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Water-borne transmission of *Dermocystidium salmonis* in the laboratory

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ABSTRACT: *Dermocystidium salmonis* is a gill pathogen of salmonid fishes in the U.S. Pacific Northwest where it has been associated with mortality of adult and juvenile chinook salmon *Oncorhynchus tshawytscha*. The previously unknown mode of *D. salmonis* transmission was determined and demonstrated in the laboratory. Uniflagellated zoospores developed within spores obtained from gill cysts and produced infections in pink salmon *O. gorbuscha* fry. These infections were lethal, and histological examination of infected gill tissue revealed large numbers of *D. salmonis* cysts in gill epithelia. Electron microscopic examination of immature spores from experimental infections showed that they were identical to immature spores in naturally infected juvenile chinook salmon.

INTRODUCTION

The genus name *Dermocystidium* has been applied to a variety of pathogenic organisms that infect aquatic animals. The systematic placement of these pathogens has been difficult to determine and it is likely that several taxonomic groups are represented. The *Dermocystidium* spp. that infect fish locate either in epithelial tissues of skin and gills (Dunkerly 1914, Davis 1947, Elkan 1962, Wootten & McVicar 1982) or in visceral organs (McVicar & Wootten 1980, Moer et al. 1986, Hedrick et al. 1989, Nash et al. 1989). Those infecting molluscs have been shown to have affinities to the coccidians (Phylum Apicomplexa) (Perkins 1976) and have been placed in a new genus, *Perkinsus* (Levine 1978).

Among the gill-infecting *Dermocystidium* species is a pathogen of Pacific salmon in the U.S. Pacific Northwest. This organism was first described as *D. salmonis* from an adult chinook salmon *Oncorhynchus tshawytscha* in California by Davis (1947). A pathogen conforming to the description of *D. salmonis* has been associated with mortality in both juvenile and adult chinook salmon as well as with infection of coho *O. kisutch* and sockeye *O. nerka* salmon in the Columbia River system (Pauley 1967, Allen et al. 1968). Hoskins et al. (1976) reported that *Dermocystidium* sp. was the primary cause of a massive fish kill on Vancouver Island (Canada) in 1973. In recent years, the organism has been increasingly documented as a lethal patho-

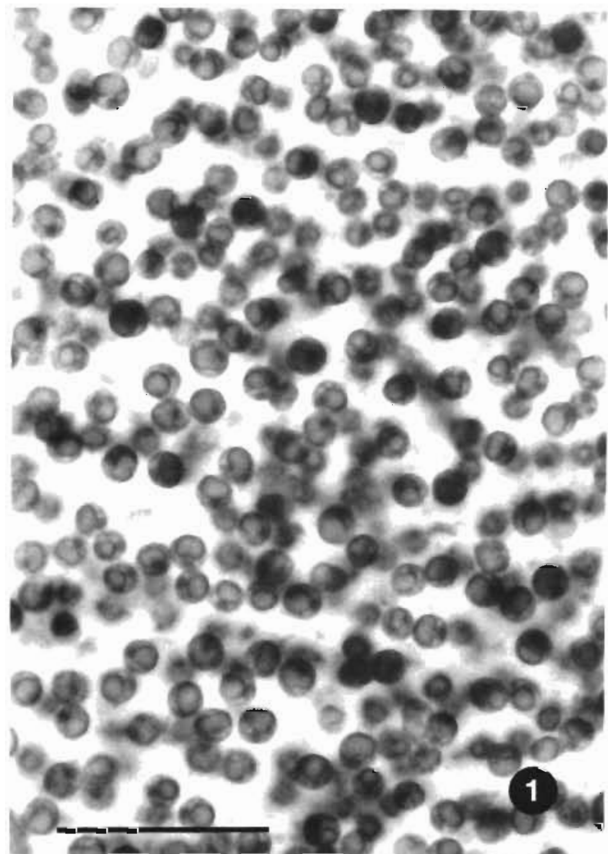

Fig. 1. *Dermocystidium salmonis* in *Oncorhynchus tshawytscha*. Mature spores in histological section of a cyst from the gill of a naturally infected adult chinook salmon (H&E). Bar = 25 µm

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gen of salmonids in Oregon where substantial mortalities of juvenile and adult chinook salmon have occurred (Olson & Holt unpubl.). Mortality among infected chinook salmon in the Elwha River of Washington has also been observed recently (Kevin Amos, Washington Department of Fisheries, pers. comm.).

Neither the life cycle of this pathogen nor its mode of transmission has been determined. In an effort to begin to understand the conditions that lead to epizootics, this study was conducted to investigate the mode of transmission of *Dermocystidium salmonis* infection.

MATERIALS AND METHODS

Dermocystidium salmonis cysts containing mature spores were obtained from gills of naturally infected adult fall chinook salmon returning to the Trask State Fish Hatchery near Tillamook, Oregon in November 1989. Cysts containing immature spores were obtained from gills of naturally infected juvenile chinook salmon reared at the Trask Hatchery in 1988. The gill tissue containing cysts from adult fish was held on ice for ca 6 h and brought to the laboratory where cysts were removed by scraping infected tissue with a scalpel. Cysts were washed repeatedly in fresh water until little extraneous material remained. The resulting accumulation of cysts was held in fresh water at 4 °C in the absence of antibiotics until zoospores first appeared after about 2 wk. The number of zoospores increased for several days and large numbers of actively motile individuals were available for transmission experiments 3 wk after incubation in fresh water was begun.

Pink salmon (*Oncorhynchus gorbuscha*) fry for transmission experiments were hatched from eggs obtained

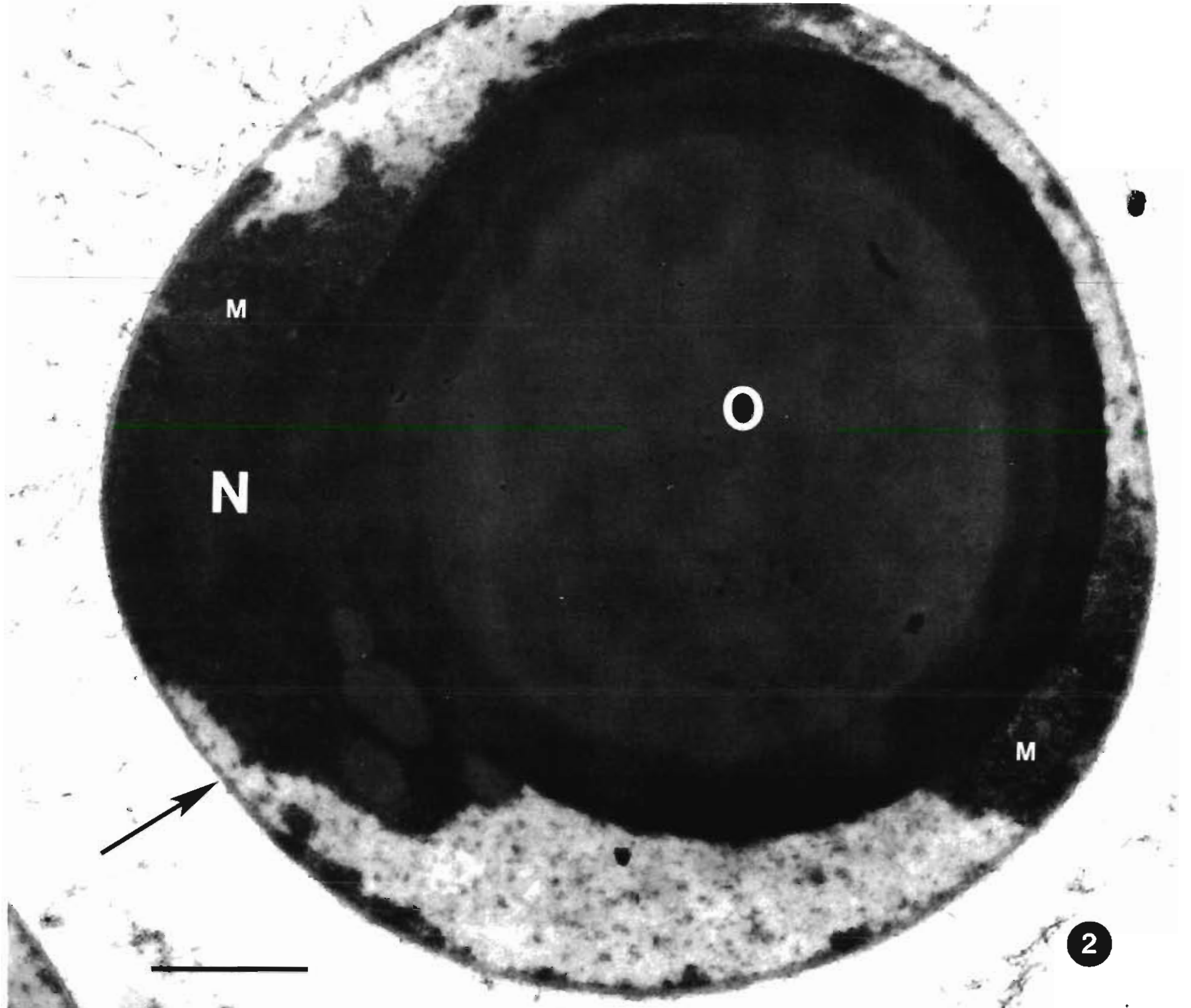


Fig. 2. *Dermocystidium salmonis*. Transmission electron micrograph of mature spore from a naturally infected adult chinook salmon. N: nucleus; M: mitochondrion; O: osmiophilic inclusion; arrow: spore wall. Bar = 1.0 µm

from the Valdez Fishery Development Hatchery in Valdez, Alaska, and reared under quarantine conditions at the Hatfield Marine Science Center Laboratory for Fish Disease Research. Fry measuring ca 30 mm in total length were exposed to putative *Dermocystidium salmonis* zoospores in two 20 l aquaria, each containing 25 fish. A third 20 l aquarium contained 25 pink salmon fry that were not exposed to zoospores and served as controls. Fish were held in aerated, static water for 15 d at temperatures varying between 12 and 15 °C. The fish were not fed during the observation period.

For electron microscopy, freshly collected *Dermocystidium salmonis* cysts from naturally infected adult and juvenile chinook salmon, cysts held in fresh water at 4 °C for several weeks, and gills from laboratory infected pink salmon were placed in 1.7 % glutaraldehyde in 0.1 M Millonig's phosphate buffer (pH 7.2) and fixed for either 16 h at 4 °C or 1.5 h at 20 °C. The samples were rinsed for 15 min in 0.14 M Millonig's phosphate buffer and post-fixed for 1.5 h with 2 % OsO_4 in 0.1 M Millonig's phosphate buffer. Tissues were dehydrated through a graded acetone series and embedded in Spurr's low viscosity resin. Thin sections (60 nm) were stained with aqueous uranyl acetate and lead citrate prior to examination with a Philips CM 12/STEM electron microscope at 60 kV.

For light microscopy, smears of zoospores were air-dried, fixed in absolute methanol and stained with Giemsa. Gills from naturally infected juvenile chinook salmon, entire exposed pink salmon, and gills from exposed pink salmon were fixed in Bouin's solution for at least 48 h before transferring them to 70 % ethanol

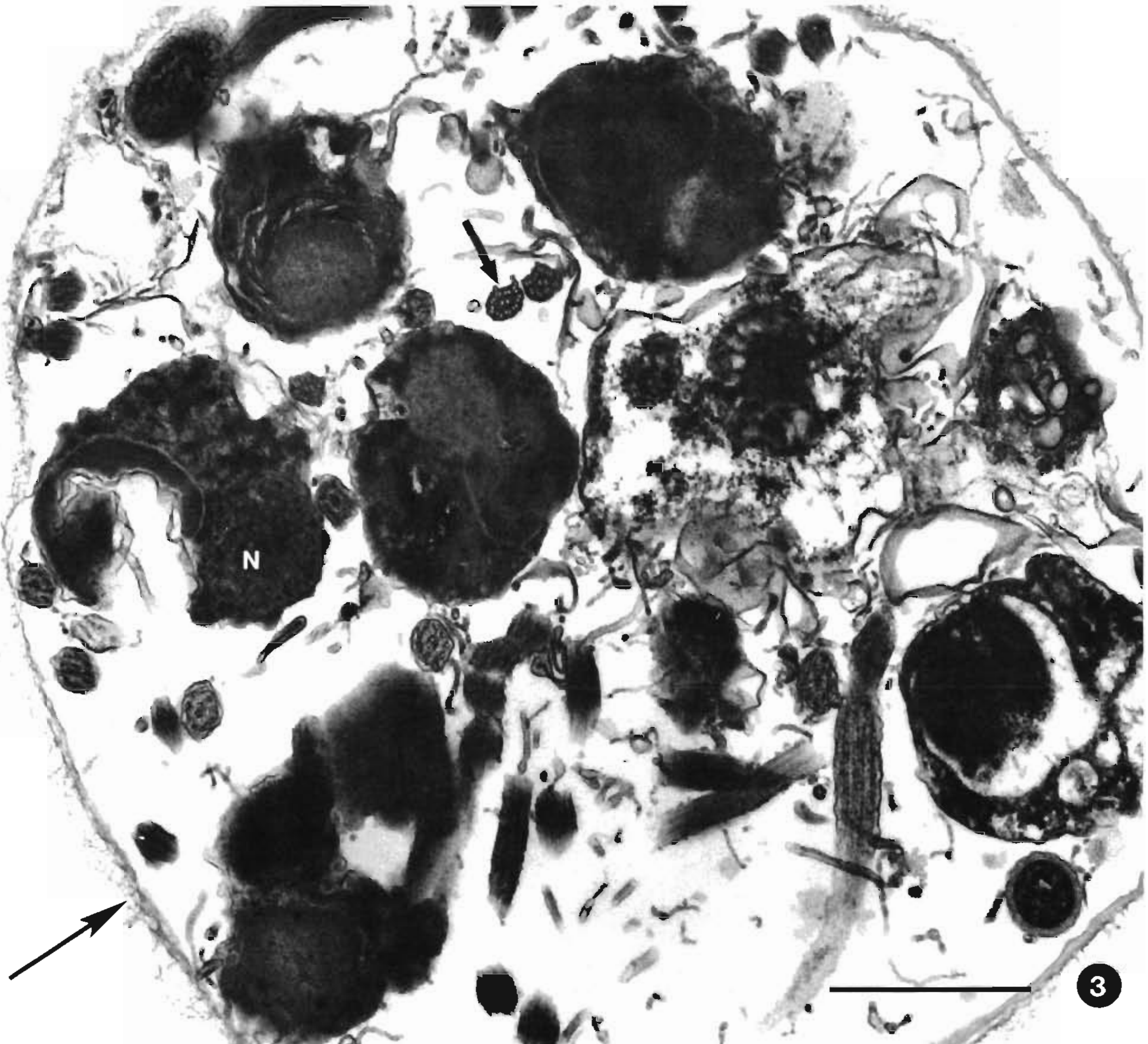


Fig. 3. *Dermocystidium salmonis*. Transmission electron micrograph of several developing zoospores within a single mature spore, after incubation at 4 °C for 14 d. Long arrow: spore wall; short arrow: cross-sectioned flagellum showing typical microtubule arrangement; N: nucleus. Bar = 1.0 µm

and processing for standard paraffin embedding and sectioning. Tissue sections were cut at 7 µm and stained with hematoxylin and eosin.

RESULTS

Gills of adult fall chinook salmon that served as a source of *Dermocystidium salmonis* cysts were infected with up to 50 cysts per primary gill lamella. The white, round cysts were up to 1.1 mm in diameter and averaged 0.5 mm. They were loosely associated with the gill epithelium and were easily removed for incubation in fresh water. Spores within cysts were spherical with a peripherally located nucleus and a large, eccentric inclusion and measured 5 to 8 µm in diameter (Fig. 1).

Electron microscopic examination of mature *Dermocystidium salmonis* spores revealed a large, osmiophilic inclusion that relegated the nucleus to its peripheral position. Structures suggestive of mitochondria were also present, but were not clearly resolved (Fig. 2). Spores were observed under a light microscope periodically during freshwater incubation, and, over a 14 d period, lost their eccentric inclusion and became granular in appearance. Electron microscopic examination of spores incubated in fresh water revealed that the granular appearance was due to the internal development of flagellated zoospores. Zoospores measured about 1 µm in diameter, had a single flagellum (9 + 2 microtubule pattern) and indistinct nuclei and mitochondria (Fig. 3). No apical complex or rhopteries were observed.

Free-swimming zoospores (Fig. 4) were first observed after 15 d at 4 °C. They became increasingly numerous and after 18 d were present in massive numbers. To confirm that these zoospores were an infectious stage of *Dermocystidium salmonis*, 50 pink salmon fry were exposed to them in aquaria as described above. After 11 d at 12 to 15 °C, a single exposed pink salmon exhibited darkening and hyperventilation and, when examined under a dissecting microscope, was found to contain numerous cysts in the gills. During the next 4 d, all exposed pink salmon fry died and all showed abundant cysts on their gills (Fig. 5). No unexposed control fish died during the 15 d observation period and all were negative for gill cysts when examined several days later.

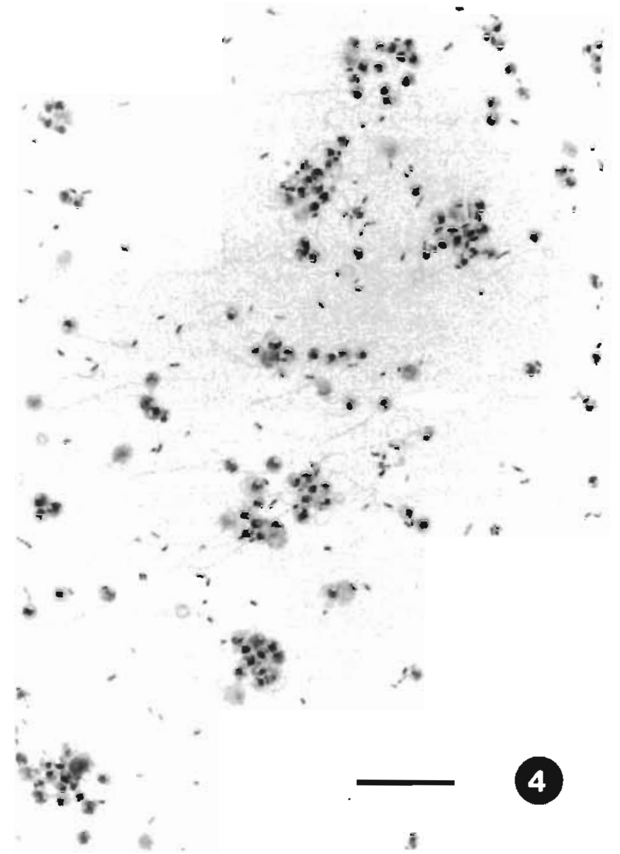
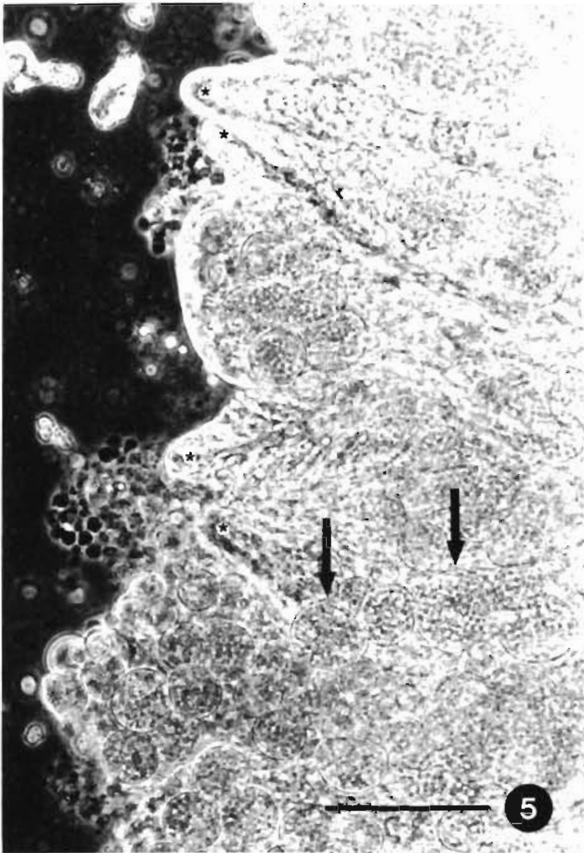


Fig. 4. *Dermocystidium salmonis*. Smear of zoospores that were free-swimming in water incubated at 4 °C (Giemsa). Arrow: zoospore. Bar = 10 µm

Examination of histological sections from infected pink salmon fry revealed large numbers of *Dermocystidium salmonis* cysts in gill epithelia. The presence of cysts induced epithelial hyperplasia, caused distortion of lamellar architecture and, undoubtedly, compromised respiratory function (Figs. 6 & 7). Although the vast majority of cysts was associated with gill epithelia, some were associated with non-gill epithelia lining the opercular cavity (Fig. 8), as well as with dermal epithelia of the head and anterior body. No cysts were associated with epithelia of posterior portions of the

Figs. 5 to 8. *Dermocystidium salmonis* in *Oncorhynchus gorbuscha*. Fig. 5. Wet mount of experimentally infected pink salmon gill, 15 d post-exposure to *D. salmonis* zoospores (phase contrast). *: tips of 2° gill lamellae; arrows: small developing cysts containing immature, proliferating spores. Bar = 100 µm. Fig. 6. Histological section of experimentally infected pink salmon gill, 15 d post-exposure to *D. salmonis* zoospores (H&E). Small cysts containing immature, proliferating spores are seen associated with 1° and 2° gill lamellae (arrows). Destruction of normal gill architecture resulting from parasite growth and epithelial hyperplasia can be seen. Bar = 100 µm. Fig. 7. Histological section of experimentally infected pink salmon gill, 15 d post-exposure to zoospores (H&E). Several walled cysts containing immature, proliferating spores are shown, as well as a blood capillary (C). Note mitotic figures in hyperplastic epithelial cells associated with cysts (arrows), and thickened gas exchange barrier which results. Bar = 10 µm. Fig. 8. Histological section of head of experimentally infected pink salmon, 15 d post-exposure to *D. salmonis* zoospores (H&E). Several small cysts containing immature, proliferating spores are seen within the dermal epithelium (arrow). Bar = 50 µm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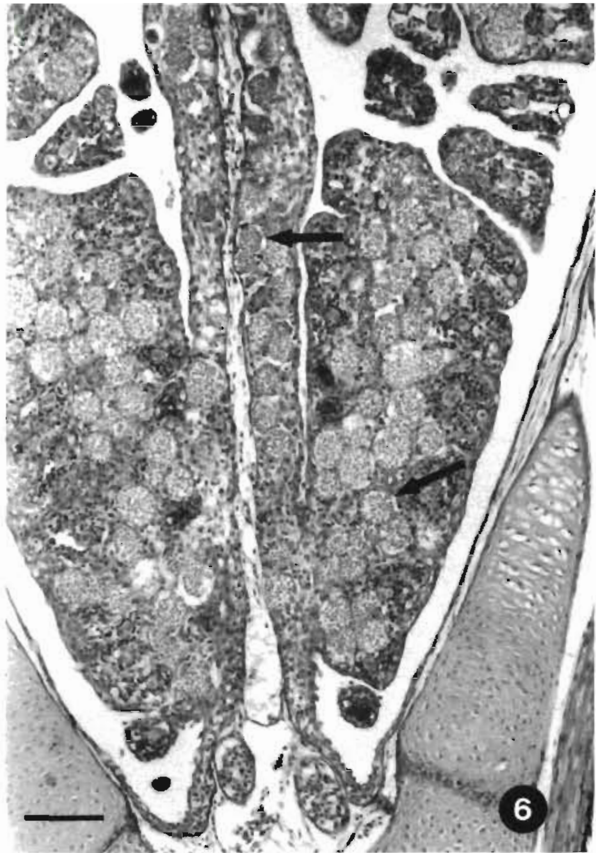
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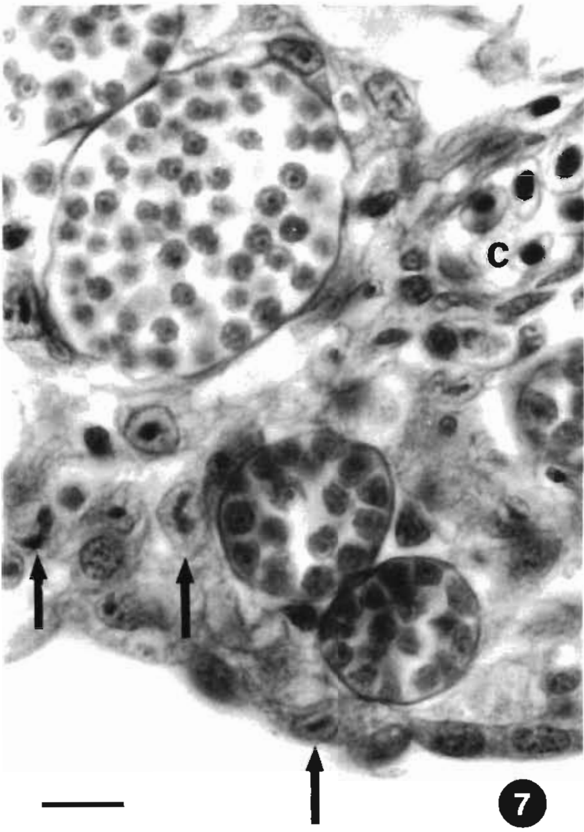
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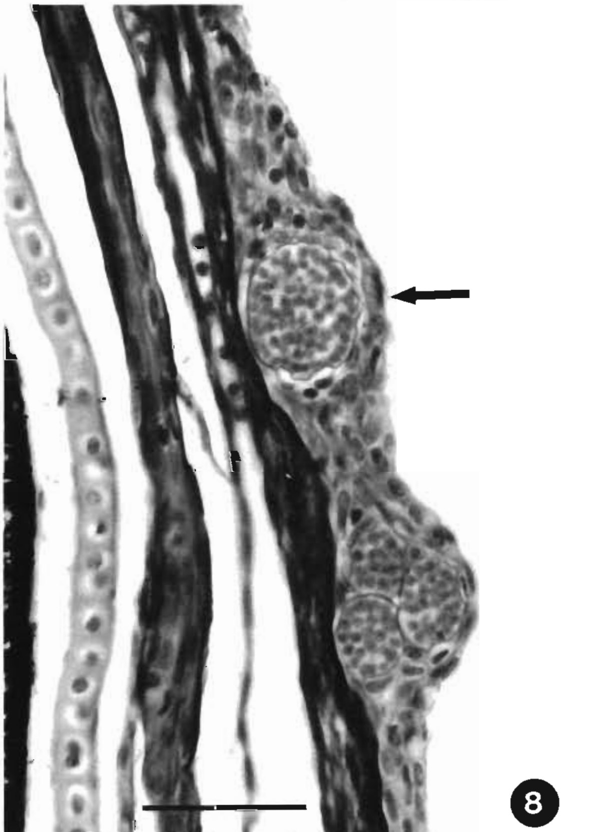
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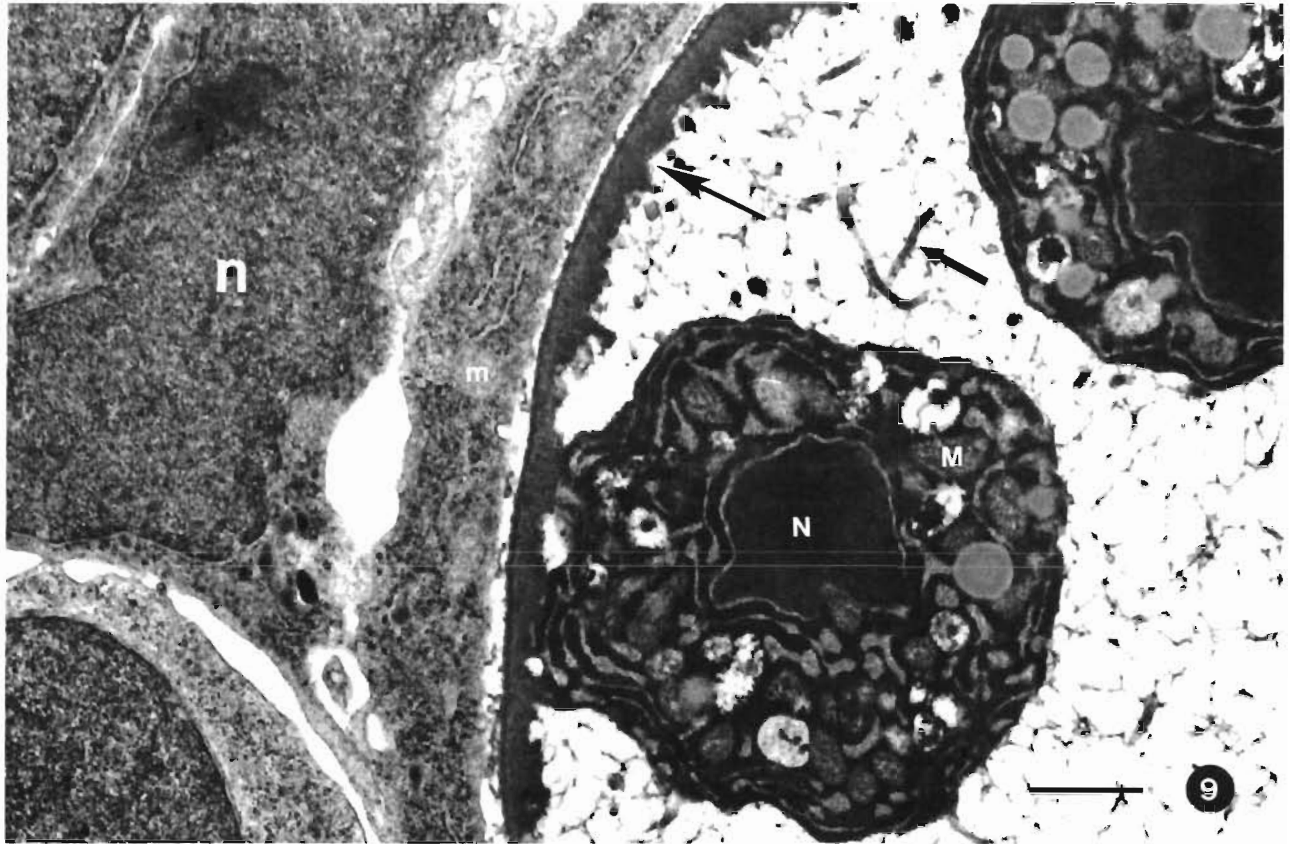

Fig. 9. *Dermocystidium salmonis* in *Oncorhynchus gorbuscha*. Transmission electron micrograph of a developing spore within a cyst on the gill of an experimentally infected pink salmon fry, 15 d after exposure to zoospores. n: host cell nucleus; m: host cell mitochondrion; N: spore nucleus; M: spore mitochondrion; long arrow: cyst wall; short arrow: pseudopodial spore projection. Bar = 1.0 µm

body, although several were found infecting the epithelial covering of visceral organs in fry that had not completely absorbed the yolk sac when they were exposed to zoospores.

Examination of gill tissue from infected fish with the electron microscope revealed developing *Dermocystidium salmonis* spores (Fig. 9) that were identical to those observed in naturally infected juvenile chinook salmon at the Trask Hatchery during a 1988 epizootic (Fig. 10). Morphological features of immature *D. salmonis* spores include well-defined nuclei and nucleoli, but an apparent absence of a nuclear membrane, probable mitochondria and an irregular spore membrane with pseudopodial projections into cyst matrix material.

DISCUSSION

The characteristics of cysts and spores observed during this study are consistent with those described for *Dermocystidium salmonis* by Davis (1947) and for *Dermocystidium* sp. by Pauley (1967). This study describes the first observation of zoospore development in a fish-infecting *Dermocystidium* species, and the first laboratory transmission of infection by a water-borne route. One stage of the pathogen was isolated from a naturally infected salmonid host. Following induction of zoosporulation, motile zoospores were shown to be infective and highly pathogenic for a second salmonid host. Gill lesions were similar in both host species, as was the characteristic morphology and ultrastructure of the pathogen within these lesions.

These data demonstrate that a motile, flagellated cell is part of the *Dermocystidium salmonis* life cycle and is the agent of disease transmission. To obtain zoospores, purified gill cysts containing mature spores were incubated at 4 °C to inhibit overgrowth by contaminating bacteria, fungi and protozoans, without recourse to the use of antimicrobial compounds. While differentiation of infectious zoospores occurred at this temperature, the influence of temperature on differentiation and competence of infectious zoospores, and thus on transmission of the disease, remains unresolved.

Monitoring of naturally infected juvenile chinook salmon in our laboratory has shown that cysts containing mature spores are sloughed from the gills during the course of the disease, and that some infected fish appear to recover (Olson & Holt unpubl.). The fate of

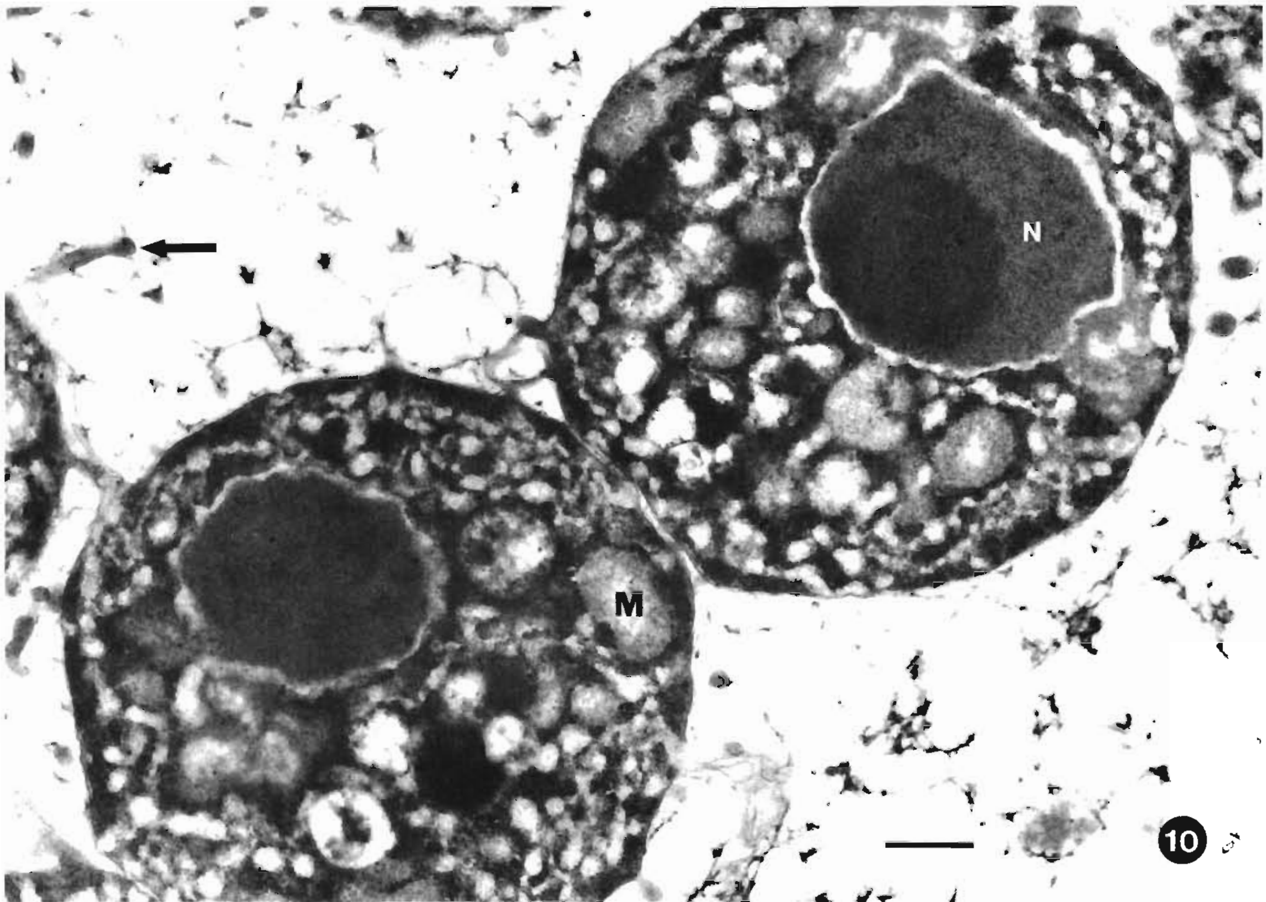

Fig. 10. *Dermocystidium salmonis* in *Oncorhynchus tshawytscha*. Transmission electron micrograph of developing spores within a cyst on the gill of a juvenile chinook salmon with a natural *D. salmonis* infection. N: spore nucleus; M: spore mitochondrion; arrow: pseudopodial spore projection. Bar = 1.0 μm

spores contained in sloughed gill cysts, or of those contained in cysts on spawning adult salmon that die in freshwater systems, is not known, but may represent a significant source of infection.

The present study also demonstrates that, in addition to chinook, coho, and sockeye salmon, reported by other authors as hosts of *Dermocystidium salmonis,* pink salmon are also susceptible. While the complete host range of this pathogen remains unknown, our data show that infections may result from disease transmission between different species and age classes of anadromous salmonids present in freshwater systems. The possibility of non-salmonid reservoirs of infection must also be considered in light of the broad range of fish species which are reported as hosts for *Dermocystidium* species (Pauley 1967).

The laboratory infection of pink salmon fry reported here followed exposure to large numbers of zoospores and was lethal to all fish that were exposed. The histopathological response to experimental *Dermocystidium salmonis* infection resembled that described by Pauley (1967) for naturally infected chinook salmon. However, the massive nature of the laboratory infection involved the entire gill structure, and fusion of gill elements due to epithelial hyperplasia was virtually complete. The intensity of infection was such that death was probably due to asphyxiation, resulting from disruption of the respiratory gas exchange barrier. The predominant distribution of cysts in gill tissue and their occurrence in the skin and fins of the anterior, but not the posterior, trunk suggests a tropism on the part of infecting zoospores. These effects have been noted by other investigators (Pauley 1967, Allen et al. 1968). The basis for such a tropism may be the ability of gill tissues to provide the nutrients and/or respiratory gases required during proliferation of vegetative spores which occurs in gill cysts prior to spore maturation. Another possibility is that respiratory currents physically concentrate zoospores in the opercular chamber.

In this report, the terms spore and zoospore have been used in the general sense to differentiate the flagellated and non-flagellated cell types of *Dermocystidium salmonis.* To date, we have no informa-

tion on the ploidy of either cell type, nor their capabilities for sexual reproduction. Our observations indicate that the spores described by Davis (1947) and Pauley (1967) as contained in gill cysts are each capable of differentiating multiple flagellated cells under the incubation conditions described here. Free-swimming zoospores are capable of penetrating the gill epithelium of a new host fish, and give rise to cysts containing large numbers of non-flagellated spores which appear to proliferate within the cyst prior to maturation. Spores within a given cyst appear to mature synchronously, and when mature are capable of differentiating a new generation of infectious zoospores. Thus, at least 2 distinct proliferative events occur in the life cycle of *D. salmonis*, but whether sexual recombination of genetic material occurs during either is not known.

To date, ultrastructural observations of *Dermocystidium salmonis* zoospores have not revealed organelles which would suggest affinities of this fish pathogen with apicomplexans such as *Perkinsus marinus* (Levine 1978). However, taxonomically useful information is expected from further ultrastructural analysis. Further studies are also required to gain an understanding of pathogen development, and of environmental conditions which promote epizootics.

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