

FHS

NEWSLETTER

FISH HEALTH SECTION - AMERICAN FISHERIES SOCIETY

Volume 24, Number 1

Page 1

January, 1996

Antigenic and Ultrastructural Characteristics of the Epitheliocystis Disease Agent in Cultured White Sturgeon (*Acipenser transmontanus*) Similar to the **Chlamydiae**

Joseph M. Groff, Scott E. LaPatra, Robert J. Munn, Mark L. Anderson, Terry L. Patterson, and Bennie I. Osburn

Department of Pathology, Microbiology and Immunology (Groff, Osburn) and the California Veterinary Diagnostic Laboratory (Anderson), School of Veterinary Medicine and the Department of Pathology, School of Medicine (Munn), University of California, Davis, CA 95616; College of Southern Idaho Aquaculture Program, P.O. Box 1238, Twin Falls, ID 83301 (Patterson), and Clear Springs Foods, Inc., P.O. Box 712, Buhl, ID 83316 (LaPatra).

A mild to moderate branchial epitheliocystis infection was diagnosed in subyearling (11 month, 250-300 g) white sturgeon (*Acipenser transmontanus*) from a private culture facility with a 4-8% mortality in the population. Infected branchial epithelial cells contained the coccoid to coccobacillary epitheliocystis organisms that appeared as cytoplasmic inclusions composed of fine, homogeneous, dense, basophilic, granular material. The infected cells were variable enlarged with spherical to oval profiles and randomly distributed throughout the branchial epithelium. The inclusions were circumscribed by a variable amount of host cell cytoplasm that consequently became less abundant with progressive enlargement of the cytoplasmic inclusion (Figure 1). The nuclei of infected cells were initially enlarged and eccentrically located with a single prominent nucleolus or multiple nucleoli but became attenuated and progressively less prominent subsequent to the progressive enlargement of the cytoplasmic

inclusion. The cytoplasmic inclusions stained positive with Macchiavello but negative with Brown and Brenn, periodic acid-Schiff and Gemenez stains. Expression of chlamydial antigen was demonstrated within the cytoplasmic inclusions using a standard peroxidase-antiperoxidase immunohistochemical technique and a primary murine monoclonal antibody specific for the chlamydial genus-specific antigen (Figure 1). Host reaction was absent or limited to a mild epithelial hyperplasia of the gills although the latter could not be definitively attributed to the infection.

Three states of coordinated intracellular development were recognized by electron microscopy. Regardless of the developmental stage, the organisms were delimited by a cell membrane that was composed of two electron-dense zones separated by an electron-lucent layer. A variable number of discrete cytoplasmic condensations that were not membrane-bound and of variable density dependent on the state of development were also a common feature. The reticulate bodies were oval to spherical and 0.4-0.8 x 0.5-1.4 μm but often exhibited a pleomorphic and convoluted appearance due to variable membrane invaginations and evaginations suggestive of uneven fission and budding. A few enlarged reticulate bodies with a maximum size of 2.2 x 3.7 μm had an exaggerated pleomorphic and convoluted appearance and

IN THIS ISSUE:

<i>Ichthyophthirius</i>	Page 6-7
FHS Annual Meeting-1996 Call for Papers	Page 10-11
<i>Enterocytozoon salmonis</i>	Page 12-13
Virus isolated from largemouth bass	Page 14

contained multiple cytoplasmic condensations. The pleomorphic appearance of the enlarged reticulate bodies was due to multiple membrane evaginations consistent with the process of budding. Separate host cells contained intermediate bodies that were spherical to oval and $0.2-0.4 \times 0.3-0.6 \mu\text{m}$ although often observed in the process of apparent uneven division (Figure 2). These cells contained a single, compact, cytoplasmic condensation that were completely circumscribed by an electron lucent zone or halo. The presence of a cap or plaque composed of hexagonally arrayed, fibrillar, surface projections was initially recognized at this stage. Tangential sections demonstrated that the cap was composed of up to 30 surface projections. An homogenous population of elementary bodies that were oval and $0.3-0.4 \mu\text{m}$ also occurred separately in individual host cells. This developmental stage had a single, dense, compact, eccentrically located, cytoplasmic condensation and a single cytoplasmic vacuole that was not membrane-bound. The hexagonally arrayed fibrils were more distinct at this stage and occurred opposite to the eccentrically located, cytoplasmic condensation. The cell membrane associated with the fibrils constituted approximately 25% of the cell circumference and displayed a prominent electron-density relative to the remainder of the cell membrane.

Ultrastructural characterization of the infected host cells was precluded due to the advanced state of infection but were consistent with epithelial cells due to the presence of apical, plasmalemmal projections that occurred at regular intervals similar to adjacent non-infected epithelial cells. The cytoplasm and organelles of the host cell were peripherally compressed although organellar lesions were not observed nor was the cytoplasmic inclusion delineated by a distinct capsule. The peripherally located nuclei contained a loose nuclear matrix and a variable number of nucleoli.

Morphological characteristics of the epitheliocystis organism in these white sturgeon were similar to previous descriptions in other teleost species and extends the species catalogue of epitheliocystis infection. Furthermore, the ultrastructural similarities to the chlamydiae and the

immunohistochemical detection of chlamydial antigen provides further evidence that the epitheliocystis agent is related to members of the Chlamydiales. The source of infection was not determined although transmission via fomites or through the water supply was considered the most likely explanation. The high-density culture of these sturgeon would further promote the transmission and exacerbation of infection within the population. The sturgeon examined in this study were randomly selected and may not have been representative of the severity of infection responsible for the low-level mortality in this population. Therefore, epitheliocystis infection cannot be ruled-out as the primary cause of mortality in this population especially since other infectious disease agents were not identified nor were water quality or other environmental parameters considered a limiting factor. Although the infection was considered mild to moderate and could not be definitively attributed to the mortality in this population, the potential adverse impact of epitheliocystis infection on sturgeon culture especially in high density operations should be considered.

The demonstration of chlamydial-specific antigen expression in this case of sturgeon epitheliocystis has renewed the interest concerning the taxonomic classification of the epitheliocystis agent.

Immunohistochemical studies are presently in progress in our laboratories to demonstrate epitheliocystis expression of chlamydial-specific antigen in other teleost species infected with epitheliocystis. In this regard, anyone interested in submitting samples or becoming involved as a cooperator in these efforts should contact Dr. Scott LaPatra. Samples may be either live fish infected with epitheliocystis and/or wet or paraffin-embedded tissue samples. Interested cooperators should contact:

Dr. Scott LaPatra
Clear Springs Foods, Inc.
P.O. Box 712
Buhl, ID 83316
Phone: (208) 543-8217
FAX: (208) 543-4146

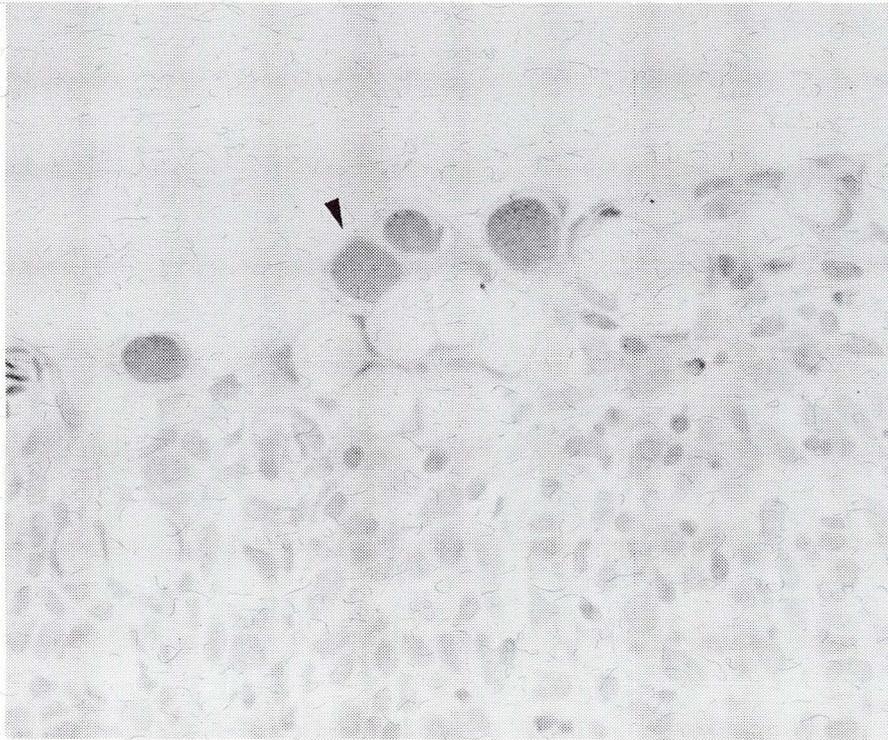


Figure 1. Photomicrograph of white sturgeon branchial epithelial tissue infected with the epitheliocystis organisms. Infected epithelial cells contain masses of the epitheliocystis organisms that appear as cytoplasmic inclusions and express chlamydial genus-specific antigen using the PAP immunohistochemical technique (arrowhead). Aminoethylcarbazole with Mayer's hematoxylin counterstain. 500x.

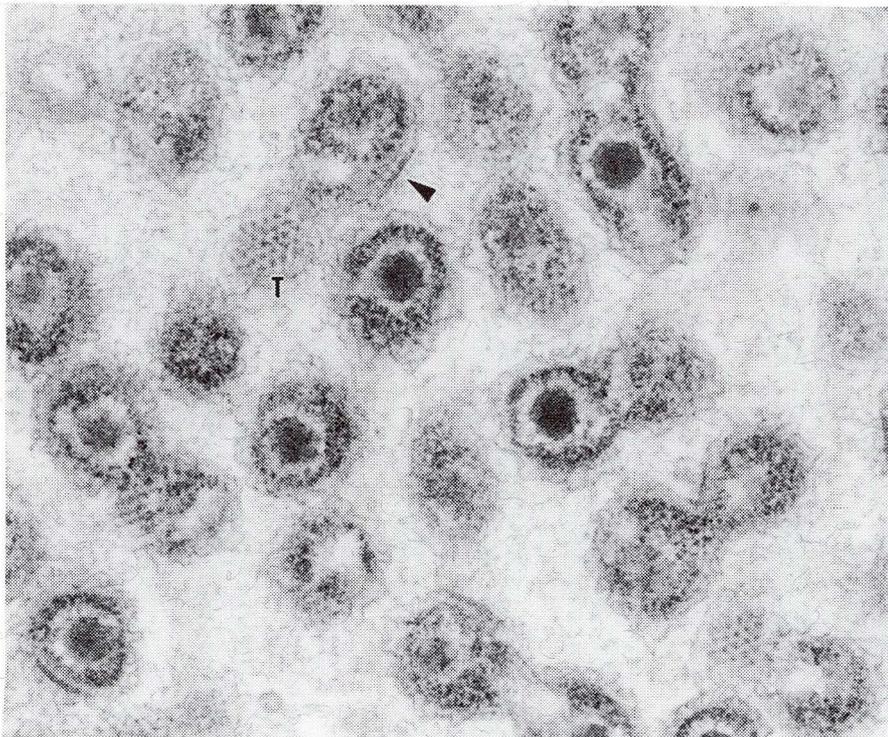
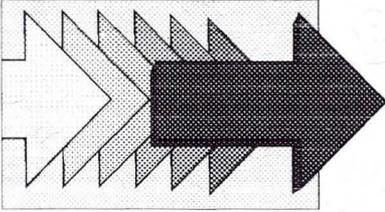


Figure 2. Transmission electron micrograph of epitheliocystis intermediate bodies often observed in the process of division that contain a single, dense, centrally located, cytoplasmic condensation circumscribed by an electron-lucent zone and a cap of hexagonally arrayed, fibrillar, surface projections (arrowhead) initially recognized at this stage of development. Tangential sections (T) through the cap demonstrate up to 30 surface projections. Uranyl acetate and lead citrate. 45,000x.

**Correction:*****Parasites of Fishes in Wyoming*
by Doug Mitchum**

is available for an even lower price than the "unbelievable low price" printed in the last issue of the newsletter! The actual price of the book is \$20.00 not \$30. The book is available from Alternative Enterprises, Wyoming Game and Fish Department, 5400 Bishop Blvd, Cheyenne, Wyoming 82006. 1-800-548-9453.

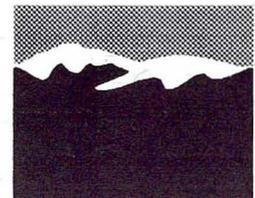
*******ANNOUNCEMENT*********Oregon State University Salmonid Disease Workshop
June 12-21, 1996**

This workshop is designed for professionals working in the fish health field and will emphasize recent advances and developments in our understanding of salmonid diseases. The workshop will be held at the OSU Hatfield Marine Science Center in Newport, Oregon. The workshop is limited to 20 participants. Cost of the workshop is \$625 plus \$125 for housing at the HMSC. For further information call or write:

Dr. Robert E. Olson, Associate Director, OSU Hatfield Marine Science Center,
Newport, Oregon 97365-5296.

Telephone: 541-867-0251

Email: olsonr@ccmail.orst.edu

**WESTERN FISH DISEASE WORKSHOP
JUNE 25-27, 1996
CORVALLIS, OREGON**

The 1996 Western Fish Disease Workshop will be hosted by Oregon Department of Fish and Wildlife and Oregon State University Center for Salmonid Disease Research at Corvallis, Oregon. On June 25, a **Continuing Education Session** will be held at the Department of Microbiology, OSU. The proposed topic for this session is hematology, but other possibilities include PCR methods to detect fish pathogens; contact **Craig Olson (360-438-1181)** with questions. **Technical sessions** will be conducted on June 26 and 27. More information about the meeting will appear in the next issue of the newsletter. Please contact **Rich Holt (541-737-1863)** or **Tony Amandi (541-737-1855)** if you have any questions, suggestions for agenda topics, or need additional information.

ASSOCIATION INTERNATIONALE
DE STANDARDISATION BIOLOGIQUE



INTERNATIONAL ASSOCIATION
OF BIOLOGICAL STANDARDIZATION

IABS TASK FORCE ON VACCINES

FIRST ANNOUNCEMENT

International Symposium on Fish Vaccinology

June 5th to 7th 1996
at Soria Moria Hotel, Oslo, Norway.

Nearly 15 years have elapsed since the first IABS symposium on fish vaccines («Fish Biologics: serodiagnostics and vaccines, Leetown W.Va. April 26-30, 1981). Since then, vaccination has become the major method of controlling bacterial diseases in farmed fish. Effective vaccines now contribute significantly to the success of fish farming by improving fish health and welfare, reducing the use of antibiotics in aquaculture and improving the industry's cost-effectiveness.

The aim of the conference is to review important aspects of fish vaccinology, and the present status of immunoprophylaxis in aquaculture.

Topics to be covered include:

- antigens and adjuvants
- immune response
- regulatory aspects
- standardization
- safety and efficacy
- vaccine strategies

The conference will bring together workers from scientific institutions, regulatory institutions and the vaccine industry. Representatives from the aquaculture industry will also benefit from this conference.

The symposium will include presentations by invited international experts as well as discussions. Oral presentations and posters are invited.

International Symposium on Fish Vaccinology

Preliminary registration from

Name:

Mailing address:

.....

Country:

Telephone: Fax: E-mail:

I plan to submit an abstract for oral presentation Yes

I plan to present a poster Yes

PLEASE SEND THIS FORM TO VESO, PO BOX 8109 DEP, 0032 OSLO, NORWAY. FAX: 47 2256 6254

An Examination of Sodium Chloride for Control of *Ichthyophthirius multifiliis* in Channel Catfish Raised in Closed Recirculating Systems

Herman H. Jarboe, Aquaculture Research Center, Northwestern State University of Louisiana, Box 157, Route 1, Lena, Louisiana 71447 Phone: 318-357-8380

A nutrition experiment, investigating the effects of feeding time on channel catfish, *Ictalurus punctatus*, growth in closed recirculating raceway systems was abruptly interrupted by an *Ichthyophthirius multifiliis* epizootic. The study had been underway for two weeks and prior to the *I. multifiliis* outbreak, no mortalities had been recorded in any of the culture tanks.

Channel catfish averaged 27 g and were stocked at a density of 3g/L. A 35% protein, floating feed was being fed at a rate of 4% of body weight, once daily. At the time of the epizootic, water temperature was 24°C, pH was 7.5, total hardness and alkalinity were 20 and 137 mg/L as CaCO₃, respectively and dissolved oxygen averaged 6.0 mg/L. Total ammonia nitrogen and nitrite nitrogen in the systems were less than 0.02 mg/L. Photoperiod was 14 hL:10 hD, and water exchange rate was 18 exchanges/ hour. Channel catfish were being raised in 693-L fiberglass raceway tanks (112 cm L x 112cm W x 56 cm D), arranged in series, each having three tiers. All raceway series drained into a common 757-L fiberglass sump tank. The culture water was circulated through rearing tanks to the sump and pumped to a 6 ft³ bead biofilter located at the head of each raceway series. Following biofiltration, water re-entered the system at tier one of each series.

On day one of the epizootic, channel catfish were off-feed and congregated in the tank corners. Affected individuals were listless, floating head up in the water and failed to respond to any external stimuli. Necropsy indicated no internal abnormalities and cultures of muscle, liver, blood, and trunk kidney were negative for bacteria. Visual signs indicative of *I. multifiliis* were confirmed with microscopic examination of fins, epidermal scrapes, and gill filaments.

On day two, system water temperatures were increased to 29-30°C with 900-W heaters and unionized, food grade sodium chloride was added until a concentration of 5 g/L was achieved. Water temperature and sodium chloride concentration in the systems remained

static for the following ten days. Dead and moribund channel catfish were removed from the culture tanks as they appeared.

Channel catfish mortalities were observed in the systems through day seven (Figure 1). No mortalities or moribund channel catfish were further observed, and the *I. multifiliis* epizootic appeared to have ended in all systems by day eight. Cumulative percentage mortality was raceway tier specific. Total percentage mortality averaged 34% in tier one, 86% in tier two, and 92% in the bottom tanks (tier three).

Sodium chloride (salt) is an unapproved new animal drug of low regulatory priority for the Food and Drug Administration for use as an osmoregulatory aid in stress relief or a short-term parasiticide treatment (10-30 minutes) at levels of 0.5-1.0% and 3.0%, respectively (1). At concentrations of 5 g/L, sodium chloride has been used to successfully control *I. multifiliis* infestations on four species of Australian warmwater fish, including the catfish, *Tandanus tandanus* (2).

Salt is currently seeing widespread use in the channel catfish industry as a general therapeutic and a vehicle to alleviate nitrite toxicity. Under the conditions of this case history, at concentrations of 5 g/L, sodium chloride is ineffective for the control of ichthyophthiriasis in channel catfish. If effective therapeutic concentrations were clinically established and proven through field tests, salt would be a desirable fish parasiticide because of its low regulatory nature and cost. Ectoparasite control on commercially valuable species of fish using sodium chloride merits further investigation.

References

1. Federal Joint Subcommittee on Aquaculture. 1994. Guide to Drug, Vaccine, and Pesticide Use in Aquaculture. Texas Agricultural Extension Service, Texas A&M University System, College Station, Texas, USA.
2. Selosse, P. M. and S. J. Rowland. 1990. Use of common salt to treat ichthyophthiriasis in Australian warmwater fishes. *Progressive Fish-Culturist* 52: 124-127.

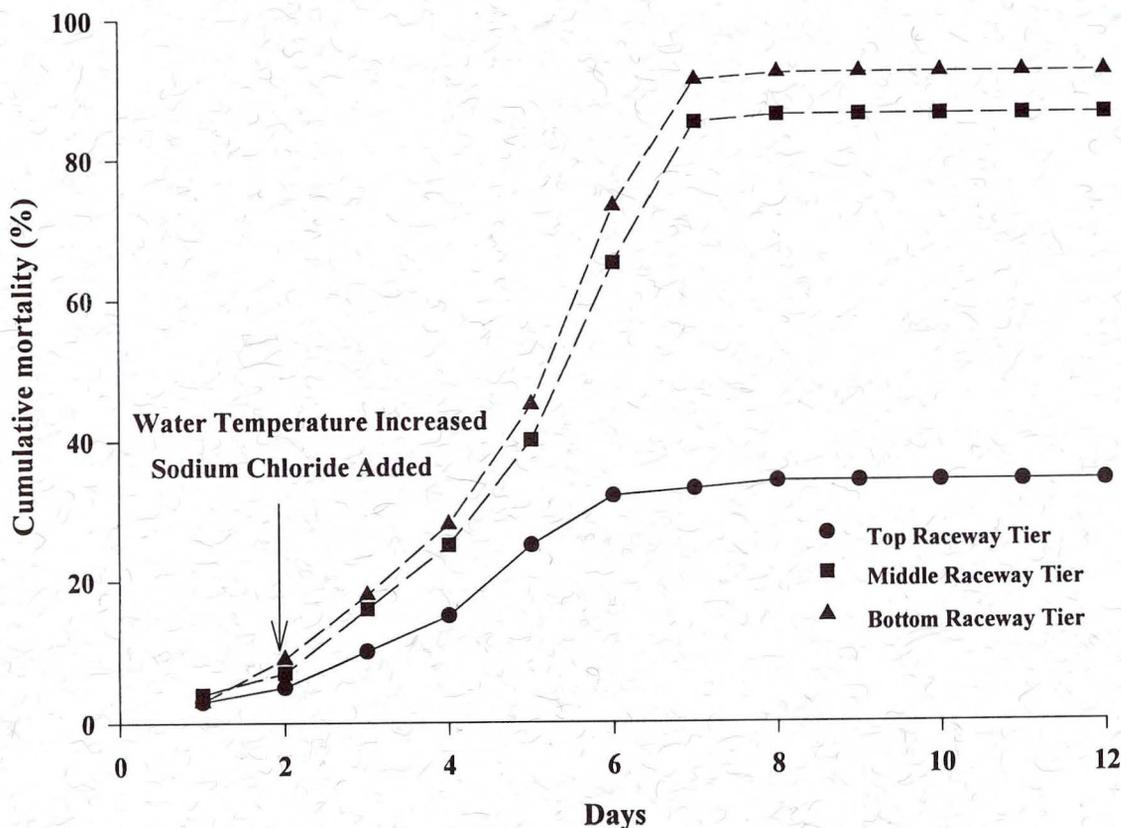


Figure 1. Channel catfish cumulative mortality for each raceway tier over a 12 day period.

FHS President's Annual Report...Erratum

In Volume 23 (4) pages 6 thru 9, the Annual Report from Ted Meyers was printed. The overview of significant reports of fish pathogens during the year was part of that report, some people were confused so I just wanted to mention it. Also, there was an omission on page 8, first column, last paragraph. It should have read as follows, "Sea lice have not been a problem in Maine but outbreaks in nearby New Brunswick, Canada have caused the concern of netpen farmers. The ciliate, *Ichthyophthirus* sp. was responsible for extensive prespawning chinook salmon mortality in the lower Shuswap River and in sockeye salmon returning to the Fulton and Pinkut River spawning channels in British Columbia last fall. The sockeye were also stressed by warm water temperatures and concurrent infections by IHNV and *Aeromonas salmonicida*."

Correction: Andree and Hedrick, Vol 23 (4) page 2, Column 1, Paragraph 2, last line Should have read " This contrasts to only 65.7 and 73.1% similarity in comparison of *M. cerebralis* to the two other *Myxobolus* spp. described by Smother et al., 1994".

I am sorry for any confusion. I had a tremendous amount of trouble with the program preparing the last issue...especially spacing... I believe I have it under control now. Remember Bev and I are human and we make mistakes. If you find significant errors, please bring them to our attention and we will correct them. Again thanks for your support. Our addresses and numbers are listed on the back cover of this issue.*****

POSITION AVAILABLE

Aquatic Animal Medicine. The Department of Microbiology and Immunology, College of Veterinary Medicine seeks candidates for a tenure track position expected to be at the Assistant Professor level. The successful applicant will develop an independent competitively-funded research program focusing on fish diseases and will teach veterinary and graduate students. We are particularly interested in candidates with established expertise in molecular approaches to disease research. Applicants must have a DVM/PhD or a PhD and Post-doctoral experience. Applications including a curriculum vitae, outline of research interests and the names of three referees should be sent to Dr. Roger J. Avery, Department of Microbiology and Immunology, College of Veterinary Medicine, C5 173 Veterinary Medical Center, Cornell University, Ithaca, NY 14853-6401. Screening of candidates will begin immediately and will continue until the position is filled. Inquiries will be welcomed (Telephone 607-253-3400, e-mail rja5@cornell.edu). Cornell University is an equal opportunity/affirmative action employer.

*****MEETINGS*****

March, 1996

International Symposium and Workshop on Stocking and Introduction of Fish in Freshwater and Marine Ecosystems. *March 25-29.* University of Hull International Fisheries Institute, Hull, United Kingdom. Contact: Ian G. Cowx, University of Hull International Fisheries Institute, Hull HU6 7RX, UK; Phone: 01482-466-421; Fax: 01482-470129.

May, 1996

Forest-Fish Conference: Land Management Practices Affecting Aquatic Ecosystems. *May 1-4.* For more information contact: Kerry Brewin, Conference Steering Committee, c/o Trout Unlimited Canada, Box 6270, Station D, Calgary, AB CANADA, T2P 2C8; Phone: 403-221-8369; Fax: 403-221-8368.

International Association for Aquatic Animal Medicine. *May 11-15.* Comfort Hotel River Plaza, 407 Chestnut St. , Chattanooga, TN 37402 (615-756-5150). For general questions regarding the meeting contact: Jackson Andrews, Tennessee Aquarium, P.O. Box 11048, Chattanooga TN 37401-2048 USA. Phone : 423-785-4006, Fax : 423-267-3561, Email: JCA@tennis.org.

June, 1996

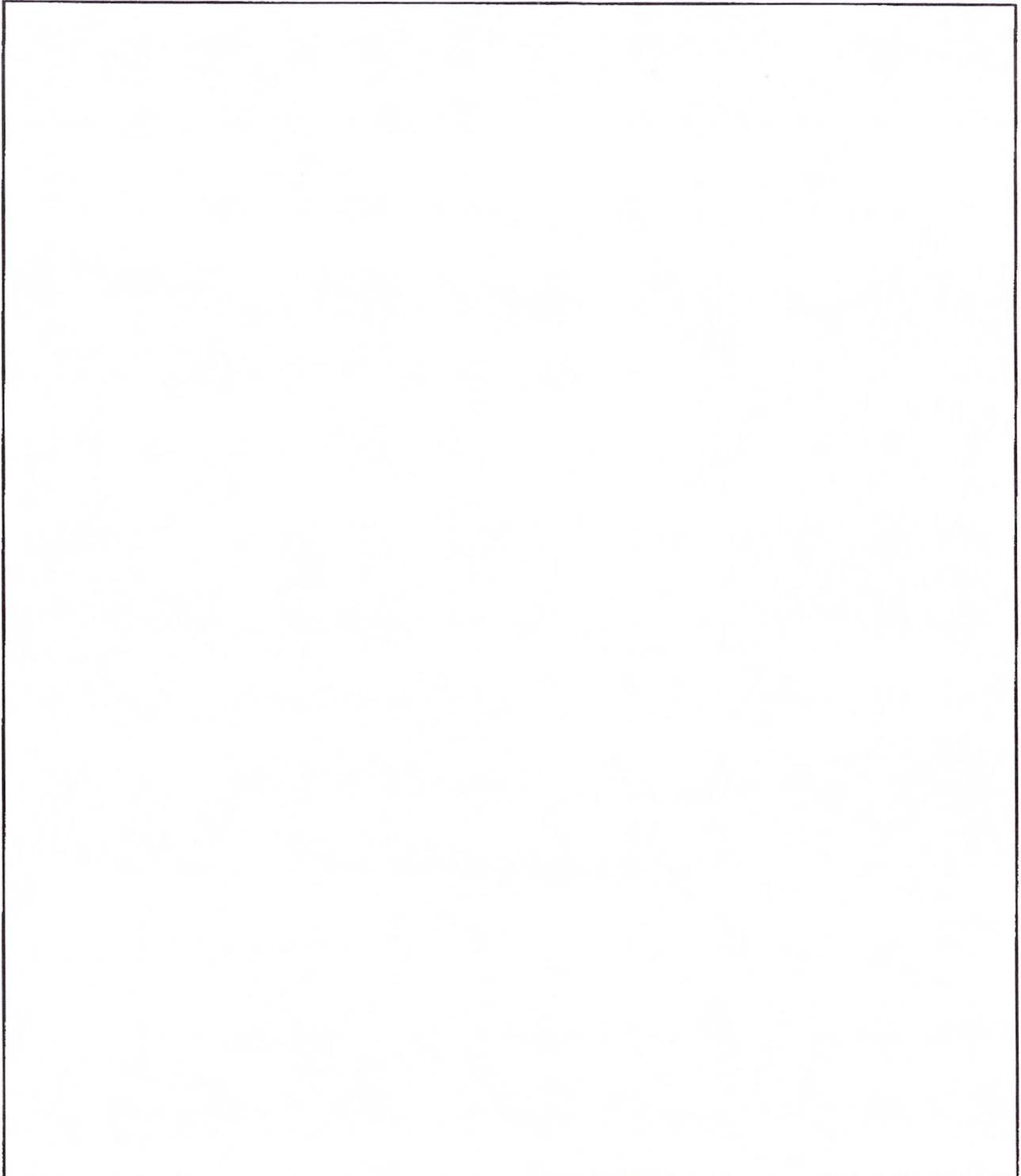
International Symposium on Fish Vaccinology. *June 5-7.* IABS Task Force on Vaccines. Contact: Veso, PO Box 8109, DEP 0032, Oslo, Norway. Fax: 47-2256-6254.

ABSTRACT

AMERICAN FISHERIES SOCIETY FISH HEALTH SECTION MEETING

MADISON, WI

AUGUST 7-9, 1996





Applications
for the 1996
S.F. Snieszko
Student
Travel
Awards
are now
being accepted!!

Application Instructions

The **S.F. Snieszko Student Travel Award** was established to **help** defray travel costs to the FHS annual meeting for students who are presenting papers. **The applicants must be members of the AFS/FHS.** It is possible that the awards committee will be able to grant more than one award; last year four awards were given.

To apply submit the following:

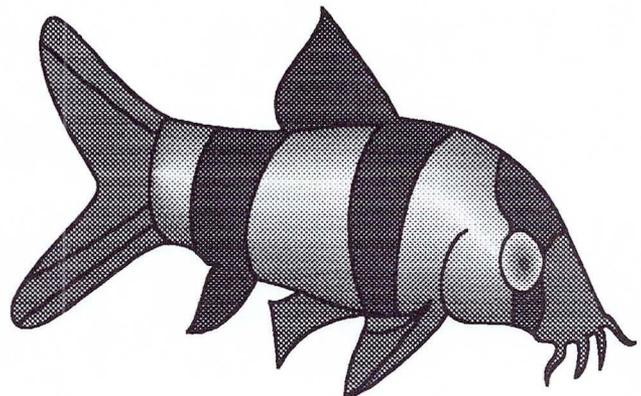
- 1) letter of application and statement of any special financial circumstances (i.e., not supported by a stipend, etc...)
- 2) curriculum vitae
- 3) three letters of recommendation
- 4) itemized budget including travel, meals, lodging and registration
- 5) copy of abstract of paper to be presented.

The applicants will be judged on quality of the abstract, significance of / interest in the research, academic achievement, professional achievement and financial need.

The deadline for receiving applications is May 15, 1996

Send all application materials to:
Dr. Larisa Ford
Awards Committee Chair, FHS
Department of Fish & Wildlife Resources
University of Idaho
Moscow, ID 83844-1136

Larisa is taking a position with the University of Idaho starting in March, 1996, but a mail box has already been established for her in the Department so please send your applications to her at the above address.



Diseases of Warmwater Fish Two Week Course

- Where:** Department of Fisheries and Aquatic Sciences, University of Florida, Gainesville, FL
- When:** June 3-14, 1996
- Sponsors:** University of Florida, College of Veterinary Medicine, Department of Fisheries and Aquatic Sciences, and the Whitney Marine Lab
- Topics:** Water quality and aquaria; fish necropsy procedures; bacterial, viral, fungal, parasitic, nutritional and environmental diseases of fish; treatment
- CEU's:** Participants may earn up to 20 hours of Continuing Education Units by attending this course.
- Contact:** Dr. Ruth Francis-Floyd, Department of Fisheries and Aquatic Sciences, University of Florida, 7922 NW 71st St., Gainesville, FL 32653.
Telephone: (904) 392-9617 ext. 229 Fax: (904) 846-1088.

Call for Nominations

The Awards Committee is soliciting nominations from the **Fish Health Section** membership for the **S.F. Snieszko Distinguished Service Award** and for **Special Achievement Awards**.



Individuals nominated for the **S.F. Snieszko Distinguished Service Award** must be nominated by a current member of FHS. The person making the nomination should obtain **six** letters of recommendation from fish health professionals that support the nominee's dedication to research, teaching and/or service to the field of fish health. The recommendation letters should be submitted with a **current curriculum vitae** for the nominee, and a **letter of nomination** to the awards committee. The S.F. Snieszko Distinguished Service Award is the **highest award** for the FHS, presented for the purpose of honoring individuals for outstanding accomplishments in the field of fish health. **Nominations will be accepted until February 1, 1996.**

The **Special Achievement Award** is presented to a FHS member who has made a significant accomplishment in the fish health field regarding a new discovery, diagnostic method, publication, etc...The achievement must meet high standards of science and survive peer review. Individuals to be considered for this award must be nominated by a current member of the FHS. The letter of nomination should clearly state: 1) the accomplishment; 2) the significance of the accomplishment to the field; 3) the implication of the accomplishment (local, regional, national, or worldwide). Copies of any articles or other documents relating to the work should be included. Nominations for this award should be made within one year of the accomplishment and may be submitted to the Chair of the Awards Committee at any time.

Please submit nominations for both awards to: Dr. Larisa Ford, Awards Committee Chair, NFHRL, NBS, 1700 Leetown Rd, Kearneysville, WV 25430.

FIRST CALL FOR PAPERS

This is the first call for papers for the American Fisheries Society Fish Health Section meeting to be held at the University of Wisconsin-Madison's Wisconsin Center on August 7-9, 1996. Co-sponsored by the Bureau of Fish Management, Wisconsin Department of Natural Resources and the La Crosse Fish Health Center, U.S. Fish and Wildlife Service.

ABSTRACT SUBMISSION INSTRUCTIONS

Please type the abstract on the attached abstract form, keeping all printed material within the "Box". Please note: (1) the title should be capitalized; (2) use superscript numbers, if necessary, to denote affiliation of authors; (3) place an asterick (*) following the author who will make the presentation; and (4) please use a high quality printer. You may also submit abstracts in the above format on computer disks utilizing wordperfect. (Please no faxed abstracts.)

Please complete the following form and submit it with your abstract by June 7, 1996 to:

La Crosse Fish Health Center
U.S. Fish and Wildlife Service
555 Lester Avenue
Onalaska, WI 54650



.....
I am submitting an abstract for the Fish Health Section Annual meeting.

Name _____

Address _____

Title _____ Telephone _____

I prefer that my presentation be: an oral presentation _____ a poster _____

Any questions regarding meeting program can be directed to Terry Ott, Becky Lasee or Richard Nelson at the La Crosse Fish Health Center, 608/783-8444 (Fax 608/783-8450). Information on registration will be in the spring FHS Newsletter.

Sample Abstract

American Fisheries Society-Fish Health Section Meeting
Madison WI
August 7-9, 1996

An Abstract form is included as an insert
in this edition of the newsletter

HOST-PARASITE RELATIONSHIPS BETWEEN BROOK TROUT *Salvelinus fontinalis* AND *SALMINCOLA EDWARDSII* (COPEPODA). Becky A. Lasec^{1*}, John D. Noble, Jr.² and Richard C. Nelson¹, ¹U.S. Fish and Wildlife Service, Fish Disease Control Center, La Crosse, WI 54602-1595 and ²Department of Army, Natural Resources Management Division, Fort McCoy, Sparta, WI 54656-5000.

Several streams located within the boundaries of the Fort McCoy military installation support healthy feral brook trout populations. Anglers at the fort reported that numerous brook trout were infected with "gill maggots." The purpose of this study was to

Sample

Rosette Agent NOT Detected in Winter-run Chinook Salmon of Sacramento River:

Correction to: FHS President's Annual Report - 1995, FHS/AFS Newsletter, October, 1995, Vol 23(4): page 8.

J. Scott Foott

U.S. Fish & Wildlife Service, CA-NV Fish Health Center, 24411 Coleman Hatchery Road, Anderson, CA 96007

Interagency concerns for management of the Endangered Sacramento River Winter-run chinook prompt me to correct President Meyer's statement that the Rosette agent has "produced severe systematic infections in 26% of winter run chinook returning to the Sacramento River in California". In 1995, the California-Nevada Fish Health Center conducted a survey of over 220 adult salmonids from the Upper Sacramento River for the parasite referred to as the Rosette agent (Harrell, L.W., Elston, R.A., Scott, T.M., Wilkenson, M.T. 1986 A significant new systemic disease of net-pen reared chinook salmon *Oncorhynchus tshawytscha*. Aquaculture 55: 249- 262). We have examined both histological specimens of various tissues and P.A.S.-stained kidney imprints from adult chinook comprising 3 different Sacramento River chinook stocks (Fall, Late-Fall, and Winter-run). The Rosette parasite was detected in 14 of 55 kidney imprints (26%) of adult **Late-Fall run** chinook as well as histological specimens from these fish. No obvious signs of infection were observed in these post-spawning adults. The parasite has not been detected in either Fall-run or Winter-run chinook adults sampled in 1994 or 1995.

President Meyer's statement that "Fish less than 18 months of age were apparently uninfected as were naturally exposed rainbow trout." may also mislead the reader. While the Rosette agent has **not been detected** in Captive Broodstock chinook younger than 18 months of age, it is likely that infection occurred at a much earlier stage. Our current method of diagnosis **must be considered to be quite insensitive**. I appreciate the President's effort in providing the membership with a overview of significant fish health issues and this opportunity to provide a correction.

Enterocytozoon salmonis in Chile

Sandra Bravo, Casilla 47, Puerto Montt, Chile

Enterocytozoon salmonis is an intracellular microsporidian that until now has been associated with losses among salmonids in North America (Hedrick et al., 1990). However in March of 1995, the parasite was detected in one stock of Atlantic salmon (*Salmo salar*) reared in a seawater netpen at a fish farm near Puerto Montt, Chile. The affected stock was introduced to Chile in December, 1993 as eggs from the United States. No unusual mortality or abnormalities suggestive of a pathogen were observed during rearing in freshwater. However, when this stock was transferred to seawater in November of 1994, mortality began increasing until August of 1995 when cumulative mortality approached 64%. This is higher than mortality reported for fish infected with this parasite in the United States and Canada.

Sick fish showed erratic swimming at the surface of the cages. The main external signs were dark body coloration, exophthalmus, hemorrhagic eyes and pale gills. Internally, the most obvious signs were severe swelling of the kidney and the spleen was enlarged in most cases. Smears of kidney, spleen and liver stained with Giemsa revealed spores of this microsporidian inside the white cells. The intracellular parasite was confirmed as *Enterocytozoon salmonis* by Dr. Ronald Hedrick of the University of California, Davis using PCR. Although there is information about the use of Fumagillin to control this parasite (Hedrick et al., 1991), this drug is not yet available in Chile so there is not an effective treatment for this chronic disease.

No behavior alterations or signs of the disease were observed in another stock of fish reared in neighboring cages. These fish remained healthy without the presence of this parasite indicating that horizontal transmission in seawater may be inefficient. This is the first report of this intracellular parasite in Chile. According to some information, it could have been present for some years in another stock of Atlantic salmon of the same origin because the progeny of this stock, that was transferred to seawater in June of 1995, showed the presence of this microsporidian; however, the level of mortality in this group of fish remained low (less than 1% per month), and the susceptibility of the fish appeared related to size and physiological condition because the disease appeared after smoltification.

Acknowledgements

I thank Dr. Ronald Hedrick for assistance with the identification of this microsporidian and Dr. Jim Winton of the Northwest Biological Sciences Center for the review of the manuscript.

References

- Hedrick, R.P., J.M. Groff, T.S. McDowell, M. Willis, and W.T. Cox. 1990. Hematopoietic intracellular microsporidian infections with features of leukemia in chinook salmon *Oncorhynchus tshawytscha*. Dis. Aquat. Org. 8: 189-197.
- Hedrick, R.P., J.M. Groff and D.A. Baxa. 1991. Experimental infection with *Enterocytozoon salmonis* Chilmonczyk, Cox, Hedrick (Microsporea): an intracellular microsporidium from chinook salmon *Oncorhynchus tshawytscha*. Dis. Aquat. Org. 10: 103-108.

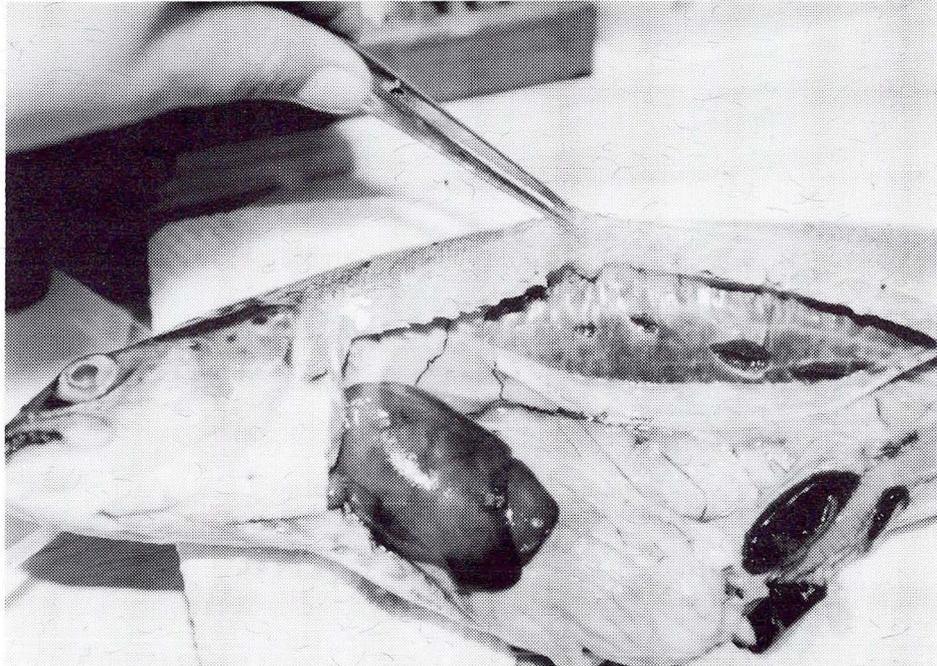


Figure 1. Fish showing the main clinical signs of disease caused by *Enterocytozoon salmonis*.

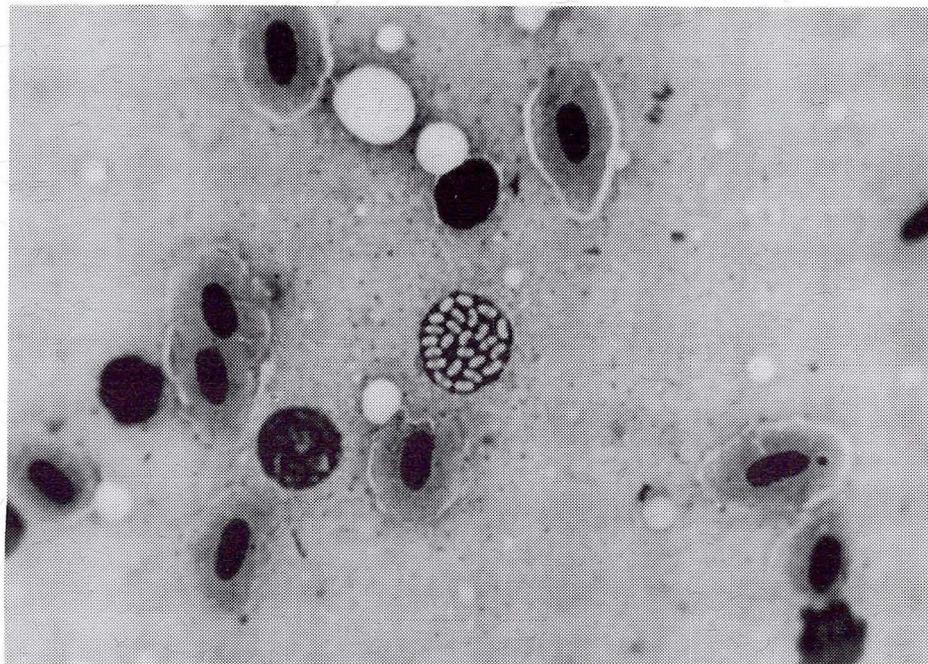


Figure 2. Spores of *Enterocytozoon salmonis* inside of a leukocyte.

Virus Isolated from Largemouth Bass

John A. Plumb¹, John M. Grizzle¹ and Scott Lamprecht²

¹Southeastern Cooperative Fish Disease Laboratory, Department of Fisheries and Allied Aquacultures, Auburn University, Alabama 36849, ²South Carolina Department of Natural Resources, Bonneau, South Carolina, 29431.

Except for lymphocystis virus and experimental virus infections, systemic viruses have not been found in any species of the family Centrarchidae. The aquareovirus 13p₂ was isolated from oysters and later shown to be infectious to bluegills by injection. Lymphocystis virus is common among centrarchids including largemouth bass, smallmouth bass, and bluegill, as well as fish in several other families, but this virus generally does not produce a viremia and seldom if ever kills infected fish.

About 1000 adult largemouth bass (2 kg and larger) died in Santee-Cooper Reservoir, South Carolina, between early August and late September 1995. Moribund fish had no external lesions; however, most of these fish had lost equilibrium, and floated at the surface probably because of an excessively inflated swim bladder. Two moribund largemouth bass, each weighing about 2 kg and measuring about 50 cm, were collected from the reservoir on August 30 (Fish 1) and on September 22, 1995 (Fish 2), placed on ice and shipped to Auburn University, Alabama, for necropsy. Upon necropsy, gills were still red but soft, eyes were clear, and skin and fins retained normal coloration. Each fish was examined for parasites, bacteriemia and viremia. Internally both fish appeared normal except for an inflamed ventral swim bladder. No parasites were found, and no bacteria were isolated on brain-heart infusion agar cultured aerobically at 30°C for 96 hours. Fathead minnow (FHM) cell cultures inoculated with 10⁻² filtered homogenates from the swim bladder of Fish 2, showed focal CPE 48 hours after inoculation. Initial CPE consisted of cellular pyknosis that progressed into rounded cells which contracted to the margins of cleared circular areas. Titration of filtered homogenate of swim bladder from Fish 1, which was refrigerated 4 days, indicated that this tissue contained approximately 10^{5.5} TCID₅₀/g. The culture fluid from the primary inoculated cells contained 10^{8.9} TCID₅₀/mL of culture fluid. It was concluded that a filterable agent, presumably a virus, was isolated from both largemouth bass.

Six largemouth bass were used for experimental transmission of the suspected virus. These fish averaged 405 g (307 to 490 g) in weight and 32.3 cm (30 to 34.5 cm) in total length. Two fish were injected intraperitoneally

with 0.2 mL of HBSS, or 0.2 mL of a solution containing 7.6 X 10⁵ or 7.6 X 10⁶ TCID₅₀/mL from the cell cultures showing CPE following primary inoculation from Fish 1. One fish from each treatment was virologically assayed at both 3 and 9 days following inoculation. The four largemouth bass injected with the filtered sell culture fluid showed no signs of disease, other than an inflamed lesion about 0.5 X 1.0 cm at the injection site on both fish assayed at day 9. However, a filterable agent was isolated from all fish injected with the suspected viral agent; no CPE occurred in cultures inoculated with filtered homogenates from control fish. Three days after injections, virus was isolated from liver, spleen, trunk kidney, ovary and swim bladder of fish injected with both concentrations of virus. After 9 days, virus titers in tissues of fish injected with 7.6 X 10⁶ TCID₅₀/mL ranged from 10^{4.5} TCID₅₀/g of liver and kidney tissue to 10^{6.5} TCID₅₀/g of spleen and ovary tissue. Tissues from the 7.6 X 10⁵ TCID₅₀/mL injected fish were frozen for 6 days, thawed and the 10⁻² dilutions of liver, trunk kidney, spleen, ovary and swim bladder yielded a CPE causing agent. Electron microscopy revealed icosahedral viral particles in large numbers in the cytoplasm of infected FHM cells. The unenveloped virion measured about 135 nm in diameter. The virus morphologically resembled an iridovirus, and preliminary nucleic acid analysis indicates DNA, but characterization of the virus has not been completed.

A virus was isolated from two largemouth bass from Santee-Cooper Reservoir. This is the first virus implicated in killing wild centrarchids. Isolation of virus from two largemouth bass collected 2 weeks apart from the same reservoir indicates that the virus was probably established in the largemouth bass population. Clearly, a viremia occurred in all fish that were injected with infected cell-culture fluid; however, there was little indication of clinical disease in the experimentally infected fish so the pathogenic effect of this agent is problematical. Our experimental transmission shows that the virus can be transmitted from cell culture by injection into smaller, naive individuals of the same species. We have tentatively named the virus "largemouth bass virus" (LMBV) pending further characterization.

IMPORTANT WORKSHOP MARK YOUR CALENDAR

Whirling Disease - Where do we go from here?

- *Historic Overview and Biology of Whirling Disease
- *Whirling Disease Distribution in the USA
- *Experiences with Whirling Disease
- *Existing Policies and Regulations
- *Whirling Disease Management Strategies
- *Needs and Future Direction

WHERE? Denver, Colorado
WHEN? February 6-8, 1996

PURPOSE:

To bring together concerned public resource agencies, coldwater angling organizations, interested members of the outdoor press, as well as interested individuals, to increase common understanding of the Whirling Disease problem and examine how best to address Whirling Disease, in a cooperative manner to ensure the long term protection, management, and use of public fishery resources.

SPONSORS:

Colorado Division of Wildlife, USFWS, Trout Unlimited, BLM, NBS, USFS, AFS, American Sportfishing Association, Colorado Aquaculture Association, Montana Fish Wildlife & Parks, Whirling Disease Foundation, Various state agencies.

CONTACT:

Eric Bergersen, Arrangements Chairman, immediately.
303-491-5396 or fax 491-1413. Attendance will be limited.
Registration fee is \$75 through 12/31/95. Late registration \$100.

The **J. Frances Allen Scholarship Committee** is accepting applications for its 1996 award. The qualified applicant must be a **female PhD** student who is an AFS member as of 31 December. Application procedures are very specific, so even though the deadline for the applications is not until **1 March 1996**, it's not too early to start getting your materials together. For complete application guidelines contact Susan Monseur at AFS headquarters, 5410 Grosvenor Lane, Bethesda, MD 20814.

Announcing the Fifth Biennial Fish Diagnosticians Workshop

The Fifth Biennial Fish Diagnosticians Workshop will be held at the College of Veterinary Medicine, Mississippi State University, Starkville, MS, on **March 13-14, 1996**. This will be an open forum to discuss applied aspects of aquatic animal health management. Focus will be on warm water aquaculture species. Discussion topics will include standardization of reporting, developments in new drug approval and current and developing diagnostic technology. For more information or topic suggestions contact **Larry Hanson** (601-325-1202; Email: hanson@acad.cvm.msstate.edu) or **S.W. Jack** (601-325-1311), College of Veterinary Medicine, P.O. Box 9825, Mississippi State MS 39762.

Canadian Society of Microbiology 46th Annual Meeting 16-20 June 1996

**University of Prince Edward Island,
Charlottetown, P.E.I.**

Tentative topics:

- *Pathogenic Mechanisms of *Aeromonas salmonicida*
- *Recent Developments in Microbial Products in Canada
- *The Marine Ecosystem
- * High Profile Bacterial Pathogens
- *Viruses of Agricultural and Economic Importance
- *Membrane Vesicles for Drug/Immunogen Delivery
- *Bioremediation

For more information contact:

Dr. F.S.B. Kibenge
Department of Pathology & Microbiology
UPEI, Atlantic Veterinary College
550 University Avenue
Charlottetown, PEI, C1A 4P3
902-566-0967, fax 902-566-0958

Fish Health Section Newsletter

The editors of the FHS Newsletter thank the members for their support regarding their enthusiasm in submitting contributions for publication in the newsletter. The **prohibitive** cost of mailing more than a 20 page newsletter; however, imposes limits for the length of each article so we are implementing **new guidelines** for authors. Articles should not exceed 4 single-spaced typed pages so that the maximum length would not exceed 6 newsletter columns. Also, please note that articles will continue to be accepted with the understanding that the material will be published without peer review. Articles should be submitted on disk in **Word perfect 5.1** or in **generic form that can be read on WP5.1**. Disks will be returned if a SASE is included with your submitted article. Again, thank you all very much for your continued support, which allows for the publication of a high quality and informative newsletter. The Fish Health Section Newsletter is a quarterly publication of the Fish Health Section of the American Fisheries Society. Submissions should be addressed to the editors listed below:

Dr. Beverly Dixon
 Department of Biological Sciences
 California State University at Hayward
 Hayward CA 94542
 510-885-3422 (phone)
 510-885-4747 (fax)
 E-mail BDIXON@DARWIN.SCI.CSUHAYWARD.EDU

Dr. Larisa Ford
 National Fish Health Research Laboratory
 1700 Leetown Road
 Kearneysville WV 25430
 304-725-8461 x225 (phone)
 304-725-7061 (fax)

Please note that Larisa's address will be changing in March. For items being submitted for the next issue (April) please send them to the address above. Her new address, phone, fax and Email numbers will be listed on the back of the April issue.

FHS/AFS Newsletter
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 1700 Leetown Road
 Kearneysville WV
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 Larisa Ford
 Natl Fish Health Res Lab
 1700 Leetown Rd
 Kearneysville, WV 25430