

# FISH HEALTH SECTION

# AFS

# NEWS LETTER



Volume 16, Number 1

January, 1988

## FROM THE PRESIDENT

All indications point toward excellent attendance for our international meeting in Vancouver in July. Session topics and co-chairs are now in the process of shaping their sessions by sending invitations to individuals they feel might not otherwise be able to attend without some formal request. This is particularly important for many of our colleagues outside of North America so that they can procure needed travel funds. The sessions will therefore be a mixture of the contributed and invited papers with all limited to 12 min with 3 min for questions. A poster session is also planned for those wishing to present their information in that format.

We are hoping to publish papers from the meetings in the first two issues (to be published concurrently) of the new North American Journal of Fish Health. There, however, will be no requirement for speakers to publish. As soon as we have final approval by the executive committee of AFS we will notify those planning to submit papers on the proper format for their preparation. We anticipate this will be similar to that presently used by other AFS journals such as Transactions of the American Fisheries Society.

During one evening of the meeting we will have our FHS annual business meeting to report on the activities of the Section over the last year. The executive committee will meet prior to this business meeting (probably as a working lunch).

Dr. John L. Fryer, professor and chairman of the Department of Microbiology, Oregon State University, who has been a key figure in fish health education and research in North America, will be our guest speaker at the banquet.

Trevor Evelyn has been dealing with the most difficult task of organizing all of the local details and all is on track for an excellent meeting in Vancouver. I would like to thank Trevor for his hard work and also Jim Winton (USFWS) for his help in getting the program organized.

As you might recall from an earlier newsletter, we have received a vote of confidence from the executive committee of the AFS for the concept of the new North American Journal of Fish Health. Unfortunately, I did not receive a final budget from managing editor Robert Kendall in time to present a complete proposal at the Annual AFS meeting in North Carolina. The excom supported the idea unanimously by vote and asked to see a final proposal with budget for the midterm meeting in Seattle in March. I have prepared that proposal and it has been sent to Stan Moberly (AFS president), Carl Sullivan (executive director) and Bob Kendall (managing editor) and have received their support and will be placed as an agenda item for the March meeting. Pending final approval in March, all systems will be ready so that we might publish the proceedings of our International Conference on Fish Health by the end of 1988.

Chairs of our standing and ad hoc committees might begin thinking in terms of having your reports prepared and in Dr. Vicki Blazer's (Secretary/Treasurer) possession two weeks prior to the start of the meeting in July. Last year we failed to get reports from all committee chairs.

Best Wishes,  
Ron Hedrick

## Tentative Agenda International Conference of Fish Health Vancouver, BC, Canada July 1988

Session Topics	Co-Chairs
Bacterial/Fungal Diseases	T. Evelyn, A. Munro, J. Plumb, R. Kusuda
Viral Diseases	J. Winton, W. Ahne, T. Sano, B. Hill
Protozoan Diseases	R. Hedrick, J. Lom, K. Molnar
Parasitic Diseases Caused by Metazoans	R. Heckman, L. Margolis, G. Hoffman
Nutritional/Environmental Diseases	V. Blazer, H. Ferguson, R. Richards
Neoplasms and Other Proliferative Disorders	D. Hinton, J. Harshbarger
Immune Responses	S. Kaattari, D. Anderson, M. Dorson
Stress	C. Shreck, G. Peters
Diseases of Molluscs/ Crustaceans	R. Elston, D. Lightner
Diagnosis/Detection Control	E. Shotts, T. Kimura G. Bullock, P. de Kinkelin

### Publication of Proceedings

It is our intention to publish the proceedings of the conference in the new North American Journal of Fish Health (NAJFH). Final approval for the journal is pending but we anticipate that we will have this by March of 1988. Participants will be notified at that time as to "instructions for manuscript preparation". The first two, or perhaps more, issues of the NAJFH will contain these reviewed manuscripts.



## FHS OFFICERS AND COMMITTEES 1987-88

### EXECUTIVE COMMITTEE

#### Voting Members

Ron Hedrick, Chair and President, FHS  
 Doug Anderson, President-Elect  
 Bill Rogers, Immediate-Past President  
 Vicki Blazer, Secretary-Treasurer  
 Charlie Smith, Chair, Nominating Committee

#### Non-Voting Members (Chairs of Standing Committees)

John Rohovec, Newsletter and Publications Committee  
 Ron Hedrick, Awards Committee  
 Randy MacMillan, Membership and Balloting Committee  
 John Schachte, Professional Standards Committee/  
 Board of Certification  
 Ron Goede, Technical Procedures Committee  
 Roger Herman, Archives Committee  
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 Craig Banner (2 years)  
 Marshall Bealeu (3 years)

#### Newsletter and Publications

John Rohovec, Chair  
 Jim Winton  
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 Doug Anderson  
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#### Technical Procedures

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 Kevin Amos  
 Dennis Anderson  
 Rod Horner  
 Jim Warren

#### Professional Standards/ Board of Certification

John Schachte, Chair  
 Marshall Bealeu (1 year)  
 Paul Bowser (1 year)  
 Joe Sullivan (2 years)  
 Drew Mitchell (2 years)  
 Ted Meyers (3 years)

#### Finance

Vicki Blazer, Chair  
 Randy MacMillan (Membership)  
 John Rohovec (Newsletter)

#### Awards

Ron Hedrick, Chair  
 Pete Bullock (2 years)  
 John Fryer (3 years)

#### Archives

Roger Herman, Chair  
 Margaret Ewing (2 years)  
 Tony Amandi (3 years)

#### Time and Place

Ron Thune, Chair  
 Paul Reno (2 years)  
 Ron Hedrick (3 years)

### AD HOC COMMITTEES

#### International Meeting

Trevor Evelyn, Chair  
 Kevin Amos  
 John Plumb  
 John Rohovec  
 Richard Heckmann

#### Program (1989 Meeting)

Paul Reno  
 Ron Goede

#### Pathogen Evaluation Criteria

Dennis Anderson, Chair  
 (To be named)

#### Procedures Evaluation

Emmett Shotts, Chair  
 John Hawke  
 Yolanda Brady  
 Phyllis Barney  
 Cliff Starlipper  
 Howard Jackson  
 Ron Hedrick  
 Diane Elliot  
 Robert Durborow

## Nominations For Snieszko Award Sought

Nominations for the S.F. Snieszko Distinguished Service Award are being solicited. This award is the highest given by the Fish Health Section and is presented for the purpose of honoring individuals for outstanding accomplishment in the field of fish health. Members of the Award Committee for 1988 are Ron Hedrick, chairman, Pete Bullock and John Fryer. Nominators should contact Ron Hedrick, Aquaculture and Fisheries Program, Department of Medicine, School of Veterinary Medicine, University of California, Davis, CA 95616, telephone (916) 752-3411.

## BENZOCAINE IS AN EFFECTIVE ANESTHETIC FOR SALMONID FISH

Philip A. Gilderhus  
 National Fisheries Research Center  
 P.O. Box 818  
 La Crosse, WI 54602-0818

Fish anesthetics are widely used to facilitate handling of fish for marking and tagging, and for artificial spawning procedures. At the present time, only one anesthetic, MS-222, is registered and legal for use on fish in the United States. Following its use, the fish must be confined for 21 days before they can be released or used for food. Use of MS-222 on fish such as Pacific salmon, that are killed during the spawning process, prevents use of the carcasses for human or animal food. Because of the restrictions on use of MS-222, there is an immediate need for an anesthetic that can be used with little or no withdrawal period. Benzocaine was identified as one of the best candidate anesthetics by Gilderhus and Marking (1987) so it was chosen for further study and development.

The anesthetic was tested in the laboratory under controlled temperatures in reconstituted waters of various alkalinities, hardnesses, and pH's. Tests were conducted with chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout (*Salmo gairdneri*) to define the concentrations that rendered the fish handleable in 3 minutes or less and that permitted recovery of swimming ability in about 10 minutes or less after 15 minutes of exposure.

Benzocaine effectively anesthetized both species under all conditions tested at concentrations of 25 to 45 mg/L. There was no difference between the species. Efficacy of the chemical was not affected by changes in water hardness, alkalinity, or pH. However, at higher temperatures, less benzocaine was required to produce anesthesia; 10 mg/L less benzocaine was required at 17°C than at 7°C. Testing of different size groups of rainbow trout showed that effective concentrations were about 10 mg/L lower for fish 5 cm long than for fish 47 cm long. There was less safety margin between effective (30 mg/L) and toxic (35 mg/L) concentrations at 17°C than at lower temperatures in 15-minute exposures. Fish survived much longer exposures to effective concentrations at 7° than at 17°C.

It appears that benzocaine is an effective anesthetic for salmonids. It works at lower concentrations than MS-222 but is slightly less effective in blocking all reflexes in the fish. The use of benzocaine requires that it first be dissolved in ethanol because the chemical is not directly soluble in water. One of the advantages to registering benzocaine as a fish anesthetic would be that little or no withdrawal period may be required.

Thus far, testing shows that benzocaine should be a good candidate for development and registration for fishery use. Because of its use in over-the-counter human drugs, benzocaine can probably be developed for fishery applications with less cost and fewer restrictions on its use than any of the other candidates. Benzocaine is not yet legal to use in production operations, but the Interregional Research Project No. 4 (IR-4 group) within the U.S. Department of Agriculture and the U.S. Food and Drug Administration have encouraged us to develop further data that might be used to support a registration of benzocaine for fishery use.

The National Fisheries Research Center soon will be seeking a limited number of field trial sites for on-site testing of benzocaine on Pacific salmon. These trials must be conducted under carefully controlled circumstances to make certain that the data will meet FDA requirements. As personnel time and resources permit, trial sites for additional species will be needed.

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FDA assigned INAD number 4890 on November 18, 1986 to the USFWS submittal on investigational uses of benzocaine as an anesthetic in trout and bluegill. Studies currently being conducted include toxicity to fish and efficacy tests. Benzocaine is being considered because there is need for anesthetic that has a zero withdrawal time, especially for the spawning of Pacific salmon. The only registered anesthetic, MS-222, has a 21-day withdrawal time. Rosalie A. Schnick, Technical Information Specialist, National Fisheries Research Center-La Cross, La Cross, Wisconsin.



## STUDIES ON THE ROLE OF TUBIFICID WORMS AND ACTINOSPORA IN THE LIFE CYCLE OF *MYXOBOLUS COTTI* AND *MYXOBOLUS CEREBRALIS*

M. El-Matbouli, R. Hoffman  
 Institut für Zoologie und Hydrobiologie  
 der Universität München  
 Kaulbachstrasse 37, 8000 München 22, FRG

Recently Wolf and Markiw, 1984 reported that *Myxobolus cerebralis* not only needs an intermediate host (tubificid), but that it transforms in this host to an actinosporean (*Triactinomyxon*) which only is infective for the trout.

To study the life-cycle of *Myxobolus cotti* in bullhead (*Cottus gobio*) and *M. cerebralis* in rainbow trout, we performed the following experiments.

- Fresh spores of *M. cotti* and *M. cerebralis* as well as the spores, which are ripened for 5 months were never infectious for bullhead and rainbow trout, respectively.
- M. cotti* and *M. cerebralis* spores (fresh or after 5 months in mud) are taken in by tubifex worms.

A development of these spores in the gut epithelium cells to actinosporea of the genus *Triactinomyxon* could be demonstrated in squash preparations and histological slides.

- Triactinomyxon* spores get to the water either by the digestive canal or after the death of the worms.
- There are two possible ways for the infection of fish: either via the water or by feeding of infected tubificids.
  - For *M. cotti* in bullhead the way of infection through water could be proven.
  - For *M. cerebralis* in rainbow trout both ways could be demonstrated.

The results of our experiments demonstrate the life cycle of *M. cotti* and confirm the hypothesis of Wolf and Markiw, 1984. In both *Myxobolus* species, actinosporean and myxosporean have been shown to be alternate life stages of a single organism.



### MYXOBOLUS CEREBRALIS IN WASHINGTON STATE

Steve Roberts, Fish Pathologist  
 Washington Department of Wildlife  
 580 Nelson Place  
 East Wenatchee, WA 98801

Last June a list of *M. cerebralis* inspections that were conducted by federal and state fish pathologists from 1970 to present in Washington was compiled. The fish examined numbered more than 15,000 and represented brook, cutthroat, chinook, coho, rainbow, and steelhead. The fish were primarily from federal and state hatcheries and represented 52 sites in Washington.

Additional hatchery and wild fish from a number of sites in southeastern Washington have been examined and to date we have not confirmed any *M. cerebralis* in any of the fish. A sample of wild steelhead from Cottonwood Creek, a tributary of the Grande Ronde River, were found to have spores of similar morphology as *M. cerebralis* but histopathology did not confirm the presence of the spores in the bone or cartilage.

The USFWS and WDF fish pathologists have also examined fish without any positive findings in Washington State.

## WHIRLING DISEASE IDENTIFIED IN COLORADO

Phyllis Barney and Dennis Anderson  
 US Fish and Wildlife Service

Peter Walker  
 Colorado Division of Wildlife

Fish Disease Control Center  
 PO Box 917  
 Fort Morgan, CO 80701

*Myxobolus (Myxosoma) cerebralis* has been confirmed at four facilities in Colorado. The first presumptive identification occurred on November 25, 1987 from samples from one private and one state fish hatchery located side by side on the Arkansas River. It was identified at a second private hatchery in the Rio Grande River drainage on December 3, 1987. Fish from a private, tourist fish-out facility on the South Platte River were examined on December 7, 1987 and found positive for spores. Fish at all four locations showed whirling behavior. All four identifications have been confirmed by histological examination.

This is the first identification of this organism in Colorado and in our federal Region 6. The Colorado Division of Wildlife is in the process of reviewing all the shipment records from the affected hatcheries to try and determine both the source of the infection and where the disease may have been dispersed. They will be surveying the hatcheries and impoundments in the state over the next six months to investigate how far the disease has spread. Our inspection services are being expanded region-wide to include more intensive *M. cerebralis* testing.



### DETECTION OF MYXOBOLUS (MYXOSOMA) CEREBRALIS IN ADULT SALMONIDS

H.V. Lorz and J.S. Rohovec  
 Department of Microbiology  
 Oregon State University  
 Corvallis, OR 97331

The published procedures (Fish Health Section Blue Book 1985) for the diagnosis of whirling disease are designed for the detection of *Myxobolus (Myxosoma) cerebralis* spores from tissues of fish that are less than one year old. These methods have also been used to examine adult salmonids for *M. cerebralis*, but they are labor and time intensive when the tissue mass of a single fish's head is more than that of a 60 juvenile fish sample.

Recently, *M. cerebralis* was detected in salmonid populations in northeastern Oregon (Fish Health Section Newsletter 15(1) 1987) and in certain regions of Washington and Idaho. In an effort to facilitate epidemiological studies which involved the examination of returning adult salmon and steelhead, we compared different methods for detection of *M. cerebralis* in these fish. A group of 40 adult chinook salmon (*Oncorhynchus tshawytscha*) were examined. One lot of 20 fish was examined by grinding the entire skull of each animal and then processing using the enzyme digest method. From the other 20 fish, a core sample from the skull of the fish was taken for examination. The sample was obtained using a 19mm dia. cork borer which was inserted into the head dorsal and perpendicular to the long axis of the fish approximately 10 mm behind the eye. This procedure insured that both otolith areas were included in the sample. The cores were then individually processed using the enzyme digest method. Upon microscopic examination of the resulting pellets, *M. cerebralis* spores were detected in 2 of 20 skull samples and in 6 of 20 core samples. Although the sample size used in this test was small, these data indicate that subsamples of adult salmon heads may be appropriate for detection of *M. cerebralis* in large fish. Not only could the technique be used in epidemiological studies, but may also be useful in examining fish for compliance of laws regulating international trade in salmonid fish.



## ENHANCEMENT OF PLAQUE ASSAY FOR DETECTION OF FISH VIRUSES BY PRETREATMENT OF CELLS WITH POLYETHYLENE GLYCOL

W.N. Batts and J.R. Winton  
U.S. Fish and Wildlife Service  
National Fisheries Research Center  
Bldg 204, Naval Station  
Seattle, WA 98115

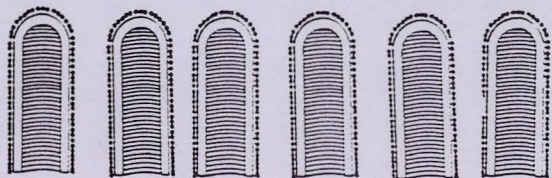
In an effort to maximize the ability to detect very low levels of infectious hematopoietic necrosis virus (IHNV) in samples of tissues, sex fluids and natural water supplies, we examined methods for enhancing the sensitivity of the plaque assay system. Pretreatment of cell monolayers with polyethylene glycol (PEG) enhanced sensitivity, increased mean plaque diameter, and allowed recovery of virus from samples of mean or ovarian fluid that were below previous detection limits.

Initial experiments used IHNV and the epithelioma papulosum cyprini (EPC) cell line to optimize the effect of PEG pretreatment. Several concentrations (4, 7, and 10%) of 6000, 8000 and 20,000 molecular weight (m.w.) PEG were compared. When EPC cells were pretreated with PEG, the largest m.w. and highest concentrations gave the best enhancement. Pretreatment of the cells for 1, 15 or 30 min did not affect the results. Because the enhancement from addition of 7% PEG was similar to that at 10%, we used 7% (w/v) 20,000 m.w. PEG (Fisher Scientific) in Eagle's minimum essential medium containing Tris (MEM-Tris) for further experimental work. The solution was added to the cell sheet at a rate of approximately 10ul/cm<sup>2</sup> and allowed to remain for 30 min while virus dilutions were prepared. The plaque assay was performed without removing the PEG and virus was allowed to adsorb for 30 min at room temperature before the cells were overlaid with methylcellulose (0.75% in MEM containing 5% fetal bovine serum).

We used PEG-treated and untreated monolayers of the chinook salmon embryo cell line (CHSE-214) to determine the plaque assay titers (p.f.u./ml) for selected fish viruses. A significant increase in p.f.u./ml was observed for IHNV (10.9X), viral hemorrhagic septicemia virus (7.8X) and chum salmon virus (90.0X). A slight increase (2.6X) was noted for the chinook salmon paramyxovirus, and infectious pancreatic necrosis virus (gum tragacanth overlay; 1.2X) while a slight decrease (0.9X) was observed for *Oncorhynchus masou* virus.

We compared the enhancement of IHNV plaque assay titers on PEG-treated and untreated monolayers of five fish cell lines. An increase in p.f.u./ml was recorded on the EPC (10.4X), CHSE-214 (3.9X), fathead minnow (FHM; 17.2X), and bluegill fry (BF-2; 7.7X) cell lines. The net effect of the increase was to enhance the titers of the cell lines that were less efficient at detecting the virus so that following treatment with PEG, the cell lines gave more similar titers. The rainbow trout gonad (RTG-2) cells did not form IHNV plaques under methylcellulose. Another effect of PEG pretreatment was to increase mean plaque diameter. On monolayer cultures of EPC cells incubated at 15°C, the mean diameter of 50 IHNV plaques on PEG-treated cell sheets was larger at 6 days than on untreated cells at 8 days. This means that the incubation period for IHNV plaque assay may be shortened.

In field experiments, PEG-pretreatment of EPC monolayers showed a consistent enhancement of IHNV titers in water samples and in samples of ovarian fluid from salmonid fish. The increase typically ranged from 3-10 fold. This allowed the detection of IHNV in certain samples where standard assays could not detect the virus. For example, of 51 spawning spring chinook at the Leavenworth National Fish Hatchery, IHNV was detected in the ovarian fluid of 11 fish plaqued on untreated EPC monolayers while 17 were positive on PEG-treated cells.



## IHN MORTALITY OF KOKANEE IN CAMERON LAKE, BRITISH COLUMBIA

G.S. Traxler  
Department of Fisheries and Oceans  
Biological Sciences Branch  
Nanaimo, B.C., Canada V9R 5K6

Mortality of 2-year-old kokanee (a non-anadromous form of *Oncorhynchus nerka*) occurred in Cameron Lake, Vancouver Island, British Columbia during July 1987. The losses were due to infectious hematopoietic necrosis (IHN). Losses were numerous enough so that no difficulty was experienced in collecting freshly dead specimens on the lakeshore.

Factors contributing to the outbreak are unknown. Cameron Lake is inaccessible to anadromous fish, being isolated from the ocean by impassable falls. Surface water temperatures measured 17.5°C during the die-off.

Gills and kidney samples were examined individually by titration. Of the 42 fish processed, 40 were positive for IHNV virus, all 40 being positive in gills and 38 in the kidneys. The virus titers of the kidneys ranged from  $1.9 \times 10^4$  -  $1.0 \times 10^8$  PFU/g of tissue (mean  $6.9 \times 10^7$ ). Viral titers in the gills ranged from  $1.2 \times 10^3$  -  $4.7 \times 10^7$  PFU/g (mean  $2.8 \times 10^6$ ). Virus titers in the gills were generally 2 logs less than those in the kidney of the same fish. Despite this, the high prevalence of virus in the gills suggests that horizontal transmission may have accounted for the spread of the disease. The lower titers found in the gills may reflect the fact that the proportion of tissue able to produce virus in the gills is less than that in the kidneys.

This is the third documented case of IHNV in feral populations of 2-year-old kokanee in British Columbia. Once again the observation of IHNV in 2-year-old fish is surprising because generally older fish are refractory.

## INTRODUCTION OF CILIATES FROM NORTH AMERICA TO EUROPEAN PART OF USSR

I.D. Trombitsky and A.J. Moshu  
Moldavian Fisheries Research Station  
Kishinev, USSR 277043

During the early 1970s, *Ictalurus punctatus* was introduced from North America into the USSR. Concurrent with the piscine introduction was noted a ciliate protozoan infestation due to *Ambiphyra ameiri*. The host range subsequently has been extended from *I. punctatus* to common carp, *Cyprinus carpio*, silver carp, *Hypophthalmichthys molitrix*, grass carp, *Ctenopharyngodon idella*, and bigmouth buffalo, *Ictiobus cyprinellus* in fish ponds of the USSR. The infestation is especially prominent in larvae and fry with numbers greater than 500 per fish. The ciliate is probably involved in the destruction of fin folds. Specimens of *A. ameiri* were compared from fishes from North America (channel catfish) and those from the Moldavian region of the USSR. Ciliates from Moldavian fishes were significantly larger than those from North America. Specimens from the various fish hosts in Moldavia for *A. ameiri* showed no difference in size. *Ambiphyra* infection was correlated with increased water temperature and organic pollution of the pond water. Common with *A. ameiri* infestations was the presence of *Trichodina acuta* and *Hemiphyra branchiarum*.



Researchers at the Fishery Research Branch (FRB), FDA, have been examining the bioavailability, tissue distribution and clearance of selected antimicrobials and other xenobiotics in channel catfish. In an upcoming issue of the journal *Xenobiotica*, S.M. Plakas and coworkers report the very low bioavailability (less than 5%) of tetracycline in this fish. Low bioavailability appears typical for this class of drugs in other fishes also, as demonstrated by J.L. Grondel and coworkers in The Netherlands. More recently completed studies at FRB by S.M. Plakas, R.W. Dickey and A.M. Guarino have examined the disposition of the antimicrobial, ormetoprim, in catfish. The bioavailability was found to be considerably higher (approximately 50%) while the tissue clearance was generally more rapid compared with tetracycline when administered at the same dosage. Steven M. Plakas, Fishery Research Branch, FDA, Dauphin Island, AL 36528.



**SECOND INTERNATIONAL SYMPOSIUM  
OF ICHTHYOPARASITOLOGY  
"Actual Problems in Fish Parasitology"**

**Sept. 27 to Oct. 3, 1987: Tihany, Hungary**

Richard Heckmann  
Department of Zoology  
Brigham Young University  
Provo, UT 84602

There were 200 plus people representing 31 countries in attendance at the 2nd International Symposium of Ichthyoparasitology. The Symposium was held at the Balatan Limnological Research Institute at Tihany, Hungary. The majority in attendance were from eastern European countries and Russia. I was the only representative from the United States. There were 93 papers presented and 18 posters displayed pertaining to fish parasite research throughout the world. Also, there were 1/2 day workshops on the Monogeneans, Myxosporidians and Markiw's life cycle studies for *Myxosoma cerebralis* (abstract included in this issue of the Fish Health Newsletter). O.H. Bauer reported on the publication of volume 3 of "Parasites of Fishes in Russia" which concludes the series. He asked for volunteers to translate and publish the 3 volumes in English. Visits were coordinated to both eel and trout farms in the immediate region of Tihany. I have abstracts of papers presented. The next symposium is scheduled for Leningrad, Russia in four years. Fliers for the FHS International Fish Health Conference, Vancouver, July 19-21 were distributed to participants of the Ichthyoparasitology Symposium.

**DNA POLYMERASE ACTIVITY IN PROLIFERATIVE  
CUTANEOUS LESIONS OF THE WALLEYE  
(*Stizostedion vitreum*)**

D. Martineau\*, P. Bowser\* and J. Williams\*\*  
\*Department of Avian and Aquatic Animal Medicine  
\*\*Department of Microbiology  
NYSCVM, Cornell University  
Ithaca, NY 14853

Four proliferative lesions of the walleye skin have been described in North America. The first one affects the dermis and consists of single or multiple and coalescent nodules composed of fibroblast-like cells separated by varying amounts of collagen fibers or bone and displaying a wide range of anaplastic changes. Although these nodules do not invade the surrounding tissue nor do they metastasize, they have been named dermal sarcoma. By contrast, a second type of cutaneous proliferation is hyperplastic and affects only the epidermis. Accordingly, it has been named (discrete) epidermal hyperplasia. Two particles, morphologically consistent with retroviruses and distinct by their size and subtle morphological features, have been observed by electron microscopy in these two lesions.

The two other proliferative processes, the well known lymphocystis (in fact a cellular hypertrophy) and the diffuse epidermal hyperplasia have been associated with an iridovirus and a herpesvirus, respectively.

So far, attempts to isolate retroviruses or to demonstrate viral reverse transcriptase activity in the first two lesions have been unsuccessful. Since November 1987, we have been able to demonstrate polymerase activity in the so-called dermal sarcoma in 6 fishes and in discrete epidermal hyperplasia occurring simultaneously in one fish using an RNA template and a DNA primer. The reaction was strongly manganese dependent and was most active at 25°C. No polymerase activity could be detected in normal skin of these animals. No virus could be seen by electron microscopic examination of the lesions.

We are currently investigating polymerase activity of different fractions of sucrose gradients in which resuspended pellets obtained from the lesions are layered. We also intend to assay for the preference of the reaction for the different template-primer combinations most typical of viral reverse transcriptase activity.

Should all characteristics of a reverse transcriptase activity be demonstrated, the walleye would become the second fish in which reverse transcriptase is present, the first one being the northern pike (*Esox lucius*) affected by lymphosarcoma.

**AN EARLY IN VITRO SAMPLING METHOD  
FOR FISH IMMUNIZATION PROGRAMS**

Doug Anderson and Ora Dixon  
U.S. Fish and Wildlife Service  
National Fish Health Research Laboratory  
Box 700  
Kearneysville, WV 25430

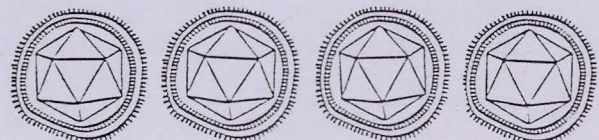
A new method of excising the spleen from fish the day after immunization and placing a section or the whole organ into in vitro culture allows early analysis of the efficacy of administering a bacterin. Ongoing research at our laboratory now shows that if rainbow trout are given an injection of the standardized *Yersinia ruckeri* O-antigen, the spleen can be excised and placed in tissue culture medium (Eagle's minimum essential medium supplemented with 2% normal calf serum), held for 9 days at 14°C and then sampled for numbers of specific antibody-producing cells.

The early in vitro method is an important addition to the analysis of field immunization programs, because biologists are often hampered now by the difficulty of knowing of and following the animal's acquisition of protection against the disease after immunization. At the fish hatchery or aquaculture facility, there is rarely enough time or trained personnel available to hold immunized and nonimmunized, control fish stocks to test for the efficacy of the bacterin. Protection tests that involve challenging the fish with live pathogens give erratic results given species, strain, size, and environmental differences. In addition, releasing the pathogens necessary for challenge outside of a carefully controlled aquaria is not recommended.

Another feature of the early in vitro method, is that the excised spleen sample can be placed in the culture medium and transported or mailed to a laboratory where trained technicians can perform the analysis for counting antibody-producing cells. This assay is presently done by the passive hemolytic plaque assay (Jerne assay), which uses labeled sheep red blood cells, species-specific complement, and an agar base to detect the lymphocytes producing antibody. While the present assay is a complex, but very sensitive technique, it can be greatly simplified for routine use, and even other assays such as ELISA or passive hemagglutination could theoretically be used for detecting the release of antibody in vitro. Presently, the in vitro spleen sections are sampled 10 days after the immunization of the trout; under certain conditions, this time may be shortened by holding the samples at warmer incubation temperatures or greatly lengthened by using cooler temperatures, thus allowing the assays to be done at a most convenient time for technicians.

The in vitro assays can also be applied to trout immunized by bath, shower, or flush exposures; however, at this time we fail to detect the antibody-producing cells unless the fish are held 5 days after immunization before the spleen is placed into in vitro culture. Evidently, by the contact exposures of administering the bacterin such as with a 2-minute dip in the *Y. ruckeri* bacterin, the immunogens are taken up by the gill pavement cells and other epidermal surfaces, transferred to the circulating monocytes for transportation, and deposited and processed in the melano-macrophage centers of the spleen and anterior kidney. The time, antigen load and regulation of these steps in the afferent immune pathway is not well understood, but these variables probably influence the reasons for not detecting earlier in vitro antibody-producing cells when the bacterin is given by bath.

The shortening of the time between immunization of the fish and excising the spleen will greatly aid the early in vitro methods. In some cases, we are considering immunizing the fish during the transportation in truck tanks to minimize multiple handling stress on the fish. An opportune time to excise spleens for the in vitro assays would be just before releasing the fish from the truck into other holding areas or into the wild environment.





## HISTORY OF COLDWATER DISEASE IN NEW YORK HATCHERIES FROM 1963 to 1987

John H. Schachte, Jr.  
NYS Department of Environmental Conservation  
Fish Disease Control Unit  
Rome, NY 13440

Coldwater disease (CWD) has apparently been a problem in lake trout in New York since approximately 1963. At that time, an epizootic of a disease caused by a "myxobacterium" was observed in the Adirondack strains of lake trout in the Fortsville State Fish Hatchery near Glens Falls, New York. No positive identification was made at that time. Again in 1970 and 1973 similar outbreaks were recorded in lake trout at the same facility. Some doubt exists as to positive identifications, but records indicate a "columnaris-like" infection. In 1977 a major epizootic occurred and we were successful in isolating typical CWD colonies on cytophaga agar from peduncle lesions. Shortly thereafter sample cultures were sent to Dr. Pete Bullock at the National Fish Health Research Laboratory at Leetown, West Virginia. He verified that the isolate was *Cytophaga psychrophila*. Through all of these outbreaks no effective means of therapy could be found. Lacking any further information as to the source of the disease, it was decided to move the rearing of the Adirondack strains of lake trout to the Chateaugay State Fish Hatchery. This occurred in the fall of 1978 and no problems were encountered that year. In January of 1980 an epizootic occurred at this hatchery. It should be pointed out that intermittently, throughout all of these epizootics, Finger Lake strains of lake trout from the Bath State Fish Hatchery were present at the affected hatcheries. The Chateaugay epizootic did respond to some extent to antibiotic and chemotherapy. The disease occurred again in 1981 at Chateaugay but expanded its range to the Bath State Fish Hatchery following the transfer of adult lake trout from the Allegheny National Fish Hatchery. We were unable to establish any concrete relationship between these adults and the infected fish at the hatchery. However, small fingerling lake trout in the Bath Hatchery building on a closed water supply were not infected in spite of the presence of the disease in the same year class in outside concrete ponds. We have been successful in our laboratory in transmitting the disease from infected yearling lake trout to '82 year class fingerlings by simple exposure. We have continued to isolate organisms with both colonial and cellular morphology that is consistent with *C. psychrophila*. Using the Leetown antisera against this organism, we have obtained positive indirect fluorescent antibody (IFAT) results against both isolates and material removed from lesions of clinically infected fish. However, unlike early descriptions of the disease, we have not demonstrated the presence of a systemic infection either by bacterial isolations or histological sections of both H and E or Giemsa stained material.

A major concern from a fish health management point of view is the source of the disease and its mode of transmission. An informal collaborative effort between the Fish Disease Control Unit and Dr. Rocco Cipriano of the USFWS at Leetown demonstrated the presence of circulating humoral antibodies in adult spawning Seneca Lake lake trout in the fall of 1983. Since lake trout are at least five to seven years old before they spawn, the presence of these antibodies indicated exposure in the lake versus the hatchery. Antibody titers acquired from hatchery exposure would not persist for this length of time in the absence of a booster exposure to the organism. In 1985, we collaborated with staff at the Wellsboro, PA USFWS laboratory to demonstrate

the presence of the same yellow pigmented *Cytophaga* organism inside of eyed lake trout eggs from Lake Ontario fish. Subsequently, lake trout eggs held at Rome from Cayuga Lake also showed infection with the same organism.

During the fall spawning operation on Seneca Lake in September 1987, the Fish Disease Control Unit sampled pooled seminal fluid representing 30-50 male fish. We have isolated yellow pigmented cytochrome oxidase positive, gram negative rods. These isolates have reacted positively with the Leetown anti-*C. psychrophila* serum (#331). Consequently, there is strong circumstantial evidence to support vertical transmission of the disease. It is interesting to note however, that there have been no epizootics of this disease at the Allegheny National Fish Hatchery which has lake trout from Lake Ontario. Additionally, the preceding evidence is further in doubt because the members of the genus *Cytophaga* are considered to be ubiquitous soil and water inhabitants. If in fact the disease is transmitted vertically, iodophor disinfection preceded by water hardening in erythromycin phosphate has had no demonstrable effect on the disease.

In 1987, the '87 year class of lake trout has been plagued by this disease at three different hatcheries: Bath, Chateaugay and Caledonia. Rome SFH is the only facility holding lake trout that has remained free of the disease. It is interesting to note at this point, that of the four hatcheries only Rome is unaffected by either chronic or seasonal nitrogen gas supersaturation. Is this a critical environmental parameter in the development of the disease? At present we do not know. In the three affected facilities, losses to CWD have been considerable. The most severe was at Chateaugay where approximately 85-90% of some 80,000 fingerlings were lost during a two-month period. As stated previously, no chemical or antimicrobial therapy was effective. We did, however, treat experimentally 15,000-20,000 lake trout with oxolinic acid at 50mg/l for 60 sec in a tank truck dip treatment. Losses were reduced from 700-800+ per day to less than 10 fish/day three days post treatment. It appears that this compound may hold some promise as a feed additive to treat the disease. The 15,000 survivors at Chateaugay were subsequently transferred to another facility, and ponds disinfected to protect the Adirondack strain of lake trout resident at Chateaugay.

It is apparent from the foregoing summary of our experiences, that we are dealing with a complex infectious disease process attributed to an organism that causes a disease of salmon on the West Coast but bears little, if any, resemblance to that disease as it occurs in lake trout. It appears to be very host-specific, does not respond readily to therapy, and the clinical course of the disease, lesions, etc. are quite different from classical coldwater disease. Additionally, from our contact with staff at the Iron River NFH, Wisconsin, and in light of their use of Seneca Lake lake trout sex products, is there any relationship between their severe losses of lake trout and our problems in New York hatcheries? Questions concerning the characterization of the pathogen, its mode of transmission, therapy and relationship to other serious lake trout problems in other Great Lakes Basin hatcheries need to be answered.

### PASSAGES

Jim Nelson has returned to the U.S. and taken a job with BioMed in Seattle. His new address and telephone number are: BioMed Research Laboratories, 1720 - 130th Ave. N.E., Bellevue, WA 98005-2203, telephone (206) 882-0448.

\* \* \* \* \*

Pat Chapman is now working as a fish pathologist for the Washington Department of Fisheries, 115 General Administration Building, Olympia, WA 98504, telephone (206) 753-6640.

\* \* \* \* \*

Dr. Jan M. Spitsbergen has accepted a position as an Assistant Professor with the College of Veterinary Medicine at Cornell University, Ithaca, New York 4853. Her new phone number will be (607) 253-3365.

Scott Foott has moved from California to take a job with the Idaho Dept. of Fish and Game as a fish pathologist. He may be reached at: Rt 1 Trout Road, Eagle Fish Health Laboratory, Eagle, ID 83616, phone (208) 939-2413.

\* \* \* \* \*

Bruce Stewart has returned to the Northwest and is a fish pathologist for the Northwest Indian Fisheries Commission. His address is: 6730 Martin Way East, Olympia, WA, telephone (206) 438-1180.

\* \* \* \* \*



## POSITION ANNOUNCEMENTS

**Position:** Aquatic Animal Health Scientist

**Location:** College of Veterinary Medicine, Mississippi State University

**Responsibilities:** Participation in the College research program with emphasis on the interaction of water quality with animal health in warm water aquatic species (catfish). Teaching responsibilities would involve didactic and laboratory training for professional and graduate students.

**Qualifications:** D.V.M. and/or Ph.D. degrees.

**Salary & Rank:** Dependent on qualifications and experience.

**Application Procedures:** Applications will be accepted until March 15, 1988, or until a suitable candidate is found. Qualified applicants are invited to submit a letter of application, a current curriculum vitae and/or academic transcripts, and names of three references to:

Dr. J.V. Kitzman, Chairman  
Department of Basic & Applied Sciences  
College of Veterinary Medicine  
Mississippi State University  
P.O. Drawer V  
Mississippi State, MS 39762

Mississippi State University is an Equal Opportunity, Affirmative Action Employer.

**Position:** Director and Research Scientist, Mountain Aquaculture Center of Western Carolina University. The newly created Mountain Aquaculture Center is seeking applications for the two positions of Director and Research Scientist. Western Carolina University is one of the sixteen constituent institutions of the University of North Carolina and is located in the southern Appalachians, twenty miles from the Great Smoky Mountains National Park. Western Carolina University has been designated the center for trout aquaculture in North Carolina. The Center, through research and education in trout aquaculture, is committed to furthering the development of the North Carolina trout industry and to making a major contribution to economic development in the region.

**Qualifications:** Qualifications for the Director include national stature as a scholar and leader in the field of fisheries biology, significant experience as an administrator and proven ability to build upon a substantial financial base to further program development and to acquire external funds. Ph.D. required.

We seek a Research Scientist with recognized ability in the basic disciplines of genetics, nutrition, developmental biology, or physiology. Preference will be given to a scientist whose research program can lead to practical application in the trout industry. The Research Scientist will be expected to supplement a considerable personnel/equipment budget by obtaining external funds. Ph.D. required.

**Application Procedure:** Applicants for the position of Director or Research Scientist should send a curriculum vitae, names and addresses of three references, and a statement of interests by February 1, 1988 to:

Dr. Frederick W. Harrison  
MAC Search Committee  
Department of Biology  
Western Carolina University  
Cullowhee, NC 28723

Western Carolina University is an Equal Opportunity/Affirmative Action Employer.



Biomed Research Labs Inc. of Seattle has moved its administrative, research and manufacturing operations to expanded new facilities in Bellevue, Washington. The new 18,000 square foot, \$1.3 million dollar facility was designed by the NBBJ Group, Seattle, and built by William Lease Lewis Construction, Seattle. In addition to operating full service research and testing laboratories, BIOMED manufactures fish bacterins for the aquaculture industry. BIOMED's new address is: 1720 - 130th Avenue N.E., Bellevue, WA 98005-2203, telephone (206) 882-0948.

## FUTURE EVENTS

**July 17-18, 1988 Western Fish Disease Workshop.** The 1988 workshop will be held at the Holiday Inn in Vancouver, B.C. in conjunction with the International Fish Health Conference. Evening discussions in virology, bacteriology, diagnostic methods or other areas of interest will be planned for Sunday evening. A session for contributed papers and an open round table discussion will occur on Monday.



**July 19-21, 1988 International Fish Health Conference.** This conference will be the first international meeting sponsored by the Fish Health Section, AFS. It will be held at the Holiday Inn in Vancouver, British Columbia, Canada. Abstracts will be due May 15, 1988 and hotel reservations should be made early. For registration materials, contact Dr. Trevor Evelyn, Dept. of Fisheries and Oceans, Pacific Biological Station, Nanaimo, B.C. V9R 5K6, Canada. Telephone (604) 756-7066.



**August 22-25, 1988 International Symposium on Viruses of Lower Vertebrates.** This symposium will be held in Munich, Germany and is the first devoted to the viruses of lower vertebrates. The meeting will be organized and hosted by Dr. Winfried Ahne, Institute of Zoology and Hydrobiology, University of Munich, Kaulbachstrasse 37, D-8000 Munich 22, Federal Republic of Germany.

## SHORT COURSES AND WORKSHOPS

**March 21-25, 1988. Introduction to Fish Health.** This short course will be held at Mount Hood Community College, Gresham, OR and is sponsored by the U.S. Fish and Wildlife Service and the aquaculture program of Mount Hood Community College. Applications are due by February 1, 1988 and application materials may be obtained by calling Jim Warren at 503-230-5972.

**May 15-June 11, 1988. Aquavet I - An Introduction to Aquatic Animal Medicine** will be presented at the Marine Biological Laboratory at Wood's Hole, Massachusetts. The course is designed for veterinary students and veterinarians who wish to work with aquatic animals. Applications are due January 15, 1988. Further information may be obtained from Dr. Donald Abt 215-898-5783.

**May 15-28, 1988. Aquavet II** course will be presented at the Marine Biological Laboratory in Wood's Hole, Massachusetts. The course is designed for those veterinarians or veterinary students who have completed Aquavet I and wish experience in marine invertebrates and fin fish held in aquaria. Applications are due January 15, 1988. For information contact Dr. Donald Abt at 215-898-5783.

**May 16-27, 1988. Disagnosis and Treatment of Diseases of Warmwater Fish.** This short course will be taught at Mississippi State University and is to provide instruction in the methods of diagnosis and treatment of parasitic, bacterial, viral, nutritional, and environmental diseases of warmwater fish. Undergraduate or graduate credit of four semester hours is given for successful completion of the course. Tuition for the course is \$193.00, however, this is subject to change.

Students will be expected to provide their own compound microscope and dissecting kit for use in the laboratory. However, a limited number of microscopes will be available. Instructors for the course will be Dr. Randy MacMillan, Associate Professor, College of Veterinary Medicine, Mississippi State University, and Dr. Craig Tucker, Associate Fisheries Biologist, Delta Branch Experiment Station, Stoneville, MS.

Applications must be received on or before March 1, 1988. Persons interested in taking the course should apply by writing Dr. Randy MacMillan, Drawer V, College of Veterinary Medicine, Mississippi State, MS 39762. All applicants will be advised whether or not they have been accepted to attend before April 1, 1988.

**June 20-29, 1988. Salmonid Disease Workshop.** This nine day course will be held at the Oregon State University Marine Science Center, Newport, OR and is designed for professionals working in the fish health field. For applications and additional information contact Dr. Robert Olson 503-867-3011.



**FISH HEALTH NEWSLETTER**

The Fish Health Newsletter is a quarterly publication of the Fish Health Section of the American Fisheries Society. Submissions of any length on a topic of interest to fish health specialists are encouraged and should be addressed to one of the editorial staff or to a member of the publication committee.

**Editors:**

James R. Winton  
National Fisheries Research Center  
Building 204  
Naval Support Activity  
Seattle, WA 98115  
206-526-6282

John S. Rohovec  
Department of Microbiology  
Oregon State University  
Corvallis, OR 97331  
503-754-4441

**FHS NEWSLETTER**

Department of Microbiology  
Oregon State University  
Corvallis, Oregon 97331

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JAMES W WARREN  
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