, type of river bottom, and many other possible factors could have been important in the results of a test.

Ultrastructure of the Spore

The ultrastructure of C. shasta appears to be similar to that observed in other myxosporidians. It was found, however, that difficulties in fixation and embedding have made comparisons difficult. The problem was first apparent when spores with a jelly-like consistency were found in thin sections. Many had the sporoplasm completely torn away. Attempts to vary the fixation and embedding procedure failed to produce satisfactory results until it was noted that Cheissen et al. (1961) had fixed the spores of Myxobolus for six months in 4% formalin. A modification in this technique (one month in 10% formalin) allowed enough modification of spore wall consistency or thickness for proper embedding. The embedding process left the spore valve with a distorted shape which had not been noted in light microscope examinations. In some micrographs, the spore embedded properly, but not the electron dense globular bodies.

Except for the irregular contour, valves of the spore appear similar to those of Myxobolus, Henneguya psorospermica, and Henneguya pinnae as presented by Cheissen (1961), Lom (1964), and Schubert (1968). They all appear to form a knob at the suture line.

The connection of the two valves appears as two mirror image shells cemented to one another at the edges. Based on similar morphological structures, it is probably safe to assume that the pattern of development of the valves of C. shasta (and most likely the whole spore) is similar to that proposed by Lom (1964) and by Schubert (1968). Degeneration (vacuolation and granulation) seems to be evident in the valves at the ends of the spores. Some intact structure is still present. Degeneration within spores of other myxosporidian species has also been reported.

The septate desmosome between the two connecting valves in protozoans has not been reported before in the previous literature. The exact composition of this lattice along with its function throughout the spore stage of the life cycle must be studied more thoroughly. These studies may open new doors to certain facets of transmission which are so far unexplained. This septate desmosome was first observed to connect cells of Hydra by Wood (1959). The multicellular character of myxosporidian trophozoites and the parallel development of myxosporidian polar capsules and coelenterate nematocysts led previous authors to suggest the metazoan character of myxosporidians. The septate desmosome seems to add additional support to this possibility.

It is difficult to compare the sporoplasm of C. shasta to other myxosporidians. Not many micrographs are available. Cheissen et al.

(1963) found a Golgi apparatus similar to those of eucaryotic cells in Myxobolus uniporus. None was seen in C. shasta. Well defined, classical shaped mitochondria were found in Henneguya pinnae, but in C. shasta only peculiar granular bodies which may be mitochondria were present. The ribosome packets appeared similar to those of Henneguya in that they appeared mostly around the outer fringes of endoplasm.

In C. shasta, one finds large electron dense bodies which may be fat bodies or other forms of nutritional material. These very pronounced structures are not present in the other myxosporidians which have been examined. There are, however, morphologically dissimilar round bodies in H. pinnae which may be of similar function. They are not quite as electron dense and not as large as the round bodies of C. shasta. The nuclei of the myxosporidian sporoplasm (including C. shasta) are vesicular in structure and are present in pairs (from two sporoplasmogenic cells).

The polar capsule in C. shasta is similar in structure to those of other myxosporidians. From the presence of the capsulogenic cell (Figure 6), the development of the capsule and filament of C. shasta is probably parallel to the development of the polar capsules of species examined by both Lom (1964) and Schubert (1968).

SUMMARY AND CONCLUSIONS

Distribution Studies in the Deschutes Basin

1. The infective stage of C. shasta is in the lower Deschutes River and is present in all three of its major tributaries.
2. In the Metolius branch of the Deschutes River the source of the infectious stage is Suttle Lake.
3. In the upper Deschutes branch of the Deschutes River, the infective stage flows from Crescent Lake. It enters the upper Deschutes by way of Crescent Creek and the Little Deschutes River.
4. Crooked River carries the infective stage in its lower stretches. Disease probably originates from contaminated irrigation water from the upper Deschutes River.
5. The Odell Lake-Davis Lake complex contains the infective stage of C. shasta and probably constitutes a closed system.

Ultrastructure of the Spore

1. The ultrastructure of C. shasta is similar in most cases to that of other myxosporidians studied with the electron microscope.
2. It is necessary to alter the spore wall by fixation in 10% formalin for at least one month before the spore will embed properly.
3. The valves of C. shasta appear to be connected by a septate

desmosome that has not been observed in other myxosporidians. This structure may be further proof that the myxosporidians may be metazoans.

4. The mitochondria-like structures found in the sporoplasm of C. shasta are not similar to mitochondria found in eucaryotic cells.
5. Large electron dense round bodies are found within the sporoplasm of C. shasta. They have not been described in other myxosporidians.

BIBLIOGRAPHY

Cheissin, E. M., S. S. Shulman and L. P. Vinnitchenko. 1961. Stroenie spore Myxobolus. Citologija 3:662-667.

Conrad, J. and M. Decew. 1966. First report of Ceratomyxa in juvenile salmonids in Oregon. Progressive Fish-Culturist 28:238.

Dogiel, V. A. 1965. General protozoology. 2d ed. Oxford, Clarendon. 747 p.

Fryer, J. L. and J. Conrad. 1965. Some observations on furunculosis in adult Pacific salmon and steelhead trout. Progressive Fish-Culturist 27:99-100.

Gottschalk, C. 1958. Die Amöbosporidien. Eine vergleichende Betrachtung ihrer Entwicklungszyklen unter Berücksichtignung ihrer systematischen Stellung im Tierreich. Zeitschrift für Wissenschaftliche Zoologie 160:1-38.

Grassé, P. P. 1960. Les Myxosporidies sont des organisms pluricellulaires. Comptes Rendus des Seances de l'Académie des Sciences 251:2638-2640.

Grell, K. G. 1956. Protozoologie. Berlin, Springer. 284 p.

Kudo, R. R. 1966. Protozoology. 5th ed. Springfield, Thomas. 1174 p.

Lom, J. 1964. Notes on the extrusion and some other features of myxosporidian spores. Acta Protozoologica 2:321-327.

Lom, J. and P. de Puytorac. 1965a. Observation sur l'ultrastructure des trophozoites de Myxosporidies. Comptes Rendus des Seances de l'Académie des Sciences 260:2588-2590.

_____ 1965b. Studies on the myxosporidian ultrastructure and polar capsule development. Prohistologica 1:53-65.

Lom, J. and J. Vávra. 1963. Fine morphology of the spore in Microsporidia. Acta Protozoologica 1:279-283.

Lom, J. and J. Vávra. 1965. Parallel features in the development of myxosporidian polar capsules and coelenterate nematocysts. In: Proceedings of the Third European Conference on Electron Microscopy, Prague, 1964. Prague, Publishing House of the Czechoslovak Academy of Sciences. p. 191-192.

_____ 1965. Notes on the morphogenesis of the polar filament in Henneguya (Protozoa, Cnidosporidia). Acta Protozoologica 3:57-60.

Noble, E. R. 1950. On a myxosporidian (protozoan) parasite of California trout. Journal of Parasitology 36:457-460.

Oregon. State Game Commission. Fishery Division. 1961. Annual Report. Portland. 355 p.

Sanders, James Edward. 1967. Occurrence of the protozoan parasite Ceratomyxa shasta among salmonid fishes in Oregon waters. Master's thesis. Corvallis, Oregon State University. 60 numb. leaves.

Sanders, James Edward, J. L. Fryer and R. W. Gould. 1969. Occurrence of the myxosporidian parasite Ceratomyxa shasta, in salmonid fish from the Columbia River basin and Oregon coastal streams. In: American Fisheries Society Symposium on Diseases of Fish and Shellfish. (In press)

Schafer, W. E. 1968. Studies on the epizootiology of the myxosporidian Ceratomyxa shasta (Noble). California Fish and Game 54:90-99.

Schubert, G. 1968. Elektronenmikroskopische Untersuchungen zur Sporenentwicklung von Henneguya pinnae (Schubert). Zeitschrift für Parasitenkunde 30:57-77.

Society of American Bacteriologists. Committee on bacteriological techniques. 1957. Manual of microbiological methods. New York, McGraw-Hill. 315 p.

Wales, J. H. and H. Wolf. 1955. Three protozoan diseases of trout in California. California Fish and Game 41:183-187.

Wood, J. W. 1960. Fish disease investigations. In: 70th Annual Report of the Washington Dept. of Fisheries. Seattle. p. 106-107.

Wood, J. W. and J. Wallis. 1955. Kidney disease in adult chinook salmon and its transmission by feeding to young chinook salmon. Research Briefs of the Fish Commission of Oregon 6(2):32-40.

Wood, R. L. 1959. Intercellular attachment in the epithelium of Hydra as revealed by electron microscopy. Journal of Biophysical and Biochemical Cytology 6:343-351.