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Increased Dilution of Fluorescein Isothiocyanate Conjugates Decreases Nonspecific Reactivity in the Detection of Bacterial Kidney Disease

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Recent studies have reported that available fluorescein isothiocyanate (FITC) conjugates used in direct fluorescent antibody tests (DFAT) for detection of *Renibacterium salmoninarum* react nonspecifically with other bacteria (Foot et al., 1992) Also, Barbash (1992) reported that conjugates cross react with certain fish feed components. We currently use DFAT to detect *R. salmoninarum*, therefore our objectives of this study were to determine if: 1) nonspecific activity to gram-negative bacteria could be decreased by dilution of the conjugate, 2) normal rabbit and normal goat sera contain antibodies to common commensal or environmental bacteria and, 3) cross-reactivity between anti-*R. salmoninarum* conjugates and components of fish feed could be decreased by dilution of the conjugates.

Procedure:

The conjugates chosen for Experiment #1, to compare and determine if nonspecific activity could be decreased by dilution, were: 1) National Fish Health Research Laboratory (NFHRL) FITC-labeled goat anti-*R. salmoninarum* (BKD) (Lot #152-162), 2) NFHRL FITC-labeled rabbit anti-BKD (Lot #1 - 5/88), and 3) Kirkegaard and Perry Laboratories, Inc. (KPL) FITC-labeled affinity purified antibody to *R.*

salmoninarum. Ten bacterial species (Table 1) were grown for 2 days at 28 C in 10 ml of trypticase soy broth and then killed by adding 0.06 ml of 37% formaldehyde solution. Each isolate was washed three times with 0.85% NaCl, resuspended to an optical density of 0.8 at 525 nm and then 10 l of the suspension was added to each ring of a double-ringed etched slide. Slides were air-dried and heat-fixed. The three conjugates were diluted to 1:40, 1:100, and 1:150 using phosphate buffered saline (PBS, pH 7.4). DFAT staining was done in a dark, moist chamber for 10 min, rinsed and covered with PBS for 5 min, then counterstained with 0.01% Evans Blue in PBS for 5 min. Slides were then rinsed with PBS, air-dried and mounted using FA Mounting Fluid pH 9.0 (Difco) and examined at 1000X using an episcopic-fluorescent illuminator.

Experiment #2 was performed using pooled normal goat serum from Sigma (Lot #102H0080), and pooled normal rabbit serum from Sigma (Lot #101H-0500). The serum was diluted to 1:40 and 1:100 with PBS and reacted with ten bacterial species to determine if antibodies to these organisms already existed in normal goat and rabbit sera. IFAT staining was accomplished by labeling with KPL FITC-labeled Protein A (Lot #QC45-5). Protein A, diluted 1:25 in PBS, was added to the bacterial slides after the primary reagent (normal sera) and before counterstaining with .01% Evans Blue. Slides were mounted and examined as above.

Cross-reactivity between the three conjugates and Oregon Moist Pellet (OMP) was tested in Experiment #3. A suspension of OMP was prepared by grinding 1.0 g of diet with a mortar and pestle and adding 10 ml of PBS. The suspension was allowed to stand for 5 min. Slides were prepared by pipetting 20 l onto double-ringed etched slides, then air-dried and heat-fixed. The three conjugate were diluted to 1:40, 1:100, and 1:150 with PBS. DFAT staining

In This Issue:

Dilution of BKD Conjugate	1
Aquatic Birnavirus	5
Piscirickettsia in Freshwater.....	6
Enterocytozoon rRNA Sequence	7
VHSV in British Columbia Herring.....	8
Use of Peroxide for Fungus.....	9
IHNV in Feral Salmonids	10
Fish Health Section Business	
Announcements.....	13
1994 Committees.....	14
Upcoming Meetings	16
Presidents Message.....	17
Committee Reports.....	18

was performed as above in Experiment #1. Slides were mounted and examined as above. Levels of fluorescence in all experiments were compared and rated on a scale of 0-4: 0 = nothing visible, 1 = red; negative, 2 = weak positive, 3 = green; positive, and 4 = strong positive (equivalent to known positive BKD cells).

Results:

Nonspecific activity was observed with both NFHRL-goat anti-BKD and NFHRL-rabbit anti-BKD. KPL-goat anti-BKD, at all dilutions, did not produce any nonspecific activity (Table 2). NFHRL-goat anti-BKD at 1:40 reacted weakly (=2) with *K. pneumoniae* but did not react with the remaining nine isolates. NFHRL-goat diluted 1:100 and 1:150 did not react with any of the bacterial species. NFHRL-rabbit at 1:40 produced weak positive reactions with four bacterial isolates: *E. aerogenes*, *E. tarda*, *K. pneumoniae*, and *A. hydrophila*. At 1:100, NFHRL-rabbit produced a weak positive reaction with *P. aeruginosa* and *K. pneumoniae*, and at 1:150, *K. pneumoniae* remained weakly positive.

Normal goat serum at 1:40 produced a weak positive reaction (=2) with two species: *A. sobria* and *E. coli* (Table 3). At 1:100, only *E. coli* was reactive (=2). Normal rabbit serum at 1:40 reacted weakly (=2) with two species: *A. sobria* and *E. coli*. At 1:100, only *A. sobria* remained weakly positive.

KPL-goat anti-BKD diluted 1:40 produced weak positive fluorescence with bacteria-sized objects in OMP (Table 4). Fluorescence was not observed when KPL-goat anti-BKD was diluted to 1:100. NFHRL-goat anti-BKD at 1:40 reacted with OMP revealed cocci and various rod forms ranging in brightness from weakly positive to strongly positive and at 1:100, short rod, rod and long rod forms remained weakly positive. OMP reacted with NFHRL-rabbit at 1:40 contained fluorescing cells that varied in morphology: cocci (=4), short rods, rods and long rods (=2-4), and large rods (=3). At the 1:100 dilution, less activity was observed but all of the various bacterial shapes reacted positively, especially the large rods. At the 1:150 dilution, short rods and rods had a reaction (=2).

Discussion:

Evidence was not found for antigenic similarity between *R. salmoninarum* and 10 species of environmental or commensal bacteria using the KPL conjugate. Cross-reactivity with the short rod, rod and long rod forms in OMP decreased with increased dilution, while positive BKD controls remained positive. Since the KPL conjugate

undergoes affinity adsorption, this may explain the lack of nonspecific reactivity. Bandin et al. (1993) detected a common antigen among *R. salmoninarum*, *Corynebacterium aquaticum*, and *Carnobacterium piscicola* by the Western Blot technique, but did not find any cross-reactivity among the three bacterial species using the DFAT. We found that both the NFHRL-goat and NFHRL-rabbit conjugates possessed some reactivity with the 10 bacterial isolates, and that they are especially reactive with various bacteria-sized objects found in OMP. The spore-forming, DFAT positive bacteria, which Barbash (1992) isolated from fish feed, may be similar to the large rod forms present in the OMP which reacted with the conjugates used in this study. The nonspecific reactivity of the NFHRL conjugates may be due to the presence of antibodies to commensal bacteria of rabbits and goats. Some evidence that laboratory-reared animals have such antibodies is shown by the results of Experiment #2. From the results of these experiments, we feel confident using the KPL conjugate at higher dilution factors such as 1:100 or 1:150. At these concentrations, the possibility of false positives is reduced, even in specimens contaminated with fish feed.

Future studies involving species producing false positive reactions, such as *Pseudomonas fluorescens* (Foot et al., 1992), should be performed using dilution factors of 1:100 or 1:150 to determine whether the DFAT and available conjugates can be used with continuing confidence.

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Table-1. Species and strains of bacteria used in study.

Species	Strain	Source
<i>Aeromonas hydrophila</i>	ATCC ^a 35654	unknown ^b
<i>Aeromonas sobria</i>	ATCC 9071	Frog
<i>Citrobacter freundii</i>	ATCC 8090	unknown
<i>Edwardsiella tarda</i>	none ^c	Snalmo
<i>Enterobacter aerogenes</i>	ATCC 35028	unknown
<i>Enterococcus durans</i>	ATCC 6056	Human
<i>Escherichia coli</i>	ATCC 25922	Human
<i>Klebsiella pneumoniae</i>	ATCC 13883	Industrial
<i>Plesiomonas shigelloides</i>	ATCC 14029	Human
<i>Pseudomonas aeruginosa</i>	ATCC 10145	Industrial

^aAmerican Type Culture Collection^borigin uncertain; widely used for quality control^cOregon State UniversityTable-2. Relative brightness of 11 species of bacteria stained with three fluorescein-conjugated anti-*Renibacterium salmoninarum* reagents.

Conjugate	KPL			NFHRL-Goat			NFRHL-Rabbit		
	1:40	1:100	1:150	1:40	1:100	1:150	1:40	1:100	1:150
Species									
<i>A. hydrophila</i>	0	0	0	1	0	0	2	1	0
<i>A. sobria</i>	1	0	0	1	1	1	1	0	0
<i>C. freundii</i>	0	0	0	1	1	0	1	1	1
<i>E. aerogenes</i>	0	0	0	1	1	1	2	1	1
<i>E. coli</i>	0	0	0	1	1	0	1	0	0
<i>E. durans</i>	0	0	0	1	1	0	1	1	1
<i>E. tarda</i>	0	0	0	1	1	1	2	1	1
<i>K. pneumoniae</i>	0	0	0	2	1	1	2	2	2
<i>P. shigelloides</i>	0	0	0	1	1	0	0	0	0
<i>P. aeruginosa</i>	0	0	0	1	1	1	1	2	1
<i>R. salmoninarum</i>	4	4	4	4	4	4	4	4	4

0=nothing visible, 1=red, 2=plus minus, 3=green, positive, 4=strong positive

Table-3. Relative brightness of 11 species of bacteria incubated with pooled normal goat or rabbit sera and stained with fluorescein-conjugated protein A to detect adsorbed immunoglobulin.

Dilution	Goat Serum		Rabbit Serum	
	1:40	1:100	1:40	1:100
Species				
<i>A. hydrophila</i>	1	0	0	0
<i>A. sobria</i>	2	1	2	2
<i>C. freundii</i>	0	0	1	1
<i>E. aerogenes</i>	1	0	1	1
<i>E. coli</i>	2	2	2	1
<i>E. durans</i>	1	0	1	1
<i>E. tarda</i>	0	0	0	0
<i>K. pneumoniae</i>	0	0	1	1
<i>P. shigelloides</i>	1	1	1	1
<i>P. aeruginosa</i>	1	0	0	0
<i>R. salmoninarum</i>	0	0	0	0

0=nothing visible, 1=red (counterstain), 2=plus/minus

Table-4. Relative brightness of bacteria-sized objects in smears prepared from homogenized Oregon Moist Pellet, after staining with three fluorescein-conjugated anti-*Renibacterium salmoninarum* reagents.

Dilution	Conjugate								
	KPL			NFHRL-Goat			NFHRL-Rabbit		
	1:40	1:100	1:150	1:40	1:100	1:150	1:40	1:100	1:150
objects									
cocci	0 ^a	0	NT	2	0	0	4	2	0
short rods	1,2	0	NT	2-4 ^b	1,2	1	2-4	1,2	1,2
rods	1,2	0	NT	2-4	2	1	2-4	1,2	0
long rods	1,2	0	NT	2,3	2	0	2-4	1,2	0
large rods	0	0	NT	2,3	0	0	3	2,3	0
<i>R. salmoninarum</i>	4	4	NT	4	4	4	4	4	4

^a 0 = nothing visible, 1 = red, 2 = +/-, 3 = +green, 4 = strong + ^b indicates bacteria with relative brightness of 2, 3, and 4 were observed in the smear

Detection of an Aquatic Birnavirus in an Anadromous Coho Broodstock, *Oncorhynchus kisutch*, in Puget Sound, Washington.

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The Lummi Indian Nation operates a salmon enhancement program at Lummi Bay Sea Ponds located on a spit in Lummi Bay in northern Puget Sound. Adult coho return to this facility during September through October. They are segregated by sex and held in large concrete circulars until spawning in December and January. During this time the fish are supplied first with seawater from Lummi Bay and then gradually changed over to fresh water piped from the Nooksack River. Juvenile progeny are reared at the tribe's Skookum Creek hatchery 51 river miles up the Nooksack River. The Northwest Indian Fisheries Commission provides fish health services for the Lummi tribe which includes broodstock viral testing.

On December 8, 1993, kidney and spleen samples along with ovarian fluids were collected in pools of five from the 60 females spawned. Samples were processed and inoculated onto CHSE-214 and EPC cell lines at 15 C for routine virus testing. Cytopathic effect (CPE) typical of Infectious Pancreatic Necrosis Virus (IPNV) developed in 7 of the 12 samples between the fourth and ninth days of incubation on CHSE-214 cells. Starting on December 27, kidneys and spleens were sampled from 100% of the remaining female and male fish spawned. These samples were all "blind passed" on the 14th day of incubation. In the end, 371 females were tested and 10 of the 75 pooled samples were positive for virus; 83 males were tested with 6 of the 18 pooled samples positive; and 3 mortalities were tested individually and virus was not detected. Initial appearance of CPE varied between four and 15 days, and in one case CPE was not visible until day 4 of the blind pass. Virus was only detected in kidney-spleen homogenate samples inoculated onto CHSE-214 cells. CPE was not seen in the same kidney-spleen samples inoculated onto EPC cells nor was it seen in ovarian fluid samples from the same fish inoculated onto CHSE-214 and EPC cells. Broodfish did not exhibit any signs of disease and there was no related mortality. Samples were not collected for histological examination.

The virus was identified based on characteristic CPE, serum neutralization and reactivity with a panel of monoclonal

antibodies. Infected CHSE-214 cells were first spindle shaped, then rounded and eventually detached from the cell sheet. Neutralization of the virus was obtained with both rabbit polyclonal polyvalent anti-IPNV serum prepared by at National Fish Health Research Laboratory at Leetown and with polyclonal antiserum prepared against the VR-299 IPNV type strain [serotype "1" (Wolf, 1988) or "A1" (Hill and Way, 1983)]. A subculture of the virus was submitted to Dr. Paul Reno for testing against his panel of monoclonal antibodies to 11 aquatic birnavirus epitopes using the immunodot technique (Lipipun *et al.*, 1989; Caswell-Reno, *et al.*, 1989). He found the pattern of reactivity most closely resembled that of viruses belonging to the "A9" (Jasper, Alberta) serotype (Hill and Way, 1983). The Lummi isolate had the same reaction pattern as the virus found in asymptomatic Atlantic salmon post-smolts in seawater in British Columbia in 1989, (Kieser, *et al.*, 1989); this isolate differed from the VR-299 strain at 4 epitopes and from the Jasper strain at only one epitope. The British Columbia isolate was found to be avirulent when used to challenge coho, chinook, Atlantic salmon and rainbow trout fry, and could not be recovered from any of the challenged fish (Traxler and Evelyn, 1991). Dr. Reno has pathogenicity testing and further monoclonal characterization testing of the Lummi isolate in progress. Stay tuned to a subsequent issue of the Fish Health Newsletter for his results. Given the wide host and geographic distribution of multiple strains of aquatic birnaviruses and the unclear definition of IPNV within the group the more general birnavirus designation is used here.

The Lummi tribe made the painful decision to destroy all 2.5 million of the eggs from this broodstock rather than put their Skookum Creek hatchery and the Nooksack watershed at risk. Several factors entered into the final decision: 1) IPNV has not previously been found in the Nooksack watershed, 2) no facilities are available at the Sea Ponds facility to rear these eggs, 3) concern for vertical transmission in spite of iodophor water hardening, and 4) the potential for this virus to develop virulence for coho, trout or other salmon given the opportunity. The decision was made considerably easier for the tribe because the Washington Department of

(Continued on page 6)

(Continued from page 5)

Fisheries offered replacement coho eggs from nearby watersheds provided the Lummis did not move the suspect eggs to their upriver hatchery.

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First Report of *Piscirickettsia salmonis* in Freshwater

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In December 1993, an outbreak of piscirickettsiosis caused by *Piscirickettsia salmonis* occurred among a stock of rainbow trout, *Oncorhynchus mykiss*, (mean weight 1.6 kg) reared in cages at Lake Llanquihue (southern Chile). Mean water temperature in the lake at the time the disease occurred was 17 C. Infected rainbow trout were imported into Chile as eggs from the United States in January 1993 and reared in freshwater - first in a fish hatchery supplied with water from a mountain river, and then moved to cages in Lake Llanquihue where they have remained.

Infected fish showed pale gills and were lethargic, tending to seek quiet water at the surface or in corners of the cages. Internally, the main signs were: mottled liver, swollen kidney, enlarged and mottled spleen and empty gut. These signs are also exhibited by salmonids infected with *Piscirickettsia salmonis* in saltwater. Tissue smears of affected organs were stained with Giemsa and showed presence of many intracellular bacterial cells morphologically similar to *P. salmonis*. In addition the FA test for *P. salmonis* was positive in each animal examined by this method. Growth was not obtained when infected tissue was inoculated on to five bacteriological media.

This is the first report of this pathogen in fish held only in freshwater and is an important observation. Llanquihue is the largest lake in Chile and provides the highest production of smolts. The lake is located near 14 hatcheries or farms and several rivers flow into this lake.

Experimentally, horizontal transmission of rickettsia in freshwater was not possible to demonstrate and all other stocks of salmonids reared in the same place tested FA negative for presence of *Piscirickettsia salmonis*.

Enterocytozoon salmonis small subunit rRNA sequence

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Microsporidia are obligate intracellular, spore forming parasites that infect many major animal groups, especially insects, fishes and mammals. Many genera are important pathogens of fishes, and 5 genera have been associated with human disease. The unusual genus *Enterocytozoon* contains two species; *E. bienewsi* reported from AIDS patients (Wittner et al. 1993) and *E. salmonis* from chinook salmon (Chilmoneczyk et al. 1991). The justification for placing *E. salmonis* in this genus was based on the presence of organelles, originally described in *E. bienewsi*, and their unique involvement in the formation of sporonts and spores. *Enterocytozoon salmonis* infects the nuclei of lymphoblasts or plasmablasts of chinook salmon, and has been associated with leukemia-like diseases (e.g., plasmacytoid leukemia [PL]) (Morrison et al. 1990; Hedrick et al. 1990; Kent et al. 1990). However, fish with PL are also infected with a retrovirus, and evidence at this time suggests this virus (SLV) is the cause of PL in British Columbia (Eaton and Kent 1992; Kent and Dawe 1993).

Relman et al. (1990) described techniques for the identification of the non-cultivable organism responsible for bacillary angiomatosis using conserved SSU-rRNA (16S rRNA) gene oligonucleotide primers and the polymerase chain reaction (PCR). Utilizing the conserved SSU-rRNA sequences and the known microsporidian rRNA sequence of *Vairimorpha necatrix* (Vossbrinck et al. 1987), we were able to amplify by PCR and subsequently clone and sequence the SSU-rRNA of *E. bienewsi* (Zhu et al. 1993b) and another microsporean from humans, *Septata intestinalis* (Zhu et al. 1993a). Both of these microsporeans are non-cultivable; however the SSU-rRNA, intergenic spacer region, and a fragment of the large subunit rRNA were cloned from infected human tissue using phylogenetically conserved rRNA primers in the SSU-rRNA gene (530f and 228f) and in the large rRNA subunit (580r) (Zhu et al. 1993a, 1993b, 1993c; Vossbrinck et al. 1993).

Using the above techniques we were able to amplify and sequence rRNA from *E. salmonis*. The amplified fragment using these conserved primers demonstrated an intergenic spacer region of over 200 bp. In microsporidia other than *E. bienewsi*, the intergenic spacer region is less than 80 bp in length. This provides molecular evidence of the correct placement of *E. salmonis* into the genus *Enterocytozoon*. Furthermore, preliminary sequence data demonstrates that *E. salmonis* and *E. bienewsi* are highly related but are distinct organisms, with about 90% homology. It is thus unlikely that salmon infected with *E. salmonis* are the environmental source for infection of humans

with *E. bienewsi*.

We plan to develop a PCR-based diagnostic for *E. salmonis* using this sequence data. This test would be very useful for determining the true prevalence of this microsporean in various populations of chinook salmon (i.e., those with and without PL), and thus would be useful for determining the role of *E. salmonis* in PL and related diseases.

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Isolation of the North American strain of Viral Hemorrhagic Septicemia Virus (VHSV) from herring (*Clupea harengus pallasii*) in British Columbia

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The North American strain of viral hemorrhagic septicemia virus (VHSV) was recently isolated from Pacific herring collected at several locations in British Columbia. Virus isolates were identified as the North American strain of VHSV by researchers at the National Biological Survey laboratory in Seattle, Washington.

The first sample of juvenile herring was collected from Prince Rupert Sound on September 9, 1993 following reports of a diesel oil spill and dead herring in the area on August 29, 1993. Virus, which subsequently was identified as the North American strain of VHSV, was isolated from 8 of 10 fish using standard virological tests. Several herring had external lesions and hemorrhages. It is not known whether the mortalities and surface lesions were due to the presence of the virus or to the diesel spill.

After initial isolation of VHSV from the Prince Rupert area, herring populations from 4 other regions of British Columbia were sampled. The first group of herring was obtained on October 10, 1993 from a bait supplier located in Campbell River on Vancouver Island. Fish length varied from 15-20 cm. Except for some mechanical damage in one fish, no external lesions were seen. The stock had experienced no excessive mortalities during the holding period in bait ponds. Analysis of pooled tissue samples (gill, kidney, spleen, and pyloric caeca) from individual fish, revealed that 13 of 60 fish were positive for VHSV.

Another 60 herring of similar size were collected on October 19, 1993 from a bait supplier near Sechelt. Again, no losses were apparent in the ponds, and fish were free of any external lesions. Twenty-five of the 60 fish were positive for VHSV.

Another group of juvenile herring (mean wt. 4.2 g) was captured from Departure Bay in September 1993 and held in tanks at the Pacific Biological Station. Over the next two months, 70 of the fish were individually assayed and all were negative for VHSV. Finally, a sample of 15 herring caught in December 1993 near Thresher Rock off of Gabriola Island also tested negative.

These isolations of VHS virus from a wide geographic area of British Columbia, in addition to those reported from Alaska and Washington State (Meyers et al. 1993, Batts pers. comm.) indicate a widespread distribution of the virus in herring populations along the north Pacific coast of North America. It would appear that the current range extends from Kodiak Island in Alaska south to Puget Sound in Washington state. Pathogenicity tests are being conducted to determine the possible threat of this virus to herring, Pacific salmon, and Atlantic salmon. We are particularly interested in the susceptibility of the Atlantic salmon to the virus because it is widely farmed in British Columbia and because it may not have evolved in contact with the virus, being non endemic to the Pacific Northwest.

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La Crosse Center Discovers Fungicide for Immediate Use

Verdel K. Dawson, Rosalie A. Schnick, Jeffery J. Rach and Theresa M. Schreier, National Biological Survey, National Fisheries Research Center-La Crosse, P.O. Box 818, La Crosse, WI 54602-0818.

The National Fisheries Research Center at La Crosse, WI (La Crosse Center) has found a replacement fungicide for malachite green. Information provided by the La Crosse Center has led the FDA to conclude that hydrogen peroxide is a low regulatory priority (LRP) drug when used at concentrations of 250-500 I/L (ppm) to control fungi on all species and life stages of fish, including eggs.

Preliminary tests conducted at the La Crosse Center suggest that prophylactic treatments of 250-500 ppm (based on 1000% active ingredient) for 15 minutes every other day will protect healthy rainbow trout eggs from fungal infections. This treatment regime also seems to inhibit fungal development and increase hatching success among infected eggs; additional confirmatory tests are being done at the La Crosse Center.

The LRP ruling allows the use of hydrogen peroxide as a fungicide on both eggs and fish; however, caution must be used when treating fish because they tend to be more sensitive to hydrogen peroxide than eggs. In preliminary studies, exposures to 1,000 ppm of hydrogen peroxide for up to 1 hour were not lethal to sac fry of rainbow trout; however, the available data supporting fungicidal applications of hydrogen peroxide, especially to fish, are minimal, and any intended applications of hydrogen peroxide should first be tested on a small batch of fish or fish eggs. The La Crosse Center is developing additional efficacy and safety data to further delineate the use pattern for all fish species and life stages. These data will be disseminated as soon as possible.

The LRP status of hydrogen peroxide should not be considered an affirmation of its efficacy or safety by FDA. This FDA ruling allows the use of hydrogen peroxide in aquaculture without an investigational new animal drug permit (INAD) or new animal drug application (NADA). It is essential that all users of hydrogen peroxide apply this compound in a responsible manner so that the LRP status is not jeopardized.

Presently, the FDA is not likely to object to the use of hydrogen peroxide if the following five conditions are met:

1. It is used as a fungicide on fish and eggs.
2. It is not used at concentrations exceeding 500 ppm.
3. It is used according to good management practices.
4. There are not likely to be any adverse effects on the environment.
5. It is of an appropriate grade for use on food animals.

Fish culturists may obtain hydrogen peroxide from many commercial sources; however, vendors who advertise the product for use in aquaculture must meet certain FDA requirements. The La Crosse Center used a 35% solution obtained from Eka Nobel, Inc., Marietta, Georgia.¹ The 35% formulation is suggested for use due to availability and safety. Users should wear rubber gloves and eye protection at all times when handling hydrogen peroxide.

Fish culturists now have the option to use hydrogen peroxide as a fungicide as well as formalin, which is the only fungicide with an approved NADA. Estimated costs for fungicidal use of hydrogen peroxide are comparable to those of formalin.

¹ Mention of company or trade names does not constitute endorsement.

Infectious Hematopoietic Necrosis Virus (IHNV) Found in Four Geographically Distinct Feral Populations of Salmonids in Oregon

H. Mark Engelking and John Kaufman. Oregon Department of Fish and Wildlife, Center for Salmon Disease

Wild salmonid fish in Oregon have been surveyed for presence of Infectious hematopoietic necrosis virus (IHNV) for several years. IHNV has been reported in feral fish from British Columbia to Alaska in both sockeye salmon, *Oncorhynchus nerka*, and kokanee salmon, non-anadromous *O. nerka*, (Traxler and Rankin, 1989). IHNV has been found consistently in fish examined from four areas in Oregon during the last three years. This report documents the presence of IHNV from dead kokanee salmon at Lake Billy Chinook in central Oregon; in spawning fall run chinook, *O. tshawytscha*, in the Applegate and Rogue rivers in southern Oregon; in spawning winter run steelhead trout, *O. mykiss*, in the South Santiam River in the mid-Willamette Valley and in the Clackamas River in northwestern Oregon. Other locations have been surveyed and IHNV has been detected. In particular, wild fall run chinook in the south Oregon coastal streams have had a high prevalence of IHNV over a number of years. These results will be reported on in a more inclusive discussion.

In winter of 1991, large numbers of bald eagles were seen in the area around Lake Billy Chinook by D. Ratliff, PGE biologist. Birds were attracted to the area by dead two and three year old kokanee salmon that were present in large numbers along the shore. These fish were examined for signs of disease and both IHNV and *Cytophaga (Flexibacter) psychrophila* were found. In February, 1991, on two separate occasions, sixteen fish each were examined for virus. In March, seven additional fish were examined. All fish had IHNV present in their tissues. Virus titers in tissues ranged from 1.4×10^8 to 2.6×10^3 plaque forming units per gram. The virus strain was Type 1 as determined by electropherotyping and non-reactivity to monoclonal antibody 2NH105B in immunofluorescent assays (Ristrow and Arnzen, 1989). the cause of mortality could not be assigned directly to one specific pathogen (Banner, et al., 1991).

On April 24, 1992 a moribund female kokanee salmon with an external lesion was examined from Lake Billy Chinook. The gill sample contained IHNV and this isolate was also a Type 1 strain. In May of 1993, two dead adult kokanee salmon from Lake Billy Chinook were examined for presence of virus. Both fish had IHNV in spleen, kidney and brain samples. Again, this was a Type 1 IHNV isolate.

Spawning ground samples, from wild spawning fall run chinook salmon, were obtained by biologists from the Central Point ODFW office and sent to the Corvallis ODFW laboratory at Oregon State University for examination. Sampling begins along the Rogue and Applegate rivers in October and continues until January. In 1991, 110 fish were sampled and IHNV was found in two sample groups from December. Twenty percent of the December 6 samples and 17% of the December 12 samples contained virus. This IHNV isolate was determined to be a Type 3 strain by electropherotyping.

In 1992, 87 fish were sampled over six occasions. Type 3 IHNV was found in a single sample of fifteen individual fish samples during November. Other virus positive samples were not found that year.

IHNV was recovered in six of thirteen 1993 examinations of wild, fall run chinook salmon from the Rogue River system. One hundred and seventy-two fish were sampled from October 1993 through January 1994. In November, the Type 3 IHNV strain was isolated from 17% of the fish in one sample group. During December IHNV, at a prevalence ranging from 20 to 60%, was found in four examinations. In January 33% of the fish assayed were IHNV positive.

Twelve winter steelhead from the South Santiam River were sampled ten times from March through May 1992. Forty-three fish were sampled in six exams taken from March and April with no evidence of virus in the samples. In the first two exams in May three of five fish were detected IHNV positive. The isolate was determined to be Type 2 by reactivity with monoclonal antibody 2NH105B in an immunofluorescent antibody assay (IFAT). The last two exams in May consisting of two fish each did not detect IHNV. In 1993, five exams of twelve fish were performed on wild winter steelhead trout from the South Santiam River. One IHNV positive fish was found and this virus strain was determined to be Type 2.

(Continued from page 10)

Seventeen of thirty wild adult Clackamas River steelhead sampled on five occasions in May and June of 1991 had IHNV present. The first two samplings had no evidence of virus, but in the last three spawnings there were virus positive fish. IHNV was found in all wild Clackamas River spawning winter run steelhead in June. Five spawned fish were determined to be IHNV positive by the coagglutination assay (Bootland and Leong, 1992). Plaque assays determined the virus titer to range from 1.6×10^4 to 1.5×10^8 plaque forming units per ml of ovarian fluid or gram of tissue (Table 1). The virus strain was determined to be Type 2 by a positive reaction with the selective monoclonal antibody 2NH105B in IFAT.

In May of 1992, a similar result was obtained when the wild winter steelhead in the Clackamas River were spawned. In two spawnings, eleven of twenty fish were found to be IHNV positive. The isolate reacted with the 2NH105B monoclonal antibody in IFAT indicating it was a Type 2 strain similar to the previous year's isolate. Thirty winter steelhead from the Clackamas river were spawned on four occasions in May of 1993; evidence of virus was not found.

Infectious Hematopoietic necrosis virus was found in four geographically distinct watersheds in three different feral species of salmonids in Oregon. This extends the known range of IHNV in feral fish to southern Oregon. For the first time, IHNV has been reported in two other wild species of salmonids, fall run chinook salmon and winter run steelhead trout. It has been found annually in all these stocks of fish in the past three years, with the exception of the winter steelhead of the Clackamas River in 1993 and the winter steelhead of the South Santiam River in 1991. The low prevalence of virus and small sample sizes may have contributed to situations where virus was not found. The virus strain has not altered in biochemical type in these areas. Each area has a distinct electropherotype of IHNV, which correlates with those previously described for these areas (Hsu, et al., 1986). Interestingly, the electropherotype associated with those areas, which have been previously studied, has been stable for more than ten years. This may indicate that the virus is under evolutionary pressure to maintain some type specific characteristics and variants are less adapted for survival. The presence of pathogenic virus and other pathogens in wild stocks of fish must be taken into consideration in plans to protect threatened and endangered salmonids. Wild stocks of fish will continue to be surveyed in Oregon. Continued monitoring of these fish will aid in understanding the ecology of this virus and its life cycle.

The support and assistance in obtaining these samples by the ODFW hatchery managers and crews and the field fish

(Continued on page 12)

Fish Sample	Sample Tissue	Titer
Female	Pyloric caeca, kidney, spleen (PKS)	1.23×10^8
	Ovarian fluid	5.20×10^7
Female	PKS	2.93×10^5
	Ovarian fluid	5.65×10^3
Female	PKS	1.51×10^8
	Ovarian fluid	3.60×10^6
Male	PKS	6.45×10^4
Male	PKS	1.64×10^4

(Continued from page 11)

biologists is gratefully acknowledged. Funding for this work was in part from Federal Aid in Sport Fish Restoration Program administered by the U.S. Fish and Wildlife Service and from the State of Oregon.

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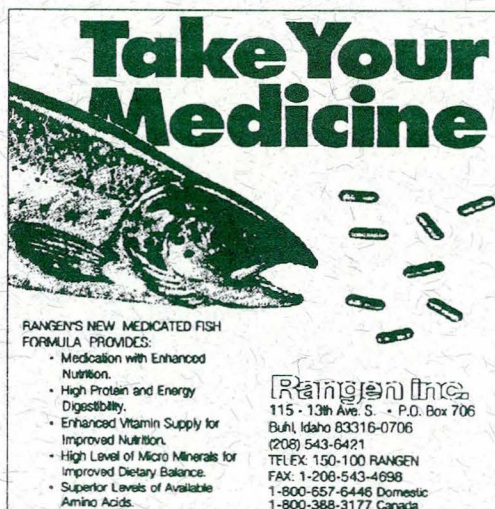
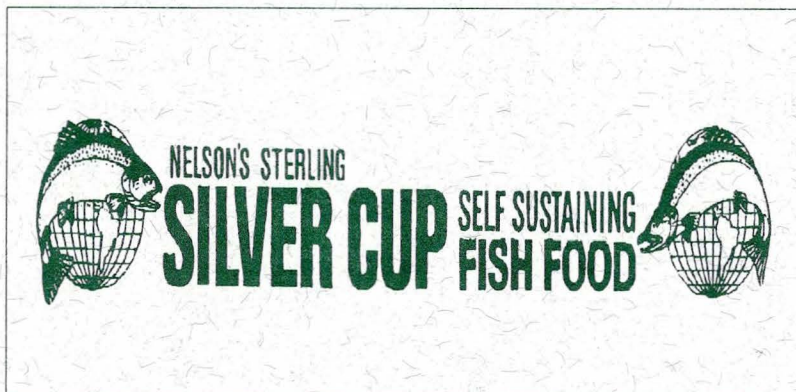
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ANNOUNCEMENTS

Brush Up On Viral CPE. In conjunction with the **Western Fish Disease Workshop, June 22-24** in Bozeman, you will have the opportunity to refresh your acquaintance with various viral CPE on various cell lines. This instructional lab session is being sponsored under the Professional Standards Committee by the fledgling *ad hoc* Continuing Education Committee. If you'd like to refresh your familiarity with CPE of viruses you don't often see, make sure you register for the Continuing Ed. Lab when you register for the Workshop.

Continuing education is an important part of maintaining our professionalism. Indeed, the forthcoming standards for Fish Pathologist and Fish Health Inspector will require continuing education for recertification. The CE Committee goals include locating, recruiting, coordinating, and publicizing educational opportunities, facilitating their availability to the profession, and operating a system of credits for attendance to be used for certification. These are ambitious goals which will need the involvement of a number of people to make them operational. If you even think you might be interested in contributing some time, educational materials, and/or suggestions to this cause, please call, FAX, or write for more specific information. A list is being assembled of interested people and what kinds of involvement and resources they'd like to offer. You can get in on the ground floor of this effort and help shape its future with any involvement level you want.

Contact: Craig Olson
NW Indian Fisheries Commission
6730 Martin Way E.
Olympia, WA 98506
(206) 438-1180
FAX (206) 753-8659

CALLING ALL SHELLFISH PEOPLE:

TECHNIQUES IN FISH IMMUNOLOGY-4 will be a special edition on techniques used in shellfish and aquatic invertebrate immunology, immunopathology and pathology. Also included will be a section on the care and handling of shellfish in laboratory situations. Titles should be submitted by April, 1994 with first drafts due July, 1994.

Write Dr. Joanne Stolen at SOS Publications, 43 DeNormandie Avenue, Fair Haven, NJ 07704-3303, (908) 530-3199, (908) 530-5893 Fax

CALL FOR PAPERS:

Aquaculture '95: The triennial meeting of the Fish Culture Section of the American Fisheries Society, the World Aquaculture Society, and the National Shellfisheries Association. FEBRUARY 1-4, 1995, Town and Country Hotel, San Diego, CA.

DEADLINE FOR ABSTRACTS: JULY 15, 1994

For Abstract transmittal form and more information contact: Aquaculture '95, c/o Sea Fare Expositions, Inc., 850 NW 45th Street, Seattle, WA 98107.

AQUAMED'94: AN AQUATIC ANIMAL PATHOBIOLOGY COURSE. June 6- July 1, 1994. Galveston, TX and Baton Rouge, LA.

The Gulf States Consortium of Colleges of Veterinary Medicine at Auburn University, Mississippi State University, Louisiana State University, Texas A&M University, Tuskegee University and the University of Florida is offering a four-week aquatic animal pathobiology course (AQUAMED). The objective of AQUAMED is to develop and understanding of health management techniques and pathological processes relating to the etiology, diagnosis, and treatment of important aquatic animal diseases. Ornamental fish, commercially important warmwater fish and shellfish will be emphasized. An overview of reptiles, marine mammals and aquatic laboratory animals will be presented. College credit and continuing education credits are available. Contact: Dr. R.F. Sis, AQUAMED, Department of Veterinary Anatomy and Public Health, Texas Veterinary Medical Center, TAMU, College Station, TX 77843-4458; (409) 740-4528

SALMONID DISEASE WORKSHOP. June 8-17, 1994. Oregon State University Hatfield Marine Science Center, Newport, OR.

This workshop is designed for professionals working in the fish health field and will emphasize recent advances and developments in our understanding of salmonid diseases. Examples of topics to be covered in the workshop include:

- *Current immunological techniques for detection and identification of salmonid pathogens.
- *Current status of salmonid virus subunit vaccine development
- *Histopathology associated with salmonid diseases.
- *Cell culture techniques, including maintenance of cultures, mycoplasma detection, and viral identification.
- *Disease associated with net-pen cultures of salmonids.
- *Current status of important viral, bacterial, and parasitic pathogens.

The workshop is limited to 20 participants on a first come first serve basis. Applications are due at the Center by **May 13, 1994**. For further information, please call or write: Dr. Robert Olson, Hatfield Marine Science Center, 2030 Marine Science Dr., Newport, OR, 97365. (503) 867-0251.

INTERNATIONAL SYMPOSIUM ON AQUATIC ANIMAL HEALTH

On behalf of the Fish Health Section of the American Fisheries Society and our co-sponsors, the Organizing Committee for the International Symposium in Aquatic Animal Health would like to extend an invitation to attend our meeting in Seattle, Washington, USA, on **September 4-8, 1994**. Please join us for what should be an excellent exchange of scientific information and a chance to meet new colleagues and renew friendships. **Because of limited space for presentations and hotel rooms, early registration is strongly suggested.**

Registration forms, payment (to qualify for early registration fee) and abstract are due **May 15, 1994** to:

Ms. Lorna McAdam
Department of Medicine
School of Veterinary Medicine
University of California
Davis, California 95616 USA

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MEETINGS

International Association of Aquatic Animal Medicine. May 11-14, 1994. Napa, CA. Contact: Brad Fenwick, Department of Veterinary Pathology, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506; (913) 532-4412.

International Workshop on the Biological Bases for Aquaculture of Siluriformes. May 24-27, 1994. Montpellier, France. For more information contact: Organizing Secretariat, Ste Internationale de Congres et Services, 10, rue Charles Amans, 3400 Montpellier FRANCE. (33) 67-58-59-03.

Eastern Fish Health Workshop. May 25-28, 1994. Blacksburg, VA. Contact: Steve Smith, Department of Pathobiology, VA/MD Regional College of Veterinary Medicine, VPI, Blacksburg, VA 24601; (703) 231-5131.

Virulence Mechanisms of Bacterial Pathogens. June 6-8, 1994. Ames, IA. Contact: Dr. James A. Roth, Professor, Department of Microbiology, Immunology and Preventative Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA 50011; (519) 294-8459.

Genetics in Aquaculture. June 19-25, 1994. Dalhousie University, Halifax, Nova Scotia. Contact: Pamela Gaines, Marine Gene probe laboratory, Life Sciences Center, Dalhousie University, Halifax, NS, Canada B3H 4J1; (902) 494-3736 Fax.

High Performance Fish: An International Fish Physiology Symposium. July 16-21, 1994. University of British Columbia, Vancouver, Canada. Contact: Don D. MacKinlay, Fisheries and Oceans, 555 West Hastings St., Vancouver V6B 5G3 CANADA; (604) 666-3520, (604) 666-3450 Fax

American Fisheries Society. August 21-25, 1994. Sheraton Hotel and World Trade Centre. Contact: Paul Brouha, AFS, 5310 Grosvenor Lane, Suite 110, Bethesda, MD 20814-2199; (301) 897-8616, (301) 897-8096 Fax.

International Symposium on Aquatic Animal Health. September 4-8, 1994. Seattle, WA. Contact: Ms. Lorna McAdam, Meeting Secretary, Dept. of Medicine, School of Veterinary Medicine, University of California, Davis, CA 95616. (916) 752-0414 Fax.

Fish Parasite Roundtable Discussion/Symposium ICOPA VIII. October 10-14, 1994. Izmir, Turkey. Contact: Professor, Dr. M. Ali Ozcel, Chairman of the Organizing Committee, ICOPA VIII, P.K. 81 35042, Bornova, Izmir, TURKEY.

POSITION AVAILABLE

Post Doctoral Fellowship, Department of Fisheries & Oceans, Pacific Region, Biological Sciences Branch

We anticipate that funds for a 2-year post-doctoral fellowship at DFO's West Vancouver laboratory will be available in April 1994, under the program Visiting Fellowships in Canadian Government Laboratories. Using molecular techniques, research will be directed towards developing DNA-based diagnostic tests for certain myxosporean and microsporean pathogens of salmonid fishes, and elucidating the relationship of various developmental stages of myxosporeans from different host species.

Candidates should have a Ph.D. and training in molecular biology. Interest and experience in parasitology and/or fish pathology would be an asset. Stipend: approximately Can.\$38,000/year. Non-Canadian citizens are also eligible to apply. Interested candidates should contact either: Drs. Michael Kent or Leo Margolis, Dept. Fisheries and Oceans, Biological Sciences Branch Pacific Biological Station, Nanaimo, B.C. V9R 5K6 tel. (604) 756-7119 or 756-7032 FAX (604) 756-7053.

FHS President's Message

As Bob Dylan wrote, "the times they are a changing". The last issue of the FHS newsletter contained a proposal to toughen up the Certified Fish Pathologist standards. I hope many of you took the time to carefully evaluate the proposal and return comments. This issue of the FHS newsletter contains a list of proposed bylaw changes for comment. Please take the time to look these over with a critical eye and make comments. I know time is short for everyone, but the future direction of the section is being shaped by many of these changes. Maximum membership input is needed to ensure that the section evolves in response to the needs and concerns of its members. Take some time, give some thought, and send in your comments on both proposals.

On another note, our discussions in Denver relative to fish health professionals/veterinary interaction were very interesting. There are some lyrics from an old Buffalo Springfield song that were brought to mind during the discussion. They go something like this:

*"There's something happening here.
What it is ain't exactly clear.
There's a man with a gun over there,
Telling me I've got to beware."*

Believe it or not, this tune was first sung (as far as I can tell) in the Fish Health Section Newsletter in 1977, when Joe Sullivan, the editor, inquired "I wonder how many of us without veterinary degrees would feel threatened if DVMs in great numbers began 'competing' with us?" This question was raised in response to an article by Marc Dulin in the June 1977 issue of Veterinary Medicine/Small Animal Clinician entitled, "Aquatic animal medicine: a new specialty for veterinarians". Lee Harrell responded that "competing is best left to used car salesmen" and, that if he were threatened professionally, it would not speak very highly of his professional confidence. Dr. Dulin responded that the competition would not materialize because veterinarians do not have the training in fisheries/aquaculture and because the salaries paid to fisheries types are all too low. However, he also thought that small animal veterinarians with some aquatic training would be generally more available to the individual tropical fish hobbyist than the more isolated fish biologist. George Klontz summed it up by saying that "our profession has to grow up and cease to think that we are the only ones who can solve our problems (in fish health)". Although the emphasis has changed somewhat, this sounds very similar to much of what I heard in Denver.

In reality, we should welcome any qualified fish health specialist regardless of the letters following their name. Members of the Fish Health Section, be they PhDs, DVMs, MSs, BSs, or SOBs, have only the responsibility and obligation of ensuring that the Fish Health Section remains an association committed to protecting the health of our aquaculture/fisheries resources. Hopefully this can be done in a congenial, cooperative manner with the veterinary profession. As many of you may know, I was recently asked to serve as a non-veterinary representative on the AVMA's Aquaculture and Seafood Advisory Committee. The appointment of a non-DVM representative to the committee was in response to suggestions from members of the FHS, both DVM and non-DVM. Although my first meeting is not until April 16, I think this appointment indicates that the AVMA wants to ensure communication and cooperation within the fish health community.

Ronald L. Thune

BYLAWS REVIEW COMMITTEE REPORT

Ted Meyers, Committee Chairman February, 1994

Ron Thune, current AFS FHS President, assigned the formation of a FHS Bylaws Review Committee to President-Elect Ted Meyers. As chairperson he and six additional members agreed to participate including Spike Beleau, Vicki Blazer, John Cvitanich, Ron Hedrick, Randy MacMillan and John Plumb. Committee members contacted many other FHS members including past presidents and committee chairs and submitted their recommendations for FHS Bylaws changes to the the Bylaws Review Committee Chair. These recommendations are presented here for membership comment and any additional recommendations by the members at large are encouraged. After the comment period a ballot will be sent to the membership for a formal vote on any proposed changes to the FHS Bylaws. Comments and additional suggestions for proposed changes to the Bylaws should be sent to Ted Meyers, ADF&G, CFMD Division, P.O. Box 25526, Juneau, AK 99802-5526 (907) 465-3577.

1. The term for FHS President should be extended to two years.
2. An additional clarification under the President's responsibilities of "appoint all committees" should also include "send a list of committee members to the Newsletter editor by deadline for summer volume" or this could be stated in Section 8 under Standing Committees.
3. Duties listed for the President-Elect should include "shall work closely with the President in all Section matters, shall be responsible for providing an annual plan for adoption by the Executive Committee immediately preceding assumption of presidency".
4. The Nominating Committee should include the immediate past-President to allow persons knowledgeable about membership to help recruit candidates.
5. Clarify the responsibility for sending "Section News" to the AFS Fisheries staff by so stating in the Bylaws.
6. The deadline for providing announcements to editors regarding awards should be no later than the deadline for the Fall edition rather than December 1.
7. The Bylaws should be changed to reflect the current practice of presentation of awards by the President rather than by the Awards Committee Chair.
8. Awards listed should also include Student Travel Awards.
9. Section 8 (g): Awards Committee - The Awards Committee should nominate the Special Achievement Award in addition to the S.F Snieszko Award.
10. Bylaws require up-to-date recording of materials in the Archives which is not currently done. Either this be done routinely or the function should be removed from the Bylaws.
11. Rewording to more accurately reflect that The Bluebook Advisory Committee will not be responsible for the distribution of the new edition.
12. Section 7: Executive Committee - A provision for the Chairperson of the Blue Book Committee to become a voting member of the Executive Committee was passed (Maryland Mtg 1989) but never incorporated into the Bylaws.
13. Language for The Newsletter and Publications Committee should be changed to state that the committee shall be composed of at least a chairperson and four members.
14. Professional Standards Committee should be changed to include an AFS Inspector or Pathologist who is also a veterinarian.
15. Change Presidential appointment of The Chair of the Professional Standards Committee to once every two years rather than for an indefinite period of time.
16. The Chair of the Board of Certification should be elected for two years rather than one and Bylaw language should clarify the mechanism for the election that is to be initiated by the current Chair. A process suggested would be to have a statement that "among the members elected to the Board that whomever has 2 years (or 3 if none with 2) left to serve on the Board shall be appointed or elected (if two or more people) by the Board to the Chair position when the current Chair's tenure is up. Formal anniversary dates must be established for each member in order to track the need for elections.

(Continued from page 18)

17. Limit the membership on the Board of certification to 3 people rather than 5. That would still allow a majority vote and would facilitate soliciting volunteers for election, paperwork and turnaround time.
18. Section 8: Standing Committees - Eliminate Technical Procedures standing committee due to redundancy by The Bluebook Advisory Committee or if effort is not duplicated language should clearly state the differences.
19. Section 8 (j): Standing Committees - correct the title of the Blue book to "Suggested Procedures for the Detection and Identification of Certain Finfish and Shellfish Pathogens".
20. Remove the requirement for full membership vote to create or delete standing committees.
21. The current Professional Standards Committee and Board of Certification would be replaced with a single, elected Professional Standards Board (PSB). The PSB would be elected by currently certified Fish Health Inspectors and Fish Pathologists. The PSB would be responsible for maintaining the certification program, including screening of applications, administration of exams, updating the Standards and Procedures for certification, etc. Changes in the Standards and Procedures would be subject to approval of the currently certified Fish Health Inspectors and Fish Pathologists.
22. Section 4: Membership - Change the Bylaws to allow membership in the FHS without being a member of AFS by creating an affiliate membership. This membership would not vote, not hold office and would not participate on any FHS committee but would receive the Newsletter and all other benefits of the FHS. This change would make the FHS available to non-fishery people such as veterinarians and others who do not wish to belong to the AFS for one reason or another. Affiliate membership may increase the FHS role but could also reduce AFS membership - a serious consideration.
23. The charge of the Time and Place Committee be changed to require that the list of sites and dates for the annual meeting that is provided to the Executive Committee will reflect sites for the meeting two years in advance. Thus, sites would be presented and approved for the 1996 meeting at the 1994 meeting, for the 1997 meeting at the 1995 meeting, etc

AWARDS SOLICITATION

The Awards Committee is soliciting nominations from the Fish Health Section membership for the 1994 S.F. Snieszko Distinguished Service Award, the 1994 Special Achievement Award and the 1994 Student Award.

The S.F. Snieszko Award is the highest award given by the Section to recognize fish health scientists for outstanding contributions to the profession. Individuals to be considered for this award must be nominated by a current member of the Section. Persons making nominations should obtain six letters of recommendation from fish health professionals that support the nominee's dedication to research, teaching and/or service to the field of fish health. The six letters along with a curriculum vitae for the nominee should be sent along with the letter of nomination. Nomination packages should be sent to the Awards Committee prior to May 15, 1994.

The Special Achievement Award is to provide timely recognition for one-time accomplishment that have a significant impact on the management or control of fish health problems. This award may be given for 1) a unique contribution to the fish health field (such as a new diagnostic tool, a new technique to control disease, etc.), 2) a significant research accomplishment, or 3) outstanding leadership in resolving a major fish health problem. The achievement must meet high standards of science and survive peer review. Individuals to be considered for this award must be nominated by a current member of the Section. The letter of nomination should clearly state 1) the accomplishment; 2) the significance of the accomplishment to the field; 3) the implication of the accomplishment to aquaculture (local, regional, national, or worldwide). Copies of any articles or other documents relating to the work should be included. Nominations for the Special Achievement Award should be made within one year of the accomplishment and may be submitted to the Chairman of the Awards Committee at any time.

These awards are an important part of our Fish Health Section and our chosen profession. Peer recognition for dedication, hard work, and professionalism is important to all of us. While compiling nominations may take a little time away from our individual dedication and hard work, it is an integral part of our professionalism to recognize appropriate achievements.

For submitting nominations or further information, contact:

Peter W. Taylor, Chairman
Awards Committee, FHS
Southeastern Fish Cultural Laboratory
Route 3, Box 86
Marion, AL 36756 (205) 683-6199

*WESTERN FISH DISEASE WORKSHOP
JUNE 22-24, 1994
BOZEMAN, MONTANA*

MEETING ANNOUNCEMENT and FIRST CALL FOR PAPERS

The 1994 Western Fish Disease Workshop will be held at the Holiday Inn in Bozeman, Montana. The workshop will begin at 8:00 am Wednesday June 22nd and end at 12:00 noon Friday June 24th. Bozeman, Montana is located 80 miles north of Yellowstone National Park. Currently, Bozeman is serviced by Delta, Horizon, and Northwest airlines. Shuttle service to the Holiday Inn (8 miles from the airport) is available at the airport.

A block of rooms has been set aside at the Holiday Inn (800-366-5101) for June 21-23. Room rates are \$64.00 per night, single and \$72.00 per night, double. Federal government rates are available for a block of 20 rooms. These rooms will be held until May 21, 1994. Make your reservations directly with the Holiday Inn and indicate that you are attending the Western Fish Disease Workshop. **MAKE YOUR RESERVATIONS NOW.** Bozeman is a very popular tourist destination and rooms will be difficult to find if you wait. A list of alternate accommodations is attached.

A registration fee of \$15 will cover meeting costs, lunch on Wednesday June 22nd, and the registration packet. A BBQ has been arranged for the evening of June 23rd and will cost \$18 per person, guests are welcome. Rangen, Inc. has agreed to cohost the meeting.

In the interest of making this an informal meeting; the format will include formal presentations, panel discussions, roundtable sessions, and poster presentations. Roundtable and/or poster sessions will be scheduled for Thursday afternoon for attendees that do not participate in the continuing education session.

Suggested agenda topics include (but are not limited to): BKD - managing for the pathogen vs. disease; results of ELISA - FAT comparative studies; unsolved mysteries - new findings - undiagnosed cases; fish health management - new alternatives and pathogen vs. disease vs. policies; vaccines - efficacy and availability; how come we are still dealing with the same pathogens as 40 years ago, should we be?; "North American" VHSV; new viruses; Enterocytozoon salmonis; and long range planning for AFS-FHS.

****CONTINUING EDUCATION OPPORTUNITY****

Sponsored by AFS FHS Continuing Education ad hoc and Professional Standard committees on fish viruses found in the western U.S. will be offered Thursday afternoon (June 23rd). This will be a hands-on lab demonstrating the various cytopathic effects caused by several viruses, common and uncommon, on different tissue culture cell types. Dr. Jim Winton will be the primary contributor of materials and instruction. Separate registration is required for this session to design the course for the number of participants. A nominal fee may be charged. CE credits will be issued to attendees - when the CE committee gets that system in place. If you have or can provide good teaching materials for this session, Craig Olson would be glad to hear from you (206-753-9010).

A raft trip will be scheduled for Saturday June 25th, if there is enough interest (see attached sheet). BFTC staff have agreed to serve as local fly fishing guides for those interested.

PLEASE FILL OUT THE ENCLOSED REGISTRATION FORMS ASAP and return no later than April 30, 1994 to: Beth MacConnell, Bozeman Fish Technology Center, 4050 Bridger Canyon Rd, Bozeman, Montana 59715. Please contact Beth or Lynn Arment (406)587-9265 if you have any questions or need additional information.

BOZEMAN MOTELS AND ROOM RATES (prices include all taxes)

<u>Motel</u>	<u>Phone</u>	<u>Gov't Rate</u>	<u>Non-Gov't Rate</u>
Holiday Inn*+	(406)587-4561	\$39.50 S	\$66.56 S
5 Baxter Lane	(800)366-5101	56.16 D	74.88 D
GranTree Inn	(406)587-5261	\$39.99 S/D	\$84.00 S
1325 N. 7th	(800)624-5865		88.00 D
(next door to Holiday Inn)			
Prime Rate ¹	(406)587-2100	\$31.20 S	\$37.44 S
805 Wheat Dr.	(800)800-7089	44.72 D	48.36 D
Super 8 ¹	(406)586-1521	n/a	\$40.44 S
800 Wheat Dr.	(800)800-8000		51.88 D
Bozeman Inn ¹	(406)587-3176	n/a	\$46.80 S
1235 N. 7th	(800)648-7515		67.60 D
Days Inn ¹	(406)587-5251	\$41.60 S	\$46.80 S
1321 N. 7th	(800)325-2525	65.52 D	72.80 D
Fairfield Inn ¹ +	(406)587-2222	\$57.00 S	\$63.39 S
828 Wheat Dr.		65.48 D	72.75 D

*Block of rooms are available until 5/21/94

S=single - 1 person, 1 bed

D=double - 2 persons, 2 beds

+to receive these rates say with Western Fish Disease Meeting

¹ within walking distance of Holiday Inn

Montana State University - located approx. 6 miles from Holiday Inn.

We are still trying to arrange use of dorm... please call us for more info if interested.

Dorm rates are approximately \$10.00 S \$15.00 D.

WESTERN FISH DISEASE WORKSHOP 1994
PRESENTATION NOTIFICATION

Oral Presentation YES___ NO___

Poster YES___ NO___

Presenter_____

Please send: title, author(s), and short abstract that fits in box below

RETURN BY APRIL 30th, 1994 TO: Beth MacConnell
USFWS Fish Technology Center
4050 Bridger Canyon Road
Bozeman, MT 59715

**BULK RATE
U.S. POSTAGE PAID
NON-PROFIT ORG.
LOGAN, UT
PERMIT # 76**

REGISTRATION
WESTERN FISH DISEASE WORKSHOP
JUNE 22-24, 1994
BOZEMAN, MONTANA

NAME _____

ADDRESS _____

TELEPHONE _____ FAX _____

Please indicate your interest in attending/participating in the following:

June 23, 1994 CONTINUING EDUCATION OPPORTUNITY _____

June 23, 1994 BBQ (\$18.00/PERSON) _____ GUESTS (HOW MANY) _____

June 25, 1994 RAFT TRIP* _____ GUESTS (HOW MANY) _____

June 25, 1994 FLY FISHING _____

?want info on golf courses in the area? _____

RETURN BY APRIL 30th, 1994 TO:

Beth MacConnell
USFWS Fish Technology Center
4050 Bridger Canyon Road
Bozeman, MT 59715

*cost for raft trip - \$35.00 or less (includes lunch), depending on number of participants.

Fish Health Section Newsletter

The Fish Health Section 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. Submissions (files on diskette from most PC word processors preferred) should be addressed to the editors listed below:

Dr. Larisa Ford
National Fish Health Research Laboratory
NBS, 1700 Leetown Rd.
Kearneysville, WV 25430
304-725-8461 ext. 225 Phone
304-725-8461 ext. 237 FAX

Dr. Chris Wilson
Fisheries Experiment Station
1465 West, 200 North
Logan, UT 84321-6262
801-752-1066 Phone
801-752-6977 FAX
Compuserve (71024,2467)

FHS/AFS Newsletter
Fisheries Experiment Station
1465 West 200 North
Logan, UT 84321-6262

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Glenn L. Hoffman
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