FHS NEWSLETTER

FISH HEALTH SECTION - AMERICAN FISHERIES SOCIETY

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FROM THE PRESIDENT

I want to take this opportunity to address the membership on several issues of importance to the Section and to initiate some discussion in advance of the Annual Meeting of the Fish Health Section in Madison. This is an exciting period for the FHS that will shape our future. Around the world, aquatic animal health is receiving increased attention, whether to protect natural stocks of animals or to better serve the growing aquaculture industry.

Development of a National Aquatic Animal Health Strategy

An increasing number of countries and international organizations are developing aquatic animal health regulations including the Office of International Epizootics (OIE), an international veterinary organization in Paris, France. The Fish Diseases Commission of the OIE has been tasked with developing an Aquatic Animal Health Code and Manual of Standard Methods to assist in harmonizing regulations and controlling the worldwide spread of aquatic animal diseases. A principal difference in the OIE approach is the concept of "zoning" rather than certification of individual lots. Once established, this approach is significantly less burdensome to maintain and requires the examination of fewer animals. It does, however, require that a federal governmental agency (or group of agencies) termed a "Competent Authority" be in charge of imports, movement, and exports of live or unprocessed aquatic animals to insure the integrity of the zones. Under the terms of the Uruguay Round of GATT, the World Trade Organization will resolve trade disputes and matters of animal health may be referred to the OIE. The significance of this development is that, in order to engage in international trade in aquatic animals, each country will need to designate a "Competent Authority" that will have the required legal powers to implement the OIE Aquatic Animal Health Code. For some countries (especially Europe) this will be a national veterinary authority, for others (most of Asia) this will be a fisheries agency. In the United States, and many other countries, the "Competent Authority" for aquatic animal disease control has not been defined.

Several months ago, the Animal Plant Health Inspection Service (APHIS) of the U.S. Department of Agriculture (USDA), developed proposed legislation that would have given APHIS authority to control imports, certify exports, and regulate interstate commerce in live or dead aquatic animals. The legislation was opposed by the majority of the private aquaculture industry as being too broad and by the U.S. Departments of Commerce and Interior as failing to adequately address the authority of various state and federal agencies to protect natural resources. The legislation was withdrawn, but it is clear that a coordinated, national strategy on aquatic animal health is needed. At the federal level, this strategy must include the Departments of Commerce, Interior and Agriculture to create a unified program to protect both wild and cultured fish, shellfish, and crustaceans in public and private waters. The national strategy will also require input from the states and private sector to insure a single national policy can be implemented that will serve private sector aquaculture without jeopardizing natural stocks

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of aquatic organisms. The Joint Subcommittee on Aquaculture (JSA) provided such an initial approach to development of a national aquatic animal health strategy. This coordinated approach should be encouraged. Otherwise, the states and federal resource agencies will continue to adopt independent regulations that will be a burden to industry and inconsistent nationally. At the annual meeting of the FHS in Madison, a panel discussion will be held to address this timely issue. Representatives from APHIS, Interior, Commerce, state resource agencies, the private sector, and the FHS will participate in this important debate. This forum will be an excellent place to inform the membership about these developments and to begin to draft an improved legislative approach to include all parties. At the national level, a formal agreement is needed between the Departments of Agriculture, Commerce, and Interior to lay out a coordinated approach and respective areas of responsibility. This would constitute an effective "Competent Authority" for the future.

Also needed will be a shared vision of a workable transition strategy to insure that the APHIS laboratories are set up and certified while allowing the private sector to be served and the natural resources protected. The FHS and the JSA should play critical roles in this transition. During this period (estimated to be 3-7 years), joint APHIS and FHS fish health inspector credentials will be required in the APHIS laboratories to allow them to provide services that comply with the various state and country requirements (APHIS for Europe and FHS for states and many countries in Asia). At the end of this transition period, APHIS would be effectively positioned to serve the private sector while federal, state, and tribal natural resources agencies would be able to maintain programs based upon the FHS certification procedures and methods if they chose.

A critical component of the transition strategy will be to build in a method for continued coordination following implementation of the plan. This could be an important function of the JSA and will insure that the fish health inspection programs of the various entities remain unified so that neither the private sector nor natural stocks of aquatic animals were being put at risk while providing a national program in fish health that would satisfy the international community, facilitate trade, and protect natural resources.

Journal of Aquatic Animal Health

Also of importance to the membership is a letter in this issue of the Newsletter from John Plumb and Margaret Ewing, co-editors of the Journal of Aquatic Animal Health (JAAH). The letter is self-explanatory, but I want to mention several additional points relative to the JAAH and to explain a change in this Newsletter. The JAAH was started during the Presidency of Ron Hedrick as a needed place to publish fish health research papers. Under the able editorship of John Plumb, Bill Rogers, John Grizzle, and Margaret Ewing, the journal has done well, managing to slightly more than break even; nonetheless, the journal was briefly targeted for elimination last year and only survived due to the strong support of several FHS members and their friends. It is imperative that we make some changes in order to insure its continuation and growth.

While I regret the impending retirement of John Plumb as co-editor of the JAAH during this critical period, I am delighted to announce that Ron Hedrick has agreed to assume this important job. In a recent conference call that included Bob and Sally Kendall from AFS, John Plumb, Margaret Ewing, and Ron Hedrick (the JAAH editors), and myself, we began to explore ways in which the JAAH could be improved or strengthened. Some of these are significant and will be discussed at Madison. Immediately, however, I want to announce the following change in the FHS Newsletter that addresses a growing problem. When John Rohovec, David Ransom, and I were Newsletter editors some time ago, we began to encourage submissions that were essentially preliminary research reports with the idea that these would be, or had been, submitted to a peer-reviewed journal. Some of these submissions appeared to be "mini-papers" that were in a regular journal format and included significant numbers of references. To our concern, many of these non-peer-reviewed papers began to be cited in the primary literature. Often, the full manuscripts were never sent to a peer-reviewed journal. In order to alleviate this problem and to encourage submissions to the JAAH, the Newsletter will begin, immediately, to return "mini-papers" of more than two Newsletter pages or that have more than five references to the authors with a

letter encouraging them to send the manuscript to the JAAH. The FHS Newsletter will be delighted to publish the abstracts of any manuscript that has been submitted to JAAH (whether accepted or not).

Bylaws and Procedures Manual

For some time, the FHS has been working to update the by-laws in several significant ways and to remove procedural material. Under the leadership of Ted Meyers and Ron Thune, this job is nearly complete. I hope to have a copy of the proposed by-laws changes and new procedure manual available at the Annual Meeting in Madison so that a vote of the membership can occur soon after.

I hope to see all of you in Madison. Meanwhile, thank you for your continued support and assistance.

Jim Winton

Short Course on Marine Fish Diseases

6-8 January 1997. Held at Hubbs Sea World Research Institute, San Diego, California. The course will emphasize diagnosis, treatment, and control of important marine fish disease of captive and wild marine fishes. The course will include lectures, laboratory dissections and demonstration material. Cost is $150.00. For further information contact either Dr. Martin Chen at 619-245-4076 (fax 9142) or Dr. Michael Kent at 604-756-7119 (fax 7053).

Letter To the Membership

To: Members of the Fish Health Section of the American Fisheries Society

From: Coeditors, Journal of Aquatic Animal Health

We are writing this open letter because we are concerned about the future of the Journal of Aquatic Animal Health. The journal was begun in 1989 because the aquatic animal health community in North America did not have a subject-specific scientific outlet for the extensive research that was taking place. For the first 7 years of the Journal we received between 70 and 80 manuscripts per year, about 75% from North America. This number was not overwhelming, but was adequate to fill four issues per year. During the first five months of 1996, we have experienced a dearth of manuscript submissions. We know there have been some problems in terms of review time, editorial disagreements, and inadequate communication, but we have worked diligently to resolve these, and improve the review and publication process while providing a high quality journal.

The Journal of Aquatic Animal Health is an extension of the Fish Health Section and we hope all of you are proud of this publication. However, we are at a critical point in the evolution of the Journal. In order for the Journal to continue to serve the FHS, it needs your personal support in terms of manuscript submission and subscription. Similarly, we need to know how the Journal can better serve the fish health community as a whole (North America and internationally) and what we can do to make the journal more "contributor friendly" and more "reader friendly". We are now considering several bold moves to generate these changes and will discuss them at the Annual FHS meeting in Madison, Wisconsin, in August. Meanwhile we are asking you to support the Journal by encouraging manuscript submission whenever possible. Also, if there are general or specific problems that we can address, let the editors, associate editors or the American Fisheries Society Editorial Office know about them. We believe that the Journal of Aquatic Animal Health is a strong extension of the Fish Health Section and the American Fisheries Society and we want it to remain so. With your help, it will.

Sincerely,

John A. Plumb
Coeditor
phone: 334-844-9215
fax: 334-844-9208

Margaret S. Ewing
Coeditor
phone: 405-744-9689
fax: 405-744-7824

or

Email: jplumb@ag.auburn.edu

Email: msewing@okway.okstate.edu

White Sturgeon Viruses isolated from Columbia River Wild White Sturgeon

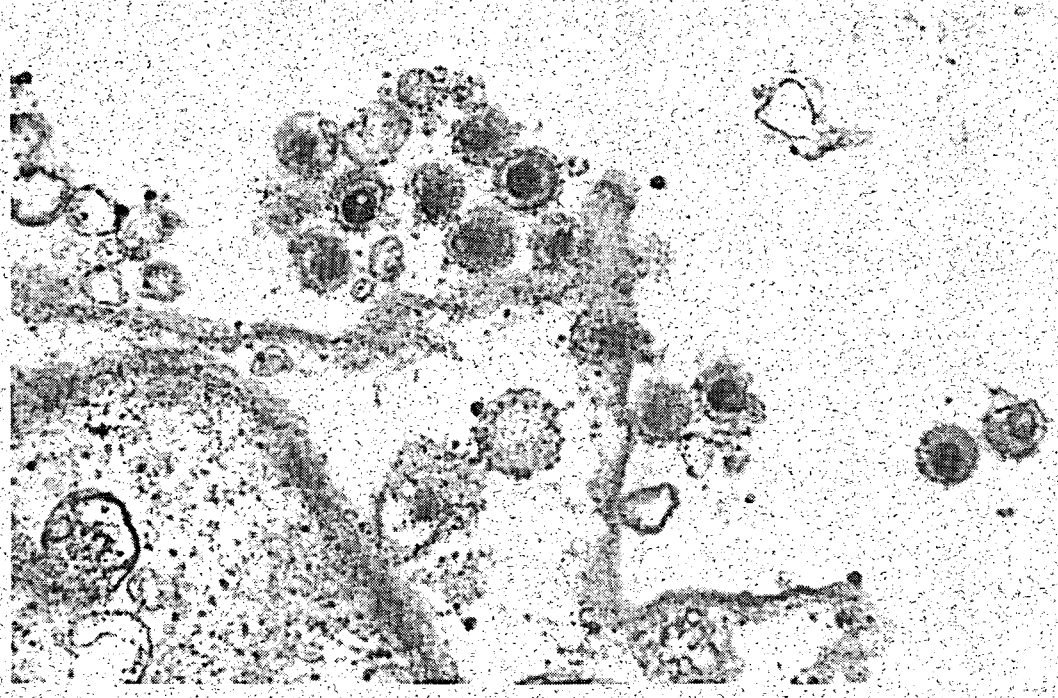
H. Mark Engelking and John Kaufman, Oregon Department of Fish and Wildlife. Center for Salmon Disease Research, Department of Microbiology, Oregon State University, Corvallis, Oregon, 97331-3804. email engelkih@bcc.orst.edu

White sturgeon (*Acipenser transmontanus*) from various locations in Oregon are occassionally transplanted to other locations or artifically spawned to enhance natural production or for commercial uses. At these times fish are sampled and assayed for two viruses; White Sturgeon Iridovirus (WSIV) and Herpes Virus-2 (WSHS-2) in white sturgeon from the Columbia River.

In July of 1992 fingerling white sturgeon from a commercial producer were sampled before release into various watersheds. These fish were "payback" for being allowed to catch and spawn native sturgeon from the Columbia River for the commercial market. Unusual signs and mortality were not exhibited by these fish. In a sixty fish sample of twelve whole fish pools, four pools gave evidence of virus by cytopathic effect (CPE) on white sturgeon spleen cells (WSS-2). The samples were sub-cultured and again cytopathic effects were noted. By indirect fluoresecent antibody assay (IFAT), WSIV was identified as being present. The immune serum was a gift of Dr. Ron Hedrick, University of California Davis (UCD) (Diseases of Aquatic Organisms 12:75-81, 1992). Another sample was taken in mid-August of thirty six fingerling sturgeon. Presumptive cytopathic effect was noted on ten of twelve pools inoculated onto WSS-2 cells. Sturgeon skin cells (WSSK) showed no evidence of virus. The samples were sub-cultured and five of eleven pools were positive by IFAT for WSIV.

In the fall of 1994 juvenile sturgeon were trapped in the Columbia River below Bonneville Dam and brought to the Clackamas Oregon Department of Fish and Wildlife (ODFW) Research Laboratory for acclimation prior to transfer to the upper Columbia River. Some of these fish appeared to stop eating and swimming and subseqently died. These juvenile sturgeon were approximatley two years old and presented few signs other than listlessness and weight loss. Samples of gill, kidney, and spleen were taken and sent for viral examination. No bacteria or parasite infections were observed. The samples produced CPE on two white sturgeon cell lines; WSS-2 and WSSK-1. The agent was subculture passed on to WSS-2 cells. No CPE was seen on CHSE 214 or EPC cells. Specific antiserum to WS iridovirus was used in an IFAT but was inconclusive. Antiserum to a white sturgeon hepres virus (WSHV-2) recently found in California was obtained again from R. Hedrick at UCD (Diseases of Aquatic Organisms 22: 199-210, 1995). This antiserum completely neutralized the virus (more than four log reduction in titer). Electron microscopy revealed a virus particle of similar morphology and size to that of a herpes virus. The plaque morphology and time of appearance was consistent to that of the California isolate. Titration of the virus gave about 106 plaque forming units (pfu) per gram of skin. This report gives further details of the characterization of this WSHV-2 isolate (Oregon Columbia River, OCR).

With antiserum prepared in yearling sturgeon (about 500 g) to the virus isolated from these fish (OCR), a comparison was made to the virus isolated at UCD. Although the viruses appeared to be similar, the isolation in California came from an ovarian fluid sample of a healthy adult female. This anitserum prepared to the OCR isolate cross neutralized the UCD isolate effectively, providing evidence that the two viruses are serologically identical. The cytopathic effects produced by both virus isolates on WSS, WSSK, and WS Liver (WSLV) cell lines are grape-like clusters at the foci of plaques with heteromorphic single and fused cells. With time the cells float off the plastic substrate. These plaques form in five to seven days similar to that described by Hedrick and co-workers. By electron microscopy, a virion of 78 nm in diameter was observed and an enveloped particle of about 160 nm in diameter. This corresponds to the sizes determined by Hedrick of 107 nm and 177 nm (Figure 1).



Attempts to infect other species were not successful. Using 10^3 and 10^4 $TCID_{50}$ per ml of both WSHV-2 isolates to challenge winter and summer run steelhead trout (*Oncorhynchus mykiss)* and spring run chinook salmon (*O. tshawytscha)* no losses occurred. Apparently these species are refractory to infection from the virus.

Further work to characterize WSHV-2 OCR by Restriction Fragment Length Polymorphisms (RFLP) and other methodology is underway. This report, along with the reports of a Largemouth Bass Virus and aquareovirus in Alaska in 1996 Fish Health Newsletters, suggests that much more remains to be learned about viruses in wild fish. The need for continued surveillance for emerging or re-emerging viruses is evident as more areas and species are being manipulated to enhance or maintain wild fish stocks.

ACKNOWLEDGMENTS

We acknowledge the help of Terry Kreps, ODFW pathologist, in obtaining the sturgeon. Funding for these studies were provided in part by USFWS project AFS-78, State of Oregon wildlife and general funds.

Fourth International Symposium of Ichthyoparasitology

I just received a copy of the abstracts for the "Fourth International Symposium of Ichthyoparasitology" held at the University of Munich : Munich, Germany. I was unable to attend, which may have been the situation for many fish parasitologists in the United States. There was a definite lack of US scientists at the meetings. The European countries were well represented during the five day conference. R. W. Hoffman (Germany) and C.R. Kennedy (UK) gave opening remarks and the plenary lecture. J. Lom presented another plenary lecture on "New Developments in the Research on Protozoan Parasites of Fishes" (*Myxobolous cerebralis* and other myxosporidans are now part of the phylum Cnidaria). Hedrick (USA) and Holstein (Germany) gave lectures on the microsporidans and myxozoa.

There were over 70 papers presented at two parallel sessions and 47 posters. I would be happy to copy the abstracts for anyone interested in the presentations. All major groups of fish parasites were represented at the symposium.

Dr. Sergei Spiridonov (Russia) is organizing the 2nd International meeting on free-living and parasitic nematodes to be held in Moscow during 1997. He has asked me to organize a session pertaining to nematodes of fishes. Anyone interested in participating, please write to me.

Dr. Richard Heckmann
Dept of Zoology/ Brigham Young University
Provo, UT 84602
Email: heckmanr@acd1.byu.edu

The 19th American Fisheries Society Fish Health Section Meeting

The 1996 AFS Fish Health Section Meeting will be held August 6-9 at the Wisconsin Center on the University of Wisconsin campus in Madison, Wisconsin. Registration will be the evening of August 6, formal papers will be presented August 7 and 8, and a continuing education workshop on hematology will be held from 8 a.m. to 2 p.m. on August 9. The meeting is co-sponsored by the Wisconsin Department of Natural Resources and the USFWS LaCrosse Fish Health Center. In these times of reduced budgets and downsizing of state and federal governments, we have tried to keep costs as low as possible, hoping this will encourage attendance and participation. Madison is a great place to spend a few days, especially during the summer. We've also included a small pitch for the Department of Tourism-what a great idea to combine a professional meeting with your family vacation!

The Program

The program (papers, posters, etc.) will be organized by the LaCrosse Fish Health Center; registration and local arrangements will be handled by the Wisconsin DNR. All presentations will be at the Wisconsin Center, 702 Langdon St. (608) 262-0881 (The phone number is only listed as a way for people to contact you at the conference; the Center staff **will not** be handling any part of registration so please do not call them).

Lodging

Hopefully we have something for every budget: Twenty-five rooms have been reserved at the Edgewater Hotel (608) 256-9071 which is two blocks from the Wisconsin Center. Rates at the Edgewater are the same for a single or a double- $85.00 (yes, it is the tourist season). A list of other hotels within walking distance is attached, however a block of rooms could not be reserved (individual rooms may be available).

As is the case on most campuses, staying at a dorm is a less costly lodging alternative. The Elizabeth Waters dorm is available to us from August 6 through August 9. The rooms are air conditioned, linens are furnished, and there is a cafeteria for breakfast. Smoking is not allowed in this dorm. It is about a 15 minute walk from the dorm to the Wisconsin Center. A separate registration form is attached for those who plan to stay at the dorm. When you arrive, rooms may be charged (Mastercard, Visa), paid by check (U.S. currency/U.S. Bank), traveler's checks, or cash. **There is a July 1 deadline for dorm reservations.**

Transportation

Madison has a moderately-sized airport for direct flights; it may be less expensive flying into Chicago's O'Hare airport and taking the Van Gelder bus to Madison-the bus stops at the Memorial Union which is just a few blocks from the dorms and the Edgewater Hotel; Madison is located at the confluence of Interstates 94 and 90 for those who plan to drive. If you fly into Madison, the Edgewater has a limo service available from 3 p.m. Sunday to 4 p.m. Friday. Call (1-800-922-5512) to make arrangements; cabs are available for those staying at the dorm.

Registration/Social

Registration will begin on Tuesday night (August 6) from 7 to 9 p.m. at the Wisconsin Center. During this time, a cash bar will be available as will various hors d'oeuvres. Registration will continue on Wednesday morning (7:30 to 8:30 a.m.) just outside our conference room.

Banquet

The banquet is Wednesday night (August 7). We plan to give you a taste of Wisconsin, outdoor picnic-style: brats, grilled chicken/steak sandwiches, all the other "stuff", preceded by a social hour/cash bar. This is the only meal that we will eat together. Lunches and Thursday dinner are on your own. Madison has a wide variety of ethnic restaurants/food carts within walking distance of the Wisconsin Center.

Registration Form for the AFS Fish Health Section Meeting
August 6-9, 1996

Name _____

Address _____

_____

_____

Phone (day) _____

Please check the boxes below and enter fees as appropriate:
The deadline for registration is July 15. The late registration fee is $30.00

[ ] Registration fee ($20.00)
[ ] Banquet ($14.00/person)
[ ] Check here if you prefer a vegetarian meal (same cost)
[ ] Continuing Education Workshop on Hematology ($20.00)

Please make checks out to "AFS Fish Health Section" and send this form with your check to:

Marybeth Loibl
Wisconsin DNR- FM/4
Box 7921
Madison, WI 53707
Phone: (608) 267-7499

First Detection of Infectious Hematopoietic Necrosis Virus in Marine Fishes

G.S. Traxler and Jon Richard, Department of Fisheries and Oceans, Pacific Biological Station
Nanaimo, British Columbia, Canada V9R 5K6

We report the first isolation of infectious hematopoietic necrosis (IHN) virus from marine fishes. The isolations were made from two species of marine fishes caught adjacent to net pens containing Atlantic salmon undergoing losses due to IHN virus. The virus was isolated from kidney/spleen samples obtained from healthy appearing fish: one of two shiner perch (*Cymatogaster aggregata*) and two of eight tubesnouts (*Aulorhynchus flavidus*) assayed on EPC cells. The isolates were confirmed as IHN virus by DNA probe. Virus titers in the perch were 8.8×10^3 pfu/g and 2.0×10^3 and 5.8×10^2 pfu/g in the tubesnouts. Further work is being conducted to determine the role of marine fishes as possible vectors or reservoirs of IHN virus.

Taxonomy of *Kudoa* species (Myxosporea; Multivalvulida) using 18S ribosomal DNA sequence

D. Hervio[1], M.L. Kent[2], J. Sakanari[3], H. Yokoyama [4], J. Khatra[1] and R.H. Devlin[1]

[1]West Vancouver Laboratory, Department of Fisheries and Oceans, 4160 Marine Drive, West Vancouver, B.C. V7V 1N6, Canada; [2]Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, B.C.[3] University of California V.A. Medical Center 113b 4150 Clement St., San Francisco, California 94121 USA; [4]Department of Fisheries, Faculty of Agriculture, The University of Tokyo, Yayoi, Bunkyo-ku, Tokyo 113 JAPAN.

Myxosporeans in the genus *Kudoa* infect the flesh of many marine fishes and often cause unsightly lesions and softening of the flesh. Most myxosporeans are host specific, but *Kudoa thyrsites* has been reported from many fish species around the world, and thus it is possible that "*K. thyristes*" is actually an assemblage of several closely related species. We are particularly interested in this species because it is associated with softflesh in seawater-reared Atlantic salmon (*Salmo salar*). Using the universal primers 18e and 18g (Hillis and Dixon 1991), we have obtained sequence from 18S rDNA (about 1,650 bp) from the following *Kudoa* species: *Kudoa miniauriculata* from bocaccio (*Sebastes paucispinis*) from California, *K. paniformis* from Pacific hake (*Merluccius productus*) from British Columbia, *K. amamiensis* from yellowtail (*Seriola quinqueradiata*) from Japan, and *K. thyrsites* from tubesnout (*Aulorhynchus flavidus*) and Atlantic salmon from British Columbia .

Sequence comparisons of these isolates using Jukes-Cantor and neighbor-joining analyses indicated that *Kudoa* species cluster by host and geographic location rather than by morphology of spores. *Kudoa paniformis* and *K. miniauriculata* were 97.7% similar, whereas *K. amamiensis* (from Japan) was about 91% similar with these species. Sequence comparisons of 447 bp of the 18S rDNA of *K. thyrsites* from Atlantic salmon and tubesnout revealed no differences, whereas differences between *K. thyrsites* and *K. paniformis*, *K. miniauriculata* and *K. amamiensis* were 3.4%, 2.7%, and 10.3 % respectively.

Intragenic comparisons of other myxosporeans examined to date (i.e., *Myxobolus* spp., *Henneguya* spp., *Kudoa* spp.) show marked differences in the 18S region, and thus they are referred to as "fast clock" organisms in regards to evolution of the ribosomal gene (Siddall et al 1995). Nevertheless, the 18S is the most evolutionary conserved region of this gene, and thus closely related species often do not show differences in this region. It is, therefore, premature to conclude that the two *K. thyrsites* isolates are the same species, but at least they are much more closely related to each other than the other *Kudoa* species we have examined. The next step of our research is to examine a more variable region of the gene (i.e., the ITS region) in attempt to identify possible strain differences in *K. thyrsites*. *Kudoa thyrsites* has been reported from many fishes from other regions of the world, and it would also be useful to include some of these isolates in rDNA studies to clarify the *K. thyrsistes* species assemblage.

The class Myxosporea comprises two orders, the Multivalvulida and the Bivalvulida (cf. Lom and Dykov 1992). Using Jukes-Cantor/neighbor joining analysis, we compared the 18S region of *Kudoa* with that of the other available myxosporean genera (*Myxidium, Henneguya, Myxobolus*) (cf Smothers 1993). *Kudoa* has been assigned to the Multivalvuida, whereas the latter genera belong to Bivalvulida. Futhermore, *Myxidium* belongs to the suborder Variisporina, and *Henneguya* and *Myxobolus* are in the suborder Platysporina. In agreement with this taxonmic scheme, we found that *Kudoa* spp. branched separately from the other genera. *Myxidium*, *Henneguya* and *Myxobolus* clustered together, with the latter two being most closely related to each other.

We have developed a sensitive and specific PCR test for *K. thyrsites* using differences between the 18S of this species with other *Kudoa* species and other myxosporean genera. We plan to use this test to detect early or light infections, and to identify the source of infection for Atlantic salmon. Based on research conducted with freshwater myxosporeans, it appears that most if not all, myxosporeans require an annelid worm as an alternate host to complete their life cycles. We are using the PCR test for *K. thyrsites* to screen invertebrates (especially annelids) from the sediment under netpens, from nets, and from plankton samples in an attempt to identify the presumed alternate host and the infectious stage of the parasite.

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Siddall, M.E., D.S. Martin, D. Bridge, S. Desser, and D.K. Cone. 1995. J. Parasitol. 81: 961-967.
Smothers, J.F. 1993. Phylogenetic analysis of five species of myxozoans using 18S rRNA gene sequences. MS Thesis, Idaho State University. 29 p.

21st Annual Eastern Fish Health Workshop
September 5 and 6, 1996

The 1996 Eastern Fish Health Workshop will be co-sponsored by the Virginia Institute of Marine Science/School of Marine Science (Gloucester Point, VA) and the National Fish Health Research Laboratory (Kearneysville, WV). The workshop will be convened in the auditorium in Waterman's Hall on the campus of the Virginia Institute of Marine Science. There will be an informal welcome reception on Wednesday evening, September 4, followed by two full-day sessions on September 5 and 6. Come early or stay late to take advantage of the cooler fall weather and tour the many attractions throughout Colonial Williamsburg, Busch Gardens, and the Yorktown area. For more information contact:

Dr. Stephen Kaattari
Co-chairman, EFHW
VA Institute of Marine Science
School of Marine Science
Gloucester Point, VA 23062-1346
804-642-7260
Fax: 804-642-7186
Email: kaattari@vims.edu

Dr. Rocco Cipriano
Co-chairman, EFHW
NFHRL
1700 Leetown Road
Kearneysille WV 25430
304-725-8462 X238
Fax: 304-725-7061
Email: rocco_cipriano@nbs.gov

CALL FOR PAPERS: WILD TROUT VI "Putting the Native Back in Wild Trout". Montana State University, Bozeman, August 18-20, 1997.

Anyone with an interest in wild trout is invited to submit an abstract to present a contributed paper or poster. Suggested seessions are listed below, however, we welcome papers concerning any aspect of wild trout ecology and management. Abstracts must follow the required format and be received by October 1, 1996. Please submit abstracts to **Bob Gresswell, Wild Trout VI Program Co-chair, by email at gresswer@ccmail.orst.edu, or send a floppy disk 3 1/2 inch preferred, 5 1/4 inch acceptable) to Bob at Pacific Northwest Research Station, 3200 SW Jefferson Way, Corvallis OR 97331.** Symposium sessions, August 18-20, will include panels on various aspects of wild trout management. Potential session topics include:

1. Evolution and biological organization of native trouts in North America
2. Large-scale threats to the future of wild trout (e.g. urbanization and global warming)
3. Ecological restoration of native trout and their habitats
4. Issues associated with native trout restoration
5. Human values associated with wild trout
6. The role of market valuation in the protection of wild trout
7. Developing public awareness about wild trout
8. Effects of government cutbacks and environmental backlash on the future of wild trout.

Isolation of an Aquareovirus from Rainbow Trout in N.W. Spain

C.P. Dopazo[1], C. Rivas[1], I Bandin1, M. Noya[2], J.M. Cutrin[1] and J.L. Barja[1].

[1]Departmento de Microbioloxia e Parasitoloxia. [2] Departmento de Bioloxia Fundamental. Facultade de Bioloxia,. Universidade de Santigo de Compostela. 15706 Santiago. SPAIN.

Since the late 1970s members of the family *Reoviridae* have been isolated from different aquatic animals in North America, Europe, and Asia. Although these viruses share some characteristics with the genera *Rotavirus* and *Orthoreovirus*, they have distinctive features. For this reason a new genus named *Aquareovirus* was proposed to group these viruses isolated from poikilothermic animals (Francki et al. 1991).

In the summer of 1995, a group of rainbow trout (250 g average weight) was analyzed in our laboratory during a routine examination of a fish farming facility. The first indication of a possible aquareovirus present in this group was the development of focal areas of syncytia in Chinook salmon embryo (CHSE-214) cells after five days post-inoculation with a kidney-spleen homogenate. The supernatant of infected CHSE-214 cells was inoculated onto the following cell lines: rainbow trout mesotheliome (RTM), rainbow trout gonad (RTG-2), fathead minnow (FHM), bluegill fry (BF-2) and epithelioma papillosum cyprini (EPC), and cytopathic effect (CPE) was detected in RTM, RTG-2, FHM, and BF-2, but not in EPC. The CPE in RTM was characterized by focal syncytia similar to that observed in CHSE-214, but in the remaining cell lines the cytopathology was different, infected monolayers tended to retract, cells adopted a round morphology and granular debris was observed.

For characterization, virus was propagated in monolayers of CHSE-214 cells grown in 150 cm^2 tissue culture flasks at a multiplicity of infection of 0.001 to 0.1. After CPE was extensive, cells were mechanically harvested and pelleted at 3000 x *g* for 20 min. The virus was purified from the supernatant as previously described (Dopazo et al. 1992).

To exclude the possibility that this viral isolate was a member of the family *Birnaviridae*, usually detected in fish farms from our area in both fresh and marine waters, the viral agent was examined for relatedness to strains of infectious pancreatic necrosis virus (IPNV) as previously described (Lupiani et al. 1989). The neutralization test performed with the three anti-IPNV sera (West Buxton, Sp and Ab) failed to affect infectivity of the viral isolate. The physio-chemical characterization was performed by assaying the susceptibility of the viral isolate to chloroform, thermostability and stability to extreme pH as previously described (Vestergard-Jørgensen, 1972; Meyers 1979; Plumb et al. 1979). The results of these tests revealed that the viral isolate was resistant to treatment with chloroform, stable at both acid and alkaline pH as well as at high temperatures (500 C), indicating that the virus was non-enveloped. Electron microscopy confirmed this finding by showing non-enveloped double-shelled icosahedral particles with an average diameter of 60-65 nm (Figure 1).

To determine whether the viral isolate contained DNA or RNA, the effect of the DNA inhibitor bromo-2'-deoxyuridine (BDU) on the virus was examined following the procedure described by Plumb et al (1979). Significant losses of viral titer did not occur in the presence of BDU, which indicated that the genome was composed of RNA. Analysis by polyacrilamide gel electrophoresis of the nucleic acid demonstrated the presence of 11 distinct bands, which is a unique feature of virus from the family *Reoviridae* (Francki et al 1991) and the genus *Aquareovirus*. It has been widely reported that aquareoviruses are isolated from salmonid species such as chum salmon (*Oncorhynchus keta*), Atlantic salmon (*Salmo salar*), landlocked salmon (*O. masou)*, coho salmon (*O. kisutch)* (Hetrick et al. 1992; Hsu et al. 1989; Moore and McMenemy, 1988; Winton

et al. 1989; Winton et al. 1981). However, to our knowledge this is the first time that a virus of these characteristics is isolated from rainbow trout and we propose the name of rainbow trout aquareovirus (RTA). Further studies must be conducted in order to compare this new isolate with other salmonid aquareoviruses.

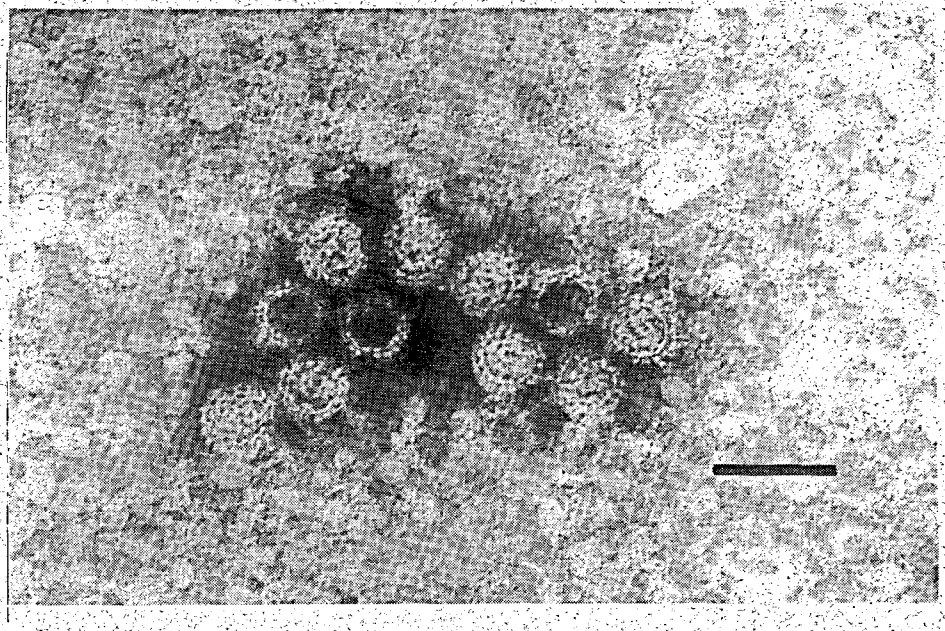

Figure 1. Electron micrograph of the rainbow trout aquareovirus (RTA). Bar represents 100 nm.

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Fish Health Section Newsletter

The Fish Health Section Newsletter is a quarterly publication of the Fish Health Section of the American Fisheries Society. Submissions on a topic of interest to fish health specialists are encouraged with the understanding that material is not peer reviewed. Articles should not exceed two newsletter pages and should not have more than five references. Submissions should be submitted on disk in Word perfect 5.1 or in a generic form that can be read on WP5.1 for IBM. Disks will be returned if a SASE is included with your submitted article. Also, we will be glad to publish any abstract of a paper that has been submitted to the *Journal of Aquatic Animal Health* (whether accepted or not by JAAH) . Submissions should be addressed to the editors listed below:

Dr. Beverly Dixon
Department of Biological Sciences
California State University at Hayward
Hayward CA 94542

Phone: 510-885-3422
Fax: 510-885-4747
Email: BDIXON@DARWIN.SCI.CSUHAYWARD.EDU

Dr. Larisa Ford
Department of Fisheries & Wildlife Resources
University of Idaho
Moscow ID 83844-1136

Phone: 208-885-7608
Fax: 208-885-9080
Email: lford@uidaho.edu

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Andrew J. Mitchell
PO Box 860
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