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Title: PHYSICAL, BIOCHEMICAL, AND BIOLOGICAL CHARACTERISTICS OF MYXOBOLUS INSIDIOSUS, A MYXOSPORIDAN PARASITE OF SALMONID FISHES

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Myxobolus insidiosus was found only in white muscle bundles of salmonid fish. The parasite's geographic range was extended to the Coos and Yaquina River systems. Myxobolus insidiosus was found in two new hosts, rainbow and steelhead trout (Salmo gairdneri). Exposures of various salmonids to this myxosporidan indicated that chinook salmon (Oncorhynchus tshawytscha) were very susceptible. Susceptibility to M. insidiosus was dependent on length of exposure period. Coho salmon (Oncorhynchus kisutch) were not infected with M. insidiosus after an 8 d exposure but were parasitized when exposed for 50 d. Susceptibility also depended on the numbers of infectious stages present in the water supply. Chinook salmon from the same lot were exposed at two sites where M. insidiosus is endemic. All those exposed in the Aumsville Rearing Ponds became parasitized, whereas only 10% of those exposed in a McKenzie River canal were infected.

Development of M. insidiosus was temperature dependent and occurred in chinook salmon maintained at water temperatures of 6-18°C. No M. insidiosus was found in chinook salmon held in 3°C water and no development occurred in those held in 21°C water. A host response, enlarged plasmodia infiltrated with host cells, occurred only against mature stages of the parasite. Autoinfection with M. insidiosus occurred.

Sera specific for M. insidiosus or Ceratomyxa shasta did not react with uninfected tissues or with spores of other Pacific Northwest myxosporidans when tested with immunodiffusion techniques. Similar tests indicated that M. insidiosus obtained from various host species collected throughout the geographic range of the parasite were closely related. Trophozoite and spore stages of M. insidiosus and C. shasta were detected by immunofluorescence. No cross reactions were noted among labeled C. shasta antisera and four myxosporidans, whereas a reaction occurred between M. insidiosus antisera and Myxosoma cerebralis spores. Morphometric studies of M. insidiosus from selected hosts and localities indicated that plasmodial and spore variability occur in this parasite.

Glutamic acid, aspartic acid, and lysine comprised over 40% of the amino acid composition of M. insidiosus and C. shasta spore valves. Other amino acid differences between the two parasites suggested that amino acid composition of myxosporidan spore valves may be a valuable taxonomic tool. The protein content of M.

insidiosus and *C. shasta* spore valves was 9.5 and 7.8%, respectively.

PHYSICAL, BIOCHEMICAL, AND BIOLOGICAL CHARACTERISTICS OF *MYXOBOLUS INSIDIOSUS*, A MYXOSPORIDAN PARASITE OF SALMONID FISHES

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Typed by Connie Zook for Antonio Amandi

To Anthony and Adrian

May Your Futures be Bright
Your Lives Healthy and Happy

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PHYSICAL, BIOCHEMICAL, AND BIOLOGICAL CHARACTERISTICS OF *MYXOBOLUS INSIDIOSUS*, A MYXOSPORIDAN PARASITE OF SALMONID FISHES

INTRODUCTION

Myxobolus insidiosus was originally described by Wyatt and Pratt (1963) from chinook salmon (*Oncorhynchus tshawytscha*) in Oregon's McKenzie River. Subsequently, the parasite was found in coho salmon (*Oncorhynchus kisutch*) in the Lewis River, Washington (Wood 1974). This myxosporidan infects fish during winter and early spring. Even though hosts become heavily parasitized, *M. insidiosus* causes no mortality or overt pathology. A subspecies *Myxobolus insidiosus clarki* was described on the basis of variation in plasmodial and spore morphology from the type species (Wyatt 1979).

The major objectives of this study were to: 1) detect the distribution of *M. insidiosus* in selected wild fish populations, 2) test susceptibility of salmonid species and strains to the parasite, 3) determine effects of temperature on development of *M. insidiosus* within the fish host, 4) define the sequential development of the parasite and its effects on the host, 5) compare *M. insidiosus* from various hosts and geographic areas, and 6) compare *M. insidiosus* and *Ceratomyxa shasta*, another myxosporidan infecting salmonids of the Pacific Northwest, with each other and various other salmonid myxosporeans.

Wild fish were collected from three Oregon coastal river systems and two rivers in the Willamette Valley. Eight salmonid

species, including two strains of coho salmon, were tested for their susceptibility to M. insidiosus at two sites in Oregon. Chinook salmon parasitized by M. insidiosus were subjected to temperature regimes ranging from 3 to 21°C, at 3°C intervals, and effects on the parasite noted. Weekly examinations of parasitized chinook salmon by wet mount, histological, and electron microscopy methods were performed to detect the developmental stages of M. insidiosus and host parasite effects. Morphometric examinations, amino acid analyses, immunodiffusion, and immunofluorescence were used to make comparisons among the myxosporidans studied.

LITERATURE REVIEW

Introduction

Myxosporidans are microscopic, multicellular, obligate parasites which primarily infect fishes and are believed to have originally parasitized the coelozoic fluids of the biliary and urinary systems of marine fishes. Evolutionary trends have led to histozoic parasitism and also invasion of freshwater hosts (Shulman 1966; Meglitsch 1970). Myxosporidans now occur in fish living near the surface to those found at 4,100 m (Noble and Collard 1970). Histozoic plasmodia are usually walled off from the host tissue by a parasite-induced and host-originated membrane, whereas coelozoic forms are not restricted by the host and are free in the fluids (Meglitsch 1970). Myxosporidans parasitize most fish tissues and cavities (Petrushevski and Shulman 1958).

The complete life cycle of myxosporidans is unknown, and the spore is the most frequently observed and studied stage. Spore shapes and sizes vary and consist of one to three (rarely more) shell valves. The valves meet in a sutural plane that can be curved, twisted or straight. The spore shell may be smooth or striated with markings, extensions, processes, or cilia. Spores contain from one to eight polar capsules each with a coiled polar filament. The capsules occur in the anterior end of the spore except in the Myxidiidae where they are positioned laterally. One or two sporoplasms are found posterior to the polar capsules except for the Myxidiidae where one is centrally located (Kudo 1966).

Numerous reviews dealing with myxosporidan taxonomy and biology have been written. Reviews on the biology of myxosporidans covering the period from their discovery in 1825 to the 1920's have been presented (Morrison 1974; Fendrick 1980). Monographs describing myxosporidan taxonomy were published by Gurley (1894), Thélohan (1895), Labbé (1899), Auerbach (1910), Kudo (1920), and Mitchell (1977). Reviews, lists, and dichotomous keys of myxosporida from certain geographic regions have been published by Fujita (1923, 1927), Kudo (1934), Fantham et al. (1939), Tripathi (1951), Shulman and Shtein (1962), Shulman (1966), and Lalitha Kumari (1969). Reviews restricted to a single family or species were published by Meglitsch (1960), Hoffman et al. (1965), Halliday (1976), Johnson et al. (1979), and Hine (1980).

Myxosporidan hosts

Although fishes are the principal hosts several species of myxosporidans parasitize other lower vertebrates. Amphibians are parasitized with at least six genera of myxosporidans (Lutz 1889; Joseph 1905; Auerbach 1910; Johnston and Bancroft 1918; Kudo 1920, 1922, 1943; Guyénot and Naville 1922; Morelle 1928; Weill 1929; Ray 1933; Kudo and Sprague 1940; Lakhotia and Chakravarty 1968; Clark and Shoemaker 1973; Ewers 1973; Théodorides et al. 1981; Sarkar 1982). Reptiles are hosts for four species of Myxidium (Bosanquet 1910; Kudo 1920; Ray 1933; Johnson 1969) and for Zschokkella lissemysi (Chakravarty 1940).

Five myxosporidans have been described which infect invertebrate hosts. The annelid, Nais lacustris, and the insect, Tostrix viridana, are parasitized by Myxobolus sp. and Chloromyxum diploxys, respectively (Kudo 1920). A cochineal bug, Dactylopius indicus, is a host for Symmetrula cochinealis (Sundara Rajulu and Radha 1966). Overstreet (1976) and Weidner and Overstreet (1979) described Fabespora vermicola, a hyperparasite of the trematode Crassicutis archosargi, a parasite of fishes. An unusual characteristic of this myxosporidan is the ability of the spores to move by undulating the shell valves. Siau et al. (1981) observed Fabespora sp. in the trematode Allopodocotyle chrysophryii, a parasite of the fish Sparus aurata. A sixth myxosporidan, Myxobolus uvuliferus, associated with an invertebrate was found in the fibrous capsules surrounding the metacercariae of Uvulifer amploplitis, a parasite of fish (Cone and Anderson 1977). The myxosporidan was not observed in any other fish tissue or in direct contact with the digenean. Two myxosporidans are hosts for microsporidans. Sphaerospora polymorpha and Leptotheca coris, both parasites of fishes, are infected by Nosema notabilis (Kudo 1944) and Nosema marionis (Stempell 1919), respectively.

Life cycles and transmission

Life cycle studies of various myxosporidans have given further understanding to the parasitic process of these organisms. Dunkerley (1925) and Mitchell (1977) presented generalized life cycles of myxosporidans. Davis (1923) and Noble (1944) gave

detailed descriptions of the stages from sporoplasm to spore for several myxosporidans. The nuclear cycles of several stages have also been described (Georgevitch 1916, 1917, 1935; Erdmann 1917b; Naville 1930; Noble 1941b; Uspenkaja 1976).

Several transmission experiments have been attempted in order to complete the entire life cycle of myxosporidans. Most of these experiments have yielded equivocal or negative results. Even though the spore is the most often studied stage of the myxosporidan, it is probably not the infectious unit. Therefore, attempts to transmit the spore as well as searches for vectors, secondary hosts and the actual infectious unit have taken place. Bond (1936, 1938b) could not transmit Myxosoma funduli, Myxosoma subtecalis, Myxidium folium, nor Myxobolus bilineatum when infected tissues were fed to fish or placed in incisions on fish. Feeding infected tissues containing Myxosoma cartilaginis to fish or allowing them to feed on cladocerans and algae containing spores failed to transmit the parasite (Hoffman et al. 1965). Neither Myxidium oviforme infected tissue fed to fish (Walliker 1968b) nor Myxidium chelonarum fed to mammals, birds, fish, and turtles (Johnson 1969) established infections with these parasites. Ceratomyxa shasta was not transmitted by "aged" spores (Schafer 1968; Fendrick 1980) or in laboratory exposure of fish to mud believed to contain the infectious agent or mud which was sterilized and seeded with spores (Johnson et al. 1979; Johnson 1980). Mitchell (1970) fed and injected fresh or aged spores of

Myxobolus muelleri and Myxobolus dujardini to fish. He also allowed fish to feed on Paramecium which had ingested spores and fed fish spores of three myxosporidans passed through the intestinal tracts of kingfishers. In no case was experimental transmission achieved. Fish allowed to contact infected mud or fed tissue infected with Myxobolus insidiosus were not parasitized (Wyatt 1971). Feeding or injecting spores and exposure to spores or the myxosporidan infectious stage by contact with infected fish or mud failed to transmit Henneguya sp. to susceptible fish (Minchew 1973). Spall (1973) was unsuccessful in transmitting Myxosoma pharyngeus by feeding fish infected tissue or fresh or aged spores. Negative results were obtained when he injected spores or plasmodia into fish and when he fed spores to cladocerans and mosquito larvae that were subsequently fed to fish. No transmission was observed by Lester (1974) when he fed tissue containing Myxobilatus gasterostei to fish. Aged or fresh spores of Myxobolus pavlovskii fed alone or mixed in food did not transmit the parasite to fish (Molnar 1979).

Reports of experimental transmission of various myxosporidans have been published, although few of these would qualify as actual transmission of the parasite. Shiba (1934) reported the transmission of Henneguya macropodi but did not state the experimental conditions or source of hosts. Myxosoma funduli and Myxosoma subtecalis were transmitted by feeding infected tissue to Fundulus heteroclitus which had been raised from eggs and held in

running water (Bond 1939). Transmission of four myxosporidans by exposing Fundulus diaphanus to infected F. heteroclitus was reported by Bond (1939). Some F. diaphanus, however, were found to be infected with one of the myxosporidans before the experiment. Also, the source of the water supply was not specified. Wagh (1961) transplanted Myxosoma ovalis from the small mouth buffalo, Ictiobus bubalus, to the golden shiner, Notemigonus crysoleucas, by injecting plasmodial contents intramuscularly. He observed trophozoites and pansporoblasts only around the injection sites. The stage of development of the plasmodia used for injecting the shiners was not reported by Wagh and it is possible he injected prespore stages that survived in the shiner and did not develop further or developed only into the observed trophozoites and pansporoblasts.

Various experiments have relied on the use of contaminated water or substrate and probably closely resemble natural transmission of the myxosporidans. Infection of silver carp, Hypophthalmichthys mollitrix, by Myxobolus pavlovskii was achieved when the carp were exposed to muddy pond water in an aquarium (Molnar 1979). Parasitism of Cirrhina mrigala was noted by Seenapa and Manohar (1981a) when the fish were placed in mud contaminated with Myxobolus vanivilasae. Ceratomyxa shasta, the causative agent of ceratomyxosis, was first transmitted experimentally by Schafer (1968) when he injected ascites fluid from infected fish into parasite-free fish. Similar results were obtained by Johnson

(1975) who found that infectivity of the fluid was lost after 3 d at 4°C in tightly closed containers. He also exposed fish to membrane filters where the infectious stage of C. shasta was trapped and was able to transmit the parasite. Ratliff (1983) noted that the infectious stage of C. shasta remained viable for 10 d in aerated, river temperature controlled water.

Myxosoma cerebralis, the causative agent of whirling disease, appears to be easily transmissible under natural and experimental conditions. This appears to be the only myxosporidan at the present time where experimental transmission actually occurs. Spread of the parasite to salmonid populations in which it was previously unknown has been documented (Halliday 1976; Hoffman 1976). The agent can be spread through contaminated eggs or containers (Keiz 1964) but transovarian transmission does not occur (O'Grodnick 1975). Uspenkaja (in Walliker 1968b) reported that transmission of the parasite occurred after spores were aged for four months in water. Putz and Hermann (1970) obtained the same results by aging the spores in mud.

Birds have been implicated as vectors for M. cerebralis. Meyers et al. (1970) found that spores passed unchanged through the intestinal tracts of birds and Taylor and Lott (1978) fed spores to birds and then added the spores contained in the feces to mud. After a period of aging, they transmitted whirling disease by exposing fish to the mud. Thus, birds can disseminate the parasite to nonendemic areas.

Recently, Markiw and Wolf (1983) suggested that tubificid oligochaetes were necessary to effect transmission of M. cerebralis in mud. An actinomyxidian of the genus Triactinomyxon parasitized only tubific worms which had been previously exposed to M. cerebralis spores. Actinomyxidians belong to the class Actinosporea and the phylum Myxosoa to which myxosporidans also belong. This parasite reacted by immunofluorescence with anti-M. cerebralis sera (Wolf and Markiw 1981). Wolf and Markiw (1981) suggested that M. cerebralis spores infect aquatic tubificids and that the infection results in the formation of Triactinomyxon which becomes waterborne and is responsible for the parasitism of fish and eventually for M. cerebralis spore formation.

Host parasite interactions

Host specificity of myxosporidans can be monotypic, e.g., Agarella gracilis in Lepidosiren paradoxa (Dunkerley 1925); monogeneric, e.g., Myxoproteus abyssus in Coryphaenoides spp. (Moser and Noble 1977b); monofamilial, e.g., Chloromyxum trijugum in Centrarchidae (Mitchell et al. 1980), Ceratomyxa shasta (Johnson et al. 1979) and Myxosoma cerebralis (Bogdanova 1968; Halliday 1976) in Salmonidae; and nonspecific, e.g., Myxobolus muelleri, which can parasitize over 250 species of fish (Kudo 1920; Shulman and Shtein 1962; Davies 1968). Myxosporidan tissue specificity is also variable. Some species infect only one tissue or organ, e.g., Myxosoma squamalis in scale pockets (Iversen 1954), Myxidium serotinum in the gall bladder (Kudo 1943; Clark and Shoemaker

1973), and Myxosoma cerebralis in cartilage (Halliday 1976). Also, most myxosporidans which infect gall bladders are not found in other tissues (Mitchell 1977). Other myxosporidans can parasitize two or more tissues in the same or different hosts. Ceratomyxa shasta (Noble 1950) and Myxoproteus rosenblatti and Myxoproteus abyssus (Moser and Noble 1977b) infect several organs of their hosts, and Myxobolus muelleri parasitizes a variety of tissues (Kudo 1920; Shulman and Shtein 1962; Davies 1968).

Myxosporidan adaptations to their hosts include affinities for the host's physiology and physical changes by the parasite which improve the chance of infecting certain hosts. Noble (1941a) saw no morphological differences between myxosporidans that parasitize only one host and those found in several hosts. He concluded that host specificity by a myxosporidan has a physiological basis. Further support for the host's physiology affecting myxosporidans was given by Clark and Shoemaker (1973). They reported that sporogony of Myxidium serotinum took place only when the salamander began its metamorphosis. They postulated that sporulation was induced by host metamorphic hormones.

Physical adaptation to hosts by myxosporidans can best be exemplified by the genus Myxobolus where the size variability of the iodinophilous vacuole could aid members of the genus to occupy several niches and enlarge their range of hosts by having different sinking rates (Donec et al. 1978). The actual function of the iodinophilous vacuole has been debated for some time. Gurley

(1894) postulated that the glycogen in the vacuole was a nutrient for the spore stage of the parasite. Podlipaev (1974) found that the glycogen increased as the spores matured and then decreased. He also considered the vacuole to be a storage depot for nutrients. Kudo (1921a) reported that the vacuole of Henneguya salminicola reached maximum size when the spores were mature and thus served as a food source. Donec et al. (1978) postulated that the iodinophilous vacuole was mainly a hydrostatic apparatus and it acted secondarily as a nutrient depot. By centrifugation, they found the vacuole had a higher specific gravity than the sporoplasm, and thus its presence could increase the sinking rate of the spore.

The ability of the vacuole to act as a hydrostatic organ gains credence when the evolution of the Myxobolidae is considered. About 65% of all freshwater myxosporidans are in the genus Myxobolus while 26% belong in Henneguya and 3% are other myxobolideans (Shulman et al. 1978). Shulman (1966) suggested that the evolution of the Myxobolidae was related to that of the Cypriniformes, their primary hosts. The cypriniforms are infected by 170 myxobolidean species, whereas 20 other orders of fish are parasitized by 116 species (Shulman 1966). The cypriniforms are primarily benthic or plant feeders. In shallow waters with dense populations of these fish it would have been beneficial to parasite survival for spores to sink rapidly. Shulman et al. (1978) speculated that if the iodinophilous vacuole were primarily an

energy storage depot, the same pattern of vacuole formation should have developed in other myxosporidans because they all contain polysaccharides in the sporoplasm. Thus, the development of the vacuole aided in the widespread parasitism of the cypriniforms by myxobolideans. Other morphological differences associated with host specificity have been reported for the Myxobolidae. The presence of fat in the spore (Podlipaev 1972), markings and appendages on the spore valves, and spore size can be used by the parasite to increase or decrease the spores rate of descent through the water column (Donec et al. 1978; Shulman et al. 1978).

Cytochemical characteristics of myxosporidan spores

Cytochemical stains specific for deoxyribonucleic acid (DNA) have given further evidence of the multicellularity of myxosporidans and have further elucidated the processes of sporulation and spore maturation. Bond (1937b) reported that DNA in the spore valves of Myxosoma funduli increased as the spores matured, probably from incorporation of degenerated valvular nuclei into the spore valves. Chakravarty et al. (1962) observed extranuclear Feulgen-positive granules on spore valves of Myxobolus bengalensis, Myxobolus catlae, and Thelohanellus catlae. They also noted that the spore valves did not form until valve nuclei degeneration had occurred. Polar filaments of the above species were also Feulgen-positive as they were partly formed from degenerated capsulogenic nuclei. A decrease in nuclear DNA was noted as the myxosporidan progressed from sporoplasm to sporoblast

stage. The high DNA content in the sporoplasm was probably due to fusion of the two sporoplasmic nuclei (Maiti et al. 1964). Extranuclear Feulgen-positive granules in the cytoplasm of the trophozoites and sporonts of the above three species of myxosporidans were reported by Maiti et al. (1964). DNA was found in developmental stages, shell valves, polar capsules, and polar filaments of Zschokkella auerbachi by Lakhotia and Chakravarty (1968).

Cytochemical reactions specific for ribonucleic acid (RNA) have indicated that most of the protein synthesis in myxosporidan spores occurs early in the sporulation process. Concentrations of RNA were greater in immature spores and decreased with maturity of Myxobolus bengalensis, Myxobolus catlae, and Thelohanellus catlae (Chakravarty et al. 1962). This decreased RNA content suggested that protein was being synthesized rapidly in immature spores. Intense reactions for RNA were also seen in the posterior end of the shell valves and in the polar capsules, whereas only a slight reaction was observed in the sporoplasm and none in the polar filaments of immature spores.

Some chemical components of myxosporidan spores have been determined by cytochemical procedures. Kudo (1921a) ruled out cellulose and chitin as components of the spore valves of myxosporidans and that the polar filaments were not composed of glycogen, as previously suggested by Erdmann (1917a). Bond (1937b) and Lom (1964) detected no mucopolysaccharides, polysaccharides,

nor chitin and determined that the major component in the spore valves was protein. An iodinophilous vacuole present in the sporoplasm of all Myxobolidae has been detected by specific stains. The vacuole was first noted in Myxobolus spp. (Thélohan 1892). The calcium and sulfur content of Myxobolus exiguus spores was determined by Siau (1977a). He reported that the suture line, sutural ridge, and the central spore region were higher in calcium than the polar capsules and surrounding areas. The spores contained more calcium than sulfur.

Myxosporidan morphometrics

Spore shape and size are the main characteristics used for myxosporidan species identification. Morphometrics, especially size, have been the basis for the establishment of many new myxosporidan species even though only small differences existed. Myxosporidans have been described from fresh, frozen, fixed, and stained preparations. Long and Meglitsch (1969) noted that fresh spores were the best for morphometric description of myxosporidans. They also observed that freezing, chilling, or storage at room temperature for up to four weeks did not change the size of spores of various species. Thus, when fresh spores are not available, they suggested using frozen or chilled material. In many cases, the parasites were described without noting the condition of the tissues and/or spores. These procedures have caused numerous taxonomic problems because fixation and staining methods can radically change spore measurements. Kudo (1921b)

observed a 22% decrease in the width of Leptotheca ohlmacheri spores after preservation in formalin. Parker and Warner (1970) tested seven fixatives and reported that they all caused spore shrinkage. Formalin caused the least shrinkage, but size reductions of 10-18% still occurred in some spores. Other reports of spore shrinkage resulting from fixation and staining are those made by Bond (1938c), Fish (1938), Iversen (1954), Wyatt and Pratt (1963), and Lewis and Summerfelt (1964).

Size variation of myxosporidan spores

Some myxosporidan species remain relatively constant in size and shape, whereas others appear to vary throughout their host and geographic ranges. Moser (1977) suggested that spore size was determined by the physiology and morphology of the host and shape was controlled by the presence of physiologically and behaviorally suitable fish. Moser (1977) and Moser and Noble (1976, 1977a, 1977b) found only small size differences in Myxoproteus spp., Zschokkella spp. and Ceratomyxa spp. from different deep sea fishes and organs. In contrast to the relatively constant size of myxosporidans of deep sea macrurid fishes, many authors have reported differences in spore size and shape for the same parasite species from different hosts, different organs of the same host and from individual plasmodia. Bond (1938a) repeatedly observed two types of spores of Myxosoma hudsonis in individual plasmodia. Similar findings were made for Myxosoma heterospora where three types of spores were present in every plasmodium (Baker 1963).

Lalitha Kumari (1969) reported a multiple infection with Myxobolus species in the fins of Barbus kolus. Spores of three sizes were present in each plasmodium and they were named M. ampullaceus, M. koli and Myxobolus sp. Undoubtedly, they represented a single pleomorphic species. Walliker (1969) reported macro and micro spores of Myxobolus serrasalmi in every plasmodia from different organs of Serrasalmus rhombeus and Kundu and Haldar (1981) described macro and micro spores of Thelohanellus jiroveci from individual plasmodia in the gills of Labeo bata.

Some differences in spore morphology have been attributed to occurrence of the parasite in atypical hosts. Bond (1937a) observed that spores of Myxosoma grandis from the liver of Rhinichthys astronasus, an unusual host for the parasite, were elongated or rounded and distorted. The polar capsules were often misplaced within the spores. Abnormal polar capsules numbers (2-8) were reported by Meglitsch (1942) for Chloromyxum opladeli from the gall bladder of Opladelus olivaris.

Host age, size, sex, and physiological condition, physical and chemical environmental factors, and seasonal changes influence myxosporidan development (Meglitsch 1960). He reported that spores of Ceratomyxa spp. from different hosts were not uniform in size and attributed the differences to host, parasite and environmental factors. Sanders (1967) reported large size variations of Ceratomyxa shasta spores. Davies (1968) observed differences as large as 3 µm (20%) among spores of Myxobolus muelleri from

different organs of Leuciscus cephalus. She concluded that the larger spores had developed in more favorable sites (mesenteries and intestinal wall) than had the smaller ones. She also reported size variations among spores of M. muelleri from the gills of several cyprinids. Tailless and tailed spores were also seen in the same plasmodia suggesting that aberrant tail formation described by others as being due to host influences was instead intrinsically controlled by the parasite. Similar tail formations were reported by Lom (1961) in M. muelleri spores.

Control of myxosporidan sporulation either through the environment or the host's physiology (partly environmentally controlled) has been reported for some myxosporidans. Davies (1968) noted that spore tails of Henneguya zschokkei were present only in early summer, whereas mature spores at other times had short spikes. Host control over H. zschokkei was apparent in the type of plasmodium the parasite formed in different fish species. In Coregonus sp., the plasmodia were white, large, and protruded from the body, whereas in Leuciscus sp. the plasmodia were small and yellow. Similar observations were made by Mitchell (1970) on Myxobolus muelleri plasmodia in several cyprinids.

Host and environmental influences on sporulation and normal shape and size variations that occur in myxosporidans should be considered in classifying species. Hine (1978, 1979, 1980) found variations in sites of infection, spore shape and size, the number and arrangment of striations on the spore surface, and plasmodia of

Myxidium spp. parasitizing anguillids. He found no relationships between plasmodium size and spore size but concluded that the shape and size of these myxosporidans was intrinsically determined, although it could be modified by the host's physiology and the site of sporogony. Hine (1979) speculated that physical space at the site of sporogony was of little importance, but a supply of nutrients and means of waste removal were the actual factors controlling the differences observed during sporulation. Because of the variation in spore size, number of shell striations, and infection sites Hine (1980) synonomized five species of Myxidium with Myxidium giardi. Likewise, Mitchell (1970) for similar reasons grouped several species of Myxobolus under Myxobolus muelleri.

Taxonomy

The taxonomic status of myxosporidans as a group has been disputed since their discovery. Thélohan (1892) classified myxosporidans and microsporidans as Protozoa. He created four myxosporidan classes based on spore shape, number of polar capsules, and presence of an iodinophilous vacuole. Gurley (1893, 1894) separated the microsporidians from the myxosporidans and established four myxosporidan families using the iodinophilous (glycogenous) vacuole as a generic characteristic for Myxobolus. The presence of an iodinophilous vacuole in the sporoplasm is currently used as the means of differentiating between members of Myxobolidae (vacuole present) and Myxosomatidae (vacuole absent).

It is used taxonomically to separate Myxosoma from Myxobolus as other genera of Myxobolidae can easily be differentiated from Myxosoma by other characteristics. Another characteristic used to differentiate myxosporidans is the presence of a mucus envelope around some spores (Lom and Vavra 1961, 1963). The shape and size of the evelope was constant and characteristic for the individual species. The envelope was not visible when the spores were embedded in tissue but when free spores contacted water the envelope swelled up and became apparent. Kudo (1920, 1933) reviewed myxosporidan taxonomy and later listed three suborders and nine families in the order Myxosporida. Meglitsch (1957) objected to the use of spore shape and size as the only criteria for species descriptions, and he suggested that host species, location in the host, and geographical range also be considered. Lom (1961) described certain problems in establishment of myxosporidan species. Often, descriptions were incomplete and drawings inadequate. Authors have described a new species from an infection in a single host and several have completely failed to consider previous descriptions. Thus, the same name was used for two different species or two different names were given to the same parasite (for examples see Appendix Table 11).

Since protozoans are defined as unicellular organisms and because it is known that myxosporidans are multicellular, Grassé (1960) suggested these parasites be given phylum status separate from the Protozoa. Levine (1961) discarded the class Sporozoa,

which contained the Myxosporida and created several protozoan subphyla with the myxosporidans in the Cnidospora. Subsequently, the Committee on Taxonomy and Taxonomic Problems of the Society of Protozoologists redefined the Protozoa and placed the myxosporidans and microsporidians in Cnidospora, one of three protozoan subphyla, due to polar filament similarities (Honigberg, et al. 1964). Sprague (1966) rejected the subphylum Cnidospora on the basis that polar filament similarity among the Myxosporida, Microsporidia, and Helicosporida (also in this subphylum) was superficial. He later separated the microsporidians from the myxosporidans and placed the latter in the subphylum Amoebogena (Sprague 1969). Subsequently, the subphylum Myxospora was created to include the myxosporidans (Levine 1969, 1970; Lom 1973; Baker 1977; Canning and Vavra 1977).

Grassé and Lavette (1978) created the phylum Myxosoa for Myxosporida and Actinomyxida. They believed that myxosoans are organisms in an evolutionary blind alley in which multicellular organization occurs without cell layers. The evolution of the Myxosoa probably ended with adoption of parasitism (Grassé and Lavette 1978). The Committee on Systematics and Evolution of the Society of Protozoologists gave the Protozoa subkingdom status in the kingdom Animalia (Protista), and divided the protozoans into seven phyla accepting the Myxosoa as one of them (Levine et al. 1980).

The validity of using the iodinophilous vacuole as a taxonomic characteristic has been challenged by various authors. Bond (1940)

observed particulate glycogenous sustances with definite patterns in spores of Myxosoma subtecalis, Myxosoma funduli and Myxidium folium. Walliker (1968a) suggested that the vacuole was of no taxonomic value because of variation in appearance of the vacuole after staining. Therefore, he suggested that the genus Myxosoma (and thus the family Myxosomatidae) be eliminated with its species transferred to Myxobolus. In Myxobolus pseudodispar spores obtained from a single host, he noticed that the glycogen either appeared as a distinct vacuole, or was particular, or diffuse, or did not stain. Similar results were found with spores of Myxobolus cyprini, Myxobolus muelleri and Myxosoma heterospora (Walliker 1968a). Lom (1969a) agreed with Walliker when he noted size variation or absence of the vacuole in spores from individual mature plasmodia of Myxobolus and thus Lom (1969b) described a species with no vacuole as Myxobolus undulatus.

Other authors believe that the vacuole has taxonomic value and have retained the two families. Podlipaev and Shulman (1978) found that spores had two kinds of polysaccharides, an observation that could explain some of the variable results of Bond (1940), Walliker (1968a), and Lom (1969a). One type of polysaccharide was confined to the sporoplasm, was easily digested by saliva, and thus, was of glycogenous nature. The other type was concentrated in small granules near the polar capsules, was not digested by saliva, and thus, was not glycogenous. The granules appeared to be the remains of polysaccharide inclusions in the cytoplasm of the capsulogenic

cells (Podlipaev and Shulman 1978). They also found these pericapsular granules in the Myxobolidae as well as in genera of other myxosporidan families such as Myxosoma, Kudoa, and Myxidium that do not have an iodinophilous vacuole. The presence of these granules would explain Bond's (1940) observations of glycogenous substances in genera other than Myxobolus.

Shulman et al. (1978) noted that polysaccharides exist as globules in the sporoplasm of all myxosporidans, and that the vacuole reported by Walliker (1968a) in Myxosoma heteromorpha was probably a collection of large globules. Podlipaev and Shulman (1978) also found that, as Myxobolus spores matured, the polysaccharides formed larger particles and gradually concentrated within the iodinophilous vacuole. At maturity, the polysaccharides slowly passed from the vacuole to the sporoplasm where they stained in a diffuse pattern. These observations, in conjunction with the known variable maturation rate of Myxobolus spores within individual plasmodia, would account for the conflicting staining results obtained by these and other authors.

Shulman et al. (1978) admitted that detection of the iodinophilous vacuole in the Myxobolidae is not always possible, but they believed that synonymizing Myxosoma with Myxobolus would create taxonomic confusion by increasing the range of Myxobolus. They concluded that the vacuole should be used in taxonomy because of its morphologic and physiologic reality as well as its adaptive significance.

The following is a short synopsis from Levine et al. (1980) of current myxosporidan taxonomy. It is modified for brevity and by addition of families. Differentiating characteristics among classifications are bracketed and a representative family and two genera are given for each myxosporidan family.

Phylum Myxosoa - all parasitic with spores of multicellular origin and containing from one to eight polar capsules and one to several sporoplasms.

Class Myxosporea - one to eight polar capsules and [one or two sporoplasms] with two but occasionally up to eight valves.

Order Bivalvulida - [two valves] present.

Suborder Bipolarina - [polar capsules laterally] at opposite ends of spore.

Family Myxidiidae - Myxidium, Sphaeromyxa.

Suborder Eurysporina - two to four [polar capsules anteriorly located and perpendicular to sutural plane].

Family Ceratomyxidae - Ceratomyxa, Leptotheca.

Suborder Platysporina - one or two [polar capsules located anteriorly in the sutural plane].

Family Myxobolidae - Myxobolus, Henneguya.

Order Multivalvulida - [three or more valves] present.

Family Kudoidea - Kudoa, Hexacapsula.

Class Actinosporea - three polar capsules, three valves and [several sporoplasms].

Pathology

Pathology caused by myxosporidans can range from tissue occupancy and displacement with no noticeable adverse effects to tissue destruction leading to death of the host. General reviews of some myxosporidans and the diseases they cause were presented by Lom (1970) and Rogers and Gaines (1975). Histological changes associated with tissue damage caused by several myxosporidans have been described (Nigrelli and Smith 1938; Nigrelli 1948; Noble 1950; Jakowska and Nigrelli 1953; Johnson 1975; and Dykova and Lom 1978).

One of the tissues infected by myxosporidans is the gill and Dykova and Lom (1978) suggested a two-step host response to attack by gill-infecting myxosporidans. The first reaction was either displacement, atrophy, or hyperplasia of host tissue around the trophozoite. In heavy infections, organ functions were impaired but the host did not respond to the infection. In the second step, the host response to the parasite was an inflammatory reaction with leucocyte infiltration and replacement of the mature plasmodium by granulomatous tissue. Gill tissue displacement can sometimes interfere with gas exchange and heavily infected fish die from suffocation. Several epizootics resulting from this type of parasitism have been described (Hoshina 1952; Shulman 1957; Delisle 1972; McCraren et al. 1975; Sanaullah and Ahmed 1980).

In addition to the damage caused to gills, histozoic myxosporidans can also cause degeneration and liquefaction of muscle tissue of fish (Davies and Beyers 1947; Arai and Matsumoto 1953; Matsumoto 1954; Patashnik and Groninger 1964; Okada et al. 1981). The trophozoite stages of other myxosporidans are also known to cause tissue destruction. Davis (1916) reported that trophozoites of Sphaerospora dimorpha phagocytized and digested host erythrocytes. Trophozoites of Myxosoma cerebralis phagocytized cartilage cells and delaminated the cartilage tissue (Uspenkaja 1979). Attachment of Chloromyxum trijugum trophozoites to the mucosal layer of the gall bladder and feeding on bladder contents via villi on their free surface was reported by Mitchell et al. (1980). Other myxosporidans are known to induce high rates of death in their hosts. Boil disease of barbel (Barbus sp.) with subsequent heavy mortalities caused by secondary infections was described by Keysselitz (1908a, 1908b) in Europe. Other myxosporidans that cause high mortalities are Mitraspora cyprini in goldfish, Carassius auratus, (Fujita 1912; Hoffman 1981), Myxobolus drjagini in the carp H. molitrix (Baohua et al. 1975, 1979), Henneguya exilis in channel catfish, Ictalurus punctatus, (McCraren et al. 1975; Smith and Inslee 1980), Ceratomyxa shasta in salmonids (Noble 1950; Johnson 1975) and Myxosoma cerebralis in salmonids (Halliday 1976; Hoffman 1976).

Coelozoic myxosporidans generally cause less damage than histozoic species. A few cases of pathological changes caused by

coelozoic species in the gall bladders of marine fishes have been reported. The walls of infected gall bladders became hard, thickened, and opaque and the bile increased in viscosity and changed in color from the normal green to yellow, orange, or brown. As infection progressed, the walls of the gall bladder became thicker, the cavity smaller, and a general desquamation of the epithelial lining was observed (Fantham and Porter 1912, 1948; Fujita 1923; Fantham et al. 1940). Mortality caused by a coelozoic myxosporidan Chloromyxum truttae was reported by Bauer et al. (1969). They described a chronic gall bladder disease of brown trout (Salmo trutta) that always ended in death of the host.

Control

Control of myxosporidans is primarily restricted to avoidance and prophylaxis. Avoiding myxosporidan infections can be accomplished by raising fish in parasite-free water (Johnson et al. 1979). Some seasonal myxosporidans, such as Myxobolus insidiosus, can be avoided by rearing fish when the infectious stage is not present (personal communication R. A. Holt, State Fish Pathologist, Oregon Department of Fish and Wildlife). Prophylactic control of myxosporidans has been achieved by sanitation, physical and chemical treatment of water supplies and substrate. Treatment of water supplies by filtration and subsequent ultraviolet radiation or chlorination killed the infective stage of Ceratomyxa shasta (Bedell 1971; Sanders et al. 1972), Myxosoma cerebralis (Hoffman 1974, 1975), and Myxobolus insidiosus (Wyatt 1978b). Treatment of

mud contaminated with Myxosoma cerebralis by air drying, chlorination, or with hydrated lime killed all exposed spores but not those that were buried (Hoffman and O'Grodnick 1977).

When parasite avoidance or prophylactic water treatment are not feasible, infections can be reduced by placing resistant stocks of fish in infectious waters (Zinn et al. 1977; Johnson et al. 1979), and for Myxosoma cerebralis by placing older fish in such waters (Halliday 1976). Drugs and chemicals used for other parasite and bacterial diseases of fish apparently have little or no effect on myxosporidans. These substances though are known to prevent secondary infections and thus reduce losses. Taylor et al. (1973) fed six compounds for 12 months to rainbow trout and exposed them simultaneously to Myxosoma cerebralis. They noted that furazolidone inhibited parasite sporulation, whereas oxytetracycline, sulfamerazine, nicarbazin, amprolium, and Merck experimental drug number 930 had no effect. But they also observed a 50% reduction in growth of fish fed furazolidone; the food was probably unpalatable as large amounts were not eaten. The poor physiological condition of these fish could have had an effect on sporulation of the parasite. Mitchell (1977), citing Hoffman and Meyer (1974), incorrectly reported some activity of acetarsone against whirling disease.

Serology

Serological techniques have just recently been applied to detect and identify myxosporidans. The methods used have been

hypersensitivity reactions, agglutination and precipitation tests, immunodiffusion, complement fixation, and immunofluorescence (Halliday 1974; Pauley 1974; Markiw and Wolf 1977, 1978; Griffin and Davis 1978; Siau 1980; Banner et al. 1982; McArthur and Sengupta 1982).

Mitchell (1970), using hypersensitivity tests in rabbits, reported that antigens of Myxobolus muelleri and Myxobolus dujardini were closely related to those of their fish hosts. Supernatant from spore washings gave positive reactions in rabbits indicating that some myxosporidan antigens were surface located. Precipitating or agglutinating antibodies were not detected when anti-M. muelleri or anti-M. dujardini rabbit serum was added to broken or whole spores.

Antigenic similarities were found between Myxosoma cerebralis spores and rainbow trout cartilage by Pauley (1974) using immunodiffusion. However, by electrophoretic mobility the cartilage and spore proteins were shown to migrate at different rates and the immunodiffusion reaction was reported to be due to shared antigens between the spores and cartilage. Using immunodiffusion and electrophoresis, McArthur and Sengupta (1982) indicated that Myxobolus sp. antigens cross reacted with skin and muscle tissue antigens of their fish host. Siau (1980) did not detect a reaction between spores and anti-Myxobolus exiguus rabbit or fish sera by gel precipitation.

Complement fixation was used to detect antigen-antibody binding between specific rabbit sera and Myxobolus exiguus (Siau 1980). Using this test, he obtained positive reactions when serum from fish previously injected with myxosporidan antigens was reacted with serum from rabbits similarly injected. The reaction was not due to a rabbit serum-fish antigen complex. Siau (1980) concluded that the reaction was caused by either a non-parasite substance capable of activating complement or by myxosporidan antigens derived from the spores and present in the fish serum.

Fluorescent antibody techniques have been used to detect Myxosoma cerebralis. Using the indirect fluorescent antibody test (IFAT), Halliday (1974), reported a reaction between spores of M. cerebralis and specific rabbit antisera. No fluorescence was detected with spores of Myxobolus spp., or when the serum of naturally or experimentally infected rainbow trout was tested by IFAT on M. cerebralis spores. Markiw and Wolf (1978) tested the efficacy of both the direct fluorescent antibody test (DFAT) and IFAT with anti-M. cerebralis rabbit sera. Using DFAT, cross-reactivity occurred only with Myxosoma cartilaginis. When IFAT was used, cross-reactions were observed between genera and with myxosporidans invading tissues other than cartilage. The IFAT has also been used to detect antibodies in rainbow trout sera that reacted against M. cerebralis. Sera from both infected fish and a few uninfected fish gave a positive reaction for antibodies specific for M. cerebralis (Griffin and Davis 1978).

Maintenance and cultivation of myxosporidans

Most in vitro research has been directed toward maintaining myxosporidans in a viable state and recent work has emphasized artificial culture of the parasites. Although viability of spores maintained in vitro is difficult to evaluate, the following criteria have been used to determine spore survival: extrusion of polar filaments, rounding or vacuolation of the sporoplasm, pyknotic degeneration of sporoplasmic nuclei, absorbance or exclusion of vital stains, respiration rates (McKinney and Bradford 1970), culture of sporoplasms released from spores, and artificial transmission. Only the last two criteria conclusively demonstrate spore viability. Release of intact sporoplasms from myxosporidans by temperature cycling (Patashnik and Groninger 1964) and by spore disruption (Siau 1977b) has been achieved. At present transmission has been performed only with spores of Myxosoma cerebralis. The most frequently used criterion, namely, polar filament extrusion after alkali treatment, is probably the least reliable. Lom (1964) reported that spores which had been dried or fixed in alcohol for several years extruded their polar filaments when treated with alkali.

Survival of myxosporidan spores appears to increase by their presence in a protecting environment. Bond (1938c) reported that spores of Myxosoma subtecalis, Myxosoma funduli, Myxidium folium, and Myxobolus bilineatum survived (no degeneration of spore components visible by light microscopy) in water or 1% saline for

only 12-15, 20, and 28 days, respectively. In contrast, spores of Myxosoma cerebralis remained viable, as shown by transmission studies, after freezing for several years (Lom 1964; Hoffman and Putz 1969) or stored in moist mud for 4 to 5 years (Hoffman and O'Grodnick 1977).

Recently, the in vitro culture of life stages of myxosporidans has been reported. Wolf and Markiw (1976) reported that trophozoites and other prespore stages of Myxosoma cerebralis sporulated when cultured in chicken plasma clots or in Eagle's minimal essential culture medium for a minimum of 21 d at 15 or 20°C. Sporoplasms removed from spores of Myxobolus exiguus have been cultured in rainbow trout gonad 99 cell monolayers and in Stocker's culture medium with 10% calf serum (Siau 1977b). The sporoplasms reached the first stage of sporogenesis but did not complete development.

Myxosporida of Pacific Northwest salmonids

Ceratomyxa shasta

A myxosporidan found in 1948 by Wales and Wolf (1955) in dead and dying fingerling rainbow trout at Crystal Lake Hatchery, Shasta County, California was described as Ceratomyxa shasta by Noble (1950). This was the only report of Ceratomyxa in fresh water fishes and the first report of histozoic parasitism by the genus. The parasite is limited geographically to certain river systems and lakes in northern California, central and western Oregon, and

southwestern Washington. In Canada it has been found in the head waters of the Fraser River and in the Capilano River and James Creek (Johnson et al. 1979).

Ceratomyxosis develops only when fish have been exposed in endemic areas to water temperatures exceeding 10°C (Schafer 1968; Sanders et al. 1970). Following infection, the development of ceratomyxosis in rainbow trout and coho salmon was temperature dependent, and occurred faster in warmer waters (Udey et al. 1975). No deaths due to C. shasta occurred in rainbow trout held at 3.9°C but the disease developed after transfer of the fish to 17.8°C water. No deaths were reported for coho salmon held at or below 6.7°C.

The susceptibility of nine salmonid species and several strains of coho salmon and chinook salmon to ceratomyxosis has been reported. Minor losses due to C. shasta in three of seven strains of coho salmon exposed in Crystal Lake water were found by Schafer (1968). Brook trout (Salvelinus fontinalis), cutthroat trout (Salmo clarki), rainbow trout, chum salmon (Oncorhynchus keta), and fall chinook salmon were most susceptible to C. shasta, while coho salmon were moderately susceptible and brown trout, Atlantic salmon (Salmo salar), kokanee salmon (Oncorhynchus nerka), and spring chinook salmon were the least susceptible to the parasite (Zinn et al. 1977). Susceptibility of chinook salmon strains to C. shasta varied from mortalities of 0-13%, in strains obtained where the parasite is endemic, to 88-100%, in stocks originating in waters

where the myxosporidan does not occur (Zinn et al. 1977). This natural resistance can be overcome by a host-parasite imbalance created by larger numbers of infectious units of C. shasta (Ratliff 1981). An increase in survival of Deschutes River spring chinook salmon exposed to C. shasta occurred if these fishes had been previously exposed to the parasite for short periods of time when few infectious units were present in the water. From these results, Ratliff (1981) postulated that resistance to C. shasta by immunity can develop in fish stocks.

Myxobolus insidiosus

A myxosporidan parasite found in spindle shaped plasmodia in the musculature of spring chinook salmon from the McKenzie River, Oregon was initially reported by Wyatt (1961) as Myxobolus sp. and later described by Wyatt and Pratt (1963) as M. insidiosus. The subspecific name clarki was suggested by Wyatt (1979) in his description of M. insidiosus from McKenzie River cutthroat trout based on the following characteristics: plasmodia were present in the connective tissue between muscle bundles (interfibrillar); oval shaped plasmodia; and smaller spores. Myxobolus insidiosus has been reported from coho salmon in the Lewis River, Washington (Wood 1974; Wyatt 1978a). More recent findings of the parasite include Aumsville canal and Aumsville Rearing Ponds (Holt et al. 1978), North Fork Alsea River, Roaring River, and Salmon Creek (Sweet 1978) in Oregon. The current geographic and host ranges are summarized in the results section.

Chinook salmon become infected in late winter or early spring when mean water temperatures exceed 8.9°C (Wyatt 1978a). In late spring, when the mean water temperature is approximately 12.3°C, infection no longer occurs (Wyatt 1978a; R. A. Holt, State Fish Pathologist, Oregon Department of Fish and Wildlife, unpublished data). The susceptibility of chinook salmon to M. insidiosus is not related to age or size. Wyatt (1978b) found that chinook salmon fingerlings, juveniles, and smolts can become parasitized. Heavy infections of fish by M. insidiosus appear to be correlated with areas where dirt bottom substrates exist such as at Aumsville and at the old McKenzie Hatchery. The incidence and severity of M. insidiosus have decreased at McKenzie Hatchery, after the ponds were resurfaced with cement (personal communication, R. A. Holt, State Fish Pathologist, Oregon Department of Fish and Wildlife).

Fish heavily infected with M. insidiosus are not killed but the parasite's effects on the salt water phase of its anadromous hosts are unknown. Wood (1974) reported lower returns of adult coho salmon in the Lewis River than of fish in nearby streams where the parasite is not endemic. In light infections, the fish appear normal, but with heavier parasite loads they swim with difficulty (Wood 1974), or they can have external lesions of slightly raised areas devoid of scales (Wyatt and Pratt 1963).

MATERIALS AND METHODS

Test animals

Hatchery fish

The salmonid fish used in the experiments were obtained from Oregon Department of Fish and Wildlife (ODFW) and Washington Department of Fisheries (WDF) hatcheries. They were: chinook salmon (Bonneville Hatchery), coho salmon (Fall Creek and Speelyai Hatcheries), kokanee salmon, brown trout, and Atlantic salmon, (Wizard Falls Hatchery), cutthroat trout (Roaring River and Leaburg Hatcheries), rainbow trout (Oak Springs and Wizard Falls Hatcheries), and brook trout (Fall River Hatchery). All the above hatcheries are operated by ODFW except for the WDF Speelyai Hatchery. All fish used in the experiments were held at the Oregon State University Fish Disease Laboratory (FDL) in fish pathogen free water. Additionally, chinook and coho salmon being reared at Springfield, Oregon by Weyerhaeuser Company Research and Development personnel were examined. Yearling coho salmon parasitized with *M. insidiosus* were obtained frozen from Speelyai Hatchery for immunological studies.

Roaring River, Leaburg, and Speelyai Hatcheries are in areas where *M. insidiosus* is endemic and fish from these locations could have been naturally infected. To obtain *M. insidiosus*-free fish from these sources, fertilized coho salmon eggs from Speelyai Hatchery were hatched and reared in 12°C pathogen-free, flowing water. Cutthroat trout fingerlings from Roaring River and Leaburg

Hatcheries were maintained in the same water supply for 180 d to determine that the parasite was absent. At this time fifty cutthroat trout from each group were examined for the presence of M. insidiosus.

All fish used throughout this work were maintained in pathogen-free, flowing water at 12°C (except where other temperatures are noted) at the FDL and fed Oregon Moist Pellets (OMP) daily. Beginning fourteen days before and through the duration of each experiment all fish were fed OMP containing 4% oxytetracycline (TM50) (Pfizer, New York) as prophylaxis against bacterial infection. All fish were bathed with malachite green (Chemwest, Seattle, Washington) at 1.0 mg/L for 1 h for 3 consecutive days, as prophylaxis for external parasites, when they were brought back from the field after exposure to the myxosporidans.

Wild fish

Fish from the Yaquina, Coos, and Rogue River systems, Aumsville Canal, and Willamette River were examined for natural infection with M. insidiosus. These animals were collected by electroshocking, seining, gill netting, or angling and when possible, were kept alive and transported to the FDL for examination, others were killed and transported on ice.

Description of field sites

Two field sites were used during these studies. One, the Aumsville Rearing Ponds, is operated by ODFW from early January

through late February to rear fall chinook salmon fry. The ponds have an earth bottom and the water is supplied through an earth lined irrigation canal originating in the North Santiam River. The other was an earthen canal near the McKenzie Hatchery (ODFW), which carries water to a Eugene Water and Electric Board (EWEB) power station. The canal originates in the pool behind Leaburg Dam on the McKenzie River (Wyatt 1971).

Fish examination

To examine wild and hatchery fish for the presence of M. insidiosus, longitudinal sections, approximately 5 x 5 x 1 mm thick, of epaxial muscle below the dorsal fin were cut from fresh or frozen fish. Sections were placed on a slide with 0.85% saline, squashed, and examined microscopically at 125 X. Presence of an iodinophilous vacuole was determined by staining spores with Lugol's iodine (Wyatt and Pratt 1963). Staining of spores with India ink was used to detect the presence of mucus envelopes (Lom and Vavra 1961). Spores were measured at 1,250 X using an ocular micrometer in a Zeiss microscope. Photographs were taken with a Leica C35 camera and Kodak Plus-X-pan or Ektachrome film.

To determine localization of M. insidiosus cysts, chinook salmon were infected with M. insidiosus at Aumsville Rearing Ponds. The animals were then killed, fixed in 10% formalin, dehydrated through an ethanol series, and stored in 95% ethanol. They were subsequently placed in 2% potassium hydroxide until the skeleton became visible and then passed through a series of

potassium hydroxide-glycerol clearing solutions (Rau and Gordon 1977). The fish were stored in 100% glycerol for later examination.

Susceptibility of salmonids to *Myxobolus insidiosus*

Chinook [60 mm mean total length (MTL)] and coho salmon (60 mm MTL) were held for 9 and 21 months, respectively in floating net pens in a slough of the McKenzie River at the Weyerhaeuser Company pilot salmon hatchery near Springfield, Oregon to determine the natural transmission of *M. insidiosus*. A year later a second group of coho salmon (60 mm MTL) was held at the same site for 11 months (Table 1). Ten fish from each of these groups were examined at monthly intervals for the presence of *M. insidiosus*.

To test the susceptibility of other strains and species of salmonids to *M. insidiosus*, 125 chinook (80 mm MTL) and 100 coho salmon (69 mm MTL) and 100 cutthroat (146 mm MTL) and 200 rainbow trout (108 mm MTL) were placed in suspended cages in the Aumsville ponds for 8 d and returned to the FDL (Table 1). The fish were sampled at 36, 61, 67 d, and weekly thereafter, for 235 d. Control fish were kept at the FDL and examined at days 1 and 235. Day 1 for this and subsequent field exposure experiments began when fish were placed in the liveboxes.

To test different salmonid species for susceptibility to *M. insidiosus* at two sites, 200 fish lots of chinook (89 mm MTL), two strains of coho (59 and 77 mm MTL), kokanee (111 mm MTL), and

Table 1. Exposure of eight species of salmonids to *Myxobolus insidiosus* at three sites in Oregon.

Exposure site	Exposure period	Fish species	Mean Total length (mm)	Fish source-Hatchery
Mckenzie River slough	9 months	Chinook salmon	60	Weyerhaeuser
	21 months	Coho salmon	60	Weyerhaeuser
	11 months	Coho salmon	60	Weyerhaeuser
Aumsville Rearing Ponds	8 days	Chinook salmon	80	Bonneville
		Coho salmon	69	Fall Creek
		Cutthroat trout	146	Roaring River
		Rainbow trout	108	Oak Springs
McKenzie River power canal	8 days	Chinook salmon	89	Bonneville
		Coho salmon	59	Fall Creek
		Coho salmon	77	Speelyai
		Kokanee salmon	111	Wizard Falls
		Atlantic salmon	51	Wizard Falls
		Brown trout	49	Wizard Falls
		Cutthroat trout	141	Leaburg
		Rainbow trout	80	Wizard Falls
		Brook trout	147	Fall River
Aumsville Rearing Ponds	8 days	Chinook salmon	89	Bonneville
		Coho salmon	59	Fall Creek
		Coho salmon	77	Speelyai
		Kokanee salmon	111	Wizard Falls
		Atlantic salmon	51	Wizard Falls
		Brown trout	49	Wizard Falls
		Cutthroat trout	141	Leaburg
		Rainbow trout	80	Wizard Falls
		Brook trout	147	Fall River
Aumsville Rearing Ponds	50 days	Coho salmon	59	Fall Creek
		Coho salmon	77	Speelyai

Atlantic salmon (51 mm MTL), brook (147 mm MTL), brown (49 mm MTL), cutthroat (141 mm MTL), and rainbow trout (80 mm MTL) were placed in cages resting on the bottom of the EWEB power canal for 8 d (Table 1). One hundred and twenty-five fish from the same lots were also placed in suspended boxes in Aumsville ponds for 8 d (Table 1). Additionally, one lot (containing 125 fish) of each strain of coho salmon were held in suspended cages for 50 d at Aumsville (Table 1). After each exposure period the fish were transported to the FDL and kept in 12°C water. Fish held in the EWEB canal were examined at 79 d and weekly thereafter for 156 d; those from Aumsville ponds were examined at 75 d and weekly thereafter for 159 d. Five fish were examined at each period. Coho salmon kept at Aumsville ponds for 50 d were held at the FDL for another 100 d in 12°C water. At this time, 50 fish from each group were examined and the rest were acclimated in a 24 h period to 18°C water, maintained for an additional 43 d, killed, and examined. Control fish from each species were kept at the FDL and examined at day 1 and at the termination of individual experiments.

Parasitism of chinook salmon by *Myxobolus insidiosus*

Effect of exposure time

To test the effect of exposure length on infection with *M. insidiosus* 225 chinook salmon (73 mm MTL) were placed in a cage in Aumsville ponds for 0.25, 0.5, 1, 3, 6, 8, 12, 36, or 168 h. After

each exposure period, 25 fish were then removed from the livebox and placed in pathogen-free water for transport to the FDL. All fish were killed and examined at 124 d. Unexposed controls were also killed and examined at the same time.

Effect of water and substrate

In an attempt to transmit M. insidiosus in the laboratory, 3 L of water and 5 cm of mud obtained from Aumsville ponds during a period when fish could be infected with M. insidiosus were added to each of seven 3.7 L aerated glass containers. The containers were placed in separate aquaria containing 3, 6, 9, 12, 15, 18, or 21°C water. After 48 h, 10 chinook salmon (73 mm MTL) were added to each container and exposed for 48 h. The fish were then removed from the jars and held in aquaria with flowing water at the same temperature used for exposure. Twenty-five chinook salmon exposed for 8 d at Aumsville served as positive controls, whereas unexposed fish kept at the FDL served as negative controls. Fish held in water at 12, 15, 18, and 21°C were killed and examined at 111 d while those at 3, 6, and 9°C were examined for infection at 160 d because of slower parasite development at these lower water temperatures.

Effect of temperature

Fish infected with M. insidiosus were held at selected water temperatures to test the effect of temperature on parasite development. In trial 1, chinook salmon (80 mm MTL) exposed in Aumsville ponds for 8 d, were distributed at random into 15 68 L

aquaria containing water at 12°C. Unexposed control fish were distributed into five identical aquaria. Three aquaria containing a total of 150 experimental fish and one aquarium with 50 control fish were supplied with flowing water at 12°C. The remaining aquaria in groups of 4 (3 experimental, 1 control) were supplied with flowing water at temperatures of 6, 9, 15 or 18°C. Fish were acclimated to these temperatures during a 24 h period. Fish held at 18°C were examined at 38 and 43 d and every 7 d thereafter for 78 d. Fish from the other groups at 15, 12, 9, and 6°C were examined at 62 and 67 d, and every 7 d thereafter for 102, 116, 102, and 151 d, respectively until all fish were examined in each group.

A similar experiment was performed one year later (trial 2) with two additional temperatures, 3 and 21°C included. Chinook salmon (73 mm MTL) held in water at 21°C were examined at 56 d and every 7 d thereafter for 105 d. The other groups of fish at 18, 15, 12, 9, 6, and 3°C were examined at 64 d and every 7 d for 120, 127, 106, 134, 176, and 183 d, respectively.

In a further experiment, chinook salmon (76 mm MTL) were exposed in Aumsville ponds for 8 d. The fish were brought back to the FDL and held in 18°C water. After 14 d half of the fish were transferred to 21°C water while the remainder were kept in 18°C water. After an additional 70 d at these temperatures the fish were killed and examined for the presence of M. insidiosus.

Histological examination

Histological sections were prepared from fish parasitized by *M. insidiosus* to examine the development of parasitic stages and to determine host responses to the myxosporidan. Chinook salmon (80 mm MTL) held in Aumsville ponds for 8 d were examined for *M. insidiosus* at 36, 61, 67 d and every 7 d thereafter for 235 d. A cross section approximately 1 cm in length from each of the five fish examined at these time intervals was obtained. This tissue was cut from the area between the dorsal and adipose fins and placed in Bouin's fixative. After 72 h, the tissue samples were put through two 24 h 50% ethanol washes and stored in 70% ethanol. Later, the tissues were dehydrated, cleared in an ethanol-toluene series, and embedded in paraffin. Longitudinal sections, 10 µm thick, were stained with Giemsa for 45 min and examined microscopically.

Spore purification

To obtain spores for biochemical analysis, immunological procedures, and scanning electron microscopy (SEM), chinook salmon muscle heavily infected with spores of *M. insidiosus* was collected. After blending the muscle for 3 min, the homogenate was sieved through Tyler screens (175 and 88 µm pore size) and the filtrate centrifuged at 1464 x g for 10 min. The supernatant containing liquid and muscle tissue was decanted and discarded; the pellet containing spores and some tissue debris was resuspended in distilled water. This procedure was repeated five times and the spore mixture stored in distilled water at 4°C.

A 10-55% sucrose gradient was layered over 70% sucrose in a 150 ml centrifuge tube. The spore mixture was slowly added to the surface of the gradient and the preparation centrifuged at 1732 x g for 45 min. Spores were collected from a band in the gradient by pipette, washed in distilled water, pelleted by centrifugation at 1464 x g for 10 min, resuspended in distilled water, and added to a second sucrose gradient. After centrifugation, the spores were collected by pipette, washed twice as above and stored in distilled water at 4°C.

Spores from another myxosporidan, Ceratomyxa shasta, were obtained from the intestines of 13 steelhead trout infected in the Nehalem River, Oregon. The intestines were cut longitudinally and their contents placed in distilled water. The resulting mixture was treated as described for M. insidiosus.

Electron microscopy

To determine the ultrastructure of various stages of M. insidiosus, tissues taken from the fish used for histological examination were also prepared for electron microscopy. Muscle tissues were placed in Hawke's fixative and the procedure outlined by Rohovec and Amandi (1981) was followed. Fixative was decanted after 6 h and the tissues stored in buffer at 4°C. The samples were postfixed in osmium tetroxide for 1.5 h, dehydrated in a graded acetone series, infiltrated with Spurr's medium, embedded, cured, sectioned, and placed on grids. Examinations were done with a Phillips model 300 transmission electron microscope.

Spores of M. insidiosus for examination by SEM were purified as previously described. The spores were frozen in liquid nitrogen and critical point dried. The samples were then affixed to SEM specimen mounts, and examined with an International Scientific Instrument's Mini-SEM MSM-2 at 10 Kv accelerating potential and 100 µamp beam current.

Amino acid analysis of purified spore valves

In order to obtain pure spore valve preparations for amino acid analysis, purified spores of M. insidiosus or C. shasta were suspended in distilled water in a centrifuge tube immersed in an ice bath. Spore valves were separated by six 1 min periods of ultrasonic oscillation alternated with 2 min periods to allow for cooling of the suspension. The sonicate was centrifuged at 1464 x g for 10 min, the supernatant fluid discarded, and the pellet containing the spore valves resuspended in distilled water.

Nucleic acids were removed by the addition of 10 mg of ribonuclease and 5 mg of deoxyribonuclease (Sigma Chemicals, St. Louis, MO) to 20 ml of the spore valve suspension. The mixture was incubated for 24 h at 18°C, centrifuged at 1464 x g for 10 min, washed twice in distilled water, and frozen at -20°C.

Amino acid analysis of purified spore valves of M. insidiosus and C. shasta was done after a 24 h hydrolysis in 6N hydrochloric acid at 110°C. Cystine concentration was determined after performic acid oxidation of spore walls before acid hydrolysis. The tryptophan concentration was calculated after a 48 h alkaline hydrolysis at 135°C by the method of Hugli and Moore (1972).

Cultivation of *Myxobolus insidiosus*

In vitro cultivation of *M. insidiosus* was attempted to experimentally study the life cycle of this myxosporidan. In order to concentrate *M. insidiosus* spores the muscle of parasitized chinook salmon was homogenized, sieved, and centrifuged as previously described. The preparation was resuspended in a solution of 200 units/ml penicillin, 200 µg/ml streptomycin, and 300 units/ml mycostatin and incubated for 4 h at 20°C and then 24 h at 4°C. After incubation in the antibiotic solution spores were separated from the muscle by centrifugation in sucrose gradients as previously described. The spore fraction was washed twice in saline, resuspended in the antibiotic solution, incubated for 6 h at 20°C, centrifuged at 1464 x g for 10 min, and resuspended in a small volume of antibiotic solution. The spores were aseptically added to a sterile tissue homogenizer containing sterile alumina and ground by hand or with the aid of a motor. The resulting preparation was then added to flasks containing Eagle's minimal essential medium (MEM) with 10% calf serum and incubated at 15°C. The cultures were examined microscopically at frequent intervals for up to 90 d.

Immunological procedures

Antisera production

For preparation of antisera specific for either *M. insidiosus* or *C. shasta* purified spores of each myxosporidan were counted in a

hemocytometer and disrupted by passage through a pressure cell at 15-17,000 PSI. The disrupted preparations were mixed with equal parts Freund's complete adjuvant and injected into New Zealand white rabbits. Each rabbit received 0.3 ml in each rear foot between the two center toes and 2.4 ml subcutaneously in the interscapular region. Each of two rabbits received the antigenic components from 3.37×10^8 C. shasta spores. The M. insidiosus preparation consisted of 4.28×10^8 spores into each of two rabbits. Blood was collected 30 days later, the serum was removed, filter sterilized (0.45 µm), tested in thioglycollate broth, and stored frozen at -20°C.

Gel diffusion

The Ouchterlony method of gel diffusion was used to detect antigen-antibody reactions between specific antisera and myxosporidan antigens as outlined in Table 2. All muscle samples were suspended in borate buffered saline teased apart and sonicated for 1 min while the whole and broken spore preparations were obtained by previously described methods. The agar medium described by Garvey et al. (1977) was used. Ion agar No. 2 (Consolidated Laboratories Inc., Chicago Heights, IL) was added to borate buffered saline containing a final concentration of 0.01% thimerosol. Wells in the agar were punched with a Shandon template consisting of a 9 mm diameter central well surrounded by four 6 mm diameter peripheral wells located 7 mm from the central well.

Table 2. Immunodiffusion tests reacting anti-*Myxobolus insidiosus* or anti-*Ceratomyxa shasta* sera with various myxosporidan antigens extracted from six species of fish collected at several sites in Oregon and Washington.

Antiserum in Central Well	Antigen in Peripheral Wells	Antigen Source	Host Location
Myxobolus insidiosus	*M. insidiosus* spores	Chinook salmon	Aumsville Ponds
	M. insidiosus broken spores	Chinook salmon	Aumsville Ponds
	Ceratomyxa shasta spores	Steelhead trout	Nehalem River
	C. shasta broken spores	Steelhead trout	Nehalem River
C. shasta	*M. insidiosus* spores	Chinook salmon	Aumsville Ponds
	M. insidiosus broken spores	Chinook salmon	Aumsville Ponds
	C. shasta spores	Steelhead trout	Nehalem River
	C. shasta broken spores	Steelhead trout	Nehalem River
M. insidiosus	Uninfected muscle	Chinook salmon	Bonneville Hatchery
	Muscle with *M. insidiosus* trophozoites	Chinook salmon	Aumsville Ponds
	Muscle with *M. insidiosus* spores	Chinook salmon	Aumsville Ponds
	M. insidiosus spores	Chinook salmon	Aumsville Ponds
M. insidiosus	*M. insidiosus* spores	Chinook salmon	Aumsville Ponds
	C. shasta spores	Steelhead trout	Nehalem River
	Henneguya salminicola spores	Chinook salmon	Rogue River
	Myxosoma squamalis spores	Coho salmon	Siletz Hatchery
C. shasta	*M. insidiosus* spores	Chinook salmon	Aumsville Ponds
	C. shasta spores	Steelhead trout	Nehalem River
	H. salminicola spores	Chinook salmon	Rogue River
	M. squamalis spores	Coho salmon	Siletz Hatchery
M. insidiosus	Uninfected muscle	Chinook salmon	Bonneville Hatchery
	Muscle from exposed fish with no visible *M. insidiosus* trophozoites	Chinook salmon	Aumsville Ponds
	Muscle with *M. insidiosus* trophozoites	Chinook salmon	Aumsville Ponds
	Muscle with *M. insidiosus* spores	Chinook salmon	Aumsville Ponds

Table 2. (Concluded)

Antiserum in Central Well	Antigen in Peripheral Wells	Antigen Source	Host Location
M. insidiosus	*M. insidiosus* spores	Chinook salmon	Aumsville Ponds
	M. insidiosus broken spores	Chinook salmon	Aumsville Ponds
	Muscle with *M. insidiosus* spores	Chinook salmon	Aumsville Ponds
	Muscle with *Myxobolus* sp. spores	Northern squawfish	Willamette River
M. insidiosus	Uninfected muscle	Chinook salmon	Bonneville Hatchery
	Muscle with *M. insidiosus* spores	Chinook salmon	Aumsville Ponds
	Muscle with *M. insidiosus* spores	Chinook salmon	McKenzie Hatchery
	Muscle with *M. insidiosus* spores	Coho salmon	Speelyai Hatchery
M. insidiosus	Uninfected muscle	Chinook salmon	Bonneville Hatchery
	Muscle with *M. insidiosus* spores	Chinook salmon	Aumsville Ponds
	Muscle with *M. insidiosus* spores	Rainbow trout	Aumsville Canal
	Muscle with *M. insidiosus* spores	Cutthroat trout	Aumsville Canal

Immunofluorescence

The fluorescent antibody method was used to test the specificity of the two anti-myxosporidan sera prepared and to detect prespore stages of the parasites. The procedure of Banner et al. (1982) was used to directly label the anti-*C. shasta* serum. Anti-*C. shasta* rabbit serum was dialyzed in tris-sodium chloride buffer; then the dialysate was passed through a DEAE-affigel blue column. Purified IgG was collected and concentrated by ultrafiltration. The IgG was dialyzed in a bicarbonate-carbonate buffer, labeled with 10% celite-fluorescein isothiocyanate (Calbiochem-Behring Corporation, LaJolla, CA), and centrifuged to clarify the solution. The conjugate was passed through a Sephadex G-25 column, collected, and stored frozen at -20°C.

Intestinal scrapings, muscle preparations, or myxosporidan spores were placed on glass slides, air dried, and heat fixed. The preparations were treated by adding either labeled anti-*C. shasta* rabbit IgG for the direct fluorescence antibody test (DFAT) or unlabeled anti-*M. insidiosus* rabbit serum. The latter preparations were then exposed to isothiocyanate-labeled goat anti-rabbit IgG (Miles-Yeda Limited, Israel) for the indirect fluorescence antibody test (IFAT). The reactions were done in a dark humidified chamber for 10 min at 20°C. Antiserum was washed off with phosphate buffered saline (PBS), and the smears counterstained with 0.1% Evans blue in PBS. After a final wash with PBS, the slides were

dried by blotting and a drop of buffered glycerol (pH 8.0) mounting medium placed on the smear. A coverslip was added and the preparation examined microscopically for fluorescence at 500 and 1,000 X using a Zeiss standard microscope utilizing an IVF1 epi-fluorescence condensor, 12 V 100 watt halogen-tungsten light source, a KP450 excitation filter, an RT 510 chromatic splitter, and an LP 520 orange barrier filter.

RESULTS

Fish examination

Muscle tissue from 430 wild fish, belonging to seven families, was examined for M. insidiosus plasmodia and spores. Only Salmonidae (33 of 189) were found to harbor the parasite while members of Cyprinidae (128 fish), Cottidae (79 fish), Petromyzontidae (18 fish), Catostomidae (6 fish), Ictaluridae (4 fish), and Poeciliidae (4 fish) were not parasitized by this myxosporidan (Tables 3-7).

Cutthroat trout (100%), coho salmon (54%), and rainbow trout (25%) from three tributaries of the Yaquina River were parasitized by M. insidiosus (Table 3). In the Coos River system this parasite was only found in 24% of the rainbow trout examined while coho and chinook salmon were not infected (Table 4). Myxobolus insidiosus was present in rainbow (58%) and cutthroat trout (25%) collected in the Aumsville irrigation canal (Table 5) and all chinook salmon examined which had escaped from the Aumsville Rearing Ponds into this canal were heavily parasitized. Plasmodia and spores of this myxosporidan were found in 54% of the Roaring River Hatchery steelhead trout collected from Aumsville Lake, whereas; fish from this same group but remaining at the hatchery were negative for the parasite. No fish from the Rogue River system (Table 6) nor from the Willamette River were parasitized by M. insidiosus (Table 7). The host and geographic ranges of M. insidiosus are given in Figure 1.

Table 3. Examination of wild fish from the Yaquina River system for *Myxobolus insidiosus*.

Location	Fish Species	Total Length (mm) Mean (Range)	Number Examined	Number Parasitized
Yaquina River	*Rhinichthys osculus*	104 (90-130)	4	0
	Cottus sp.	93 (50-147)	5	0
	Lampetra sp.	94	1	0
Thornton Creek	*Cottus* sp.	72 (62-85)	5	0
	Salmo clarki	156	1	1
Little Elk Creek	*R. osculus*	52	2	0
	Oncorhynchus kisutch	112	1	0
Hayes Creek	*R. osculus*	81 (60-98)	4	0
	Cottus sp.	71 (56-85)	4	0
	S. clarki	141 (98-168)	4	4
	O. kisutch	110 (79-163)	10	7
	Salmo gairdneri	132	1	0
Wright Creek	*Cottus* sp.	68 (49-83)	26	0
	O. kisutch	125 (120-133)	3	1
	S. gairdneri	240	1	1
Tributary of Big Elk Creek	*R. osculus*	95 (92-98)	2	0
	Cottus sp.	71 (41-90)	5	0
	O. kisutch	142 (123-161)	2	0
	S. gairdneri	129 (126-132)	2	0

Table 4. Examination of wild fish from the Coos River system for *Myxobolus insidiosus*.

Location	Fish Species	Total Length (mm) Mean (Range)	Number Examined	Number Parasitized
Millicoma River East Fork	*Rhinichthys osculus*	61 (56-73)	4	0
	Cottus sp.	64 (46-74)	6	0
	Lampetra sp.	101 (82-114)	9	0
	Catostomus macrocheilus	103	1	0
	Richardsonius balteatus	65 (50-84)	9	0
	Oncorhynchus kisutch	62 (51-72)	4	0
	Salmo gairdneri	67 (62-72)	2	0
Coos River South Fork	*R. osculus*	66	1	0
	R. balteatus	68 (51-92)	5	0
Coal Creek	*Cottus* sp.	49 (46-51)	3	0
	S. gairdneri	116 (66-158)	11	6
	Salmo clarki	120 (117-122)	2	0
	Oncorhynchus tshawytscha	76 (62-83)	4	0
Cox Creek	*Cottus* sp.	66 (48-84)	4	0
	O. kisutch	84 (74-96)	7	0
	S. gairdneri	133 (72-197)	21	4
	O. tshawytscha	77 (76-78)	2	0
Marlow Creek	*R. osculus*	80 (67-104)	4	0
	Cottus sp.	101 (72-121)	5	0
	Lampetra sp.	130 (124-134)	3	0
	O. kisutch	73 (56-112)	15	0
	S. gairdneri	117 (99-136)	7	1
	S. clarki	137 (106-192)	12	0

Table 5. Examination of wild fish from the Aumsville irrigation canal for *Myxobolus insidiosus*.

Fish Species	Total Length (mm) Mean (Range)	Number Examined	Number Parasitized
Rhinichthys osculus	52 (51-53)	2	0
Cottus sp.	72 (44-116)	18	0
Lampetra sp.	108 (87-128)	5	0
Richardsonius balteatus	86 (72-94)	4	0
Gambusia affinis	27 (23-32)	4	0
Salmo gairdneri	127 (101-137)	12	7
Salmo clarki	161 (103-233)	8	2

Table 6. Examination of wild fish from the Rogue River system for *Myxobolus insidiosus*.

Location	Fish Species	Total Length (mm) Mean (Range)	Number Examined	Number Parasitized
Rogue River	*Oncorhynchus tshawytscha*	105 (96-120)	28	0
Applegate River	*O. tshawytscha*	98 (93-111)	25	0

Table 7. Examination of wild fish from the middle fork of the Willamette River[a] for *Myxobolus insidiosus*.

Fish Species	Total Length (mm) Mean (Range)	Number Examined	Number Parasitized
Ptychocheilus oregonensis	288 (249-362)	86	0
Catostomus macrocheilus	262 (241-290)	5	0
O. tshawytscha	288 (226-307)	3	0
Acrocheilus alutaceus	296	1	0
Ictalurus melas	226 (198-262)	4	0
Salmo gairdneri	201 (196-206)	2	0

[a]Fish were collected above the ladder in Dexter Ponds and in Dexter and Lookout Point Reservoirs.

Figure 1. Known host and geographic ranges of Myxobolus insidiosus in Oregon and southwest Washington.

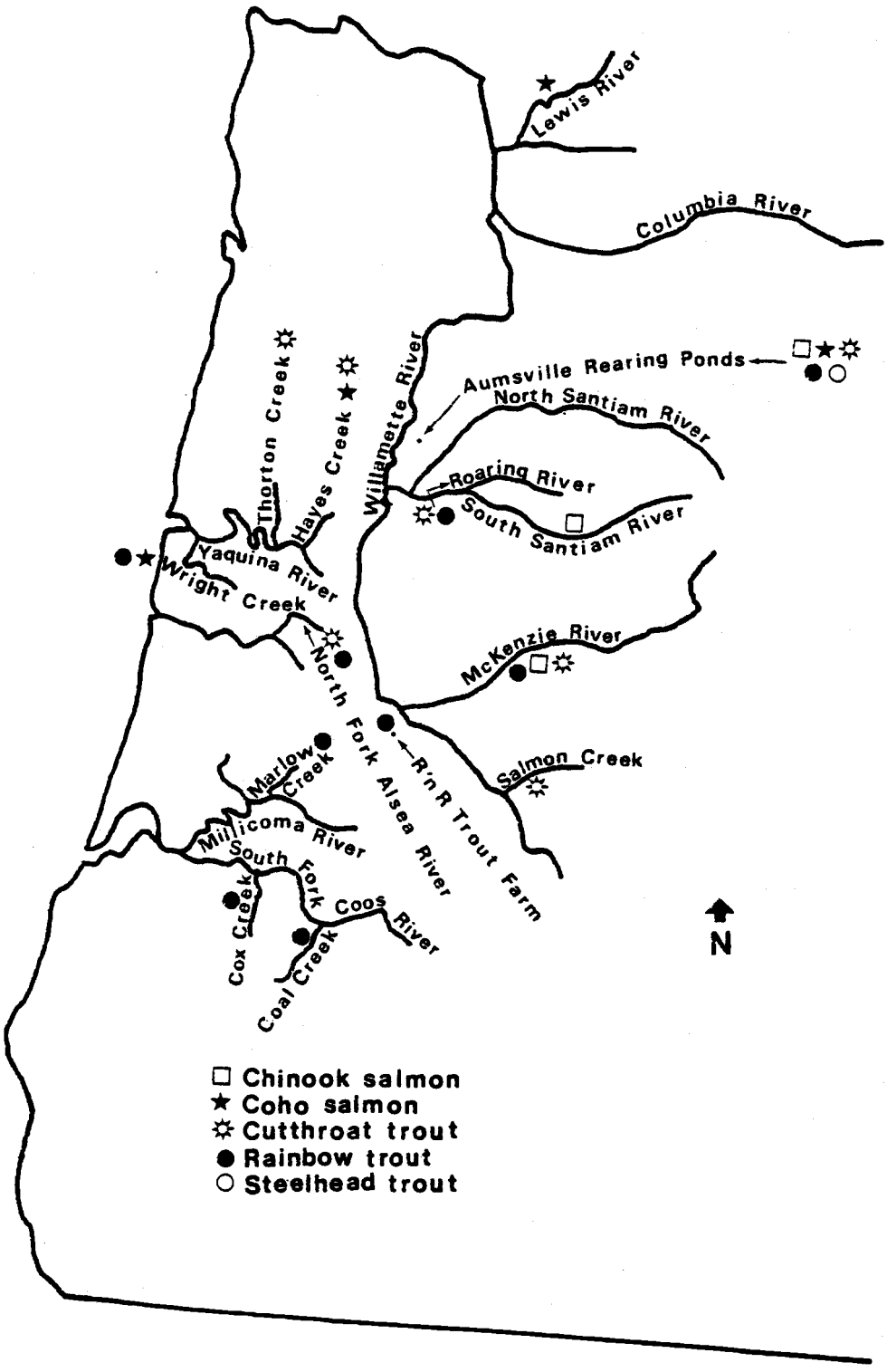

Lewis River
Columbia River
Aumsville Rearing Ponds
North Santiam River
Roaring River
South Santiam River
Willamette River
Thorton Creek
Hayes Creek
Yaquina River
Wright Creek
McKenzie River
North Fork Alsea River
R'n R Trout Farm
Salmon Creek
Marlow Creek
Millicoma River
South Fork Coos River
Cox Creek
Coal Creek
N
Chinook salmon
Coho salmon
Cutthroat trout
Rainbow trout
Steelhead trout

Figure 1

Variability in size and shape of M. insidiosus spores was found among different host species and within fish of the same species residing in the same or different streams (Table 8). A coho salmon harboring plasmodia of M. insidiosus with giant spores was collected in Hayes Creek (Yaquina River system). Mean measurements for these spores were 22.4 µm long (20.9-24.2 µm) by 16.1 µm wide (14.3-17.6 µm). Mean spore lengths in all other hosts ranged from 12.8 to 17.1 µm while mean widths varied from 8.0 to 11.9 µm (Table 8). Mean lengths of M. insidiosus polar capsules ranged from 6.8 µm to 10.0 µm while widths were 3.3 µm (Table 8). Polar capsule length was not associated with spore length. Spore shapes varied from oval to piriform and were consistent within individual hosts but not among all rainbow trout examined. Salmonids parasitized by M. insidiosus which were collected at Aumsville contained spores with less size variation than spores collected from fish in other sites (Table 8).

Plasmodia of M. insidiosus varied widely in size within the same and different host species. In rainbow trout collected in the Coos River system plasmodia averaged 148 µm long (63-170 µm) by 62 µm wide (45-84 µm) while those in the same species collected at Aumsville averaged 90 µm long (63-170 µm) by 42 µm wide (28-64 µm). Plasmodia in cutthroat trout from Aumsville averaged 70 µm long (51-90 µm) by 44 µm wide (27-56 µm) and those in steelhead trout from Aumsville Lake averaged 111 µm long (69-150 µm) by 57 µm wide (33-90 µm). Plasmodium shapes varied from oval (78 µm long by

Table 8. Spore dimensions of *Myxobolus insidiosus* from individual hosts collected in selected Oregon streams.

Host	Mean Total Host Length (mm)	Spore Length (µm) Mean (Range)	Spore Width (µm) Mean (Range)	Polar Capsule Length (µm) Mean (Range)	Polar Capsule Width (µm) Mean (Range)
Fish collected from Coal Creek-Coos River system					
Salmo gairdneri	158	15.0 (14.3-15.4)	9.5 (8.8-9.9)	7.7 (7.7)	3.3 (3.3)
S. gairdneri	112	13.5 (13.2-14.3)	8.0 (7.7-8.8)	7.7 (7.7)	3.3 (3.3)
S. gairdneri	104	12.8 (12.1-13.2)	10.6 (9.9-11.0)	7.5 (6.6-7.7)	3.3 (3.3)
Fish collected from Cox Creek-Coos River system					
S. gairdneri	143	15.4 (13.8-16.5)	11.4 (11.0-12.1)	7.8 (6.6-8.3)	3.3 (3.3)
S. gairdneri	123	14.5 (13.2-15.4)	10.8 (8.8-12.1)	6.8 (6.6-7.7)	3.3 (3.3)
S. gairdneri	90	13.0 (12.1-13.2)	9.7 (8.8-9.9)	7.5 (6.6-7.7)	3.3 (3.3)
Fish collected from Marlow Creek-Coos River system					
S. gairdneri	136	13.4 (13.2-14.3)	10.1 (9.9-11.0)	7.0 (6.6-7.7)	3.3 (3.3)
Fish collected from Thornton Creek-Yaquina River system					
Salmo clarki	156	13.9 (13.2-14.3)	10.1 (8.8-11.0)	7.1 (6.6-7.2)	3.3 (3.3)
Fish collected from Hayes Creek-Yaquina River system					
Oncorhynchus kisutch	118	22.4 (20.9-24.2)	16.1 (14.3-17.6)	10.3 (9.9-11.0)	4.8 (4.4-5.5)

Table 8. (Concluded)

Host	Mean Total Host Length (mm)	Spore Length (µm) Mean (Range)	Spore Width (µm) Mean (Range)	Polar Capsule Length (µm) Mean (Range)	Polar Capsule Width (µm) Mean (Range)
O. kisutch	114	14.0 (12.6-15.4)	10.3 (9.8-11.2)	a	a
Fish exposed in Aumsville Rearing Ponds					
O. kisutch	89	17.1 (15.4-17.6)	11.9 (11.0-13.2)	10.0 (8.8-11.0)	3.3 (3.3)
Fish collected from Aumsville canal and Aumsville Lake					
S. clarki	103	14.9 (14.3-15.4)	10.2 (8.8-11.0)	a	a
S. gairdneri	123	14.9 (13.2-15.4)	10.7 (9.9-11.0)	a	a
S. gairdneri	126	15.2 (13.2-16.5)	11.3 (9.9-13.2)	a	a
S. gairdneri	136	14.3 (13.2-15.4)	10.3 (9.9-11.0)	a	a
S. gairdneri (steelhead)	142	14.9 (14.3-15.4)	10.7 (9.9-11.0)	a	a
S. gairdneri (steelhead)	149	16.1 (15.4-16.5)	11.7 (9.9-12.1)	9.1 (8.8-9.9)	3.3 (3.3)

a - not measured

60 µm wide) to spindle shaped (170 µm long by 53 µm wide) and a range of shapes were found within individual cutthroat, rainbow, and steelhead trout. The majority of the M. insidiosus plasmodia found in these three species were located in the connective tissue between muscle bundles, but several plasmodia were also observed within the muscle bundles. As with chinook salmon all the plasmodia found in coho salmon (wild fish from the Yaquina River system, experimental fish from Aumsville Ponds, and yearlings obtained from Speelyai Hatchery) were within muscle bundles. Plasmodia of M. insidiosus in chinook salmon cleared with potassium hydroxide appeared to be present in the entire musculature when whole fish were examined. The plasmodia were seen as parallel, opaque bands located along the length of individual muscle bundles (Figure 2).

Myxobolus sp. in northern squawfish muscle tissue

Plasmodia and spores of a myxosporidan closely resembling one previously described from fish in Montana as Myxobolus muelleri (Mitchell 1970) were found in the musculature of adult northern squawfish (Ptychocheilus oregonensis) collected in the Willamette River, Oregon. The name M. muelleri has not been accepted for this myxosporidan (personal communication, Dr. L. G. Mitchell, Dept. of Zoology, Iowa State Univ., Ames, Iowa) and thus the parasite will be referred to as Myxobolus sp. in this text. Sixty-three percent (54 of 86) of the squawfish examined contained this myxosporidan.

Seventy-four percent (52 of 70) of the parasitized fish were found immediately below Dexter Dam while 20% (1 of 5) and 9% (1 of 11) of the parasitized squawfish were in Dexter and Lookout Point reservoirs, respectively. The fish collected below Dexter Dam were trapped at the ODFW Dexter Ponds ladder. Mitchell (1970) observed this parasite in various catostomid organs. None of the five Catostomus macrocheilus examined from the Willamette River contained the myxosporidan in muscle and no other tissues were examined. Spores were also found by Mitchell in the kidney of all squawfish containing muscle plasmodia. No tissues other than muscle were examined in the Willamette River adult squawfish and no juvenile squawfish were captured in the Willamette River.

Early developmental stages of Myxobolus sp. were not found but trophozoites in various stages of sporulation were detected (Figure 3). Plasmodia of this myxosporidan were spindle shaped (Figure 4) and averaged 264 µm long (216-340 µm) by 49 µm wide (40-68 µm). Two plasmodia, which were excluded from the above mean, measured 0.56 and 1.94 mm in length. These were probably composed of two or more overlapping plasmodia. The majority of the Myxobolus sp. plasmodia were found within muscle bundles (Figure 4) with a few occurring in the connective tissue between muscle bundles (Figure 5). Melanin granule deposition was associated with several of the plasmodia found in the squawfish (Figure 6).

Myxobolus sp. spores obtained from the squawfish averaged 13.2 µm long (12.1-16.5 µm) by 10.1 µm wide (8.8-11.0 µm). The spores

contained polar capsules of unequal sizes (Figure 7). The larger capsule was 7.3 µm long (6.6-8.8 µm) by 4.0 µm wide (3.3-4.4 µm) while the smaller capsule was 6.5 µm long (5.5-7.7 µm) by 3.2 µm wide (2.2-4.4 µm). Several indentations were visible along the internal surface of the spore valves (Figure 8). An iodinophilous vacuole was detected within the sporoplasm when the spores were treated with Lugol's iodine. No aberrant spores or spores with tails or other valvular projections were noted. No mucus envelope was discernible on fresh or frozen spores after treatment with India ink.

The Myxobolus sp. found in northern squawfish is similar to the one described by Mitchell (1970) from five species of fish, including the northern squawfish, in Montana. Similarities of the myxosporidan isolates include the presence of interfibrillar and intrafibrillar plasmodia, unequal polar capsules, and spore valve indentations. Spore and plasmodial sizes and shapes were also correlated. Several differences were noted between the Willamette River Myxobolus sp. and that described by Mitchell (1970). No giant Myxobolus sp. spores similar to those described from Montana were seen in squawfish from the Willamette River and neither were there any spores with tails, envelopes, or other valvular extensions. The association of melanin granules with several of the Willamette River Myxobolus sp. plasmodia was not reported for the Montana myxosporidan.

Figure 2. *Myxobolus insidiosus* plasmodia (arrows) in muscle bundles of chinook salmon (*Oncorhynchus tshawytscha*) cleared with potassium hydroxide, 25 X.

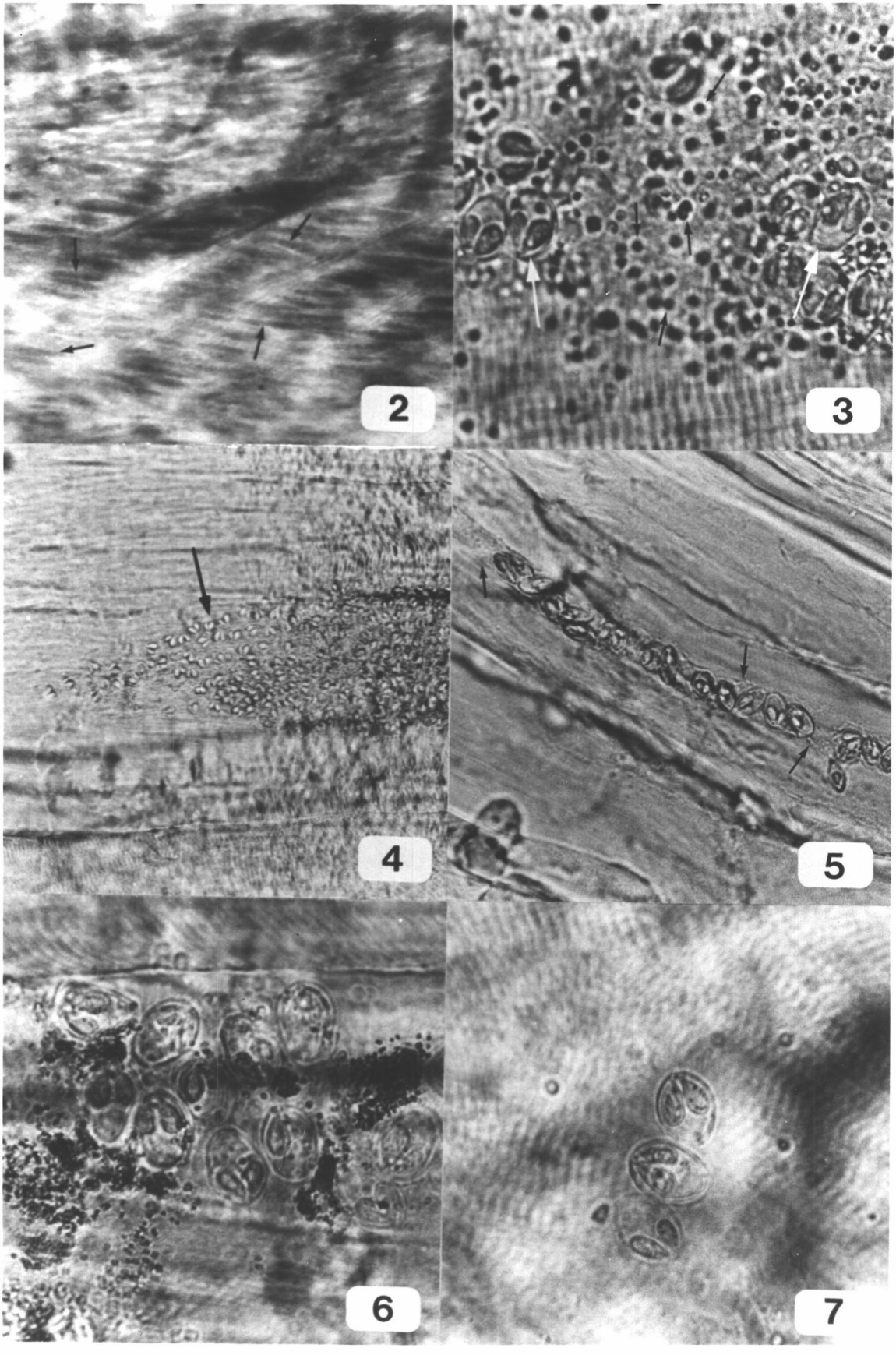
Figure 3. *Myxobolus* sp. trophozoite in northern squawfish (*Ptychocheilus oregonensis*) muscle tissue. Note the presence of spores (white arrows) and nuclei of nonsporulated stages (black arrows), 1,000 X.

Figure 4. Spindle shaped plasmodium of *Myxobolus* sp. (arrow) within a muscle bundle of northern squawfish (*Ptychocheilus oregonensis*), 125 X.

Figure 5. Plasmodium of *Myxobolus* sp. in connective tissue separating muscle bundles of northern squawfish (*Ptychocheilus oregonensis*). Note that the plasmodium is completely enclosed in the connective tissue strand (arrows), 250 X.

Figure 6. Melanin granules associated with a mature plasmodium of *Myxobolus* sp., 1,000 X.

Figure 7. *Myxobolus* sp. spores containing polar capsules which are typically unequal, 1,000 X.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Parasitism of selected salmonid species by Myxobolus insidiosus

Twenty-three percent of the chinook salmon exposed for 9 months in a slough of the McKenzie River became lightly parasitized by M. insidiosus, but the myxosporidan was not detected in two groups of coho salmon reared at the same site for 11 and 21 months, respectively. Exposure of fish in the EWEB canal for 8 d resulted in 10% of the chinook salmon and one rainbow trout becoming parasitized by M. insidiosus, whereas brook, brown, and cutthroat trout, kokanee, Atlantic, and both stocks of coho salmon were not found to harbor the parasite.

At Aumsville Ponds all of the chinook salmon placed in suspended cages for 8 d during the initial experiment became parasitized by M. insidiosus, whereas similarly exposed coho salmon and rainbow and cutthroat trout remained uninfected. In the second experiment at Aumsville 100% of the chinook salmon became parasitized with M. insidiosus. The myxosporidan was not detected in brook, brown, rainbow, and cutthroat trout, nor in kokanee, Atlantic, and two stocks of coho salmon, exposed for the 8 d period. Three of 50 Speelyai Hatchery coho salmon exposed for 50 d at Aumsville and maintained in 12°C water for an additional 100 d at the FDL contained a few M. insidiosus plasmodia. The parasite was not found in 50 Fall Creek Hatchery coho salmon treated identically to the Speelyai fish. The detection rate or parasite load also did not increase in coho salmon exposed for 50 d then held for 100 d at 12°C, followed by 43 d in 18°C water (193 d total

period). After this interval, a few M. insidiosus plasmodia were detected in only 1 of 39 Speelyai Hatchery and 1 of 65 Fall Creek Hatchery coho salmon.

Parasitism of chinook salmon by Myxobolus insidiosus

Effect of exposure time

Chinook salmon exposed for periods of 0.25, 0.5, 1, 3, or 6 h at Aumsville were not parasitized by M. insidiosus. One fish exposed for 8 h and one exposed for 12 h became lightly infected with the myxosporidan. Fish exposed for 36 h were parasitized when examined at the conclusion of the experiment. Chinook salmon exposed for 168 h were heavily infected with the myxosporidan.

Effect of water and substrate

When exposed for 48 h to water and canal substrate collected at Aumsville one chinook salmon became parasitized by M. insidiosus. This fish was exposed to water and substrate maintained at 18°C and held for 111 d in water of the same temperature. The myxosporidan was not detected in chinook salmon similarly exposed to water and mud at 3, 6, 9, 12, 15, or 21°C and held at the same temperature as that for exposure.

Effect of temperature

Development of M. insidiosus was temperature dependent and occurred in chinook salmon maintained at water temperatures of 6-18°C (Table 9). In trial 1, parasite development occurred in fish held in 6, 9, 12, 15, and 18°C water. Trophozoites were detected

Table 9. Trophozoite and spore development of *Myxobolus insidiosus* in chinook salmon (*Oncorhynchus tshawytscha*) exposed for 8 days at Aumsville Rearing Ponds, Oregon and held at selected temperatures in pathogen-free water.

Water Temperature (°C)	Initial Trophozoite Observation (Days Post-Exposure)	Initial Spore Observation (Days Post-Exposure)	Termination (Days Post-Exposure)
Trial 1			
18	44	71	na
15	68	81	na
12	69	88	na
9	88	a	102
6	123	b	151
Trial 2			
21	c	c	105
18	59	71	na
15	62	85	na
12	64	95	na
9	113	a	134
6	c	c	176
3	c	c	183

na - Not applicable.
a - Fish died before spores were detected.
b - All fish examined before spores were detected.
c - None detected.

first in fish held in 18°C water and last in those held in 6°C water. Sporulation occurred first in fish held in 18°C water and last in those held in 12°C water. No spores were detected in plasmodia of fish held in 6 and 9°C water during the 151 and 102 d respective examination periods (Table 9). No myxosporidan development was detected in fish held in 3, 6, and 21°C water in trial 2, whereas the pattern of trophozoite and spore development was similar to that in trial 1 for fish held in 9, 12, 15, and 18°C water (Table 9).

Myxobolus insidiosus parasitism occurred in chinook salmon exposed for 8 d at Aumsville and then held at the FDL in 18°C water for 84 d. Fish separated from the above group after being in 18°C water for 14 d and then placed in 21°C water for an additional 70 d were negative for the myxosporidan.

Myxobolus insidiosus development

Various developmental stages of M. insidiosus were detected by light microscopy examination of chinook salmon muscle wet mounts. These stages were similar to those described for other myxosporidans (Davis 1916, 1923; Dunkerly 1925; Mitchell 1977). A few binucleated amoeboid cells which did not appear embedded in the muscle tissue were found at the earliest period of parasite detection (Figure 9). The earliest encysted stages observed and detected concurrently with the amoeboid cells, were binucleated or quadrinucleated trophozoites found in spindle shaped plasmodia

within the muscle bundles. Trophozoite growth by cell division and elongation of the plasmodium then occurred over a period of several days to form multicellular stages (Figures 10-11). No host reaction to any of these early stages was detected.

Formation of polar capsules and the presence of faint spore valve outlines indicated early stages of sporulation (Figure 12). In early sporulation the disporoblastic nature of M. insidiosus was noted (Figure 13). Sporulation continued with the numbers of spores increasing with time (Figure 14) until mature plasmodia full of spores were present (Figure 15). In several instances, aberrations were seen in which a part of a plasmodium did not develop whereas the rest of the plasmodium sporulated. Several plasmodia appeared to be deteriorating and were being replaced by mesenchymal cells at a time when mostly mature plasmodia were present in the musculature (Figure 16).

Histological examination

The development of M. insidiosus was observed in histological sections of parasitized chinook salmon muscle tissue. No unencysted amoeboid stages were found in any of the sections examined. A trophozoite containing two cells (probably the same stage observed as a binucleated trophozoite in wet mount observations) located in a plasmodium within a muscle bundle was the first stage detected (Figure 17). Tissue displacement with no apparent host reaction was noted around these early stages of the parasite (Figure 18). Development continued by repeated nuclear

Figure 8. Indentations (arrows) along the internal surface of the spore valves of Myxobolus sp., 1,250 X.

Figure 9. Binucleated amoeboid cell on muscle tissue of chinook salmon (Oncorhynchus tshawytscha). Possibly the earliest detected stage of Myxobolus insidiosus, 1,000 X.

Figure 10. Early trophozoite stages of Myxobolus insidiosus. Nuclei (arrows) are visible within the plasmodium, 1,000 X.

Figure 11. Myxobolus insidiosus spindle shaped plasmodium containing a multicellular trophozoite, 400 X.

Figure 12. Early sporulation stages of Myxobolus insidiosus. Faint spore valve outlines and polar capsules are present (arrows), 400 X.

Figure 13. Disporoblast of Myxobolus insidiosus (arrow) in a trophozoite beginning to sporulate, 400 X.

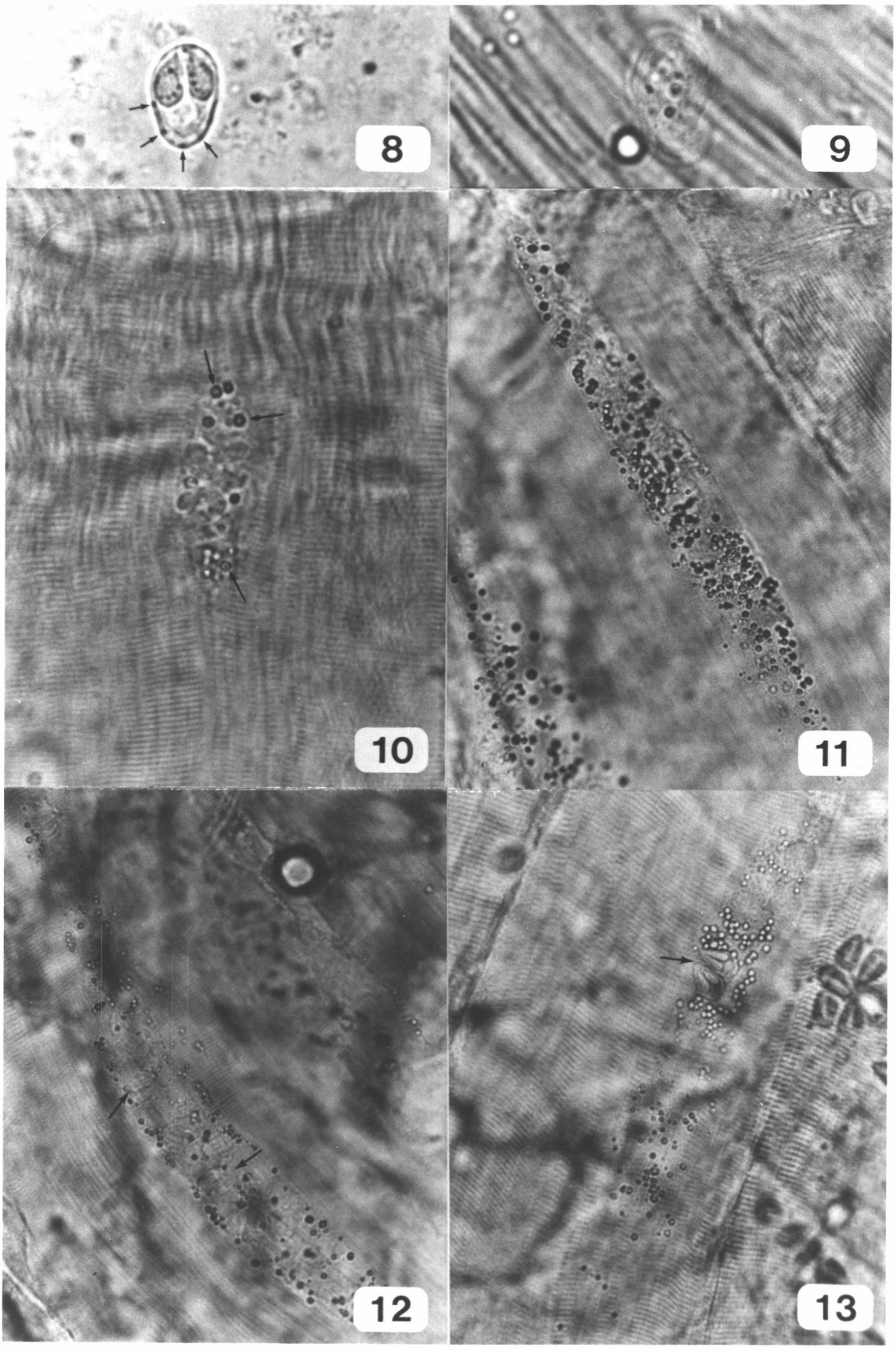
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Figure 14. Sporulating plasmodium of Myxobolus insidiosus containing spores (white arrows) and undifferentiated cells (black arrows), 400 X.

Figure 15. Mature plasmodia (P) of Myxobolus insidiosus filled with spores, 250 X.

Figure 16. Deteriorating plasmodium of Myxobolus insidiosus with degraded spores (arrows), 1,000 X.


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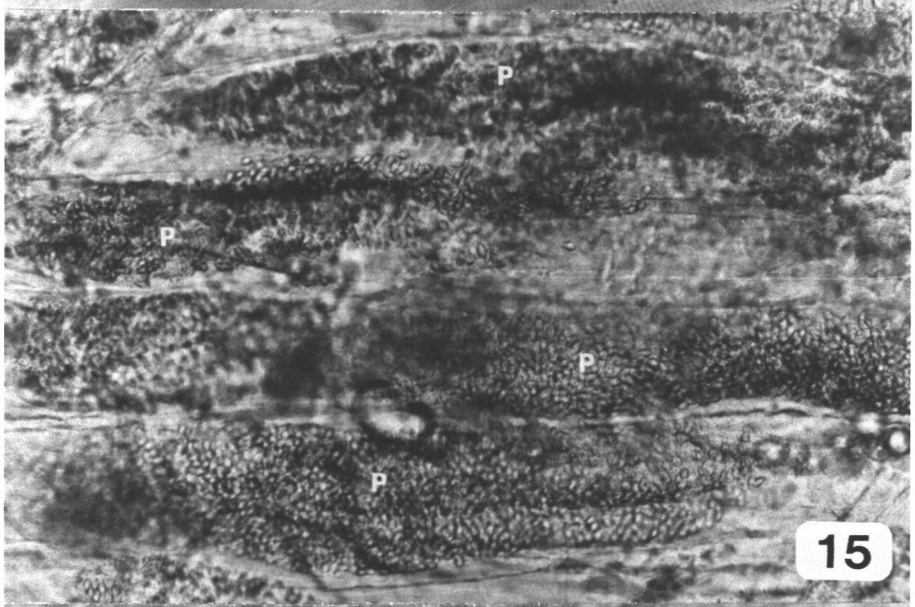
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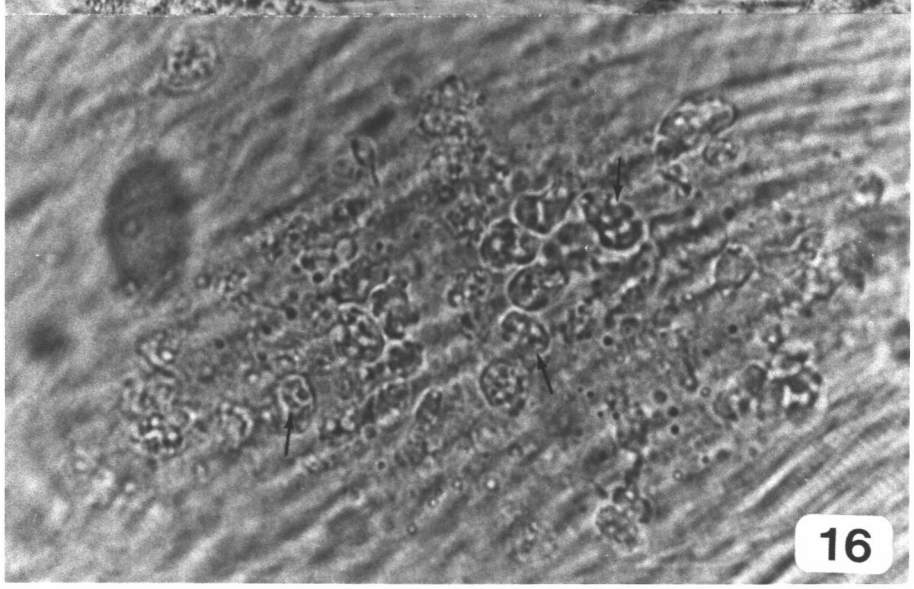
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divisions and cytoplasmic growth of the parasite (Figures 19-21) finally ending with a multicellular trophozoite (Figure 22). During growth of the trophozoite, generative cells divided and differentiated into multicellular sporonts each of which contained two sporoblasts. Maturation of the sporonts resulted in spore formation and mature M. insidiosus plasmodia (Figure 23). As previously noted, some trophozoites do not sporulate completely with one end remaining in the multicellular stage while spores are formed at the opposite end of the plasmodium (Figure 24).

Sporulation of M. insidiosus in chinook salmon held in 12°C water started at approximately 88 d post exposure and was almost complete by 100 d. After a period of time (100-120 d), in which only sporulating plasmodia were present, trophozoites were again detected and were found in fish until the end of the experiment (173 d) (Figure 25). In some cases the trophozoites were found adjacent to mature plasmodia within the same muscle bundle (Figure 26). Myxobolus insidiosus plasmodia were found scattered throughout the white muscle bundles of chinook salmon, whereas none were detected in the red muscle bundles (Figures 27, 28).

No host reaction to trophozoites or sporulating stages of M. insidiosus was detected in chinook salmon held in 12°C water, whereas a host response to mature plasmodia was observed after 102 d post exposure. In most cases, single plasmodia became enlarged and infiltrated by host cells (Figure 29) but occasionally groups of plasmodia in several muscle bundles became affected

Figure 17. Binucleated trophozoite of Myxobolus insidiosus, the first parasite stage detected in histological sections. Giemsa stain, 1,250 X.

Figure 18. Muscle tissue displacement (arrow) associated with growth of Myxobolus insidiosus plasmodium. Giemsa stain, 1,250 X.

Figure 19. Eight cell stage of Myxobolus insidiosus, note the arrangement of the cells into two groups. Giemsa stain, 500 X.

Figure 20. Developing Myxobolus insidiosus trophozoite. Plasmodium elongates as further cell division occurs, but cell groups are still evident. Giemsa stain, 500 X.

Figure 21. Myxobolus insidiosus plasmodia filled with undifferentiated parasite cells. Giemsa stain, 500 X.

Figure 22. Sporulating Myxobolus insidiosus plasmodium. Two spores (arrows) can be seen on the edge of the multicellular trophozoite. Giemsa stain, 1,000 X.

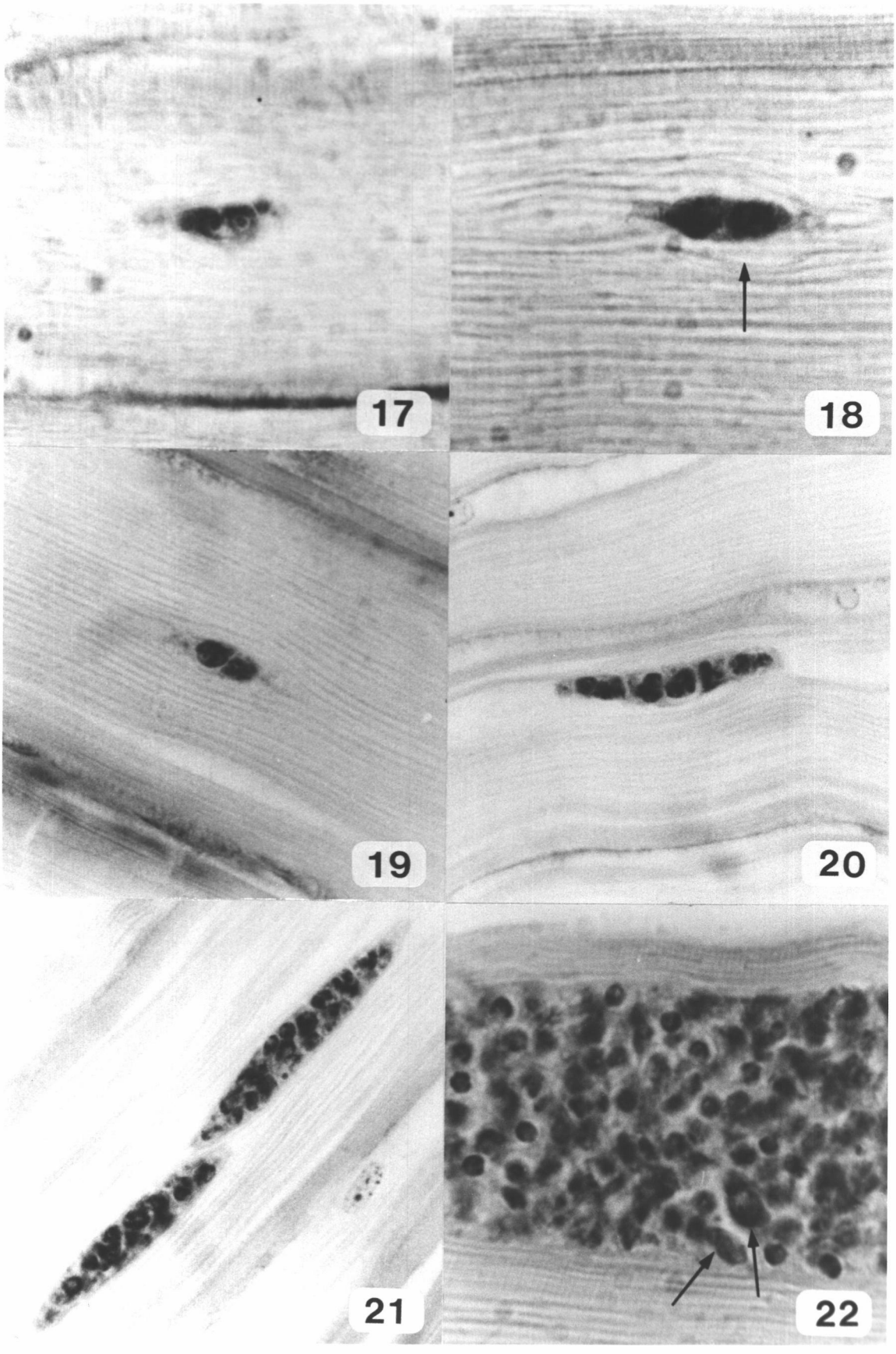
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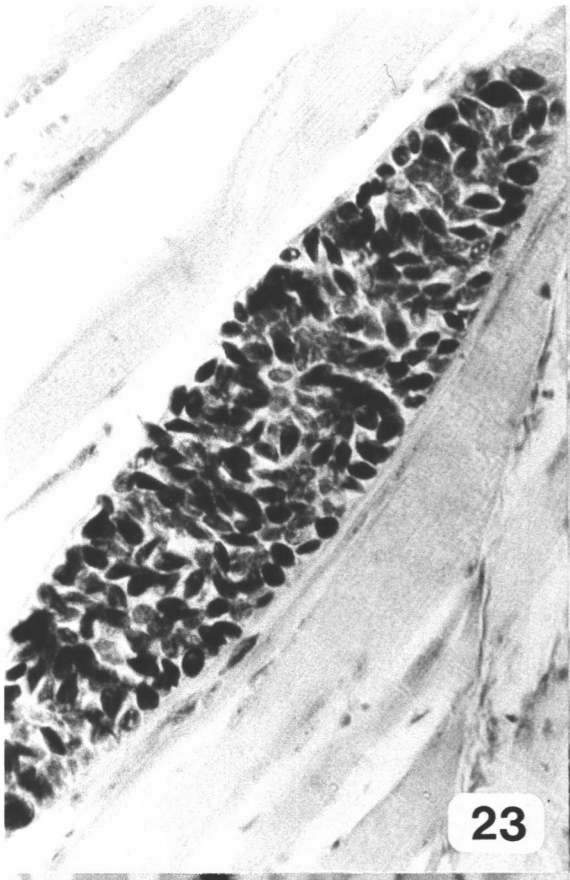
Figure 23. Mature Myxobolus insidiosus plasmodium containing only spores. Giemsa stain, 500 X.

Figure 24. Myxobolus insidiosus trophozoite with an undeveloped multicellular section (white arrow) at one end and mature spores (black arrow) at the other. Giemsa stain, 500 X.

Figure 25. Section of chinook salmon (Oncorhynchus tshawytscha) muscle tissue containing a mature plasmodium (white arrow) and two newly formed plasmodia (black arrows) of Myxobolus insidiosus with early trophozoite stages. Giemsa stain, 125 X.

Figure 26. Mature (white arrow) and immature (black arrow) plasmodia of Myxobolus insidiosus within the same muscle bundle of chinook salmon (Oncorhynchus tshawytscha). Giemsa, 125 X.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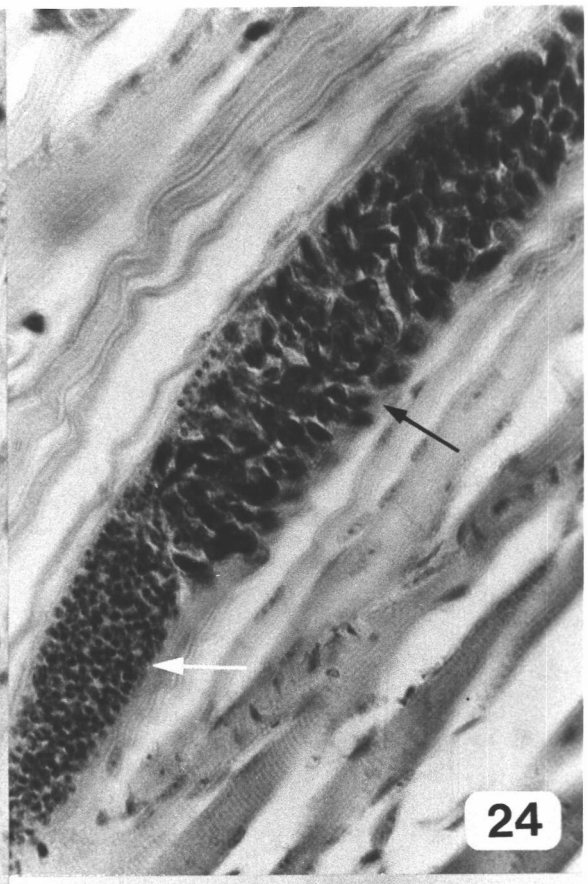
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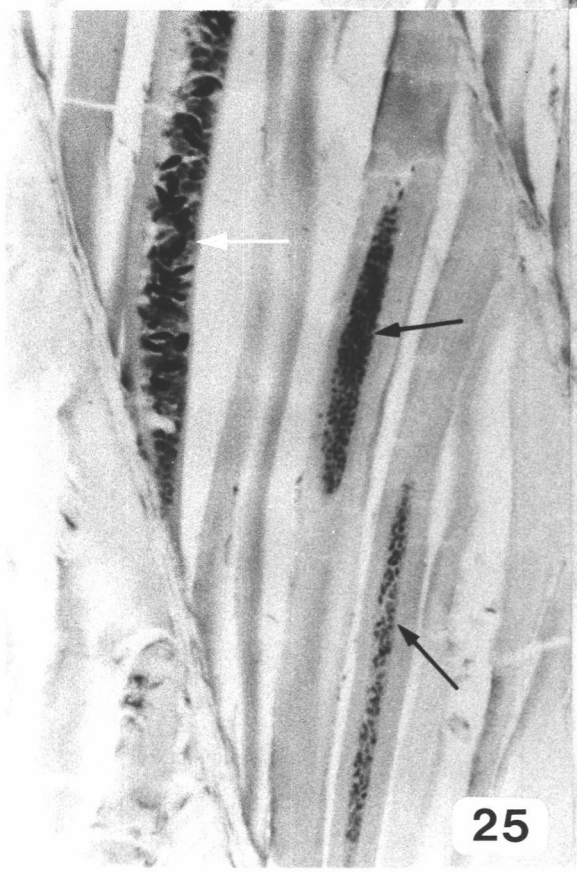
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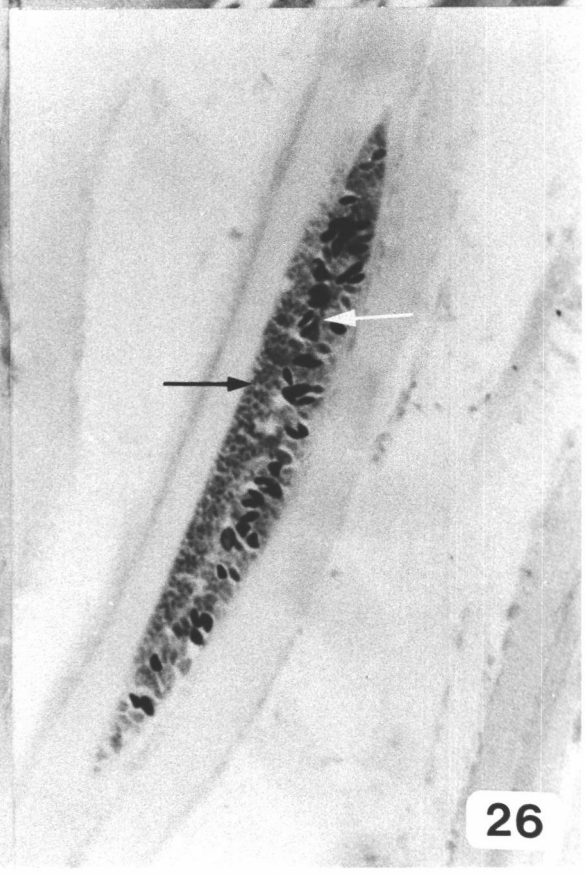
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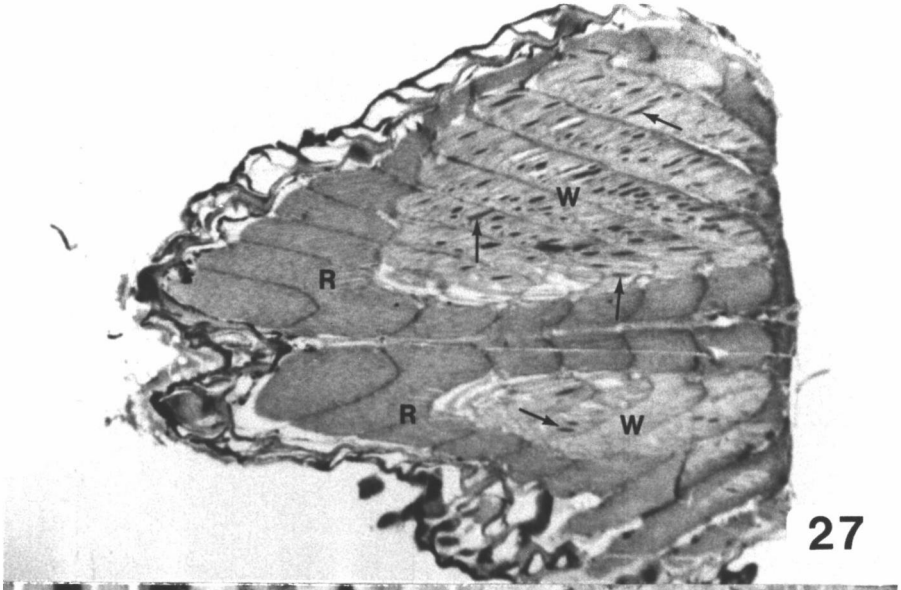
Figure 27. Myxobolus insidiosus plasmodia (arrows) in the white muscle tissue (W) of chinook salmon (Oncorhynchus tshawytscha). Note the absence of parasites in the red muscle tissue (R). Giemsa stain, 84 X.

Figure 28. White (W) and red muscle tissue (R) of chinook salmon (Oncorhynchus tshawytscha). Plasmodia of Myxobolus insidiosus (arrows) are present exclusively in white muscle. Giemsa, 125 X.

Figure 29. Enlarged plasmodium of Myxobolus insidiosus infiltrated by host tissue (white arrow). Note the normal size of unaffected plasmodia (black arrow). Giemsa, 125 X.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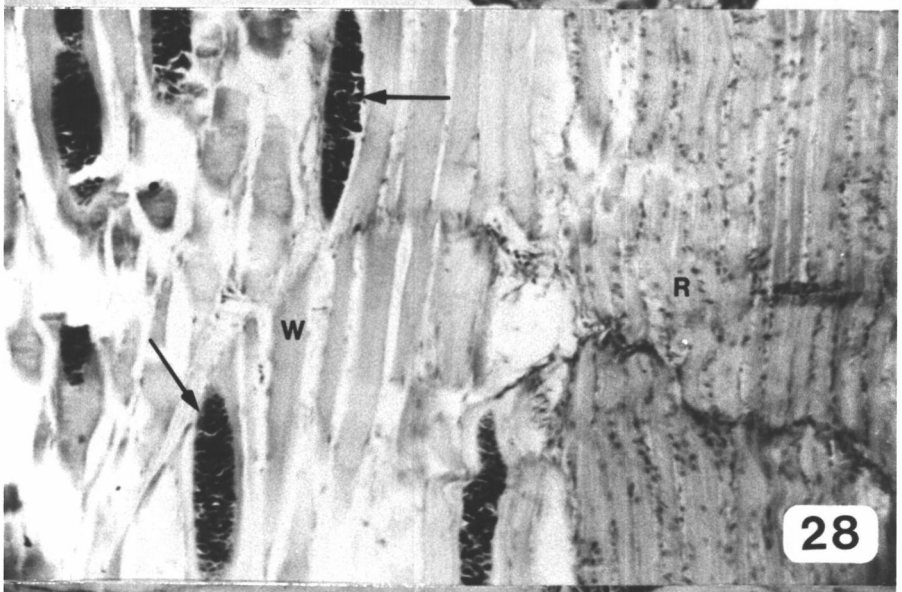
W
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(Figure 30). In some instances a few spores could still be found (Figure 31) but in the majority of the affected plasmodia no remains of spores were detected. The composition of the enlarged plasmodia was variable. Some were filled with an amorphous matrix, a few blood cells and mesenchymal cells, whereas the presence of fibroblasts was noted in others. A few plasmodia did not contain a matrix and were instead composed of orderly arrays of host cells (Figure 32).

Ultrastructure of *Myxobolus insidiosus*

Transmission electron microscopy

Sporogenesis of *M. insidiosus* was asynchronous and resulted in the formation of disporoblasts. Vegetative cells were found in early undifferentiated plasmodia (Figure 33). The first recognizable sporogenic stages were spherical generative cells containing several large mitochondria, a Golgi apparatus in close proximity to the nuclear membrane, and numerous cytoplasmic ribosomes. Generative cells had an eccentric nucleolus, a narrow zone of chromatin directly beneath the well developed nuclear membrane, and small aggregations of chromatin scattered throughout the nucleoplasm (Figure 34). Myeloid figures typical of many myxosporidans were observed at this stage of development (Figure 35).

The first cellular association in the sporogenic process occurs when two generative cells come in close contact with each

Figure 30. Group of Myxobolus insidiosus plasmodia being infiltrated by chinook salmon (Oncorhynchus tshawytscha) cells. Giemsa stain, 125 X.

Figure 31. Infiltrated plasmodium of Myxobolus insidiosus with a few intact spores (arrows) remaining. Giemsa stain, 500 X.

Figure 32. Orderly array of host cells within an infiltrated Myxobolus insidiosus plasmodium. Giemsa stain, 500 X.


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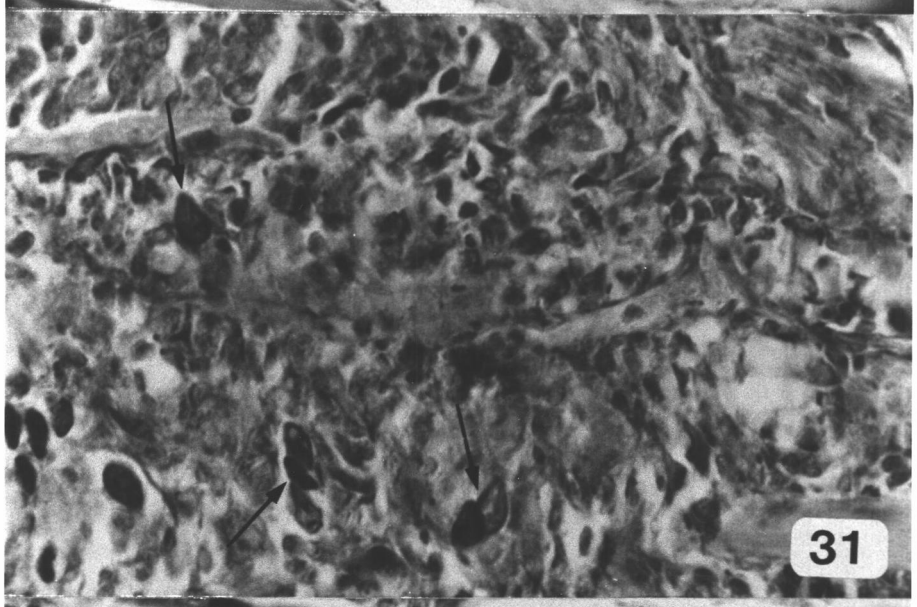
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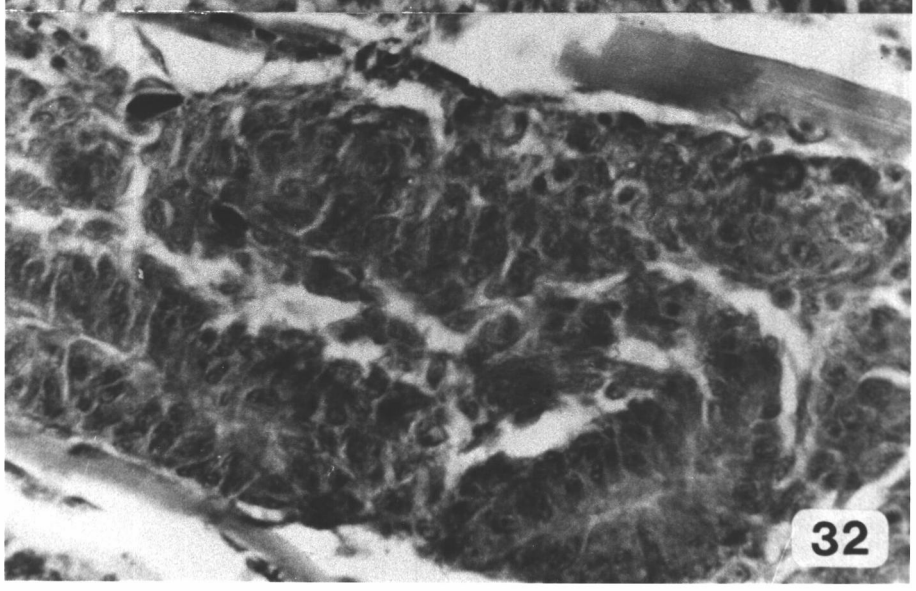
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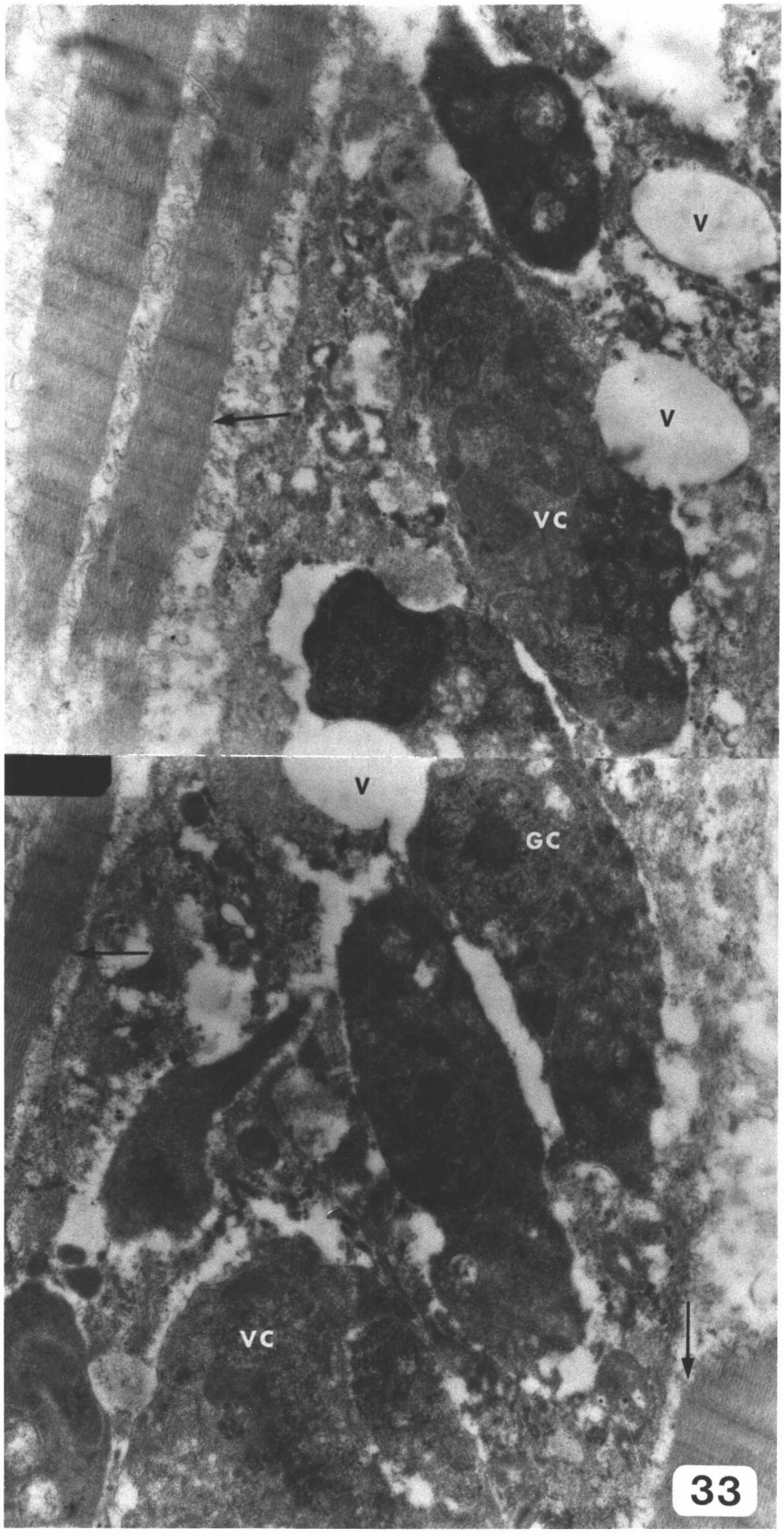
Figure 33. Early stage of development of a Myxobolus insidiosus plasmodium. Note the presence of normal muscle tissue (arrows) in proximity to the plasmodial wall (PW). Generative cells (GC), vegetative cells (VC), and several vacuoles (V) are present in the plasmodial endoplasm, 6,500 X.


V
V
VC
V
GC
VC
33

other (Lom and DePuytorac 1965a). Such pairs of generative cells were observed in early trophozoites of M. insidiosus (Figure 36). The smaller of these cell pairs became the envelope cell while the larger cell, destined to form the sporont, divided repeatedly to form the disporoblast. No host tissue reaction to immature plasmodia or plasmodia which contained pansporoblast stages was noted (Figure 37).

A section through a developing plasmodium showed the disporoblastic nature of M. insidiosus. Peripherally located valvogenic cells enclose capsulogenic cells and a sporoplasm. For each spore produced, each of a pair of valvogenic cells surrounds the inner cells and forms half of the spore valve. Where the valvogenic cells meet thickened crests form and crests from apposing cells are joined by a continuous septate junction forming the sutural ridge and suture line. Two capsulogenic cells, each containing a large capsular primordium are enclosed by a pair of valvogenic cells. A portion of the envelope cell cytoplasm is also visible at this stage (Figure 38).

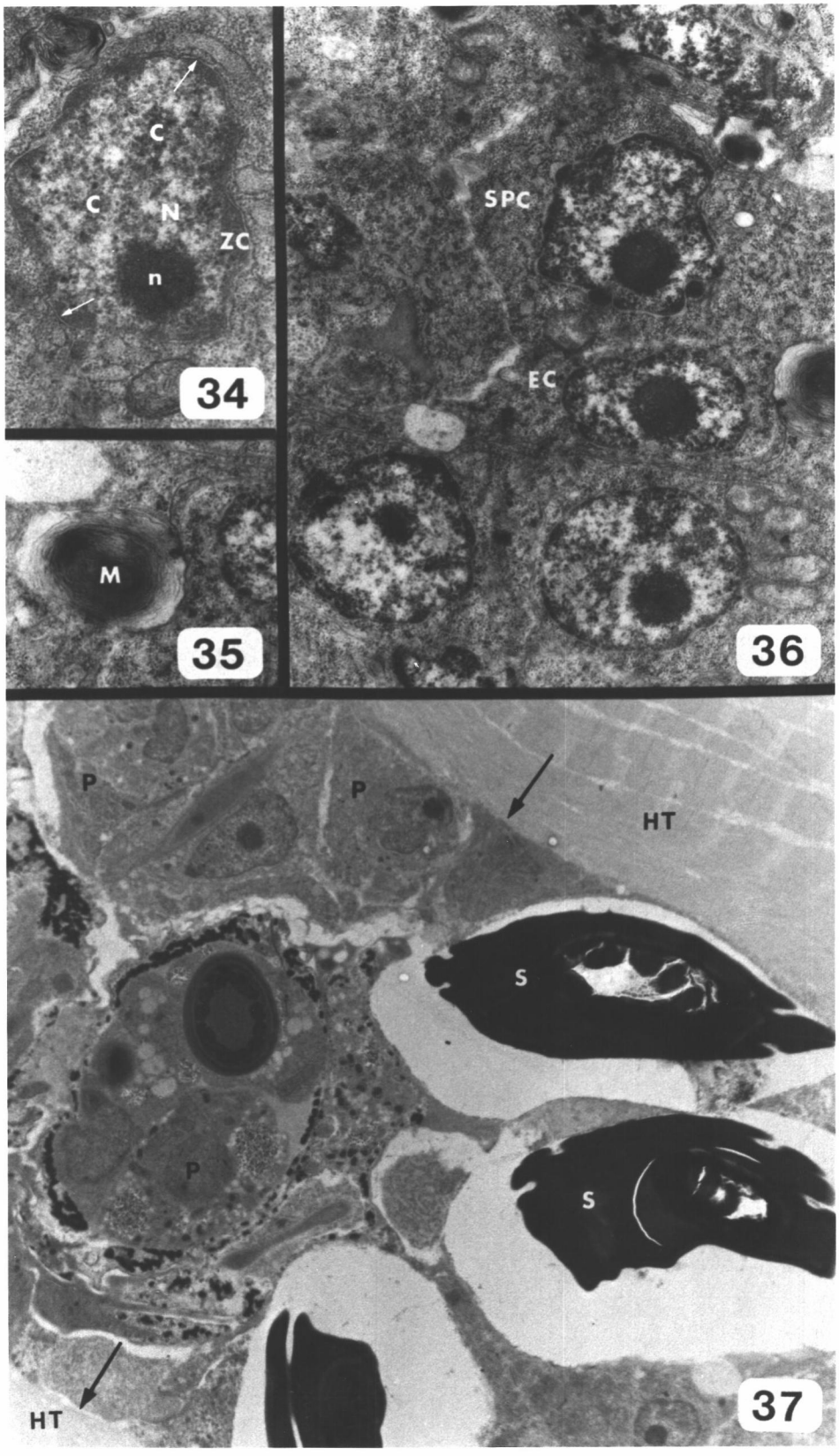
The capsular primordia are enclosed by a membrane which overlies three zones: an electron lucent zone followed by a moderately electron dense zone, and finally another electron lucent zone (Figure 39). These zones surrounded the homogeneous granular matrix of the capsular primordia which usually contains an electron dense inclusion (Figure 40). During early capsulogenesis external tubules are present in the cytoplasm of the capsulogenic cells

Figure 34. Myxobolus insidiosus generative cell nucleus (N) containing an eccentric nucleolus (n), a narrow chromatin zone (CZ) beneath the nuclear membrane (arrows), and small aggregations of chromatin (C) scattered throughout the nucleus, 10,000 X.

Figure 35. Myeloid figure (M) present in many early trophozoites of Myxobolus insidiosus, 8,000 X.

Figure 36. A closely associated pair of Myxobolus insidiosus generative cells. The larger cell is the sporont precursor (SPC) while the smaller cell becomes the envelope cell (EC) and surrounds the larger cell, 8,000 X.

Figure 37. Plasmodium of Myxobolus insidiosus with both pansporoblast stages (P) and maturing spores (S). Note the absence of a host response at the parasite-host tissue (HT) interface (arrows), 2,600 X.


C
C
N
ZC
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SPC
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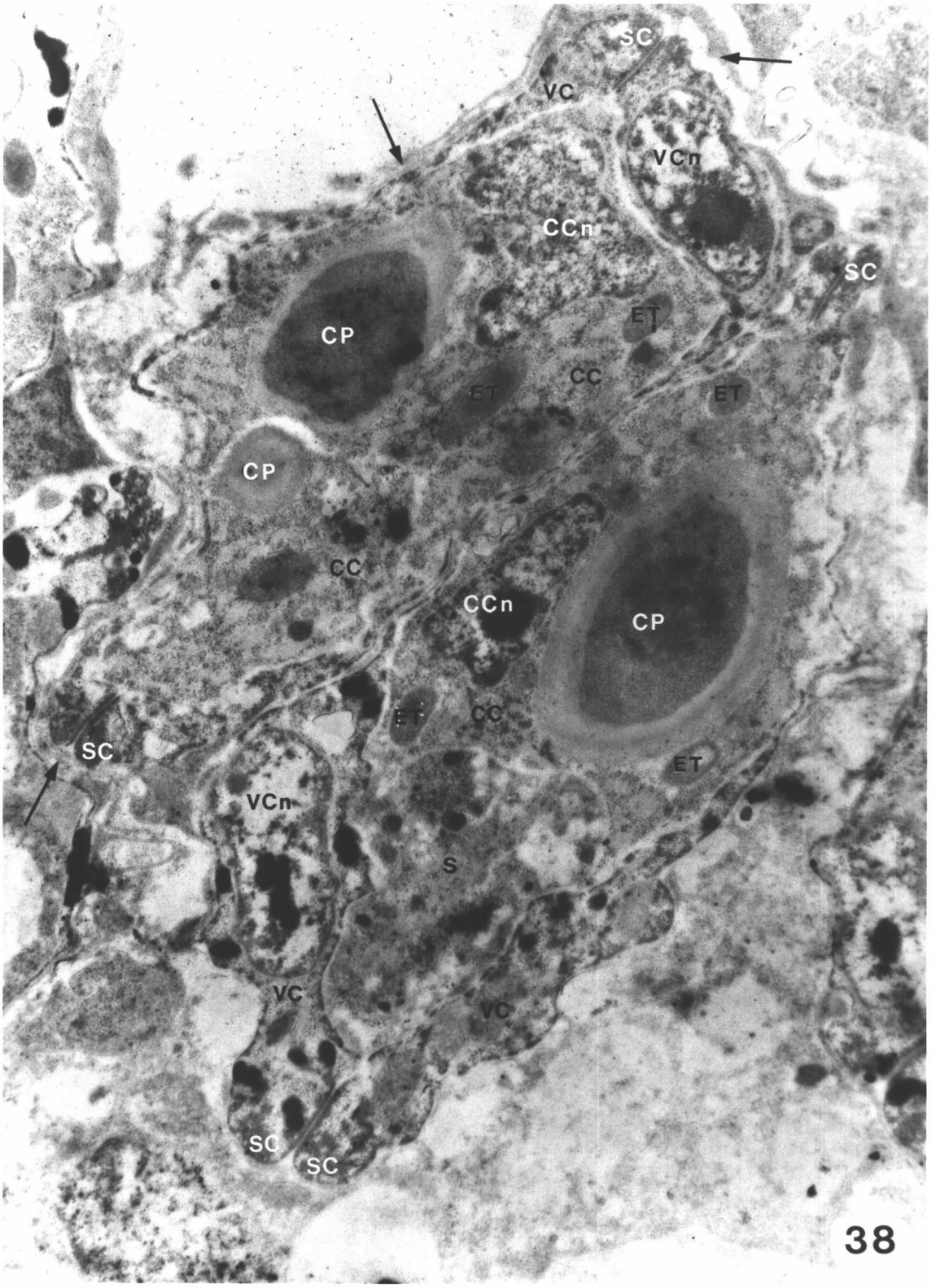
M
35
P
P
HT
S
S
P
HT
37

Figure 38. Developing disporoblast of Myxobolus insidiosus. Note the peripherally located valvogenic cells (VC) which have large nuclei (VCn) at this stage of development. Thickened sutural crests (SC) form where valvogenic cells meet. Each pair of valvogenic cells encloses two capsulogenic cells (CC) and a sporoplasm (S). The capsulogenic cells contain a nucleus (CCn), external tubules (ET), and a capsular primordium (CP). Portions of the envelope cell (arrows) are seen surrounding the disporoblast, 4,700 X.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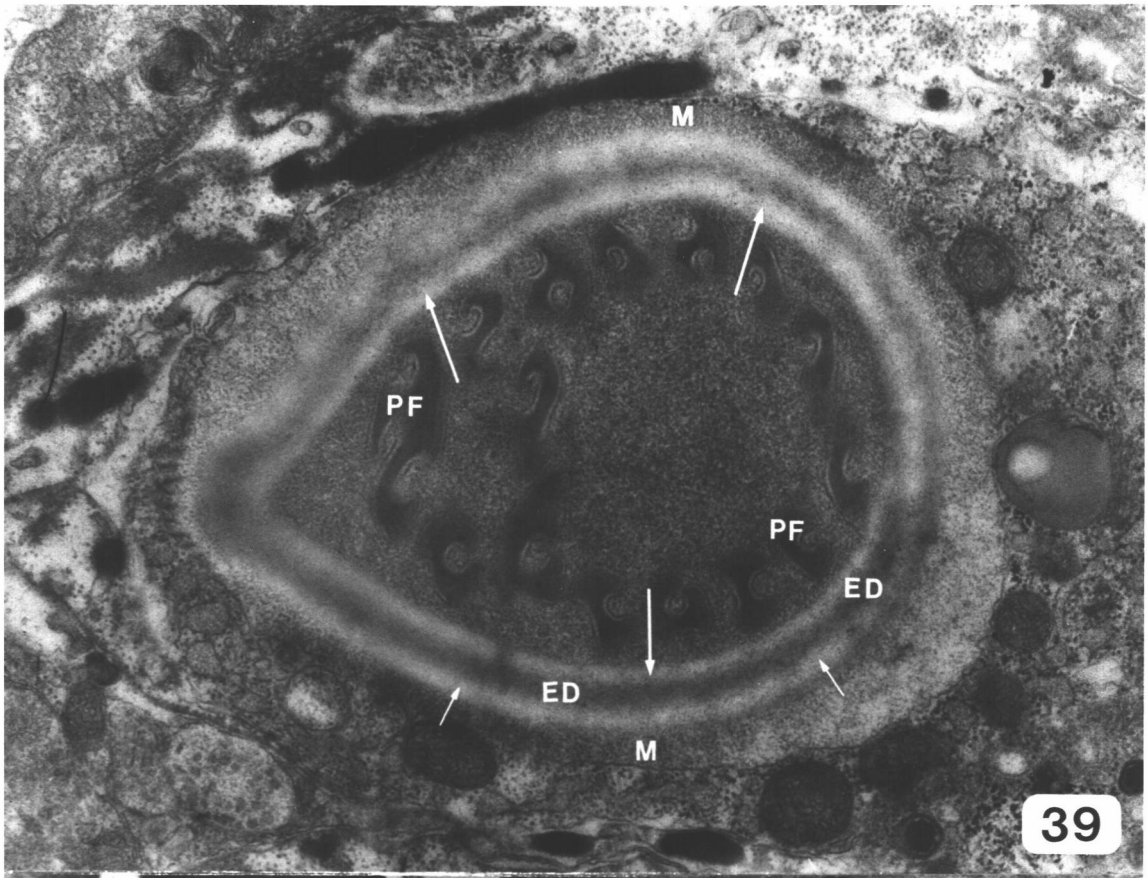
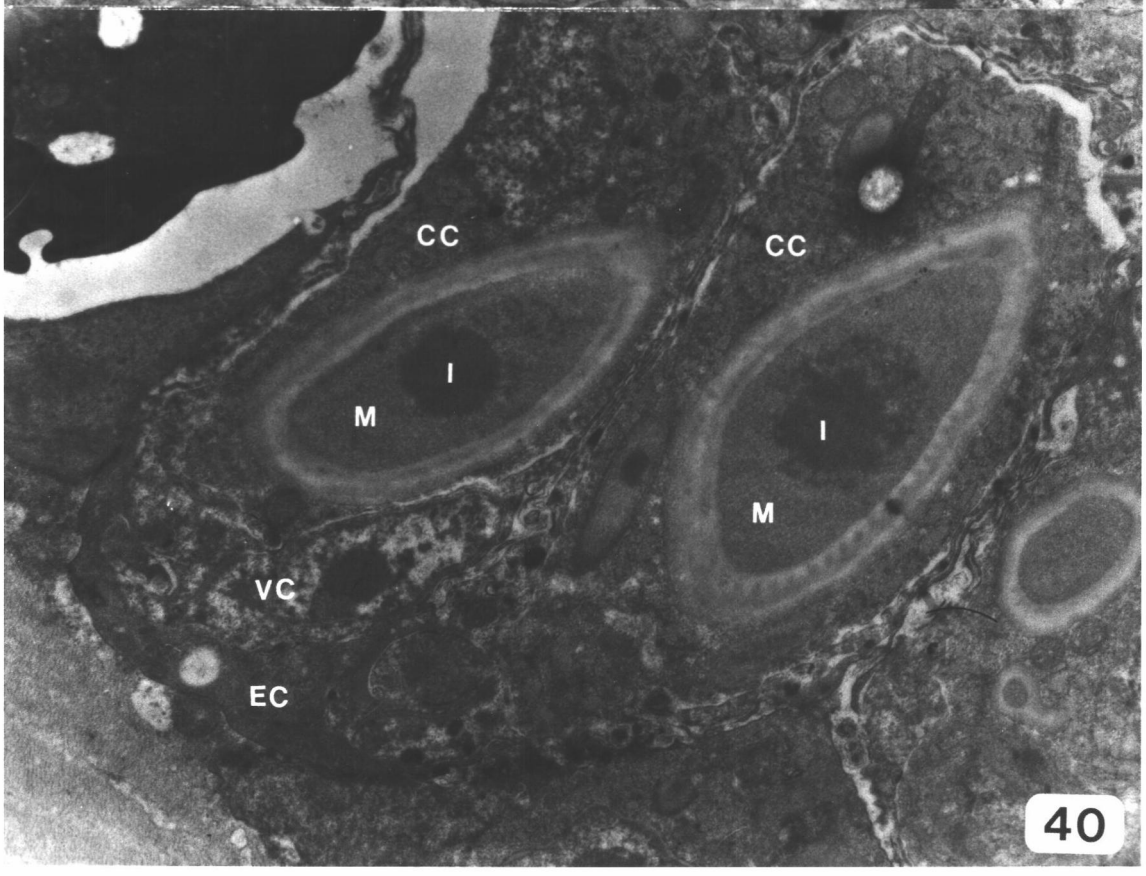
VC
SC
SC

38

Figure 39. Capsular primordium of Myxobolus insidiosus enclosed by a membrane (M) which overlies three zones: an electron lucent zone (short arrows), an electron dense zone (ED), and another electron lucent zone (long arrows). Sections of the polar filament (PF) are visible within the primordium, 13,000 X.

Figure 40. Developing capsulogenic cells (CC) of Myxobolus insidiosus. Note the homogeneous matrix (M) which typically contains an electron dense inclusion (I). Sections of the envelope cell (EC) and a valvogenic cell (VC) are also present, 6,500 X.


M
PF
PF
ED
ED
M
39


CC
CC
I
M
I
M
VC
EC
40

(Figure 41). Polar filament precursor membranes can sometimes be found within a primordium as the external tubules disappear from the cytoplasm (Figure 42). As the spores mature, the valvogenic cells form a narrow wall around the spore and their nuclei degenerate and become incorporated into the valves (Figure 43). At this stage in development the sutural crest and ridges are fully formed (Figure 44). Glycogen granules were found in the sporoplasm's cytoplasm and in the area surrounding the polar capsules of fully formed spores (Figure 45). Mature spores were usually surrounded by a vacuole which seemed to form from the degeneration of the enveloping cell observed in the early sporoblast (Figure 46).

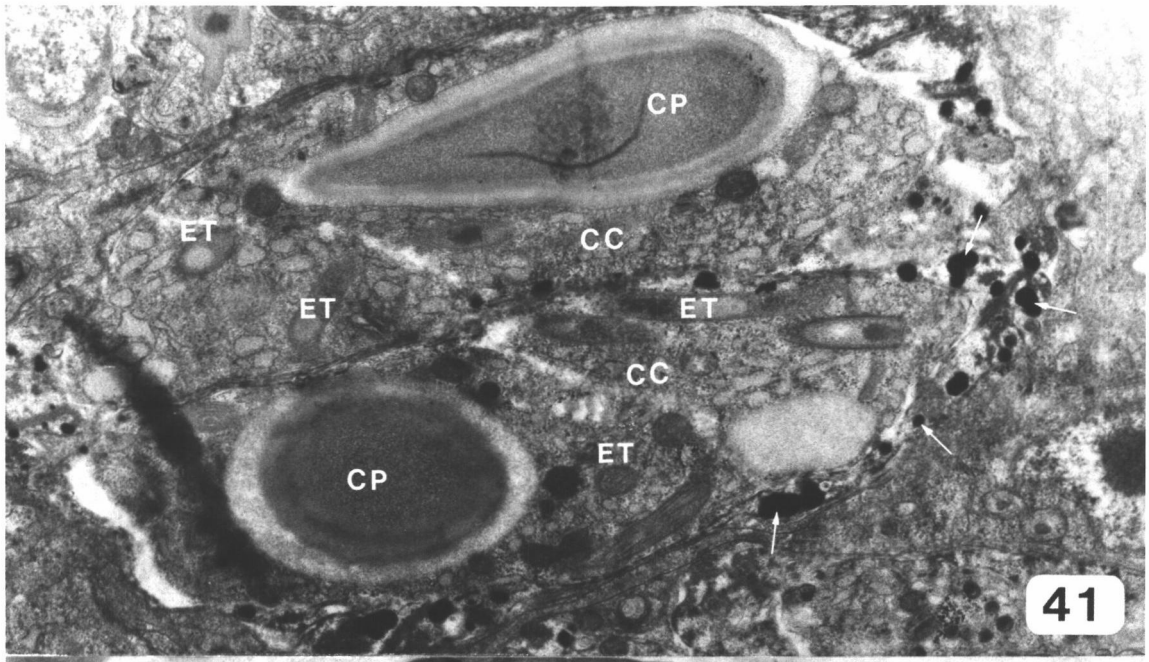
Scanning electron microscopy

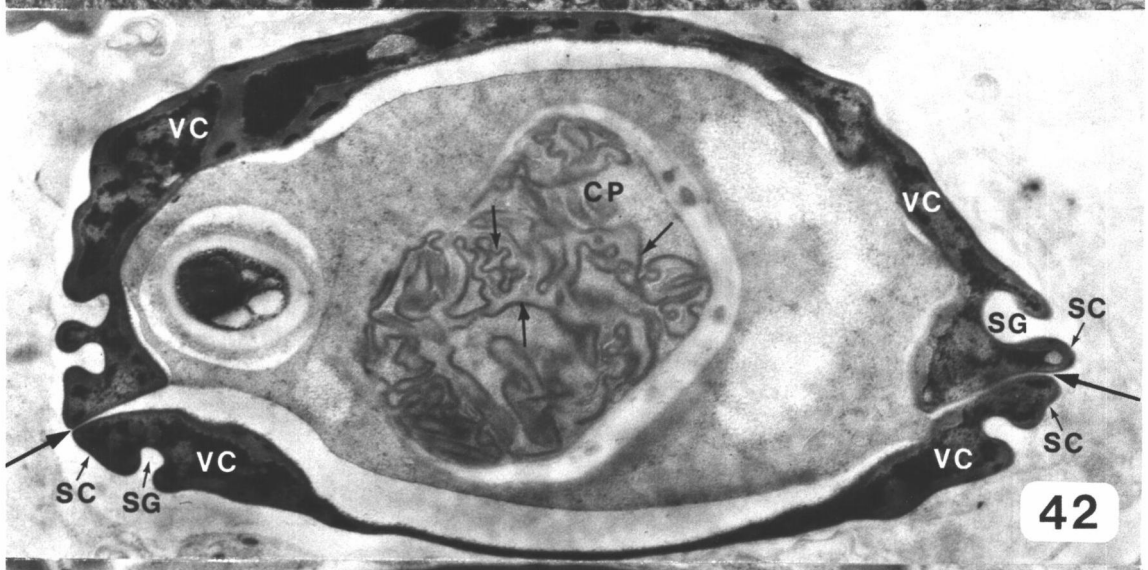
The piriform shape of M. insidiosus spores was observed in scanning electron microscopy examinations (Figure 47). The absence of a mucus envelope such as the one described from M. cerebralis (Lom and Hoffman 1971) and from Myxobolus sp. (Desser and Patterson 1978) and the structure of the sutural crests and ridges were clearly discerned (Figures 48, 49). The extended polar filaments (Figure 48) and the discharge pores (Figure 49) were similar in appearance to those described for other myxosporidans (Lom and Hoffman 1971; Canning and Vavra 1977; Mitchell 1977; Fendrick 1980).

Figure 41. External tubules (ET) present in the cytoplasm of capsulogenic cells (CC) in early stages of Myxobolus insidiosus capsulogenesis. Capsular primordia (CP) and liposomes (arrows) are also present, 5,300 X.

Figure 42. Polar filament precursor membranes (small arrows) within a capsular primordium (CP) of Myxobolus insidiosus. Still developing are the valvogenic cells (VC), sutural crests (SC), sutural grooves (SG), and suture line (large arrows), 6,500 X.

Figure 43. Mature spore of Myxobolus insidiosus. Nuclei of valvogenic cells (VC) are degenerated and the fully formed coiled polar filament (PF) is found within the polar capsule (PC), 8,000 X.


CP
ET
CC
ET
ET
CC
ET
CP
41


VC
CP
VC
SG
SC
SC
SC
SG
VC
VC
42

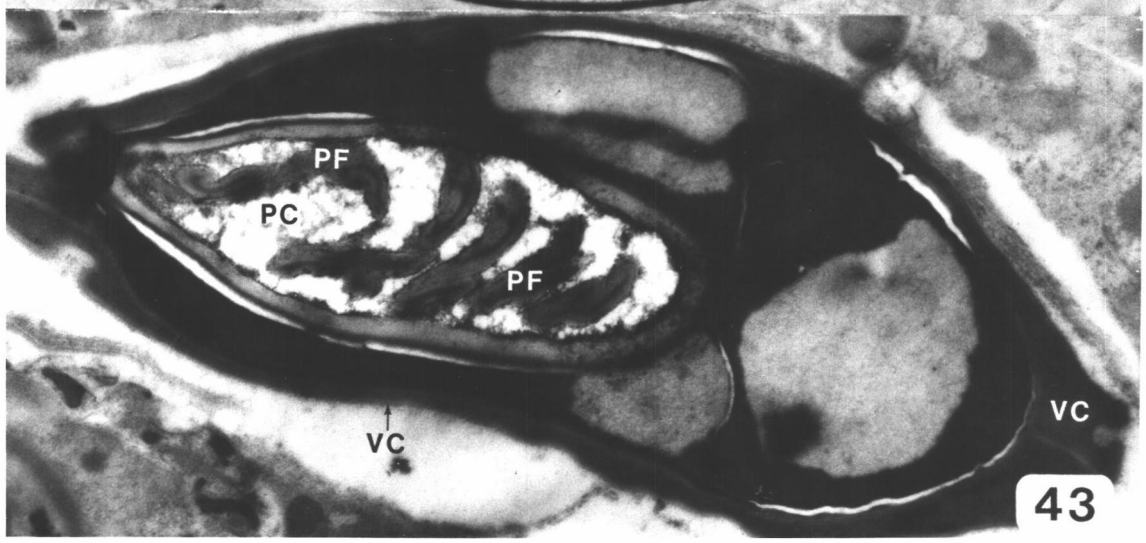
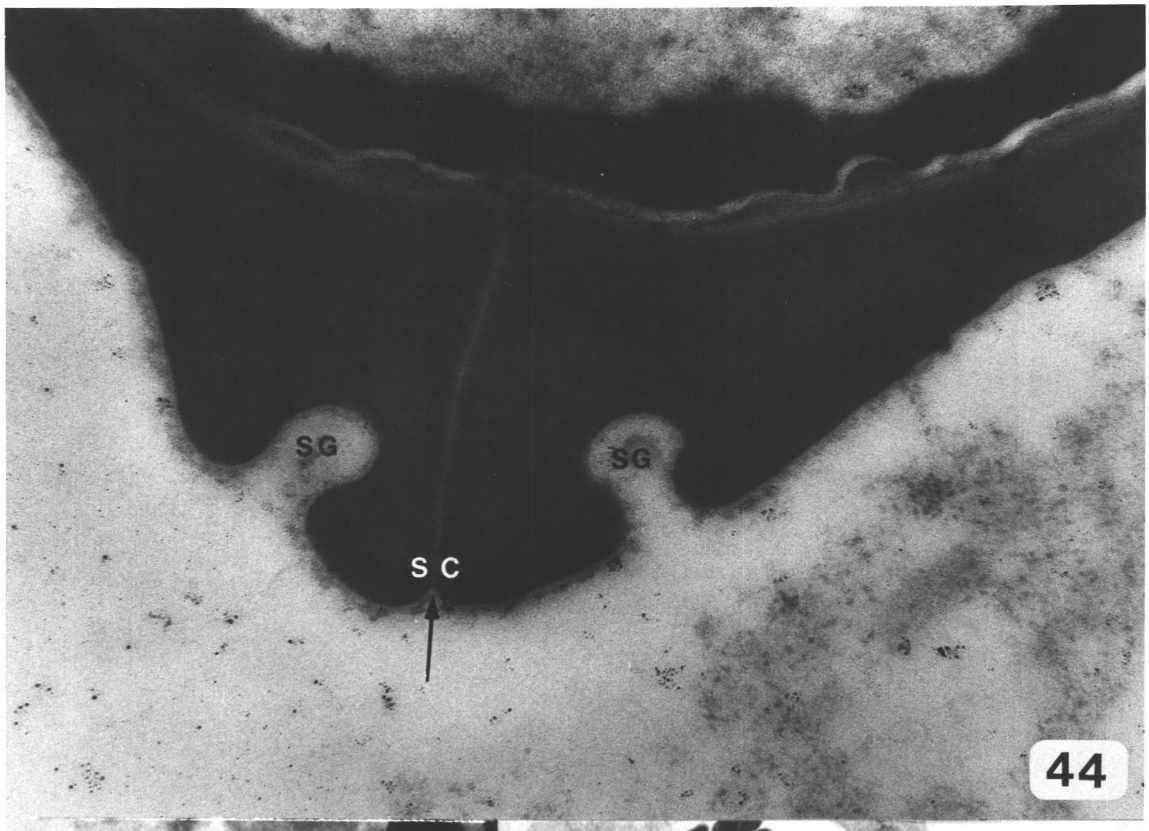
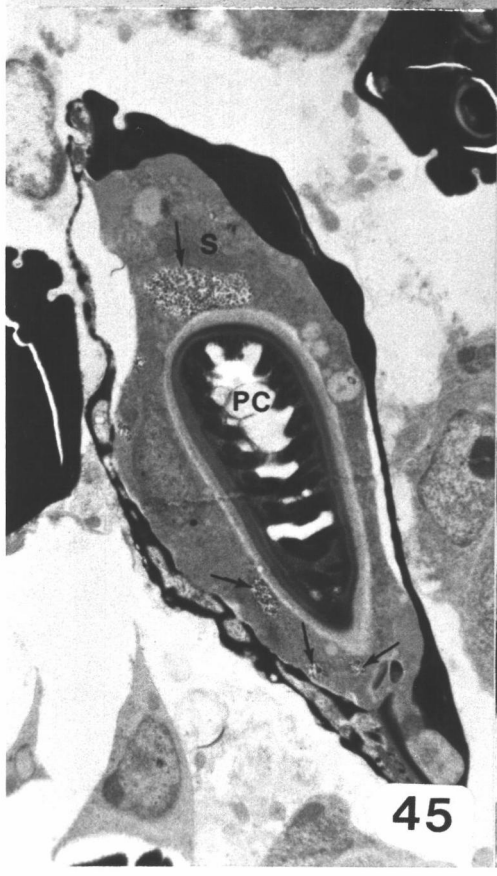

PF
PC
PF
VC
VC
43

Figure 44. Sutural crests (SC) and grooves (SG) of Myxobolus insidiosus spores. Sutural line marked by an arrow, 27,000 X.

Figure 45. Glycogen granules (arrows) are present in the sporoplasm (S) and in pockets around the polar capsules (PC) of Myxobolus insidiosus, 3,600 X.

Figure 46. Vacuoles (V) surround mature spores (S) of Myxobolus insidiosus. Parasite development is asynchronous and both spores and presporulating stages (PS) can be found in close proximity to each other, 2,600 X.


SG
SG
S C
44


S
PC
45

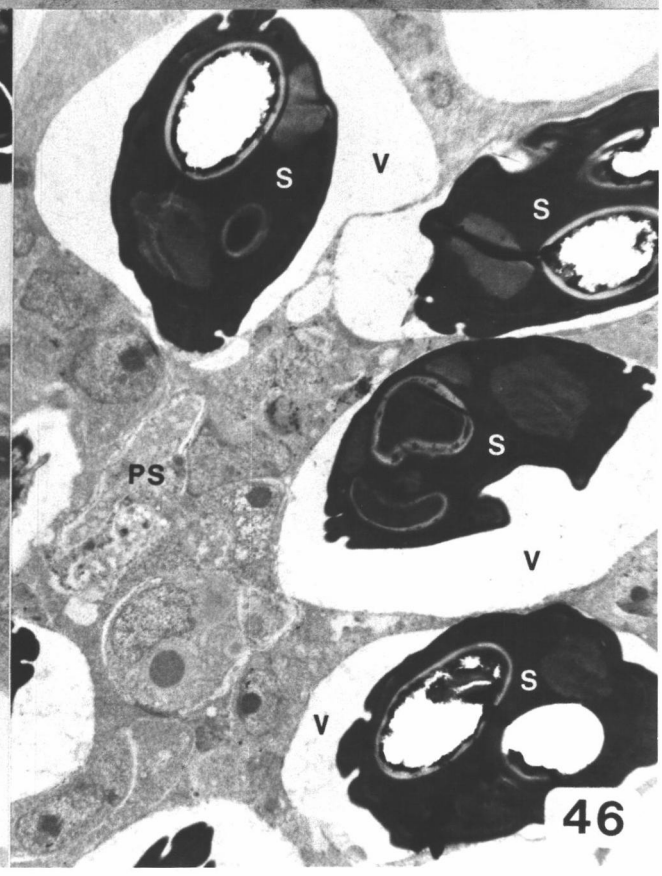
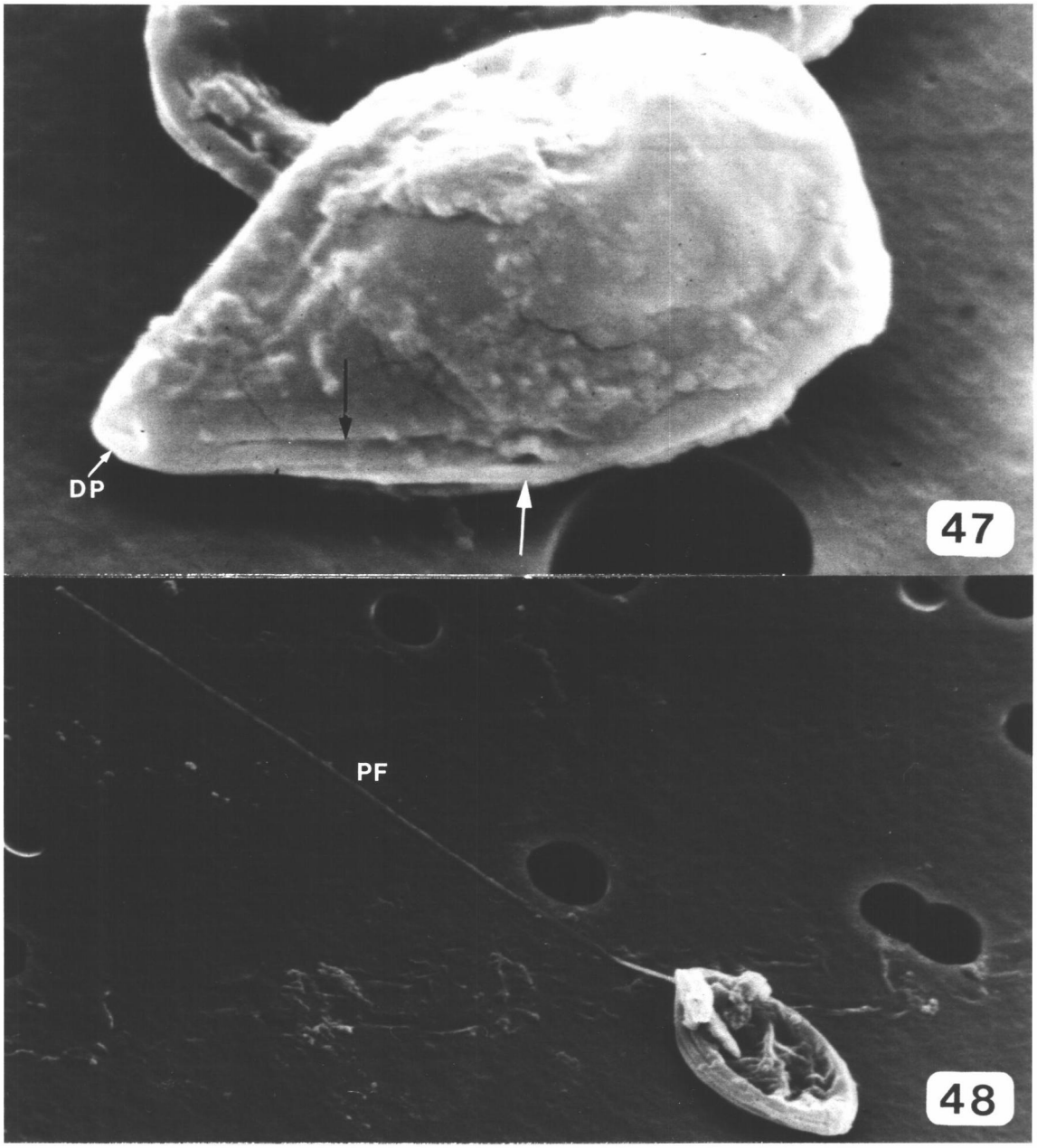

S
V
S
S
PS
V
S
V
46

Figure 47. Spore of Myxobolus insidiosus. Note the piriform shape of the spore, polar filament discharge pore (DP), sutural crest (white arrow), and sutural groove (black arrow), 6,000 X.

Figure 48. Discharged polar filament (PF) of a Myxobolus insidiosus spore, 2,000 X.


DP
47
PF
48

Amino acid analysis of purified spore valves

Purified spore valves of M. insidiosus and C. shasta were obtained after sonication and nuclease treatments. Spore valve analysis indicated that 18 amino acids were present in both preparations (Table 10). The total amino acid concentrations for M. insidiosus and C. shasta were 95.1 and 78.2 µg/mg of spore valve, respectively. Glutamic acid, aspartic acid, and lysine made up 43.7 and 40.0% of the amino acid composition of M. insidiosus and C. shasta spore valves, respectively (Table 10). A large difference in cystine concentration was noted between the two parasites. The concentration of cystine was higher in M. insidiosus (9.3 µg/mg), than in C. shasta where only 3.4 µg/mg was detected. Molar ratio comparisons indicated that aspartic acid and lysine were equimolar, whereas the concentration of glutamic acid was higher for both myxosporidans. Further molar ratio comparisons indicated the presence of higher concentrations of valine, serine, leucine, isoleucine, threonine, and proline in C. shasta spore valves than in those of M. insidiosus (Table 10). Results from the amino acid analysis indicated that protein is a minor component of the spore valves of these two myxosporidans. The valves of M. insidiosus and C. shasta contained 9.5 and 7.8% protein, respectively.

Table 10. Amino acid composition[a] of spore valves of Myxobolus insidiosus and Ceratomyxa shasta.

Amino Acid	Myxobolus insidiosus micro g/mg	Myxobolus insidiosus micro moles/g	Myxobolus insidiosus molar ratios[b]	Ceratomyxa shasta micro g/mg	Ceratomyxa shasta micro moles/g	Ceratomyxa shasta molar ratios[b]
Glutamic acid	17.2	116.9	1.4	13.3	90.4	1.5
Aspartic acid	12.3	92.4	1.1	9.0	67.4	1.1
Lysine	12.1	82.8	1.0	9.0	61.2	1.0
Cystine[c]	9.3	38.5	0.5	3.4	14.2	0.2
Valine	4.3	36.4	0.4	3.4	28.9	0.5
Serine	3.7	35.6	0.4	4.6	43.5	0.7
Leucine	4.4	33.5	0.4	4.9	37.4	0.6
Isoleucine	3.9	29.4	0.4	4.0	30.5	0.5
Threonine	3.3	28.0	0.3	3.9	32.4	0.5
Proline	3.0	26.3	0.3	3.9	33.5	0.6
Arginine	4.6	26.1	0.3	2.9	16.4	0.3
Alanine	2.1	24.0	0.3	2.3	25.4	0.4
Tyrosine	4.2	23.1	0.3	4.1	22.5	0.4
Phenylalanine	3.6	21.9	0.3	3.3	19.9	0.3
Histidine	3.0	19.2	0.2	2.2	14.2	0.2
Glycine	1.2	15.2	0.2	1.5	19.4	0.3
Methionine	2.1	14.1	0.2	1.8	12.1	0.2
Tryptophan[d]	0.8	3.9	0.1	0.7	3.3	0.1

[a]Hydrolysis for 24 h at 110°C in 6 N-HCl; serine micro g/mg and threonine micro g/mg increased by 10 and 5%, respectively to compensate for destruction by acid. One crystal of phenol added before acid hydrolysis.

[b]Molar ratios expressed in relation to lysine.

[c]Performic acid oxidized prior to acid hydrolysis. The micro g/mg calculated from cysteic/alanine ratio.

[d]Alkaline hydrolysis at 135°C for 48 h by the method of Hugli and Moore (1972). The micro g/mg calculated from tryptophan/histidine ratio.

Cultivation of *Myxobolus insidiosus*

Efforts to cultivate *M. insidiosus* in Eagle's MEM with calf serum were unsuccessful. Few spores were broken open with the tissue homogenizer and the few sporoplasms liberated after the procedure failed to develop further. The tough external structure of *M. insidiosus* spores was also noted when sonication and passage through a pressure cell were performed during other procedures. Intact spores did not change in appearance while in the culture medium during the 90 d observation periods.

Immunological procedures

Gel diffusion

Immunodiffusion tests in which either anti-*M. insidiosus* or anti-*C. shasta* sera were reacted with various antigen preparations are listed in Table 2. From one to three antigen-antibody precipitin bands were observed when anti-*M. insidiosus* serum was reacted with whole or broken spores of *M. insidiosus* or with muscle tissue containing spore filled plasmodia of this myxosporidan. In all cases where multiple precipitin bands occurred they were present when the antigen was obtained from chinook salmon which had been exposed to *M. insidiosus* at Aumsville Ponds. No precipitin bands occurred in any tests with anti-*M. insidiosus* sera reacted against chinook salmon uninfected muscle tissue. No cross reactions were detected when sera specific for *M. insidiosus* was tested with whole or broken spores of *C. shasta* nor with partially

purified spores of H. salminicola or M. squamalis. A single precipitin band was noted when muscle tissue of northern squawfish parasitized by Myxobolus sp. was reacted with anti-M. insidiosus serum.

Muscle tissue from chinook salmon parasitized by M. insidiosus at a stage not yet detectable by light microscopy observation (all fish from the same lot examined at a later date were found to be heavily parasitized) or with trophozoite stages of the myxosporidan did not react with anti-M. insidiosus sera. Preparations of muscle tissue containing spore filled plasmodia of M. insidiosus obtained from chinook and coho salmon, and rainbow and cutthroat trout gave a band of identity when reacted with sera specific for M. insidiosus. No cross reactions were noted when anti-C. shasta sera was reacted with purified whole or broken spores of M. insidiosus or with partially purified spores of H. salminicola or M. squamalis in gel diffusion tests. Whole C. shasta spores did not elicit a reaction and a precipitin band was only noted when anti-C. shasta sera was tested against broken C. shasta spores.

Immunofluorescence

Myxobolus insidiosus and C. shasta were detected by fluorescent antibody techniques. Trophozoites and spores of M. insidiosus gave strong fluorescent reactions when the IFAT was performed on parasitized tissue or purified spores (Figure 50), whereas no fluorescence occurred when antisera was reacted with muscle tissue of chinook salmon containing M. insidiosus in a stage

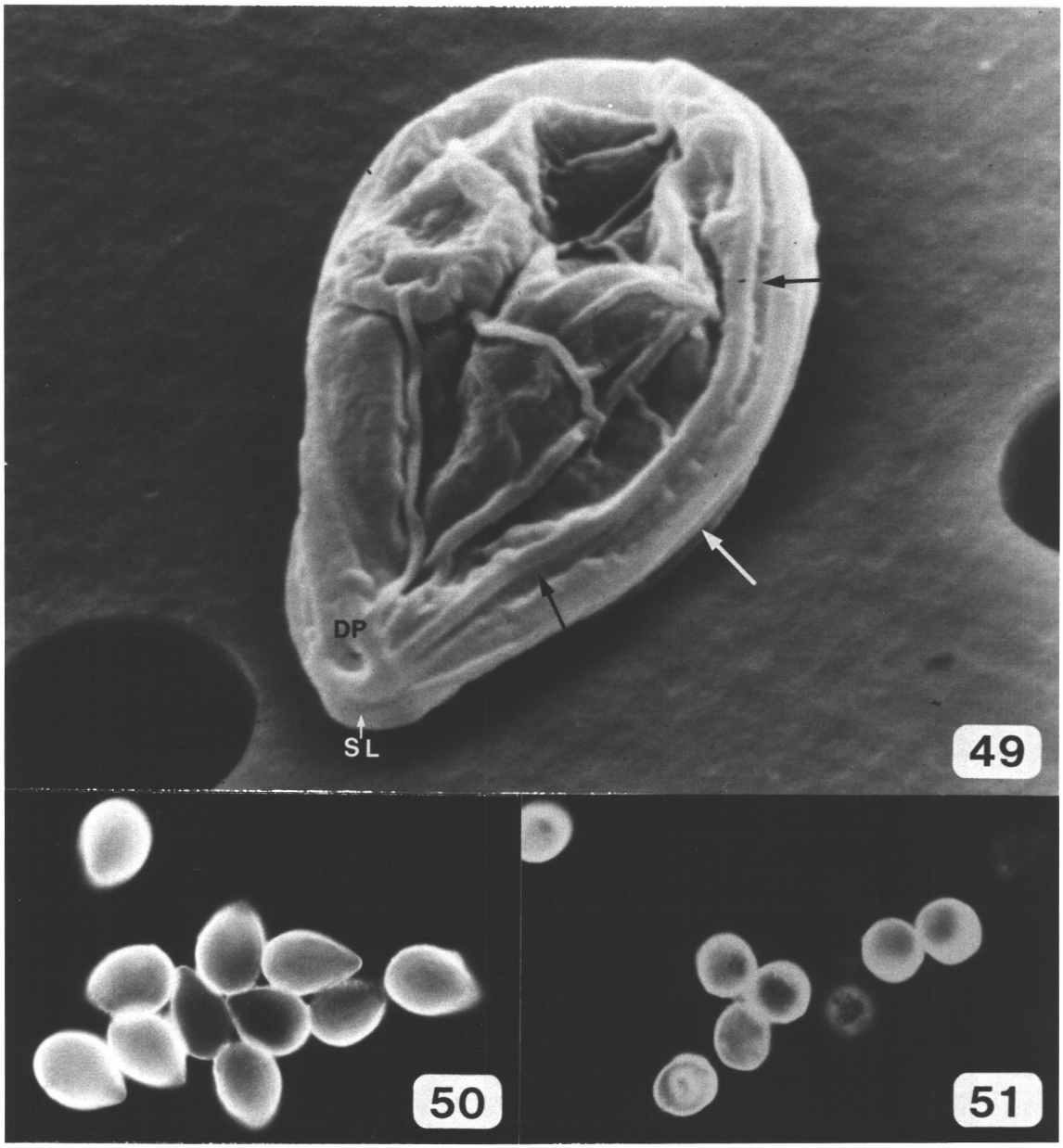
not yet detectable by light microscopy. No fluorescence was observed when uninfected muscle tissue was tested with anti-M. insidiosus serum by the IFAT. Labeled sera specific for C. shasta showed fluorescence with C. shasta spores and trophozoites by the DFAT. No cross reaction was noted when labeled C. shasta serum was applied to uninfected intestinal smear preparations.

Strong fluorescence indicating cross reactivity occurred when M. cerebralis spores were allowed to react with anti-M. insidiosus serum followed by incubation with labeled goat anti-rabbit serum (Figure 51). No cross reaction was found when spores of C. shasta, H. salminicola, or M. squamalis were treated with the same procedure. No fluorescence of M. insidiosus, H. salminicola, M. cerebralis, or M. squamalis spores occurred when labeled anti-C. shasta sera was applied.

Figure 49. Myxobolus insidiosus spore with a distinct suture line (SL) and deep sutural grooves (black arrows). A well developed sutural ridge (white arrow) and the polar filament discharge pore (DP) are also evident, 6,000 X.

Figure 50. Myxobolus insidiosus spores reacted with Myxobolus insidiosus antiserum and subsequently stained with fluoroscein labeled goat anti-rabbit serum, 500 X.

Figure 51. Myxosoma cerebralis spores reacted with Myxobolus insidiosus antiserum and subsequently stained with fluorescein labeled goat anti-rabbit serum, 500 X.


DP
SL
49
50
51

DISCUSSION

Wyatt and Pratt (1963) described M. insidiosus from muscle tissue of fingerling chinook salmon reared at McKenzie Hatchery in Oregon. The subspecific name M. insidiosus clarki was given to a myxosporidan found in the muscle tissue of cutthroat trout in the McKenzie River (Wyatt 1979). The present study focused on various physical, biological, and biochemical aspects of M. insidiosus. Size and shape variations of plasmodia and spores parasitizing salmonid species were examined. Salmonid species susceptibility to M. insidiosus was tested at three sites in Oregon and the development of the parasite in chinook salmon was documented by light microscopy examination of wet mounts and histological sections as well as electron microscopy studies of parasitized tissues. Immunodiffusion and immunofluorescence techniques were used to study M. insidiosus from various hosts and locales and to compare this parasite with other myxosporidan species.

During this study M. insidiosus was described for the first time from rainbow and steelhead trout. The parasite's geographic range was extended to the Coos and Yaquina River systems and infected cutthroat trout and coho salmon were found in areas other than those described by Wood (1974) and Wyatt (1978a, 1979). Further indications of M. insidiosus specificity for salmonids were shown in the present work. All non salmonids collected in areas where M. insidiosus occurred did not contain the parasite. Other myxosporidans specific for salmonids are C. shasta (Johnson et al. 1979) and M. cerebralis (Bogdanova 1968; Halliday 1976).

The variable rate of M. insidiosus parasitism observed in salmonids in this study could be due to fish species or strain variability or to the number of infectious units present in different areas of the parasite's range. Hatchery reared chinook salmon were heavily parasitized after exposure to M. insidiosus at Aumsville, but much less when exposed at the EWEB canal and at the Weyerhaeuser facility. The few wild chinook salmon collected in Coal and Cox creeks were not parasitized by the myxosporidan. The presence of M. insidiosus in wild coho salmon, rainbow and cutthroat trout in some streams and not in others of the Coos and Yaquina River systems indicated varying levels of susceptibility to the parasite by different populations of these salmonids.

Results from experimental exposure tests of various salmonid species to M. insidiosus indicated that chinook salmon were the most susceptible species. Exposures of 8 d duration at Aumsville resulted in only chinook salmon becoming parasitized. High numbers of this myxosporidan were found in all these fish. Exposure of fish at the EWEB canal for 8 d resulted in parasitism of chinook salmon and one rainbow trout. All chinook salmon exposed at Aumsville were heavily parasitized by M. insidiosus, whereas fish from the same lot exposed in the EWEB canal for an identical time period were moderately infected and only had a 10% incidence of parasitism. Examinations of wild rainbow and cutthroat trout and coho salmon from various locations, including Aumsville canal, indicated that M. insidiosus can parasitize these hosts. The

variable susceptibility of salmonid species to M. cerebralis (O'Grodnick 1979) and C. shasta (Schafer 1968; Zinn et al. 1977) has been reported. Exposures of salmonid strains to other myxosporidans have given variable infection results. A strain of lake trout (Salvelinus namaycush) was reported to be refractory to M. cerebralis by O'Grodnick (1979) whereas, Hoffman and Putz (1969) found a different strain of the same fish species to be susceptible. Exposure of brook trout strains to C. shasta has also given variable results. Wales and Wolf (1955) reported the species refractory, whereas Schafer (1968) and Zinn et al. (1977) found 24 and 100% loss to ceratomyxosis in the strains they tested. Variable responses also occur when the same strain of fish is used for exposure to myxosporidans in different areas. Schafer (1968) reported that Klaskanine River coho salmon were not parasitized by C. shasta at Crystal Lake, whereas Sanders et al. (1970) observed spores in adult fish of the same strain at Klaskanine Hatchery. Similarly, Alsea River coho salmon exposed at Crystal Lake were negative for C. shasta (Schafer 1968), whereas 42% mortality due to ceratomyxosis occurred in the same stock exposed in the Willamette River (Zinn et al. 1977).

Increasing the exposure length to 50 d for coho salmon at Aumsville resulted in a low incidence and level of M. insidiosus. Wyatt (1978a) reported the absence of M. insidiosus in chinook salmon after an 8 d exposure to EWEB canal water at McKenzie Hatchery. After this time period he noted that as the exposure

period was extended an increasing number of fish became parasitized. Thus the salmonid species which appear to be refractory to M. insidiosus in this study could become parasitized by exposing the fish to greater numbers of parasites through extended exposure. One can also conclude that chinook salmon should be the species of choice when performing tests to detect the presence of M. insidiosus infectious stage. Exposure results thus indicated that parasitism with M. insidiosus was related to species susceptibility, exposure time, and numbers of parasites present.

Wyatt (1978a) discussed the association of dirt bottom canals and ponds with parasitism by M. insidiosus. Both the EWEB and Aumsville canals have dirt bottoms but the Aumsville canal is considerably longer than the EWEB canal. The Aumsville canal is also shallower and narrower and thus carries less water at a much reduced water flow when compared to the EWEB canal. The presence of more substrate and decreased water velocities in the Aumsville canal may enable larger numbers of M. insidiosus infectious units to occur and parasitize susceptible fish.

The size and shape of myxosporidan plasmodia and spores are variable and appear to be partially controlled by the parasitized host. Variations in size of specific myxosporidan spores from different host species (Meglitsch 1960; Hine 1979) as well as differences within individual hosts (Hine 1979) have been reported. Different myxosporidan plasmodial shapes and sizes have also been noted (Schuurmans Stekhoven 1920; Davies 1968; Mitchell

1970). During this study variations in size and shape of spores and plasmodia occurred in fish of the same species. These variations were also apparent in other species of fish collected in the same or different water bodies. The variations were more evident in rainbow and cutthroat trout and coho salmon, whereas plasmodia and spores in chinook salmon were more uniform.

Intrafibrillar and interfibrillar plasmodia of M. insidiosus were found within the muscle of individual rainbow and cutthroat trout. A similar observation was made by Sweet (1978) for M. insidiosus plasmodia in trout. However, Wyatt (1979) described M. insidiosus clarki from cutthroat trout in the McKenzie River on the basis of interfibrillar plasmodia, ovoid plasmodia, and smaller spores even though he speculated that these variations could have been due to host influences and not parasite differences. Results of the present work indicate that development of M. insidiosus in trout differs from that in salmon and that the presence of interfibrillar, ovoid plasmodia and smaller spores do not warrant subspeciation of the parasite.

No chinook salmon were parasitized by M. insidiosus when exposed for 6 h or less to water containing the infectious stage and only 1 fish was parasitized during an 8 h exposure. A longer exposure period of susceptible hosts is required for parasitism by M. insidiosus than for certain other myxosporidans. Schafer (1968) observed C. shasta spores in fish exposed for 15 min and Putz and Herman (1970) reported that M. cerebralis can parasitize fish in 40 min.

The presence of M. insidiosus in one chinook salmon exposed for 48 h to water and mud collected at Aumsville and kept at 18°C was the first successful attempt at infecting fish with M. insidiosus in the laboratory. Natural M. insidiosus infections occur between 8.9 and 13.3°C (Wyatt 1978b). No parasitism was found in chinook salmon exposed for 429 d to mud seeded with M. insidiosus spores "aged" for 210 d and transmission did not occur when fish were exposed for 190 d to mud collected from McKenzie Hatchery rearing ponds (Wyatt 1978b). Both experiments were carried out in water maintained at 12.3°C. Once infectious stages are formed at lower water temperatures the possibility exists that movement to warmer water temperature (18°C) used in the present study is not detrimental to their survival and may even enhance infectivity. Laboratory transmission of myxosporidans has occurred when susceptible fish were exposed to lake bottom sediments (Fryer and Sanders 1970), muddy pond water (Molnar 1979), and mud contaminated with spores (Seenapa and Manohar 1981a). Routine laboratory infections of salmonids with M. cerebralis occur when spores are aged 4 months in sediment containing tubificid worms (Markiw and Wolf 1983).

The disease process characteristic of M. insidiosus was temperature dependent, infecting fish when water temperatures averaged 8.9°C and no longer parasitizing fish when average temperatures exceeded 13.3°C (Wyatt 1978b). Wyatt (1978b) noted that M. insidiosus development accelerated when parasitized fish

were moved from 8.9 to 12.2°C. Similar temperature dependent development was reported for M. cerebralis (Halliday 1973; O'Grodnick 1978), Henneguya sp. (Awakura and Tonosaki 1978) and C. shasta (Udey et al. 1975). Results of the present work indicated that once fish are parasitized the development of M. insidiosus was also temperature dependent. Development of M. insidiosus at 6 and 9°C was greatly retarded and sporulation did not occur during the examination period. Had the experiments continued, sporulation would have probably occurred. A longer incubation period may also have enabled M. insidiosus trophozoites to become patent in fish held at 3°C. These experiments indicate that development of M. insidiosus occurs faster as temperature is increased up to 18°C.

The absence of M. insidiosus in fish held at 21°C could have resulted from several possible factors; temporary inactivation of the parasite, inability of the parasite to tolerate the higher temperature, and/or increased host defenses leading to parasite destruction. Udey et al. (1975) reported no deaths due to C. shasta in fish held at 3.9°C; however, ceratomyxosis occurred when these same fish were acclimated to 17.8°C. It is possible a reverse pattern may occur with M. insidiosus and that if water temperatures are lowered on fish held at 21°C the infection may become patent. Destruction of M. insidiosus at 21°C either by the higher temperature or by a heightened host response is most likely. Degeneration of myxosporidan trophozoites (Lom 1970; Dykova and Lom 1978) and mature plasmodia (Awakura and Tonosaki

1978) are known to occur at elevated water temperatures. Andrews (1979) speculated that the absence of H. psorospermica plasmodia on perch was due either to a direct temperature effect on the parasite or host reactions to the infection.

The inability to detect M. insidiosus in the musculature of salmonids after exposure, for at least 44 days, indicated the parasite was probably present in other tissue(s) during this period. A similar delay in observation of the first stages of M. cerebralis after exposure was documented by Halliday (1973) and O'Grodnick (1978). In contrast, Johnson et al. (1979) detected C. shasta trophozoites 3 d post-exposure on the mucoid surface of the pyloric caeca. If myxosporidans entered their hosts by an oral route, these differences may be explained. Ceratomyxa shasta parasitizes the epithelial surface of the intestinal tract and is detected shortly after exposure in the pyloric caeca and with time progresses down the intestine (Johnson et al. 1979). The time delay between exposure and detection of trophozoites of M. insidiosus and M. cerebralis would indicate a period of transit between the site of penetration, possibly the alimentary tract, and the definitive tissue. The gills could also serve as a site of myxosporidan penetration of their fish hosts, but some gill infecting myxosporidans are known to arrive at the gill tissue from another site in the host. Cone (1979) suggested that trophozoites of Henneguya doori reached the gills of perch via the circulatory system. Greven (1956) noted that H. psorospermica plasmodia were

present only in the septal tissue surrounding the afferent artery but absent in the septal tissue surrounding the efferent artery and thus probably arrived at the gills from somewhere else in the body.

The reappearance of M. insidiosus trophozoites after a period during which only mature plasmodia could be detected suggested autoinfection. This type of infection by myxosporidans has been described on several occasions (Kudo 1920, 1926; Debasieux 1922; Ganapathi 1941). In contrast to the present work, Wyatt (1971) did not observe autoinfection with M. insidiosus in fish held at the McKenzie River Hatchery for 13 months, moved to spring water and examined 156 and 713 d later. In a separate examination of fish held at the hatchery for 4 months, moved to spring water and examined 237 d later no immature stages of M. insidiosus were noted (Wyatt 1971). It is possible autoinfection occurs early in the infection period and that after a certain time, the fish is able to destroy new infectious stages. Wyatt's examinations were performed much later after infection than in the current work. Fish strains reacting differently to the parasite may also be a factor. The trophozoite stages described were in fall chinook salmon, whereas Wyatt was working with spring chinook salmon.

A variety of host tissue reactions to myxosporidan parasites has been documented (Nigrelli and Smith 1938, 1940; Nigrelli 1948; Jakowska and Nigrelli 1953; Guilford 1963; McCraren et al. 1975). The destruction and infiltration of M. insidiosus plasmodia by mesenchymal-like cells and fibroblasts was described from chinook

and coho salmon (Wyatt 1971). Phagocytosis of myxosporidan spores has been reported to occur as a host defense mechanism (Kudo 1923; Dykova and Lom 1978). In chinook salmon infected with M. insidiosus at Aumsville no phagocytosis associated with affected plasmodia was observed but infiltration of parasitic plasmodia by host cells was readily seen. Enlarged masses of connective tissue and chinook salmon host cells, which in some cases contained a few M. insidiosus spores, were similar to those described by Mitchell (1970) in squawfish muscle.

The ultrastructure of developmental stages of M. insidiosus closely parallels that for other myxosporidans (Cheisin et al. 1961; Lom and dePuytorac 1965a, 1965b; Current and Janovy 1976, 1977; Desser and Patterson 1978; Yamamoto and Sanders 1979; Pulsford and Mathews 1982). The manner in which capsular primordia and external tubules of developing spores are formed and the process by which the amorphous capsular primordia and external tubule become transformed into the polar capsule with its coiled polar filament have been difficult to determine by electron microscopy. Lom (1969c) suggested that Sphaeromyxa sp. capsular primordia were formed from smooth endoplasmic reticulum or Golgi apparatus. Schubert (1968) indicated that granular endoplasmic reticulum was the source of the primordium in Henneguya pinnae. Desser and Patterson (1978) noted the presence of abundant granular endoplasmic reticulum and lack of Golgi complexes in Myxobolus sp. capsulogenic cells. Capsular primordia of Myxobolus exiguus

appeared to develop from fusion of electron lucent material within the cisternae of endoplasmic reticulum (Pulsford and Mathews 1982). In the present study, granular endoplasmic reticulum was present in the cytoplasm of M. insidiosus immature capsulogenic cells and appeared the most likely source of amorphous capsular primordia.

The external tubule found in cytoplasm of immature capsulogenic cells of myxosporidans is thought to grow out and elongate from the capsular primordium. Polar filament formation within the external tubule follows later. Invagination and coiling of the polar filament and incorporation of the proximal end of the external tubule into the neck region of the polar capsule occurred upon maturation of the capsulogenic cell (Lom and dePuytorac 1965b; Desser and Patterson 1978). Many sections of external tubules were detected within the cytoplasm of capsulogenic cells of M. insidiosus but tubule elongation from the primordium and formation and invagination of the polar filament were not detected. Polar filament precursor membranes within the capsular primordia similar to those described by Desser and Patterson (1978) were present in capsulogenic cells of M. insidiosus when external tubules were no longer visible in the cytoplasm.

Amino acid contents of cell or spore walls of Protozoa have been reported (Perov and Taldrik 1975; Stotish et al. 1978; Johnson and Cross 1979; Vivares et al. 1980; Tibbs 1982). The present study reports the first amino acid analysis of spore valves from

Myxosporea. Quantitative examination of purified M. insidiosus and C. shasta spore valves indicated that protein was not a major component, comprising less than 10% of the preparation. In contrast, Lom (1964) reported that protein was the major component of myxosporidan spore valves analyzed with histochemical methods. He observed weak reactions for histidine, tryptophan, and sulfhydril groups and no reaction for disulfide groups and tyrosine on spore valves of Myxobolidae.

Amino acid composition of cell or spore walls of other Protozoa has been shown to differ between organisms. Perov and Taldrick (1975) reported that oocyst walls of different species of Coccidia contained different amino acids. The spore coat of Colpoda steinii was composed almost entirely of glutamic acid, glycine, serine, and cysteic acid (Tibbs 1982), whereas the oocyst wall of Eimeria tenella contained glutamic acid, proline, alanine, serine, and valine as the major constituents (Stotish et al. 1978). The main components of spore walls from four microsporidians were aspartic acid, glutamic acid and lysine (Vivares et al. 1980). These results are almost identical to those reported in the present work and may indicate that spore walls of myxosporidans and microsporidians are similar. Vivares et al. (1980) also noted that relative amounts of the next most abundant amino acids varied and suggested these variations may serve as a systematic criterion at the genus level. Differences in amino acid

composition of cell or spore walls of Protoza indicate that amino acid analysis may be of use in taxonomic differentiation. Whether differences in amino acid composition observed with spore valves of M. insidiosus and C. shasta can be associated with familial, generic, or specific characteristics of myxosporidans remains to be determined.

Immunological techniques have been used on several occasions for detection of myxosporidan antigens or antibodies. Pauley (1974), using gel diffusion tests, noted bands of identity between uninfected rainbow trout cartilage and M. cerebralis spore preparations. He suggested that M. cerebralis mimicked the host cartilage antigens and thus gained some protection from immunological reactions of the host. Possible mimicry of eel antigens by another myxosporidan was also reported by McArthur and Sengupta (1982). Mitchell (1970) could not differentiate between antigens of M. muelleri or M. dujardini and host antigens by means of hypersensitivity reactions in rabbits. In the present study, purified spore preparations of M. insidiosus and C. shasta injected into rabbits resulted in the formation of antisera highly specific for these myxosporidans. No reaction by either immunodiffusion or immunofluorescence methods was observed when sera specific for M. insidiosus was allowed to react with uninfected salmonid muscle tissue. Similarly, no reaction was noted when sera specific for C. shasta was tested by immunofluorescence on intestinal smears of non parasitized salmonids. The occurrence of host reactions against

many myxosporidans (Kudo 1923; Nigrelli and Smith 1938, 1940; Nigrelli 1948; Jakoveska and Nigrelli 1953; Guilford 1963; Wyatt 1971; McCraren et al. 1975; Dykova and Lom 1978; Andrews 1979; the present study) would indicate that mimicry of host antigens by myxosporidans is not a common occurrence. It is possible the pepsin-trypsin digest by ether extraction method for host tissue removal from spores of M. cerebralis employed by Pauley (1974) either altered the antigenic composition of the spores or was not totally effective in the removal of host antigens. Mitchell (1970) disected host material from the myxosporidan plasmodia and possibly did not remove all host tissue.

Mitchell (1970) noted skin responses in rabbits injected with spore washings of M. muelleri or M. dujardini and suggested that some myxosporidan antigens located on the spore surface were soluble. Using M. insidiosus antisera, precipitin band formation occurred when either purified whole spores or muscle tissue containing plasmodia and spores of M. insidiosus were used. These results indicated the presence of soluble antigens on spores of M. insidiosus. No soluble antigens were detected when whole spores of C. shasta were reacted with sera specific for the parasite, but were detected when crushed spores were used as the antigen. These reactions could indicate differences in the presence of surface antigens in myxosporidan spores and in specificity of antibodies for such antigens. Absence of precipitin band formation between M. insidiosus antisera and muscle tissue heavily parasitized with

trophozoites of this myxosporidan could indicate lack of soluble antigens in trophozoites or the presence of amounts too low to form detectable bands.

Trophozoites of M. insidiosus reacted specifically by indirect immunofluorescence with serum from the same lot used for the immunodiffusion tests, indicating trophozoite and spore stages of M. insidiosus share some somatic antigens. The presence of a band of identity among individual muscle tissue preparations, containing M. insidiosus spore filled plasmodia obtained from salmonids collected in different geographic areas, indicated the serological relatedness of this parasite. Thus the host species parasitized and morphogenic differences (spore and plasmodial shape and size) appear less important for the taxonomy of M. insidiosus.

Three precipitin bands were usually noted in immunodiffusion tests between anti-M. insidiosus serum and spore-tissue preparations of chinook salmon parasitized at Aumsville. Similar spore-tissue preparations of M. insidiosus from other hosts in the same and other geographic areas reacted with only one precipitin band. These differences could be explained by one or more of the following: 1) the presence of a larger number of soluble surface antigens on plasmodia and spores obtained from chinook salmon exposed at Aumsville than on plasmodia from other preparations; 2) increased specificity of antisera to its homologous antigen; and 3) increased amounts of soluble antigen present in preparations from chinook salmon exposed at Aumsville as compared to other

preparations. The antisera was prepared against crushed M. insidiosus spores collected from chinook salmon parasitized at Aumsville. Thus, it is possible that these spores had antigens not present in spores used for other preparations and thus could only be detected in the homologous test. The larger numbers of spores in the musculature of chinook salmon exposed at Aumsville compared to other preparations could have caused the observed differences in band formation. Perhaps identical antigens are present in all M. insidiosus plasmodia and spores regardless of host species or geographic area but not enough antigen may be present in light infections to form precipitin bands.

Cross reactivity between antisera specific for one myxosporidan and spores of other species occurs infrequently. Halliday (1974) reported no significant cross reaction between anti-M. cerebralis sera and Myxobolus spp. spores when tested by indirect immunofluorescence and Pauley (1974), using immunodiffusion, did not detect cross reaction between anti-M. cerebralis sera and crushed M. cartilaginis spores. By contrast, Markiw and Wolf (1977, 1978) observed a cross reaction by direct immunofluorescence between anti-M. cerebralis sera and M. cartilaginis spores. When they used the indirect immunofluorescence test (IFAT) cross reactivity did not occur with M. cartilaginis but was noted with other myxosporidans. Results of the present work showed no cross reactivity in immunodiffusion tests conducted between anti-M. insidiosus or anti-C. shasta sera

with spores of four myxosporidans from Pacific Northwest salmonids indicating the absence of common soluble antigens between these myxosporidans. With direct fluorescence, no cross reactions were found between fluorescein labeled anti-C. shasta sera and four myxosporidan species, however, strong fluorescence was noted when spores of M. cerebralis and M. insidiosus were tested with anti-M. insidiosus serum by the indirect fluorescence method.

Wolf and Markiw (1978) suggested that the fluorescence observed with the IFAT using M. cerebralis antisera was probably due to a reaction with an outer mucoid layer of the spores. One cross reaction occurred with M. insidiosus spores collected from chinook salmon exposed at Aumsville. In the present study absence of a mucus envelope on M. insidiosus, determined by background stain and SEM, suggested the fluorescence observed by Wolf and Markiw with M. insidiosus spores did not result from a mucoid substance.

The taxonomic importance of immunofluorescence methods have been noted by Halliday (1974) and Markiw and Wolf (1978). However the fluorescence of M. cerebralis spores treated by the IFAT with M. insidiosus antisera is difficult to explain. The two myxosporidans parasitize salmonids but infect different tissues. Furthermore, they are members of different myxosporidan families. Results of identical IFAT tests gave no fluorescence with H. salminicola, a myxosporidan which also parasitizes muscle and is in the same family as M. insidiosus, or with M. squamalis, a parasite

in the same family as M. cerebralis. Autofluorescence of M. cerebralis spores was ruled out by exposing untreated spores to ultraviolet light and also from negative result obtained when the parasite was treated with labeled anti-C. shasta sera. In future studies antigen analyses of myxosporidans with subsequent sera preparation against antigens specific for individual species may aid in solution of this problem.

Fluorescent antibody techniques could be used to detect infectious stages of myxosporidans and possible association with secondary hosts. Detection of the portal of entry for myxosporidans and localization of early stages of these parasites within the host by using histological techniques coupled with immunofluorescence would yield valuable information on the life history of these parasites.

SUMMARY AND CONCLUSIONS

1. Myxobolus insidiosus was found only in salmonid fish. No fish from the six other families collected in areas endemic for the parasite were infected.

2. The geographic range of M. insidiosus was extended to the Coos and Yaquina River systems; rainbow and steelhead trout were described as new hosts for the myxosporidan.

3. Pleomorphic features of M. insidiosus among and within host species indicated that subspecific recognition of this parasite is not warranted.

4. Chinook salmon are most susceptible to M. insidiosus, but other salmonids refractory during short exposures (8 d) became parasitized during longer exposure periods (50 d).

5. An exposure of 8 h or longer was required for chinook salmon to become parasitized with M. insidiosus at Aumsville Ponds.

6. Rate of development of M. insidiosus increased as temperature was elevated up to 18°C. No development occurred in fish held at 21°C.

7. Autoinfection occurred in chinook salmon parasitized with M. insidiosus.

8. Wet mount, histological, and ultrastructural examinations of chinook salmon indicated that M. insidiosus development was similar to that described for various myxosporidans.

9. The first amino acid analysis of spore valves of Myxosporeans was reported. Glutamic acid, aspartic acid, and lysine composed over 40% of the amino acids of M. insidiosus and C. shasta spore valves. Differences in other amino acids between the two myxosporidans suggested that such analyses may be of taxonomic value. The valves of M. insidiosus and C. shasta contained 9.5 and 7.8% protein, respectively.

10. Myxobolus insidiosus from various hosts and geographic areas possessed common antigens. No cross reactions between host and parasite antigens occurred in immunodiffusion tests.

11. Trophozoites and spores of M. insidiosus and C. shasta were detected by immunofluorescence. Cross reactivity between M. insidiosus antisera and M. cerebralis spores occurred but none was observed with other myxosporidans or when C. shasta labeled antisera was used.

12. A Myxobolus sp., similar to one described in Montana from cyprinids and a catostomid, was found for the first time in northern squawfish from the Willamette River.

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APPENDIX

Table 11. Genera and species of myxosporidans parasitizing the musculature[a] of fishes[b].

Parasite	Host(s)	Reference
Myxobolus baueri	Tinca tinca	Chernova 1970
M. bhadhrensis[c]	Labeo rohita	Seenapa and Manohar 1981b
M. calbasui	Cirrhina mrigala	Chakravarty 1939
M. carassi	Carassius carassius	Barysheva and Bauer 1957
M. catostomi	Catostomus commersoni	Fantham et al. 1939
M. cheni[c]	Mugil spp.	Shulman and Shtein 1962
M. chondrostomi[c]	common savetta	Shulman and Shtein 1962
M. cordis[c]	Barbus fluviatilis B. barbus	Kudo 1920 Schuurmans Stekhoven 1920
M. cycloides[d]	Leuciscus leuciscus	Barysheva and Bauer 1957
M. cyprini	Cyprinus carpio T. tinca Rutilus rutilus Abramis brama	Markevich 1951
M. destruens[c]	Scardinius erythrophthalmus	Schuurmans Stekhoven 1920
M. dispar	several cyprinids	Shulman and Shtein 1962
M. dogieli[c]	several cyprinids	Shulman and Shtein 1962
M. ellipsoides	several cyprinids	Shulman and Shtein 1962
M. funduli	Fundulus heteroclitus F. majalis	Kudo 1920
M. galaxii	Galaxias maculatus	Walliker 1969
M. grandiintercapsularis	Hypseleotris swinchonis	Shulman and Shtein 1962
M. grandis[c,e]	Leuciscus idus	Kulemina 1969
M. hosadurgensis	C. mrigala	Seenapa and Manohar 1981b

Table 11. (Continued)

M. homeospora	Ctenopoma multifilis	Paperna 1973
	C. murieri	
	Synodontis membranaceus	
M. indicum	C. mrigala	Tripathi 1951
M. inornatus[c]	Huro floridana	Fish 1938
M. insidiosus[c]	Oncorhynchus tshawytscha	Wyatt and Pratt 1963
	O. kisutch	Wyatt 1978a
	Salmo clarki	Wyatt 1979
	S. gairdneri	this author
M. iucundus[c]	Galaxias maculatus	Hine 1977
M. koi	Cyprinus carpio	Kudo 1920
	several cyprinids	Shulman and Shtein 1962
M. kubanicum	C. carpio	Shulman and Shtein 1962
	Carassius auratus	
M. lintoni[c,d]	Cyprinodon variegatus	Nigrelli and Smith 1938
M. muelleri[f,g]	Catostomus macrocheilus	Mitchell 1970
	Mylocheilus carinus	
	Richardsonius balteatus	
	Ptychocheilus oregonensis	
M. moxostomi	Moxostoma aureolum	Nigrelli 1948
M. musculi[g]	several cyprinids	Shulman and Shtein 1962
M. nodularis[c]	Rasbora daniconicus	Kudo 1920
M. notatus[c]	Pimephales notatus	Kudo 1920
M. notropis[c]	Notropis cornutus	Fantham et al. 1939
	N. heterolepis	
M. obliquus[c]	Carpiodes velifer	Kudo 1934
M. obpyriformis	Phoxinus phoxinus	Shulman and Shtein 1962
M. orbiculatus[c]	Notropis gilberti	Kudo 1920
	N. blennius	
M. oviformis	several cyprinids	Shulman and Shtein 1962

Table 11. (Continued)

M. percae	*Perca flavescens*	Fantham et al. 1939
M. pfeifferi[g]	*B. barbus*	Kudo 1920
M. pleuronectidae[c]	*Pseudopleuronectes americanus*	Kudo 1920
M. pseudodispar	*R. rutilus* *A. brama* *S. erythrophthalmus*	Shulman and Shtein 1962 Denham 1982
M. pseudorasborae	*T. tinca* *Rhodeus amarus* *Gobio albipenatus* *Saurogobio amurensis* *Pseudogobio rivularis*	Shulman and Shtein 1962
M. punctatus	*Ophicephalus punctatus*	Mukherjee and Haldar 1981
M. rasborae[c]	*R. daniconicus*	Schuurmans Stekhoven 1920
M. salmonis[c]	*Salmo ischcham*	Donec et al.1973
M. sandrae[h]	*Lucioperca lucioperca* *L. volgensis* *Acerina cornua* *L. sandrae*	Markevich 1951 Kudo 1920
M. spherocapsularis[c]	*Acheilognathus chankaensis*	Shulman and Shtein 1962
M. sprostomi	catfish	Shulman and Shtein 1962
M. subcircularis[c]	*C. commersoni*	Fantham et al. 1939
M. suturalis[c]	*P. phoxinus*	Shulman and Shtein 1962
M. talievi	cottids	Shulman and Shtein 1962
M. tauricus	*Barbus tauricus*	Miroshnichenko 1979
M. teres[c]	*Notropis whippli*	Kudo 1934
M. transversalis[c]	*N. cornutus*	Fantham et al. 1939
M. vanivilasae	*C. mrigala*	Seenapa and Manohar 1980
M. volgensis[h]	*L. lucioperca*	Reuss 1966
Myxobolus sp.[c]	*R. daniconicus*	Kudo 1920

Table 11. (Continued)

Myxobolus sp.[c]	*C. variegatus*	Rigdon and Hendricks 1955
Myxobolus sp.[c]	*S. erythrophthalmus*	Kozicka 1959
Myxobolus sp.[c]	several cyprinids	Shulman and Shtein 1962
Myxobolus sp.[c]	*Pimephales promelas*	Krall et al. 1982
Myxobolus sp.[c]	*Etheostoma nigrum*	Dechtiar 1972
Myxobolus sp.[c]	*Fundulus diaphanus*	Wiles 1975
Myxobolus sp.[c]	*N. cornutus*	Mitchell 1978
Henneguya diversis	*Ictalurus punctatus*	Minchew 1977
H. electrica[c]	*Electrophorus electricus*	Jakowska and Nigrelli 1953
H. ophicephali	*O. punctatus*	Chakravarty 1939
H. otolithi[c]	*Otolithus ruber* *O. maculatus*	Ganapathi 1941
H. psorospermica	*Esox lucius* *Perca flavescens* *L. lucioperca*	Shulman and Shtein 1962
H. salminicola[c]	*O. kisutch* *O. keta* *O. nerka* *O. tshawytscha*	Kudo 1920 Fish 1939
H. schizura	*E. lucius*	Shulman and Shtein 1962
H. sebasta[c]	*Sebastes* spp.	Moser and Love 1975
H. tegidiensis[c]	*Coregonus clupeoides*	Nicholas and Jones 1959
H. travassori[c]	*Astyanax fasciatus* *Leporinus copelandi*	Guimaraes and Bergamin 1933
H. visceralis	*E. electricus*	Jakowska and Nigrelli 1953
H. zschokkei	*Coregonus* spp. *O. nerka* *O. kisutch* *Salvelinus* sp.	Kudo 1920 Shulman and Shtein 1962

Table 11. (Continued)

	E. lucius P. flavescens A. brama catfish Prosopium williamsoni P. coulteri	Newell and Canaris 1969
Henneguya sp.[c]	Pomatomus saltatrix	Meyers et al. 1977
Unicauda monura[c]	Aphredoclerus sayanus	Kudo 1920
Thelohanellus fuhrmanni	Nemachilus barbulatus other loaches several cyprinids	Shulman and Shtein 1962
T. gangeticus[c]	Chela bacaila	Tripathi 1951
T. notatus	C. commersoni N. cornutus Pfrille neogoeus Hyborhynchus notatus	Fantham et al. 1939
T. pyriformis	several cyprinids	Shulman and Shtein 1962
T. swellengrebeli[c]	R. amarus	Schuurmans Stekhoven 1920
Myxosoma andhrae[c]	O. punctatus	Lalitha Kumari 1969
M. bibullatum[c]	C. commersoni	Kudo 1934
M. branchialis	B. barbus Ctenopharyngodon idella R. rutilus R. amarus P. phoxinus	Shulman and Shtein 1962
M. catostomi[c]	C. commersoni	Dechtiar 1972
M. dermatobia	Anguilla dermatobia	Copland 1982
M. homeospora[c]	Tilapia sp.	Baker 1963
M. lairdi[c]	Liza macrolepis	Narasimhamurti and Kalavati 1979b

Table 11. (Continued)

M. karnatakae[c]	*Barbus chola*	Hagargi and Amoji 1981
M. multiplicata[c]	*Idus melanoticus* *L. leuciscus*	Kudo 1920 Markevich 1951
M. pseudorasborae[c]	*Pseudorasbora parva*	Hoshina 1952
Myxosoma sp.[c]	*Fundulus similis*	Rigdon and Hendricks 1955
Kudoa amamiensis[c]	*Abudefduf sexfasciatus* *A. vagiensis* *Chromis isharai* *C. notatus* *Chrysiptera assimilis* *Seriola quinqueradiata*	Egusa and Nakajima 1980
K. bengalensis[c]	*Tachysurus platystomus*	Sarkar and Mazumder 1983
K. bora[c]	*Mugil cephalus*	Egusa and Nakajima 1980
K. chilkaensis[c]	*Strongylura strongylura*	Tripathi 1951
K. clupeidae[c]	*Clupea harengus* *Pomolobus pseudoharengus* *P. aestivalis* *P. mediocris* *Brevoortia tyrannus* *Stenotomus chrysops* *Tautogolabrus adspersus*	Kudo 1920
K. crumera[c]	*Scombromerous maculatus*	Egusa and Nakajima 1980
K. funduli[c]	*F. heteroclitus* *F. diaphanus*	Kudo 1920
K. histolytica[c]	*Scomber scomber* *Salmo salar*	Perard 1928 Prudhomme and Pantaleon 1959
K. musculoliquefaciens[c]	*Xiphias gladius*	Matsumoto 1954
K. paniformis[c]	*Merluccius productus*	Kabata and Whitaker 1981
K. pericardialis[c]	*S. quinqueradiata*	Nakajima and Egusa 1978
K. quadratum[c]	*Syngnathus acus* *Trachurus trachurus* *Nerophis aequorens*	Kudo 1920

Table 11. (Continued)

	Callionymus lyra Coris julis Ariodes polystaphilidon Zeus capensis Merluccius capensis Thyrsites atun	Davies and Byers 1947
K. rosenbuschi[c]	Merluccius gayi	Gelormini 1944
K. sphyraemi[c]	Sphyraema jello	Narasimhamurti and Kalavati 1979a
Kudoa sp.[c]	M. cephalus	Rigdon and Hendricks 1955
Kudoa sp.[c]	Microstomus pacificus Hippoglossus stenolepis Parophrys vetulus Eopsetta jordani Platichthys stellatus	Patashnik and Groninger 1964
Kudoa sp.[c]	Chaetodon sp.	Voelker et al. 1978
Kudoa sp.[c]	Tennodon saltator	Egusa and Nakajima 1980
Kudoa sp.[c]	Thunnus thynnus	Egusa and Nakajima 1980
Kudoa sp.[c]	Limanda aspera	Konagaya 1980
Kudoa sp.[c]	M. gayi	Okada et al. 1981
Kudoa sp.[c]	Arnoglossus thori A. imperialis	Lom and Dykova 1982
Neochloromyxum cruciformum[c]	Lateolabrax japonicus	Matsumoto 1954
Unicapsula muscularis[c]	H. stenolepis	Davis 1924
U. pflugfelderi[c]	Maena smaris	Schubert et al. 1979
U. seriolae[c]	Seriola lalandi	Lester 1982
Unicapsula sp.[c]	Polynemus sheridani	Lester 1982

Table 11. (Concluded)

Hexacapsula neothunni[c]	*Neothunnus macropterus*	Arai and Matsumoto 1953
Pentacapsula sp.[c]	*Chaetodon collare*	Cheung et al. 1983
Myxidium serum	*Anguilla dieffenbachii*	Hine 1975
M. giardi	*Anguilla* spp.	Hine 1980
Leptotheca perlata	*Acerina cornua*	Kudo 1920

[a]Includes skeletal, cardiac and alimentary tract muscles.

[b]No myxosporidans which infect muscle tissue of amphibians, reptiles or invertebrates have been described.

[c]Muscle tissue is the only described site of infection.

[d]Synonymous with *Myxobolus muelleri* (Shulman and Shtein 1962).

[e]Name not valid as it was used to describe a myxosporidan from *Notropis cornutus* by Fantham et al. (1939).

[f]Only description of this species from muscle tissue and from North America.

[g]Mitchell (1970) synonymized *Myxobolus musculi* and *Myxobolus pfeifferi* with *Myxobolus muelleri*.

[h]Synonymous and known as *Myxobolus sandrae* (Shulman and Shtein 1962).