JOURNAL OF INVERTEBRATE PATHOLOGY 53, 278–280 (1989)

A Hepatopancreatic Microsporidian in Pond-Reared Tiger Shrimp, *Penaeus monodon*, from Malaysia

Microsporidian parasites are known from a number of different wild and cultured penaeid species in North America (J. A. Couch, *Fish. Bull.* **76**, 1–44, 1978; V. Sprague, 1977 *in* "Comparative Pathobiology," L. A. Bulla, Jr., and T. C. Cheng, Eds., Vol. 2, pp. 335–386, Plenum Press, New York). More recently, M. L. C. Baticados and G. L. Enriques (*Nat. Appl. Sci. Bull.* **34**, 255–271, 1982) described a microsporidian infection of *Penaeus merguiensis* from the Philippines affecting the ovary of adults. This communication reports a microsporidian infection of *P. monodon* hepatopancreatocytes associated with low production in brackishwater pond culture.

P. monodon juveniles were collected from two shrimp farms for diagnostic examination during September and October 1987. Both farms, in Sarawak, East Malaysia, and Selangor, Peninsular Malaysia, had histories of low production, slow growth rates, and occasional mortalities. Specimens collected were darkened, small, and lethargic, with no gross tissue lesions. Tissues were fixed in Davidson's solution, stored in 50% ethanol, and processed routinely for light microscope examination.

Histological examination revealed 3 out of 9 and 2 out of 6 specimens, from each farm, had marked hepatopancreatic changes (Fig. 1). Numerous tubules in proximal hepatopancreatic areas were dilated and lined with flattened epithelial cells, and their lumens contained pale granular debris or necrotic cells and small, oval refractile spore-like bodies. Over 40% of all hepatopancreatocytes were observed to contain similar intracytoplasmic spore-like bodies (Fig. 2). These bodies, diffuse throughout the cytoplasm, did not appear to be enclosed in vacuoles. Several tubules containing intraluminal spore-like bodies were completely necrotic, with a multiple melanized hemocytic encapsulation replacing the epithelium. Edema and an increased number of hemocytes in interstitial tissue was a generalized change in affected hepatopancreases.

Electron microscopy of thin-sectioned Agar 100/Araldite-embedded hepatopancreas recovered from Paraplast Plus embedded specimens confirmed the presence of typical thick-walled microsporidian spores (Fig. 3). Due to fixation inappropriate for electron microscopy, details of sporoplasm and extrusion apparatus were not readily apparent, nor could microsporidial developmental stages be determined in the cell profiles examined. In stained sections, at the center of the oval spores, a stained band separated unstrained areas at either end. Spores measured 1.9 ± 0.2 μm by 1.4 ± 0.2 μm ($n = 60$) in differential interference contrast microscopy of stained sections. The microsporidian infection was entirely restricted to hepatopancreatocytes. While further electron microscope studies are necessary, the absence of a pansporoblast membrane and diffuse distribution of many single spores in infected host cells are indicative of a microsporidian in the genus *Ameson*.

In two specimens, tissues other than the hepatopancreas were also abnormal. There was necrosis and marked hemocytic inflammation of midgut epithelium, similar to lesions described in hemocytic enteritis (HE) following toxic algae ingestion (D. V. Lightner, *J. Invertebr. Pathol.* **32**, 139–150, 1978). Severe *P. monodon* baculovirus (MBV) infections were also present (D. V. Lightner, R. M. Redman, and J. A. Bell, *Aquaculture* **32**, 209–233, 1983).

The source of the microsporidian is un-

0022-2011/89 $1.50

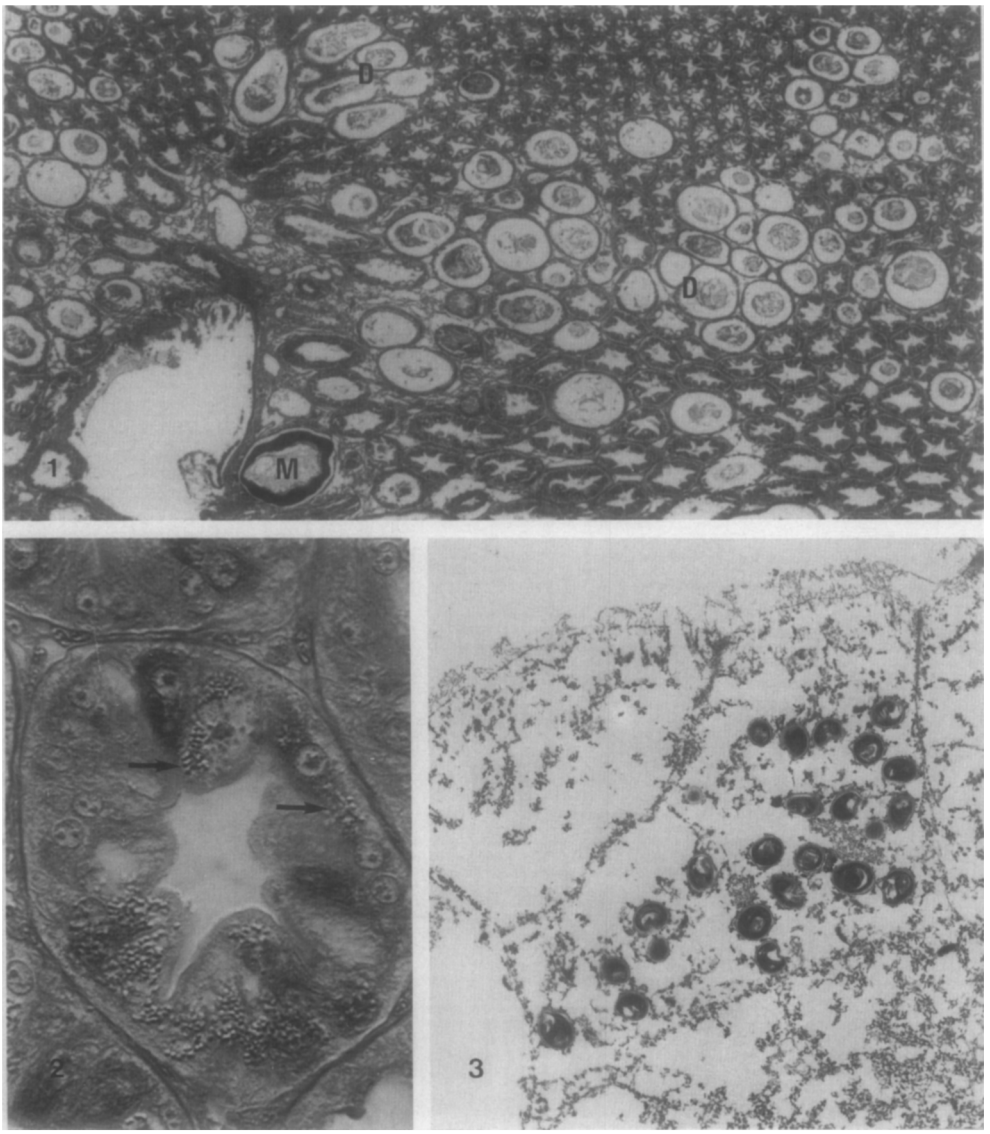

FIG. 1. *Penaeus monodon* hepatopancreas infected with microsporidia. Dilated, degenerating tubules (D), lined with flattened epithelial cells and containing necrotic debris and/or numerous refractile spores, are present. Note that one tubule is necrotic and infiltrated by melanized hemocytes (M). H and E stain. 43×.

FIG. 2. An intact tubule with microsporidial spores (arrow) diffuse throughout hepatopancreatocyte cytoplasms. Giemsa stain. 500×. Differential interference contrast microscopy.

FIG. 3. Electron photomicrograph of intracytoplasmic microsporidial spores in a hepatopancreatocyte. Lead citrate and uranyl acetate stain. 4600×.

known, as is the relative importance of HE-like inflammatory lesions and MBV infections associated with this hepatopancreatic microsporidian disease condition. Undoubtably the microsporidian affected hepatopancreatic function and thus shrimp health. Possibly low intensity infections are endemic, previously undetected due to the

small spore size and lack of distinct gross signs. Environmental stressors and/or other pathogens could have altered the physiological homeostatic integrity of shrimp in these instances, allowing microsporidian replication without restriction.

KEY WORDS: Tiger shrimp; *Penaeus monodon;* microsporidia; hepatopancreas infection.

We thank O. K. Ho and Rosli Aslim for assistance with the electron microscopy and histological preparations. Support for this study was received from EEC Science and Technology Development, Tropical Agriculture Grants TSD A.200 UK(H) and TSD A.287 MAL(H).

I. G. ANDERSON[1]
M. SHARIFF

Faculty of Fisheries and Marine Science
Universiti Pertanian Malaysia
43400 UPM Serdang
Malaysia

G. NASH

Institute of Aquaculture
University of Stirling
Stirling, Scotland

Received June 10, 1988; accepted September 12, 1988

[1] Address for correspondence: Animal Research Institute, 665 Fairfield Road, Yeerongpilly Q., Australia 4105.