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POXVIRUS-LIKE AGENT ASSOCIATED WITH EPIZOOTIC MORTALITY IN JUVENILE KOI (*CYPRINUS CARPIO*)

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Our laboratory has been investigating causes of fry and juvenile mortality among fancy carp or koi. In 1996 we transported, on three separate occasions, groups (n = 5,000 - 10,000) of juvenile koi (2.3 - 3.5 g ave wt. and 2 - 3 mo. in age) directly from the producer's pond to the fish health laboratory. Fish were placed into 650 L circular tanks receiving 15 C well water (same temperature as the pond). With all three groups of young koi, mortality

began 3 d post transport and continued for 5 - 7 d at which time the cumulative mortality had reached 98 - 99 %. Dying fish showed signs of hypoxia, lethargy, and pallor of the gills. Upon necropsy, no evidence was found for external or systemic parasites nor was a consistent bacterium isolated from internal organs or gills. An abnormal proliferation and sloughing of epithelial cells of the gills and epidermis was evident in moribund fish. Electron microscopy demonstrated virions (360 nm in diameter) resembling poxviruses in the cytoplasm of infected epithelial cells. Virions were ovoid with one flattened surface. The rounded aspect of the virion was covered with projections (capsomeres) clearly arranged in a symmetrical pattern around a "kidney bean" shaped nucleoid. Only one viral agent, *Herpesvirus cyprini*, is known (from experimental infections only) to induce a systemic disease and mortality in koi of this size. The poxvirus-like agent we have observed is clearly not an herpesvirus based on size, morphology and location for virus replication in the cell. This represents to the best of our knowledge the first observation of a true poxvirus from fish. The source of the virus is still unknown but poxiviruses, in particular

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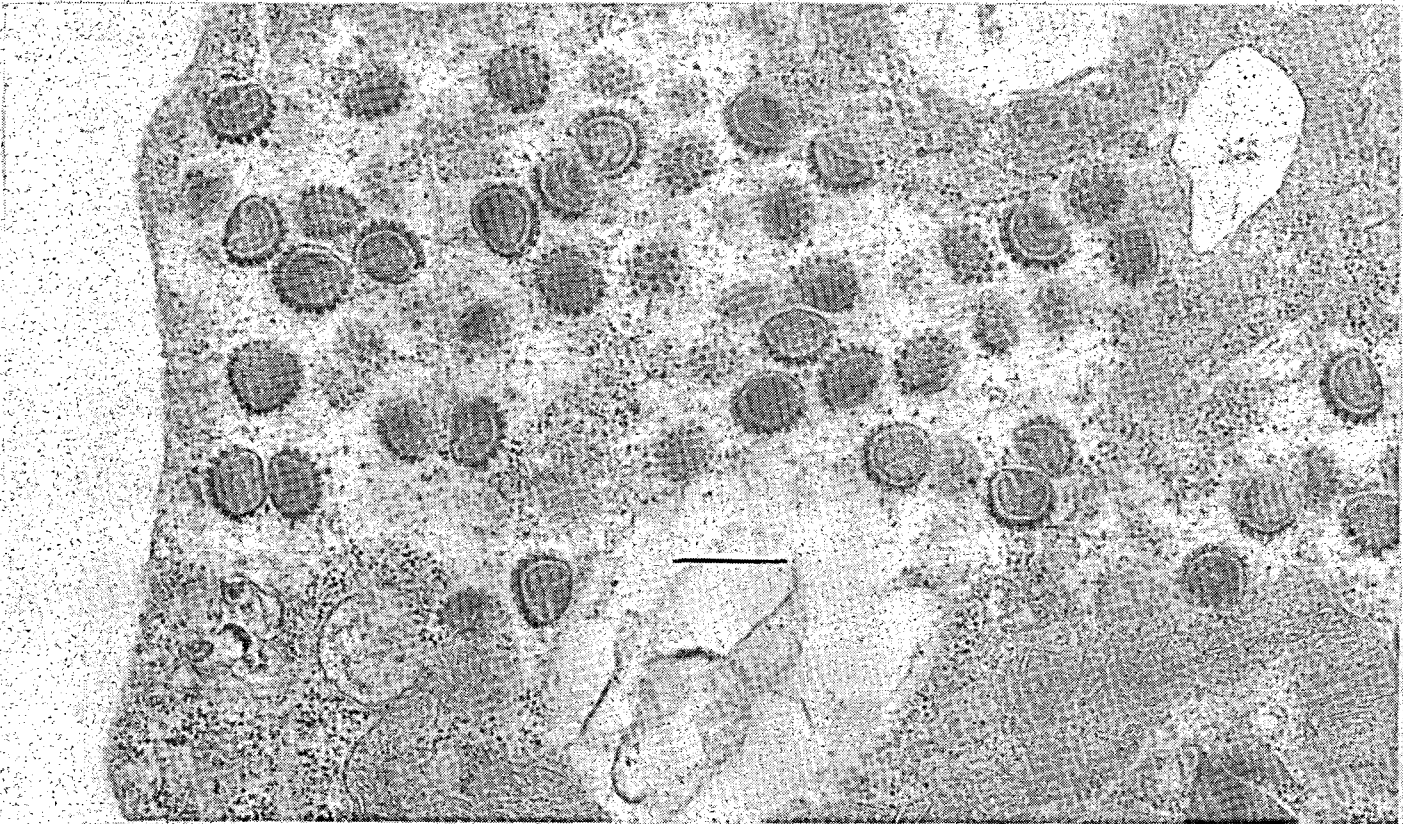
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entomopoxviruses are found in several insects including aquatic species. It is tempting to speculate that we are observing a potential movement of viruses from their insect hosts to fish, a step critical in the evolution of new fish pathogens.

Figure 1. Poxvirions as found in the cytoplasm in the gill epithelium of juvenile koi. Bar = 100 nm.



Graduate Assistantship Opportunity
Department of Fish and Wildlife Resources
University of Idaho, Moscow, Idaho 83844-1136

Funding has been made available by the Western Regional Aquaculture Center for a MS thesis research project on the interaction between coldwater disease and infectious hematopoietic necrosis in cultured rainbow trout. The project will involve laboratory studies of immune system function and disease resistance in rainbow trout. Applicants should have completed a B.S. degree in microbiology, biology or fisheries science or have completed a veterinary degree program. Experience with viral cell culture and bacteriology is desirable but not essential. For information, contact one of the principal investigators: Dr. Jim Congleton, Idaho Cooperative Fish & Wildlife Research Unit, 208-885-7521 (phone), 208-885-9080 (fax), jcongelt@uidaho.edu OR Dr. Larisa Ford, Department of Fish & Wildlife, 208-885-7608 (phone), 208-885-9080 (fax), lford@uidaho.edu.

ANNOUNCEMENTS

Third International Symposium on Aquatic Animal Health August 30 -September 3, 1998 Baltimore, Maryland, USA

The 3rd International Symposium on Aquatic Animal Health will be held in Baltimore on the east coast of the USA from August 30 until September 3, 1998. The symposium will be the first major international meeting to focus comprehensive presentation and discussion on the health of a diversity of aquatic animals including shellfish, fish and marine mammals.

The symposium will be sponsored by the American Fisheries Society -Fish Health Section, Asian Fisheries Society, European Association of Fish Pathologists, International Association for Aquatic Animal Medicine, Japanese Society of Fish Pathology, and the National Shellfisheries Association. The meeting is supported by the John Hopkins University and the University of Maryland at Baltimore.

For further information please contact: Dr. Sarah L. Poynton, Division of Comparative Medicine, John Hopkins University School of Medicine, Ross Research Building 4th Floor, 720 Rutland Avenue, Baltimore, MD 21205 USA, ph: (1) 410-955-3273, Fax: (1) 410-550-5068, E-mail: spoynton@welchlink.welch.jhu.edu.

The Fourth International Symposium on Viruses of Lower Vertebrates 12-15 May, 1998 Weymouth, UK

This symposium will be **limited to 60 participants**. To be placed on the mailing list to receive further information about the Symposium and a Second announcement, please contact: Mrs H. Cobbold, Symposium Secretariat, International Symposium on Viruses of Lower Vertebrates, CEFAS Weymouth Laboratory, Barrack Road, Weymouth, Dorset, DT4 8UB, UK.

13th Annual U.S. Landscape Ecology Meeting Michigan State University 17-21 March, 1998

The next meeting of the US Regional Association, International Association for Landscape Ecology, "Applications of Landscape Ecology in Natural Resource Management", will be held at MSU, East Lansing, Michigan. Fisheries, human dimensions, planning, range, soils, timber, water, wildlife and other resource subjects will be emphasized. The deadline for abstracts is 31 October 1997. For information about abstract submission, symposium proposal submission, local arrangements and travel, please refer to the meeting web site: www.fw.msu.edu/iale98. Local Hosts: Jianguo Liu and William W. Taylor, Department of Fisheries and Wildlife, 13 Natural Resources Bldg, Michigan State University, East Lansing, MI 48824; 517-355-1810 (phone); 517-432-1699 (fax); iale98@perm3.fw.msu.edu.

GRADUATE RESEARCH ASSISTANTSHIPS

WHIRLING DISEASE INVESTIGATIONS

MONTANA STATE UNIVERSITY BOZEMAN

Four(4) graduate research assistantships are available immediately at Montana State University for studies concerning salmonid whirling disease. Applications for both MS and PhD degree programs are being accepted. Minimum Qualifications: GPA>3.0, verbal + quantitative GRE >1100; BS in fisheries or related field for MS assistantships, MS in fisheries or related field for PhD assistantships.

1. Relation of Life History Type to Whirling Disease Susceptibility in Missouri River Rainbow Trout. This project will examine life history, types of rainbow trout in the blue ribbon section of the Missouri River near Helena, Montana, where recent sampling has confirmed the presence of whirling disease. Distribution studies of the oligochaete (*Tubifex tubifex*), the other known host of the whirling disease causing parasite (*Myxobolus cerebralis*) indicate that tubifex are rare in some spawning tributaries. Thus, life history types that spawn and rear in these tributaries for at least the first summer prior to migration to the mainstem may be at reduced risk to the disease. Objectives of the study are: determine timing and location of spawning using spawning weirs and redd surveys; determine contribution of various fry life history types to avel adult recruitment in the mainstem; test methods for determining life history type using scales and otoliths; determine fry production changes as whirling disease spreads through the system; and identify possible rainbow trout life history types that could be used to bypass effects of whirling disease in other river systems. An overall goal of the study is to test the utility of various techniques (batch marking of outmigrant fry, scale and otolith banding patterns, otolith microchemistry) for determining life history type in wild salmonids. PhD student preferred. Experience in ageing scales and otoliths, fish trapping, and strong chemistry background desirable. To apply, send letter of interest, resume, transcripts, GRE scores, and names of three references to Thomas McMahon, Fish and Wildlife Program, Department of Biology, Montana State University, Bozeman, MT 59717. Email: ubitm@msu.oscs.montana.edu (no telephone calls please).

2. The Role of Tubificid Worms in Salmonid Whirling Disease. Whirling disease is a parasitic disorder that can be fatal to salmonid fishes. The parasite that causes the disease, *Myxobolus cerebralis* (Myxozoa: Myxosporidia), has been implicated in severe declines of wild rainbow trout (*Oncorhynchus mykiss*) populations in Montana and Colorado. The life cycle of *M. cerebralis* alternates between a salmonid fish and an aquatic worm, *Tubifex tubifex* (Oligochaeta: Tubificidae) is known to be one host. Whether other worms can be hosts is unclear. It is the purpose of this study to investigate, using controlled, laboratory experiments, the effect of environmental conditions on the ecology of infection of *T. tubifex* and other species of tubificids by *M. cerebralis*. Specifically, worm infection levels, release of particles that cause infection in fish, and life cycles of infected and uninfected worms under two different temperature regimes, will be compared among worm species and variants of *T. tubifex*. This work will begin to elucidate the role of tubificids in whirling disease and aid in our understanding of why whirling disease seems to be a problem in some areas and not in others. MS student preferred. Experience in invertebrate and worm culture and identification and laboratory histological techniques of other squishy, icky things desirable. To apply, send letter of interest, resume, transcripts, GRE scores, and names of three references to Billie Kerans, Fish and Wildlife Program, Department of Biology, Montana State University, Bozeman, MT 59717. Email: ubibk@msu.oscs.montana.edu (no telephone calls please).

3. Restoration of Wild Rainbow and Cutthroat Trout to the Upper Madison River in the Face of Whirling Disease. This project is part of a multipronged effort to restore wild trout to their former abundances in the upper Madison River. Wild rainbow trout have declined 90% due to the effects of whirling disease over the past 5 years in this section of one of the most productive and popular wild trout fisheries in the U.S. One possibility for maintaining wild trout populations is via maintenance/introduction of life history types with specific behavioral characteristics that reduce exposure to whirling disease infection during the highly vulnerable young fry stage. Phase One of this restoration effort will be to identify tributaries that have low incidences of whirling disease infection and that have suitable spawning and rearing habitat for possible reestablishment of wild rainbow or cutthroat trout. This project will involve habitat surveying of potential spawning and rearing tributaries and radiotracking to determine current spawning locations and life histories of surviving rainbow and cutthroat trout in the upper Madison River. MS student preferred. Experience in habitat surveying and/or radiotelemetry desirable. To apply, send letter of interest, resume, transcripts, GRE

scores, and names of three references to Thomas McMahon, Fish and Wildlife Program, Department of Biology, Montana State University, Bozeman, MT 59717. Email: ubitm@msu.oscs.montana.edu (no telephone calls please).

4. Effects of Age, Dose, and Environmental Stress on Development of Whirling Disease in Rainbow Trout. The development and severity of pathology of whirling disease in salmonids is known to be dependent generally on the age of fish when exposed to the infective triactinomyxon stage of *Myxobolus cerebralis* and on the density of triactinomyxons to which the fish are exposed. Nevertheless, no systematic determination of the effects of these factors, and their interaction, has been conducted. Accordingly, fishery managers remain unsure of the ages at which young trout may become resistant to development of whirling disease, especially when exposed to different parasite doses. This uncertainty precludes effective management of wild trout fisheries, including managing for life history types that spawn and rear in *M. cerebralis* free habitats, but that later migrate to infected waters. To remedy these information deficiencies, comparative testing of disease susceptibility of different ages of rainbow trout *Oncorhynchus mykiss* exposed to different parasite doses will be conducted in a replicated factorial experiment under standardized laboratory conditions (in MSU's new Wild Trout Research Laboratory). Complementary assessments of the effects of stress on the development of whirling disease and infection on stamina will also be performed. Other aspects of susceptibility (e.g., species susceptibility,) will be examined in subsequent segments of the project, gaining a better understanding of this parasite and the disease it manifests, management activities can be designed to enhance viability and productivity of wild trout populations in *M. cerebralis* positive river systems. This is a laboratory study and will be conducted in collaboration with Elizabeth MacConnell, Fish Pathologist, Bozeman Fish Technology Center, U.S. Fish & Wildlife Service. PhD student preferred. Experience in fish diseases and laboratory maintenance of experimental aquatic organisms desirable. To apply, send letter of interest, resume, transcripts, GRE scores, and names of three references to A Zale, Montana Cooperative Fishery Research Unit, Department of Biology, Montana State University, Bozeman, MT 59717. Email: ubiaz@msu.oscs.montana.edu (no telephone calls please). For information on the MSU Biology Department and graduate program, visit our website at www.montana.edu/wwwb/

MORE POSITIONS AVAILABLE

Postdoctoral Position. Two - year postdoctoral position available August 25, 1997 to develop molecular methods for the reliable and sensitive detection and identification of mycobacterial pathogens (e.g., *Mycobacterium marinum* and *M. fortuitum*, etc.) in fish and water samples from intensive aquaculture systems. Individual applying should have documented experience in microbiology and molecular biological techniques (e.g., DNA sequencing, PCR, DNA probes, DNA fingerprinting). Salary of \$24,000 per year plus fringe benefits with second year of employment dependent upon successful completion of first year. Please send cover letter, resume, and names of three references to either Stephen A. Smith, DVM, PhD, Virginia-Maryland College of Veterinary Medicine, Virginia Tech, Blacksburg, VA 24061-0442; E-mail: stsmith7@vt.edu or Joseph O. Falkinham, III, PhD, Fralin Biotechnology Center, Virginia Tech, Blacksburg, VA 24061-0345; Email: jofiii@vt.edu. Applications will be accepted until position is filled. Virginia Tech is an equal opportunity/affirmative action employer.

Graduate Studies Position in Aquatic Medicine. Graduate student position available August 25, 1997 for PhD degree in the area of aquatic medicine/fish health. Areas of research include but are not limited to pathology, toxicology, immunology, diagnostics and evaluation of chemotherapeutics for freshwater and marine finfish. Desired applicant should have a DVM and/or Masters degree and experience in related field of aquatics. Please send letter of intent, curriculum vitae, and names of three references to Dr. Stephen A. Smith, Virginia-Maryland College of Veterinary Medicine, Virginia Tech, Blacksburg, VA 24061-0442; E-mail: stsmith7@vt.edu. Applications accepted until position is filled. Virginia Tech is an equal opportunity/affirmative action employer.

Histopathology associated with an Irido-like virus infection of Tilapia

Charlie E. Smith & Doug Ramsey

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&

C.A. Speer & Andy Blixt

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Montana State University
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While iridoviruses have been identified from several species of fish, the most notable viral disease caused by an iridovirus is epizootic hematopoietic necrosis virus disease in redbfin perch, *Perca fluviatilis* (Langdon & Humphrey, 1987). Our report describes histopathological changes in tilapia, *Tilapia nilotica*, infected with an irido-like virus.

Tilapia fingerlings experiencing high mortality were submitted to Rangen's diagnostic laboratory for routine disease examination. Fish had recently been imported as fry into Idaho from Florida. Affected fish were 3.5 to 5.0 cm in length, were dark in color, lethargic, bloated and some gulped at the water surface for air. Gills were pale as were internal organs. Kidneys were sometimes swollen. Severe abdominal ascites was present in most fish. Mild corneal opacity was apparent in some. Mortality rate was approximately 50% in group of fish we sampled. However, affected fish in some adjacent tanks only suffered a 10 - 20% mortality. In addition to routine bacteriological, parasitological and virological examinations, 5 fish were also preserved for histopathological examination.

Cultures of kidney on trypticase soy agar produced moderate bacterial growth from only one of four fish. No other pathogens were seen in significant numbers in Giemsa-stained sections suggesting that the bacteria cultured were probably opportunistic secondary pathogens. Attempts to culture virus on cell culture were unsuccessful. Although rounding of a few epithelium papulosum cyprini (EPC) cells occurred initially, no cytopathic effect occurred after transferring to new cells.

Histopathological examination of longitudinal sections of fish revealed pathological changes consisting primarily of necrosis and inflammation in several organs including choroid of the eye, heart, intestine, kidney, liver and spleen. The most severe changes were present in hematopoietic tissue of the spleen and kidney, and in the heart where necrosis had occurred. Renal tubules were seldom involved. Associated with the pathological changes were large, apparently infected cells containing finely granular cytoplasmic material and degenerate, or eccentrically placed nuclei. Some cells had hypertrophied nuclei and appeared to be in earlier stages of infection. Infected cells were most numerous in the spleen and had often replaced both red and white pulp (fig.1). In kidneys, they were often seen occluding glomerular capillaries, occasionally in association with cellular necrosis (fig.2). Infected cells were common in hearts of infected fish as was myocardial necrosis and necrotic cellular debris (fig.3). Extreme congestion with necrotic debris, presumably blood and hematopoietic cells, was apparent in the atrium.

Fatty change was apparent in livers of most fish. Perivascular cuffing was also common. Necrotic changes, while apparent, were generally mild and focal. Infected cells were uncommon.

Because infected cells were so numerous in the spleen, spleens from five fish showing clinical signs and sacrificed at a later date, were dissected and minced into fine pieces, fixed in Karnovsky's fluid and prepared for ultrastructural examination by transmission electron microscopy. Infected cells contained numerous polyhedral viral particles (fig.4), 150-175 nm in diameter suggestive of an iridovirus (Ron Hedrick, personal communication). The disease was also confirmed histopathologically from these same fish.

Fortunately, fish were being held in small tanks in a building at the lower end of the hatchery. To prevent potential spread of the disease to other fish, they were all destroyed and tanks disinfected with chlorine shortly after the virus was identified. Since disinfecting, disease outbreaks have not occurred in any fish held in the tanks used to rear fry/fingerlings. The disease has never been found in older tilapia originating from broodstock held on the hatchery and reared in raceways.

A disease outbreak in tilapia, similar to this one has been identified in tilapia fry imported into Ontario, Canada from Florida (Dr. Hugh Ferguson, personal communication). A paper describing his findings has been submitted for publication in J. Fish. Diseases. To our knowledge these are the first reported outbreaks of a virus disease in cultured tilapia.

Reference: Langdon, J.S. & J.D. Humphrey. 1987. Epizootic hematopoietic necrosis, a new viral disease in redfin perch, *Perca fluviatilis* L, in Australia. J. Fish Diseases 10:297-298.

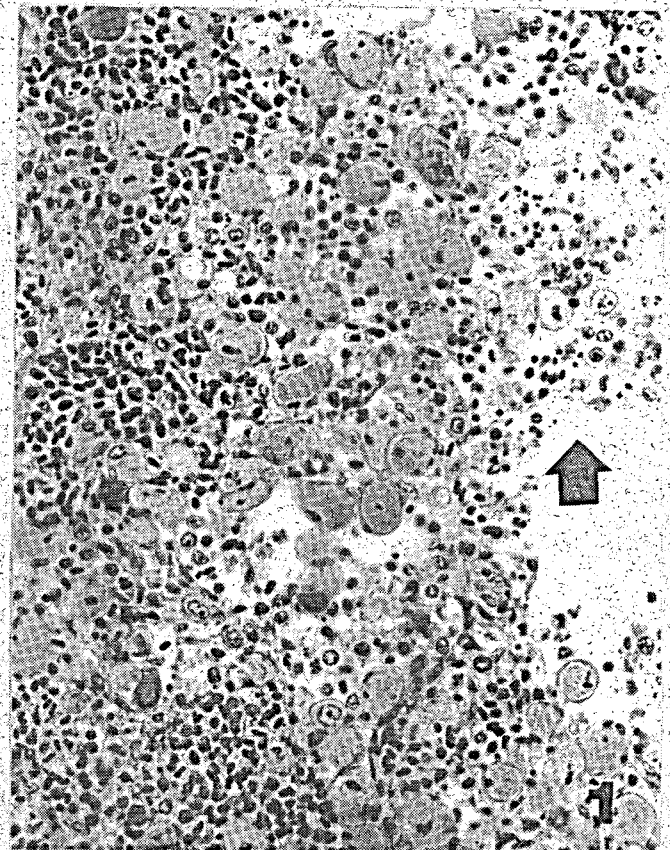


Figure 1. Numerous hypertrophic, infected cells in spleen have replaced normal tissue. Necrotic debris is seen adjacent to and above arrowhead. Hematoxylin & Eosin (H&E) X450.

Figure 2. Two glomeruli show infected cells (arrows) lodged in glomerular capillaries. Both show degenerative changes. H&E, X450.

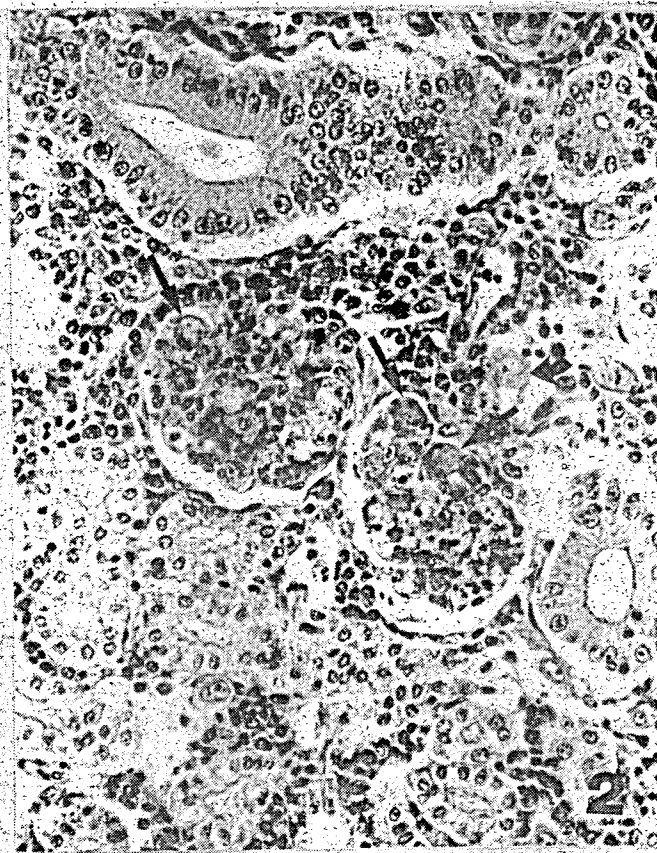


Figure 3. Heart shows infected cells scattered throughout (arrows). Necrotic cellular debris (n) is present in lower half of photo. H&E, X450.

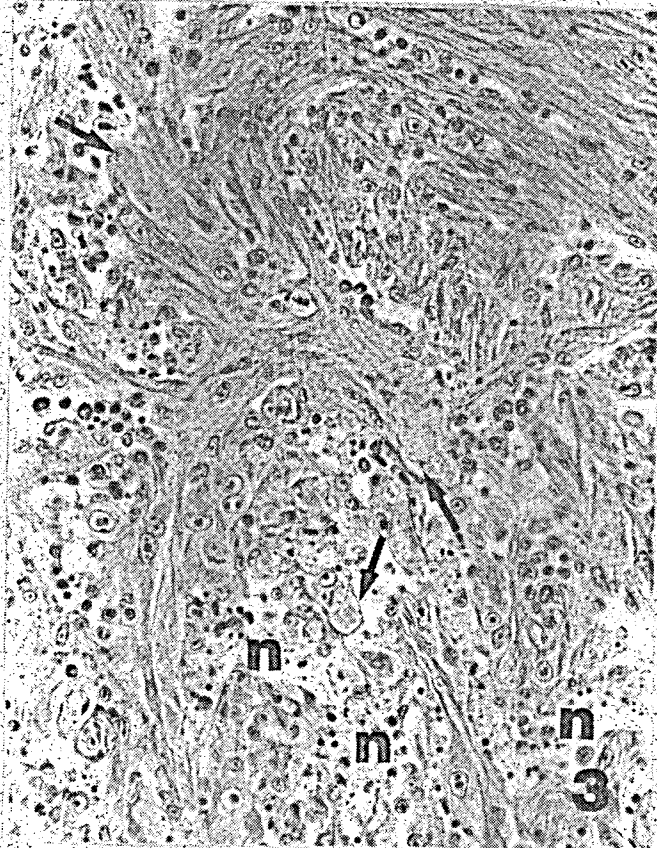
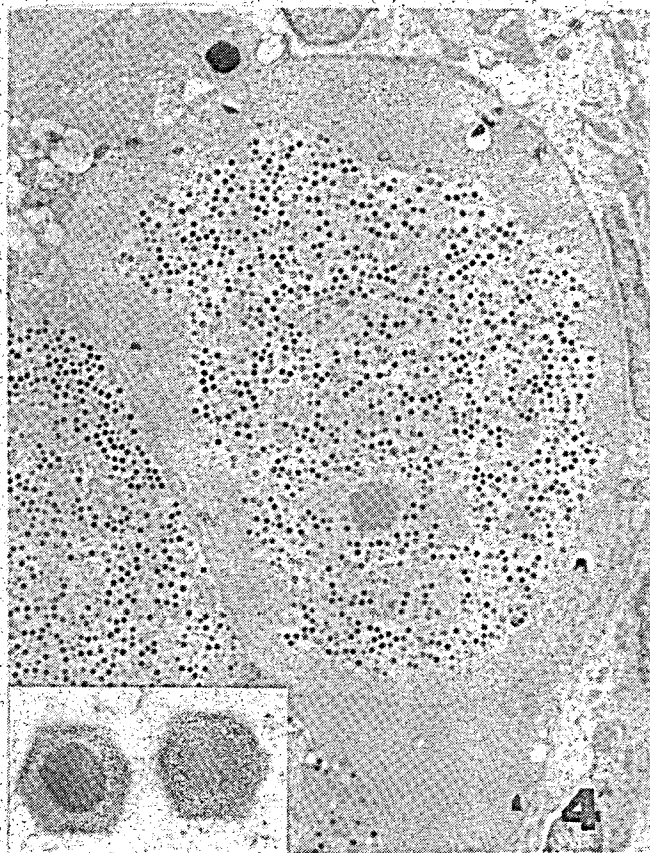


Figure 4. Electron micrograph of splenic tissue shows infected cell filled with virons. Lead citrate, uranyl acetate, X5,400. Insert shows two individual irido-like virons. X99,000.



1997 ANNUAL MEETING OF THE AMERICAN FISHERIES SOCIETY FISH HEALTH SECTION

SEPTEMBER 4-6. JUNEAU, ALASKA

Meeting and hotel

The 1997 meeting will be held at the Westmark Baranof Hotel in Juneau, Alaska September 4-6. The meeting will begin at 8:00 AM on Thursday the 4th and continue through about 5:00 PM on ~~Saturday~~ the 5th. A block of 100 rooms has been reserved at rates of 89\$ (Baranof) and 98\$ (Westmark) for singles or doubles per night. Rooms will be held under "Fish Health Section Meeting" at this rate until July 10, 1997. Room reservations can be made by calling 1-800-544-0970 or 1-907-586-2660. Complimentary shuttle service from the Juneau International Airport is provided by the Baranof and its sister hotel, the Westmark.

Registration

Conference registration will be handled by Ted Meyers, Alaska Department of Fish and Game, CFMD Division, P.O. Box 25526, Juneau Fish Pathology Lab, Juneau, AK 99802-5526 (telephone 1-907-465-3577). A registration fee of \$100 will cover the costs of break refreshments, meeting rooms, A/V equipment, meeting packet with abstract/agenda booklet and a prime rib/fresh halibut banquet on the evening of September 5th. Please fill out the attached registration form and make checks payable to "FHS Annual Meeting". The registration deadline is June 1, 1997.

Fish Health Section Newsletter

The Fish Health Section Newsletter is a quarterly publication of the Fish Health Section of the American Fisheries Society. Submissions on a topic of interest to fish health specialists are encouraged with the understanding that material is not peer reviewed. Articles should not exceed two newsletter pages and should not have more than five references. Submissions should be submitted on disk in Word perfect 5.1 or in a generic form that can be read on WP5.1 for IBM. Disks will be returned if a SASE is included with your submitted article. Also, we will be glad to publish any abstract of a paper that has been submitted to the *Journal of Aquatic Animal Health* (whether accepted or not by JAAH). Submissions should be addressed to the editors listed below:

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