

FISH HEALTH NEWSLETTER

American Fisheries Society/Fish Health Section

Volume 31 Number 2 April 2003

In this issue:

President's Report.....	Page 1
Meetings / Announcements	Page 2
FHS Nominations and Ballot of Candidates for 2003-2004.....	Page 8
Call for Fish Health Section awards.....	Page 13
FHA Participation in the AAVLD/ USAHA Annual Meetings.....	Page 14
Notes from "The Drug Lady".....	Page 16
Current Highlights of VIMS Mycobacteriosis Research.....	Page 19
Scientists blame fish deformities on fertilizers.....	Page 20
Opinion: The feeding of wild fishmeal to farmed fish and recycling of fish with regard to the risk of TSE.....	Page 20
Bactericidal Antibiotic Effective Against Methicillin-Resistant <i>S. aureus</i>	Page 21
Raritan Bay Shellfish Transplant Program cancelled for 2003.....	Page 22
An update on the antigenic diversity in <i>Tenacibaculum maritimum</i> strains isolated from marine fishes.....	Page 24

President's Report:

The Fish Health Section has been active on a number of projects over the past months. Among them are efforts associated with the Inspection Manual, the development of Continuing Education opportunities and the transition of the Journal of Aquatic Animal Health to a system of electronic submission of manuscripts.

The Inspection Manual, officially titled: "Standard Procedures for Aquatic Animal Inspections," has been completed. This document was co-authored by the USFWS and the AFS Fish Health Section. Within the Inspection Manual effort is a mechanism for annual reviews and revisions as necessary. The Inspection Manual has been provided to APHIS for their examination. APHIS is currently coordinating the development of a national fish health plan. It is hoped that the Inspection Manual will play a role in that plan. The Inspection Manual is also part of a larger effort by the Fish Health Section to distribute the Blue Book in an electronic CD format. Originally published in loose-leaf format, the Blue Book, as an updated,

searchable CD will contain both the familiar chapters of the "Suggested Procedures for the Detection and Identification of Certain Finfish and Shellfish Pathogens" and the newly developed "Standard Procedures for Aquatic Animal Health Inspections". This new format allows the user to easily access information and contains numerous color photographs and video clips. The CD will be published by the Fish Health Section. Specific details on these distribution mechanisms will be provided in the near future.

Several efforts are underway to provide Fish Health Section members with Continuing Education opportunities. These have been developed to provide current Certified Fish Pathologists and Certified Fish Health Inspectors with training that may be of use in re-certification procedures. An additional objective is to provide more in depth coverage of some timely topics in aquatic animal health to the Fish Health Section Membership at large. A CE presentation titled "Molecular Biology - The Basics" will be presented on 25 April 2003, in conjunction with the Eastern Fish Health Workshop scheduled for Gettysburg, PA on 21-24 April. The course will be taught by Jim Casey and Ted Clark of Cornell University. The original target enrollment for this course was 40. As a result of the response to this offering, the class size has increased to 54. During the upcoming Annual Meeting of the Fish Health Section/AFS and Western Fish Disease Workshop (14-17 July 2003) in Seattle, WA, there will be a CE presentation titled: Current Topics in Fish Virology (14 July). Presenters at this CE offering include: Andy Goodwin, Jim Keleher, John Grizzle and Scott LaPatra. Given recent events in fish virology in North America, this looks to be another most timely educational presentation. We greatly appreciate the efforts of the presenters at both of these CE sessions for their willingness to provide these unique training opportunities.

As you are probably aware, the Journal of Aquatic Animal Health is now accepting manuscripts via an electronic mechanism. I strongly encourage you to consider the Journal as an outlet for publication of your manuscripts. The electronic submission mechanism will provide many advantages in terms of reducing time and cost of publication. As someone who might be described as "moderately challenged" by some of the new technologies, I found the submission mechanism to be fairly easy to negotiate when I made a recent submission.

Paul Bowser

Meetings and Announcements:

Wisconsin Aquaculture Veterinary Medicine Course

May 22 - 23, 2003

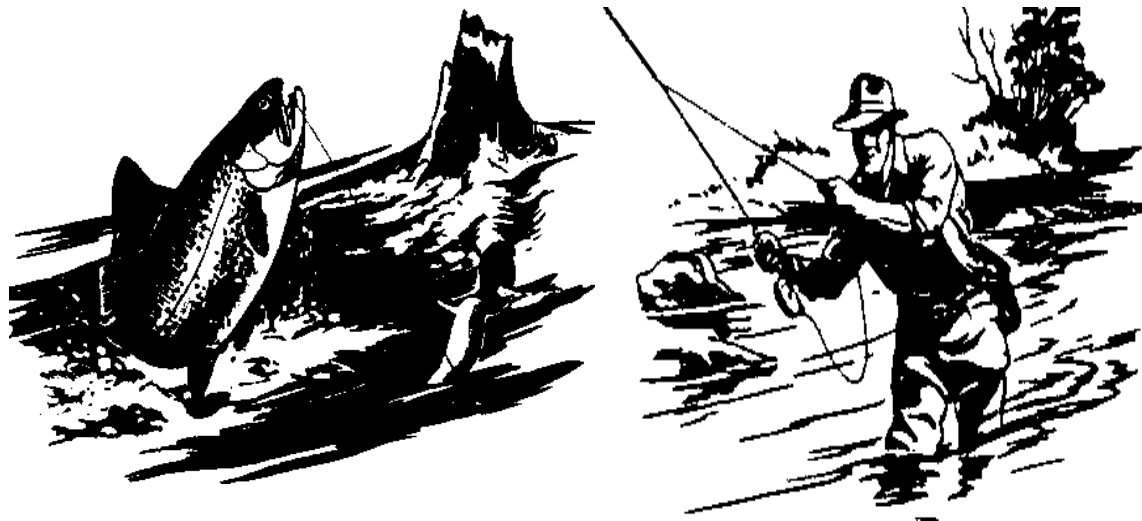
Madison, Wisconsin

An intensive program designed to provide practical training in the area of fish diagnostics. This course complements didactic courses offered in other parts of the country. Participants will be exposed to several species of warmwater and coolwater fish. Emphasis will be placed on providing hands-on exposure to techniques that are essential for fish veterinarians. Hands-on training will take place on a commercial fish farm in southern Wisconsin.

Course materials will be mailed to participants prior to the course. We request that participants read over the material and come prepared to discuss and review the materials during the orientation session on Thursday.

The brochure will be posted on <http://www.vetmed.wisc.edu> - look under continuing education.

9TH Annual Investigational New Animal Drug Workshop
July 30 - 31, 2003
Bozeman, Montana



The U.S. Fish and Wildlife Service's Bozeman National INAD Office (NIO) is pleased to announce that it once again plans to host an Annual INAD Workshop in Bozeman, MT. This year's workshop will be our 9th Annual INAD Workshop...how time flies when you're having fun!! The Workshop will be held July 30 - 31, 2003, and will be held at the Holiday Inn in Bozeman, Montana. The Workshop is scheduled to last 2 full days. Workshop format will be similar to that of previous year's workshops, with overviews, technical presentations, and discussion of nearly all aspects of the INAD/NADA process. This year we hope to be able to provide a bit more time for interactive group discussion. We are fortunate that once again representatives from FDA's Center for Veterinary Medicine are planning on attending. It is a tremendous opportunity for those of us in field-related aquaculture to be able to discuss with CVM our progress and strategies with respect to INAD/NADA objectives.

Lodging accommodations have been reserved at the Holiday Inn and the nearby Days Inn. Phone numbers for the two hotels, room rates, number of rooms reserved, and amenities are listed below. Rooms have been reserved for July 29 - August 3, 2003. Rooms may be reserved for any portion of this period. Please note that the hotels will release all unreserved rooms by June 29, 2003, and that cancellations must be made no later than one day prior to arrival. Also listed below are room rates and phone numbers of several downtown hotels and a bed and

breakfast for folks who want to be closer to downtown. Be forewarned!....this year's workshop is again scheduled to be held at the same time as Bozeman's Annual Sweet Pea Summer Festival, so available rooms will be at a premium. Also, local hoteliers suggest booking rooms through the weekend if you have even the slightest interest in spending any extra time in Bozeman. Attempts to get a reservation extension once you arrive will likely be unsuccessful. Please note that the NIO will be happy to help arrange transportation for participants between the airport and hotels, and between hotels and the Workshop conference room at the Holiday Inn.

As most of you are well aware, INADs, NADAs, data packages, data submissions, data and report reviews, etc., etc. can be an exhausting endeavor. Therefore, as has been the case with past Workshops, this years Workshop will also include an "INAD Retreat" for those suffering from severe mental consumption. Past retreats have not only functioned to "revive the spirit", but have also been found to facilitate additional INAD/NADA discussions in a more "open and frank manner." At this time, we're planning on having a Bar-B-Q (similar to last year) at a scenic spot in the mountains on Thursday evening. If nothing more, it will help us alleviate residue levels after two days of drug talk.

We encourage all past workshop participants, as well as first-timers (locally known as "pilgrims") to attend. If you are interested in the approval of new animals drugs for use in aquaculture, this Workshop is for you!! If you have any questions, or would be interested in giving a presentation at the Workshop, please call or email Jim Bowker at (406) 587-9265 ext. 126; jim_bowker@fws.gov.

Bozeman area hotels at or near Workshop Conference site

(rooms have been reserved under "INAD Workshop")

Holiday Inn	5 Baxter Lane (Corner of N. 7 th Ave. & I-90)	406-587-4561	\$93
	coffee pots, hair driers, pool		
	10 rooms reserved		
Days Inn	1321 N. 7 th Ave. (next to Holiday Inn)	406-587-5251	
		single \$55	double \$65
	hot continental breakfast		
	25 rooms reserved		

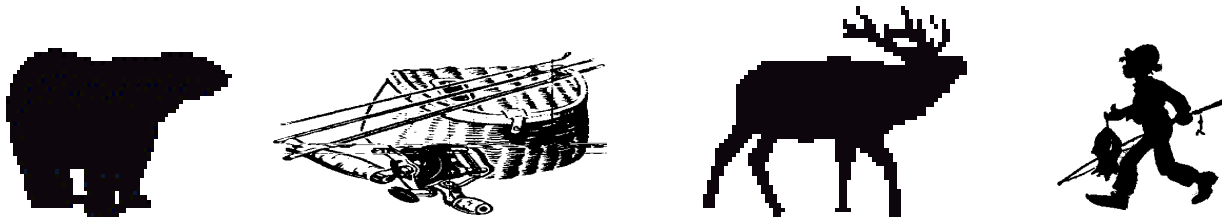
Downtown Bozeman

Best Western City Center	507 W. Main St. (location of previous workshops)	406-587-3158	\$55
	pool, downtown location		
Lewis and Clark	8 th Ave. & Main St. (~1 mile from Holiday Inn)	406-586-3341	\$55
Lehrkind Mansion B & B	719 N. Wallace Ave. (close to downtown)	406-585-6932	\$79-159

Other

Microtel Inn I-90 & N 7th Ave. (< _ mile from the Holiday Inn) 586-3797 \$55
continental breakfast, pool, hot tub
Room rates are higher on the weekend

If you cannot procure lodging accommodations at one of the above listed hotels, please call us and we'll help you arrange lodging accommodations



SHRIMP HEALTH MANAGEMENT TRAINING WORKSHOP: August 18-23, 2003 Thailand

This course is co-organized annually by NACA and the Aquatic Animal Health Research Institute of Thailand. The course runs for six days and includes lectures, practical, case studies, visits to farms and adequate time for discussion. The lectures are based on the information contained in the book "Health Management in Shrimp Ponds" and are illustrated with an extensive range of photographic slides. Emphasis is placed on the benefits of maintaining healthy stock and preventing disease through appropriate management of the pond. The use of chemical treatments will be covered but only as part of an integrated management system. Topics addressed include: Update on shrimp culture systems; pond environment; pond preparation; water management; farm records; disease; larval assessments; chemical treatments; and the current situation in Thailand.

Registration fee is US\$ 750. It covers the cost of tuition, a copy of "Health Management in Shrimp Ponds" and the workshop dinner. Participants will be responsible for the cost of hotel accommodation and subsistence during the workshop.

Key resource speakers include Dr Pornlerd Chanratchakool (AAHRI), Dr. James F. Turnbull (Stirling University), Dr Chalor Limsuwan (Kasetsart University) and Mr Dan Fegan (National

Centre for Genetic Engineering & Biotechnology). For further information contact the Training Officer training@enaca.org or download the brochure: <http://www.enaca.org/Training/ShrimpHealthWorkshop.pdf> [PDF, 223 KB]

American Fisheries Society 133rd Annual Meeting
Québec City, Québec, Canada
August 10-14, 2003

"Worldwide decline of wild fish populations"

Preregistration begins April 2003.

For more information on the meeting or to register online, please go to www.fisheries.org and click on "Annual Meeting"

Aquatic Animal Models of Human Disease:
September 29- October 2, 2003
Manassas, Virginia

The University of Miami and the American Type Culture Collection (ATCC) are pleased to announce a conference entitled "Aquatic Animal Models of Human Disease." This meeting is sponsored by the National Center for Research Resources (NCR) of the National Institutes of Health and will be held at the ATCC facility in Manassas, VA, from September 29 through October 2, 2003. Topics for scientific sessions will include the use of aquatic animal models for the study of comparative genomics, gene expression, transgenesis, carcinogenesis, toxicology, infectious disease, neurological disorders and aging. Workshops are planned on transgenesis and gene expression, as well as on current technologies for resource development and funding mechanisms. Submissions of abstracts for oral and poster presentations are invited with a deadline of July 1, 2003. Registration will be limited. For information, please see the conference website at http://pasteur.atcc.org/aquatic_conference/

4th World Fisheries Congress
May 2 - 6, 2004
Vancouver, BC Canada

The Congress theme, "Reconciling Fisheries with Conservation: The Challenge of Managing Aquatic Ecosystems," will be addressed by a world class list of Keynote speakers, session topics, posters, limited presentations, round table discussions, forums, workshops and debates.

Online Abstract Submittal for the Fourth World Fisheries Congress will open April 2003.

Please visit www.worldfisheries2004.org for details

The Hutton Junior Fisheries Biology Program

The Hutton is an educational program for high school students designed to develop interest in a career in fisheries among groups underrepresented in the fisheries profession, including minorities and women. AFS encourages its members to consider mentoring a student during the summer of 2003.

For information on how you can get involved, go to <http://www.fisheries.org/Hutton.shtml>, or contact Christine Fletcher at cfletcher@fisheries.org phone: 301-897-8616, ext. 213.

New Book Releases from AFS:

Population Genetics: Principles and Applications for Fisheries Scientists
Eric Hallerman, editor

The principles of population genetics have important bearing on the practice of fisheries science. However, fisheries managers do not typically receive training in population genetics. This is due, in large part, to lack of course materials in population genetics relevant to fisheries science. This book was born of a need perceived by many fisheries geneticists for the availability of a textbook for a course in fisheries genetics. Qualified instructors too busy to develop their own course from scratch can use this title as a ready resource text for teaching such a course. Population Genetics is an excellent resource for making the field of population genetics relevant and accessible to students and practitioners of fisheries science.

507 pp., hardcover, March 2003

Stock Number: 550.34

List price: \$69.00

AFS member price: \$48

ISBN 1-888569-27-1

Nutrients in Salmonid Ecosystems: Sustaining Production and Biodiversity
John Stockner, editor

The proceedings of the 2001 conference "Restoring Nutrients to Salmonid Ecosystems" and the first book of its kind, this volume presents recent information on the role and importance of marine-derived nutrients in salmonid ecosystems. The authors examine how this research can be used effectively to assist in rebuilding salmonid stocks in the Pacific Northwest.

The book contains: (1) Description and management of historical and current nutrient regimes in salmonid ecosystems; (2) Ecological linkages between salmon and productivity of freshwater ecosystems and the ecological impacts of a diminished salmon nutrient shadow; (3) Dispersal mechanisms of marine-derived nutrients in Pacific Northwestern freshwater ecosystems; (4)

The effects of hatcheries, harvest, and other resource management regimes on nutrients and their dispersal; and (5) An incorporation of nutrient management into ecosystem restoration.

AFS Symposium 34
302 pp., paper, February 2003
Stock Number: 540.34
List price: \$60
AFS member price: \$42

ISBN 1-888569-44-1

To order either of these titles:
Online: www.fisheries.org/cgi-bin/hazel-cgi/hazel.cgi
Phone: (678) 366-1411, or Fax: (770) 442-9742
Email: afspubs@pbd.com

FISH HEALTH SECTION ELECTION for 2003-2004!

It's time again to vote! This year we elect a Vice-President of the Section, and one new member for each of three committees: the Nominating and Balloting; Technical Standards; and Professional Standards Committees. If you wish to refresh your memory about what these positions entail, pull up the Jan 2003 issue of the *Fish Health Newsletter* (Vol. 31, number 1) on the FHS website. On page 6 you will find short descriptions of duties.

Short biographies of nominees for these positions follow, as does the ballot.

Please download the ballot, vote, and mail, email or fax the ballot to:

Margaret S. Ewing
Department of Zoology
Oklahoma State University
Stillwater, OK 74078
msewing@okstate.edu
Fax: (405) 744-7824

by May 15, 2003.

These fellow members are willing to contribute significant time and energy to the work of the Section; please take a few minutes to vote.

Thanks,
Margaret S. Ewing, for the
Nominating and Balloting Committee

NOMINEES FOR FHS POSITIONS:

Vice-President

John Phillip Hawke

B.S. Auburn University, 1972

M.S. Auburn University, 1974

Ph.D. Louisiana State University, 1996

Department of Pathobiological Sciences

School of Veterinary Medicine

Louisiana State University

Baton Rouge, LA 70803

John Hawke is an Associate Professor with the Department of Pathobiological Sciences (PBS), School of Veterinary Medicine, Louisiana State University, Baton Rouge, Louisiana. Dr. Hawke serves as Chief Diagnostician for the Louisiana Aquatic Diagnostic Laboratory (LADL) and has been involved in fish diagnostics and research for over 25 years. The LADL receives specimens of diseased fish from over 30 states as well as several foreign countries and is a full service diagnostic laboratory. Over 100 different species have been examined by the lab from commercial fish, crawfish and alligator farms, recreational fishing ponds, ornamental fish ponds, ornamental aquaria, bioassay laboratories, and research labs. The LADL has participated in the documentation of many emerging bacterial fish and crustacean pathogens. The lab also has an extensive repository of archived bacterial and viral isolates. Dr. Hawke participates in the team teaching of courses in the PBS department including Diseases of Aquatic Animals and Aquamed and gives lectures in a variety of other courses. Dr. Hawke has directed several graduate students and conducts research in the general areas of pathogenesis of bacterial diseases of warm water fish, bacterial taxonomy, fish vaccines, and molecular biology of microbial fish pathogens. The production of live attenuated vaccine strains from specific genetic mutants of *Photobacterium damsela* subsp. *piscicida* and characterization of strains of *Flavobacterium columnare* from various geographic locations and hosts are current areas of research interest. Dr. Hawke has served as Associate Editor and ad hoc reviewer for the Journal of Aquatic Animal Health, was co-chair of the Aquaculture Working Group of the Veterinary Subcommittee on Antibiotic Susceptibility Testing (VAST) of the NCCLS, and helped coordinate several meetings including the 7th Fish Diagnosticians Workshop, March 2000, at Louisiana State University and the 4th International Symposium on Aquatic Animal Health, September 2002, in New Orleans, LA.

Nominating and Balloting Committee

Julie Bebak-Williams

Dr. Julie Bebak-Williams is Director of Aquatic Animal Health at the Freshwater Institute in Shepherdstown, West Virginia. Julie received her veterinary degree and PhD from the University of Pennsylvania School of Veterinary Medicine. She has nine years experience as an aquaculture veterinarian and works extensively with both traditional serial reuse and with recirculating fish production systems. Her previous research experience includes investigation of the contribution of fish density and pathogen concentration to outbreaks of a trout viral disease (IPN), the fate of oxytetracycline antibiotic in a recirculating system, early rearing of Arctic char and viral diseases affecting Arctic char. She publishes and lectures about biosecurity in recirculating and serial reuse systems. Current research and extension projects include work on the epidemiology of bacterial gill disease on rainbow trout farms, fish health certification of

salmonids reared on private and state facilities in WV and investigation of a chlamydia-like/rickettsia-like bacteria that infects gill of Arctic char.

Laura L. Brown

National Research Council of Canada, Institute for Marine Biosciences
1411 Oxford Street, Halifax, Nova Scotia, B3H 3Z1, Canada

Dr. Laura Brown pursued her post-secondary studies at McGill University (B.Sc. 1982), Simon Fraser University (M.Sc. 1990) and University of British Columbia (Ph.D. 1995). She was an NSERC Post-Doctoral Fellow 1995 – 1996 under the supervision of Dr. Paul Levine at the Hopkins Marine Station, Stanford University in California. Dr. Brown joined the National Research Council, Canada in 1996 at the Institute for Marine Biosciences (IMB). In 2002 she was made a Senior Research Officer, and in 1999 Dr. Brown assumed the responsibilities of Group Leader for the Genome Sciences Group at IMB, and in 2003, was made Acting Director of the Bioinformatics and Strategic IT division of IMB.

Dr. Brown is internationally recognized for her work on infectious diseases of salmonids. Her current research includes biochemical, immunological and molecular biological studies of host/pathogen relationships in fish. Dr. Brown has published numerous papers in peer-reviewed journals, has co-authored a book chapter, and has been an invited speaker at several international conferences. Dr. Brown is an Associate Editor for the international journals, *Diseases of Aquatic Organisms* and *Journal of Aquatic Animal Health*, and she is also Chair of the Canadian Natural Sciences and Engineering Research Council's (NSERC) Cell and Molecular Biology Scholarships and Fellowships Selection Committee. Dr. Brown is an Adjunct Professor at Dalhousie University and is also a Principal Investigator within the Network Center of Excellence, "AquaNet". Dr. Brown is the Project Leader for a multi-disciplinary, multi-institute project underway on the molecular mechanisms of interactions between Atlantic salmon *Salmo salar*, and the pathogen *Aeromonas salmonicida*. This project involves over 40 NRC personnel and collaborators from academia and industry and is worth ca. \$1M per year.

Technical Standards Committee

Marcia House

I am currently a Fish Pathologist for the Northwest Indian Fisheries Commission in Olympia, Washington. I provide diagnostic and fish health management services for the western Washington tribal fish enhancement and restoration programs. Prior to taking this position, I was involved in research investigating several aspects of fish and shellfish health. I completed my B.S. degree in Biology at Cornell University in 1988, and earned my Ph.D. in Microbiology at Oregon State University in 1997. My dissertation research examined disseminated neoplasia in the soft shell clam. I worked for the next 3 years at the Western Fisheries Research Center in Seattle on projects investigating *Piscirickettsia salmonis* and management strategies for IHNV. I then returned to Newport, Oregon, to work as part of a team on a NMFS project researching the ecology of juvenile salmon in the ocean, specifically, using PCR to screen field samples for the presence of *Renibacterium salmoninarum*. In utilizing the available techniques for detection of a variety of pathogens in different types of samples, I have gained an awareness of the strengths and limitations of these techniques and an appreciation for how they can be most efficiently applied.

Keith A. Johnson

Fish Pathologist Supervisor, Idaho Department of Fish and Game

B.S. in Fisheries Management (U Idaho, '66)

M.S. in Zoology (Montana State, '68)

Ph.D. in Pathogenic Microbiology (Oregon State, '75)

I have split my career between culture of salmonids, mostly four species of Pacific salmon, in Washington State, Alaska, and most recently with Idaho ESA-listed sockeye and chinook captive broodstock programs; and with a second emphasis in vaccine research and development (Tavolek, Inc. and Connaught Labs, Ltd.) and fish pathology.

I supervise the Eagle Fish Health Laboratory of the Idaho Department of Fish and Game which provides diagnostic and inspection functions for eleven hatcheries which rear resident species and twelve anadromous species facilities. My staff consists of two fish pathologists, three fish health technologists, and clerical assistance. Additional opportunities at Eagle include a wet lab to support whirling disease research, wild fish examinations, and limited inspections of live fish transports into Canada. I envision our future direction will include more effort into cool and warm water species. Our lab also administers the transport and import permitting process for the Department.

I am a Fish Health Official (Canada), been an AFS annual member forever, and a member of the FHS since 1974. I have served on the FHS Nominations Committee and am the Department's technical representative to the PNFHPC.

My FHS Technical Standards Committee vision:

The FHS Blue Book is a series of documents that must remain technically current, professionally credible, and validated. There remains additional work to have it remain an international standard by which other similar documents are compared. The challenge in this continued effort will be to attain collaboration, compromise and motivation. It is necessary to remain mindful that laboratories performing diagnostic and inspection functions have to remain cost-effective while providing a valuable resource function.

**FISH HEALTH SECTION
Election 2003
Ballot of Candidates**

Please signify your choice with an **X**

Vice-President

John Hawke _____

Other _____

Nominating and Balloting Committee (vote for one)

Julie Bebak-Williams _____

Laura Brown _____

Technical Standards Committee (vote for one)

Marcia House _____

Keith Johnson _____

Professional Standards Committee (vote for one)

Andy Goodwin _____

Bruce Stewart _____

THANK YOU for your participation.

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 31, 2003. For a list of previous awardees, go to the FHS website at: <http://www.fisheries.org/fhs/snieszko.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.

S.F. Snieszko Student Travel Award - award to provide funding for a student to attend and to present a research paper at the annual national FHS meeting. Student should send the abstract of the paper to be presented, a travel budget, and a letter of support from the sponsoring faculty member.

FHS Student Paper Award - an award will be presented to a student whose paper is being presented at the International Meeting held in New Orleans. 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 your paper judged.

Send nominations for all awards by May 31 to:
Dr. Vicki Blazer, FHS Awards Committee,
National Fish Health Research Laboratory
11700 Leetown Road
Kearneysville, WV 25430
E-mail: vicki_blazer@usgs.gov

FHS PARTICIPATION IN THE AAVLD / USAHA ANNUAL MEETINGS

Scott LaPatra

As you know, the Fish Health Section (FHS) has committed to becoming more involved on issues of importance to the membership. For the last six years I attended and participated in the United States Animal Health Association (USAHA) and the American Association of Veterinary Laboratory Diagnosticians (AAVLD) annual meeting. Last year the meeting was held in Hershey, Pennsylvania and this year it was held in St. Louis, Missouri. For 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 objectives, interests and goals regarding animal health are very similar to the USAHA. One of the reasons we were in attendance was to offer our expertise and established programs in aquatic animal health and maintain visibility with other groups also interested in aquatic animal medicine. This year the AAVLD and the USAHA Aquaculture Committees met jointly and were chaired by Dr. Randy White representing the AAVLD and myself representing USAHA. As in past years, I updated the committee about the Sections activities including the changes that were made in the certification categories in order to acknowledge the general training that veterinarians receive and encourage their participation. I also spoke about the collaborative project with the USFWS to develop a “Standard Procedures for Aquatic Animal Health Inspections” manual that was recently completed.

In the past the committee has been very successful at passing resolutions which are then forwarded to the Executive Committee of the USAHA. Three resolutions were supported by the Committee in 2001 and forwarded to USDA/APHIS for comment and included:

Resolution 02: Significance of Aquatic Animal Pathogens in Aquaculture Effluents

Resolution 03: Development of a National Aquatic Animal Health Plan

Resolution 04: Control Strategies for Infectious Salmon Anemia in the Northeastern U.S.

This year, in addition to normal committee business two resolutions were introduced including:

- 1) **Resolution Subject:** Standard Processes for the Approval of Diagnostic and Pathogen Identification Tests and Methods, Diagnostic Reagents, and Diagnostic Laboratories

Background Information

The USAHA recognizes the leadership and authority mandated to USDA-APHIS by the passage of the Animal Health Protection Act of 2002 and the Virus-Serum-Toxin Act of 1913, as amended. Accurate and timely disease diagnostics and pathogen identification are pivotal

cornerstones for all responses to animal diseases, including prevention, control, and/or eradication of endemic, emerging, and foreign animal diseases. A uniform approval process for disease diagnostic and pathogen identification tests, reagents and reference materials would substantially contribute to the health and welfare of aquatic animals, and enhanced public health, food safety and environmental health. In order to advance these principles it is vital that approaches to, and processes for, the approval of tests, test methods, reagents and reference materials, and diagnostic laboratories are harmonized at the international, national, state, and local levels.

While this resolution focuses on aquatic animal diseases, the USAHA/AAVLD Aquaculture Committee recognizes the application of these principles to all animal disease diagnostics, in particular to diseases in all wildlife.

Resolution:

The USAHA encourages USDA-APHIS to work with other agencies, organizations and entities to develop a uniform process concerning aquatic animal disease diagnostics and pathogen identification, including: 1) Validation and approval of diagnostic and identification tests, and test methods; 2) Approval of standardized diagnostic reagents and reference materials; and, 3) Quality assurance, quality control, and approval of aquatic animal diagnostic laboratories.

2) Resolution Subject: Standard Procedures for Aquatic Animal Health Inspections

Background Information

In June of 2001, members of the Fish Health Section (FHS) of the American Fisheries Society and the United States Fish and Wildlife Service (USFWS) met to discuss the possibility of jointly producing an update of the portion of the FHS Bluebook that covers procedures for aquatic animal health inspections. The USFWS had already put in considerable effort to produce a comprehensive Fish Health Handbook that would address standard procedures for providing consistent, standardized information to natural resource managers. Both groups realized the need to update the current Bluebook, as new information and technologies have emerged since its revision in 1985, and it no longer met the needs of persons in a regulatory capacity. It was also felt that it was a duplication of efforts to produce similar manuals, and this would result in confusion on the part of inspectors. Therefore, the committee decided to start with the draft USFWS document and revise it to meet the needs of all parties.

The group assembled three committees made up of both FHS members and USFWS employees, that included DVM and non-DVM aquatic animal health professionals, to review and revise the bacteriology, virology and parasitology sections. Criteria that were used in the selection of appropriate assays included, 1) the sensitivity of the assay, 2) the specificity of the assay, 3) the cost of the assay, 4) availability of reagents, 5) availability of technology, 6) manpower requirements, and 7) scientific defensibility (they must be referenced). Additional sections were added to detail sampling methods, PCR, and methods for revision of this document. The committees recognize that there will be changes and the process put in place for an annual review so that new technologies and clarifications on existing methods can be incorporated on a more timely basis.

The documents were sent out for initial review to a select group of fish health inspectors and their comments were reviewed by the committees and a number of revisions made. The document was subsequently revised and posted on the FHS website for a minimum of 30 days for general review by aquatic animal health professionals. The inspection manual was presented and accepted by the FHS and USFWS on September 4, 2002 at the 4th International Symposium of Aquatic Animal Health that was held in New Orleans, Louisiana. The title of the manual is **Standard Procedures for Aquatic Animal Health Inspections**.

These inspection techniques represent a minimal acceptable standard. The techniques are inspection techniques, used to detect the presence of certain selected fish pathogens and they are NOT diagnostic techniques. The **Standard Procedures for Aquatic Animal Health Inspections** is a protocols manual and not a policy manual. State and federal governments will stipulate which pathogens should be inspected for, what aquatic animal species are to be examined, and may well wish to define their own conditions for sample sizes and inspection frequency. This manual is meant only to provide appropriate methods for fish inspection, not to specify when and where and to which animals they should be applied.

Resolution:

The USAHA endorses the **Standard Procedures for Aquatic Animal Health Inspections** manual and encourages APHIS to adopt this manual as part of the Aquatic Animal Health Task Force on Aquaculture.

The first resolution was approved by the committee and forwarded to the USAHA Executive Committee which subsequently approved it. This resolution has been forwarded to USDA/APHIS for comment. The second resolution was tabled because the committee members wanted to make an informed decision, and most had never seen the document. This resolution will be revisited at the next annual AAVLD/USAHA Meeting which is scheduled for October, 2003 in San Diego. If you have any ideas, questions or need for additional information please don't hesitate to contact myself or any of the other members of the FHS Executive Committee.

NOTES FROM "THE DRUG LADY"

This column is the first one written for the Fish Health Section (FHS) Newsletter. I plan to provide as much information as possible in the coming FHS Newsletters regarding the current status of drug approvals for U.S. aquaculture. If you have any questions in particular that you would like answered, please contact me at RozSchnick@centurytel.net.

NEW SUPPLEMENTAL NADA FOR FORMALIN

The Center for Veterinary Medicine (CVM) approved a supplemental New Animal Drug Application (NADA # 137-687) on November 25, 2002 for Natchez Animal Supply Company (Natchez, Mississippi) for its formalin product, Formalin-F®, to control certain fungi on the eggs of **all finfish**, certain external protozoa and monogenetic trematodes on **all finfish**, and external

protozoan parasites on **penaeid shrimp**. CVM announced in the *Federal Register* on February 4, 2003 that the agency was amending its regulations to reflect this change in the original NADA from Natchez Animal Supply Company. The aquaculture industry now has two formalin products that can be used on all finfish and penaeid shrimp. The other formalin product is Parasite-S® (NADA #140-989) from Western Chemical Inc. (Ferndale, Washington).

GUIDANCE ON CLOVE OIL AND EUGENOL AS ANESTHETICS IN AQUACULTURE

CVM recently issued a guidance document (Guidance for Industry #150) providing information on the status of clove oil and eugenol for use as anesthetics in U.S. aquaculture. Both clove oil or eugenol are unapproved animal drugs that may not be used in any form on fish that could possibly be consumed by humans, even if the treatment occurs in a laboratory setting. This includes endangered species, or species that otherwise may be released into public waters where they would be available for human consumption. The only exception would be under the auspices of an Investigational New Animal Drug (INAD) exemption in which a treatment authorization, including an appropriate investigational withdrawal time, has been obtained from FDA (<http://www.fda.gov/cvm/index/updates/gl150.htm>). As of this time, no known INAD exists for these unapproved drugs.

Isoeugenol is a possible substitute for clove oil or eugenol. At this time, there is one disclosable INAD exemption for the use of isoeugenol as a fish anesthetic and the US Fish and Wildlife Service holds that INAD. The product called AQUI-S® has been given an investigational withdrawal time of 21 days for the dosing regimen being used in the current studies. If you are conducting studies that could be cited to help demonstrate the safety or effectiveness of isoeugenol in fish, you may contact Dr. Dave Erdahl to discuss working under this INAD. He can be reached by email at dave_erdahl@fws.gov.

The only other options for anesthetizing fish are (1) approved tricaine methanesulfonate products (Finquel® and Tricaine-S®) with a 21-day withdrawal time, (2) carbon dioxide and sodium bicarbonate as Low Regulatory Priority drugs with no withdrawal time. Information on these anesthetics can be found on CVM's website <http://www.fda.gov/cvm/index/aquaculture/aqualibtoc.htm>.

COMPLETION OF THE FEDERAL-STATE AQUACULTURE DRUG APPROVAL PARTNERSHIP PROJECT

After eight years of effort, tremendous progress has been made and 13 new label claims for six drugs are nearing completion under the International Association of Fish and Wildlife Agencies' Federal-State Aquaculture Drug Approval Partnership Project (Project). The Project is now completed in its present form. NADA submissions to CVM to support original or supplemental approvals for Project drugs are the result of efforts by the U.S. Geological Survey's Upper Midwest Environmental Sciences Center (UMESC), U.S. Department of Agriculture's Harry K. Dupree Stuttgart National Aquaculture Research Center (SNARC), U.S. Fish and Wildlife Service's (FWS) Bozeman National Investigational New Animal Drug (INAD) Office (NIO), other public agencies such as state natural resources agencies, the private aquaculture sector,

company sponsors, and the National Coordinator for Aquaculture New Animal Drug Applications (National NADA Coordinator).

Building on this important foundation, work will continue on additional label claims as well as new drugs, using appropriated funds for UMESC, SNARC, NIO, and others, including the pharmaceutical and chemical company sponsors. Multi-State Conservation Grant funds have also supported aspects of this work, and may provide more future funding. UMESC will continue involvement in drug approvals as long as the states request it. The National NADA Coordinator will continue to coordinate all aquaculture drug approvals. A larger expanded future effort is also being planned for the FWS Division of Fisheries and Habitat Conservation. Drug sponsors are starting to step forward to play a more active role in the drug approval process.

Label Claim Approvals

1. Formalin—control of fungi on the eggs of all finfish
2. Formalin—control of external protozoa and monogenetic trematodes on all finfish

Label Claims Nearing Successful Completion

1. Chloramine- T--mortality from bacterial gill disease on all freshwater-reared salmonids
2. Chloramine- T--mortality from external columnaris disease on all freshwater-reared coolwater finfish
3. Copper sulfate--*Ichthyophthirius* on channel catfish in earthen ponds
4. Florfenicol--mortality from furunculosis in salmonids
5. Florfenicol--mortality from enteric septicemia in catfish
6. Formalin--mortality from saprolegniasis on all finfish
7. Hydrogen peroxide--mortality from saprolegniasis on all freshwater-reared finfish eggs
8. Hydrogen peroxide--mortality from saprolegniasis on all freshwater-reared finfish
9. Hydrogen peroxide--mortality from bacterial gill disease on all freshwater-reared salmonids
10. Hydrogen peroxide--mortality from external columnaris disease in all freshwater-reared coolwater finfish
11. Oxytetracycline--mortality from systemic columnaris disease in all freshwater-reared salmonids
12. Oxytetracycline--mortality from systemic coldwater disease in all freshwater-reared salmonids, and
13. Oxytetracycline--otolith marking of all finfish by immersion

As developments on these label claim occur, I will provide updates on these and other label claims for these six drugs plus AQUI-S® and potassium permanganate that are also part of this Project.

Rosalie (Roz) Schnick
National Coordinator for Aquaculture New Animal Drug Applications
Michigan State University
Website: <http://ag.ansc.purdue.edu/aquanic/jsa/aquadrugs/index.htm>

Current Highlights of VIMS Mycobacteriosis Research

<http://www.vims.edu/myco/index.html>

A new VIMS report shows that a molecular technique for detecting bacterial disease in striped bass is faster than current laboratory methods. Use of the new technique will allow researchers and managers to better monitor the prevalence and geographic range of mycobacteriosis in Chesapeake Bay stripers. VIMS scientists have discovered that a new species of bacteria is largely responsible for a disease outbreak among Chesapeake Bay striped bass. The new species, which they named *Mycobacterium shottsii*, is closely related to *M. marinum*, a species known to infect both fish and humans.

Read the VMRC Final Report... (39 pages; 1.2 MB pdf)
<http://www.vims.edu/myco/pdfs/VMRC.pdf>

Mycobacteriosis in Striped Bass of the Chesapeake Bay:
Expansion of Studies Emphasizing Cultural and Rapid Molecular
Diagnostic Methods to Evaluate Disease Prevalence

EXECUTIVE SUMMARY

Researchers in Virginia and Maryland have recently documented an epizootic of mycobacteriosis in striped bass, *Morone saxatilis*, from the Chesapeake Bay. Utilizing histological techniques, prior research at the Virginia Institute of Marine Science (VIMS) confirmed the presence of acid-fast bacilli within granulomas, implicating mycobacterial infections in skin and internal tissues of a large number of wild striped bass (Vogelbein et al., 2001; Cardinal, 2001). Histological studies relied upon fixation of fish tissues in a preservative, followed by specialized stains which allowed for direct detection of the mycobacteria or the typical lesions associated with mycobacteria, called granulomas. In addition, microbiological studies consisting of isolation and phenotypic characterization of the *Mycobacterium spp.* were undertaken for a subsample of these fish (Rhodes et al., 2000). Microbiological methods initially employed traditional processing techniques, including tissue decontamination to eliminate any rapidly growing "contaminant" bacteria. Selective media and incubation at temperatures of 30°C or higher were used. Such routinely used methods, however, were subsequently determined to have a detrimental effect on recovery of mycobacteria from striped bass. For example, one of the common disinfectants for decontamination, ZephiranT, not only destroyed nonmycobacteria, but killed over 99% of the mycobacteria in pure suspensions. In addition, the predominant mycobacterial isolate from wild striped bass, *M. shottsii*, (Rhodes et al., 2001a; b; c; 2002) seldom would grow at 30°C, so incubation at a lower temperature, 23°C, was essential. Even at their optimal low incubation temperature, many *Mycobacterium spp.* grow very slowly, requiring weeks to months for initial colony detection. Molecular techniques began to be utilized in an effort to quickly detect mycobacteria in fish (Kaattari et al., 1999; 2000; 2001). Amongst the molecular techniques available, polymerase chain reactions (PCR) and a further amplification technique called nested PCR were utilized. The PCR and nested PCR techniques use oligonucleotide primers that detect and amplify a portion of the *Mycobacterium* genus-specific gene, the 16S rRNA gene. This gene is highly conserved amongst all *Mycobacterium spp.* and thus represented a reliable type of DNA to serve as an indication of mycobacterial infections in striped bass tissues.

Surveys of wild striped bass from the Potomac, Rappahannock, and York Rivers (N = 1899) were initially conducted during spring, 1998 through fall, 1999 (Vogelbein et al., 2001; Cardinal, 2001). Histological examination of skin and spleen samples from these fish was analyzed. Prevalence of splenic infection by mycobacteria appeared to be much higher than for dermal (skin) infections. Splenic infections ranged from 31.5% in the Rappahannock River in summer, 1999, to 62.7% in the York River in fall, 1999. There seemed to be no significant variance in prevalence spatially (from site to site).

Scientists blame fish deformities on fertilizers: substances that remove oxygen from water seem to reduce marine creatures' ability to reproduce

March 15, 2003

The Ottawa Citizen

A14

Tom Spears

Scientists were cited as saying in this story that manure and fertilizers that strip away the oxygen in lakes and rivers could deform the fishes' sex organs and cripple their ability to reproduce. And, one of the researchers was cited as saying, as the global amount of oxygen-poor water increases, damage to many fish species will spread. The new discovery adds hypoxia -- low oxygen levels in water -- to the list of "gender-bender" chemicals that create fish with low testosterone levels and tiny sex organs. Until now, most known gender-benders have been synthetic chemicals such as DDT, which can mimic the action of the female hormone estrogen. Many such pollutants are widely banned. This time scientists are pointing to much more old-fashioned pollution from chemicals in manure, sewage and many fertilizers.

European Commission Health & Consumer Protection Directorate- General Scientific Steering Committee opinion on:

The Feeding of wild fishmeal to farmed fish and recycling of fish with regard to the risk of TSE

Adopted by the Scientific Steering Committee at its meeting of 6-7 March 2003

OPINION

MANDATE:

Mammalian MBM and other mammalian products have historically been fed to farmed fish. Furthermore, intra-species and intra-order recycling via feed is common practice in fish farming. It is therefore important to address the question whether the latter practice could enable mammalian TSE agents to establish themselves in fish and for species adaptation of such agents to occur. This could lead to the development of a TSE in fish that might lead to a TSE epidemic in fish and/or create a health risk for the consumer. The outcome of the assessment would improve the scientific basis for the possible updating of the animal waste disposal legislation and other legislative texts in the field of veterinary public health. The Scientific Steering Committee (SSC) was therefore invited:

- (1) to advise whether the feeding of wild fishmeal to farmed fish presents any risk to animal or human health vis-à-vis TSE's;
- (2) if appropriate, to suggest examples of conditions under which intra-species or intra-order recycling of fish could be allowed.

The SSC asked the TSE/BSE ad hoc Group to prepare a scientific report to serve as basis for an opinion on the two questions. The report, finalized by the TSE/BSE ad hoc Group at its meeting of 5 September 2002, is largely based on various SSC opinions and reports of the TSE/BSE ad hoc group related to animal waste disposal and intra-species recycling, on elements from the (draft) report of the Scientific Committee on Animal Health and Welfare on "The use of fish waste in aquaculture" and on the interim results of the FAIR CT97 3308 project entitled "Separation, identification and characterization of the normal and abnormal isoforms of prion protein from normal and experimentally infected fish".

This opinion provides the possible scientific reasons for a general feed ban of meat-and-bone meal, applicable to all farmed animals including cattle, pigs, poultry, farmed fish and pet food.

Bactericidal Antibiotic Effective Against Methicillin-Resistant *S. aureus*

MedScape, March 11, 2003

By Will Boggs, MD

NEW YORK (Reuters Health) Mar 03 - MC21-A, an antibiotic produced by a newly identified marine bacterium, is effective against methicillin-resistant *Staphylococcus aureus* (MRSA) in vitro, according to a report in the February issue of *Antimicrobial Agents and Chemotherapy*.

Increasing evidence of MRSA resistance to vancomycin and teicoplanin, the antibiotics of last resort for these infections, drives the search for alternative antibiotics after these have failed, the authors explain. Dr. Yuto Kamei and Dr. Alim Isnansetyo from Saga University in Saga, Japan identified MC21-A through a screening program for anti-MRSA substance-producing marine bacteria. The substance was produced by *Pseudoalteromonas phenolica* sp. nov. O-BC30(T), a newly identified marine bacterium.

The antibacterial activity of MC21-A was similar to that of vancomycin against methicillin-sensitive *S. aureus* (MSSA), MRSA, *Escherichia serolicida*, *E. faecalis*, *E. faecium*, and *Bacillus subtilis*, the authors report, but was less active against *Streptococcus pneumoniae*, *S. pyogenes*, and *S. mutans*. MC21-A effectively killed a clinical isolate and a reference strain of MRSA after 12 hours of exposure at 4 micrograms/ml and after 8 hours of exposure at 8 micrograms/mL, the report indicates, and the killing rates of MC21-A for MRSA were much higher than those of vancomycin. The postantibiotic effect of MC21-A was significantly longer at the minimum inhibitory concentration (MIC) than was that of vancomycin, the researchers note, though they were similar at twice and four times their respective MIC.

MC21-A rapidly permeated cell membranes of MRSA, the results indicate, but did not lyse MRSA cells. In toxicity studies, MC21-A did not lyse human erythrocytes and was not

cytotoxic to human fibroblasts, rat pheochromocytoma cells, or monkey kidney cells, though the substance showed moderate cytotoxic activity against human leukemic cells and significant cytotoxic activity against canine kidney cells.

This compound will be useful in treating drug-resistant *S. aureus* nosocomial infections, especially the most serious MRSA, Dr. Kamei told Reuters Health. "This compound is dramatically more effective than other anti-MRSA compounds reported so far." "We know of two other anti-MRSA compounds that are produced by *Pseudoalteromonas phenolica*," Dr. Kamei added. His laboratory is in the process of characterizing these other new compounds.

Antimicrob Agents Chemother 2003;47:480-488.

RARITAN BAY SHELLFISH TRANSPLANT PROGRAM CANCELED FOR 2003

DEC Takes Action to Protect Resource After Discovery of Hard Clam Disease

The New York State Department of Environmental Conservation (DEC) today announced a decision to cancel the Raritan Bay Shellfish Transplant Program for 2003 due to the diagnostic finding of the hard clam parasitic disease Quahog Parasite Unknown (QPX) in wild hard clam populations in Raritan Bay off Staten Island, New York.

DEC has administered a hard clam transplant program in the uncertified state waters of Raritan Bay since 1987. The program involves the harvest of hard clams from uncertified (polluted) waters and their relay to certified (clean) waters for bacterial cleansing and eventual marketing as a food product. Shellfish transplanting is normally undertaken on a seasonal basis from April through October, when water temperatures are high enough to allow the shellfish to remain active and to adequately pump and cleanse themselves during a minimum 21-day period.

"The discovery of QPX represents a serious threat to this commercially important hard clam fishery," DEC Commissioner Erin M. Crotty said. "DEC's extensive monitoring and research shows that any movement of clams from Raritan Bay would pose an unacceptable risk of transmission to wild clams in Peconic Bay and other receiving waters of the state. We are very concerned about the long term impacts of the QPX disease on the hard clam resources of Raritan Bay and our shellfish industry. Because there is still much unknown about transmission of the disease, DEC will continue to monitor clam beds in Raritan Bay, Peconic Bays and other areas of the state in order to reduce the risk of transfer of QPX to non-infected clam beds."

QPX is a protozoan parasite that infects the soft tissue of hard clams causing an inflammation in the tissue which prevents the clams from closing their shell and pumping food and water to the gills. The disease is normally observed in the mantle and gills of the clam. The hard clam disease was first reported in wild clams from New Brunswick, Canada during the 1960's and hatchery reared clams from Prince Edward Island during the 1980's and has been documented in New Jersey, Massachusetts and Virginia. QPX is not harmful to humans and does not represent

a public health concern but is fatal to hard clams, causing significant mortalities in an infected clam bed. There is no known treatment or cure for QPX in hard clams.

DEC heard reports from shellfish harvesters of dead and dying clams in the transplant harvest area in Raritan Bay last summer. Based on these reports, DEC collected a sample of 30 clams from Raritan Bay in August and delivered them to the State of Connecticut Pathology Laboratory for analysis. In September, DEC was notified that the hard clams showed heavy infestation of the shellfish disease QPX. This preliminary QPX diagnosis was confirmed by the Marine Biological Laboratory at Woods Hole, Massachusetts in late September. This was the first time QPX has been found in hard clams in New York waters. Following consultation with experts on QPX, DEC immediately terminated the Raritan Bay transplant program on September 6 in order to prevent the potential introduction of the parasite to the cleansing sites and waters of Peconic Bays.

In September, DEC began conducting QPX monitoring of hard clams at three relay cleansing sites in Peconic Bays and an adjacent wild clam population in North Sea Harbor, Southampton, in order to determine if there was any transmission of the QPX parasite to Peconic Bays. Ten hard clam samples (30 clams per sample) were collected and shipped to the Virginia Institute of Marine Science (VIMS) for analysis. The pathology report completed by VIMS indicated low prevalence of QPX positive infections in 4 out of 10 samples which may represent background levels of the parasite rather than an emerging QPX epizootic.

In order to determine the extent and distribution of QPX in the transplant harvest area in Raritan Bay, DEC completed an extensive monitoring program in late fall 2002. The QPX sampling involved the collection of more than 600 clams from 20 designated sampling sites in Great Kills Harbor and Raritan Bay.

The pathology report documented the finding of QPX positive infections at 16 out of 20 sampling sites examined. Although there were no QPX lesions found in clams from four of the sites sampled, the sites are located adjacent to positive QPX sites and cannot be considered for transplant purposes. More than half of the QPX positive clams had multifocal and severe visceral infections which are considered a more intense form of QPX than those with mantle-only infections. This analysis represents the first documentation of a widespread epizootic of QPX in subtidal populations of wild clams in New York.

DEC is working with pathologists at the Marine Animal Diagnostic Laboratory at SUNY Stony Brook, Cornell University and other experts to develop strategies and monitoring programs for determining prevalence of QPX in Raritan Bay and other areas of the state and mitigation measures that can be undertaken to reduce the intensity of the QPX disease in infected clam beds.

AN UPDATE ON THE ANTIGENIC DIVERSITY IN *Tenacibaculum maritimum* STRAINS ISOLATED FROM MARINE FISHES

Submitted by

RUBÉN AVENDAÑO, BEATRIZ MAGARIÑOS, JESÚS L. ROMALDE, ALICIA E. TORANZO

Departamento de Microbiología y Parasitología. Facultad de Biología. Universidad de Santiago de Compostela. 15782, Santiago de Compostela, Spain.

Marine flexibacteriosis caused by *Tenacibaculum maritimum* (formerly *Flexibacter maritimus*) is a disease with potential to cause severe mortalities in different cultured fish. Initially, *T. maritimum* was isolated from red sea bream (*Pagrus major*) and black sea bream (*Acanthopagrus schlageli*) cultured in Japan [11]. The presence of this fish pathogen in Europe was first demonstrated in Scotland from a Dover sole (*Solea solea*) suffering from “black patch necrosis” [3]. Since 1990 this microorganism has also been isolated from farmed turbot (*Scophthalmus maximus*), sea bass (*Dicentrarchus labrax*), gilthead sea bream (*Sparus aurata*) and salmon (*Salmo salar*) [1, 2, 8]. Recently, commercial production of sole (*Solea senegalensis*) in the Spanish coasts has been impaired by mortalities due to flexibacteriosis.

Because the economic importance of the disease, the aquaculture industry is increasingly relying on vaccines for its control. The development of these vaccine formulations implicates the precise biochemical, serological and genetical knowledge of the pathogen. *T. maritimum* isolates constitute a biochemically homogeneous group sharing common traits with other phenotypically similar yellow-pigmented bacteria of the genera *Flavobacterium* and *Cytophaga* [1]. However, with regard to the serological characteristics of the strains, different results have been reported. Whereas Ostland et al. [5] and Pazos et al. [6] mentioned the existence of differences in the cell wall composition, which implies a different antigenic composition, Wakabayashi et al. [11] using the same techniques describes *T. maritimum* as a serological homogeneous group. Therefore, in the present report the antigenic diversity of a wide group of *T. maritimum* strains isolated from different strictly marine fish species such as sole (11 isolates), turbot (11 isolates), gilthead sea bream (3 isolates), and sea bass (1 isolate) was studied. The reference strain NCIMB 2153 from black sea bream was also included.

The antigenic analysis was performed by slide agglutination test [10] as well as by dot blot assay [4]. The tests were carried out using “O” antigens of each strain obtained after heating the bacterial suspensions in phosphate buffered saline (pH 7) at 100°C for 1 h and washing once in the same saline solution.

The strains PC 503.1 and PC 424.1 isolated from sole and turbot respectively, were selected and used to obtain immune sera in rabbits according to the methods described by Sørensen & Larsen [9]. With the objective to determine the possible antigenic variability, reciprocal absorption of antisera was performed [7].

The slide agglutination assays using “O” antigens revealed cross-reaction for all the strains, regardless of their host origin and serum employed. However, when the dot blot assays were

performed the existence of antigenic heterogeneity was demonstrated (Table 1). In fact, three patterns of serological reactions were evidenced: the *T. maritimum* strains isolated from sea bream and sea bass reacted only with the antiserum obtained against the sole isolate (PC 503.1), the turbot isolates only showed reactivity with the serum against the turbot strain (PC 424.1), and finally, the sole isolates that although showed a strong reaction with the antiserum against the sole strain, also displayed cross-reactions with the serum against the turbot strain. However, these cross-reactions were eliminated when the turbot strain antiserum was absorbed with a heterologous sole strain (Table 1).

All these results allowed to propose the existence of at least three serological groups in *T. maritimum* from strictly marine fishes which could be associated with the host origin: the first group compiles the strains isolated from sole, group 2 the isolates from sea bream and sea bass and the group 3 corresponds to the turbot isolates. The reference strain displayed a similar pattern to the group 3.

In conclusion, the antigenic variability found here must be taken into account when developing effective fish vaccination programs against marine flexibacteriosis caused by this microorganism. Two approaches can be considered to formulate the vaccine composition: i) to design a polyvalent vaccine including all the serological types detected until now which can be useful for a wide range of marine fish species, or ii) different monovalent bacterins specific for each serological group to be used in the appropriate fish cultures.

Table 1. Reactivity showed by the *T. maritimum* strains in the serological assays using O antigens.

Isolates	Slide agglutination with serum		Dot blot assay with serum		
	Anti-PC 503.1	Anti-PC 424.1	Anti-PC 503.1	Anti-PC 424.1	Absorbed ^a
Sole isolates (11 strains)	++	++	++	+	–
Turbot isolates (11 strains)	++	++	–	++	++
Gilthead seabream isolates (3 strains)	++	++	++	–	–
Sea bass isolates (1 strain)	++	++	++	–	–
NCIMB 2153	++	++	–	++	++

^a Serum anti-PC 424.1 absorbed with the heterologous sole strain PC 503.1.

1. **Bader, J. A., Starliper, C. E.** 2002. The Genera *Flavobacterium* and *Flexibacter*. In: Cunningham, C. O. (Ed). *Molecular Diagnosis of Salmonid Diseases*. Kluwer Academic Publ. The Netherlands, pp: 99-139.
2. **Bernardet, J. F.** 1997. Immunization with bacterial antigens: *Flavobacterium* and *Flexibacter* infections. In: Gudding, R., Lillehaug, A., Midtlyng, P. J., Brown, F. (Eds.) *Fish Vaccinology*. Dev. Biol. Standard. basel, Karger, pp: 179-188.
3. **Campbell, A. C. Buswell, J. A.** 1982. An investigation into the bacterial etiology of ‘black patch necrosis’ in Dover sole, *Solea solea* L. *J. Fish Dis.* 5, 495-508.
4. **Cipriano, R. C., Pyle, J. B., Starliper, C. E., Pyle, S. W.** 1985. Detection of *Vibrio anguillarum* antigen by the dot blot assay. *J. Wildlife Dis.* 21, 211-218.
5. **Ostland, V. E., LaTrace, C., Morrison, D., Ferguson, H. W.** 1999. *Flexibacter maritimus* associated with a bacterial stomatitis in atlantic salmon smolts reared in net-pens in British Columbia. *J. Aquat. An. Health.* 11, 35-44.
6. **Pazos, F., Santos, Y., Núñez, S. Toranzo, A. E.** 1993. Increasing occurrence of *Flexibacter maritimus* in the Marine Aquaculture of Spain. *AFS/FHS Newslett.* 21, 1-2.
7. **Romalde, J. L., Magariños, B., Barja, J. L., Toranzo, A.E.** 1993. Antigenic and Molecular characterization of *Yersinia ruckeri*. Proposal for a new intraspecies classification. *System. Appl. Microbiol.* 16, 411-419.
8. **Santos, Y., Pazos, F. Barja, J. L.** 1999. *Flexibacter maritimus*, causal agent of flexibacteriosis in marine fish. In: Ollivier G. (Ed.) *Ices Identification Leaflets for Diseases and Parasites of Fish and Shellfish*. No. 55. International Council for the Exploration of the Sea. (ICES) Denmark.
9. **Sørensen, U.B.S. Larsen, J.L.** 1986. Serotyping of *Vibrio anguillarum*. *Appl. Environ. Microbiol.* 51, 593-597.
10. **Toranzo, A.E., Baya, A.M., Roberson, B.S., Barja, J.L., Grimes, D.J. Hetrick, F.M.** 1987. Specificity of the slide agglutination test for detecting bacterial fish pathogens. *Aquaculture* 61, 81-97.
11. **Wakabayashi, H., Hikida, M. Masumura, K.** 1984. *Flexibacter* infection in cultured marine fish in Japan. *Helogolaender Meeresuntersuchungen* 37, 587-593.

Fish Health Newsletter – Editorial Policy

The *Fish Health Newsletter* is a quarterly electronic publication of the Fish Health Section of the American Fisheries Society and is available for downloading in Adobe pdf file format. Submissions on any topic of interest to fish health specialists and preliminary case reports are encouraged with the understanding the material is not peer- reviewed. Abstracts submitted to the *Journal of Aquatic Animal Health* are also encouraged. Submissions must be formatted in Microsoft Word, WordPerfect, or Rich Text Format, 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.**

Editor

Lora Petrie-Hanson (lora@cvm.msstate.edu)
 College of Veterinary Medicine
 P.O. Box 6100
 Mississippi State University, MS 39762

