

**FHS
NEWSLETTER
FISH HEALTH SECTION
AMERICAN FISHERIES SOCIETY**

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Page 1

April, 1995

Prospects for Fish Drugs: Aquaculture '95

Randy MacMillan, Director, Research and Development, Clear Springs Foods, Inc., P.O. Box 712, Buhl, ID 83316.

A special session, "Aquaculture Drugs and Therapeutants: Where We Are and Where We Need To Go", was held at the San Diego Aquaculture '95 meeting. Government (FDA, USDA, NBS), professional (AFS), academic (Michigan State University) and commercial industry representatives summarized the current fish drug situation and provided directional insights. While there are some promising developments, considerable challenge to obtaining needed drugs remains.

Considerable research is ongoing at governmental and commercial facilities. The National Biological Service at LaCrosse, WI continues to be the focus for US fish drug approval research. Research efforts are focused on chloramine-T, formalin, copper sulfate, potassium permanganate, hydrogen peroxide and the crop grouping concept. Crop grouping, in which similar fishes are grouped together for drug approval research, would help minimize research requirements for the plethora of fish species needing approved drugs. Sarafloxacin, an aryl-fluoroquinoline, is on the back-burner pending FDA resolution of its status. The commercial industries focused research is directed at potassium permanganate, HCG, and a new quaternary ammonium compound,

BARDAC. The NRSP-7 project, formerly IR-4, continues to help fund research on some fish therapeutants. It was announced that a National New Animal Drug Application (NADA) coordinator was being funded to facilitate aquatic animal drug approvals.

The FDA reported efforts toward consolidation of INADs for greater efficiencies. While FDA is sensitive to the needs of aquaculturists, budgetary constraints will require reduction in FDA efforts. Consequently, FDA is considering a policy restricting compassionate INADs to the top 10 or so fish drugs under development. This does not mean that traditional INADs will be constrained only that all drugs must have drug company sponsors. FDA did invite comment on their efforts.

The USDA reported on the history of drug development and educational efforts. They announced the availability of a publication written by the federal JSA Working Group on Quality Assurance entitled, "Guide to Drug, Vaccine and Pesticide Use in Aquaculture". This publication is available from the Aquaculture Information Center, National Agricultural Library, USDA, 10301 Baltimore Blvd., Rm 304, Beltsville MD 20705. Telephone: 301-504-5558.

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Representatives of the commercial industries also presented perspectives of where we need to go. It was suggested that while drugs are valuable, the industry needs to examine other fish health management tools such as vaccines. Additionally, the commercial industry has devoted considerable resources towards drug registration. A drug company representative described an ideal drug registration scenario and concluded that industry size (small) and the cost (high) of drug approval would continue to limit drug availability. The final presentation suggested ways to fund drug research. Joint efforts between various state fisheries agencies through the International Association of Fish and Wildlife Agencies and the USFWS have been helpful. It was suggested that the commercial industry tax itself to generate additional funds.

Summary

The outlook for additional fisheries drugs is clouded. Several groups continue research efforts on select compounds and methods to expedite research. Reductions in funding, shifts in governmental agency missions and increased costs for drug approval do not encourage user groups. Alternative fish health management tools must be continually examined.

*******CALL FOR APPLICATIONS*******

Applications for Snieszko Student Awards are due 5-15-95. Applicants for the Snieszko Student Awards should send a curriculum vitae and 3 letters of recommendation to the Awards Committee. Please send all materials to: Larisa Ford, Awards Committee Chair, NFHRL, NBS, 1700 Leetown Rd., Kearneysville WV 25430 phone:304-725-8461 x225, fax: 304-725-7061.

SULLIVAN MEMORIAL MEMBERSHIP AWARD

The Carl Sullivan Memorial Membership Award was established in 1991 by the late Carl R. Sullivan, who served many years as the Executive Director of the American Fisheries Society, to support membership for non-North American fishery scientists. At Mr. Sullivan's request, preference is given to Irish, Australian, English and other candidates from English-speaking countries, although all candidates from other countries will be considered. The award is administered by the American Fisheries Society, and includes an annual membership in AFS and a year's subscription to one of the AFS peer-reviewed journals.

To qualify, applicants must submit a one-page letter describing professional goals and current efforts towards those goals. A brief statement of how membership in AFS would assist in meeting those goals should be included. While not required, a recommendation from a member if a professional fisheries organization or university is desirable.

Applications for the 1996 AFS Memorial Membership Award should be submitted to:

J. Larry Hutchinson, International Fisheries Section, 601 Teakwood Drive, Lincoln, NE 68510 (FAX 402-471-5554 or by E-mail to lhutch@ngp.ngpc.state.ne.us)

or address to:

American Fisheries Society, 5410 Grosvenor Lane, Suite 110, Bethesda, MD 20814. All applications need to be received by August 1, 1995. The successful applicant will be announced in September at the 1995 Annual Meeting of the American Fisheries Society in Tampa, Florida.

Pathogenesis of Select Aquareovirus Isolates in Rainbow Trout

S. E. LaPatra¹ and R. P. Hedrick²

¹ Clear Springs Foods, Inc., Buhl, Idaho 83316

² Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis 95616

Reoviruses (respiratory enteric orphan viruses) are being examined for their ability to prevent infectious hematopoietic necrosis (IHN). They were originally called reoviruses because they were often isolated from subclinical animals. We now know that at least some reoviruses can cause mild diseases (Winton et al. 1989). During the course of our studies mortality in fish exposed to the chum salmon reovirus (CSV) was observed. Gross signs of a potential viral infection included abdominal distention and exophthalmia and CSV was isolated from mortalities with high concentrations (10^6 to 10^7 PFU/mL) of virus detected. Histologically liver tissues exhibited a moderate to severe multi-focal necrosis, however, anterior kidney tissue appeared unaffected. A CSV challenge was subsequently performed on 0.5 g rainbow trout exposed to 10^2 and 10^4 TCID₅₀/mL. Cumulative mortality was 13% (12/94) in the high dose and 1% (1/100) in the low dose 28 d post-infection. Mean number of days to death in high and low dose groups was 20 and 25 d, respectively. A pathogenesis study was recently completed that tested other aquareovirus isolates for virulence and their capability to produce microscopic tissue changes in target organs. Duplicate groups of 50 rainbow trout (mean weight, 0.4 g) were exposed to 10^4 TCID₅₀/mL of each isolate. One group was monitored for mortality and the other group was sampled at weekly intervals for histology.

The chum salmon reovirus (CSV) produced the highest number of mortalities (Table 1), in agreement with our previous studies, and also induced the

Table 1. Aquareovirus challenges of rainbow trout.

| Label | Year | Location | Species | CPM ^a |
|-------|------|-----------------|---------|------------------|
| CSV | 1978 | Hokkaido, Japan | Chum | 14% (7/51) |
| CSR | 1985 | Oregon, USA | Coho | 0 (0/49) |
| ICR | 1989 | California, USA | Chinook | 0 (0/49) |
| LCR | 1990 | Washington, USA | Chinook | 0 (0/51) |
| NCR | 1993 | N. Dakota, USA | Chinook | 0 (0/49) |

^a CPM = cumulative percent mortality.

greatest amount of microscopic changes in renal and hepatic tissues (Table 2).

Table 2. Results from microscopic pathology associated with aquareovirus challenges of rainbow trout.

| Day Post-Infection | CSV | | NCR | | CSR | | ICR | | LCR | | Control | |
|--------------------|----------------|----------------|-----|---|-----|---|-----|---|-----|---|-----------------|----|
| | H ^a | R ^a | H | R | H | R | H | R | H | R | H | R |
| 14 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 |
| 21 | 3 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0/4 | 0 | 1 ^b | 0 |
| 28 | 5 | 3 | 2 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| 35 | 5 | 1 | 4 | 0 | 0 | 0 | 1/3 | 0 | 1 | 0 | 0 | 0 |
| 42 | 4/4 | 1 | 1 | 0 | 0 | 0 | 3 | 2 | 0 | 0 | NS ^c | NS |
| 48 | 5 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 56 | 4 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

^a Number of fish with hepatic (H) or renal (R) lesions per 5 fish sampled.

^b Small foci.

^c NS = no sample.

Focal to multifocal hepatonecrosis were the first lesions observed. These lesions continued to enlarge through 21-28 d post-infection and progressively involved increasing numbers of macrophages. Beginning at 35 d post-infection mononuclear cell infiltration (presumably lymphocytes) increased. By 56 d, early stages of hepatic regeneration was evident with a focus of mononuclear cells still present. Microscopic changes were also observed in renal tissues and began as a diffuse necrosis of the endothelium of the sinusoids. Larger areas of hematopoietic necrosis were not observed but increased macrophage concentrations around melanomacrophage centers were present but not numerous. During the most significant periods of hepatonecrosis there was some hematopoietic cell depletion but conversely during recovery there was an increase in these cell populations. Overall a nice transition from early necrosis to the beginnings of resolution of the lesions was observed. In some of the specimens the lesions encompassed approximately 25% of hepatic parenchyma. By 56 d post-infection the hepatic lesions had not completely resolved and may have required 2-4 weeks.

"New" aquareoviruses are being isolated more frequently than any other replicating agent; at least 25 new isolations in the state of Washington, USA, have occurred since 1990. In each case the virus originated from an asymptomatic adult salmonid. However, the results reported herein suggest aquareovirus isolates exist that can cause mortality and/or induce microscopic tissue changes in hepatic and renal tissues of rainbow trout fry.

References

- Winton, J.R., C.N. Lannan, M. Yoshimizu, and T. Kimura. 1989. Response of salmonid fish to artificial infection with chum salmon virus. Pages 270-278 in Viruses of Lower Vertebrates. W. Ahne, E. Krustak eds, Springer-Verlag, Berlin-Heidelberg-New York.

Book Review: Techniques in Fish Immunology-3 (1994). J.S. Stolen, T.C. Fletcher, A.F. Rowley, J.T. Zelikoff, S.L. Kaattari, S.A. Smith (eds). SOS Publications, 43 DeNormandie Ave., Fair Haven, NJ 07704-3303. ISBN 0-9625505-7-4. LCC 89-92807.

"Techniques in Fish Immunology-3 is a new laboratory manual edited by J.S. Stolen and 5 other eminent scientists in the field of fish immunology, physiology, genetics and disease diagnostics. This third book containing over 214 pages, expands this outstanding series with carefully outlined instructions on methods such as obtaining blood from various species of fishes, preparation and purification of antibodies, cell descriptions and explanations of steps involved in new techniques of DNA analysis. The book also contains a special section on immunopathological and pathological techniques.

Fish blood can be obtained on a continuous basis by cannulation. The anesthetized fish is held in an apparatus that allows continuous irrigation of the gills. Physiologists/Immunologists Iwama (Vancouver, B.C.) and Ishimatsu (Nagasaki, Japan) describe and illustrate different cannulation procedures and anesthetics used. Obtaining different blood cell populations is described in 3 chapters. Secombes (Aberdeen, Scotland) describes panning methods using anti-immunoglobulins to separate cells. Adams and Morris (Stirling, Scotland) report on methods using the phagocytic cells ability to capture magnetic beads as a tool for then attracting the cells to a magnet. Fisher and Kollner (Reims, Germany) use the cells preference to adherence to nylon wool, plastic, or glass to separate cell populations. Once separated, these cells can be cultured as described by Morimoto and Wantanabe (Kameino, Japan), using carp cells. Coll (Madrid, Spain) uses fibrin clots for cell support, followed by the addition of growth inducers such as PHA or Con A or antigens. Both of these methodologies discuss the ability to induce proliferation and prolong cell viability. Tamai et al., (Japan) use oncogene transfection to immortalize fish cells. Going further with DNA technology they outline cloning the DNA of Interleukin 2 and interferons from flatfish. Secombes describes the production and detection of fish interferons using rainbow trout (RTG-2) cells and infection with the IPN virus. Neutrophilic granules are an important defense line against diseases. Verberg-van Kemenade (Wageningen, the Netherlands) describes a method for detecting the respiratory burst activity of these cells *in vitro* by flow cytometry. Kaatari (Gloucester Point, Virginia) and Shapiro (Corvallis, Oregon) present methods for determination of serum antibody affinity distributions using ELISA-based technology. Polycyclic aromatic hydrocarbons (PAH) are major contaminants of the aquatic environment. Rutan and Faisal (Gloucester Point, Virginia) use fish leukocytes and hematopoietic organs to show physiological changes and detection methods for PAH metabolites. Basic hematological procedures with normal range values are presented by Klontz (Moscow, Idaho). Adams and Marin de Mateo give methods for staining cells with immunoperoxidase and monoclonal antibodies for the detection of fish pathogens, using PKX as an example. Ristro and Arzen de Avila (Pullman, Washington) and Lorenzen (Arhus, Denmark), specialists with fish rhabdoviruses, present methods using monoclonal antibody to detect fish viruses. Flow cytometry can also be used to separate virus-infected leukocytes. Perez et al. (Madrid, Spain) used fluorescent dyes for DNA and RNA or monoclonal antibody to separate these cells and thus detect carrier fish. Using Western Blot analysis, rabbit anti-rat and rat anti-P450 antibodies can be used to establish the presence of nitrosodialkylamine-metabolizing enzymes in fishes (Kaplan, Henniker, New Hampshire). Laughlin et al. (Frederick, Maryland and Blacksburg, Virginia) give procedures for determining multilocus DNA fingerprints of fishes with oligonucleotide probes and direct hybridization in agarose gels.

The world is growing to appreciate the importance of these sophisticated techniques in use for real fish culture applications. While many outside the field of fish immunology may regard our information esoteric, deeper analysis shows these techniques have important applications in aquarium fish culture, preservation of fish in their precarious environments, and use of fish as tools for toxicological studies. A very important "for instance" appears in the appendix section under "Care and Maintenance of Fish for Experimentation". These tips are hard fought for information from experts on juvenile Chinook salmon, channel catfish, tilapia, angelfish, striped bass, rainbow trout, medaka and red drum. The price of FITC-3 is \$70; a bargain for this valuable information. Dr. Stolen has done a magnificent job of organization and original diagrams will help students and researchers following complicated procedures. This laboratory manual is a necessity in fish health classes and laboratories.

Dr. Douglas P. Anderson, Salmon Bay Biologics, 2805 NW Golden Drive, Seattle, Washington
98117

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

July 19-22, 1995
Syracuse, New York USA

The Joint Meeting of the Fish Health Section of the American Fisheries Society and Eastern Fish Disease Workshop will be held in Syracuse, New York from July 19-22, 1995. It is not too early to begin consideration of submission of an abstract for this meeting. The general meeting schedule has been arranged to allow air-travelers to arrange for supersaver airfare. The program will have Wednesday July 19, serve as a travel day with a reception in the early evening. Formal presentations will be on Thursday, Friday and in the morning on Saturday, July 20-22. An optional field trip will be arranged to tour the Oneida Fish Hatchery of the New York State Department of Environmental Conservation on Saturday afternoon, July 22. This state-of-the-art walleye hatchery went through its first full production year in 1994. Although the nature of the overall program of the scientific meeting will be influenced by the type of abstracts submitted, we are looking to develop a meeting that will have a balance of basic and applied studies/presentations.

The 1995 meetings will be coordinated by John Schachte and Paul Bowser (Local Arrangements Co-Chairmen). Frank Hetrick will serve as program chairman. Abstracts should be sent to Frank Hetrick. A general time line for the meeting is as follows:

- May 31, 1995 - Receipt deadline for abstracts (please note oral or poster presentation preference)
- June 15, 1995 - Notification of abstract acceptance

The meetings will be held at the Sheraton Inn Syracuse, located just off exit 37 of the New York State Thruway. The Sheraton Inn also provides a courtesy van service to the Syracuse Airport. A block of rooms have been reserved for the meetings for the nights of 19-22 July 1995. Reservations may be made by calling the Sheraton Inn Syracuse (toll free reservation number 800-325-3535 or 315-457-1122).

(for more information contact:

Dr. John Schachte, Jr.
Fish Disease Control Unit
New York State Department
of Environmental Conservation
8314 Fish Hatchery Road
Rome, New York 13440
(315) 337-0910

Dr. Paul R. Bowser
Department of Avian and
Aquatic Animal Medicine
College of Veterinary
Medicine
Cornell University
Ithaca, New York 14853
(607) 253-3365

ABSTRACT

FISH HEALTH SECTION/AFS AND EASTERN FISH DISEASE WORKSHOP
SYRACUSE, NEW YORK
19-22 JULY 1995

**WALLEYE DERMAL SARCOMA VIRUS: A NOVEL RETROVIRUS THAT
CAUSES TUMORS IN FISH**

PR Bowser^{1*}, JW Casey², VM Vogt³, FM Poulet¹, S
Quackenbush² and D Martineau⁴.

¹Department of Avian and Aquatic Animal Medicine,
College of Veterinary Medicine, Cornell University,
Ithaca, New York, USA; ²Department of Microbiology,
Immunology and Parasitology, College of Veterinary
Medicine, Cornell University; ³Section of Biochemistry,
Molecular and Cell Biology, Cornell University; and
⁴Faculty of Veterinary Medicine, University of
Montreal, Saint-Hyacinthe, Quebec, Canada.

The walleye dermal sarcoma virus (WDSV) is a
retrovirus that causes non-metastasizing dermal
sarcomas in walleyes (Stizostedion vitreum). Tumors
contain multiple copies per cell of the unintegrated
13.2 kB viral DNA, which is discontinuous on one strand
like that of the spuma- and lentiviruses. Together
with recent DNA sequence, analysis of the data indicate
that WDSV is unique among the Retroviridae.
Transmission is successful using homogenates of tumors
collected from adult walleyes in the spring but not in
the fall. Virus purified from tumor homogenates showed
major polypeptides of 90 kDa, 25 kDa, 18 kDa, and 10
kDa on SDS-PAGE. Tumor cells were strongly positive
for viral RNA, while surrounding tissue was mostly
negative. An antiserum to the 90 kDa protein also
reacted strongly with tumor tissue.

WESTERN FISH DISEASE WORKSHOP

June 6-9, 1995

Twin Falls, ID

HOST: The Idaho Aquaculture Association**LOCATION:**

The workshop will be held in the conference rooms of the Best Western Canyon Springs Inn, which is located at the north end of town on Blue Lakes Blvd. (Hwy 93). To reach the hotel from I-84, take Exit 173 to Twin Falls and head south. The hotel is on the right hand side about 1/3 mile from the first traffic light, which is just south of the Snake River Canyon bridge.

REGISTRATION:

The Continuing Education Session on HISTOLOGY is being conducted on June 6 by John Morrison, Charlie Smith, and Beth Mac Connell. Registration is \$15.00 which includes a full day of instruction consisting of about five hours of illustrated lecture followed by an open lab with microscopes available for examination of slides while the instructors rove about to answer questions. Participants are invited to bring slides of their own for interpretation. The lecture will cover normal tissues as well as the pathological effects of bacteria, parasites, viruses, neoplasias, toxicity, and nutritional and environmental problems. In addition the instructors have prepared 30 sets of about 50 slides that participants can take back with them for future reference. This will be offered for purchase on a first come basis on the day of the class for \$50.00.

The Western Fish Disease Workshop Technical Session will be conducted June 7 and 8. Registration is \$60.00, which includes two lunches and all coffee breaks during the workshop, a social gathering on Wednesday evening and dinner on Thursday evening. You must register in advance by sending a check or money order and the enclosed registration form to: Scott LaPatra, c/o Clear Springs Foods, Inc., P.O. Box 712, Buhl, ID 83316. Please make checks payable to AFS/Fish Health Section.

LODGING: A block of rooms **was** reserved at the Best Western Canyon Springs Inn for workshop participants, but are no longer available. You can contact the hotel directly at 1-208-734-5000 to check for vacancies. There are several other hotels within easy walking distance of the Canyon Springs Inn. The Ameritel Inn is right next door (\$59.75 single, \$64.75 double, 1-800-822-8946). The Super 8 (\$45.88 single, \$52.30 double, 1-208-734-5801), Weston Plaza Hotel/Convention Center (\$45.00 single, \$49.00 double, airport courtesy van available, 1-208-733-0650) and Motel 6 (\$34.23 single, \$40.65 double, 1-208-734-3993) are all within two blocks of the Canyon Springs Inn.

ABSTRACT SUBMISSION INSTRUCTIONS

**FISH HEALTH SECTION/AFS AND EASTERN FISH DISEASE WORKSHOP
SYRACUSE, NEW YORK
19-22 JULY 1995**

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

Please complete the following form and submit it with your abstract by May 31, 1995 to:

Dr. Frank Hetrick
Fish Disease Unit
Department of Microbiology
University of Maryland
College Park, Maryland 20742

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

Name: _____

Address: _____

Telephone: AC () _____

I prefer that my presentation be: _____ an oral presentation
_____ a poster presentation

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

**PREREGISTRATION FORM
FISH HEALTH SECTION/AMERICAN FISHERIES SOCIETY
AND THE
EASTERN FISH DISEASE WORKSHOP**

19-22 July 1995
Syracuse, New York

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

Name: _____

Address: _____

Phone: AC () _____

Preregistration Fee: \$65.00 (postmarked before 15 June 1995)

\$75.00 (postmarked after 15 June 1995)

\$ Amount enclosed

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

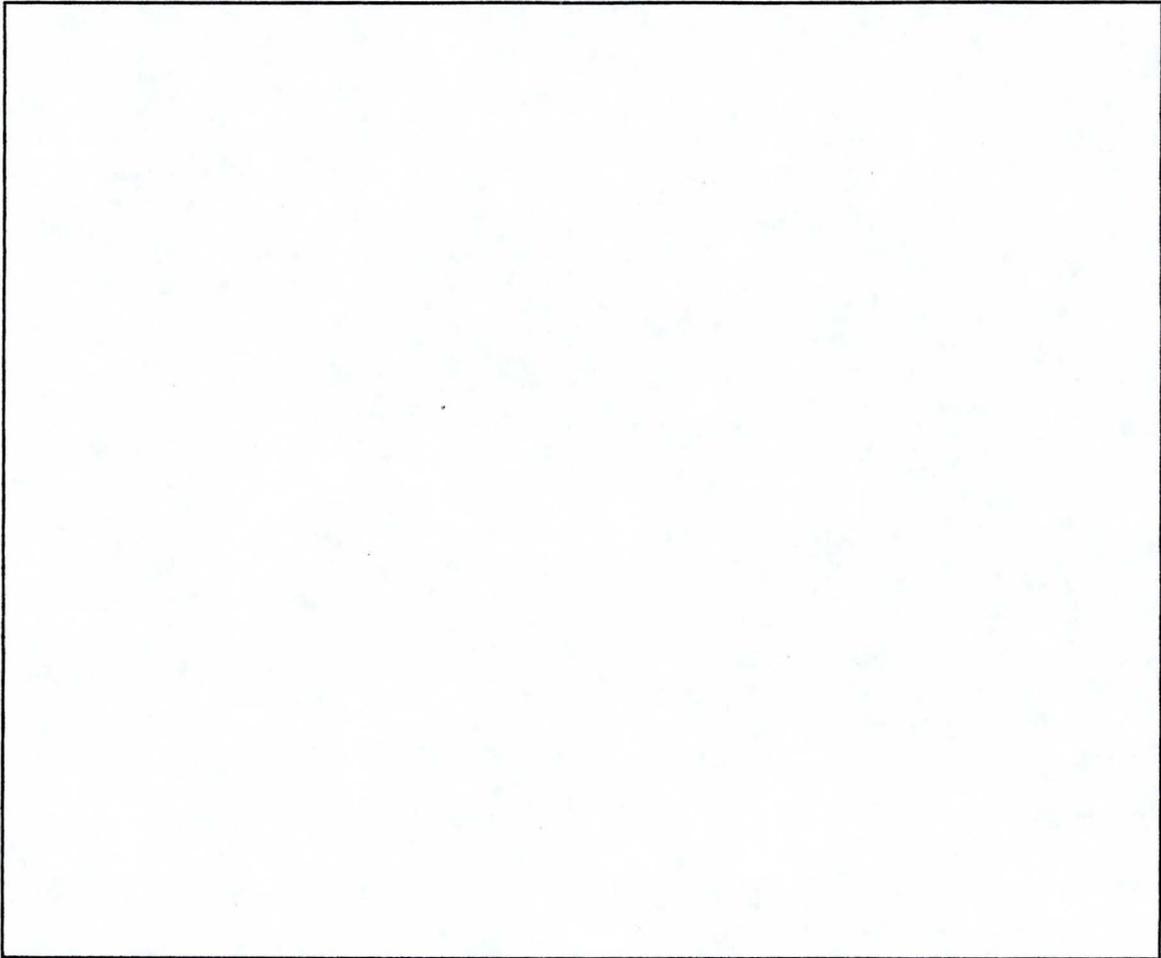
**Dr. John Schachte, Jr.
Fish Disease Control Unit
New York State Department of Environmental Conservation
8314 Fish Hatchery Road
Rome, New York 13440**

Hotel reservations should be made directly with:

Sheraton Inn Syracuse
Electronics Parkway
Liverpool, New York 13088
(315) 457-1122
(800) 325-3535

ABSTRACT

WESTERN FISH DISEASE WORKSHOP
TWIN FALLS, IDAHO
6-9 JUNE 1995



REGISTRATION
WESTERN FISH DISEASE WORKSHOP
JUNE 6-9, 1995
TWIN FALLS, IDAHO

NAME: _____

ADDRESS: _____

TELEPHONE: _____ FAX: _____

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

| | | |
|----------|---|----------|
| June 6 | Continuing Education Session: HISTOLOGY (\$15.00 per person) | \$ _____ |
| June 7-8 | Technical session, coffee breaks, lunches, social and BBQ (\$60.00 per person) | \$ _____ |
| June 9 | Industry tour (\$10.00 per person) | \$ _____ |
| | TOTAL | \$ _____ |

Return by April 30, 1995 to:

Scott LaPatra
Clear Springs Foods, Inc.
P.O. Box 712
Buhl, ID 83316

ABSTRACT SUBMISSION INSTRUCTIONS

WESTERN FISH DISEASE WORKSHOP
TWIN FALLS, IDAHO
6-9 JUNE 1995

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

Please complete the following form and submit it with your abstract by April 30, 1995 to:

Scott LaPatra
Clear Springs Foods, Inc.
P.O. Box 712
Buhl, ID 83316

I am submitting an abstract for the Western Fish Disease Workshop meeting.

Name: _____

Address: _____

Telephone: (____) _____

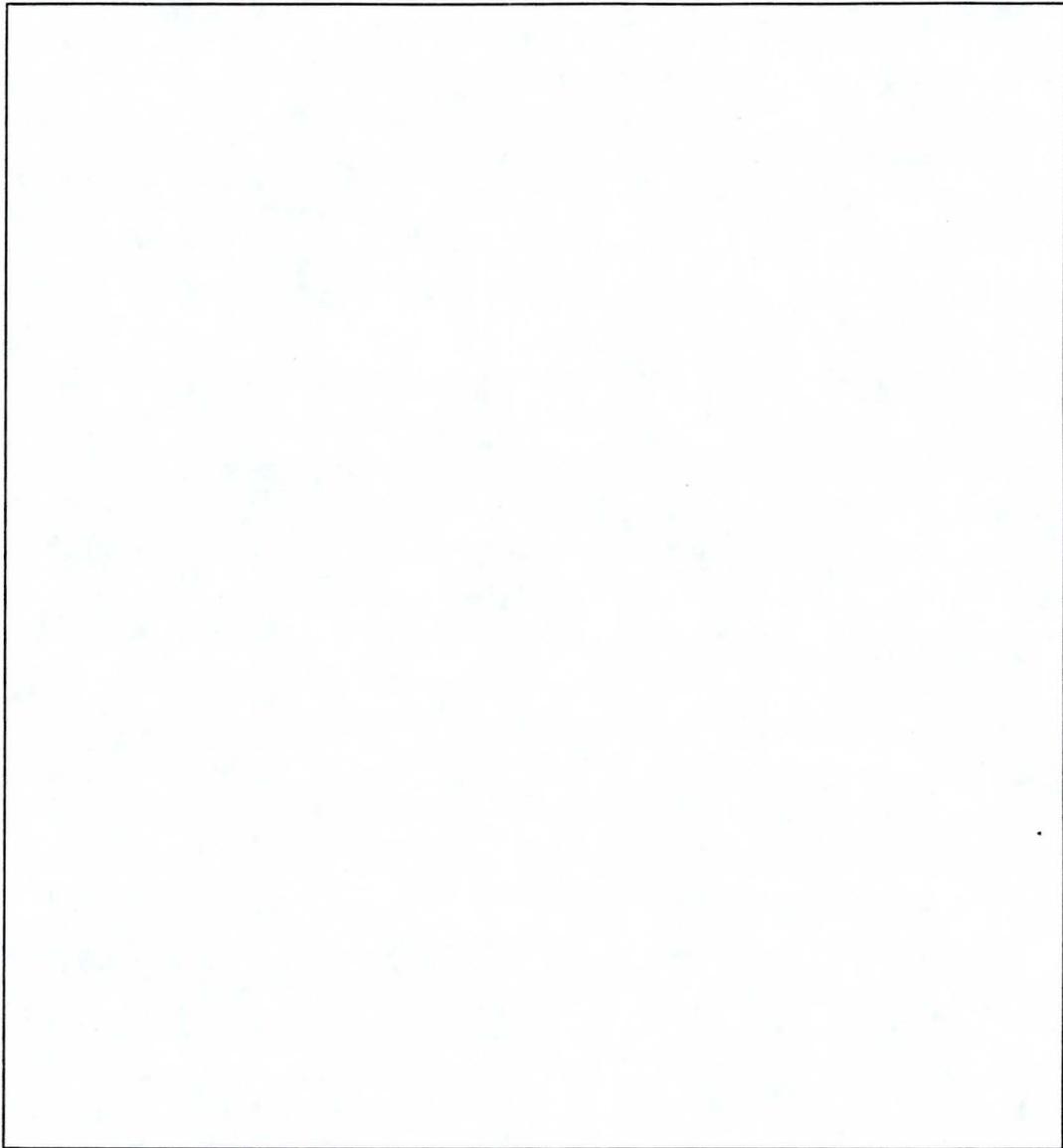
FAX: (____) _____

I prefer that my presentation be: _____ an oral presentation
_____ a poster presentation

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

ABSTRACT

FISH HEALTH SECTION/AFS AND EASTERN FISH DISEASE WORKSHOP
SYRACUSE, NEW YORK
19-22 JULY 1995



AIR TRAVEL:

You may fly directly into Twin Falls, which is serviced by commuter flights (Horizon and Skywest) via Boise or Salt Lake City. The Canyon Springs Inn operates a courtesy shuttle from the Twin Falls airport to the hotel. You can either call in advance to let them know which flight you will be arriving on, or use the courtesy phone at the airport when you arrive. You may also drive from either Boise, ID or Salt Lake City, UT, which are serviced by several major airlines. It is a two hour drive east on I-84 from Boise, and a four hour drive west on I-84 from Salt Lake City.

TOUR: The tour is an optional event. It is planned for a half day (about 8:30 am to 1:00 pm) on Friday, June 9. Cost of the tour is (\$10.00). The proposed schedule is:

June 9 - 8:30 am - leave Twin Falls, Sun Valley Stage (Tour Bus)
9:00-10:00 am - Clear Springs R&D, Snake River Hatchery
10:30-11:00 am - Bill Jones, innovative and totally enclosed facility
11:30 am - Box Canyon Hatchery drive through; potentially the
largest hatchery in the world
Noon - Seafood Specialty in Filer; hor d'oeuvres and refreshments
1:00 pm - return to Twin Falls

FOOD: Lunches will be provided during both days of the technical session. A social gathering is planned for Wednesday evening, and a fish dinner will be provided on Thursday.

OTHER ACTIVITIES:

Sun Valley is located an hour and a half north of Twin Falls. Chamber of Commerce: 1-800-634-3347. Jackpot, NV is located an hour south of Twin Falls. Hunting and fishing information is available from Idaho Fish & Game at 1-800-635-7820. This is an automated help line with a wide variety of information on hunting, fishing and other outdoor activities.

Prevalence and Distribution of Bacteria in the Mucus of Atlantic Salmon (*Salmo salar*) During 1994 Spawning Migration On the Connecticut River

Patricia A. Barbash¹ and Richard J. Van Nostrand²

¹USFWS, Northeast Fishery Center-Fish Health Unit, Lamar, PA

²Connecticut DEP, Fish Health Lab, Burlington, CT

INTRODUCTION

Efforts to restore the Atlantic salmon (*Salmo salar*) to the rivers of New England have continued for over 20 years. The restoration programs have depended entirely upon artificial propagation, of which wild, searun broodstock are the most genetically valuable source of eggs. Because of the prevalence of furunculosis documented within the sea-run salmon of the southern New England rivers, rigorous disease management protocols involving the use of antibiotic and vaccine injection have been established to reduce disease related mortality among these captured broodstock.

Annual trends of disease among groups of salmon which were not injected with antibiotic and vaccine suggest that exposure to pathogens occurs within the river and can cause mortality exceeding 50% of the population. In contrast, the data also indicates that pathogen exposure may be very low or not take place at all during some years (Barbash, 1993). To date, however, no data has been gathered which can verify the status of individual sea-run broodstock as pathogen carriers upon their capture from the river. It is unknown whether a few carriers contribute to the spread of disease to healthy fish during holding, or whether most fish are captured with some degree of infection. This submission presents the first in a series of annual data on the prevalence and distribution of bacterial species carried on the mucus of migrating Atlantic salmon at the time of their capture from the Connecticut River and two of its tributaries.

METHODS

Migrating salmon were captured during May and June, 1994, from fish lift facilities at dams located on the Connecticut River in Massachusetts, and two of its tributaries, the Farmington and Salmon Rivers in Connecticut. Fish were transported by truck in aerated 50°F well-water from capture to holding sites at the Cronin National Salmon Station in Sunderland, MA and the Whittemore Salmon Station in Riverton, CT. Upon arrival, each fish was anaesthetized in a solution of MS-222 until rendered immobilized for tagging, data collection and sampling prior to antibiotic injection.

Mucus samples were obtained and processed according to the methods of Cipriano and Ford (1992) for 51 of 52 salmon captured from the Farmington and Salmon Rivers during the spring migration and again on 52 fish in the fall prior to spawning. Unfortunately, on the pre-spawn fall samples only species identification and not quantification was performed. Samples were obtained from 50 Connecticut River sea-runs using a 10 ul inoculation loop streaked onto petri plates containing tryptic soy agar with coomassie brilliant blue (CBB). The loop inoculation as compared to the dilution-drop plating method of

Cipriano and Ford (1992) was determined to be an average of one log lower in plate counts. Although inappropriate for accurate bacterial quantification, results from this rough method of quantifying bacteria are included because they offer consistency between samples, which allows for some evaluation of the presence of pathogens and the distribution of bacteria on the mucus.

RESULTS

A. salmonicida was not detected in any of the mucus samples examined. *Pseudomonas fluorescens* was found to be the most prevalent species on the mucus of salmon captured from all three rivers, occurring on 70-90% of fish sampled at levels ranging from 10³ (in loop counts) to 10⁶ (drop plates) cell forming units per gram of mucus (cfu/gm). *Pseudomonas fluorescens* was also the most predominant species identified on the mucus of captive salmon at Whittemore 4-5 months post capture at 53.06% of isolates identified. In fact, pseudomonad bacteria accounted for 91.8% of all isolates identified. The other prevalent pseudomonad bacteria being *P. alcaligenes*. Other predominant species identified include *P. putrefaciens*, *P. alcaligenes*, *Moraxella* species, and *Acinetobacter* species.

As expected, *A. salmonicida* was never detected in the few mortalities which occurred during the subsequent holding period, nor was it detected in kidneys of post-spawn males which were lethally sampled. Only one of two fall migrating fish at the Whittemore Salmon Station was determined to have a lethal furunculosis infection. Unfortunately, no mucus sample had been obtained from this fish at capture.

DISCUSSION

All fish were treated with antibiotic injection immediately after the mucus samples were obtained. Because of the historic success of the antibiotic in controlling furunculosis in these fish (Barbash, 1993), it is not possible to relate the presence or absence of pathogens at capture to infection levels in the broodstock during the holding period. The lack of detection of *A. salmonicida* in the mucus of spring migrating Atlantic salmon appears to indicate that these fish may not have been sufficiently challenged by the pathogen to induce a carrier state while migrating upriver. The low number of fall migrating fish, however, experienced an exposure to the bacterium within the river which led to disease and death of one fish shortly after arriving at the holding facility. Seasonal fluctuations of river bacteria, or lower water levels which may congregate fish into desirable areas may be accountable for this event. Fish in closer proximity to each other may facilitate exposure to *A. salmonicida*.

The significant prevalence of *P. fluorescens*, however, may indicate that other environmental

factors contributed to the absence of A. salmonicida within mucus samples. Recent studies have strongly noted the inhibitory effect which P. fluorescens has upon A. salmonicida. Smith and Davey (1993) exhibited that A. salmonicida showed poor or no growth in liquid culture media containing by-products of P. fluorescens. In addition, the authors demonstrated that Atlantic salmon pre-smolts known to be furunculosis carriers experienced a significant reduction in stress-induced infections after being bathed for 24 hours in a solution of P. fluorescens. Other authors have noted the inverse relationship between isolation of these two organisms in fish and environments that experience periodic furunculosis (Cipriano, et al. 1992; Cornick, et al. 1969).

It has become increasingly apparent that healthy Atlantic salmon populations exhibit a high prevalence of pseudomonad type bacteria in their mucus particularly Pseudomonas fluorescens. What role P. fluorescens plays as a protector or indicator species is still undetermined. It will be necessary to continue gathering data of this sort to evaluate annual and seasonal trends of bacteria inhabiting the mucus of migrating Atlantic salmon adults, and to determine whether certain species of bacteria prevalent on captured fish can be used to indicate and/or control disease situations

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BLUE BOOK PRINTING ERRORS

We have received word from several people purchasing Blue Books that printing errors are present in their copies. All purchasers should examine their copies for such errors and inform Paul Reno, Chair of the Blue Book Advisory Committee by letter or telephone at (503) 867-0147. These errors are being tracked so that they may be corrected in future Blue Book updates.

SHORTCOURSES:

"DISEASE DIAGNOSIS AND CONTROL IN MARINE SHRIMP CULTURE"

June 19 to June 30, 1995 at the University of Arizona, Tucson, Arizona. For info contact: Donald V. Lightner or Leslie S. Jones, Department of Veterinary Science, The University of Arizona, Building 90, Room 108b, Tucson, Arizona 85721. FAX: (602) 621-6366, Tel: (602) 621-8414, E-mail: AQUAPATH@CCIT.ARIZONA.EDU or LESLIEJ@CCIT.ARIZONA.EDU

"DISEASES OF WARMWATER FISHES"

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.

WHERE: Fisheries and Aquatic Sciences
University of Florida
Gainesville, Florida

WHEN: June 5-16, 1995

SPONSORS: University of Florida, College of Veterinary Medicine, Department of Fisheries and Aquatic Sciences, Whitney Marine Lab

TOPICS: Water quality and aquaria, fish necropsy procedures, bacterial, viral, fungal, parasitic, nutritional and environmental diseases of fish, treatment.

CONTACT: Dr. Ruth Francis-Floyd, Department of Fisheries and Aquatic Sciences, University of Florida, 7922 NW 71st St., Gainesville, FL 32606
Phone: (904) 392-9617 ext. 229
Fax: (904) 846-1088

NEW SOCIETY:

A new society has been formed: **FIN (Fish Immunomodulators Network)** to enhance communication between scientists interested in immune modulation in ectothermic species, especially finfish and shellfish. Immune modulators are defined as natural regulatory substances such as cytokines as well as introduced pharmacological, biochemical and chemical substances under the general class of immunostimulators, immunosuppressants and immunotoxicological substances.

The society publishes a newsletter and sponsors conferences and workshops. Membership dues are \$20 (US, CAN), \$24 (overseas).

Contact: Dr. Joanne Stolen, SOS Publications, 43 Denormandie Avenue, Fair Haven, NJ 07704 FAX: (908) 530-5896.

Reovirus Interference Mediated IHN Resistance

Scott E. LaPatra, K.A. Lauda, and G.R. Jones

Research Division, Clear Springs Foods, Inc., Buhl, Idaho 83316

A study was conducted to evaluate the chum salmon reovirus (CSV) (Winton et al. 1981) and its capacity to non-specifically stimulate host defenses against a viral infection. Reoviruses have been shown to be potent interferon inducers in other systems and have been detected in salmonids throughout the Pacific Northwest. The first evaluation consisted of exposing one group of rainbow trout (mean weight, 8.8 g) to 10^4 TCID₅₀/ mL CSV for 1 h and mock exposing a duplicate group. These fish were put on identical feeding regimes and held 1 week. Groups of fish from both treatments were challenged with IHN using standard procedures (LaPatra et al. 1991). Subgroups from each treatment were mock exposed to virus and served as negative controls. All groups were monitored for 5 weeks and a portion of the dead fish were examined for virus. Cumulative mortality detected in CSV exposed and IHN challenged group was 9% (26/300) and 26% (79/300) in the IHN challenged only group. Relative percent survival was 65% which is consistent with results obtained in a previous experiment that investigated interference mediated IHN resistance with a picorna-like virus, the cutthroat trout virus (CTV). The protection afforded by 1 h bath exposures to CTV provided up to 69% relative percent survival following IHN challenge. This effect was present at 1, 2, and 4 wk post-exposure to CTV but was absent when fish were tested at 6 wk (Hedrick et al. in press). To test duration of protection against IHN after preexposure of fish to CSV, groups of 500 fish (mean weight, 5.2 g) were exposed to 10^4 TCID₅₀/ mL CSV for

1 h and mock exposed. Duplicate 25 fish groups from each treatment were challenged with IHN at 1, 2, 4, 6, and 8 weeks. Major differences were observed in cumulative percent mortality between the two treatment groups at each of the time points tested (Table 1).

Sixty surviving fish from each treatment and 20 mock infected controls were bled and the serum was titered for IHN neutralizing activity. No IHN neutralizing activity was detected in any of the control fish. However, CSV exposed IHN survivors appeared to have significantly ($p < 0.001$) lower neutralization titers than surviving fish exposed only to IHN. This is contrary to what was observed during the course of similar experiments with the CTV. The levels of IHN antibodies were significantly higher among fish receiving prior exposure to CTV than among non-CTV treated fish challenged with IHN (Hedrick et al. in press). These results indicated that not only was CSV capable of providing excellent protection to IHN challenge but specific immunity (serum neutralizing activity) also developed, however, the response appeared to be depressed in treated fish.

Several possibilities for the CSV stimulation of nonspecific immune functions have been postulated. These include interferon induction and/or stimulation of macrophage, or natural killer cell functions. Cytokine activity is central to these responses but unfortunately, in salmonids, many of these functions are poorly understood.

Our results have shown that CSV

provides protection for up to 8 weeks. We are now attempting to define the mechanism by which this protection may be occurring. Possibly by understanding this mechanism more effective viral control strategies such as vaccines can be developed along with generating basic fish immunology information.

References

Hedrick, R.P., S.E. LaPatra, S. Yun, K.A. Lauda, G.R. Jones, J.L. Congelton, and P. de Kinkelin. Induction of protection from infectious hematopoietic necrosis virus in rainbow trout *Oncorhynchus mykiss* by pre-exposure to the avirulent cutthro at trout virus (CTV). *Diseases of Aquatic Organisms*, (in press).

LaPatra, S.E., K.A. Lauda, G.R. Jones, and S. Walker. 1991. Standardization of infectious hematopoietic necrosis virus challenge procedures. *AFS/FHS Newsletter* 19:3-5.

Winton, J.R., C.N. Lannan, J.L. Fryer, and T. Kimura. 1981. Isolation of a new reovirus from chum salmon in Japan. *Fish Pathology* 15:155-162.

Table 1.

IHNV Challenged

| Week ^a | CSV Exposed | Mock Exposed | Relative Survival |
|-------------------|-----------------|--------------|-------------------|
| 1 | 2% ^b | 26% | 77% |
| 2 | 10% | 92% | 89% |
| 4 | 0 | 86% | 100% |
| 6 | 2% | 44% | 95% |
| 8 | 13% | 40% | 68% |

^a Week post-exposure to CSV or mock exposure.

^b Cumulative percent mortality.

UPCOMING MEETINGS

East Coast Trout Management and Culture Workshop II. May 31-June 2, 1995.

Penn State University, State College, PA. Contact: Marty Marcinko, 450 Robinson Lane, Pennsylvania Fish Commission, Bellefonte, PA 16823. (814)-359-5223.

Modulators of Immune Responses: Hiking up the Evolutionary Trail. July 8-15, 1995.

Breckenridge, CO. Contact: Joanne Stolen, SOS Publications, 43 DeNormandie Avenue, Fair Haven, NJ 07704-3303. (908)-530-3199.

Fourth Asian Fisheries Forum. October 16-20, 1995.

Beijing, China. Contact: the China Society of Fisheries, 31 Min Feng Lane, Xidan, Beijing, CHINA. (861) 602-0794.



DEADLINE FOR NEXT NEWSLETTER IS JUNE 5, 1995

The editors of the FHS newsletter thank the members for their support regarding their enthusiasm in submitting contributions for publication in the newsletter. The **prohibitive** cost of mailing more than a 20 page newsletter, however, imposes limits for the length of each article so we are implementing **new guidelines** for authors. **Articles should not exceed 4 single spaced typed pages** so that the maximum length would not exceed 6 newsletter columns. Also, please note that articles will continue to be accepted with the understanding that the material will be published without peer review. Articles should be submitted on disk in Word Perfect 5.1 or in generic form that can be read on WP 5.1. Disks will be returned if a SASE is included with your submitted article. Again, thank you all very much for your continued support, which allows for the publication of a high quality and informative newsletter. The Fish Health Section Newsletter is a quarterly publication of the Fish Health Section of the American Fisheries Society. Submissions should be addressed to the editors listed below:

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