FISH HEALTH SECTION

AFS

NEWS LETTER

Volume 17, Number 2 Spring 1989

VHSV ISOLATED IN NORTH AMERICA

FROM THE EDITORS

This issue of the Newsletter contains a series of reports describing the initial isolation and characterization of viral hemorrhagic septicemia virus from salmonid fish in North America as well as the extensive sampling and eradication plans set in motion as a result of this finding. While the reports were authored by some of the participants, the whole process has involved many persons working long hours together in a coordinated effort. Much money has been spent, several million fish have been killed, and more remains to be done. Among the agencies directly involved in the sampling and eradication efforts are: Washington Department of Fisheries, Washington Department of Wildlife, Northwest Indian Fisheries Commission, Makah Tribe, Battelle Marine Laboratory, U.S. Fish and Wildlife Service, and Department of Fisheries and Oceans-Canada. The private aquaculture industry has been exceptionally cooperative. Fisheries agencies in neighboring states and foreign countries have been notified and are increasing efforts to prevent the introduction of the virus. The Pacific Northwest Fish Health Protection Committee and pathologists in many parts of the United States and Europe were consulted and often provided itical advice and reagents. Without exception, the entire effort has represented the highest level of professionalism and cooperation by the fish health community.

The origin of the virus is not understood, but has been the subject of some speculation. Without further antigenic and genetic analysis, it is unlikely that any new information about the source will be forthcoming. The recovery of the virus from two species of anadromous fish at two locations in the same year suggests that the salmon may have been infected in the marine environment. Whether a marine reservoir of infection has been established is not known. Regardless of the origin or possible reservoir, the isolation of an emergency disease in North America calls for the strongest possible action by the responsible fisheries agencies. Emergency disease eradication plans were drafted in advance by the State of Washington and the U.S. Fish and Wildlife Service for precisely this situation and these plans should be carried out in full. Not only does VHSV pose a significant biological threat to our salmonid fish stocks, the economic impact on the aquaculture industry has already been severe. While in the midst of much work, great expense, and difficult decisions, it may be tempting to think that efforts to eradicate the virus are not warranted due to cost, ecological impacts, genetic concerns, or for other reasons. Fish health professionals must remain solidly unified in urging administrators to continue to do everything possible to eradicate this virus from the Pacific Northwest until convincing proof exists that the virus has become enzootic. Anything less does not serve to protect the public and private fisheries resources of North America.

RECRUIT NEW MEMBERS!

Kathleen Hopper, Chairman of the Membership Committee is conducting a drive to recruit new members to the section. Included in this issue of the Newsletter is a letter and brochure with information about the Fish Health section and an application for membership in the AFS and FHS. We encourage you to give this information about the Fish Health Section and an application for membership in the AFS and FHS to one of your colleagues who is not a member of the Fish Health Section.

THE ISOLATION OF VHSV FROM CHINOOK SALMON AT GLENWOOD SPRINGS, ORCAS ISLAND, WASHINGTON

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Glenwood Springs is an enhancement facility jointly operated by a private landowner and the Washington State Department of Fisheries. It is located on Orcas Island, in the San Juan archipelago (northern Puget Sound), near Vancouver Island. The facility is spring fed, utilizing a low cost rearing strategy including earthen ponds. A cement adult holding pond is situated at the base of the stream into which all ponds flow. Returning adults enter this pond directly from seawater via a short fish ladder.

An introduced run of fall chinook has been returning to Glenwood Springs since 1984. Each brood year has been sampled in the same routine manner (1984-1988). The 1988 sample included the first isolation of Viral Hemorrhagic Septicemia Virus (VHSV) in North America. Here is a brief summary of procedures used for viral broodstock screening:

Sixty spawning adults are sampled for ovarian fluid and kidney/spleen, in five or less fish per pool.

Ovarian fluid is diluted 1:2 in Hank's Balanced Salt Solution (HBSS) with an antibiotic/antimycotic mixture and polyethylene glycol (PEG; see Brunson, et al., FHS Newsletter 16(4):3). The samples are centrifuged, placed on preformed layers of EPC cells in 24 well plates (0.1 ml of inoculum per well, 2 replicates per pool), adsorbed for one hour at 15 C, overlayed with 0.5 ml of MEM-5 in 0.75% methylcellulose solution, and incubated at 15 C.

Kidney/spleen samples are homogenized in HBSS using a Stomacher 80, centrifuged, and the supernatant added to HBSS with antibiotic/antimycotic at a final dilution 1:40. This mixture is centrifuged again and placed on 96 well plates (0.05 ml. of inoculum per well, 6 replicates per pool). CHSE-214 cells in suspension are added to the wells (0.1 ml each) and the plates are incubated at 15 C.

Sixty-eight fish were sampled from a spawning population of 393. Virus was recovered from a single kidney/spleen pool out of the 19 tested. CPE was observed in only 1 well. The suspect well was replated on both EPC and CHSE-214 cells. These showed CPE typical of viral etiology. When the sample failed to be neutralized by monoclonal anti-IHNV, polyvalent anti-IHNV, or polyvalent anti-IPNV, it was referred to the USFWS National Fisheries Research Center in Seattle, Washington for further study.

After confirmation that this isolate was VHSV, the fish being reared at Glenwood Springs were buried, the water supply chlorinated, and the dirt bottom ponds were drained and lined with unslaked lime. Samples taken from ponds prior to burial (all chinook) were negative for viral pathogens.

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CHARACTERIZATION OF THE FIRST NORTH AMERICAN ISOLATES OF VIRAL HEMORRHAGIC SEPTICEMIA VIRUS

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Following isolation of the virus from returning adult chinook salmon at Orcas Island, Washington by Kathleen Hopper and the recovery of virus isolates from returning adult coho salmon at Neah Bay, Washington by Ray Brunson, the agents had been tested with both monoclonal and polyclonal antisera against infectious hematopoietic necrosis virus (IHNV) and found negative. The chinook isolate was also tested with polyclonal antiserum against infectious pancreatic necrosis virus (IPNV) and did not react. In addition, each virus caused a cytopathic effect (CPE) in fish cell lines that was unlike that caused by other viruses isolated from Pacific salmon in North America. Because the two agents were recovered from tissue samples of chinook salmon and ovarian fluid samples of coho salmon and initially appeared different from each other in the speed and nature of their CPE, the isolates were presumed to represent two new, or at least exotic, viruses and were referred to the National Fisheries Research Center in Seattle for assistance in determining their identity.

We began our study with general techniques used for viral taxonomy before we undertook a more detailed biochemical and serological analysis. We grew the new chinook and coho isolates in epithelioma papulosum cyprini (EPC) cells at 15 C, reacted the viruses and appropriate controls (IHNV and IPNV) with chloroform or ether, and tested the mixtures for the presence of infectious virus. Both new isolates were inactivated by treatment with chloroform or ether indicating the virions contained essential lipids and were enveloped particles.

We used electron microscopy (EM) to determine the morphology of the virions. Because several genera of viruses have characteristic shapes, the results from the EM investigation could provide important information on how to proceed. Chinook salmon embryo (CHSE-214) cell monolayers were infected with each isolate, incubated for 48-72 h at 15 C, fixed in cell culture medium containing 3% glutaraldehyde, embedded, and sectioned using an ultramicrotome. Examination of the thin sections revealed large numbers of bullet-shaped virions with helical symmetry that were associated with cell membranes (Figure 1). The virions ranged from 53 to 65 nm in diameter (average approximately 180 nm) and appeared to be typical rhabdoviruses.

Because fish rhabdoviruses can be assigned to either the Lyssavirus or Vesiculovirus genus on the basis of the molecular weights of the virion proteins, we compared the structural proteins of the new isolates with three known fish rhabdoviruses. Large amounts of IHNV, hirame rhabdovirus (HRV), viral hemorrhagic septicemia virus (VHSV) serotype F1, and the two new isolates were purified and the proteins analyzed using polyacrylamide gel electrophoresis. The virions of the new isolates were composed of five proteins that have molecular weights typical of the Lyssavirus genus of rhabdoviruses. The known fish viruses with this protein profile are IHNV, HRV, and VHSV. The protein profiles of the new isolates were unlike those of IHNV or HRV run in the same gel, but molecular weights of the five proteins of the two new isolates were identical to each other and to those of the F1 serotype of VHSV. The molecular weights were approximately: L (polymerase) 165,000; G (glycoprotein) 70,000; N (nucleoprotein) 40,000; M1 (matrix protein 1) 26,000; and M2 (matrix protein 2) 23,000.

We used rabbit antisera against IHNV, HRV, and VHSV serotype F1 in a serum neutralization test. The antiserum made against the F1 serotype

of VHSV was provided by Dr. Phil McAllister of the National Fisheries Research Center in Leetown. This serum was able to neutralize infectivity of both new isolates at serum dilutions of approximately 1:1000. The antiserum also neutralized the F1 serotype reference strain of VHSV but did not react with IHNV or HRV (Table 1). Conversely, antisera against IHNV and HRV recognized only the homologous viruses and not VHSV or the two new isolates. This test was repeated with identical results.

Because the fluorescent antibody assay has been used to identify fish rhabdoviruses, we used rabbit antisera against IHNV, HRV and VHSV and reacted them with CHSE-214 cells infected with each of the three reference viruses and the two new isolates. Cell monolayers infected with the new isolates reacted with VHSV antisera. This test was repeated. Although the intensity of fluorescence varied for each virus-antiserum combination, the results indicated that cells infected with the new isolates reacted only with antiserum to VHSV.

In order to provide additional evidence that the new isolates shared more than just a neutralizing antigenic epitope with VHSV, we conducted a western blot assay. The structural proteins of IHNV, HRV, VHSV, and the two new isolates were separated on four identical polyacrylamide gels. One gel was stained using Coomassie Brilliant Blue to confirm that equal amount of protein were present and that the separation was efficient. Proteins from three of the gels were transferred to nitrocellulose paper and reacted with antiserum against IHNV, HRV or VHSV. Bound antibodies were visualized by reacting the complex with anti-rabbit serum conjugated to peroxidase followed by the addition of substrate. The antiserum against VHSV reacted with all proteins of VHSV and all proteins of the new isolates. Antiserum against HRV reacted most strongly with HRV proteins and with the G and N proteins of IHNV. Antiserum against IHNV was able to recognize the G and N proteins of IHNV and the N protein of HRV.

These data, taken together, provided convincing evidence of the close biochemical and antigenic relationships between the new isolates and the F1 serotype of VHSV. On February 17, 1989 the confirmation of VHSV in North America was made and communicated to Kathy Hopper and Ray Brunson. This set into motion a previously agreed upon plan to attempt to eradicate the virus from North America and the initiation of a massive sampling program which continues to date. The origin of the virus is not known. Studies are being planned to determine the pathogenicity of the new isolates for selected species of Pacific salmon.

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Table 1. Plaque reduction titers of antisera to hirame rhabdovirus (HRV), viral hemorrhagic septicemia virus (VHSV), and infectious hematopoietic necrosis virus (IHNV) when reacted against homologous strains, the chinook salmon isolate provided by Kathy Hopper (KHV), and the coho salmon isolate provided by Ray Brunson (RBV). The values are expressed as percent plaque reduction relative to controls reacted with normal rabbit serum.

		Antibody dilution					
ANTI-SERUM	VIRUS	1:32	1:64	1:128	1:256	1:512	1:1024
HRV	HRV	100	100	100	90	55	33
	IHVN	0	0	0	36	18	0
	VHSV	0	0	0	0	0	15
	KHV	12	0	0	0	0	0
	RBV	8	10	0	6	0	0
IHNV	HRV	23	23	0	0	0	0
	IHNV	100	100	100	100	94	78
	VHSV	0	0	0	0	0	0
	KHV	11	14	0	8	0	0
	RBV	0	7	29	18	14	5
VHSV	HRV	33	29	21	27	23	28
	IHNV	26	13	0	0	0	26
	VHSV	100	96	78	60	47	39
	KHV	100	100	100	100	100	94
	RBV	100	100	100	100	100	87

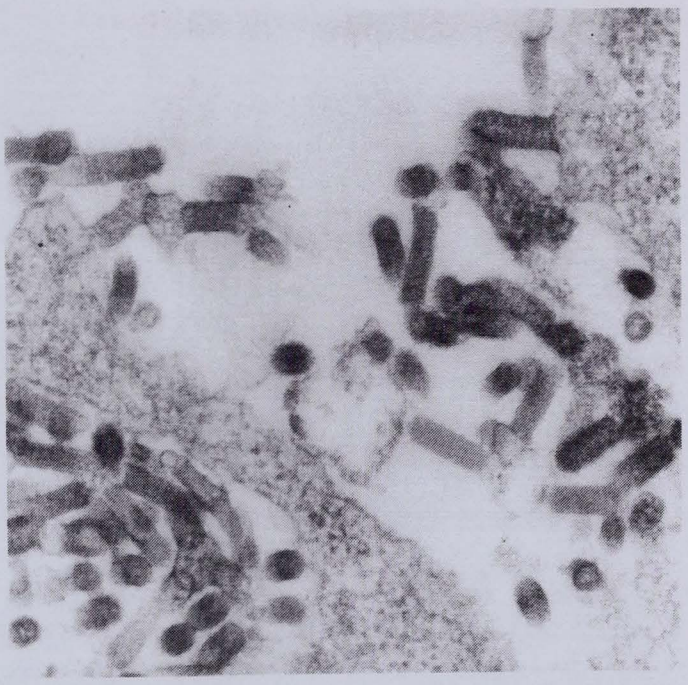

Figure 1. Electron micrograph of a thin section of CHSE-214 cells infected with the virus from adult chinook salmon at Orcas Island, Washington. Infected cells were incubated for 48 h, fixed with 3% glutaraldehyde, and sectioned. Typical bullet-shaped rhabdovirus virions are seen associated with the cytoplasmic membranes.

VHS VIRUS ISOLATED AT MAKAH NATIONAL FISH HATCHERY

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VHS virus was detected in samples from adult coho salmon returning on 12/01/88 and 12/08/88 to the Makah NFH located near Neah Bay, at the extreme northwest part of the state of Washington. Eight of ten, 5-fish pools of ovarian fluid collected on December 1, and one of four pools of ovarian fluid collected on December 8 were positive. The virus was later detected in pooled kidney-spleen tissues of yearling coho and steelhead and pooled whole body homogenates of fall chinook salmon fry sampled on 2/13/89. Samples of moribund yearling steelhead taken on 1/04/89 were negative as were all spawning steelhead tested between 1/04/89 and 1/31/89. The following tables summarize the sampling by species at the hatchery and detail the sampling of the coho broodstock.

Ovarian fluid samples were centrifuged at 2000 X RCF for 15 minutes, diluted 1:2 in a solution of penicillin-streptomycin in 14% polyethylene glycol (PEG) to yield a final concentration of 800 I.U. penicillin, 800 mcg. streptomycin and 7% PEG. Kidney-spleen samples were processed in a similar fashion, but first diluted 1:10 in Hanks' BSS and homogenized in a Stomacher-80 blender for a final dilution of 1:20 onto cell sheets. All samples were held a minimum of 2 hours at 4 C in antibiotics before recentrifugation and inoculation.

Replicate wells of CHSE-214 and EPC cell monolayers in 24-well plates were inoculated with 0.1 ml of the prepared samples. After 1 hour incubation, EPC plates were overlaid with 0.8% methylcellulose in TRIS-buffered MEM-5 (Burke and Mulcahy, 1980) with penicillin-streptomycin and fungizone to retard contamination. CHSE-214 plates were simply overlaid with TRIS-bicarbonate buffered liquid MEM-10 with the same antibiotics.

(continued next page)

VHS VIRUS ISOLATED (continued from page 3)

Kidney-spleen homogenates from 30 coho females and 30 males were also plated on EPC and CHSE-214. all adult kidney-spleens tested were negative on both cell lines. Only the ovarian fluid samples on EPC cells demonstrated evidence of the virus. Subsequent tests suggest that CHSE-214 cells are slightly less susceptible to the virus. All samples were incubated at 15-18 C for a minimum of 14 days. Cytopathic effects similar to IHN with rounding of cells and plaque formation usually occurred within 7-10 days, but the samples from the coho yearlings took 14 days for form plaques. One interesting note is that only our EPC cells showed definite virus CPE with the kidney-spleen samples from the juveniles tested. This may be explained by an apparent low level of infection and the lower sensitivity of the CHSE-214 Cell line to this virus.

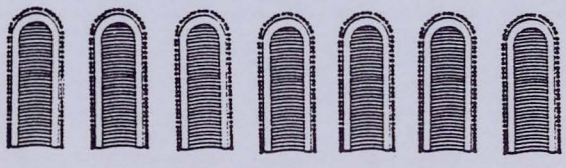
All fish in the hatchery were destroyed on February 23 following confirmation of VHS virus. Due to depopulation, no further epizootiological data could be gathered from the hatchery fish, making it impossible to determine if the fish would have experienced mortality or only shown a transient infection. During late January through early February the hatchery had experienced extremely low water temperatures and had almost lost the fish due to freezing of intake screens, and several water valves. At times, the ponds were completely frozen over and ice had formed on incubators. The hatchery staff also noted the formation of "frazzle" ice or crystals of ice forming within the flowing water column of ponds and pipes. No abnormal mortality or morbidity was observed in the yearling fish. Some early fry mortality was noted in the chinook, but histological examination of the fish did not reveal pathology typical of that described for VHS. Mortality was attributed to coagulated yolk syndrome and coldwater disease. Feral stock surveys are being conducted on freshwater streams near the hatchery and results are pending.

VIRAL SAMPLING SUMMARY BY SPECIES AND LOT AT MAKAH NFH

Species/Lot	Sample Date	No. Fish Sampled	Results
Fall chinook/ adults	9/21-10/17/88	211 females 47 males	Negative Negative
Chum/adults	11/7-12/20/88	179 females 30 males	Negative Negative
Coho/adults	11/21-12/20/88	300 females 30 males	VHSV Negative
Winter steelhead/ adults	1/4-1/31/89	73 females 10 males	Negative Negative
Winter steelhead/ yearlings, moribund	2/13/89	4	Negative
Winter steelhead/ yearlings	2/13/89	60	VHSV
Coho/yearlings	2/13/89	60	VHSV
Fall chinook/fry	2/13/89	15	VHSV

COHO BROODSTOCK VIRAL SAMPLING SUMMARY MAKAH NFH

Date	Tissue	No. of Fish	No. of Pools	Results
11/21/88	Ovarian fluid	2	1	Negative
12/1/88	Ovarian fluid	50	10	8/10 positive
	Kidney-spleen	60	12	Negative
12/7/88	Ovarian fluid	65	13	Negative
12/8/88	Ovarian fluid	19	4	1/4 positive
12/14/88	Ovarian fluid	71	15	Negative
12/15/88	Ovarian fluid	36	8	Negative
12/20/88	Ovarian fluid	57	12	Negative



IFAT FOR RAPID CONFIRMATION OF INFECTIOUS HEMATOPOIETIC NECROSIS VIRUS (IHNV)

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With the recent detection of viral hemorrhagic septicemia virus (VHSV) in Washington, USA, and the endemic range of IHNV within the Pacific Northwest, confirmatory diagnosis to distinguish these two agents becomes a requirement. These two viruses replicate on many of the same cell lines and produce similar cytopathic effect (CPE). Time to CPE, incubation temperature, and tissue of origin are also similar. Detection of IHNV or VHSV in susceptible salmonid fry or carrier adults is routinely done using standard cell culture techniques described in the Fish Health Blue Book (1985). A presumptive diagnosis is often made based on type of CPE, time to the appearance of CPE, cell line used in the isolation, incubation temperature, species and tissue of origin, and disease history of the fish stock being examined. Because of the similarities of VHSV and IHNV within a susceptible host or in cell culture, a rapid serological technique to distinguish these pathogens is advantageous. An indirect fluorescent antibody test (IFAT) has been reported previously (LaPatra and Rohovec 1988). We routinely use this IFAT for IHNV confirmation. If virus is detected at a new site, isolants are confirmed by standard serum neutralization tests. Detection of other virus positives at the same site or in fish from watersheds with histories of IHNV are subsequently confirmed by IFAT. The number examined is dependent on the number of isolations that are made for a particular fish stock. The IFAT is rapid, sensitive, and has reacted with all IHNV strains tested including different electropherotypes, those isolated from selected salmonids at different stages of their life cycles, and from different geographic regions. Specifity tests of the IFAT indicated no cross-reactivity with VHSV or other viruses or with cell lines of salmonid and non-salmonid origin. This method uses CHSE-214 or EPC cells grown on cover glasses pretreated with 7% (w/v) polyethylene glycol. The source of virus can be obtained by subculturing from cell cultures exhibiting CPE while the diagnostic assay is still in progress. Incubation for 12 to 24 h followed by a balanced salt solution rinse and 10 min acetone fixation prepares the specimen for IFAT staining. We use a mouse monoclonal antibody reagent (IgG type) followed by either a goat anti-mouse fluorescein conjugate or an equivalent biotin-avidin fluorescein system. Counterstaining for 1 min with 0.01% Evan's blue and mounting in glycerol (pH 9) allows easy visualization at 400x using a microscope equipped for fluorescent microscopy. A protocol describing the materials and method is available along with small volumes of the monoclonal reagent.

EFFECTS OF VOMITOXIN ON SERUM CHEMISTRIES OF CHANNEL CATFISH (*ICTALURUS PUNCTATUS*)

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Vomitoxin (Deoxy nivalenol) is a mycotoxin produced by *Fusarium graminearum*. This study was designed to determine the effects of vomitoxin on serum chemistries of channel catfish. Vomitoxin was dissolved in physiological saline and injected intraperitoneally at a dose of 1 mg/kg body weight and the controls were injected with physiological saline. After a 96h exposure, fish were bled and necropsied. No mortality occurred in any of those fish during a 96h period. A significant decrease in total WBC count, osmolality, serum glucose, aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase concentrations, and an increase in total RBC count and hematocrit value occurred in fish exposed to vomitoxin. Decreased serum enzyme levels suggest possible enzyme suppression or suppression of protein synthesis.

AN UPDATE ON INFECTIOUS HEMATOPOIETIC NECROSIS VIRUS (IHNV) TRANSMISSION STUDIES IN OREGON

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Two major questions are being addressed regarding IHNV and its impact on hatchery populations. The first is directed at the potential of progeny from IHNV carrier adults to contract the disease (vertical transmission). Production trials have been conducted throughout the state where progeny from carrier and virus-free parents are kept segregated and monitored for IHN. To date over 9 million fish from IHNV positive parents, representing two different anadromous species and four different stocks, have been reared with no evidence of virus. In most cases, eggs were either water-hardened or surface disinfected with iodophor and in all tests, egg incubation and early rearing was done in virus-free water.

The second question is do progeny from IHNV positive parents return as carriers of IHNV (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 prior to release. By monitoring returning adults for IHNV we hope to be able to answer this question. In 1987 at Elk River, jack salmon (2 yr adults) from the 1985 brood year returned, and in 1988, jacks from the 1986 brood year returned along with three year old adults from 1985 releases. No virus was detected in any of these fish whether they were from carrier or virus-free parents. Marked adults at these facilities will continue to be evaluated for the next five years.

Funding for these studies were provided in part by U.S. Fish and Wildlife Service, project AFS-78, Lower Snake Comp. Plan, state wildlife and general funds, Bonneville Power Adm., and National Marine Fisheries Service CRFDP.

THE PHYSIOLOGICAL IMPACT OF AFLATOXIN B1 ON CHANNEL CATFISH *ICTALURUS PUNCTATUS*

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Aflatoxin is carcinogenic in rainbow trout and perhaps in man but apparently fails to induce neoplasms in channel catfish. The objective of this study was to determine if aflatoxin B1 was nevertheless sublethally toxic to channel catfish. Aflatoxin B1 was suspended in propylene glycol and injected intraperitoneally at a dose of 2 mg/kg body weight. The controls were injected with propylene glycol. Fish were bled and necropsied 96 h after the injection. Blood was analysed for total red blood cell (RBC) count, total white blood cell (WBC) count, hematocrit and differential cell count. Serum was analysed for aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), glucose, protein concentration and osmolality. Several tissues were processed for histopathological analyses. No mortality occurred in this experiment and no gross lesions were found in either experimental or control fish. A significant increase in total WBC count and serum glucose concentration, and a significant decrease in serum AST and ALT concentrations occurred in fish injected with aflatoxin B1. Aflatoxin B1 may have hitherto undocumented sublethal toxicity.

A PICORNA-LIKE VIRUS FROM SALMONID FISHES IN CALIFORNIA

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Examinations of salmonid fish from several locations in Northern California have revealed the presence of a newly recognized virus. The virus has been isolated from ovarian fluids of cutthroat trout (*Oncorhynchus clarkii*), rainbow trout (*O. mykiss*), brown trout (*Salmo trutta*) and brook trout (*Salvelinus fontinalis*). There was no evidence of disease in any of the adult fish sampled nor was the virus isolated from progeny of the adults from which the agent was recovered.

The cytopathic effect (CPE) of the virus is characterized by areas of diffuse necrosis in CHSE-214 cells (Fig. A). The virus shares many physical properties with a recently discovered picorna-like viruses from smelt (*Osmerus mordax*) and Atlantic salmon (*Salmo salar*) but does not induce syncitia in CHSE-214 cells as the latter two agents. Viral particles appear to have an icosahedral symmetry and have a mean diameter of 37.5 nm (n = 10, std. dev. 0.41)

Initial studies in young rainbow trout, kokanee salmon (*O. nerka*) and chinook salmon (*O. tshawytscha*) show the virus does not cause any detectable disease. The agent is being further examined for its biochemical properties and antigenic relationship to the other two known picorna-like agents from fish.

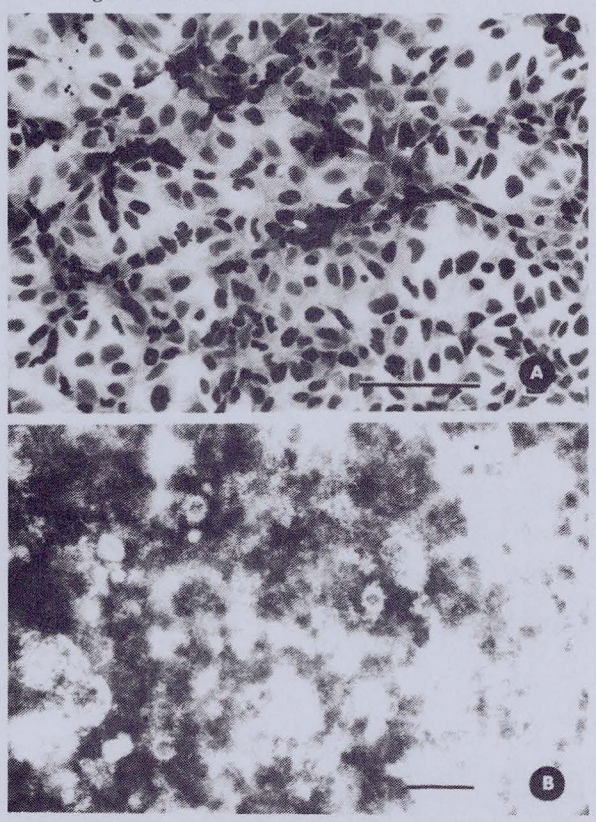

Figure A. Cytopathic effects seen in CHSE-214 cells infected with the picorna-like virus from salmonid fish.

Figure B. Virions of the picorna-like virus from salmonid fish. Stained with phosphotungstic acid. Bar = 100 nm.

USE OF p-PHENYLENEDIAMINE TO REDUCE FADING OF IMMUNOFLUORESCENCE

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Olympia, WA 98504

Current contract work under Bonneville Power Administration's Augmented Fish Health Monitoring project has resulted in a significant increase in the use of the fluorescent antibody technique (FAT) for the detection of *Renibacterium salmoninarum* by our laboratory. We recently noted rapid fading of fluorescence of known positive as well as suspect positive samples upon exposure to UV light which made prolonged examination of samples impossible. Fading of fluorescence was so rapid that a microscope field being observed became unreadable within 15 to 30 seconds, resulting in the inability to perform extended observations as is often necessary with questionable samples or when training new employees. Investigations into the cause of our problem resulted in improved fluorescence time using a technique that is described in published literature but apparently is not widely utilized in fish health laboratories.

Initial investigations indicated that neither our microscope (Nikon Labophot with 100 watt mercury UV bulb) nor the FITC-labelled *R. salmoninarum* antisera purchased from Anadromous, Inc. was the source of the problem. John Civitanich of Anadromous, Inc. suggested that the FA mounting fluid we used could be the problem, however, and he provided reprints on the use of p-phenylenediamine (PD) in immunofluorescence microscopy (Johnson et al, 1981; Johnson et al, 1982; Platt and Michael, 1983). Tests comparing mounting fluid prepared in our laboratory containing PD against commercially available pH 7.2 and 9.0 mounting fluids (Difco) confirmed that the mounting fluid we were currently using (pH 7.2) was vastly inferior for maintaining fluorescence than was either pH 9.0 fluid or fluid containing PD. We had recently inadvertently changed from pH 9.0 fluid to pH 7.2, which accounted for the increased fading rate we had noted. The longest fluorescence was obtained using fluid containing PD. Consequently, we currently use mounting fluid prepared as described below rather than either of the commercially available types. Fading of fluorescence is now reduced to the point where samples may be viewed for several minutes without significant fading of fluorescence.

Mounting fluid containing PD should be prepared in small batches and stored in the dark at -20 C between uses since it gradually acquires a brown color which reportedly reduces its effectiveness. Adjusting pH of the fluid to 8.6 (optimum for FITC fluorescence emission) was recommended in the literature but we achieved satisfactory results without doing so.

Preparation of p-phenylenediamine FAT mounting fluid

p-phenylenediamine (Sigma #P-6001	100 mg
PBS (pH 7.4)	10 ml
Glycerin	90 ml

Dissolve p-phenylenediamine in PBS, add solution to glycerine. Mix well. Adjust pH to 8.6. Store in dark at -20 C between uses.

LYMPHOMA IN AN ADULT HYBRID *MORONE*

Arunthavarani Thiyagarajah and John R. MacMillan
Department of Basic and Applied Sciences
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Formalin-fixed liver, gill, kidney, heart, and intestine from a cultured striped bass hybrid were submitted for histopathological diagnosis. Infiltration of mononuclear round cells (lymphocytes) was found in the submucosa and muscularis of intestine, hepatic sinusoids, intra and extrahepatic exocrine pancreas, mesentery, anterior and posterior kidney, and in the pericardium, myocardium, atrium, ventricle and blood vessels of heart. This condition was tentatively diagnosed as a lymphoproliferative disorder suggestive of lymphoma. Brief review of literature suggests this is the first reported occurrence of this condition in striped bass hybrid.

ISOLATION OF *FLAVOBACTERIUM* SPP. FROM GILL NECROSIS OF CHANNEL CATFISH (*ICTALURUS PUNCTATUS*) HELD AT COLD TEMPERATURE

Arunthavarani Thiyagarajah and John R. MacMillan
College of Veterinary Medicine, Drawer V
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Bacterial gill necrosis occurred in channel catfish fingerling used in an experiment to determine the role of serotonin in gill chondroplasia. During the course of this experiment, the water temperature ranged between 15-20 C. During necropsy, abundant filamentous bacteria that were not forming hay-stacking morphology but in clumps were found on fresh mount preparations of gill. Orange-yellow, smooth colonies were isolated on blood agar and Ordal's agar after 72 h of incubation at 27 C. These organisms were Gram-negative slender rods; nonmotile; and cytochrome oxidase positive. APE 20E and Minitek rapid identification system were used to identify the bacteria. These bacteria have been tentatively identified as *Flavobacterium* spp. Histologically, gill lesions consisted of inflammation and necrosis; hemorrhage; eroded filaments; filamentous bacteria associated with necrotic area and also on the surface of gill lamellae. Infectivity studies to fulfill Koch's postulate are currently being conducted.

HEAT SHOCK PROTEIN SYNTHESIS IN CHANNEL CATFISH HEPATIC CELLS

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Synthesis of heat shock proteins (hsps) is increased in response to a variety of stressors, including heat, heavy metals, oxidizing agents, viruses, and glucose deprivation. In more recent literature, hsps are sometimes termed stress proteins. Hsps are genetically conserved and are found in almost all prokaryotic and eukaryotic cells. Fundamental roles in cellular stress response mechanisms, and in homeostatic function, are inferred from their genetic relatedness among diverse species and their apparent ubiquity. Although well studied in other species, published reports concerning hsps in fish are uncommon.

A methodology has been developed to facilitate the study of these proteins in channel catfish (*Ictalurus punctatus*, Rafinesque). Single cell suspensions of hepatic cells were obtained by *in situ* perfusion of the liver with collagenase. Cells were incubated for 24 hours in nutrient media and then subjected to a number of stressors. During stressor stimulus, cells were incubated in media containing ^{35}S-labelled methionine. Proteins were recovered from cells and separated by polyacrylamide gel electrophoresis. The gels were treated with a fluor, dried and used to expose X-ray film. The films were developed, and the resultant autoradiographs demonstrated proteins which were synthesized during the period of stressor exposure.

Hepatic cells subjected to heat stress (raising ambient temperatures from 28 to 40 C) synthesized increased amounts of six proteins ranging in size from 100 to 36 kilodaltons (kD). Cells exposed to low concentrations (0.16 mM) of copper sulfate produced four proteins ranging in size from 68 to 32 kD; three of these proteins were not induced by heat stress. Cells exposed to cold stress (shift from 28 to 2 or 8 C) and consequently allowed to recover (return to 28 C) synthesized increased amounts of two proteins (87 and 32 kD). A third protein with a molecular weight of 30 kD may have also been induced, but results were equivocal.

Data collected have demonstrated that a family of stress proteins exists in channel catfish hepatic cells. These cells respond to different stressors with increased synthesis of a variety of proteins. In some instances one protein is induced by more than one stressor, suggesting that these proteins play central roles in the cellular stress response mechanism. All the stress proteins demonstrated thus far appear to be constitutively synthesized in nonstressed cells; they are therefore believed to function in homeostatic mechanisms as well. Further research is planned to study what other stressors induce hsp synthesis in channel catfish, under what conditions induction of hsp synthesis is maximized, and the duration of the hsp response in stressed cells.

A HEMIC PROLIFERATIVE DISORDER ASSOCIATED WITH SEVERE ANEMIA IN PEN-REARED CHINOOK SALMON, *ONCORHYNCHUS TSHAWYTSCHA*

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A hemic proliferative disorder of seawater-reared chinook salmon, referred to as "marine anemia", caused high mortalities at several netpen sites in the Sachelt area, British Columbia, Canada during the fall and winter of 1988. Market-size salmon that had been in seawater for at least one year were affected. The disease was characterized by pallor of the gills due to severe anemia and affected fish often exhibited exophthalmia, ascites, renal hypertrophy, and splenomegaly. Petechiae were observed in the skeletal muscle and viscera of some fish. Histological examination revealed prominent proliferation of hemoblasts in the orbit of the eye, pancreas, liver, lamina propria of the intestine, kidney interstitium, spleen, and heart. The hemoblasts were mitotically active, contained a large, often deeply cleft nucleus, and had abundant cytoplasm. Blood smears revealed a normochromic, normocytic anemia and hematocrits ranged from 2-28%. No pathogenic bacteria were consistently isolated from affected fish and several attempts at isolating viruses from affected fish using co-cultivation and standard techniques on EPC, RTS-2 and CHSE-214 cell lines maintained at 10 and 15 C were unsuccessful. The histological changes are suggestive of leukemia or a bizarre leukemoid reaction, but it is not known if these very immature cells were erythroblasts or myeloblasts.

A morphologically identical disease, diagnosed as a possible granulocytic leukemia, was observed in chinook salmon reared in fresh water at the Minter Creek Hatchery in Washington State in 1974 (Harshbarger, 1984). However, our observations are the first report of the disease in sea water.

We transmitted marine anemia by injection of crude homogenates of kidney tissue. Morbidity in the challenged chinook salmon was first observed at 8 week post-injection. All injected fish exhibited anemia, histological changes consistent with the disease, and concurrent bacterial kidney disease (BKD). The challenged and control fish were derived from a population with pre-existing, subclinical infections of *Renibacterium salmoninarum* (the causative agent of BKD), and 2 of 10 control fish developed BKD. Possibly marine anemia causes immunosuppression, which allowed for exacerbation of BKD in the exposed fish.

Although the relationship of BKD and marine anemia in clinical disease has not been completely determined, we believe that marine anemia is not directly caused by *Renibacterium* because the histological changes of the diseases are distinctly different. Furthermore, although BKD is widespread in pen-reared chinook salmon, the two diseases did not always occur together in netpens. Histological examination of 35 fish with marine anemia from netpens revealed BKD lesions in only 11 fish, and only one of these fish exhibited severe BKD. Examination of fish from netpens with marine anemia using IFAT preparations revealed *Renibacterium* infections in 9 of 15 fish. However, 4 of the positive fish exhibited very few bacteria.

The cause of marine anemia is unknown, but the histological changes and transmission experiment indicate that it is an infectious leukemia or a bizarre leukemoid reaction to an infectious agent. Further studies are underway to elucidate the relationship of BKD with marine anemia, and to determine the cause and possible neoplastic nature of the disease. We thank H.W. Ferguson, J.W. Fournie, J.M. Groff, J.G. Harshbarger, J.K. Morrison, C.E. Smith, D.J. Spear, and J.G. Zinkl for review of histological material.

ISOLATION OF A NEW VIRUS FROM ATLANTIC SALMON (*SALMO SALAR*)

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A previously unknown virus has been isolated from Atlantic salmon (*Salmo salar*) fingerlings being reared in freshwater in the Pacific Northwest. The virus was recovered from salmon undergoing a low-grade mortality for which no causes could be detected. An examination of the stained tissues from affected fish by light microscopy showed only a mild necrosis of the hematopoietic portions of the kidney and a few focal areas of mononuclear cell infiltration in the liver.

Portions of the kidney, liver and spleen were processed for virological analyses and inoculated onto monolayers of CHSE-214 cells at 10 C. Following 4 wk of incubation at 15 C, cytopathic effects (CPE) evident as syncytia were detected. Virus particles having a mean diameter of 39.5 nm (n = 10, std. dev. = 0.32) with icosahedral symmetry were observed in negative stains from concentrated fluids from infected cell cultures. The virus did not cause CPE in the Atlantic salmon (AS), rainbow trout, *Oncorhynchus mykiss* (RTG-2) or the EPC cell lines. The virus grew at temperatures of 5, 10 and 15 C. Growth was best at 15 C but inconsistent at 20 C.

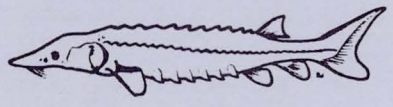
The virus is resistant to chloroform treatments and is not neutralized by hyperimmune rabbit serum prepared against infectious pancreatic necrosis or infectious hematopoietic necrosis viruses or chum salmon reovirus. Viral replication is not blocked by BUdR suggesting an RNA genome. These virus properties are consistent with those described for picorna and caliciviruses and resemble closely those described for a picorna-like virus from smelt (*Osmerus mordax*) from New Brunswick, Canada by Moore et al. (J. Dis. 11:179-184, 1988).

Preliminary rsults of in vivo testing indicate the virus causes no mortality or signs of disease in rainbow trout or Atlantic salmon. Virus could not be recovered from juvenile rainbow trout following water-borne exposures to the agent nor were there any microscopic signs of disease when stained sections of their tissues were examined. Further studies on the biochemical properties of the virus and its proper taxonomic placement are in progress.

NOMINATIONS SOUGHT

The AFS Fish Health Section Nominating Committee is looking for people to consider for nomination to fill 3 elected positions: President-Elect, Board of Certification, and Nominating Committee. If you have the name of someone you feel would be interested in one of these positions, please call me at 503-754-2852, or send your suggestions to: Craig Banner, Oregon State University, Dept. of Microbiology, Corvallis, OR 97331.

Let's get people involved!



SUPERCHILLING AT TWO FUNDY FISH FARMS

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Department of Marine Resources
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West Boothbay Harbor, ME 04575

In early March extreme spring high tides, shallow grow out sites, low water temperatures, and wind pattern changes created a superchilling event at two NE salmon farms. One farm from each side of the U.S.-Canada boundary was affected.

The New Brunswick salmon farm recorded water temperatures of -1.0 C on March 7th with very cold winds blowing. The farmer realized something was wrong at their 20 cages when they saw hundreds of harbor pollock floating on the surface. An attempt was then made to feed the salmon, but no fish appeared. A diver was called in to check the cages and all the salmon were found to be dead. Over 27,000 fish were lost including 22,500 salmon from three to six pounds, 3,500 grilse, 1500 market size fish, and 250 broodstock. The operation had been existence for six years without any prior superchilling problems. Scientists from the Department of Fisheries and Oceans visited the site, and tests are being carried out on the fish.

On the Maine side, over 7,000 adult fish were lost, but 100,000 smolts survived due to their differing locations within their particular lease site. The cages with adult salmon were located closest to shore, where during the extreme high tide of March 8th (3 feet above normal) the seawater rose up over a thick layer of ice along the edge of the bay, became superchilled, and as the tide ebbed the water flowed through the adult cages. By the time this water reached the smolt cages, the chilled water was diluted to non-lethal temperatures. Air temperatures that night were -23.5 C and water temperture was -2 C.

BRIEF REPORTS

A recently developed "Standard Method" for the measurement of dissolved gas supersaturation is included in the Supplement to the Sixteenth Edition of **Standard Methods for the Examination of Water and Wastewater,** published by the American Public Health association et al., Washington, D.C. This method will be included in the next normal edition of **Standard Methods** (17th edition) and in all following editions. John Colt, P.O. Box 5000, Davis, CA 95617

* * * * * *

Analysis of the Mississippi Cooperative Extension Service (NCES) "fish kill reports" from 1980 through 1985 revealed an increasing trend in laboratory submissions (544 to 1771) and in the proportion of cases positive for *Edwardsiella ictaluri,* 7.9% to 47.5% respectively. From 1986 to 1988, however, laboratory submissions have slowly decreased from 2927 to 2633 submissions, with an associated decrease in the proportion of *E. ictaluri* cases, from 45.7 to 43.7%. Some explanations for this decrease in spite of continued expansion in the Mississippi catfish industry are: 1) improved management and availability of effective drugs, 2) seasonal variation in maintaining the optimal temperature window of 22 to 28 C for *Edwardsiella* outbreaks, and 3) the termination of mandatory reporting of ESC for the use of ROMET-30 (1986). Of the 427 producers who submitted *E. ictaluri* cases to the laboratories, during the nine year period, 28 producers generated 66% of the total *E. ictaluri* case load. James D. Freund, College of Veterinary Medicine, Mississippi State, MS 39762, in cooperation with the MCES.

* * * * * *

Personnel from the Pathology Laboratory at the Maine Department of Marine Resources are interested in reviewing blood slides of marine birds which have been collected by other agencies in the past. These slides will be examined for the presence of cytoplasmic inclusions characteristic of a viral blood disease found in fish. If your agency has any slides that you are willing to let us borrow, please contact:

Laurie Bean, M.R.1, Department of Marine Resources, W. Boothbay Harbor, ME 04575, (207-633-5572).

All slides will be examined promptly and returned in good condition.

SPECIAL CONTRIBUTION

AQUACULTURE HEALTH MANAGEMENT HOUSE

Ralph Elston, Ph.D.
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Sequim, WA 98382

I am writing because I believe there is a need in the fish health field to better define the different roles which fish health professionals are playing today and, very importantly, to facilitate a dialogue on the training of additional professionals to fill the needs of tomorrow. Over the last several years of working with fish health professionals including fish biologists, graduate-educated researchers, veterinarians, and the aquaculture industry, I have developed a perspective which I would like to share with you and which I think you will find useful and perhaps thought-provoking.

The Aquaculture Health Management House is an analogy which neatly helps conceptualize the different capacities which fish health professionals fill as they apply their profession to issues in both aquaculture and resource management. Let's look first at the services which are needed and then consider who provides these services. Scientific research is the "foundation" on which fish health management is built. This basic information gives us the technical ability to understand how infectious diseases are harbored and transmitted and how they affect individuals and populations of animals. The "living space" of the aquaculture health management house is the application of the underlying scientific information to managing and preventing the effects of infectious diseases on both cultured and wild populations of animals. As this activity applies to intensively cultured populations of aquatic animals, it is, in reality, the practice of medicine and the ancillary supporting laboratory services. Finally, the protective "roof" of the aquaculture health management house is composed of regulations, formulated by government. Such regulations are aimed toward preventing the geographic spread and effects of infectious animal diseases.

In theory and practice then, the Aquaculture Health Management House is composed of three more or less discrete functions and services. The need to recognize these functions becomes more apparent as private industry enters the aquaculture field to an increasing extent. The foundation of the aquaculture health management house, effective research on fish health problems, is a highly focused activity in specific scientific disciplines. The successful application of this information to intensive aquatic animal husbandry, that is, the practice of medicine, is a very generalized activity requiring a broad but more superficial spectrum of knowledge. Regulation of diseases of wild and cultured populations of animals requires a knowledge of the important infectious diseases of fish populations including the ability to recognize disease entities, an understanding of how they spread geographically and most importantly, of how this spread can be prevented.

Today, the fish health field is populated by professionals with a variety of technical backgrounds. There are researchers with training in fisheries biology, microbiology, biochemistry or pathology, professional fisheries biologists, veterinarians who are functioning primarily as researchers and a few veterinary practitioners. The successful researchers are those with detailed knowledge in their particular field who can prioritize and recognize important fish health problems and design and execute (and financially support) research programs to solve these problems. Those in regulatory roles are largely, I believe, individuals with training in fisheries biology often followed by additional training in infectious diseases of aquatic animals. Finally, in the practice of medicine we find today the most diverse group including individuals with research backgrounds, professional fisheries biology backgrounds and a scattering of veterinarians. This diversity results in part from the fact that there has been little clear definition between the practice of medicine and the provision of laboratory support services for diagnosis and certification. As most of you know, the Fish Health Section has, to its credit, taken steps to professionalize these endeavors by creating and defining criteria for certification of individuals as Fish Health Inspectors and Fish Pathologists. The former are individuals involved primarily in making inspections for regulatory purposes

FISH HEALTH SECTION

AMERICAN FISHERIES SOCIETY

5410 Grosvenor Lane, Bethesda, Maryland 20814-2199

Dear Colleague:

The Fish Health Section of the American Fisheries Society was formed in 1972 by aquatic biologists, pathologists, farmers, and those involved in research and development of finfish and shellfish health.

* We promote the exchange of knowledge and new findings through the Fish Health Section Newsletter, the only widely circulated non-refereed communication of its type.

* We sponsor publication of the **North American Journal of Aquatic Animal Health.** The subscription rate is substantially reduced for Fish Health Section members.

* We offer credentials through certification as Fish Health Inspector or Fish Pathologist.

* We publish and update the FHS Blue Book, the most widely accepted guide for detection and identification of certain fish pathogens. A similar shellfish health book is nearing completion.

* We sponsor annual meetings, including the International Fish Health Conference held in Vancouver, B.C., July 1988, and the meeting to be held in Annapolis, Maryland, July 17-20, 1989.

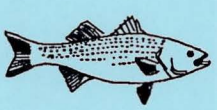
The Fish Health Section of the American Fisheries Society is comprised of people from varied disciplines with tremendous expertise in fish and shellfish health who belong because we want to be informed about what's happening in our field and tell others about our work.

This is an invitation to you to join the American Fisheries Society/Fish Health Section. I have enclosed a copy of the most recent FHS Newsletter and an application for membership. Please join and participate in this important and informative professional organization.

Sincerely.

Kathleen Hopper

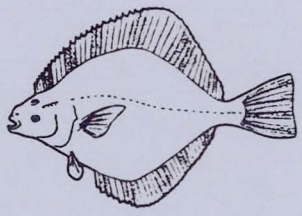
Kathleen Hopper
Membership Committee AFS/FHS



while the Fish Pathologist is involved in both regulatory activities and making judgments on diagnoses and therapeutic recommendations.

As a Fish Pathologist who has specialized in working closely with commercial fish farmers, I know that many of my colleagues who are Fish Health Inspectors and Fish Pathologists have operated very effectively and professionally in the regulatory field in particular and in making diagnostic and therapeutic judgements. They have done so and continue to do so because there is a need for this service in both public and private aquaculture. However, over the long term (perhaps the next 25 years), I see some serious limitations for Fish Pathologists engaged in what is essentially the practice of medicine, particularly in areas with substantial development of industrial aquaculture. Often, with their highly focused backgrounds, these individuals, who may be outstanding researchers, do not have the broad knowledge base to effectively solve a spectrum of fish health problems. Most of us in fish health research have seen instances where competent researchers tend to find solutions to fish health problems which reflect their own particular discipline. Another characteristic I have noticed is that often the primary focus of research people in approaching fish health problems in industrial aquaculture is to obtain information for publication in technical journals rather than what their primary goal should be —solving the problem for the aquaculturist. Professional fisheries biologists who are endeavoring to solve the day to day problems in fish health (i.e. the practice of medicine) may in some cases be effective, but again usually do not have the broad training required to recognize and treat a variety of fish health problems, many of which are non-infectious in nature. Finally, as many of you know, some veterinary practitioners have an interest in the fish health field. The primary problems which the veterinary profession has had in being effective are the following. First, little training is provided in veterinary colleges on fish health, although this has started to change over the last 10 years. Perhaps more importantly, the veterinary profession has typically failed to recognize that fish are different creatures from dogs, cats, sheep, cattle and goats and that as a consequence, there is a tremendous information void which we must fill before veterinarians can practice fish medicine at the same level as domestic animal medicine.

So where do I think these problems leave us in the fish health field and what should we be working toward? Certainly, it should be our aim to help shape the various components of the fish health management profession to best manage and prevent the effects of aquatic animal diseases. Our foremost goal should be to understand the fish health management needs of private and public aquaculture and resource management and encourage professional development which will most effectively meet those needs. While I don't think there is any reason for professional insecurity on the part of any competent fish health professionals today whether they are in research, regulation or medical practice, I do believe things need to change over the long run and that we need to cultivate a greater and more responsible integration of the veterinary profession into the fish health field. Specifically, I think that over the long term we should support the increased activity of veterinary professionals in the practice of medicine component (and research component as well) of the Aquaculture Health Management House. To support this objective, I believe that we should be encouraging veterinary training institutions to make the necessary commitment to train individuals in the fish health area. Today is an opportune time for this activity, since there are many more veterinarians currently being trained than there have been in the past. Effectively trained veterinarians will have the broad knowledge base to approach the multitude of animal health problems which are encountered in aquaculture. As a corollary to this commitment, the veterinary field must recognize the need to work closely with fish health professionals in research and applied fields in order to effectively advance the fish health management field. In regions where industrial aquaculture has developed to a substantial degree, practicing veterinarians can provide a much needed service to the industry. Certainly we must continue to train research scientists in specific disciplines. This is critically important, considering the tremendous knowledge needs we have in this fish health field. There will be important continuing needs for professional fisheries biologists with a specialization in aquatic animal health. Their roles include work for private industry performing, for example, as fish farm health managers and in diagnostic support laboratories as well as in government regulatory aspects of fish health as they apply both to aquaculture and to resource management. Ultimately, this may exclude Fish Pathologists from the activities in which they are engaged today which in effect comprise many of the components of the practice of medicine. The time frame for such changes is going to be over the next 25 years, I believe. I don't think that any of today's Fish Pathologists need to be concerned about being excluded from their professional activities. In the future, however, the Fish Pathologist may become more focused as an individual with training and certification to address regulatory needs in the transport of fish. I think we need to realize that change is inevitable, often productive and that by embracing and facilitating such change, rather than resisting it, we will productively support the evolution of our field. However, it is critically important that we encourage an active dialogue on this change so that by understanding it we can define goals for training future professionals and ensuring that they have productive careers clearly integrated into a component of the aquaculture Health Management House, thus effectively serving both the needs of natural resource management and the aquaculture industry.



PASSAGES

Bill Cox has moved from the University of California to accept a position as associate fish pathologist with the California Department of Fish and Game. His new address is: Department of Fish and Fame, Fish Disease Laboratory, 2111 Nimbus Road, Rancho Cordova, CA 95670.

* * * * * *

The editors have recently learned of the death of two of Europe's prominent fish disease specialists. Dr. Emmy Egidius from Norway and Dr. Pietro Ghittino from Italy had both contributed a great deal to our field. Memoria to these two individuals will appear in the next issue of the Newsletter.

FUTURE EVENTS

WESTERN FISH DISEASE WORKSHOP

The 30th Annual Western Fish Disease Workshop will be held at the Rosario Resort and Spa on Orcas Island in Washington on June 21-23, 1989. The meeting will be hosted by Wayne Brunson of the Washington Department of Wildlife. Sessions will commence at 1 pm on the 21st and end at noon on the 23rd. Further information on accommodations and program format will be available soon.

ANNUAL MEETING OF THE FISH HEALTH SECTION and EASTERN FISH HEALTH WORKSHOP

The Maryland Department of Natural Resources—Annapolis, the University of Maryland, Department of Microbiology—College-Park, and the National Fish Health Research Laboratory—Leetown, WV are the hosts of this year's national meeting. The joint meeting will be held at the Ramada Hotel in Annapolis, MD on July 17-20, 1989. Registration forms, hotel information and call for papers have been sent to the membership of the Fish Health Section. If you have not received this information and would like it, contact Dr. Frank Hetrick, Department of Microbiology, University of Maryland, College Park, MD 20742 USA. Telephone 301-454-5411.

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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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