FISH HEALTH SECTION AFS NEWS LETTER

VOLUME 15, Number 2 APRIL 1987

ANNOUNCEMENT OF A JOINT ANNUAL MEETING OF THE FISH HEALTH SECTION OF THE AMERICAN FISHERIES SOCIETY, THE EASTERN FISH HEALTH WORKSHOP AND THE MIDWEST FISH HEALTH WORKSHOP

July 13-16, 1987
Baton Rouge, Louisiana

The Louisiana State University Agricultural Center and the School of Veterinary Medicine cordially invites members of the Fish Health Section of the American Fisheries Society and participants in the Eastern and Midwestern Fish Health Workshop to attend a joint annual meeting on July 13-16, 1987 at the Hilton Hotel in Baton Rouge, Louisiana. Baton Rouge is located on the Mississippi River approximately 65 miles northwest of New Orleans. A program of interesting scientific presentations is anticipated for participants and several tours of South Louisiana Plantation Houses and Cajun country will be available for spouses and guests.

Registration: Registration will be $35 if postmarked before June 15, 1987; late registration will be $40. The registration will cover all breaks, the reception on Tuesday night, the Cajun banquet on Wednesday, a bound copy of the abstracts and other expenses of the meeting. Registration for spouses and guests will be $25 and will cover the banquet and reception only.

Hotel Accommodations: Rooms can be arranged directly through the Baton Rouge Hilton with special rates of $55 for a single or double. Reservations can be made by calling 1-800-221-2584 in Louisiana or 1-800-621-5116 outside of Louisiana. When making room reservations, state your affiliation with the Fish Health meeting to insure billing at the reduced rates. A block of rooms has been set aside and will be held until July 1, so please make your reservations prior to that time to be guaranteed a room.

Transportation: Baton Rouge is located about 65 miles northwest of New Orleans at the junction of highways I-10 and I-12. The city is served by four major airlines (American, Continental, Delta and Northwest). Consult your travel agent for schedules. A hotel courtesy van is available for transportation from the airport to the Hilton. Additional service is provided by most major airlines into New Orleans. Transportation between New Orleans and Baton Rouge is available via rental car companies and Greyhound Bus. Buses run regular schedules from the N.O. airport to downtown Baton Rouge for a one-way fare of $12.95 ($8.00 with a college student I.D.). If you plan to arrive by bus, please call 346-3281 before your departure to arrange transportation from the bus station to the hotel.

Tours: South Louisiana has a wide variety of interesting sightseeing alternatives. The following tours have been arranged and each include guides, transportation, lunch and admission to the attractions. A minimum of 20 people are necessary for each tour.

Tour A: Bayou Cruise: Experience the Cajun mystique and elegance of the bayou aboard the newly renovated Vermillion Queen. Glide leisurely down the meandering Bayou Vermillion in air conditioned comfort while the Captain shares some of our Cajun history and folklore. View a multitude of wildlife: alligators, beaver, racoons, heron, egret and more. Also, some of the most beautiful homes in the area are located on the Bayou Vermillion. We'll enjoy a delightful lunch while on board and a cash bar will be available for those who wish to enjoy a drink.

Tour B: Feliciana Plantations: Enjoy a leisurely ride through the scenic country roads, stopping along the way to visit antique shops, churches, historical Lawyers Row and beautiful Rosedown Plantation. We'll dine at Asphodel in a romantic setting admist the shaded live oaks complete with a delicious luncheon!

ACTIONS REQUIRED:

1. Send your completed registration form before **June 15, 1987** to avoid the late penalty.
2. Make your hotel reservations prior to **July 1, 1987** to insure room availability.

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AFS/FHS MEMBERS RECEIVE AWARDS

WELLBORN HONORED

Dr. Tom Wellborn of the Mississippi Cooperative Extension Service was honored with a distinguished service award by Catfish Farmers of Mississippi at their January convention. CFM President Bill Stephens presented the award.

HETRICK NAMED DISTINGUISHED SCHOLAR—TEACHER

Dr. Frank Hetrick was recognized by the University of Maryland for innovative research and effectiveness in the classroom. His research has involved the diseases of sportfish and shellfish of Chesapeake Bay.

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To be named

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RESPONSES TO QUESTIONNAIRE

There have been some suggestions over the last few years that the Fish Health Section promote the publication of a scientific journal in the area of fish health. President, Bill Rogers, addressed the possibility in his keynote address in July, 1986 and surveyed the membership for comments through a questionnaire in the October issue of the Fish Health Section Newsletter. A summary of the 108 responses follows:

1. Is there a need for a North American Fish Disease Journal?

Absolutely, yes	5
yes	68
probably yes	7
undecided	9
probably not	4
no	14
absolutely not	1

2. What should be the scope of such a journal?

   Fish diseases, possibly including shellfish, health problems in all aspects, new diagnostic techniques, treatments written for the FHS membership use were common responses.

3. What standard should be met in the quality of the journal?

   The majority wanted peer-reviewed articles with less critically reviewed short notes and opinions. A few responded in favor of formats similar to other American Fisheries Society Journals. Many commented that the publication should be rapid.

4. Would you submit articles?

   74 said "yes"

5. Would page charges be acceptable to you if you submit papers?

   To the majority, page charges seemed not a serious problem.

6. Would you subscribe?

yes	72
probably yes	14
undecided	4
probably not	3
no	8

7. What would be an acceptable cost to you?

   Most responses were in the range of $25.00/year, a few suggested $50.00 or above.

8. Other comments: Typical were the following:

   "Can the FHS members afford the cost of the AFS, FHS, and subscription to the journal?

   "A definite need for state-of-the-art techniques reporting to the practicing culturists"

   "It is unnecessary because of other outlets"

   "About time for a North American fish health journal"

   Some comments were more dramatically positive or negative!

PASSAGES

Dr. Peter W. Taylor has moved from Denver and is now working as an Area Fisheries Specialist at the Belzoni Fish Disease Laboratory, P.O. Box 631, Belzoni, MS. Pete's phone is 601-247-2917.

Sally J. Wechsler has moved from Gainsville, Florida where she was a member of the coop unit and now resides in Wyoming. Her new address is USDA-ARS, Arthropod-borne Animal Disease Research, P.O. Box 3965, University Station, Laramie, WY 82701.

FUTURE EVENTS

June 24-25, 1987. Western Fish Disease Conference. The 28th annual meeting will be held in Bozeman, Montana and hosted by the U.S. Fish and Wildlife Service. The first call for papers has been sent out. If you did not receive this mailing, or would like additional information, contact Beth MacConnell, USFWS, 4050 Bridger Canyon Road, Bozeman, MT 59715; telephone 406-587-9265.

June 13-16, 1987. Joint Annual Meeting of the Fish Health Section, the Eastern Fish Health Workshop, and the Midwest Fish Health Workshop. The meeting will be hosted by Louisiana State University and will be held at the Hilton Hotel in Baton Rouge, Louisiana. A block of rooms is reserved until July 1 and registration forms are due before June 15. Additional information may be obtained from Ron Thune, Program Chairman, Dept. of Veterinary Microbiology and Parasitology, Louisiana State University, Baton Rouge, LA 70803. Telephone 504-346-3308.

August 9-15, 1987. VII International Congress of Virology. This meeting will feature a symposium session on viruses of marine and aquatic animals and will be held at the Edmonton Convention Center, Edmonton, Alberta. For information, please contact Dr. Tats Yamamoto, Dept. of Microbiology, Univ. of Alberta, Edmonton, Alberta T6G 2E9. Telephone 403-432-4429.

August 31-September 3, 1987. Third International Conference of the European Association of Fish Pathologists. The meeting will be held at the University of Bergen in collaboration with the Institute of Marine Research, Bergen, Norway and will include papers, poster sessions, workshops and round table discussions on all aspects of fish and shellfish pathology. For details contact Dr. E. Egidius, Institute of Marine Research, C. Sudtsgate 37, 5000 Bergen, Norway. Telephone 5-327760.

July 19-21, 1988. International Conference on Fish Health. This meeting will be hosted by the Fish Health Section of the American Fisheries Society and will have sessions on all aspects of fish health. The conference is scheduled to be held in Vancouver, B.C. For further information contact Dr. T.P.T. Evelyn, Pacific Biological Station, Nanaimo, B.C. V9R 5K6. Telephone 604-756-7066.

SHORT COURSE SCHEDULED

Advances in Fish Disease Control, a two-week workshop designed for professionals in the fish health field, will be held at the Oregon State University Marine Science Center, August 3-14, 1987. The course will emphasize diseases of salmonids and will include morning lectures and afternoon laboratory sessions. The topics and instructors for the sessions are: Host-Microbial Relationships - J. Fryer; Viral Pathogens - J. Winton; Bacterial Pathogens - J. Rohovec; Parasite Pathogens - R. Olson; Immunology and Vaccination - S. Kaattari; Cell Culture and Diagnostic Methods - C. Lannan; Histology and Histopathology - C. Smith and J. Morrison. Applications and a $100.00 deposit are due by July 6, 1987. The cost for the two-week session will be $600.00 which includes housing at the Marine Science Center. For further details, contact Dr. Robert Olson, Marine Science Center, Newport, OR 97365. Telephone 503-867-3011.

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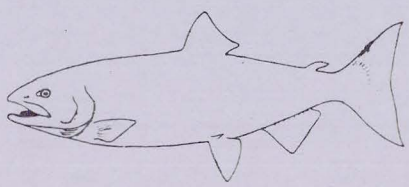
A limited number of the 1986 Fish Health Section Directory are available. Copies are available by written requests to John Rohovec, Dept. Microbiology, OSU, Corvallis, OR 97331.



FACTORS AFFECTING THE BINDING OF IHN VIRUS TO SALMONID SPERM CELLS

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Infectious hematopoietic necrosis virus (IHNV) has been shown to rapidly and efficiently bind to salmonid sperm cells (99% in 1 min.). The effect of pH on adsorption was studied using organic buffers over the range of pH 5-11. Binding occurred rapidly at pH 7.2 and above; however, at acidic pH values (below pH 6.7) a decrease in adsorption efficiency was noted. When the pH of these samples was returned to 7.6, the virus again adsorbed to the sperm indicating the virus and the binding sites were intact.

The ability to elute IHNV from the sperm was also studied. After adding IHNV to sperm and allowing adsorption, the sperm cells were pelleted by centrifugation. The pellet was resuspended in various eluents, recentrifuged to remove the sperm cells, and the virus remaining in the supernate titered by plaque assay. Poor elution occurred when solutions containing salts were used to resuspend the virus pellet, but 50-90% of the bound virus was eluted using deionized or tap water.

ICHTHYOPHTHIRIUS MULTIFILIIS IN FISHES NATIVE TO LAKE TITICACA, SOUTH AMERICA

Richard Heckmann
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Brigham Young University
Provo, UT 84602
and
Victor Hugo Inchausty B.
Benson Research Institute
Calle Chaco #719
LaPaz, Bolivia

Ichthyophthirius multifiliis Fouguet is considered one of the most wide-spread fish parasites and its host range now includes endemic or native fish from Lake Titicaca, South America. The holotrichous ciliate is the causative agent for "ICH" or "white spot disease." During January 1987 we had an opportunity to examine native fish from Puno Bay, Lake Titicaca, Peru for parasites representing part of a research program undertaken between the University of Altiplano, Puno, Peru and Brigham Young University, Provo, Utah. On the gills and body surface of 100% of 30 each *Orestias agasii* and *Orestias olivaceous* we found *I. multifiliis*. These ciliates were so common, presence on the host could be determined without magnification (white spots). While checking floating fish cages in the lake several dead fish were observed on the surface of the water. The full-time staff for the research station commented on the seasonal die-off of *Orestias* and the large number of dead fish observed in Puno Bay. Lake Titicaca is a tropical lake situated at 13,000 feet elevation on the Altiplano of Peru and Bolivia. Long water retention time causes high dissolved solid concentrations in Lake Titicaca (Richerson, P.J., C. Widmer and T. Kittel, 1977. The Limnology of Lake Titicaca (Peru-Bolivia), a large high altitude tropical lake. Inst. Ecol. Publ. 14. Univ. Calif., Davis: 78p.). Most areas of Lake Titicaca are steep-sided and deep but Puno Bay is a shallow embayment (7 m maximum depth) covered with submerged vegetation such as the emergent macrophyte, *Schoenoplectus tatora*. Also sewage collection and treatment for Puno, Peru (60,000+ population) is very ineffective. Most untreated sewage including fecal matter is conveyed directly into the Bay contributing to bay eutrophication and ichythofauna stress. Besides the holotrichous ciliate several other parasites were observed on the gill surface including: *Costia, Chilodonella, Trichodina, Costia* and a myxosporidan. There has been a reduction in numbers of *Orestias* during the last few years in Lake Titicaca. *Orestias* has been used for food by people inhabiting the shoreline. Recent introductions into Lake Titicaca such as pejerrey and trucha have also impacted the native fish species.

ANTIBIOTIC RESISTANCE TO ROMET-30 IN BACTERIAL INFECTIONS OF CATFISH

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In May, 1986, Romet-30 was cleared for use as a new antibiotic for bacterial infections in pond-raised catfish. Clearance of Romet-30 has given the fish farmer an alternative to Terramycin in combating bacterial epizootics. As with any new drug there exists opportunity for misuse and abuse in treatment applications. Fish pathologists and fish farmers alike need to be aware of the potential problems in prescribing this new drug.

From June, 1986, through December, 1986, the Extension Wildlife and Fisheries Department's Fish Disease Lab, Belzoni, MS, investigated 917 cases involving bacterial infections in farm-raised catfish. Antibiotic sensitivity to Terramycin and Romet-30 were routinely run on all bacterial isolates except *Flexibacter columnaris*. The percent of these isolates sensitive to the two drugs approved for the treatment of bacterial infections in channel catfish is given in Table 1.

Aeromonad resistance to Terramycin has long been a problem in aquaculture. Indiscriminant feeding of medication may well have been responsible for some increase in aeromonad resistance. At present aeromonad resistance to Romet-30 is not a major concern. However, resistance potential appears to exist and misuse of this drug may create problems in the future. Bacterial isolates need to be identified and tested for antibiotic sensitivity prior to a proper treatment recommendation. Romet-30 resistance needs to be monitored and recorded as an indicator of possible problems in the future.

Table 1. Percent of bacterial isolates from farm-raised catfish resistant to two drugs. The figures in parenthesis are the number of resistant isolates over the total number of isolates.

Isolate	Terramycin	Romet-30
Edwardsiella ictaluri	0.0 (0/697)	0.0 (0/697)
Aeromonas hydrophila	44.4 (16/36)	5.6 (2/36)
A. sobria	19.3 (43/223)	2.2 (5/223)
Plesiomonas sp.	60.0 (12/20)	10.0 (2/20)

STORAGE CONDITIONS OF STRIPED BASS TISSUES AFFECTS RECOVERY OF IPNV

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Striped bass can be chronic inapparent carriers of infectious pancreatic necrosis virus. The virus is recovered most frequently from the anterior kidney from striped bass, especially if the fish were injected with steroids 1-2 weeks prior to testing. However, virus infectivity is significantly affected by the conditions in which the tissues are stored before testing using the virus plaque assay. When experimentally IPNV-infected striped bass were killed and stored whole for 2-14 days, virus was detected in 9 out of 10 fish stored at 4°C, but not in any of 17 fish stored at -70°C. In contract, when homogenized striped bass tissues were stored for 2-14 days, IPNV was detected more frequently in tissues stored at -70°C than in those stored at 4°C. Virus titers were least affected in striped bass tissues stored whole at 4°C. These data demonstrate that striped bass tissues, if stored prior to IPNV assay, should be stored whole and held at 4°C.

AEROMONAS SALMONICIDA, APPARENT DRUG RESISTANCE TO ORMETOPRIM—POTENTIATED SULFADIMETHOXINE

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In July, 1986, furunculosis was diagnosed in an Icelandic stock of Atlantic salmon (*Salmo salar*) which was being reared at Oregon AquaFood's freshwater hatchery. Drug sensitivity of several of the isolates was investigated (sensitivity discs on Mueller-Hinton agar were employed) and resistance to tetracycline and triple sulfa was noted. A zone of susceptibility was observed with trimethroprim × sulfamethoxazole (Romet equivalent). Chemotherapy with Romet was prescribed and daily mortality was reduced from a high of 0.33% to 0.01%. However, two weeks after the chemotherapy ended, daily mortality started to rise again and *Aeromonas salmonicida* was isolated from moribund fish. Drug sensitivity was again investigated and a slightly different zone pattern to trimethroprim × sulfamethoxazole was observed. A zone of inhibition of sufficient diameter to be deemed "susceptible" was apparent, however, there was often some growth present within the zone. This zone of partial "inhibition" varied from isolate to isolate in both diameter and degree of inhibition within the zone. In addition, some isolates showed sensitivity to tetracycline while some, taken from the same pond, showed definite resistance. Romet was again prescribed for treating the population, however no consistent decrease in daily mortality was observed and daily mortality became as high as 4%. Another separate group of Atlantic salmon from which *A. salmonicida* had not been previously isolated, experienced a similar scenario later in August, i.e., initial "recovery" from furunculosis with Romet, subsequent relapse, appearance of this partially Romet-inhibited isolate, and finally no or little response to Romet chemotherapy. In order to minimize the opportunity of contaminating other groups of fish, these infected populations were destroyed. Furthermore, thorough chlorine disinfection was conducted in all emptied raceways and other potentially contaminated areas. Furunculosis has not been diagnosed in juvenile fish populations on site since then.

INDIRECT FLUORESCENT ANTIBODY TEST FOR IHNV

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Detection of infectious hematopoietic necrosis virus in susceptible salmonid fry is routinely done using standard cell culture techniques (Fish Health Blue Book, 1985). It has also been reported that examination of peripheral blood smears and kidney imprints taken from fish infected with IHNV usually exhibit hemotopatholgical changes. This diagnostic technique has the advantage of that it can be completed within one hour whereas cell cultures usually do not begin showing typical cytopathology until 96 hours. Rapid diagnosis of IHN is important for the containment and removal of infected fish since no chemotherapeutants are available.

Both of the diagnostic procedures only give a presumptive identification of the virus. A confirmatory diagnosis includes neutralization by specific IHNV antiserum. In an effort to combine the rapidity of blood smear examination with the confirmatory ability using specific IHNV antiserum we have developed an indirect fluorescent antibody test (IFAT). Peripheral blood smears have been made from infected juvenile fish and, by the IFAT, pathological changes seen in fluorescing cells are consistent with those reported in the literature for stained preparations. These changes include necrobiotic bodies and monocytes (macrophages) with varying degrees of cytoplasmic vaculation.

Current work involves methodology modifications, evaluation of the IFAT with different types of IHNV and hematopathological studies in juveniles. We have recently begun to apply this technique to adults using peripheral blood smears and cells from the ovarian fluid. Early results indicate that this methodology could aid in the screening of adults for the presence of IHNV.

ANOTHER ERYTHROCYTIC VIRUS FROM SALMONID FISH?

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and
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At least two viruses are known to infect erythrocytes of salmonid fishes. Viral erythrocytic necrosis (VEN) caused by a poorly understood iridovirus has been reported among numerous species of marine fish and salmonids from the Pacific Northwest. VEN can be associated with chronic and severe anemias and is presumptively diagnosed by the observation of magenta colored inclusion bodies in proximity to the nucleus in Giemsa-stained blood cells. Confirmation of VEN infection in salmonid fish is presently dependent on the observation of hexagonally-shaped virions 180 to 200 nm in diameter in infected erythrocytes.

A second erythrocytic virus of more recent discovery has been found in several stocks of salmonids in the Columbia drainage in Oregon and Washington. The cytoplasmic inclusions which may vary in size and number are usually pale blue to translucent in Giemsa-stained erythrocytes. Pinacyanol chloride stains more clearly show the inclusions and infected cells are known to contain virus particles, possibly togaviruses, with a dense core and a diameter of 60 to 80 nm (Fish Health Newsletter 12:4; 13:4). This virus can induce anemias in infected fish and is believed to be a major predisposing agent to subsequent infections with other bacterial and fungal pathogens.

Evidence for yet a third erythrocytic virus that is associated with anemias in salmonid fish has recently been found in coho salmon (*Oncorhynchus kisutch*) reared in salt water in California. The fish suffering from a severe anemia (PCVs down to 10%) of unknown cause were submitted as diagnostic samples to the laboratory. No bacterial, protozoan or fungal pathogens were detected. Histopathological signs were characterized by a diffuse degenernation of the hematopoietic tissues and abnormally abundant accumulations of hemosiderin in macrophage centers in the spleen and kidney. Numerous degenerating erythrocytes often ingested by macrophages were found in the major sinuses of the kidney, spleen and liver. Mild to moderate nephrocalcinosis was also observed in several fish examined.

Blood films prepared from affected fish showed that their erythrocytes were laden with cytoplasmic inclusions (Fig. 1a). These stained pink to red in Lieshman-Giemsa prepared blood films and they were often rod shaped, ranging in size from 1-2 um. In some fish nearly 100% of the erythrocytes showed these inclusions. An examination of erythrocytes by electron microscopy showed that numerous virus-like particles with a very consistent size (diameter X = 106 nm, SD = 2.7, n = 20) were evident within membrane-bound vacuoles in the cytoplasm (Fig. 1b,c). The particles were not observed within the nucleus but in several cells they lied in close proximity to the nuclear envelope (Fig. 1d). The particles were spherical, sometimes showing an indentation and appeared to possess regular surface projections or spikes that might be obtained with an envelope as they bud into the cytoplasmic vacuoles (Fig. 1e).

Studies are presently underway to identify the agent and to determine if it can be transmitted under laboratory conditions. Initial attempts to isolate the virus from the kidney and spleen of affected fish on established salmonid cell lines has failed. The geographical distribtuion of the agent is unknown but identical inclusions were observed in red cells from coho salmon with an idiopathic anemia from Washington state; also the source of the eggs for the stocks in California.

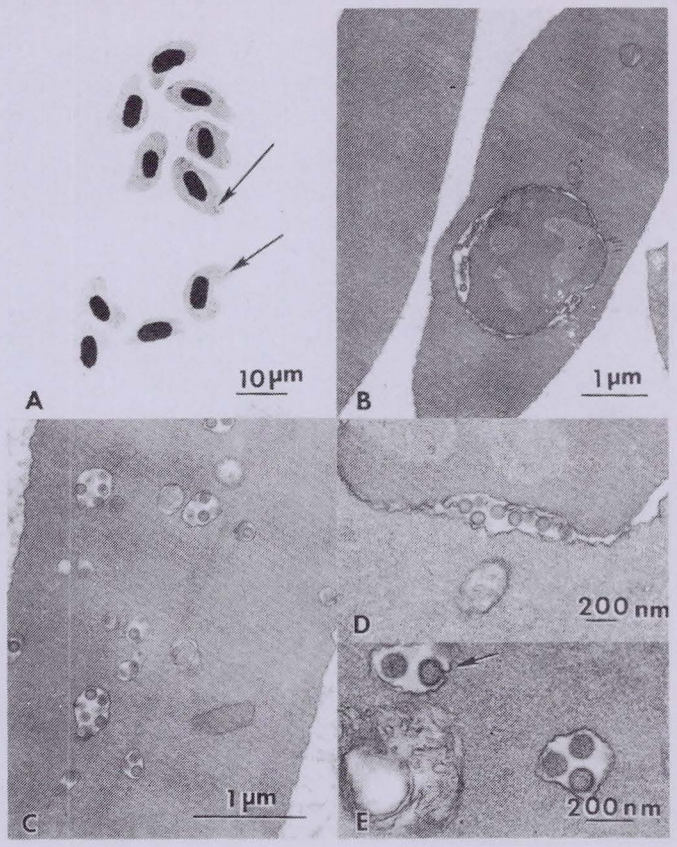

Figure 1. Erythrocytes from coho salmon suffering from an anemia. (a) Arrows show rod-like inclusion bodies in the cytoplasm, Lieshman-Giemsa stain, (b) virus-like particles in proximity to the nucleus, (c) within cytoplasmic vacuoles, (d) in the perinuclear space and (e) arrow showing a particle possibly budding into a cytoplasmic vacuole.

POSSIBLE HORIZONTAL TRANSMISSION OF EIBS

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Lower Columbia River Fish Health Center, Box 17
Cook WA 98605

During August and September 1986, losses in yearling coho salmon (COS) at Little White Salmon NFH increased to 2% per month - from 0.4% in June and 0.2% in July. Fish were weak and found heavily infected with erythrocytic inclusion body syndrome (EIBS) (50% and 48% incidence in August and September, respectively). Hematocrits were low overall, but were not correlated with the presence of inclusion bodies. Other hatcheries in the Northwest have had EIBS-related loss in COS during the spring. A scheduled release of these fish was made September 30, 1986.

Upriver bright fall chinook salmon (URB) yearling fish are commonly found to be infected with the EIBS virus at Little White Salmon NFH, and it was suspected that these fish could be a source of the virus and that coho could become infected by horizontal transmission. Consequently, a group of 110 EIBS-negative COS from Willard NFH were brought to Little White Salmon on October 31, 1986 and placed in two live boxes below two raceways of known EIBS positive URB. The remaining two million fish at Willard were used as "controls". Sample fish were tested for EIBS prior to stocking the two live boxes and were negative. Further samples on November 17, December 1, 9, 1986 and January 15, 1987 were also negative. A final sample of 24 fish on February 20, 1987 showed three fish (12.5%) infected with EIBS. One hundred fifty COS sampled in February at Willard NFH were all negative. The study was terminated February 20 because the "source" of EIBS (URB) was moved to other raceways and space for holding live boxes was not available.

These results suggest that EIBS can be transmitted horizontally from URB to COS. The incubation period for EIBS in coho cannot be determined with certainty, but infected fish were detected after 85-112 days at water temperatures ranging from 5-7°C.

A CHLAMYDIA—LIKE ORGANISM ASSOCIATED WITH HIGH MORTALITY IN HATCHERY REARED LAKE TROUT (*SALVELINUS NAMAYCUSH*)

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National Fish Health Research Laboratory
Box 700, Kearneysville, WV 25430

In January, 1986, excessive mortality began in yearling lake trout (10-11 cm) following routine tagging operations at the Iron River National Fish Hatchery (Wisconsin). Subsequently, mortality with similar clinical signs occurred in fingerling lake trout (3-4 cm). Mortality continued until both populations were essentially eliminated. Routine diagnostic examination failed to show any infectious or parasitic agents and a toxic cause of the mortality was suspected. Yearling and fingerling fish were sent to the National Fish Health Research Laboratory (West Virginia) for histological, virological, and electron microscopic examinations and for disease transmission studies.

A series of transmission trials at the NFHRL and the Iron River NFH indicated that an infectious agent was involved in the lake trout mortality. Mortality did not occur in fingerling brook, brown, or rainbow trout or Atlantic salmon, although this does not preclude the possibility that these species could become infected and carry the agent.

Histological examination showed hyperplasia of the epithelium inside the mouth, around the snout, and in extreme cases over the body (Figure 1). The epithelium of the gills showed hypertrophy and hyperplasia but no evidence of epitheliocystis. Proximal tubules of the kidneys were often dilated and contained fluid and protein in the lumina. Macrophages and monocytes with phagocytized cellular debris were common in the kidneys and were found in the liver sinusoids.

Samples of snout, gills, kidney and spleen, and blood were assayed at 9 and 15°C on CHSE-214, EPC, FHM, RTG-2, and primary lake trout cell cultures, but no cytopathic effects were observed.

Electron microscopic examination revealed ellipso-spherical particles 100 nm to 175 nm in diameter (Figure 2) in the hyperplastic tissue of the snout — the site of distinctive histological change. The particles were double membrane bound with a diffuse electron dense core. A tail piece (about 140 nm in length) was routinely seen. Particles were observed in intercellular spaces as well as in cytoplasmic aggregates. Morphologically, the particles resemble the chlamydia-like organism reported for epitheliocystis in steelhead trout (*Salmo gairdneri*), but the particles we observed are less than one-half the size of the epitheliocystis organism.

The Iron River NFH lake trout mortality involved an infectious agent, but the agent we described cannot be indicted as the cause of the mortality until the organism has been isolated and cultured and Koch's postulates fulfilled.

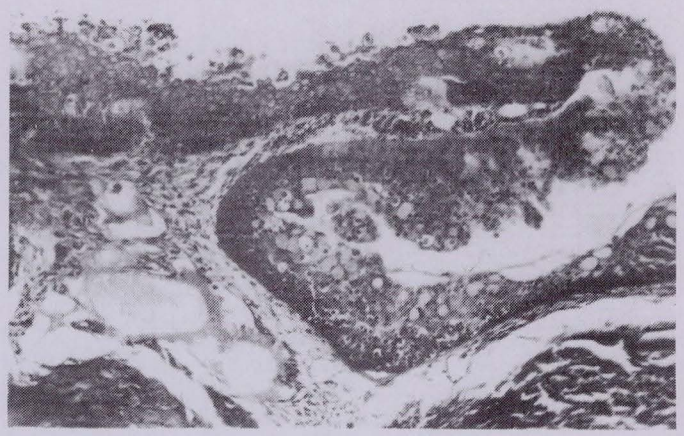

Figure 1. Saggital section through the oral flap and adjacent tissues of a lake trout fingerling infected during experimental trans mission studies showing hyperplasia, hypertrophy, necrosis, and lymphocytic infiltration of the epithelium.

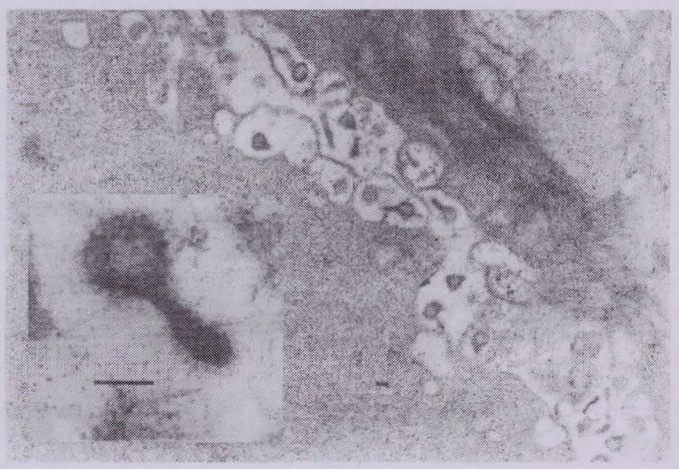

Figure 2. Thin-section electron micrograph showing chlamydia-like particles in hyperplastic tissue from the snout of an infected lake trout. Bars = 100 nm.

SCHISTOCEPHALUS sp. OF FISH INHABITING LAKE TITICACA, PERU

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During January 1987, a survey of fish parasites for the ichthyofauna from Lake Titicaca, Puno Bay, Peru was conducted in conjunction with other research projects. A high incidence of the plerocercoid, *Schistocephalus* sp., was noted in 3 species of fish, *Orestias agasii, Orestias olivaceous* and *Basilichthyes bonariensis.* Twenty fish for each species were examined for fish parasites. Eight *O. agasii,* ten *O. olivaceous* and five *B. bonariensis* contained pleroceroids of *Schistocephalus* in the body cavity (*Orestias*) and the intestine (*Basilichthyes*). The specimens of *Orestias,* a native species for Lake Titicaca, averaged 11 cm TL while *Basilichthyes,* introduced into Lake Titicaca from Argentina, measured 15 cm TL. The three species of fish are used for human food. Puno Bay receives raw sewage from the community of Puno, Peru. *Basilichthyes* is a predatory fish which often contains small *Orestias* in stomach contents while *Orestias* is both a benthofagus and planktivorous feeder. Most reports of *Schistocephalus* polerocercoids inhabiting fish record the cestode in the body cavity often compressing the viscera (Hoffman, G.L., 1967, Parasites of North American Freshwater Fishes. University of California Press and Yamaguti, S., 1961, Systema Helminthum vol II Cestodes, InterScience Publishers Inc.). The average length of the *Schistocephalus* plerocercoids in *Basilichthyes* was 1.5 times the length of the plerocercoids found in *Orestias.* Plerocercoids from both hosts were viable and active when removed from the fish. The intestinal location of *Schistocephalus* in *Basilichthyes* is probably due to the trophic habits of the host. There has been a limited amount of research published on the parasites of fishes from Lake Titicaca.

STANDARDIZATION OF STAIN USED FOR DIAGNOSING ERYTHROCYTIC INCLUSION BODY SYNDROME (EIBS)

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Erythrocytic inclusion body syndrome (EIBS), a viral erythrocytic necrosis (VEN)-like disease, has been observed in several areas in the Northwest. This virus disease is clinically diagnosed by microscopic examination of blood smears for intracytoplasmic erythrocytic inclusion bodies. Fish biologists involved in EIBS diagnostic work have been using several types of hemotological stains. It became apparent that standardization of the staining procedure was needed. Comparative tests were conducted on blood smears and kidney imprints with the following commonly used blood stains: (1) Leishman-Giesma, (2) Pinacyanol chloride, (3) Powell's Giemsa, (4) Harleco's Giemsa, (5) Diff Quik differential stain, (6) Wright's.

Pinacyanol chloride stain was found to be the most consistent. The following staining procedure is recommended:

Pinacyanol Chloride Staining Method for EIBS

A. Solutions

1. 1. Methanol, absolute
2. 95% ethanol
3. Pinacyanol chloride stain

Pinacyanol chloride	2.5 gm
(Sigma Chemical Co. - Cat.# P-0392)	
95% ethanol	367.0 ml
Distilled water	132.5 m

Dissolve pinacyanol chlorde in 95% ethanol and add distilled water. Adjust pH to 7.1-7.2 with sodium bicarbonate. Filter, as necessary, to remove precipitate.

95% ethanol is recommended instead of absolute ethanol because the former is less expensive. It is important to end up with a 70% ethanol solutuion.

B. Procedure

1. Air dry at least 30 min.
2. Fix in methanol 5-10 min. Should be fixed within several hours.
3. Air dry. At this point the slides may be stored (in slide boxes) in the refrigerator for several months.
4. Stain in pinacyanol chloride solution 1 min. The longer the methanol fixed slides are stored, the longer they should be stained; the older the solution, the longer the staining time.
5. Rinse in tap water until clear.
6. Air dry overnight. The slides should be stored away from lights to minimize fading.
7. Mount with coverslip.

We found that the staining variability was minimized when unfixed smears and imprints were air dried for at least 10 minutes and stained within 5 or 6 hours. Even under refrigeration, unfixed smears and imprints stored overnight did not store well and often exhibited numerous lysed blood cells. Refrigerated, methanol-fixed, unstained smears and imprints, however, stored well for several months, although the longer they were stored unstained, the longer they had to be stained in pinacyanol chloride before microscopic examination. Reuse of staining solution is not recommended.

Dr. Takahisa Kimura reports that in Hokkaido, Japan there was a recent epizootic involving masou salmon which was caused by the chum salmon virus. (CSV). CSV was isolated and characterized approximately nine years ago by Jim Winton and colleagues and until Kimura's recent observations it was thought that the virus had little pathogenicity for salmonids. Virulence studies had been conducted in chum, coho, and chinook salmon, but not in masou.

TRANSMISSION OF INFECTIOUS HEMATOPOIETIC NECROSIS VIRUS (IHNV) BETWEEN ADULT SPECIES OF SALMON: MANAGEMENT STRATEGIES FOR ANADROMOUS BROODSTOCK

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Evidence suggests that adult salmon become infected with infectious hematopoietic necrosis virus (IHNV). Therefore the hatchery practices involved with the handling of these adults may influence their subsequent infection. This is particularly important when the hatchery design is conducive to the horizontal transmission of IHNV between adult salmon. Horizontal transmission among adult salmon (perhaps between different species) may have occurred at the Trinity River Hatchery in northern California. An annual run of fall chinook salmon (*Oncorhynchus tshawytscha*) returns to this facility and since 1979 IHNV has been detected among broodstock examined. There is also an annually returning population of coho salmon (*O. kisutch*) which begins arriving at the hatchery in the latter part of the chinook salmon run.

Historically, coho salmon have been considered resistant to IHNV infection. However, in 1985 IHNV was detected in ovarian fluids taken from adult coho salmon at the Trinity River Hatchery. The number of coho salmon harboring IHNV declined as the chinook being held in the same vicinity were removed. It was supposed that the chinook salmon were the source of the virus that via horizontal transmission had subsequently infected the coho salmon.

Biochemical and serological properties of the IHNV isolates obtained from both chinook and coho salmon at the Trinity River Hatchery were compared. In addition, the virulence of the two virus isolates was examined in both species of fish. No significant difference was seen between the two IHNV isolates biochemically or serologically. Both isolates of IHNV remained virulent for chinook salmon fry while coho salmon fry were resistant. The results from these studies suggest that the two IHNV isolates are the same.

In 1986 at Trinity River Hatchery the same IHNV epizootiological pattern was observed in the adult chinook and coho salmon. Late in the spawning season adults of both species were sampled individually for ovarian fluid, gill, spleen and pyloric ceaca tissues and virus levels compared. The prevalence of the virus in coho salmon was 27% with only low levels of the virus being detected in a portion of the samples. The chinook salmon exhibited a 100% prevalence with high levels of virus detected in almost every sample. Again the results suggested that the chinook salmon were the reservoir of infection by which, via a horizontal or waterborne route of transmission, the coho salmon were becoming infected.

Using Trinity River Hatchery as a case study, the facility design was critically evaluated to determine where horizontal transmission could be occurring among adult salmon. Potential areas included the piping of adult holding pond effluent and spawn shed drainage into the fish ladder, the holding of adults at high densities with low water flows and the anesthetic bath water into which all adults are placed prior to sorting. Significantly, IHNV was detected in water samples taken from the anesthetic bath. Extrapolation of virus levels we detected to larger volumes of water showed there to be 20,000 IHN virus/liter or 75,700 IHN virus/gallon. These studies have identified potential sources where viral contamination could occur and led to the development of management strategies that could minimize the transmission of IHNV between adult salmon broodstock.

SUSCEPTIBILITY OF COHO AND CHINOOK SALMON HYBRIDS TO EXPERIMENTAL INFECTIONS WITH IHNV

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Sockeye or kokanee (*Oncorhynchus nerka*) and chinook (*O. tschawytscha*) salmon and rainbow or the anadromous steelhead trout (*Salmo gairdneri*) are the principal species susceptible to natural infections with infectious hematopoietic necrosis virus (IHNV) (Pilcher and Fryer, 1980). Coho salmon (*O. kisutch*) have traditionally been resistant to infections even in hatcheries where they are raised with susceptible species. However, the last two years adult coho salmon infected with IHNV have been found returning to the Trinity River Hatchery in northern California. This hatchery has had well established populations of returning coho and chinook salmon for many years and they begin entering the facility in October and continue through January. Although chinook salmon at this facility have been known to be IHNV positive each year since 1969, only recently has virus been detected in coho salmon.

After the Trinity Hatchery has taken the needed number of eggs for production quotas from returning adult coho and chinook salmon, the gates are closed and the remaining adults in the river spawn naturally at the base of the impassable dam. The high concentrations of spawning adults of both species near the hatchery may explain why hybrids between the two species have been observed at Trinity Hatchery in enzyme electrophoresis studies.

The susceptibility to virus infection of hybrids between resistant and refractive species has been studied both with viral hemorrhagic septicemia virus (VHSV) and IHNV. Dorson and Chevassus found that hybrids of brook (*S. fontinalis*) and rainbow trout were totally resistant to VHSV. Chen and Parsons et al. in similar studies with coho salmon and rainbow trout hybrids found increased resistance to IHNV. We could find no reports of studies examining the susceptibility of coho and chinook salmon hybrids to IHNV and this prompted us to conduct the study reported here.

Four groups of fish were utilized in this study. Coho (mean wt. 9.0 g) and chinook salmon (mean wt. 12.9 g) were obtained from Darrah Springs Hatchery, a facility with no history of IHNV infections. Two groups of hybrids between the species were also obtained from the same location. One group was a result of crossing a male chinook with a female coho (mean wt. 8.7 g) and the second were progeny of a male coho crossed with a female chinook salmon (mean wt. 25.0 g).

Experimental challenges with IHNV showed that chinook and both hybrid groups were susceptible to virus infections (Table 1). Mortality was associated with high concentrations of virus (10^5 - 10^6 PFU/g) and clinical signs (both gross and microscopic) indicated the susceptibility of the chinook and both hybrid groups to IHNV.

One coho salmon injected with the chinook salmon strain of IHNV died during the course of the study (Table 1). This fish died as a result of IHNV as shown by clinical signs of infection, both grossly and microscopically, and recovery of virus from the tissues. Virus concentrations of 3.0×10^6, 1.8×10^5, 6.0×10^3 PFU/g were found in the kidney-spleen, liver and brain, respectively. However, a sample of 5 coho from the same tank on day 7 failed to show any signs of infection or virus presence.

Approximately equivalent concentrations of both the coho and chinook strains of IHNV were used in the challenges. Among the four groups challenged with IHNV, the hybrids made from the coho male and chinook female were the most susceptible to virus-induced mortality with either strain of IHNV (Table 1). It is not known why this particular parental cross might have resulted in an increased susceptibility to IHNV infection. It however, demonstrates that hybridizations may result in more susceptible species than the parental stocks used for the crosses.

Resistance within and among certain species of salmonids to virus infections has been observed. The potential for using selective breeding for virus resistance has been demonstrated for IHNV in sockeye salmon (Amend and Nelson, 1977; McIntyre and Amend, 1978) but these approaches have not been fully exploited. Hybrids made between virus resistant and susceptibile species have been tested and shown to have either complete or intermediate resistances to virus infections (Chen, 1984; Dorson and Chevassus, 1985; Parsons et al., 1986).

In our study however, hybrids made between coho and chinook salmon were susceptible to IHNV infections. Next year we will examine coho adults returning to the Trinity River Hatchery for virus and enzyme electrophoresis profiles to ascertain whether naturally occurring hybrids are returning as IHNV infected adults.

Table 1. Mortality among chinook, coho and hybrid salmon following intraperitoneal injections of infectious hematopoietic necrosis virus isolates from adult chinook and coho salmon.

	No. dead* / No. total (%) Host species+			
Challenge Virus++	Coho	Chinook	Coho Male x Chinook Female	Chinook Male x Coho Female
Coho IHNV	0/24 (0)	7/15 (47)	7/32 (22)	2/21 (10)
Chinook IHNV	1/31 (3)	12/14 (86)	31/32 (97)	19/23 (83)
No virus	0/30 (0)	1/15 (7)	0/20 (0)	0/20 (0)

* The kidney and spleen from nearly all mortalities was examined for the concentrations of IHNV by plaque titration on EPC cells (see Table 2). With certain fish, titrations of the virus content of the liver and spleen were also determined.

\+ The mean weights of the four groups were 9.0, 12.9, 8.7 and 25 g, respectively, for the coho, chinook, coho male-chinook female and chinook male-coho female hybrids.

++ The viruses used in this study were isolated from adult coho and chinook salmon returning to the Trinity Hatchery in 1984 and 1985, respectively. Fish received intraperitoneal injections with 3.0×10^6 PFU of the coho strain or 3.8×10^6 PFU of the chinook strain of IHNV. Both viruses were grown in CHSE-214 cells. Control fish received an equal volume of balance salt solution with no virus. Water temperature during the 19 days following injection was a constant 12 C.

DETECTION AND DISTRIBUTION OF CCV IN TISSUES OF EXPERIMENTALLY INFECTED CHANNEL CATFISH

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Detection of channel catfish virus (CCV) by a simple and rapid method, such as the fluorescent antibody technique (FAT), has been hampered by high background staining. This background staining can be removed by passing rabbit anti-CCV serum through normal channel catfish. The rabit serum (a ml/400gm weight adult catfish) is injected via the intraperitoneal route and 24h later the fish is bled and the serum collected. The serum, which now contains anti-CCV activity, can be used directly in indirect FAT examinations of tissues for CCV antigens and background staining is reduced to negligible levels.

Using this modification for anti-sera preparation, tissues (liver, kidney, spleen, brain, and blood) were examined from fish experimentally exposed to $1x10^4$ pfu/ml CCV for 30 minutes at 27°C. This concentration of virus caused a 42.4% cumulative mortality during the course of the study. Beginning on day 1 post infection, ten fish per day were sampled. Blood from all 10 fish was examined for viral antigens by FAT. Five of the 10 fish were further used to determine concentration of infectious virus and the remainder examined for virus antigens in frozen tissue sections by FAT.

Infectious virus was recovered from day 2 through day 10 post infection. Mortalities began on day 2 and continued to day 9, with a peak at days 3 and 4. Viral antigens were first seen on day 1, and remained through day 13. The FAT was effective in detecting viral antigens in the blood and frozen sections. Although the frozen tissue sections showed viral antigens earlier and at an initially greater level (day 1, 60% of tissues positive) than the blood films (day 2, 30% positive), the blood films continued positive by FAT throughout the remainder of the active phase of the infection. Consistent detection of viral antigens in the blood even late into the infection (60-90% positive from days 4 to 10) indicate their potential usefulness in the diagnosis of channel catfish virus disease (CCVD).

The clinical use of blood films has several advantages over virus isolation: (1) samples are easily obtained in the field; (2) no special transportation needs are required; (3) rapidity of diagnosis (within three hours) and (4) accuracy (using high specificity anti-sera). Possible disadvantages are statistically low numbers of positive tests at certain stages of infection.

Overall, however, the use of blood films stained by FAT may be quite effective in rapid diagnosis of viral infections, particularly those known to involve hematopoietic tissues. Recent trials have shown that the same technique is successful when applied to blood films from salmonids with infectious hematopoietic necrosis virus (S. LaPatra, Department of Microbiology, Oregon State University, Corvallis, Oregon, this issue of Newsletter). Similar studies with viral hemorrhagic septicemia virus (VHSV) and infectious pancreatic necrosis virus (IPNV) should be initiated.

Table 1. Cumulative mortalities, recovery of infectious virus and detection of viral antigens in channel catfish following experimental infection with channel catfish virus (CCV)

Days Post Infection	Cumulative Mort. (%)	Virus Recovery (no. pos./ no exam)	Antigen Detected	
			blood	other tissues*
1	0	0/5	0.10	3/5
2	19 (4.2%)	4/5	3/10	5/5
3	67 (14.7%)	5/5	3/10	5/5
4	121 (26.9%)	4/5	6/10	1/3
5	152 (33.8%)	4/5	9/10	1/5
6	165 (36.7%)	5/5	9/10	3/5
7	176 (39.1%)	5/5	8/9	2/5
8	188 (41.8%)	4/5	8/10	0/5
9	190 (42.4%)	2/5	8/10	1/5
10	190 (42.2%)	1/5	7/10	2/5
13	190 (42.2%)	0/5	2/10	0.5
17	190 (42.2%)	0/5	0/10	0/5

CAREER OPPORTUNITIES CENTER ANNOUNCEMENT

The Education Section of the American Fisheries Society will sponsor a Career Opportunities Center, including a new interactive, computer-based job searching system, from September 14-16, 1987, at the 117th Annual Meeting of the Society to be held in Winston-Salem, NC. EMPLOYERS in state, federal and private organizations with positions in fisheries and aquatic science should send information about actual or anticipated job openings to the COC Chairman. Please send any other appropriate information or brochures about the organization. By arrangement with the COC Chairman, the Career Center may be used for interviewing potential employees. JOB SEEKERS should send a resume to the COC Chairman. Vacancy and resume submissions are encouraged throughout the year, but should arrive no later than September 1, 1987. All submissions will be available for review at the Annual Meeting. Send materials to Dr. James M. Haynes, AFS ES COC Chair, Department of Biological Sciences, SUNY College at Brockport, Brockport, NY 14420.

POSITIONS AVAILABLE

COLLEGE OF VETERINARY MEDICINE MISSISSIPPI STATE UNIVERSITY

Aquatic Toxicologist/Molecular Biologist

Location: College of Veterinary Medicine, Mississippi State University

Responsibilities: Participation in the college research program with emphasis in toxicology as related to molecular and cellular physiology in warm water aquatic species.

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

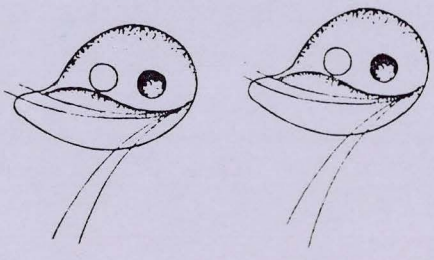
Salary and Rank: Negotiable depending on qualifications and experience.

Available: Immediately

Qualified individuals are invited to submit a letter of application, current curriculum vita and three references to: Dr. J.V. Kitzman, Chairman, Department of Basic and Applied Sciences, College of Veterinary Medicine, Mississippi State University, P.O. Drawer V, Mississippi State, MS 39762. MUS is an equal opportunity/affirmative action employer.

DEPARTMENT OF MICROBIOLOGY, OREGON STATE UNIVERSITY

The Department of Microbiology, Oregon State University seeks applicants for a Research Assistant to work at the Hatfield Marine Science Center, Newport, Oregon, and the Department of Microbiology, OSU, Corvallis. The position involves the study of infectious agents of Pacific Salmon. The applicant is required to possess experience in pathogenic bacteriology, virology, and immunology. A working knowledge of methods used to produce and test monoclonal antibodies and ability to work independently is desirable. The position is for a minimum of two years beginning June 1, 1987. Send a letter of application, curriculum vitae, and three references before May 15, 1987 to Dr. J.S. Rohovec, Department of Microbiology, Oregon State University, Corvallis, Oregon 97331-3805, Telephone 503-754-4441. Oregon State University is an Affirmative Action/Equal Opportunity Employer and complies with Section 504 of the Rehabilitation Act of 1973.



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.

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