MFR PAPER 1340

# Comments on Trends in Research on Parasitic Diseases of Shellfish and Fish

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This subject is arbitrarily limited in scope to the protozoa and in time to the past decade. It deals very briefly with recent developments in the biology and systematics of some protozoan groups that are significant as pathogens. More detailed reviews have recently been published by Couch (1978), Hazard and Oldacre (1976), Sindermann (1970), Sprague (1970a, b, 1971, 1977), and Sprague and Couch (1971). The symposium edited by Snieszko (1970) contains numerous articles pertinent to "fish health," the two by Sprague being specifically cited above as being particularly relevant to this presentation.

## RECENT ADVANCES

### Amoebae

#### *In Crabs*

Only one amoeba is believed to be a serious mortality factor in shellfish. This parasite, *Paramoeba perniciosa* Sprague, Beckett, and Sawyer, 1969, was first reported and briefly described in 1966 by Sprague and Beckett who found it in the hemolymph of dead and moribund crabs, *Callinectes sapidus,* in Chincoteague Bay, Maryland and Virginia. These authors did not recognize the parasite as an amoeba at that time, but in a later note (Sprague and Beckett, 1968) assigned it to genus *Paramoeba* Schaudinn, 1896.

The parasite occurs in the hemolymph of heavily infected crabs. Sometimes the body fluid of the host became milky white due to the presence of enormous numbers of the parasites. As the number of parasites increases, the hemocytes decrease in number. Heavy infections may be accompanied by lysis of the skeletal muscles.

*Paramoeba perniciosa* has been limited to the higher salinity waters. It has been found most frequently in "peeler" crabs suffering heavy mortalities in holding tanks, but has been found also in "hard" crabs collected in enzootic areas. Lunz (1968) found it associated with crab mortalities along the coast of South Carolina and Georgia. Sawyer (1968) gave preliminary data on the epizootiology and host-parasite relations. Newman and Ward (1973) reported a modest epizootic in Chincoteague Bay. Sawyer (1968) found the parasite in the blood only during mortalities, which occurred mainly in the spring. He postulated a cryptic tissue phase in the parasite's life cycle during other parts of the year. Very recently, Johnson (1977) discovered the "cryptic phase" in the connective tissues. She found that the parasite is present in these tissues at all seasons of the year and invades the circulating blood in the vessels only when the disease is terminal. Johnson also transmitted the infection by inoculation, with fatal results to the inoculated crabs.

The evidence that this amoeba is a very significant mortality factor in *Callinectes sapidus* is overwhelming, if largely circumstantial. The parasite has not been grown in pure culture, and its normal mode of transmission is unknown.

#### *In Fish*

Only very recently, amoebae have become generally recognized as important mortality factors in fish. Some amoebae pathogenic to man also occur in fish.

Disease conditions of epizootic proportions in rainbow trout on commercial trout farms of Italy and other European countries have been attributed by Ghittino et al. (1977) to an amoeba. Epizootics associated with the same amoeba were reported by Ferguson and McC Adair (1977) in Scotland and Ireland. It seems clear from these reports that an amoeba (apparently not yet identified) is a very important factor in widespread mortalities of trout in European fish farms.

Taylor (1977) isolated amoebae from 11 species of fish in the southeastern states. Among those isolated was *Acanthamoeba polyphaga* which Taylor regarded as the cause of a large kill, involving mainly blue tilapia, in Florida. This study has special interest because *A. polyphaga* is pathogenic to man.

Sawyer et al. (1974) described *Thecamoeba hoffmani* from the gills of fingerling rainbow trout, coho salmon, and chinook salmon. They concluded that the species is essentially a free-living form, although it seems to be a contributory factor in mortalities.

### Microsporidia

#### *In Bivalve Mollusks*

*Microsporidium* sp. has been found, but not yet reported, by Brian Jones[1] in the visceral mass of *Ostrea lutaria* in New Zealand. This is the third species known in bivalve mollusks. These species are not significant pathogens.

[1]Jones, B., Zoology Department, Victoria University, Wellington, N.Z.

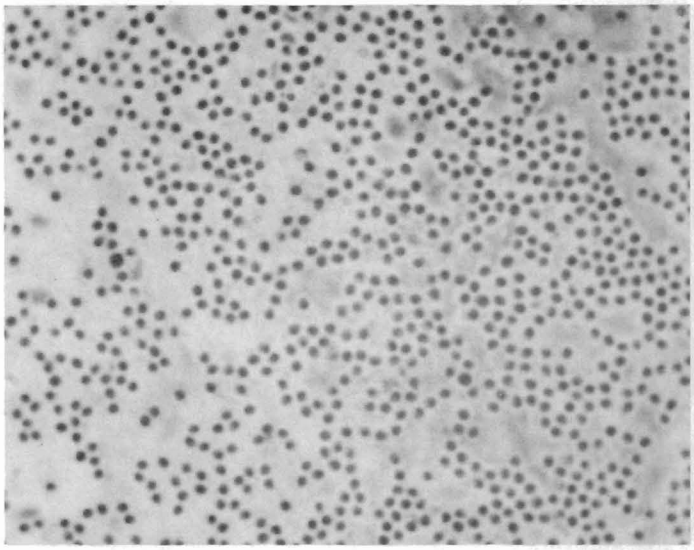

Figure 1.—*Ameson pulvis* (Perez, 1905), spores, in muscle of green crab.

*In Crabs*

Microsporidia are common pathogens of crabs and shrimp, mainly in the skeletal muscle. About a dozen species are now known in crabs, four of them in the muscles of the blue crab, *Callinectes sapidus* Rathbun. Three of the latter are rare, but one, *Nosema michaelis* Sprague, 1970a, is common, widely distributed, and lethal to its host; the impact on the host population has not been assessed. *Nosema michaelis,* being readily obtainable in great quantity, has become a useful object for studies on cytology of the parasite (Sprague et al. 1968; Weidner, 1970, 1976; Weidner and Trager, 1973) and on intracellular parasitism (Trager, 1974). This species was recently made the type of a new genus, now being *Ameson michaelis* (Sprague, 1970) Sprague, 1977. One of the four species known in *Callinectes, Thelohania* sp. Johnson, 1972, is undescribed. The first two species of microsporidia reported in crabs, *Thelohania maenadis* Perez, 1904, and *Nosema pulvis* Perez, 1905, both in the green crab *Carcinus maenas* (L.), were recently rediscovered, the first by Sprague and Vivares independently and the second by Vivares. Vivares (1975) has already published on the former, while Vivares and Sprague (in press) have redescribed the latter, now *Ameson pulvis* (Perez, 1905) Sprague, 1977 (Fig. 1).

*In Shrimp*

*Nosema nelsoni* recently became *Ameson nelsoni* (Sprague, 1950) Sprague, 1977. This species, originally found in the muscles of *Penaeus aztecus* Ives, has now been identified in six species of penaeid shrimp. *Thelohania macrocystis,* in muscles of *Palaemonetes varians,* recently became *Chapmanium macrocystis* (Gurley, 1893) Hazard and Oldacre, 1976. *Thelohania penaei,* in ovary *Penaeus setiferus* (L.), recently became *Agmasoma penaei* (Sprague, 1950) Hazard and Oldacre, 1976.

Quite recently, a relatively large number of new species have been discovered in shrimp. A new genus and new species, *Inodosporus spraguei* Overstreet and Weidner, 1974, was found in *Palaemonetes pugio* Holthius and *P. kadiakensis. Pleistophora lintoni* Street and Sprague, 1974 was described in *Palaemonetes pugio.* Several undescribed species were found in pandalid shrimp sent to me from British Columbia by T. H. Butler. One of these, *Thelohania* sp. Vernick, Sprague, and Krause, 1977, in *Pandalus jordani* Rathbun, has been described by Johnston, Vernick, and Sprague (in press) as a new species (Fig. 2). *Pleistophora penaei* Constransitch, 1970 was found in commercial shrimp. *Pleistophora crangoni* Breed and Olson, 1977 was found in four species of *Crangon.* Some undescribed species have been listed by Sprague (1977).

*In Fish*

Microsporidia are very common in fish; over 100 species of fish are now known to be parasitized. Some microsporidia are also known to be serious mortality factors in fish. Delisle, 1972 found massive mortalities of smelt, associated with this parasite, in certain Canadian lakes. *Glugea stephani* (Hagenmüller, 1899) has been found by different investigators to be an important mortality factor in young flatfish of different species. Sprague and Vernick (1968) found that *Ichthyosporidium giganteum* (Thélohan, 1895), long treated as haplosporidium, is a microsporidium. This (or a very similar species) is not uncommon in "spot," *Leiostomus xanthurus* Lacépède, in tributaries of the Chesapeake Bay. It produces a very large abdominal swelling, usually in young fish, that probably kills the host.

## Haplosporidia

There has been much interest in haplosporidia during the past decade, mainly because they are very destructive pathogens of oysters. This has led to the discovery of a few new species, much better understanding of the group, and new ideas on classification.

*In Bivalve Mollusks*

Perkins (1968, 1969) and Rosenfield et al. (1969) have contributed much to our understanding of haplosporidia by making the first electron microscope studies on some typical forms—those familiar species in *Crassostrea virginica* (Gmelin).

*Minchinia armoricana* van Banning, 1977, was found in the European flat oyster, *Ostrea edulis.* This is the only known haplosporidian that has tails on its spores like those seen in the type species *M. chitonis* (Lankester, 1885). Sprague (1970c) suggested that presence or absence of these tails be used as

the basis for distinguishing *Minchinia* Labbé, 1896 from *Haplosporidium* Caullery and Mesnil, 1899.

Two species of a previously unfamiliar kind have been found in association with oyster mortalities. They are *Marteilia refringens* Grizel et al., 1974 (type species), in the European flat oyster *Ostrea edulis* L., and *M. sydneyi* Perkins and Wolf, 1976, in the Australian oyster, *Crassostrea commercialis*. Perkins (1976a) suggested that they are related to the haplosporidia, mainly because they have "haplosporosomes."

*In Decapods*

"*Minchinia* sp." Marchand, 1974, was found in the mud crab, *Rhithropanopeus harrisii*, in France. Plasmodia of a haplosporidian were found by Newman et al. (1976) in *Callinectes sapidus* from Virginia and North Carolina.

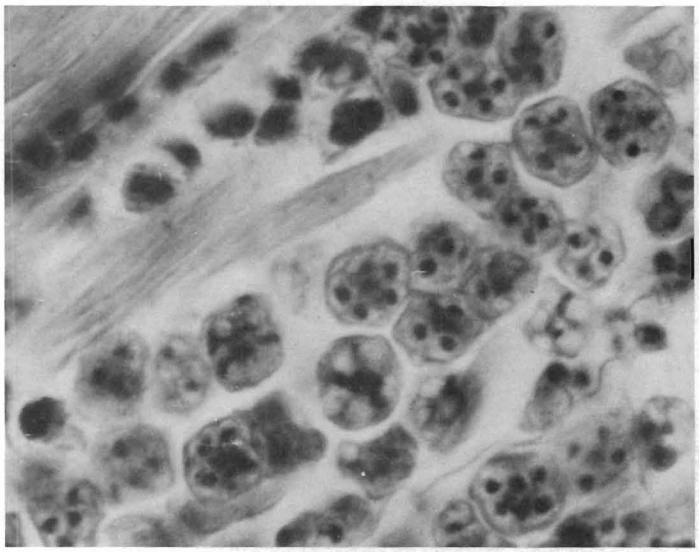

Figure 2.—*Thelohania* sp. Vernick et al., 1976, various sporulation stages, in muscle of the shrimp *Pandalus jordani*. Bouin, section, Heidenhain; 1,300×.

**Myxosporidia**

New species are frequently added to the long list of myxosporidian species in fish. Most are not noted for pathogenicity, but one highly pathogenic form, *Myxosoma cerebralis* (Hofer, 1903), has been the object of many recent studies. An interesting species recently described is *Unicapsula pflugfelderi* Schubert, Sprague, and Reinboth, 1975. It has spores with two rudimentary polar capsules, as well as the fully formed one that is characteristic of the genus. Another species, interesting because it infects an important sport fish, is *Henneguya* sp. Meyers, Sawyer, and MacLean, 1977, in the heart of the bluefish, *Pomatomus saltatrix* (L.).

**Protista Incertae Sedis**

An unidentified parasite with vague resemblance to the Coccidia was recently found by Peter Wolf (1977) in ova of the blacklipped oyster, *Crassostrea echinata*, from Australia. Another unidentified protist was found in the digestive epithelium of the pearl oyster, *Pinctada maxima*, in Australia (Wolf and Sprague, 1978).

A parasite in spleen and liver of rainbow trout, causing epizootic disease in England, was recently sent to me by John Finlay[2] for identification. I noted a resemblance to intracellular stages of *Hexamita* but, being unable to make positive identification, sent the slides to Glenn Hoffman. At first, Hoffman[3] suspected the parasite might be identical with the amoebae reported by Ghittino et al. (1977), but after further consideration, he doubted that it is an amoeba. The identity of the parasite remains undetermined.

## CLASSIFICATION

Some minor changes in classification, particularly the transfer of some microsporidian species to new genera, have already been mentioned. One of the most spectacular of the minor developments in recent years was the finding by Perkins (1976b) that the flagellated zoospore of *Dermocystidium marinum* Mackin, Owen, and Collier, 1950 has an apical complex. This led Perkins to conclude that the parasite, long regarded as a fungus, is related to the Coccidia.

Drastic changes are taking place in the classification of the higher categories of protozoa, since recent advances in knowledge indicate that the old phylum Protozoa is an artificial assemblage of several phyla. A "Committee on Classification and Nomenclature" of the Society of Protozoologists, headed by N. D. Levine, is making a revision. Microsporidia, haplosporidia, myxosporidia, and some other groups will probably become distinct phyla, as ciliates have already done. Classification of microsporidia probably will be based on that of Sprague (1977). Classification of haplosporidia will probably include a major new taxon to include *Marteilia*. Myxosporidia are now considered by many protozoologists to be metazoa with coelenterate affinities, but they will probably remain for awhile in the system of the protista.

This is a convenient occasion to take specific action upon my previous (Sprague, 1970c) suggestion and transfer from genus *Minchinia* Labbé, 1896 to *Haplosporidium* Caullery and Mesnil, 1899 species which, like the type species *H. scolopli* Caullery and Mesnil, 1899, have spores without tails. Accordingly, *Minchinia louisiana* Sprague, 1963b becomes *Haplo-*

[2]Finlay, J., Fish Diseases Laboratory, Ministry of Agriculture, Fisheries and Food, The Nothe, Weymouth, Dorset, England DT4 8UB.
[3]Hoffman, G. A., Fish Farming Experimental Station, U.S. Fish and Wildlife Service, Stuttgart, AR 72160. Pers. commun.

*sporidium louisiana* (Sprague, 1963b) comb. n. and *M. nelsoni* Haskin, Stauber, and Mackin 1966 becomes *H. nelsoni* (Haskin et al., 1966) comb. n. Other species which Sprague (1963a: 265-266) transferred (for reasons later found to be invalid) from *Haplosporidium* to *Minchinia* are now returned to *Haplosporidium*.

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MFR PAPER 1341

# Susceptibility Studies of Various Salmonids to Whirling Disease: Histological Staining and Spore Concentration Procedures

JOSEPH O'GRODNICK

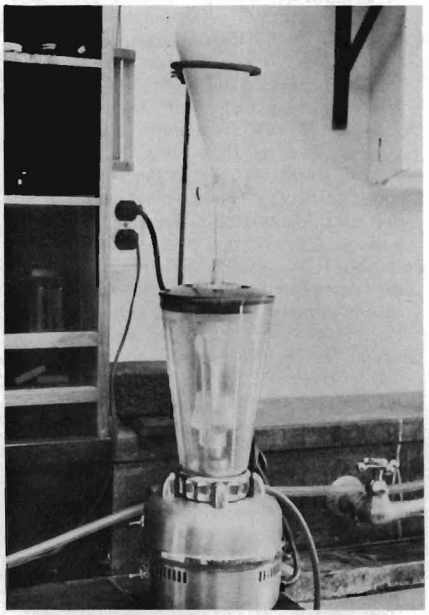

Figure 1.—The plankton centrifuge. The centrifuge removes water through the centrifugal force created by the revolving drum. The excess water is carried away through a rubber tube situated below the drum. Solid particles are deposited on the wall of the revolving drum.

Since 1968, research on the transmission, life history, and control of whirling disease in trout has been conducted by the Pennsylvania Fish Commission. Whirling disease is caused by a myxosporidian parasite, *Myxosoma cerebralis*, which invades and destroys cartilage of susceptible salmonid species. The parasite was introduced into the United States from Europe and has been established as a persistent hatchery disease in several areas.

Affected fish exhibit rapid, tail chasing behavior from the disintegration of the cartilaginous support skeleton of the organs of equilibrium. Also, severe crippling may result from destruction of the spinal skeleton if fish are exposed as fry. "Blacktail," which is caused by pressure on the nerves that control the caudal pigment cells, may be seen in some fish.

In this research, two methods were used to monitor the development of the whirling disease parasite in various salmonid species: 1) examination of histological sections, and 2) quantitative estimates of spores determined by the use of the plankton centrifuge procedure (Fig. 1). (This method involves homogenization of head skeletons, screening out tissue shreds, and concentrating with a continuous plankton centrifuge.

The life cycle of *Myxosoma cerebralis* was monitored in rainbow trout (highly susceptible species) and brown trout (highly resistant species) by the examination of histological sections. Tissues were prepared from infected fish at given intervals, averaging 3 days from initial exposure to the development of spores at 120 days. The following staining methods were used in the study: 1) Hematoxylin and Eosin, 2) Mallory Heidenhain (Casson-Modification), 3) Wright's stain, and 4) Ziehl-Neelson (acid fast staining for spores).

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