



# NEWSLETTER

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## ETIOLOGICAL AGENT IN A CHILEAN COHO DISEASE ISOLATED AND CONFIRMED BY KOCH'S POSTULATES

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An estimated 1.5 million Chilean coho salmon, roughly 200 g to market size (approx. 2 kg), died of an unknown disease. The disease occurred only in coho reared in seawater. This represents a significant decrease in coho production and more than a 10 million dollar loss to the Chilean fish farming industry. The number of farms with infected fish increased from 4 in April to at least 26 in December (1989), and essentially all in the areas around Puerto Montt and the island of Chiloe. Although fish mortalities at infected farms have been variable, cumulative totals over 70% have been reported.

*Vibrio*, *Aeromonas salmonicida*, *Renibacterium salmoninarum*, PKX, *Pasteurella*, sporozoans, mycoplasmas, chlamydia, rickettsia, and aflatoxins in feed have been implicated as causative agents. This has led to considerable confusion and controversy over the cause, treatment, and management of the disease.

In May 1989, an intensive investigation was initiated and funded

by Trouw Suralim (a fish feed company in Chile) to (1) isolate and identify the responsible agent, (2) describe the pathology caused by the agent, (3) develop a fluorescent-antibody test for diagnostic purposes, (4) demonstrate any fish immunity to the pathogen, and (5) conduct field trials with antibiotics.

Attempts to culture the organism using over 35 diverse culture media at 15°C were unsuccessful. Anaerobic and increased CO<sub>2</sub> conditions, and 27°C incubation of some media also failed to isolate the organism. In September the organism was successfully isolated from infected coho kidney tissue in 6 cell lines established from 6 fish species; 4 salmonid (RTG-2, CHSE-214, CHH-1 and CSE-119) and 2 warmwater (FHM and EPC). Tissue culture grown organisms obtained from a third passage were washed, diluted, and injected into healthy, uninfected coho in both fresh water and seawater aquaria at 15°C. Mortality from this disease occurs naturally between 9 and 16°C. All injected fish died within 14 days and, by mid-November, Koch's Postulates had been fulfilled, thereby establishing the etiological agent of this disease.

The causative agent is a bacterium, coccoid in shape but often pleomorphic (Figure 1), strongly Gram negative, non-motile, and typically variable in size (ranging from approx. 0.5 μm to greater than 2 μm in diameter). It is PAS, acid-fast, and Gimenez negative, and stains well with hematoxylin and eosin, Giemsa, and methylene blue. The organism is an obligate intracellular pathogen which is frequently found within membrane-bounded vacuoles or inclusions in the cytoplasm of host cells (Figure 1). More than one inclusion may exist per infected cell and there may be variable numbers of organisms per inclusion, either sparsely distributed or in compact morula-like clusters. Electron micrographs of the organism in naturally or experimentally infected fish tissues and infected cell lines (FHM, EPC, CHH-1, and CHSE-214) show similar prokaryotic ultrastructural characteristics. In Gram or Giemsa-stained smears from heavily infected tissues, the organism may be seen singly, in pairs, small groups, or in larger clusters either intracellularly or extracellularly.

Taxonomically, the organism appears to belong to the order Rickettsiales, family Rickettsiaceae, but not to the tribe Rickettsieae. Preliminary serological findings and morphological characteristics suggest it may belong to the tribe Ehrlichieae. The organism produces a septicemia in which anemia and

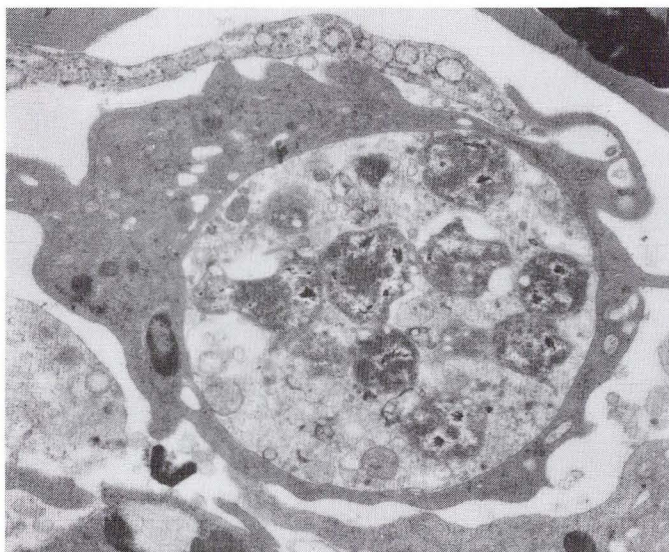


Figure 1. Electron micrograph of a spleen cell from an infected coho salmon. Note the 8 rickettsial-like organisms within the large cytoplasmic vacuole. Magnification is 9,170 X.

Rickettsiales

1990

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FHS



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kidney swelling are common signs. Hematocrits have ranged from 4 to 33% among infected fish and 35 to 50% among apparently healthy fish. Heavily infected fish sometimes manifest a liver with characteristic mottling. Histopathological examinations of selected tissue from moribund coho have shown severe necrosis and inflammation in the liver, spleen, intestine, and hematopoietic tissue of the kidney. Vascular lesions, characteristic of those caused by rickettsial organisms, were seen primarily in the liver, kidney, and spleen. These consisted of acute necrotizing vasculitis and thrombosis as well as perivascular necrosis and inflammation. Intracellular organisms were apparent in all tissues mentioned above. In the liver tissue they were scattered diffusely in the cytoplasm of hepatocytes, but were more often seen within vacuoles typical of those reported for rickettsial diseases of other animals. Mild lesions were also seen in pancreatic, heart, and ovarian tissues. Organisms were also seen within the cytoplasm of macrophages in the form of morula-like inclusions in Giemsa-stained peripheral blood smears.

The disease has only been reported to occur in sea water, however we have reproduced it experimentally in fresh water. Additionally control fish in the same "infected" fresh water or sea water aquaria died of the disease, but not control fish in a separate "uninfected" control aquaria, thereby demonstrating horizontal transmission as a means of infection. No intermediate vectors were detected.

Examination of tissues from experimentally infected fish showed the same lesions as described for naturally infected fish, the most severe being in hematopoietic tissues. The organism has also been observed in gills, brain, visceral fat, nares, skin, and muscle.

In January 1990, mortalities among Chilean, Atlantic salmon in seawater were observed for the first time with the same disease agent. Microscopic observations of infected tissues, gross clinical signs, and histopathological findings were similar to those seen in coho. The same organism is also suspected in samples from moribund rainbow trout.

Antibiotic field trials in Chile are ongoing, however, the diverse and mixed treatment regimens generally used by fish farmers in the past have not provided any clear evidence of an effective antibiotic or drug. *In vitro* studies with a number of potential therapeutics are also ongoing.

The disease has been called "Coho Salmon Syndrome" and "Huito Disease". However, in light of new information and in the interest of being more descriptive, we propose the disease be called "Salmonid Rickettsial Septicemia".

## VHS VIRUS DETECTED AT LUMMI BAY SEA PONDS, BELLINGHAM, WASHINGTON

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Viral Hemorrhagic Septicemia Virus (VHSV) has been detected again in Washington State. The isolation occurred in adult coho salmon at the Lummi Tribe's Lummi Bay Sea Pond Hatchery, a hatchery located near Bellingham, Washington in Northern Puget Sound. This detection of VHSV marks North America's third such discovery since the fall of 1988 when the virus was first found at Glenwood Springs, Orcas Island, Washington. Adult coho returned to the Lummi Bay Sea Pond saltwater trap from September 14 to November 16. They were moved into cement circular ponds, ripened in brackish water, and spawned between November 20 and December 28. We detected VHSV in one of 14 pooled ovarian fluid samples taken during the fourth spawn. The virus was detected from samples taken on December 11, 1989. All adult coho sampled before (N = 318) and after (N = 914) December 11 tested negative for VHSV.

Ovarian fluid samples taken on December 11 were centrifuged and then diluted 1:2 in MEM-O diluent media with an antibiotic/polyethylene glycol mixture



(see Brunson et al., FHS Newsletter 16(4):3). We held the prepared samples in antibiotics overnight at 4 C before inoculating 0.1 ml of the samples onto replicate wells of EPC cell monolayers. After a one-hour incubation period, we overlaid EPC plates with 0.8% methycellulose in TRIS-buffered MEM-5 (Burke and Mulcahy, 1980). After a seven-day incubation period at 15 C, cytopathic effects on EPC cells similar to IHNV appeared. Our first attempts to neutralize the virus with IHNV monoclonal (RB/B5) and polyclonal (Cedar River) antibodies proved unsuccessful, as did USFWS Fisheries Research Center-Seattle's probing of the virus with an experimental DNA probe specific for IHNV. An immunoblot assay run by USFWS' Olympia Fish Health Center on December 30 first pointed to VHSV. Positive confirmation occurred on January 4, 1990 when USFWS' National Research Center-Seattle successfully completed their serum neutralization test. Typical virions for VHS were later confirmed ultrastructurally.

To prevent the virus from spreading, the Lummi tribe destroyed all eggs and fry at the Lummi Bay Sea Ponds Hatchery. Because nondisinfected green eggs had been taken from VHSV positive parents at Lummi Bay Sea Ponds on December 11 and transferred to Skookum Creek Hatchery—thereby potentially exposing the incubation and early rearing areas to the virus—the Lummi Tribe also destroyed all eggs and fry at Skookum Creek. They destroyed a total of 6,000,000 coho eggs and fry, 5,000,000 pink salmon fry, and 150,000 fall chinook fry. Both the Lummi Bay Sea Ponds and the affected areas at Skookum Creek Hatcheries have since been disinfected, and sentinel rainbow trout fry now are live-boxed in the hatcheries effluent.

Since finding VHSV, the Tribal Fish Health Center have tested fish and shellfish in Lummi Bay; to date, we have found no further evidence of the virus. We have noticed the following similarities between this isolation and the previous two: (1) the time of year, all isolations occurred in mid-December; (2) the age and fish species, one of the two other cases involved returning adult coho; and (3) the proximity of the adults to saltwater, all infected adults were in or near saltwater. We plan to continue sampling saltwater fish and shellfish hoping to find a potential saltwater reservoir.

#### THE FOURTH (AND FIFTH?) ISOLATION OF VIRAL HEMORRHAGIC SEPTICEMIA VIRUS IN WASHINGTON STATE

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The viral fish pathogen VHSV (Viral Hemorrhagic Septicemia Virus), discovered in North America for the first time last year in Washington state, has been isolated during routine examination of fish at the Washington Department of Fisheries Soleduck Hatchery. All affected eggs have been destroyed and all suspect adult carcasses have been buried in lime.

The 1988-89 VHSV isolates, found last season at the Makah and Orcas Is. facilities and this season at the Lummi Bay sea ponds, were from hatchery-reared salmonids. The newest VHSV isolates were not from hatchery-reared fish, rather they were from wild adult coho salmon broodstock captured from the Bogachiel and Soleduck Rivers and brought to be Soleduck hatchery. Since the VHSV-positive fish came from two different rivers and were held in pathogen-free spring water at the hatchery it can be argued that these two isolates represent the fourth and the fifth isolation of VHSV in the state.

The fish were captured from the respective rivers, brought to and held at the hatchery in spring water, and spawned on December 19, 1989. Upon routine examination, virus was isolated from ovarian fluid of one five-fish pool from the Bogachiel River stock (1221-3/1.4) and milt of one five-fish pool from the Soleduck River stock (1221-4/1.7). Initial serum neutralization assays indicated neither isolate was neutralized by anti-VHSV polyclonal antiserum. Subsequent

serum neutralization assays showed both isolates were neutralized by anti-VHSV polyclonal antisera and not by anti-IHNV polyclonal antisera or the three monoclonal antibodies RB/B5, SRCV/B3, or 193-110 (Table 1). Studies conducted in our lab has shown that either the RB/B5 or the 193-110 monoclonal antibody, and often both, will react with all 20 different Washington isolates of IHNV so far tested. The serologic identity of the virus was also confirmed by a dot-blot assay, conducted by Ray Brunson and Kim True at the USFW Olympia Fish Health Center.

There are about 13.5 million fish or eggs which either are currently at the hatchery or have been removed from the facility but were present at some time when the VHSV-positive stocks of coho were also on station. Unfortunately, all these fish are now considered suspect and are quarantined to the two rivers. This most certainly will have some effect on stock management as many of these fish were destined for release into other watersheds besides the Bogachiel or Soleduck Rivers. Discussions are now in progress concerning the management of the Bogachiel and Soleduck watersheds for VHSV and potential research projects concerning the pathogenic effects and possible reservoirs of VHSV in Washington.

#### Edwardsiella ictaluri ISOLATE RESISTANT TO ROMET-30 AND TERRAMYCIN

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Two isolates of *Edwardsiella ictaluri* from the brain of two channel catfish examined during a single fish kill investigation were resistant to both Romet-30 and Terramycin, the only two registered antibiotics for treating catfish. The bacteria, grown on Mueller-Hinton sensitivity plates (1 with blood; 1 without blood), showed no zones of growth inhibition around the Romet-30 (1.2 mcg ormetoprim and 23.8 mcg sulfadimethoxine) and Terramycin (30 mcg oxytetracycline) discs. Bacterial identification with biochemical and specific polyclonal antibody tests, and sensitivity results were confirmed by Dr. John Plumb, Auburn University.

When these moribund catfish were examined (14 November 1989), water temperature was 17°C which is not in the optimum temperature range (22-28°C) for *E. ictaluri* infections. The catfish had fed poorly (less than 2% body wt. per day) during the preceding growing season and were anorectic when examined in November. A heavy infestation of *Trichodina* on the gills was diagnosed as the primary cause of mortalities. Temperatures were well below the range where *E. ictaluri* is most dangerous so the bacteria was not considered the primary cause of death. A copper sulfate treatment was applied in late November immediately after the majority (40,000 pounds) of fish in the pond were removed during harvest. The CuSO<sub>4</sub> treatment and/or reduction in fish density halted fish mortality. No further mortalities occurred until thick ice formed on the pond on December 20 and remained for over a week in late December, 1989. When the ice thawed shortly before January 1990, about 500 to 600 dead fish were discovered.

Before the November harvest, this 16-acre pond was stocked at 2,000 harvestable-size fish per acre (approximately 0.6 kg each) and 20,000 juvenile (20-277 g) fish per acre.

The pond owner estimated that 5,000 pounds of fish died during the course of the disease in fall 1989. It is speculated that *E. ictaluri* was the primary cause of mortality during this time although moribund fish had not been necropsied until November. By this time *E. ictaluri* was no longer the primary cause of mortality.

Most catfish farmers in Mississippi have learned to diagnose *E. ictaluri* infections by observing its characteristic clinical signs. Many farmers begin treating the disease with medicated feed without first having a fish sample examined by a fish disease specialist. In the past, odds have been with the farmer that



both Terramycin and Romet-30 medicated feeds would be effective in treating *E. ictaluri* infections. Now the possibility also exists that *E. ictaluri* may be resistant to Romet-30 and/or Terramycin, thus further complicating the treatment challenge. This complication makes it imperative for catfish farmers to obtain a complete and accurate diagnosis of fish suspected of having *E. ictaluri* infections to help ensure that the antibiotic used to treat their fish will be effective.

## YOUNG SALMON MAY BE ADVERSELY AFFECTED BY BORON AND SELENIUM

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Concern has been raised regarding the potential effects of irrigation-derived elements such as boron, molybdenum, and selenium on fish inhabiting rivers which receive substantial inflows of subsurface agricultural drainage water. Young salmon hatched in tributaries of the Sacramento and San Joaquin Rivers migrate downstream to rearing areas in the Sacramento-San Joaquin Delta. As young salmon move through fresh water and rear in the brackish Delta, they could be exposed to waterborne concentrations of various elements. Toxicity tests showed that boron and selenium (as environmentally relevant mixtures of selenate and selenite-inorganic selenium compounds commonly found in drainwater) had low margins of safety for young salmon in both fresh and brackish waters, and therefore could be exerting sublethal toxic effects on young salmon.

Acute toxicity tests were conducted using various waterborne concentrations of boron, molybdenum, selenate, selenite, selenomethionine (a common organic selenium compound), and mixtures of the first four elements. These tests were conducted with swimup and advanced-fry life stages of chinook salmon (*Oncorhynchus tshawytscha*) and coho salmon (*O. kisutch*) in reconstituted, site-specific waters. Coho salmon do not typically occur in the San Joaquin River but were included in tests to compare the sensitivity of the two species. Swimup fry were tested in freshwater simulating the dilution of an agricultural drainage water (containing high concentrations of salts dominated by sodium and sulfate but minus toxic elements) such as the San Luis Drain. Tests with advanced fry (about 70 mm) were conducted in a similar manner to those with the swimup life stage except that slightly brackish water (about 1 ppt salinity) was used instead of freshwater. These tests simulated environmental conditions that might be present if large amounts of irrigation return flows were discharged into freshwater at a time when young salmon would be moving downstream, or into the Delta, where young salmon are reared before smoltification and migration to the ocean. The endpoint of acute toxicity tests was fish death, and the measurement value was the concentration that killed 50% of the test fish in a 96-hour exposure period.

At both life stages, coho salmon were more sensitive than chinook salmon to both selenate and selenite (Table 1). Swimup fry of coho salmon were more sensitive than chinook salmon to boron, but there was no difference between swimup fry and advanced fry of either species (Table 1). Tests with molybdenum indicated that it is relatively nontoxic to both life stages of both species (i.e., 96-hour LC50 values were > 1000 ppm). Selenite was significantly more toxic than selenate to both species (Table 1). Sensitivity to the toxic effects of selenate and selenite was greater in swimup fry tested in freshwater than in advanced fry tested in brackish water for both species (Table 1). No deaths occurred in any of the tested concentrations of selenomethionine; however, at the highest concentration (21.6 ppm), at least 50% of the fish showed pronounced surfacing behavior, which indicated stress. In additional tests with swimup chinook salmon, differences in the salinity characteristics of the test waters did not modify the toxicities of the elements; boron toxicity values ranged from 566 to 725 ppm,

selenate values ranged from 114 to 149 ppm, and selenite values ranged from 13 to 17 ppm. Tests with environmentally relevant combinations of boron, selenate, and selenite demonstrated that the joint toxic action of these mixtures was purely additive rather than antagonistic or synergistic.

One criterion used in estimating the hazard of chemical substances to aquatic organisms is the margin of safety between expected environmental concentrations of a chemical and concentrations of the chemical that cause toxic effects. Based on acute toxicity values, safety margins less than 100 (i.e. toxic concentrations > 100 times environmental concentrations) indicate a high potential for environmental damage. The expected environmental concentrations used in our calculations were those present in the San Luis Drain (boron = 13 ppm, selenium = 0.35 ppm). In our tests with chinook salmon, the margin of safety for boron was only 56 in freshwater and 46 in brackish water. The margins of safety for selenate and selenite respectively, were 383 and 276 in freshwater and 479 and 468 in brackish water. However, the margin of safety for selenate and selenite combined in an environmentally realistic mixture was 145 in freshwater and 220 in brackish water. Similar margins of safety were calculated for coho salmon. The low margins of safety for boron indicate that this element could exert sublethal toxic effects on young salmon.

Although the margins of safety for waterborne mixtures of selenate and selenite were greater than 100, aquatic organisms (especially fish at the top of the food chains) also would be exposed to dietary sources of selenium. Previous research at our laboratory has shown that selenium concentrations as low as 5 ppm in the diet cause adverse effects in young chinook salmon. Concentrations of selenium this great would be readily bioaccumulated in aquatic food organisms even though water borne levels were as low as 0.01 ppm. Thus, the relatively low hazard indicated for waterborne selenium alone is greatly modified when dietary sources are also considered.

This study was supported by the San Joaquin Valley Drainage Program, a cooperative effort between the U.S. Department of the Interior and the State of California.

**Table 1. Acute toxicity (96-hour LC50 values in ppm) of selenium and boron to different life stages of chinook salmon and coho salmon.**

Chemical	Water Quality	Advanced	Chinook Salmon	Coho Salmon
Selenite	Freshwater	Swimup	13.8	7.8
	Brackfish	Advanced	23.4	13.6
Selenate	Freshwater	Swimup	115	32.5
	Brackfish	Advanced	149	39.0
Boron	Freshwater	Swimup	725	447
	Brackfish	Advanced	600	600

## INFECTIOUS HEMATOPOIETIC NECROSIS VIRUS (IHNV) TRANSMISSION STUDIES IN OREGON

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Two major questions are being addressed by the ODFW pathology staff regarding IHNV and its hatchery populations. The first question is whether progeny can become infected from adults carrying the virus (vertical transmission)?



Trials have been conducted throughout Oregon where progeny from carrier and virus-free adults are kept segregated and tested for IHNV. To date, over 9 million fish have been reared with no evidence for the vertical transmission of the virus. These data were collected from two different anadromous species (chinook salmon and steelhead) and four different stocks (Table 1). In most cases, the eggs were either water-hardened or surface disinfected with iodophor. In all cases, egg incubation and early rearing was done in virus-free water.

**Table 1. Production trials of rearing progeny from adult salmonids infected with infectious hematopoietic necrosis virus (IHNV).**

Facility	Species	Year	IHNV Carrier Rate		Resulting Progeny		IHNV Tests
			Female	Male	Positive	Negative	
Bonneville	Chinook	1986	63%	45%	3.5 mil.	0.5 mil.	NEV <sup>a</sup>
		1987	52%	42%	4.7 mil.	1.2 mil.	NEV
Ronde Butte	Steelhead	1984	60%	21%	64,000	64,000	NEV
		1985	71%	16%	100,000	100,000	NEV
		1988	71%	12%	60,000	19,000	NEV
Elk River	Chinook	1985	10%		70,000	50,000	NEV
		1986	37%		75,000	50,000	NEV
		1987	7%		75,000	50,000	NEV
Irrigon H.	Steelhead	1986	22%	48%	0.5 mil.	1.1 mil.	NEV

<sup>a</sup>No evidence of virus.

The message seems fairly clear. Vertical transmission of IHNV does not occur.

The second question being examined is: Do progeny from IHNV-positive adults return as carriers of the virus (covert transmission)? For the last three years at Elk River Hatchery, and in 1986, at Bonneville and Irrigon Hatcheries, progeny from carrier and virus-free parents were differentially marked before release. By analyzing the adults for IHNV as they return, we hope to be able to determine if covert transmission occurs. In 1987 at Elk River Hatchery, jack chinook salmon (2 yr old adults) from the 1985 brood year returned to the hatchery. No virus was detected. In 1988 at the same hatchery, jacks from the 1986 brood and 3-yr-old adults from the 1985 brood returned to the hatchery. No IHNV were detected in either of these groups (Table 2). Marked adults at these facilities will be evaluated over the next five years, but it seems probable at this point that covert transmission does not take place.

**Table 2. Prevalence of infectious hematopoietic necrosis virus (IHNV) in returning adults to determine of covert transmission of IHNV occurs.**

1987-88 Results of Marked Fall Chinook Jacks: 1985 Brood Year						
IHNV Status	Mark	Number Sampled	Proportion of Sample Pools IHNV (+)			Result
			Milt	Spleen	Gill	
Negative	LV <sup>a</sup>	16	0/16	0/16	0/16	NEV <sup>c</sup>
Positive	RV <sup>b</sup>	12	0/12	0/12	0/12	NEV

1988-89 Results of Marked Fall Chinook Jacks: 1986 Brood Year						
IHNV Status	Mark	Number Sampled	Proportion of Sample Pools IHNV (+)			Result
			Milt	Spleen	Gill	
Negative	LV	35	0/35	0/35	0/3	NEV
Positive	RV	20	0/20	0/20	0/2	NEV

1988-89 Results of Marked Fall Chinook Adults: 1985 Brood Year						
Males						
IHNV Status	Mark	Number Sampled	Proportion of Sample Pools IHNV (+)			Result
			Milt	Spleen	Gill	
Negative	LV	25	0/25	0/25	0/2	NEV
Positive	RV	22	0/22	0/22	0/2	NEV

Females						
IHNV Status	Mark	Number Sampled	Proportion of Sample Pools IHNV (+)			Result
			Milt	Spleen	Gill	
Negative	LV	12	0/12	0/12	0/12	NEV
Positive	RV	2	0/2	0/2	0/2	NEV

a Left ventral fin clip.

b Right ventral fin clip

c No evidence of virus

## CALL FOR NOMINATIONS

### S. F. Snieszko Distinguished Service Award

The S. F. Snieszko Distinguished Service Award is the highest award presented by the Fish Health Section of the American Fisheries Society. Dr. S. F. Snieszko was dedicated to excellence in research, teaching, and service in the fish health sciences and the first recipient of the Distinguished Service Award. The purpose of this award is to recognize a fish health scientist(s) for their continued outstanding contributions to the field of fish health.

The individual(s) to be considered for the Distinguished Service Award must be nominated by a current member of the Fish Health Section. The person nominating the individual for the award is expected to solicit and obtain six letters of recommendation from fish health scientists in support of the nominee. These letters shall address the candidate's dedication to research, teaching, and service. Letters of recommendation should be sent to the nominator. These six

letters should be accompanied by a letter of nomination that clearly states the qualities of the candidate and the specific reason(s) he or she is being nominated for this award. A current curriculum vitae for the candidate should also be obtained and sent with the nomination packet directly to **Dr. John Fryer, Department of Microbiology, Oregon State University, Corvallis, OR 97331**. It is suggested that persons nominating or providing letters of support for candidates maintain confidentiality throughout the process.

The process for selection of award recipient(s) is described in the By-laws of the Fish Health Section under the Awards Committee.

The By-laws indicate a certificate will be prepared and presented to the recipient at the annual meeting. In addition, their name(s) will be placed on a plaque with those of previous recipients. This plaque will remain at the U.S. Fish and Wildlife Service Laboratory in Leetown alongside Dr. Snieszko's portrait.



## SNIESZKO GRADUATE TRAVEL FUNDS

Travel awards will be given to graduate students needing funds to present a talk or poster at the Fish Health Section Annual meeting in Bloomington, Minnesota this summer. For the first time, these competitive awards will be granted from funds given to the FHS by Dr. S. F. Snieszko. Selection will be made by the committee consisting of Doug Anderson, West Virginia; Pete Walker, Colorado; and Alec Maule, Oregon. Candidates are requested to send a cover letter explaining their needs, a letter of recommendation from their professor or mentor and a tentative abstract to one of these committee members by April 15, 1990. Recipients will be notified by May 15.

## ISOLATION OF AN OXIDASE-NEGATIVE *AEROMONAS SALMONICIDA* FROM COHO SALMON

Patrick F. Chapman  
Washington Department of Fisheries  
115 General Administration Building  
Olympia, WA 98504

An atypical, oxidase-negative *Aeromonas salmonicida* was isolated from coho salmon fingerlings being reared at Washington Department of Fisheries' (WDF) Coulter Creek Hatchery in June, 1989. These fish had been transferred in May from WDFs' Skykomish Hatchery. Mortality increased from normal levels in early June, two weeks after the population was divided into two rearing ponds.

Necropsies of dead and moribund fish indicated systemic bacterial infections were present in the majority of fish examined, although few external or internal signs were observed. Inoculations onto Coomassie Brilliant Blue agar (CBB) from kidney tissue yielded dark blue colonies that produced a brown, diffusible pigment within two days. Previous experience with CBB indicated these colonies were characteristic of *A. salmonicida*. Other colony types were not detected. Two isolates selected for further study were biochemically and morphologically similar, except that one isolate was oxidase-negative and exhibited no zone of sensitivity to oxytetracycline by the disc diffusion technique. Both isolates gave a strongly positive FAT reaction using conjugate prepared against *A. salmonicida*. Despite the atypical oxidase reaction, both isolates were presumptively identified as *A. salmonicida* and were subsequently confirmed by Rocco Cipriano, Jeff Teska and Bane Schill of the National Fish Health Research Laboratory in Leetown, WV.

Prior to completion of drug sensitivity testing, treatment with oxytetracycline-medicated feed was initiated, which reduced mortality. Reexamination of fish in August resulted in isolation of both oxidase-negative and oxidase-positive *A. salmonicida* from most moribund and dead fish. However, only one type was cultured from any particular fish. Following five days of treatment with Romet-medicated feed, mortality returned to normal levels and no *A. salmonicida* could be isolated from the population when examined in October.

Oxidase-negative isolates of *A. salmonicida* have not been previously reported. Strict adherence to Blue Book procedures for identification of bacteria would have resulted in misidentification of this isolate of *A. salmonicida*. Caution should be exercised when using Blue Book or other schemes for identifying fish pathogenic bacteria because all rely on results of the oxidase test early in the sequence of steps leading to identification.

*Editor's note: All aerobic bacteria contain a cytochrome oxidase enzyme; however, that enzymes substrate specificity may change through various mutations. The Blue Book oxidase test uses a 1% solution of dimethyl-p-phenylenediamine for substrate. Testing other substrates, e.g. tetramethyl phenylenediamine di-HCl, should indicate the presence of an oxidase enzyme. Diagnostically it is important to standardize reagents but we must be aware that identification of infectious agents is often presumptive and not definitive until confirmatory tests are completed. Extrapolation of biological behavior or physiologic limits from these diagnostic tests may not be appropriate.*

## CCV REPLICATION IN MYCOPLASMA-CONTAMINATED AND FREE CCO CELLS

Mycoplasma contamination is one of the most serious problems that tissue culturists must deal with. Tissue cultures are easily contaminated with mycoplasmas but they are difficult to remove. Mycoplasma can adversely affect tissue culture performance affecting the cells virus replication capability, affect cellular physiology and may even kill the cell cultures. Some antibiotics, Gentamycin for example, inhibit mycoplasma reproduction in cell cultures, but does not eradicate these contaminants. Recently reagents have become available that will eradicate mycoplasma from cell culture. Channel catfish ovary cells (CCO) developed at Auburn University have been contaminated with mycoplasma for a long time. Inclusion of Gentamycin (50 mg/ml) has made these cells useful in routine channel catfish virus (CCV) assays and other non-critical CCV research. After receiving starter cultures of CCO cells, Larry Hanson of Louisiana State University successfully eliminated mycoplasma from a CCO culture. A culture of the mycoplasma free cells was returned to Auburn. We performed comparative CCV replication studies and titrations of CCV in mycoplasma free CCO cells and mycoplasma contaminated CCO (with Gentamycin) cell cultures. Titrations of CCV grown in mycoplasma free cells and titrated in contaminated CCO

**Table 1. Titrations (TCID<sub>50</sub>) of CCV from CCO cells contaminated with mycoplasma and without mycoplasma and titrated in the same and reciprocal CCO cultures.**

CCV Isolate	Titrated in		Titrated in Mycoplasma Free Cells	
	CCV From Mycoplasma Free Cells	CCV From Mycoplasma Contaminated Cells	CCV From Mycoplasma Free Cells	CCV From Mycoplasma Contaminated Cells
AL-89-299	10 <sup>6.83</sup>	10 <sup>6.53</sup>	10 <sup>7.17</sup>	10 <sup>6.83</sup>
AL-89-202	10 <sup>5.75</sup>	10 <sup>6.62</sup>	10 <sup>7.5</sup>	10 <sup>7.83</sup>
AL-89-369	10 <sup>5.68</sup>	10 <sup>6.5</sup>	10 <sup>5.62</sup>	10 <sup>7.83</sup>
AL-89-267	10 <sup>7.83</sup>	10 <sup>7.25</sup>	10 <sup>7.12</sup>	10 <sup>6.62</sup>
AL-89-254	10 <sup>5.5</sup>	10 <sup>6.62</sup>	10 <sup>6.32</sup>	10 <sup>6.5</sup>
UNKNOWN	10 <sup>6.75</sup>	10 <sup>7.5</sup>	10 <sup>7.75</sup>	10 <sup>6.5</sup>

cultures ranged from 105.5 to 107.83 and the same virus titrated in mycoplasma free cells ranged from 105.62 to 107.75. Although the titers in the noncontaminated cultures were generally higher there were no significant differences ( $p < 0.05$ ) (Table 1). The same pattern was true for CCV grown in mycoplasma contaminated cells and titrated in contaminated and non-contaminated cells. Although mycoplasma contaminated CCO cells would not be desirable for critical cell culture studies it does not appear that they affect CCV isolation or CCV replication. For more information contact John A. Plumb, Department of Fisheries and Allied Aquacultures, Auburn University, AL 36849-5419 (205-844-9215) or Larry Hanson, College of Veterinary Medicine, Dept. of Pathology, LSU, Baton Rouge, LA 70803 (504-346-3308).

## FROM THE PRESIDENT

John H. Schachte, President  
Fish Health Section/AFS

At midterm in the section's business year, many committee activities are moving forward. Plans for our next annual meeting in July are firmly in place. General meeting chairman, Paul Bowser, and local arrangements chairman, Joe Marcino



are well ahead of schedule in providing a strong program as well as comfortable and reasonable meeting facilities in Minneapolis. The joint meeting with the Midwest fish disease workshop group should present attendees with interesting options in the area of applied and research-oriented presentations. The executive committee approved a request by the Professional Standards Committee (PSC) chairman, John Cvitanich, to underwrite expenses for a special meeting of the PSC in February. The purpose of this meeting was to review and update the section's two certification programs as well as plot strategy for new initiatives within the area of professional standards. EXCOM appropriated expense funds from the Sneiszko fund with any difference coming from our general account. Work on revision of the Blue Book is progressing according to committee chairman John Thoesen. Hopefully, a draft will be ready for peer review next fall. In conjunction with this effort, work begun during former President Hedrick's term on a Blue Book for shellfish diseases, chaired by Ralph Elston, is near completion. The issue now for the section is how it should be published and disseminated. Should we have one Blue Book for finfish and shellfish or should they be separate? This is an issue that should be the subject of input broader than EXCOM. Therefore, I ask you to give it some thought and we will consider it as an item of business in July in Minneapolis. An additional item related somewhat to the Blue Book is a request from several of our members represented by John Hnath of Michigan. Specifically, John is interested in member's thoughts on the desirability of establishing a document that defines standard procedures for hatchery disinfection. Many of us, myself included, have had the opportunity to conduct one or more disinfections. For the most part we've had to rely on "hand-me-down" information or that which might be found in obscure reports. What do you think? Should we undertake such an effort, the mechanics of which would have to be worked out? Please let John know what your thoughts are. Drop him a note at the Fish Health Laboratory, Wolf Lake State Fish Hatchery, 34270 C.R. 652, Mattawan, MI 49071, (616) 668-2132. The Membership and Balloting Committee is moving forward with efforts to establish a new membership directory. Kathy Hopper has included the membership information forms in the current issue of the newsletter. This is a most useful reference for all of us in the section. It was last revised in 1986. Please cooperate with Kathy and her committee and promptly return the information forms included in this newsletter.

## EDITORIAL

Public concern for seafood safety has sparked congressional interest in mandating a seafood inspection program. This program would apply to both wild and aquaculturally produced finfish and shellfish. The concerns center around contamination of fisheries products by infectious organisms, toxins or chemicals. While seafood is basically safe and very few reports document safety problems, oversight by federal and state authorities appears appropriate since current public awareness of potential problems exists. Reduced per capita seafood consumption in 1989 has been attributed to public concern for seafood safety. For aquaculturally produced products to expand their market, the consumer must feel they are purchasing a safe and wholesome product. If a mandatory seafood inspection program is instituted and allays public concern then the aquaculture and seafood industries may be well served.

Of greatest concern to federal agencies regarding aquaculture is aquaculture's use of antibiotics or chemical therapeutants. The concern is that illegal or inappropriate use of antibiotics or other food additives or water treatments may pose a human safety problem if these fish are consumed. The Food and Drug Administration (FDA) for example, is currently examining the uses of therapeutants and is conducting inspections at aquaculture facilities to gather information needed to formulate an inspection program. Fish Health Specialists have a major responsibility to promote legal use of therapeutants by aquaculturists and try to ensure established withdrawal times are followed. While fish health specialists cannot be accountable for fish farmers who assume responsibility for diagnosing disease or deciding on treatments, we can direct our efforts towards educating these individuals in proper, legal use. We also need to ensure that we follow established federal guidelines for therapeutant use. — *Randy MacMillan, editor.*

## NOVEL ICH TREATMENT AVAILABLE

A device that generates low electric current to treat ichthyophthiriasis has been developed. Details are sparse, but 100% efficacy against tomite and trophonts is claimed. Details may be obtained from Dr. E. Ieshko, Biological Institute, Karelian Branch of the USSR Academy of Sciences, Pushkinskaja, 11: Petrozavodsk, 185610 USSR. Telex 4 422-II3. Submitted by Dr. Richard Heckman, Brigham Young University.

## PASSAGES

Hugh Mitchell, DVM, is the new corporate veterinarian/fish health manager for Ocean Products, Inc., one of North America's largest salmon farming corporations. He received his training at the University of Guelph. His address is: P.O. Box 263, Estes Head, Eastport, Maine 04631. Phone: (207) 853-6081.

Dr. Chris Wilson (formerly of Mississippi State University) has joined the staff of Utah Division of Wildlife Resources, Fisheries Experiment Station, 1465 West 200 North, Logan, Utah 84321; 801-752-1066.

## GRADUATE STUDY STIPENDS

The **Louisiana State University** School of Veterinary Medicine offers advanced training stipends for both M.S. and Ph.D. applicants for studies on the molecular biology of bacterial diseases of aquatic animals, particularly the channel catfish. Ongoing research emphasizes studies on bacterial pathogenesis, vaccine development, and host immunity to bacterial diseases. Projects utilize a variety of modern biotechnical procedures, including antigen analysis, monoclonal antibodies, and recombinant DNA. Training in the latest biotechnical techniques is conducted by a supportive, active faculty using state of the art equipment in spacious, new laboratories. Opportunities also exist for clinically related studies through the Aquatic Animal Disease Diagnostic Laboratory. Interested applicants should send a letter of inquiry, along with GRE scores and college transcripts, to Dr. Ron Thune, Department of Veterinary Microbiology and Parasitology, Louisiana State University, Baton Rouge, LA 70803. Phone 504-346-3308. LSU is an equal opportunity employer.

**Mississippi State University**, College of Veterinary Medicine is seeking applications for graduate training positions in fish immunology, bacteriology, pathology, virology, and molecular biology. Each position allows for full-time commitment to pursuit of M.S. or Ph.D. Available technologies include ELISA, monoclonal antibodies, cell culture, fluorescent activated cell sorting, mass spectroscopy, nucleic acid hybridization and many other conventional technologies. Stipends are available and are dependent on qualifications. Qualified applicants should submit a curriculum vitae, complete transcripts, a statement of career goals and interests, and three letters of reference to: Dr. Jerald Ainsworth, Coordinator of VMS Graduate Program, College of Veterinary Medicine, P. O. Drawer V, Mississippi State, MS 39762. Mississippi State University is an equal opportunity, affirmative action educator and employer.

## HONORS

**Jim Warren**, fish health manager for the U. S. Fish and Wildlife Service's Pacific Region, and a career Federal Employee for 30 years, has been selected as one of the agency's 20 most outstanding employees for 1989. *Congratulations, Jim.*



## 1990 FISH PATHOLOGIST EXAMINATION

Individuals seeking certification as an AFS/FHS Fish Pathologist must have their application submitted and approved by June 1, 1990 in order to take the qualifying exam. The examination will be scheduled during the month of July, 1990. Examinees will be notified of exact dates. Application forms and information concerning required qualifications can be obtained by calling the Chairman of the Board of Certification, Ralph Elston, at 206-683-4151 or the Chairman of the Professional Standards Committee, John Cvitanich at 503-746-1442.

## CALL FOR MANUSCRIPTS

The Journal of Aquatic Animal Health seeks fish health manuscripts for inclusion in upcoming volumes. The backlog resulting from the FHS International meeting is over and publication of accepted submissions should be expected within 6 months. Contact Dr. Bill Rogers, John Plumb or John Grizzle (Eds.), Fisheries and Allied Aquaculture, Swingle Hall Auburn University, Auburn University, AL 36849-5419

## MEETINGS

**The Second Biennial Fish Disease Diagnosticians Workshop** will be held March 20 and 21, 1990 at the Auburn University Hotel and Conference Center. Contact Dr. Yolanda Brady (205-844-9122) for details. The workshop is sponsored by the Southeastern Cooperative Fish Disease Project, Fisheries and Allied Aquacultures, Auburn University and Gulf States Consortium for Aquatic Pathobiology.

**International Association of Aquatic Animal Medicine, 21<sup>st</sup> Annual Meeting**, May 13-17, 1990. For details contact Dr. Ruth Francis-Floyd, 7922 NW 71st St., Gainesville, FL 32606. Phone 904-392-9617.

**Eastern Fish Health Workshop**, June 17-19, 1990. Charlottetown, PEI, Canada. Contact Dr. Dave Groman, Department Pathology and Microbiology, Atlantic Veterinary College, University of Prince Edward Island, 550 University Ave., Charlottetown, PEI. C1A 4P3 Canada. Telephone: 902-566-0831, FAX 902-566-0958.

**Bacterial Diseases of Fish**, June 26-29, 1990. Biennial Symposia organized by the Institute of Aquaculture at the University of Stirling in conjunction with BP Nutrition (UK) Ltd. and Scottish Salmon Growers Association. Further details from Dr. N. Bromage, Conference Secretariat: Bacterial Diseases of Fish, Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland.

**31st Annual Western Fish Disease Conference**, Shore Lodge, McCall, Idaho. June 28-30, 1990. For information regarding program or hotel arrangements, contact Dr. Keith A. Johnson, Fish Pathologist, Eagle Fish Health Lab, 1800 Trout Road, Eagle, Idaho 83616. Phone 208-939-2413.

**Joint Meeting of Fish Health Section of the American Fisheries Society and the Midwest Fish Disease Workshop**, July 16-19, 1990. Minneapolis, MN. See insert for details.

**Symposium on Diseases of Asian Aquaculture**, November 26-29, 1990, Sawer Beach, Bali, Indonesia. Information may be obtained from Dr. Mohd. Shariff, Asian Fisheries Society, Fish Health Section, c/o Faculty of Fisheries and Marine Science, Universiti Pertanian Malaysia 43400 Serelang, Selangor, Malaysia.

## SHORTCOURSE

A shortcourse, "Diagnosis and Treatment of Diseases of Warm Water Fish" (FNR6934) will be taught at the University of Florida, June 11-22, 1990. This

shortcourse is to provide instruction in the methodology of diagnosis and treatment of parasitic, bacterial, viral, nutritional, and environmental diseases of warm water fish. Four hours of college credit are available to graduate and undergraduate students. The shortcourse is also available to veterinary students as an elective clerkship. Tuition for Florida residents is \$70.70 per credit hour. For non-residents tuition is \$205.65 per credit hour. Those wishing to receive continuing education credit in lieu of college credit are invited to participate by paying a \$250.00 registration fee in place of tuition. Instructors are Dr. Ruth Francis-Floyd and Dr. Tom Wellborn.

Prospective students should apply in writing to Dr. Ruth Francis-Floyd, IFAS Extension Veterinarian for Aquaculture, 7922 NW 71st St., Gainesville, FL 32606. The deadline for receipt of a letter of application is March 15, 1990. All applicants will be advised whether or not they have been accepted to attend before April 15, 1990.

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## REMINDER:

COMPLETED MEMBERSHIP DIRECTORY  
INFORMATION DUE MAY 1, 1990.

TEAR-OUT FORM THIS NEWSLETTER.

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**ANNOUNCEMENT**

**OF THE JOINT MEETING OF THE  
FISH HEALTH SECTION  
OF THE AMERICAN FISHERIES SOCIETY AND THE  
MIDWEST FISH DISEASE WORKSHOP**

July 16-19, 1990  
Minneapolis, Minnesota USA

The Joint Meeting of the Fish Health Section of the American Fisheries Society and Midwest Fish Disease Workshop will be held in Minneapolis, Minnesota from July 16-19, 1990. It is not too early to begin consideration of submission of an abstract for this meeting. The general meeting schedule will have Monday July 16, serve as a travel day with a reception in the early evening. Formal presentations will be on Tuesday, Wednesday, and Thursday, July 17-19. Although the nature of the program will be influenced by the type of abstracts submitted, we are looking to develop a program that will have a balance of basic and applied studies/presentations.

The 1990 meetings will be coordinated by Paul Bowser (General Meeting Chairman), Rod Horner and Charlie Suppes (MWFH Workshop Co-charimen) and Joe Marcino (Local Arrangements). Abstracts should be sent to Paul Bowser. A general time line for the meeting is as follows:

- June 15, 1990 - Receipt deadline for abstracts (please note oral or poster presentation preference)
- July 1, 1990 - Notification of abstract acceptance

The meetings will be held at the Thunderbird Hotel and Convention Center (a Best Western Motel), located adjacent to the Minneapolis/St. Paul International Airport. A block of rooms have been reserved for the meetings for the nights of 16, 17, 18, and 19 July 1990. Room rates for the meetings are \$46.00 plus tax for single occupancy and \$52.00 plus tax for double occupancy. Reservations may be made by calling the Best Western toll free reservation number (1-800-328-1931).

(for more information contact: Dr. Paul R. Bowser, Department of Avian and Aquatic Animal Medicine, N. Y. State College of Veterinary Medicine, Cornell University, Ithaca, New York 14853)



## ABSTRACT

Fish Health Section/AFS & Eastern Fish Health Workshop  
Annapolis, Maryland -- July 17-20, 1989

DETECTION OF INFECTIOUS HEMATOPOIETIC NECROSIS VIRUS IN FISH MUCUS  
SCOTT LAPATRA<sup>1\*</sup>, J. L. FRYER<sup>2</sup> AND J. S. ROHOVEC<sup>2</sup>

<sup>1</sup>Oregon Department of Fish and Wildlife, Department of Microbiology,  
Oregon State University, Corvallis, Oregon 97331-3804.

<sup>2</sup>Department of Microbiology, Oregon State University, Corvallis,  
Oregon 97331-3804.

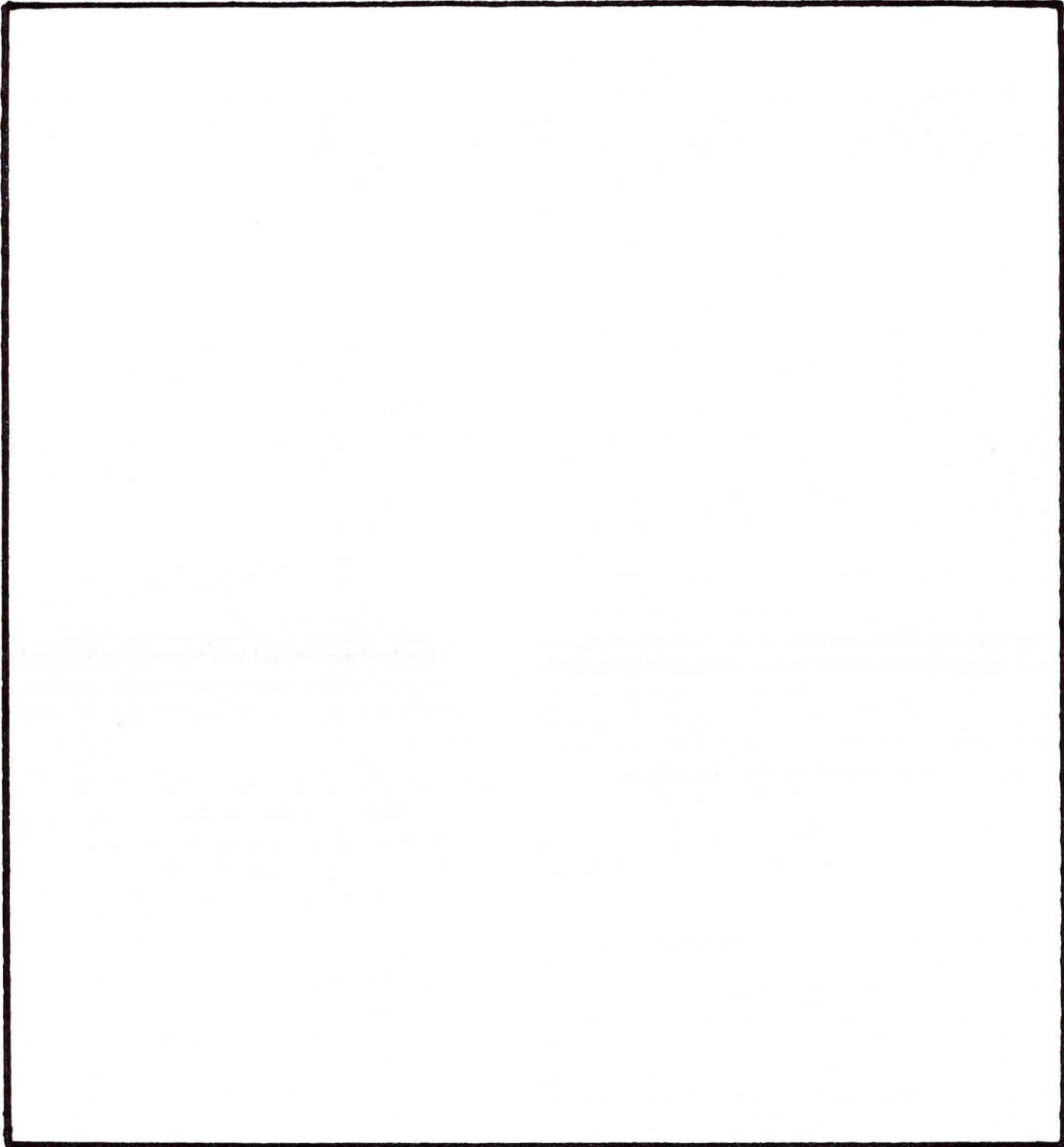
Infectious hematopoietic necrosis virus (IHNV) is usually detected by inoculating susceptible cell cultures with reproductive fluids or tissues obtained from infected or carrier fish. Studies have shown the mucus from adult fish can exhibit a higher prevalence of IHNV than standard preparations. Examination of the reproductive fluids and tissues from 36 adult salmon showed that 50% of the females and males were carriers of IHNV. If mucus, from the external surface of the same fish was tested the carrier rate increased to 96% of the females and 83% of the males. The virus was also detected by assaying mucus obtained from clinically infected juvenile fish.

Tests were conducted to determine if IHNV present in the mucus was part of the normal progression of the disease or if the mucus was adsorbing and concentrating virus present in the water. Although the virus was detected in mucus 24 h post-infection and concentrations increased in mucus over time, no virus was detected in aquaria water. The potential for using fish mucus as a source of material for virus testing may provide a technique for fish health monitoring.



**ABSTRACT**

**FISH HEALTH SECTION/AFS AND MIDWEST FISH DISEASE WORKSHOP  
MINNEAPOLIS, MINNESOTA  
16-19 JULY 1990**





PREREGISTRATION FORM

THE JOINT MEETING OF THE  
FISH HEALTH SECTION  
OF THE AMERICAN FISHERIES SOCIETY AND THE  
MIDWEST FISH DISEASE WORKSHOP

July 16-19, 1990  
Minneapolis, Minnesota USA

I will be attending the Joint Meeting of the Fish Health Section of the American Fisheries Society and the Midwest Fish Disease Workshop:

Name: \_\_\_\_\_

Address: \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

Phone: AC (      ) \_\_\_\_\_

Preregistration Fee: \$65.00 (postmarked before 15 June 1990)  
\$15.00 (OPTIONAL Mississippi River Cruise  
Tuesday evening, 17 July --  
fee must be received by 15 June  
to allow for reservations to be  
made)

\$75.00 (postmarked after 15 June 1990)

\$ \_\_\_\_\_ Amount enclosed

Please send this preregistration form and fee (check made payable to Fish Health Section/AFS) to:

Joe Marcino  
Minnesota Department of Natural Resources  
Box 25  
500 Lafayette Road  
St. Paul, MN 55155

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Hotel reservations should be made directly with:

The Thunderbird Hotel and Convention Center  
2201 East 78th Street  
Bloomington, MN 55425-1228  
(612) 854-3411  
Toll Free Reservation Number 1-800-328-1931

ABSTRACT SUBMISSION INSTRUCTIONS

FISH HEALTH SECTION/AFS AND MIDWEST FISH DISEASE WORKSHOP  
MINNEAPOLIS, MINNESOTA  
16-19 JULY 1990

Please type the abstract on the attached abstract form, keeping all printed material within the "box." Use the attached abstract from the 1989 meetings as an example. Please note: 1. The title should be in all capital letters, 2. Use superscript numbers, if necessary, to denote affiliation of authors, 3. Place a superscript asterisk (\*) following the author who will make the presentation, 4. Please use a good quality printer, as abstracts will be duplicated as they are received.

Please complete the following form and submit it with your abstract by June 15, 1990 to:

Dr. Paul R. Bowser  
Department of Avian and Aquatic Animal Medicine  
N. Y. State College of Veterinary Medicine  
Cornell University  
Ithaca, New York 14853

I am submitting an abstract for the Fish Health Section/AFS and Midwest Fish Disease Workshop Meetings.

Name: \_\_\_\_\_

Address: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Telephone: AC (     ) \_\_\_\_\_

I prefer that my presentation be: \_\_\_\_\_ an oral presentation  
\_\_\_\_\_ a poster presentation

If necessary, I would be willing to change  
the format of the presentation                    \_\_\_ yes    \_\_\_ no



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## 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 with the understanding that material is not peer reviewed and should be addressed to one of the editorial staff or to a member of the publication committee.

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DEADLINE FOR SUMMER EDITION IS MAY 15, 1990.



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