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PARASITES OF FRESHWATER FISH

II. PROTOZOA

1. Microsporidea of Fish

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The microsporidea are among the smallest protozoans parasitizing freshwater fish; the majority being 2-10 microns in length. Stages of their vegetative development usually are not recognized unless found with the mature (spore) forms. Microsporidian spores have a diversity of forms, most being oval, ovo-cylindrical, or pyriform. They are typically cytozoic parasites and undergo asexual division (schizogony) and fusion (sporogony) producing many spores within the host cells until there is an enormous hypertrophy of cells, resulting in the formation of cysts (fig. 1).

There are few detailed reports of microsporidea from North American freshwater fish which is probably due to their minuteness and not their absence.

MORPHOLOGY AND IDENTIFICATION:

Any white or opaque structure which might be suspect for a microsporidian cyst should be stained or broken open, and the contained material put on a microscope slide and viewed carefully under high dry or oil immersion lens.

The microsporidian stage most easily recognized is the spore stage which varies in

form from one species to another. The following forms have been reported: oval, ovo-cylindrical, pyriform, reniform, tubular, bacilliform, crescentic, commaform, and spherical. The spores are covered by a chitinous membrane - a single piece in most species. They are extremely refractive except for a clear space or "vacuole" at one or both ends. Internally the spores have a polar filament which in some species is contained in a definite polar capsule, while in others it lies coiled beneath the inner spore membrane as shown by electron microscopy. From the middle to the posterior region of the spore is found the sporoplasm which contains the nuclear structures. Also located internally as shown by electron microscopy is the polaroplast, which is a system of laminated structures within a ground substance extending from around the anterior (basal) portions of the polar filament into the posterior region of the spore, which, along with the sporoplasm, gives a girdle-like appearance within the spore (fig. 2).

In early infections the parasites may not have developed to the spore stage. This makes positive identification extremely difficult because the cysts are smaller and contain only development (vegetative) stages.

The following microsporidians have been reported from North American fresh-water fishes.

Glugea anomala Moniez, 1887. Reported in Gasterosteus aculeatus from Otter Creek, Anchorage, Alaska.

Glugea hertwigi Weissenberg, 1911. Reported in Osmerus mordax from Sunapee and Massabesic Lakes, and from the coastal waters of Maine; in O. mordax from Lake Edward, Quebec, Canada; and in O. mordax from Loon Pond, Gilmanton, Lake Winnisquam, and the Great Bay Region, N. H.

Nosema pimephales. Reported in Pimephales promelas from Canada.

Plistophora salmonae Putz and Hoffman, 1964. Reported in Salmo gairdneri (rainbow and steelhead trout) from California.

Plistophora sp. Bond, 1937. Reported in Fundulus heteroclitus from Chesapeake Bay.

Plistophora sp. Wales and Wolf, 1955. Reported in Salmo gairdneri, Oncorhynchus nerka and Cottus sp. from northern California. Probably synonymous with P. salmonae, Putz and Hoffman, 1964.

Plistophora cepedianae Putz and Hoffman, 1964. Reported in Dorosoma cepedianum from Ohio.

Plistophora ovariae Summerfelt, 1964. Reported in Notemigonus crysoleucas from Illinois, Kentucky, Missouri and Arkansas.

LIFE CYCLE: Upon the death of an infected fish, and/or the rupture of the microsporidian cyst, the spores are set free in the water to continue the parasite's life cycle.

After gaining entrance into a new fish host, the mature spore may attach to the intestinal epithelium with its polar filament which is extruded under the action of the fish digestive fluids. After attachment, the sporoplasm emerges from the spore as an amoebula which, by amoeboid movements, penetrates through the intestinal epithelium, and travels to the specific site of infection via the bloodstream or other means. At this point the amoebula enters the host cell and is now called a tropho-

zoite. It begins and continues division (shizogony and fusion (sporogony) until spores are formed thus completing the parasite's life cycle, (figs. 3-11).

TRANSMISSION: The exact method of transmission from an infected fish to a non-infected fish is not known, but it is believed that once the spores are freed by the death of the old host, and/or rupture of the microsporidian cyst, they are taken in by the new fish host directly from the water or via a food organism serving as a transport or intermediate host.

The viability of microsporidian spores of fish has not been adequately studied, but their chitinoid outer covering in an aquatic medium should enable them to remain viable for long periods.

PATHOGENICITY: A few serious microsporidian epizootics have been reported. Probably others have occurred, but have not been reported.

One of two epizootics in California involved 170,000 advanced steelhead fingerlings resulting in an almost complete loss, while the second epizootic involved a similar group of British Columbia rainbow fingerlings which suffered a three-quarter loss.

Large numbers of young gizzard shad were found dead around the shore of a lake in Ohio, and upon examination were found to be infected with a microsporidian causing a huge cyst protruding from the abdominal wall (fig.). A sample of 161 young of the year shad showed 107 afflicted fish.

Spawning failures of 5-year-old female golden shiner brood stock at a hatchery in Illinois were attributed to microsporidian infection of the ovaries. In two ponds the incidence of infection was 65 percent. No eggs were found in the infected fish, which suggests that brood stock may become sterile as a result of microsporidian infections.

SYMPTOMS: Clear-cut diagnosis on the basis of gross symptoms is usually not possible without finding spores by microscopic

examination. Fish involved in the previously noted epizootics swam about in a moribund condition, and in the case of the acute hatchery epizootics of salmonids, the dying fish lay quietly in the shallow water of the pond or descended to the lower end.

GEOGRAPHICAL RANGE: Microsporidian infections of freshwater fish of North America have been reported from Alaska, Arkansas, California, Canada, Illinois, Kentucky, Maine, Maryland, Missouri, New Hampshire and Ohio.

CONTROL AND TREATMENT: Biological or chemical treatments for microsporidia of fish are not known. Should an epizootic occur at a hatchery, the fish should be buried or incinerated, not shipped to other hatcheries.

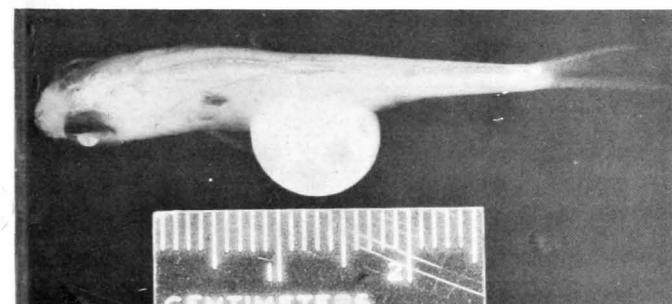


Figure 1:--Macro photograph of gizzard shad *Dorosoma cepedianum* showing a microsporidian (*Plistophora cepedianae*) cyst.

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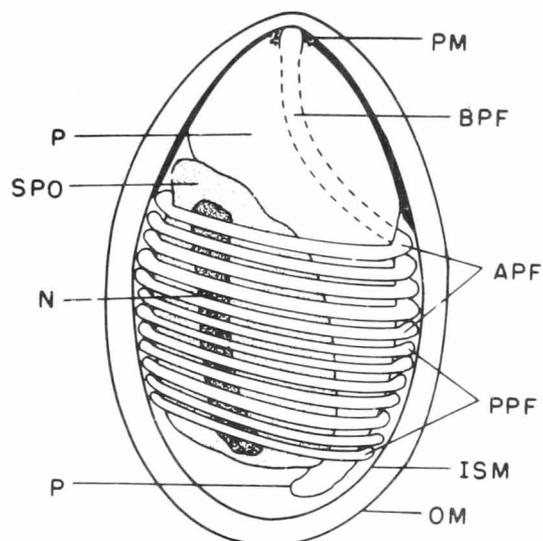


Figure 2:--Schematic drawing showing the structure of a microsporidian spore (*Thelohania californica*) after Kudo and Daniels, 1963. Abbreviations used: APF = anterior polar filament. BPF = basal portion of polar filament. ISM = inner surface membrane. N = nucleus. OM = outer membrane. P = polaroplast. PM = polar-mass and polaroplast. PPF = posterior polar filament. SPO = sporoplasm.

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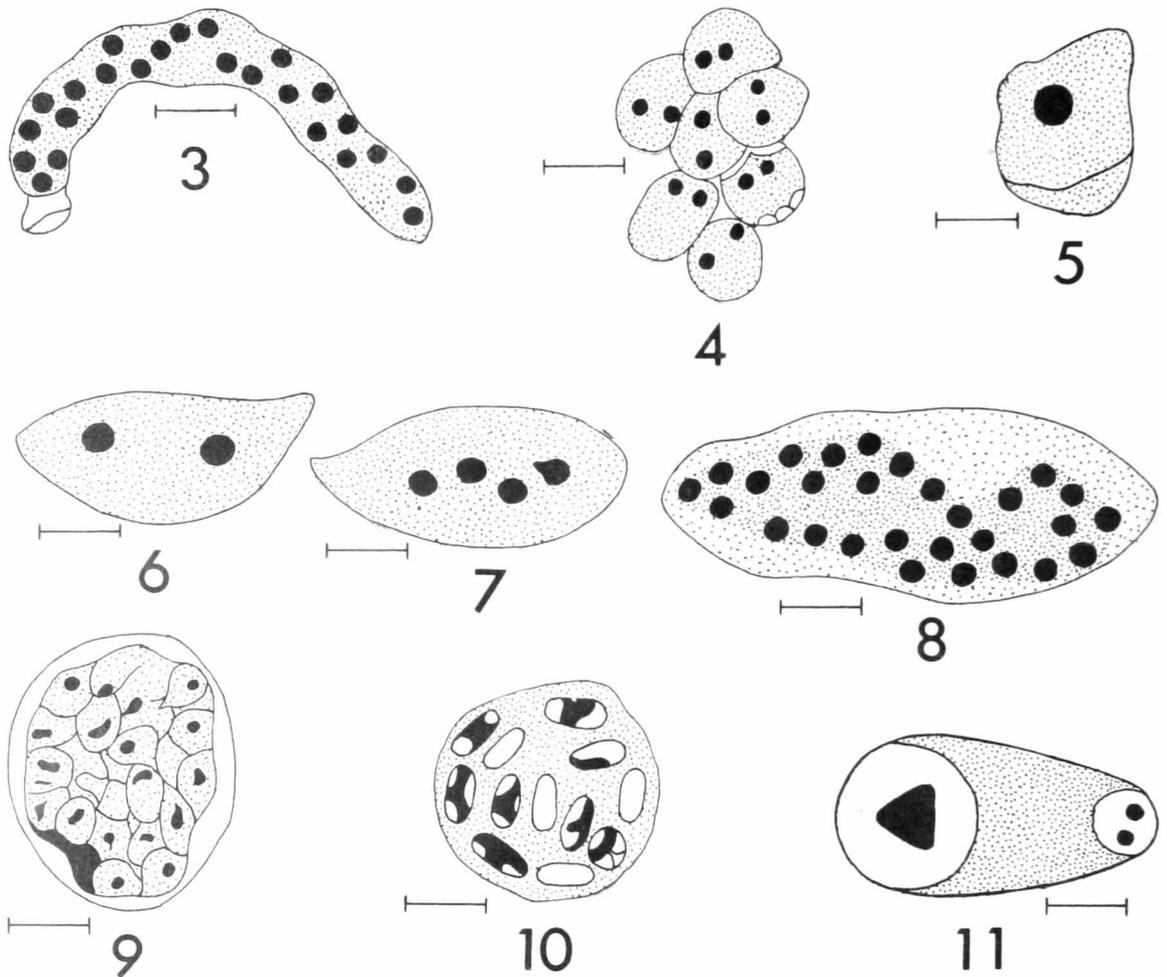
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Figures 3 - 11:--Developmental stages of the microsporidian *Plistophora cepediana* Putz and Hoffman, 1964. All scales represent 5 μ except figure 11 which represents 1 μ .

Fig. 3. Schizont.

Fig. 4. Binucleated bodies, post-schizont and pre-sporont stage.

Fig. 5. Beginning sporont.

Fig. 6. Binucleate sporont.

Fig. 7. Quadrinucleate sporont.

Fig. 8. Polynucleate sporont.

Fig. 9. Sporont with beginning sporoblasts.

Fig. 10. Pansporoblast containing nearly mature spores.

Fig. 11. Schematic drawing of a mature spore.