Fish Health Newsletter

Fish Health Section/American Fisheries Society

April 2001 **Volume 29, Issue 2**

ISOLATION OF NORTH AMERICAN VIRAL HEMORRHAGIC SEPTICEMIA VIRUS (VHSV) FROM COLUMBIA RIVER SMELT (*THALEICHTHYS PACIFICUS*)

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In recent years we have been examining wild stocks of herring, smelt and other marine species to determine if the North American strain of VHS virus may be enzootic in fish within Oregon waters. Limited sampling of pacific herring (*Clupea pallasi*) from Yaquina Bay and Winchester Bay has produced unremarkable results. Similarly, viral examination of fresh, packaged smelt obtained from a local fish market and reportedly taken from local waters also returned negative results. Small numbers of rockfish (*Sebastes* spp.) and lingcod (*Ophiodon elongatus*) obtained from commercial fisheries have also been surveyed and all produced negative results when assayed for cultivable viral pathogens.

In March 2001, a sizeable run of eulachon (*Thaleichthys pacificus*) was reported by ODFW fisheries biologists entering the Sandy River, a tributary of the Columbia River, approximately 18 miles east of Portland, Oregon. Historically, large numbers of these fish had migrated up the Columbia River at least as far as the Sandy River to spawn; however, since 1988, a return of these fish, in any significant number, has failed to occur. The size and range of this season's eulachon return to this area is the largest to take place in over a decade, and the smelt were observed as far up the Columbia River as the Bonneville Dam.

On March 13, live fish were netted by ODFW fisheries biologists to obtain scale and otolith samples. Fish were taken mid-stream in the Sandy River, in one meter of water approximately 0.25 miles from the confluence of the Sandy and Columbia rivers. The following day 80 fish were received at the Corvallis Fish Pathology Laboratory for examination. External examination showed no signs of clinical disease and internally most fish appeared to have normal gametic development although eight individuals exhibited neither ova or testes and were likely spawned out females. No internal pathology was noted. Gill, kidney and spleen (GKS) tissues were taken from individual fish and combined into 5 fish pools. Of the 80 individuals examined, 9 were gravid females and a sample of ova was taken from each and eluted using Hanks Balanced

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Salt Solution. All samples for virus assay were inoculated onto EPC cell monolayers in 12 well plates.

Typical rhabdoviral cytopathology was first observed in a single tissue culture well of GKS sample 4 days post inoculation, and an Indirect Fluorescent Antibody Test (IFAT) on the cells from the affected monolayer indicated the presence of VHSV antigen. This potential virus isolate was designated SR3.01. By day 7, monolayers of three other GKS samples were also showing progressive degeneration. Additional IFATs were conducted using antisera to known viruses as outlined in Table 1. After 14 days of incubation, 5 total GKS sample wells exhibited CPE, and no CPE was observed in any eluted ova sample. Apparent negative GKS, and the ova samples were blind passed and the incubation continued.

Table 1. Results of Additional Indirect Fluorescent Antibody Tests

Virus	Antiserum			
	Anti-VHSV	B9/C6	105B	Anti-IPNV
SR3.01	++	-	-	-
VHSV (OR1)	++	-	-	-
IHNV (Bo98)	-	+	++	-
IPNV (ID87)	-	-	-	+
Neg. Control	-	-	-	-

Notes
SR3.01= unknown virus recovered from Sandy River eulachon
OR1 = known VHSV from Marine Science Center, Oregon 1998
Bo98 = known IHNV from Bonneville Hatchery, Columbia River 1998
ID87 = known IPNV from Pahsimeroi Idaho, 1987
Neg Conrol = uninfected cells
Anti-VHSV = polyclonal serum produced in rabbits and obtained from P. McAllister
B9/C6 = universal IHNV monoclonal antibody produced at OSU
105B = monoclonal antibody produced to identify Type 2 IHNV, DiagXotics, Inc.
Anti-IPNV = polyclonal serum produced in rabbits by Karla Mason, OSU 1986

The polymerase chain reaction (PCR) was performed on media from the initial infected sample using primers to identify both VHSV and IHNV. Media from cells infected with a known isolate of VHSV was also processed. Results of the nested amplification of these two samples produced DNA segments of very similar molecular weights using the VHSV primers, but no amplification was detected using primers for IHNV. Confirmation that the VHSV isolated from the smelt was the North American strain could not be done using the available primers, and a sample was submitted to Bill Batts at the Western Fish Research Center in Seattle for further testing. Bill confirmed that this strain of VHSV is indeed of North American origin.

This is the first isolation of VHSV from smelt that we are aware of, and it is the first isolation of this virus in Oregon waters. Similar to IHNV, North American VHSV appears to be expressed in its host during periods of high stress, particularly spawning times with respect to herring and smelt. From the sample size, and the condition of the fish examined, it is not possible to conclude if the presence of VHSV has any significant

negative effect on the eulachon populations in the Pacific Northwest, however, these fish may be a part in a more subtle relationship involving the transmission of potential pathogens between marine species, *and* between marine and freshwater environments.

According to ODFW fisheries biologists in the Columbia River basin, this year's smelt run is the largest to occur in several years, but in comparison with past data based on total catch and duration, it falls only within the 'above average' range. Estimated catch in the Columbia River this year is placed at 2 million fish and this figure typically represents only about 10 percent of the total run size. Speculation about the prevalence of individuals potentially carrying virus based on this report is obviously premature, but nonetheless, provocative considering the assay results and the sample size. As all scientists of any value are trained to comment about any results, "Additional investigation is necessary."

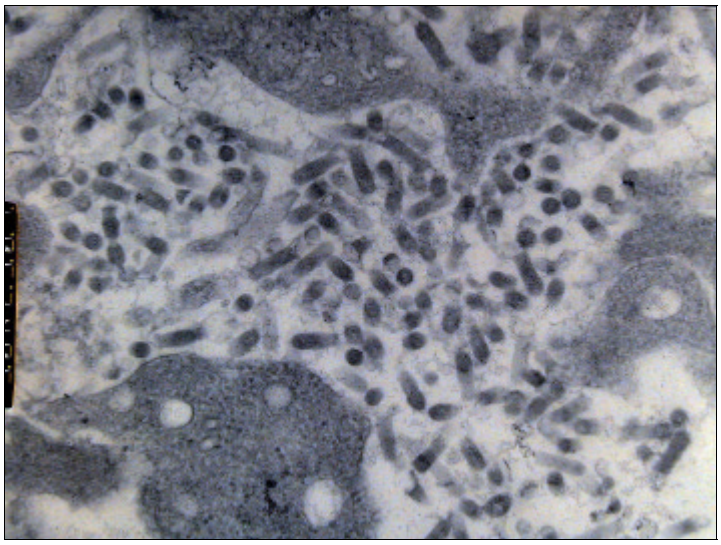

Figure 1. Ultrathin section of EPC cells infected with SR3.01. Magnification = 35K.

Acknowledgments

John Kaufman would like to thank Bill Batts, Western Fish Research Center, Seattle, WA for once again providing the 'final word' on the identification of suspect viral agents in the Pacific Northwest. Also Mark Redhead, ODFW Fish Pathology, for his organization and attention to detail, and Ben Simon, Virology Lab, Department of Microbiology, OSU, for being gentle with a PCR virgin.

FHS/AVMA MEETING

Dr. Michael Kent, President, represented the AFS/Fish Health Section at "Veterinary Medicine in Aquaculture: A Midwest Perspective Roundtable Discussion" which was coordinated by Dr. Myron Kebus, hosted by the Wisconsin Department of Agriculture, Trade & Consumer Protection, Division of Animal Health, Wisconsin. This meeting (held 23 March 2001) was held in Conjunction with the Wisconsin Aquaculture 2001 Conference Madison, Wisconsin. This well-attended meeting included other guest speakers such as Dr. Roz Schnick National Coordinator for Aquaculture New Drug Applications, LaCrosse, WI, and Dr. A. David Scarfe, Ph.D., DVM, American Veterinary Medical Association. Dr. Kebus, member of the Ad Hoc Committee on Veterinary Issues for the Section, chaired the discussion session. Drs. Kent and Kebus successfully presented the benefits of the AFS/FHS Section and Fish Inspector Certification to the veterinarians in attendance (about 30), and we anticipate this meeting will result in new members to the Section.

INCONSISTENCY OF KIRKEGAARD AND PERRY BKD ELISA ANTIBODY LOTS

Roberta Scott and Keith Johnson, Eagle Fish Health Laboratory, Idaho Department of Fish and Game, Eagle, Idaho.

The application of enzyme-linked immunosorbent assay (ELISA) for the detection of *Renibacterium salmoninarum* antigens (Pascho et al. 1991, Meyers et al. 1993) has become a valuable tool for successful culture of chinook salmon *Oncorhynchus tshawytscha* at facilities throughout the Pacific Northwest. A Columbia Basin-wide effort entitled Implementation of ELISA for Bacterial Kidney Disease (BKD) Segregation was funded by the Bonneville Power Administration from 1991 through 1995 to provide this ELISA capability to diagnostic laboratories for the management of BKD. Other concurrent practices to control BKD include intraperitoneal injection of all adults held for brood with erythromycin (20mg/kg) 60 to 30 days prior to spawning (Haukenes and Moffitt 1999), culling of eggs from females whose ELISA optical densities (ODs) were above threshold levels, and oral delivery of erythromycin during the culture of progeny (Moffitt and Haukenes 1995 University of Idaho INAD #6013). These health practices have dramatically reduced clinical episodes of BKD (VanderKooi and Maule, 1999. Pascho et. al. 1993) at the five IDFG facilities that rear spring/summer chinook. ELISA-based hatchery management practices are, therefore, dependent upon being able to obtain consistent quality antibody lots from a vendor such as Kirkegaard and Perry Laboratories (KPL), Gaithersburg, MD.

As with most resource agencies in the Pacific Northwest, IDFG has sampled all female chinook spawners of the last ten broodyears and have established our culling/segregation rearing values based on ELISA comparisons with FAT tests using duplicated samples to generate a goodness of fit between the two diagnostic techniques (Meyers et al. 1993) and also on subsequent detection of BKD in progeny. Although flexible ELISA culling criteria have been applied at IDFG facilities over the years due to variability in run strength we have generally culled eggs from chinook females having ELISA ODs exceeding 0.250. For an average year, eggs from 9 % of females have been culled and there have been very few in the "moderate" range of 0.250 to 0.800. Consequently, the stringent 0.250 cut-off criteria have not resulted in loss of many eggs but does presumably decrease the risk of vertical transmission.

At the Eagle Fish Health Laboratory we have become aware that the potency of both coating and HRP-conjugated antibody lots from KPL have varied. Contact with the manufacturer's technical representatives revealed that two "mother batches" (batch 1 and 2) had been produced and aliquots withdrawn from these were affinity-purified and a portion HRP-conjugated. In our experience, batch 1 antibody lots were those originating from the manufacturer's alphanumeric designation of UB or earlier while those of batch 2 had alphabetical letters of UL or later. We employ KPL's *R. salmoninarum* positive control standards in the "checkerboard" matrix to optimize concentration of both coating and HRP-conjugated antibodies between lots according to the manufacturer's recommendation. We agree that with this positive control that ODs from the two batches fall within KPL's 5% quality control requirement but the OD values obtained with chinook kidney tissues do not meet this standard between batches. To evaluate the extent and significance of this variation we have maintained chinook kidney samples throughout the OD range of 0.070 to 3.8 when run with batch1 lots of both coating and HRP-conjugated antibodies.

A total of 428 duplicate samples have been examined since 1996. Many of these have been examined multiple times using the same HRP-conjugated antibody lots to compare potency of subsequent lots. Samples which were frozen for up to five years at -20°C yielded the same OD values within a range normally demonstrated with the duplicate paired wells. A linear regression analysis of the same lot of batch 1 antibody, i.e. KPL lot# TA 025 as the independent variable (X) was compared to values (Y) obtained with multiple HRP-conjugated and coating antibody lots of

batch 2, i.e. KPL lot # U, V, and W alphabetical designations. The selection of which batch to designate the independent variable (X) and which the dependent variable (Y) is a matter of perspective and desired use. For our purposes, we still have a limited quantity of batch 1 products but are preparing for the time when we will have to switch to batch 2. In our case, we set batch 1 as (X) and batch 2 as (Y) to predict how much to adjust the OD values to obtain similar culling criteria results. We expect most fish health laboratories have exhausted their batch 1 antibodies and would be more interested in examining the relationship of the batches if criteria cut-off values were not changed between batches. In this case batch 2 would be designated (X) and batch 1 as (Y). The regression equations would be the inverse of each other but the ranges would differ.

This relationship was non-linear throughout the entire range of values tested with the two different antibody batches. The regression was divided into segments of OD values based upon ranges delimiting those normally employed in segregation rearing and culling at IDFG chinook facilities to generate equations to correct the "cut-off OD values" of background to 0.250, 0.251 to 0.399, 0.400 to 0.800, and > 0.800 from the batch 1 lots to those comparable values of the batch 2 lots (Figure 1). The slope of the regression equation for the range > 0.800 approached 1.0 indicating acceptable agreement between the two batches. The greatest divergence of values were in the intermediate ranges obtained with batch 1 (0.100 to 0.800) which are the most important for hatchery broodstock management purposes. The dotted line in these figures of the four ranges represents a slope of 1.0 for comparison purposes. It is noteworthy that only two of the 428 paired observations demonstrated an OD value greater for batch 2 than batch 1. The differences in performance between antibody batches are sufficient to require adjustment of the "cut-off values" by applying the regression equations presented in Table 1. These correction values are of a sufficient magnitude that there is a high probability that successful BKD management would not be attained without these adjustments. This is especially true for the values of 0.250 and 0.400 with

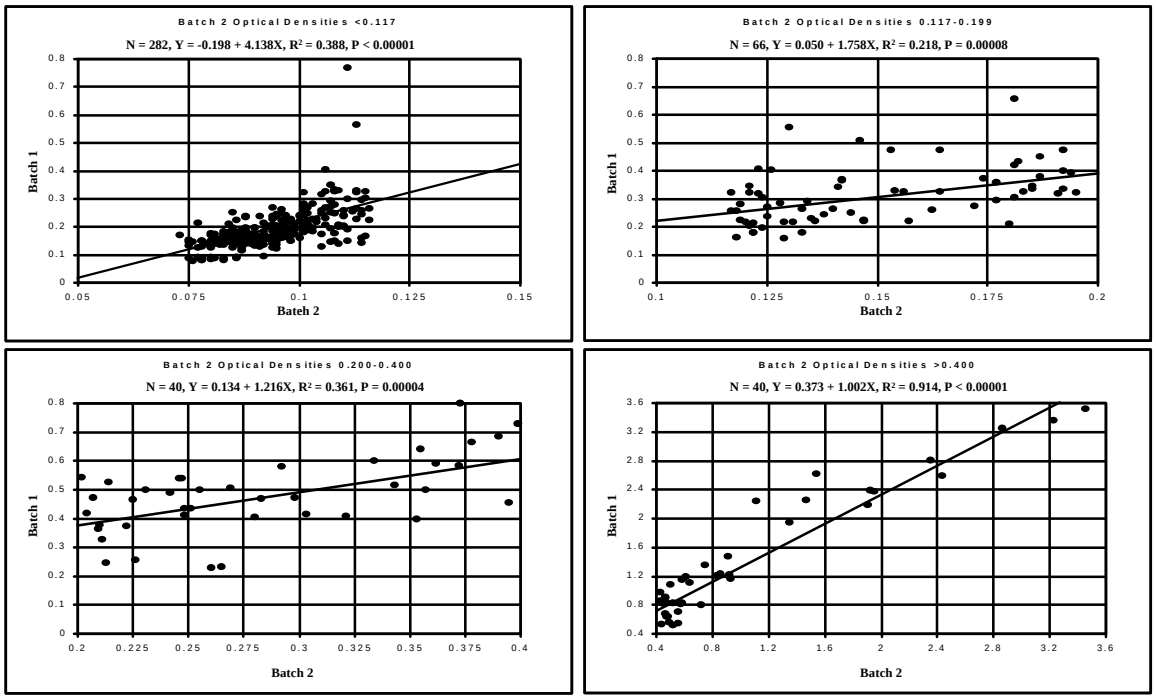

Figure1.--Comparison of two KPL *R. salmoninarum* ELISA antibody batches.

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batch 2 since the corresponding values of batch 1 are approaching the range in which the probability of vertical transmission of BKD are increasing.

The discrepancies between the two batches were also demonstrated using dilutions of *R. salmoninarum* spiked into negative kidney tissues and when diluted in saline. Furthermore antibodies of the second batch were demonstrated to have a higher affinity to purified p57 protein than those of batch 1. This was demonstrated also through absorption tests comparing both antibody batches.

The USDA/APHIS has recently solicited input concerning their role in aquaculture. The agency presently licenses vaccines for aquaculture assuring customers of their safety and potency. The results we present demonstrate the need for quality assurance of biological reagents used for diagnostics. Including this quality assurance for biological reagents would be consistent with the role of USDA/APHIS with other animal agriculture.

At the Eagle Laboratory, we have been fortunate to obtain two vials of batch 1 coating and HRP-conjugated antibodies through the kindness of Doug Ramsey (Rangen's Laboratory) and Rick Cordes (South Dakota Game, Fish, and Parks Department). We will be able to continue to use batch 1 antibodies through the 2001 spawning season. This permits our laboratory and hatchery staffs to be confident we are going to be able to continue with this very successful BKD management program. We have had repeated contact with the technical staff of KPL over the past four years to discuss these data and their implications to our BKD management program. There has been no attempt by KPL to address this distressing situation.

Table 1. Equations derived from the linear regression of optical density values obtained with KPL *R. salmoninarum* ELISA antibodies of batches 1 and 2 with chinook salmon kidney tissues.

ADJUSTING BATCH 1 (X) CUT-OFF VALUES TO BATCH 2 (Y)			
OD RANGE	**EQUATION**	**BATCH 1**	**BATCH 2**
0.100 – 0.249	Y=0.0492 + 0.271 (X)	0.250	0.117
0.250 – 0.399	Y=-0.0529 + 0.627(X)	0.400	0.198
0.400 – 0.800	Y=-0.0826 + 0.707 (X)	0.800	0.521
> 0.800	Y= -0.0445 + 0.996(X)		
ADJUSTING BATCH 2 (X) VALUES TO BATCH 1 (Y) VALUES			
OD RANGE	**EQUATION**	**BATCH 2**	**BATCH 1**
0.070 – 0.117	Y= -0.198 + 4.138(X)	0.117	0.286
0.118 – 0.198	Y= 0.050 + 1.758(X)	0.198	0.398
0.199 – 0.521	Y= 0.134 + 1.216(X)	0.250 0.400	0.438 0.620
> 0.521	Y= 0.373 + 1.002(X)	0.800	1.175

References

Haukenes, A. H. and C. M. Moffitt. 1999. Concentration of erythromycin in maturing chinook salmon after intraperitoneal injection of one of two drug formulations. Journal of Aquatic Animal Health 11:61-67.

Meyers, T.R., S. Short, C. Farrington, K. Lipson, H.J. Greiger, and R. Gates 1993. Establishment of a negative-positive threshold optical density value for the enzyme-linked immunosorbent assay (ELISA) to detect soluble antigen of *Renibacterium salmoninarum* in Alaska Pacific salmon. Diseases of Aquatic Organisms 16: 191-197.

Moffitt, C. M. and A. H. Hawkenes. 1995. Regional investigational new animal drug permits for erythromycin as a feed additive and injectable drug. The Progressive Fish-Culturist 57:97-101.

Pascho, R. J., D. G. Eliott, and J. M. Streufert. 1991. Brood stock segregation of spring chinook salmon *Oncorhynchus tshawytscha* by use of enzyme-linked immunosorbent assay (ELISA) and the fluorescent antibody techniques (FAT) affects the prevalence and levels of *Renibacterium salmoninarum* infections in progeny. Diseases of Aquatic Organisms 12:25-40.

Pacho, R. J., D. G. Elliot, and S. Achord. 1993. Monitoring of the in-river migration of smolts from two groups of spring chinook salmon, *Oncorhynchus tshawytscha* (Walbaum), with different profiles of *Renibacterium salmoninarum* infection. Aquaculture and Fisheries Management 24:163-169.

VanerKooi, S. P. and A,. G. Maule. 1999. Prevalence of *Renibacterium salmoninarum* in juvenile spring chinook salmon at Columbia and Snake River hatcheries, 1993-1996. Journal of Aquatic Animal Health 11:162-169.

EMPLOYMENT/EDUCATIONAL OPPORTUNITIES

Educational Opportunities

The University of Florida is offering an intensive two-week course, "Diagnosis and Treatment of Warmwater Fish Diseases." This class is designed to provide instruction in the methodology of diagnosis and treatment of parasitic, fungal, bacterial, viral, nutritional and environmental diseases of warmwater food fish and aquarium species.

When	May 14-25, 2001
Where:	Tropical Aquaculture Laboratory, Ruskin, Florida (May 14-18, 2001) The Whitney Laboratory, St. Augustine, Florida (May 19-25, 2001) Transportation provided from Ruskin to St. Augustine
Sponsors	University of Florida, College of Veterinary Medicine, Department of Fisheries and Aquatic Sciences, The Whitney Laboratory, and Tropical Aquaculture Laboratory; Sea World of Florida; The Florida Aquarium, Segrest Farms; All Florida Veterinary Laboratory
Topics	Water quality, freshwater and marine systems; fish necropsy procedures, bacterial, viral, fungal, parasitic and environmental diseases of fish; treatment protocols; nutrition; preventative medicine; anesthetic and surgical protocols
Cost	$800.00 U.S. ($850.00 after April 1, 2001)
CEUs:	Participants may earn up to 20 hours of Continuing Education Units by attending this course.
Contact:	Dr. Ruth Francis-Floyd, Dept. of Fisheries and Aquatic Sciences, University of Florida, 7922 NW 71st St., Gainesville, FL 32653. Telephone: (352) 392-9617 ext 229 FAX: (352) 846-1088 E-mail: rff@gnv.ifas.ufl.edu Website: http://www.ifas.ufl.edu/~conferweb/

Fish Parasitology Course on the Great Barrier Reef and Coral Sea, 3-9 Nov. 2001

The University of Tasmania and Undersea Explorer (see web page at: www.undersea.com.au) will be conducting a 6 day field course in marine fish parasitology. The course offers a series of lectures, hands on laboratories, and opportunities to SCUBA or skin dive aboard the "Undersea Explorer" research vessel.

Drs. Barbara Nowak (University of Tasmania), Robert Adlard (Queensland Museum), and Michael Kent (Oregon State University) will teach the course.

Space is limited to 15 persons. Contact Dr. Barbara Nowak, University of Tasmania B.Nowak@utas.edu.au.

The cost is A$3,500 (about US $2,000), which includes 6 days aboard ship accommodation (including meals), the course, certificate of completion, and diving (including weights, tanks, airfills).

Non-refundable A$1000 due 3 Sept. 2001.

EMPLOYMENT/EDUCATIONAL OPPORTUNITIES

The Meeting

Following the great success of the three previous International Symposia on Aquatic Animal Health, it is a pleasure to announce that the Fourth Symposium will be held at the beautiful Sheraton New Orleans Hotel in Louisiana, USA, September 2-6, 2002. Please mark your calendar and plan to attend what will prove to be an outstanding meeting in one of the most exciting and interesting cities in the world.

At present, sessions are planned for Monday-Thursday, with plenary sessions first thing in the morning, followed by 3-4 concurrent sessions for the remainder of the day. The space we have been provided will allow the poster to remain up for 3 days of the meeting, and several functions are planned in the poster exhibit area to provide ample time for poster viewing and discussion. Make sure to mark your calendar and plan to attend the 4th International Symposium on Aquatic Animal Health in New Orleans in September 2002.

Organization

The 4th International Symposium on Aquatic Animal Health is sponsored by the following professional societies:

- Fish Health Section of the American Fisheries Society
- Fish Health Section of the Asian Fisheries Society
- European Association of Fish Pathologists
- Japanese Society for Fish Pathology
- International Association for Aquatic Animal Medicine
- National Shellfisheries Association

The 2002 Symposium will be hosted by the Fish Health Section of the American Fisheries Society and the organizing committee is chaired by Ron Thune. In addition, an informal international advisory group, comprised of the Presidents of the six societies and the organizers of the previous two Symposia, Ron Hedrick, Jim Winton, Andrew Kane and Sarah Poynton, has been formed to contribute to the development of the scientific program.

For Further Information

In order to receive further information about the 4th International Symposium on Aquatic Animal Health, you will need to be included in the Symposium mailing list. Visit the Symposium web site **at http://www.vetmed.lsu.edu/isaah2002.htm** and complete the online **Request for Information** form**.**

AQUAVET 2001:
"AQUAVET I - A Program in Aquatic Veterinary Medicine" will be presented in Woods Hole, MA beginning on May 13 and ending on June 9, 2001. This four week course is designed for veterinary students and veterinarians seeking introductory training in the field of aquatic animal medicine.

"AQUAVET II - Comparative Pathology of Aquatic Animals" will be presented in Woods Hole, MA beginning on May 13 and ending on May 26, 2001. This two week course is designed for veterinary students, veterinarians, fisheries biologists, and pathologists seeking advanced training in the comparative pathology of aquatic species encountered in aquaculture, laboratory animal medicine, and aquarium environments.

Admission to both courses is by competitive application. Applications must be received not later than January 15, 2001. For application forms and additional information contact:

Donald A. Abt, V.M.D. PHN: 508-457-7969
Director of AQUAVET FAX: 508-457-7982
230 Main Street EMail: abtda@aol.com
Falmouth, MA 02540

WANTED! All AQUAVET alumni and faculty. The year 2001 marks the 25th anniversary of AQUAVET. As was the case with the 20th Anniversary, an Anniversary Conference and Reunion is planned for October 26 - 28, 2001 at the Marine Biological Laboratory in
Woods Hole. With more than 700 alumni "out there" now we need your assistance in locating you and obtaining your mailing addresses that we may send out the Conference Announcements and registration forms. Please send your current mailing address,
email address, and phone number ASAP to:

Donald A. Abt, V.M.D. PHN: 508-457-7969
Director of AQUAVET FAX: 508-457-7982
230 Main Street EMail: abtda@aol.com
Falmouth, MA 02540

We look forward to seeing you all in Woods Hole for some good science, good fellowship, and a re-awakening of fond memories. Please plan to join us.

EAFP 10TH INTERNATIONAL CONFERENCE
TRINITY COLLEGE DUBLIN, IRELAND

10TH - 14TH SEPTEMBER, 2001

Deadline for receipt of abstracts— April 20th, 2001

For more information, go to http://www.nuigalway.ie/microbiology/fdg

CALL FOR PAPERS:
THE AFS/FHS/WESTERN FISH DISEASE
2001 ANNUAL MEETING; VICTORIA, B. C., CANADA

JUNE 26 – 28, 2001
June 29 – Continuing Education Session

Hosted by: Pacific Biological Station
Association of Aquatic Veterinarians of British Columbia

Schedule: Mon, June 25 – Executive Committee meetings
Tues, June 26 –Technical Sessions
Evening Mixer and Poster Session
Wed, June 27 – Technical Sessions
Banquet
Thurs, June 28 – Technical Sessions; FHS Business Meeting
SPECIAL SESSION:THE FATE OF FISH PATHOGENS IN SPENT SALMON CARCASSES
FRI, JUNE 29 – CONTINUING EDUCATION WORKSHOP

Paper/Poster topic due by MAY 25, 2001
We encourage talks/posters on infectious and non-infectious diseases of salmonid and non-salmonid fish, diseases of shellfish, and on fish health related topics.
Email abstracts by May 25: bartholj@bcc.orst.edu
We also encourage presentation of case reports for an informal session. Abstracts will not be required for this session, but please tell us if you have a case for discussion.

Hotel Accommodations: Rooms have been reserved until May 25 at the Laurel Point Inn, 680 Montreal St., Victoria BC. The hotel is in downtown Victoria, on the waterfront. Cost for rooms is $150 Can./$97 US for either a single or double. Contact information: phone: (250) 386-8721 or 1-800-663-7667; www.laurelpoint.com or reservations@laurelping.com

Students: You are eligible for a reduced registration fee and for the award for student presentations (please indicate on the attached registration form). Members of the FHS are also eligible for the Snieszko student travel award – see the awards section of the newsletter.

Continuing Education Session: The Friday following the meeting, a one-day workshop on neoplasia in fishes will be conducted by Jack Fournie of the U.S.EPA, Gulf Breeze, FL and Mark Myers of NMFS in Seattle, WA. Lectures will cover neoplasms in multiple tissues and organ systems from fishes. A portion of the afternoon session will involve microscopic examination of representative examples of a variety of fish neoplasms. Fish Health Section CE credit certificates will be awarded for attending this class. Both this session and the workshop may be recognized for CE credits by your state or provincial veterinary association.

Contact information: If you have questions, please contact
Garth Traxler (250-756-7068;traxlerg@pac.dfo-mpo.gc.ca)
Dorothee Kieser (250-756-7069; KieserD@pac.dfo-mpo.gc.ca)
Jerri Bartholomew (541-737-1856; bartholj@bcc.orst.edu)Check web page at

http://www.fisheries.org/fhs/meeting.htm for meeting updates.

ABSTRACT SUBMISSION

Follow the below format, using 12 pt times font and set left/right margins to 1.5 in on letter-sized paper (left justification); place an asterisk after the presenter. Submit as an attachment to Jerri Bartholomew (bartholj@orst.edu) or call 541-737-1856 for other arrangements.

STRATEGIES FOR THE DIAGNOSIS OF *CERATOMYXA SHASTA*: FROM THE RESEARCH LAB TO THE FIELD

Ken Peters[2*], Oswaldo Palenzuela[3], Melanie Fox[1] and Jerri Bartholomew[1*]

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The complexity of the life cycle of myxozoans, involving dramatic morphological changes, and the similarity of the vegetative stages of even distantly related species, are traditional limitations to the accurate diagnosis of these fish parasites. The standard methods for diagnosis reliably detect and identify myxozoan parasites only when the fish are in late stages of the infection, and thus these techniques are not useful for surveillance of populations or for detecting early infections. New diagnostic technologies based on the detection of nucleic acids from the parasite, like *in situ* hybridization and the polymerase chain reaction (PCR), provide increased sensitivity and specificity when compared to more traditional, morphological approaches. A simple PCR assay has been developed for the diagnosis of *Ceratomyxa shasta* that allows detection of the different life stages using a simple and inexpensive protocol. The applicability of this PCR as a routine diagnostic and field surveillance tool was demonstrated in a small scale trial by comparing the results with examination of standard wet mount examinations. In addition, a non-lethal adaptation of the assay was developed which, although less sensitive than the lethal test, was significantly more sensitive than visual examination.

RESULTS OF FHS ELECTIONS:

Vice president:	Chris Wilson
Technical Standards:	Sue Marquenski
Professinal Standards:	Joy Evered
Nominating Committee:	Beth MacConnell

Congratulations to the winners and to all those excellent candidates who were willing to run!
Vicki Blazer

REVISION OF THE REQUIREMENTS FOR CERTIFICATION OF FISH HEALTH INSPECTOR

The FHS / AFS offers credentials through certification as a Fish Health Inspector. The Section has recognized the need for a peer review system to identify those individuals possessing the competence, training, and ethical standards required to serve the fishery resource by providing fish health inspection services as mandated by domestic and foreign regulatory agencies. Individuals meeting these requirements are eligible for certification by the FHS as "Fish Health Inspectors."

The AFS/FHS certified Fish Health Inspector is an individual who, through a regimen of appropriate training and experience, has developed the skills and knowledge necessary to examine populations of fish for the presence or absence of those specific pathogens regulated by domestic and foreign agencies. This individual can recognize signs of clinical and chronic infection involving specific pathogens and remains up-to-date on methods for detecting these pathogens among apparently health fish. He/She has access to equipment and laboratory facilities which enable samples to be tested for the presence or absence of specific pathogens in accordance with those procedures in Suggested Procedures for the Detection and Identification of Certain Fish Pathogens (1994, AFS/FHS), Fish Health Protection Regulations Manual of Compliance (1984, Fisheries & Environment, Canada) or others specified by regulatory agencies. These technical skills in concert with high ethical standards allow the Fish Health Inspector to provide current, accurate information regarding inspection of fish populations which can be used for the improved management of the fishery resource.

The objectives include: 1) To identify individuals possessing the technical skills and demonstrating high ethical standards qualifying them to conduct health inspections of fish populations, to perform recognized and acceptable detection and diagnostic procedures and subsequently to issue certificates or other such documents attesting to the presence or absence of specific pathogens in the populations inspected; 2) To establish a peer review system within the FHS for evaluating the qualifications of those applying for certification or recertification as a Fish Health Inspector; 3) To provide individuals, employing organizations, regulatory agencies, and the general public with definitive minimum standards for training, experience, and ethics required by the FHS for certification as a Fish Health Inspector.

Recently the Section approved changes to the Standards and Procedures for Certification and Recertification of Fish Health Inspector to encourage Veterinary involvement. One proposed change was to clarify that a degree in veterinary medicine fulfills the basic academic education requirement. The other proposed change allows certified fish health inspectors to use service laboratories rather than requiring direct access to equipment and laboratory facilities. The change proposed for recertification defined the type and amount of continuing education required for recertification. For additional information check the FHS website at www.fisheries.org/fhs.

REVISED AND NEW SECTIONS OF THE BLUEBOOK

The following chapters have been revised for inclusion or replacement in the 4th Edition of the Bluebook :

- Infectious salmon anemia (ISA)
- Piscirickettsia salmonis
- Bacterial Kidney Disease (appendices for ELISA protocols)
- Ceratomyxa shasta

These chapters will be available as PDF files for downloading and printing at the Fish Health Section website (www.fisheries.org/fhs), along with instructions for their insertion after May 1, 2001. The entire Bluebook (minus the new additions) is available through AFS at www.fisheries.org

CALL FOR FISH HEALTH SECTION AWARDS

S.F. Snieszko Distinguished Service Award - the highest award of the FHS. Dr. S.F. Snieszko stands as one of the most prominent figures in the establishment of the modern fish health sciences in the U.S.A. and internationally. This award is presented to individuals to honor their outstanding accomplishments in the field of fish health. This is a career achievement award. The nomination must be made by a current member of the FHS to the awards committee. The nomination should consist of a current curriculum vitae of the nominee, a letter of nomination and six letters of recommendation that support the nominee's dedication and contributions to research, teaching and/or service in fish health. **Nominations will be accepted until May 15, 2001.** For a list of previous awardees, go to the FHS website at: http://www.fisheries.org/fhs/sniezko.htm

Special Achievement Award - award for a significant accomplishment in the field of fish health. This award is presented to a FHS member who has in the past year made a significant accomplishment in basic or applied fish health. The achievement must meet a high standard of science as determined by peer review. Candidates for this award must be nominated by a current FHS member. The letter of nomination should state the accomplishment, its importance to the science of fish health, and the implications of the accomplishment (regional, national or international). Copies of articles and other supporting documents should be submitted with the nomination. The nomination may be submitted any time within one year of the accomplishment to the awards committee.

Sniezko Student Travel Awards—a sum of up to $1000 awarded to one or more students yearly to defray travel to a professional meeting to present a talk in the aquatic animal health field. The requirements and procedures for selection of student awards are as follows:

1. Letter of application and statement of any special financial circumstances (i.e., not supported by a stipend, etc).
2. Curriculum vitae must be submitted along with three letters of recommendation.
3. Itemized budget on how money is to be spent, i.e., travel, meals, lodging and registration
4. Copy of abstract of paper to be presented.
5. The student must be a member of the AFS/Fish Health Section

Send nominations for all awards by May 15 to:
Dr. Larry Hanson, FHS Awards Committee,
College of Veterinary Medicine, Drawer V,
Mississippi State University, MS 39762.
E-mail: hanson@cvm.msstate.edu

FHS Student Paper Award - an award will be presented to a student whose paper is being presented at the National Meeting to be held in Victoria, B.C, Canada. Selection will be made by 3 judges, based on (a) scientific content, (b) scientific merit of the research, (c) originality and (d) quality of presentation. Please note on your application if you wish to have you paper judged.

FHS TESTIMONY AT APHIS PUBLIC MEETING

Recently in the Federal Register, the Department of Agriculture - Animal and Plant Health Inspection Service (APHIS) notified the aquaculture industries, interested parties, and the general public that a public meeting was to be held during the World Aquaculture Society Meeting in Orlando, Florida on January 25, 2001 to discuss how and to what extent APHIS should regulate aquatic species, and discuss any other issues concerning regulation of aquaculture by the Agency. As background information, on May 4, 1999, the Animal and Plant Health Inspection Service (APHIS) published in the Federal Register (64 FR 23795-23796, Docket No. 98-085-1) an advance notice of proposed rulemaking (ANPR) titled "Aquaculture: Farm-Raised Fin Fish." APHIS published this ANPR after receiving petitions asking APHIS to regulate aquaculture in various ways. Many petitioners asked APHIS to define farmed aquatic animals as livestock. APHIS indicated that, in general, the petitioners seemed to be interested in receiving the same services that domestic producers of livestock receive for animals moving in interstate and foreign commerce. However, based on the petitions alone, APHIS had difficulty determining what segments of the industry wanted services and exactly what services they wanted. APHIS published the ANPR in an attempt to clarify the industry's needs, the nature of the services sought, and the concerns the petitioners had with regard to such regulations.

APHIS has recently indicated that negotiated rulemaking may not be suitable for this situation. The aquaculture industry is very large and diverse. Additionally, there are many parties outside aquaculture that have a substantial interest in such a rulemaking. APHIS has concluded that it would not be appropriate to pursue an aquaculture-negotiated rulemaking. However, they have not decided whether to pursue aquaculture rulemaking by other means. Before that decision is made, APHIS wants to have as much information as possible from all interested persons, and they want to provide the aquaculture industries and other interested persons with as much opportunity as possible to discuss with APHIS and inform APHIS regarding the relevant issues.

Thus, APHIS is holding a series of public meetings. There are no set agendas for the meetings. Any issues and concerns related to aquaculture and possible APHIS regulatory action can be discussed. However, there are three specific issues on which APHIS would like more information. Specifically, if APHIS does propose regulations; 1) Should their program be mandatory or voluntary, 2) Should they cover shellfish, and 3) Should they cover ornamental finfish? Information elicited at the meetings could result in a new APHIS regulatory program, or in changes to aquaculture-related services currently provided by APHIS. APHIS plans to hold additional meetings in Idaho, Maine, Mississippi, Pennsylvania, and Washington. APHIS will publish a notice or notices in the Federal Register announcing the dates, times, and locations of additional meetings.

The Executive Committee of the Fish Health Section took the initiative to sponsor a representative of the Section to attend the meeting and provide testimony and a written comment that included:

Fish Health Section of the American Fisheries Society Comment
Public Meeting held by APHIS-USDA January 25, 2001

The American Fisheries Society was founded in 1870 and is the largest and oldest professional fisheries society in the United States. It is composed of 4 Divisions, 51 Chapters, and 21 Sections. Founded in 1972, the Fish Health Section was the first specialized section in the American Fisheries Society. It was formed to give individuals involved in aquatic animal health a professional venue for communication and interaction. Today, the Fish Health Section with approximately 500 members is one of the largest

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sections in AFS with 80% of its members from the US and 20% international representing aquatic animal health specialists in federal, state and tribal natural resource agencies, the research sector, and commercial aquaculture. The goals of the Section are to 1) Maintain a professional association of persons involved in the health of fish and other aquatic animals; 2) Focus attention on fish health problems through research, education and dissemination of appropriate information; and 3) Foster effective fish health practices through communication and cooperation with government and private interests. The Fish Health Section has several publications including 1) The FHS Newsletter that covers section news, policy announcements and discussion, non-peer reviewed research findings, technical notes, and service announcements; 2) The Journal of Aquatic Animal Health which contains peer reviewed articles and communications and has become one of the leading scientific journals in the field of fish health; and 3) Suggested Procedures for the Detection and Identification of Certain Finfish and Shellfish Pathogens known as the ABlue Book@ which serves as a standard for fish health inspections and diagnostic services. The Section also offers professional credentials through certification as Fish Health Inspector and Certified Fish Pathologist. Additionally, continuing education that includes a number of short courses and workshops are offered on various topics in fish health. While open to all who are interested, these courses are designed to complement some of the continuing education requirements for re-certifications.

The Fish Health Section is committed to providing our expertise for the protection of our natural resources and the enhancement of the commercial industries. We maintain that only a joint partnership among federal, state, tribal and private sector entities that are performing aquatic animal examinations using approved standard methods (e.g. OIE, American Fisheries Society Fish Health Section) in laboratories staffed with competent, trained personnel (e.g. DVMs or those certified by the American Fisheries Society Fish Health Section) will be able to generate the data necessary to adequately and economically serve the needs of the private aquaculture industry as well as to protect the health of aquatic animals that are managed under the legislative authority of the several federal, state and tribal entities already established in this field.

We believe that it is of increasing urgency to develop a truly national aquatic animal health program that unites the expertise of APHIS, National Marine Fisheries Service and the US Fish and Wildlife Service as well as the vast infrastructure of the various states, tribes and private sector laboratories doing fish health inspections. Another important component is the Fish Health Section professional certification and continuing education programs for those doing such inspections. These, coupled with Blue Book and the OIE Code and Manual, form a powerful body of highly credible expertise that probably exceeds that of any other OIE Member country.

Ideally, such a National Aquatic Animal Health Program would occur under the auspices of the Joint Subcommittee on Aquaculture where the various private, state, tribal and federal entities involved with aquatic animal health would be consulted to draft a national program that could meet the goals of the inspection programs mandated by OIE. At a minimum, APHIS, NMFS and FWS need to sign a Memorandum of Understanding as originally outlined at the national meeting of the Fish Health Section in Madison, WI several years ago to define a joint "Competent Authority" for the US and to pool the extensive body of aquatic animal inspections that currently exists. Failing the creation of a joint national program, we will be left in the highly vulnerable position of lacking sufficient information to convincingly declare the entire US free of specific diseases, and should the causative agent be discovered within the national borders, we will have insufficient data upon which to define more limited disease-free zones that can be used to support trade.

FHS Involvement in EPA's Initiative to Develop Effluent Limitation Guidelines

The Environmental Protection Agency (EPA) continues its efforts to develop Effluent Limitation Guidelines for aquaculture. As background information, in 1989, the Natural Resources Defense Council filed suit against the EPA for lack of enforcement of the Clean Water Act. The lawsuit resulted in a settlement and Consent Decree on January 31, 1992. The Consent Decree provided for EPA to develop effluent limitation guidelines for certain specified industries and laid out a timetable for initiating guidelines for additional industries. EPA originally designated the Industrial Container Cleaning industry as a category for rule making. However, in late 1999, EPA asked the court to substitute aquaculture for the Industrial Container Cleaning industry. The reasons given by EPA for this action include the following; 1) The only relevant EPA guidance on aquaculture is over 20 years old, 2) The aquaculture industry has changed significantly in term of the types of species raised and the industrial processes employed, and 3) Aquaculture point sources appear to discharge nutrients which states regularly identify as one of the most common causes of water quality impairment in this country. EPA has also indicated that in addition to developing effluent limitation guidelines for nutrients they will also be examining effluent limitation guidelines for drugs and chemicals and aquatic animal pathogens.

The Joint Subcommittee on Aquaculture has formed a National Aquaculture Effluents Task Force to support a nationally coordinated, systematic process that will identify and report the best available and appropriate science, information and data relating to discharges from diverse aquaculture production systems and husbandry practices. Included within this infrastructure are different species groups and technical subcommittees including the Aquatic Animal Pathogen Technical Subcommittee. The FHS has taken a lead role in this subcommittee and the members include Scott LaPatra (chair), Ray Brunson, Bev Dixon, Don Lightner, Bruce Stewart, Mark Strom, Paul Waterstrat, and David Wise. The committee has provided specific answers to questions that EPA has posed, done an extensive literature review and stands ready to assist EPA on aquatic animal pathogen issues. Currently the biological significance of aquatic animal pathogens in effluents is unknown.

The FHS has also continued an active role in the United States Animal Health Association (USAHA) and the American Association of Veterinary Laboratory Diagnosticians (AAVLD) Aquaculture Committee. As background information, the USAHA is the most well established animal health organization that has approximately 1,400 members and works with a variety animal health entities both nationally, including the United States Department of Agriculture Animal Plant Health Inspection Service (USDA APHIS), and internationally. The purpose of the AAVLD, which works closely with the USAHA, is the dissemination of information relating to the diagnosis of animal disease, the coordination of the diagnostic activities of regulatory, research and service laboratories, the establishment of accepted guides for the improvement of diagnostic laboratory organizations relative to facilities, equipment and personal qualifications. The FHS's objectives, interests and goals regarding animal health are very similar to the USAHA. One of the reasons we continue to be involved is to offer our expertise and established programs in aquatic animal health and maintain visibility with other groups also concerned with animal health.

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In the past the FHS has also been very successful at passing resolutions in the USAHA Aquaculture Committee that then go before the Executive Committee of the USAHA. The Executive Committee of the FHS again took the initiative to sponsor a representative of the Section to attend the Aquaculture Committee meeting and present a resolution that was recently approved.

UNITED STATES ANIMAL HEALTH ASSOCIATION - 2000

RESOLUTION

SOURCE: AQUACULTURE COMMITTEE

SUBJECT MATTER: SIGNIFICANCE OF AQUATIC ANIMAL PATHOGENS IN AQUACULTURE EFFLUENTS

DATES: Birmingham, Alabama, October 19-26, 2000

BACKGROUND INFORMATION:
On January 21, 2000 the United States Environmental Protection Agency (EPA) announced its decision to promulgate national effluent standards for aquaculture operations. Included within this decision, EPA was to evaluate aquatic animal pathogens in effluents. Guidelines and regulations are needed to safeguard human health, habitat, and native species, however, there are no standardized procedures to determine the presence and/or the concentration of aquatic animal pathogens (if present) in effluents and there are no practices currently in use to control the discharge of aquatic animal pathogens in effluents of commercial or public aquaculture facilities. In assessing the risks of aquatic animal pathogens that may occur in aquaculture effluents, the characteristics of the pathogen must be considered including their abilities to multiply and remain viable in water, survival times outside the host, and the numbers of infectious units required to cause disease. In addition, fish species present in waters receiving discharged effluents, and their inherent susceptibility to agents present in effluents (if any) should be considered. Environmental considerations also must be included such as the effects of season, hydrography and water quality on the survivability of potential pathogens and risks of transmission to susceptible species. Hence, a complete and likely complex analysis is required to assess environmental impacts of potential pathogens in effluents. Such an analysis will be difficult given the lack of available credible scientific information and the inherent variation in agent types and numbers, aquatic animal hosts present, and the type of natural ecosystem or artificial culture environment present.

RESOLUTION:
USAHA encourages U.S. Department of Agriculture-Animal Plant Health Inspection Service (USDA-APHIS) to seek authority and funding to work with EPA and federal and state natural resource agencies to define risk-assessment procedures to determine the significance of aquatic animal pathogens in aquaculture effluents. Additionally, AAVLD encourages USDA-APHIS to utilize data generated by the U.S. Fish and Wildlife Service's national survey of pathogens present in free-ranging aquatic animals. This survey may help identify where aquatic animal pathogens already exist.

Fish Health Section Newsletter
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Deadline for next issue:
June 30, 2001

Fish Health Newsletter - Editorial Policy

The *Fish Health Newsletter* is a quarterly publication of the Fish Health Section of the American Fisheries Society. Submissions on any topic of interest to fish health specialists and preliminary case reports are encouraged with the understanding that material is not peer reviewed. Abstracts submitted to the *Journal of Aquatic Animal Health* are also encouraged. Articles should not exceed two newsletter pages and should not have more than five references. Submissions *must* be formatted in Microsoft Word, WordPerfect 6.x or other major Windows word processors, and can be sent by electronic mail or via 3.5" floppy disk to the editor's address below. Graphics files should be sent separately in jpeg format.

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