Vol. 8: 99–104, 1990 | DISEASES OF AQUATIC ORGANISMS Dis. aquat. Org. | Published June 7

A microsporidium-induced lymphoblastosis in chinook salmon *Oncorhynchus tshawytscha* in freshwater

J. K. Morrison[1], E. MacConnell[2], P. F. Chapman, R. L. Westgard[3]

[1] U.S. Fish and Wildlife Service, Olympia Fish Health Center, 2625 Parkmont Lane, Building A, Olympia, Washington 98502, USA
[2] U.S. Fish and Wildlife Service, Bozeman Fish Technology Center, Bozeman, Montana, USA
[3] Washington Department of Fisheries, Olympia, Washington, USA

ABSTRACT: Yearling chinook salmon *Oncorhynchus tshawytscha* were found to be infected with an intranuclear microsporidium. The primary pathologic response in infected salmon was a marked lymphoblastosis. Although clinical signs and gross pathology were similar to other salmonid fish health problems, the induced cellular changes and prominent nuclear inclusions permitted accurate differentiation and diagnosis. This is the first reported occurrence of this infection in freshwater

INTRODUCTION

During October and November 1987, unexplained losses occurred in yearling chinook salmon *Oncorhynchus tshawytscha* at the Washington Department of Fisheries Wells Salmon Hatchery, USA. Bacterial kidney disease was the suspected cause of mortality, and Gram-stained kidney imprints were examined to detect the presence of the presumptive causative agent *Renibacterium salmoninarum*. Instead of the expected small, Gram-positive diplobacilli bacteria, organisms with 'yeast-like' or 'spore-like' characteristics were observed.

Clinical signs of the condition were similar to those observed in many other salmonid fish health problems; namely anemia, renalmegaly, splenomegaly, exophthalmos, and intestinal swelling.

Light microscopic and ultrastructural examinations revealed organisms identical to those previously described in adult chinook salmon from saltwater net pens in the Puget Sound, Washington State, USA (Elston et al. 1987).

This report describes the occurrence, clinical and histopathological signs, and ultrastructural features associated with an intranuclear microsporidium infection in yearling chinook salmon in freshwater.

MATERIALS AND METHODS

Samples were collected for histopathological examination on 8 different days (November 11 and December 16, 1987, January 13, February 5, March 3 and 22, April 1 and 12, 1988). The total number of specimens collected for light microscopic examination was 80 (20 whole fish and the posterior intestines from an additional 60 fish). Specimens collected for light microscopy were preserved in Bouin's solution for 12 to 24 h, then transferred to 65 % ethanol. Paraffin sections of selected organs (brain, gill, intestine, kidney, liver, pyloric caeca, spleen) were cut at 5 µm and stained with hematoxylin and eosin (H & E) and Giemsa.

Kidney and spleen tissues from a moribund fish were collected for ultrastructural examination on 2 separate occasions (February 5 and April 12, 1988). These were fixed immediately in 4% formalin plus 1% glutaraldehyde buffered to pH 7.2 in phosphate buffer. They were post fixed in 1% aqueous osmium tetroxide (OsO_4), dehydrated in graded ethanols, and embedded in Spurr's embedding medium. Sections, 50 to 70 nm thick, were cut and stained with uranyl acetate and Reynolds lead citrate and examined with a JEOL-100CX transmission electron microscope.

Kidney imprints and blood films were also taken on

0177-5103/90/0008/0099/$ 03.00

each sampling date. These samples were air dried, fixed in 100% methanol, and stained by either the Leishman-Giemsa (L-G) method (Yasutake & Wales 1983) or Diff-Quik (American Scientific Products, McGaw Park, Illinois).

RESULTS

Clinical signs and gross pathology

Infection was identified in fish from the initial observation (October 1987) until release (April 1988).

Clinical signs and gross pathology of infected fish in freshwater included lethargy, anemia, renalmegaly, splenomegaly, exophthalmos and intestinal swelling. Mortality directly attributable to this organism was low (0.4 % mo^{-1}), yet the prevalence of infection, determined by the examination of a random sample of 60 apparently healthy fish on April 1, 1988, was 15 % (9 of 60).

Histopathology-hematopathology

The primary pathologic response to this organism appears as a lymphoblastosis. Initial examination suggests a lymphocytic type of inflammatory reaction. Infected cells are disseminated throughout the fish with a prominent hyperplasia occurring in the kidney interstitium, spleen, and lamina propria of the intestinal mucosa (Fig. 1). In heavily infected fish, hyperplastic cells were easily observed in all vascular areas.

Careful examination of these hyperplastic cells revealed spherical, opaque, nondescript nuclear inclusions (Fig. 2). Only one inclusion was observed within each infected cell and no conspicuous aggregates of parasites were observed in any of the fish examined. Inclusions typical of the infection were often observed within mitotic figures.

Blood films also revealed the apparent lymphoblastosis (Fig. 3). Hyperplastic cells resembled lymphocytes. They were spherical, with a diameter of 7 to 10 µm, and had a nuclear-cytoplasmic ratio of 4:1 or

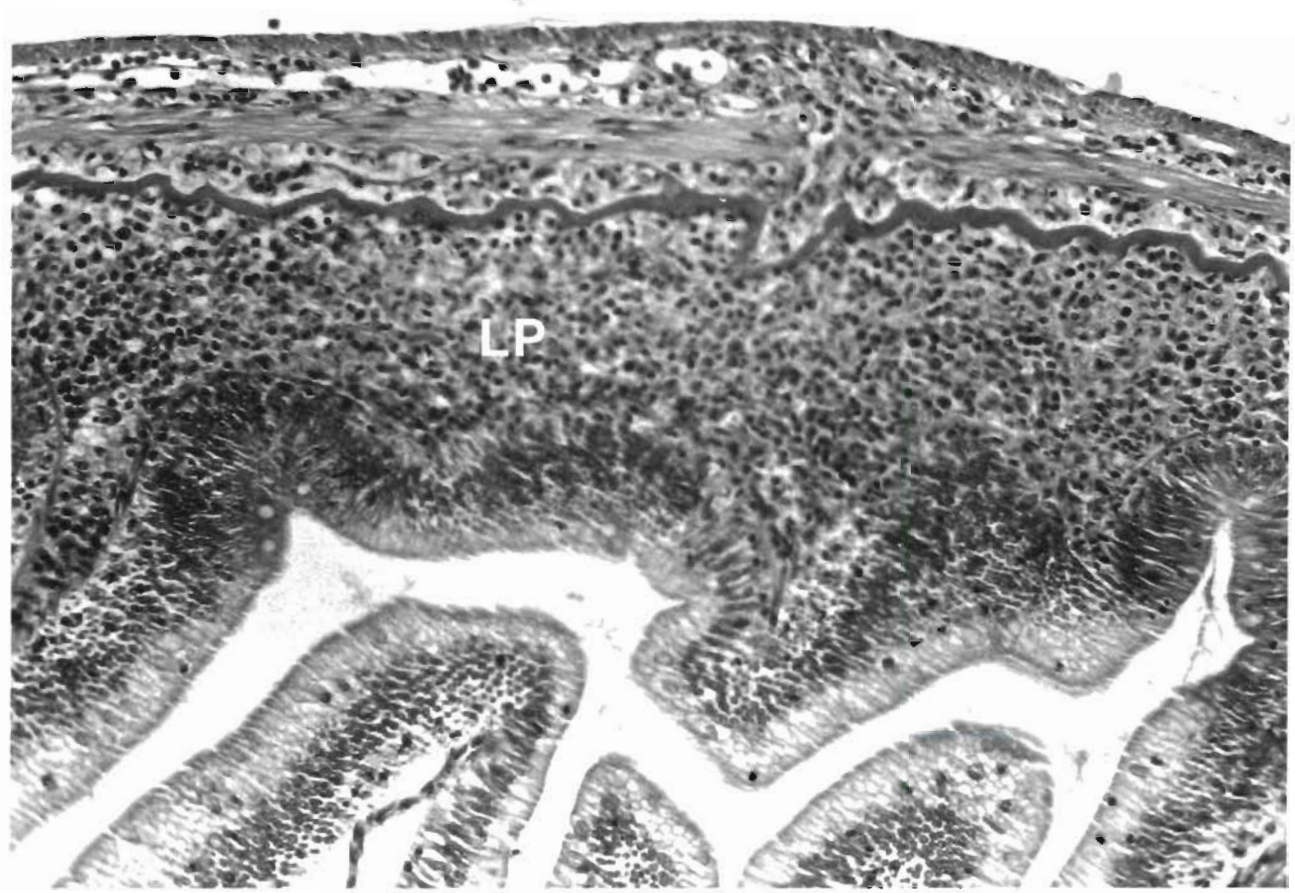

Fig. 1 *Oncorhynchus tshawytscha*. Cross section of posterior intestine. Note hyperplasia within the lamina propria (LP) of the intestinal mucosa. H & E, ×215

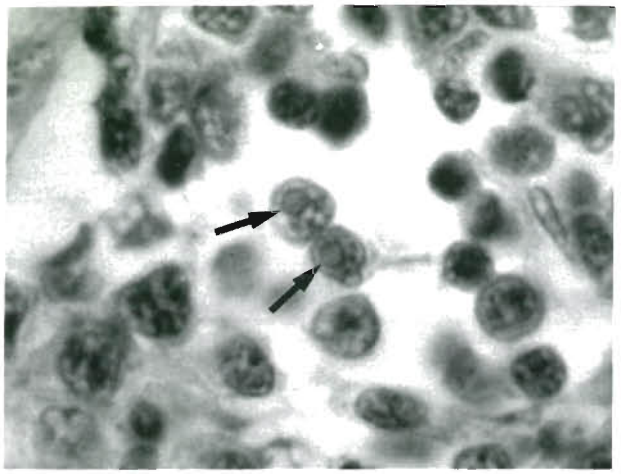

Fig. 2. *Oncorhynchus tshawytscha*. Section of kidney with 2 infected cells at center. Nuclear inclusions are identified by arrows. H & E, ×1215

greater. The nuclei were frequently indented and contained very dense chromatin. The cytoplasm consisted of a thin, blue-staining margin around the nucleus, and the cell membrane was irregular, with short pseudopodial-like projections.

Kidney imprints revealed the infection quite clearly. Nuclear inclusions were obvious, though still very nondescript, within infected cells (Fig. 4).

Only 2 of the fish collected were observed to contain the extranuclear organisms typical of those described as 'yeast-like' or 'spore-like' in the initial reporting. These organisms measured ca 1 × 2 μm, and appeared to contain a centrally located spherical polar body of less than 0.5 μm in diameter (Fig. 5).

Ultrastructural observations

Ultrastructural characteristics of the infected cell type included: (1) pseudopodial projections of the plasma membrane; (2) cytoplasmic vesicles; (3) nuclei often indented or cleft; and (4) large elongate mitochondria.

Ultrastructurally, the intracellular organisms were 1 to 2 μm in diameter with a distinct membrane-bound nucleus. They lacked mitochondria, but contained distinct cytoplasmic organelles of unknown function. These appeared as stacked discs. Each disc consisted of 2 electron-dense outer layers separated by an electron-lucent middle layer (Fig. 6). Only once was a developing spore stage (sporoblast) observed (Fig. 7).

The 'yeast-like' or 'spore-like' extracellular organisms were not observed with the electron microscope.

DISCUSSION

The clinical signs of this disease (lethargy, anemia, renalmegaly, splenomegaly, exophthalmos, and intestinal swelling) closely resemble those typically observed with other parasitic fish diseases, such as proliferative kidney disease (PKD) (Ferguson & Needham 1978, Smith et al. 1984), chinook salmon 'rosette' disease (Harrell et al. 1986), and *Parvicapsula* infections (J. Morrison pers. obs.).

The systemic parasitosis in the fish examined in this report suggests that the parasites had been effectively disseminated throughout the fish rather than localizing in specific areas. Canning & Lom (1986) state, 'Sporo-

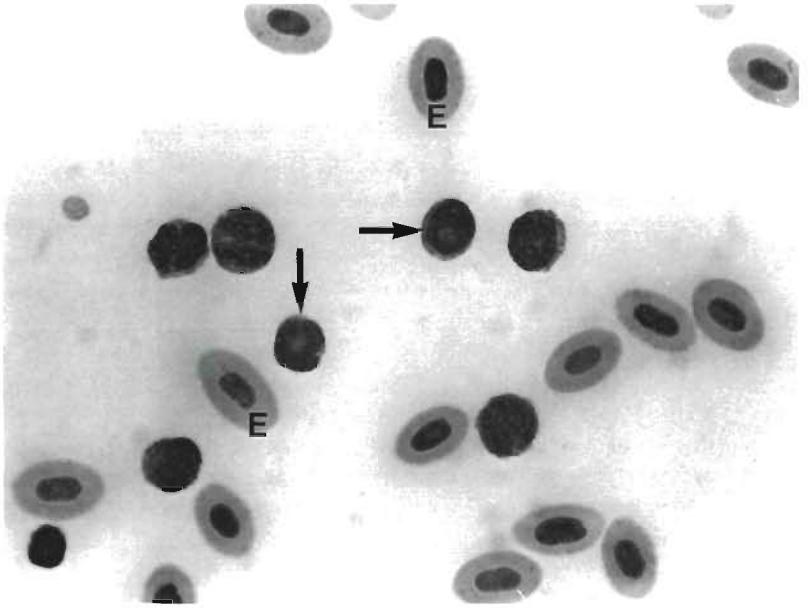

Fig. 3. *Oncorhynchus tshawytscha*. Blood film demonstrating lymphocythemia. Parasitic nuclear inclusions are seen in 2 cells (arrows); erythrocytes (E). Leishma-Giemsa, ×1220

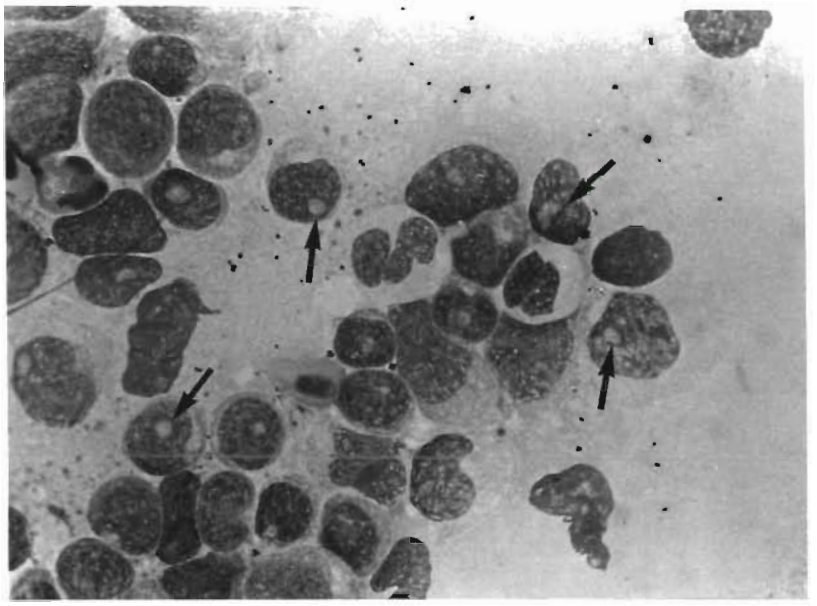

Fig. 4. *Oncorhynchus tshawytscha*. Kidney imprint. Note intranuclear inclusions (arrows). Leishma-Giemsa, ×1010

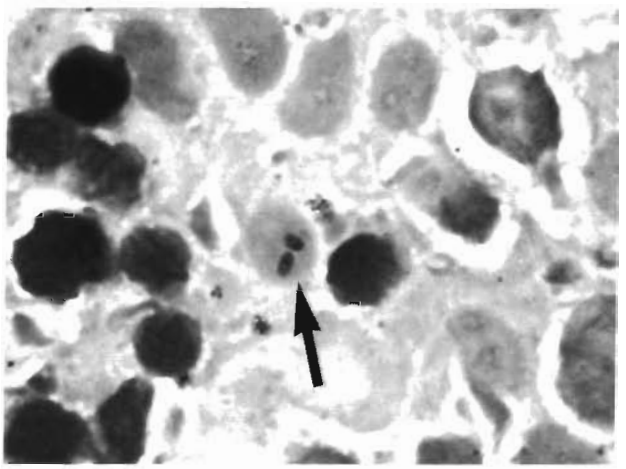

Fig. 5. *Oncorhynchus tshawytscha*. Kidney smear with minute organisms at center of photo (arrow). Gramstain, ×1285

plasms released from spores may develop in tissues far removed from the site of hatching in the gut. Little is known about the transport of early phases but wandering cells, especially undifferentiated mesenchyme cells, macrophages and bodily fluids probably aid in distribution.' In fact, on occasion, wandering cells within the intestinal epithelium were observed to be infected, suggesting this is exactly what was occurring.

The infecting organism was identified as a microsporidium for the following reasons: it lacked mitochondria; developing polar tubules were observed to be free within a sporoblast; and it was intracellular. It is also identical to the microsporidium previously described by Elston et al. (1987).

The 'stacked discs' that were observed within the cytoplasm of this parasite resembled the organelles described in the microsporidium *Enterocytozoon bieneusi* (Desportes et al. 1985). In that report, the authors allude to the fact that the organelles are polar filament precursors.

This organism appears to preferentially infect cells of mesenchymal origin. A fine line separates lymphoblasts, hemocytoblasts, and undifferentiated mesenchyme cells. In fact, at some point prior to complete maturation or differentiation, all can be considered to have multi-potential properties. Lymphocytes represent a mobile reserve of mesenchyme cells which, together with the fixed undifferentiated mesenchyme cells, constitute a group of multipotential or blast type cells (Schalm et al. 1975, Leeson & Leeson 1976).

A comparison between the infected cell characteristics and descriptions of normal blood cell types at both the light microscopic and ultrastructural levels support the lymphocyte as the preferred infection site. Yasutake & Wales (1983) describe lymphocytes as 'small spherical cells, 7 to 10 µm in diameter with an eccentrically located spherical nucleus, which sometimes has a slight indentation. They have fairly compact chromatin, which stains reddish-purple and a lightly basophilic narrow band of cytoplasm which often has pseudopodial projections.' Ultrastructural characteristics used to differentiate fish lymphocytes from the other blood cell types include elongate mitochondria, pseudopodial projections of the plasma membrane, indented or cleft nuclei, and cytoplasmic vesicles (Ferguson 1976, Cannon et al. 1980, Cenini 1984).

Morphologic evidence strongly implicates the lymphocyte, but close morphologic similarities between other cell types, especially at early or immature stages

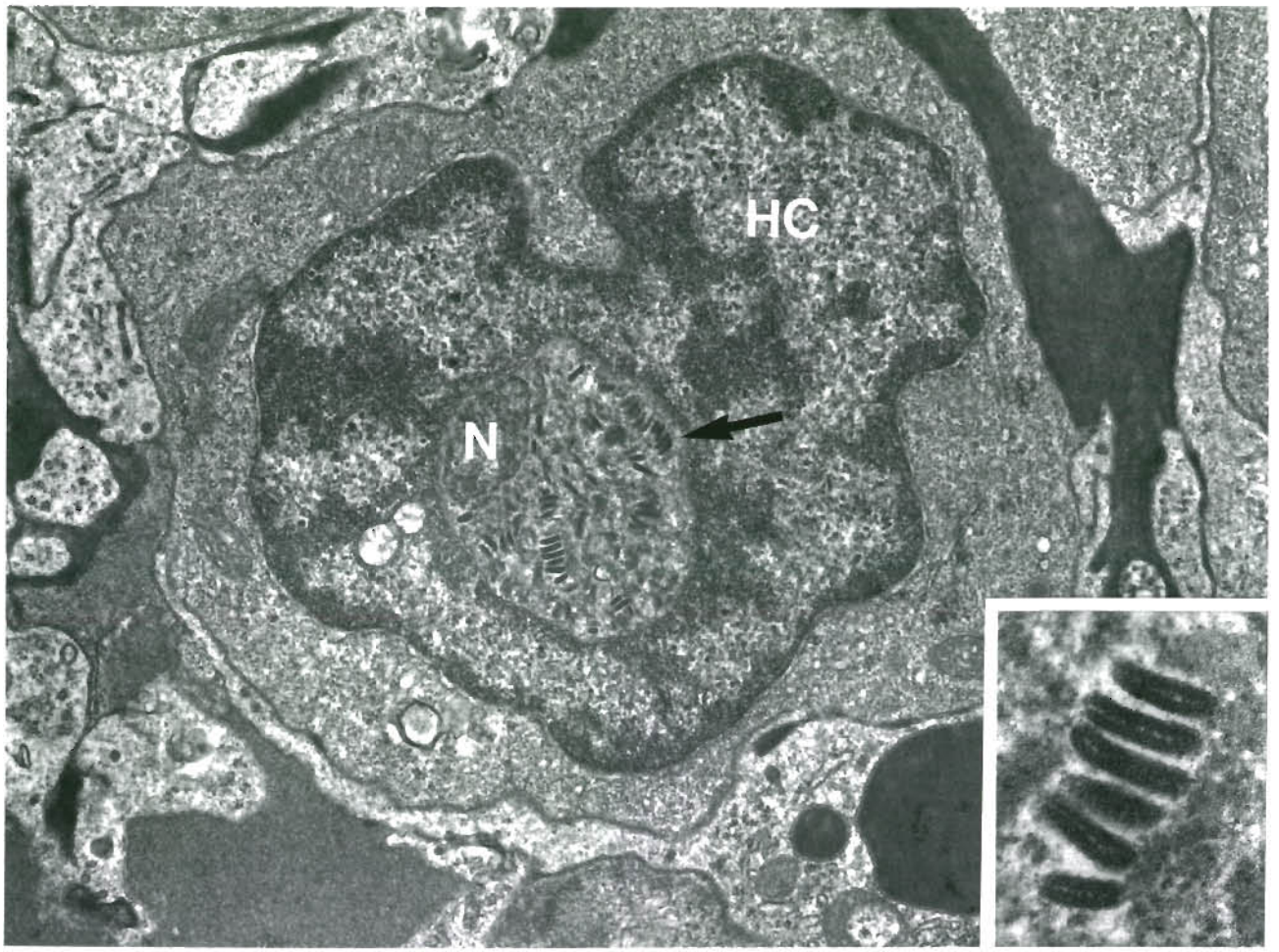

Fig. 6. *Oncorhynchus tshawytscha*. Infected host cell. Parasite within host cell nucleus (arrow), parasite nucleus (N), host cell nucleus (HC). TEM, ×18 700. Inset: High magnification (×85 000) of cytoplasmic organelles of unknown function

(blast cells, undifferentiated mesenchyme cells) preclude absolute identification at this time.

Inclusions typical of infection were observed within cells undergoing mitosis. This suggests that this parasite can stimulate host-cell mitosis and then replicate itself at the time of cell division. This type of synchronized division has been reported to occur in certain species of *Theileria* and *Eimeria* (Stagg et al. 1980, Gregory et al. 1987).

The induced cellular changes and prominent nuclear inclusions associated with this infection are unique. This allows for accurate differentiation and diagnosis, even though the clinical signs and gross pathology of infected fish closely resemble other salmonid fish health problems.

Acknowledgements. This work is a result of the Bonneville Power Administration-sponsored 'Augmented Fish Health Monitoring' Project 87-119. Special thanks go to Drs Ralph Elston, Michael Kent, Lee Harrell, and Ms Patty Michak for their assistance.

LITERATURE CITED

Canning, E. U., Lom, J. (1986). The microsporidia of Vertebrates. Academic Press, Harcourt Brace Jovanovich, Publ., London

Cannon, M. S., Mollenhauer, H. H., Eurell, T E., Lewis, D. H., Cannon, A. M., Tompkins, C. (1980). An ultrastructural study of the leukocytes of the channel catfish *Ictalurus punctatus*. J. Morph. 164: 1–23

Cenini, P. (1984). The ultrastructure of leukocytes in carp, *Cyprinus carpio*. J. Zool., Lond. 204: 509–520

Desportes, I., LeCharpentier, Y., Galian, A., Bernard, F., Cochand-Priollet, B., Lavergne, A., Ravisse, P., Modigliani, R. (1985). Occurrence of a new microsporidan: *Enterocytozoon bieneusi* n. g., n. sp., in the enterocytes of a human patient with AIDS. J. Protozool. 32 (2): 250–254

Elston, R. A., Kent, M. L., Harrell, L. H. (1987). An intranuclear microsporidium associated with acute anemia in the chinook salmon, *Oncorhynchus tshawytscha*. J. Protozool. 34 (3): 274–277

Ferguson, H. W. (1976). The ultrastructure of plaice, *Pleuronectes platessa*, leukocytes. J. Fish Biol. 8: 139–142

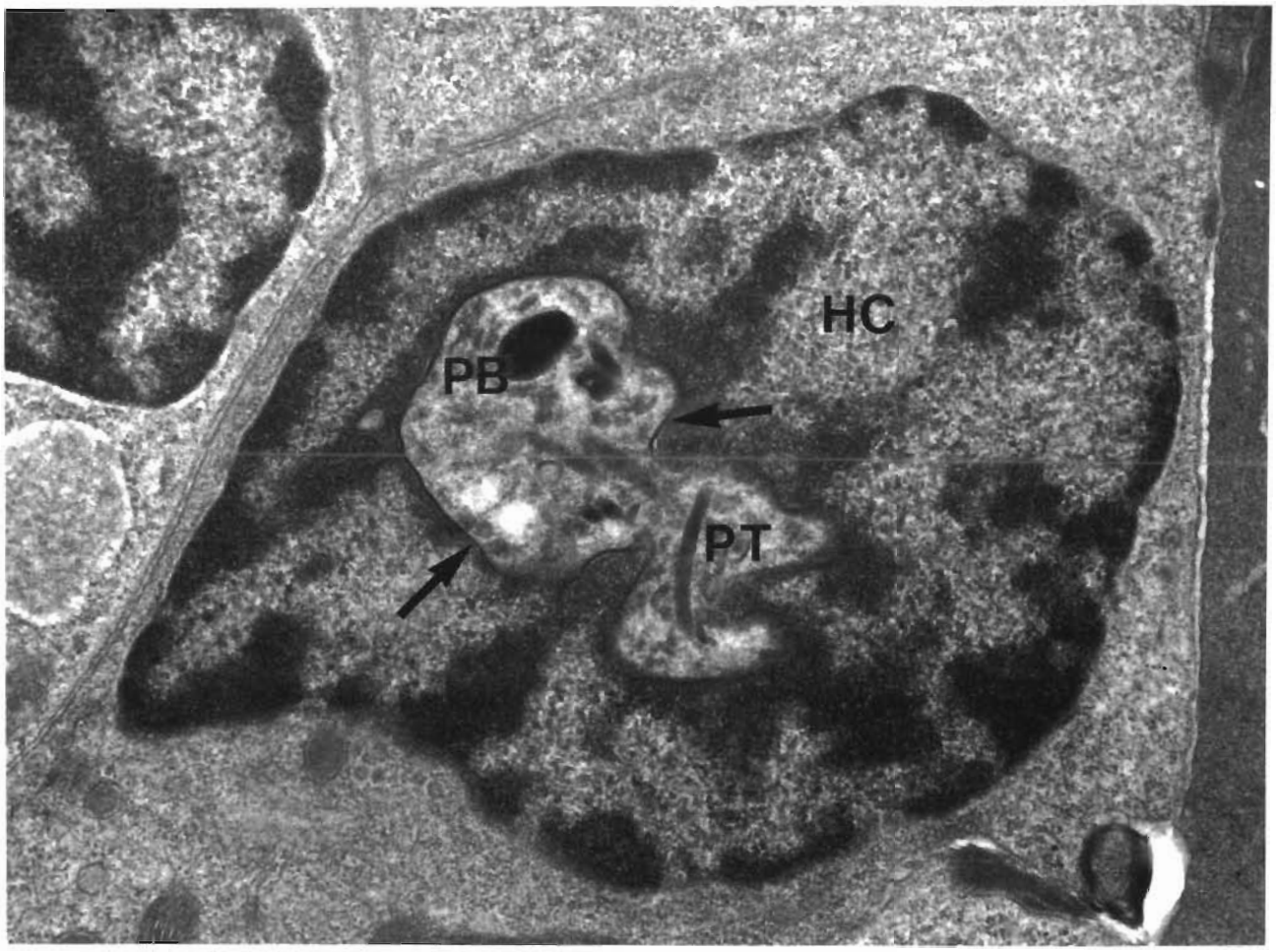

Fig. 7. *Oncorhynchus tshawytscha*. Developing sporoblast within host cell nucleus (HC). Sporoblast membrane (arrows), developing polar body (PB), and polar tubule (PT). TEM, ×22 770

Ferguson, H. W., Needham, E. A. (1978). Proliferative kidney disease in rainbow trout, *Salmo gairdneri* Richardson. J. Fish Dis. 1. 91–108

Gregory, M. W., Catchpole, J., Norton, C. C., Pittilo, R. M. (1987). Synchronized division of coccidia and their host cells in the ovine intestine. Parasit. Res. 73: 384–386

Harrell, L. W., Elston, R. A., Scott, T M., Wilkinson, M. T. (1986). A significant new systemic disease of net-pen reared chinook salmon, *Oncorhynchus tshawytscha*, brood stock. Aquaculture, Amsterdam 55: 249–262

Leeson, C. R., Leeson, T S. (1976). Histology, 3rd edn. W. B. Saunders Co., Philadelphia

Schalm, O. W., Jain, N. C., Carroll, E. J. (1975). Veterinary hematology, 3rd edn. Lea & Febiger, Philadelphia

Smith, C. E., Morrison, J. K., Ramsey, H. W., Ferguson, H. W (1984). Proliferative kidney disease: first reported outbreak in North America. J. Fish Dis. 7· 207–216

Stagg, D. A., Chasey, D., Young, A. S., Morzaria, S. P., Dolan, T T. (1980). Synchronization of the division of *Theileria* macroschizonts and their mammalian host cells. Ann. trop. Med. Parasit. 74: 263–265

Yasutake, W. T., Wales, J. H. (1983). Microscopic anatomy of salmonids: an atlas. U.S. Dept. Interior, Fish and Wildlife Service, Resource Publ. 150, Washington, D.C.

Responsible Subject Editor: Professor W. Körting, Hannover, F. R. Germany

Manuscript first received: August 21, 1989
Revised version accepted: January 16, 1990