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Isolation of North American Viral Hemorrhagic Septicemia Virus (VHSV) from Alaskan Pacific Herring, *Clupea harengus pallasii*.

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The North American strain of viral hemorrhagic septicemia virus (VHSV) was first isolated in Alaska from skin lesion material of two Pacific cod, *Gadus macrocephalus*. These fish were sport-caught in Prince William Sound (PWS), Alaska during the summers of 1990 and 1991 (Meyers et al. 1992). During April of 1993, two thirds (about 100,000 tons) of the 5 yr-old 1988 year class of Pacific herring, *Clupea harengus pallasii*, expected to return to spawn in PWS failed to appear. Hence, the commercially important seine fishery was never opened for harvest. Among the herring that did return, 15% to 43% had varying degrees of external ulceration or hemorrhage beneath the skin, at the bases of fins and around the vent accompanied by lethargic swimming behavior. A rhabdovirus identified as VHSV by serum neutralization and further identified as the North American strain of VHSV by cDNA probe methods was isolated from kidney and spleen pools from two groups of 10 of these herring, and from skin and organ samples of a single Pacific cod sport-caught nearby. Also during late April, herring with similar skin and fin hemorrhages were observed near Kodiak Island, Alaska. Subsequently, the same North

43% of 46 juvenile herring from Auke Bay, Alaska near Juneau. These fish had a concomitant VEN infection and were captured for use in a VHSV susceptibility study.

This report documents the Pacific herring as a new host species for North American VHSV which may have been responsible for the high prevalences of external skin and fin lesions and lethargic behavior observed in herring returning to PWS. VHSV was present in high titers indicative of active replication in host tissues while other likely fish pathogens were not isolated or observed. Histological lesions observed including massive passive congestion of the liver and kidney, subdermal and kidney hemorrhages, kidney tubule degeneration and active RE cell foci in the livers and kidneys of PWS herring were suggestive of a systemic viral agent.

Whether VHSV played any role in the reduced number of herring returning to PWS has not been established nor has there been verification of major herring mortality except for unconfirmed reports. As indicated by isolation of the virus from herring over wide geographic areas, VHSV may be indigenous to Pacific herring throughout Alaska and possibly the Pacific Northwest. There appears to be no relationship of VHSV isolation with the Exxon Valdez oil spill of 1989 since the virus has been isolated from areas other than PWS. The virus may be an opportunistic pathogen causing periodic occurrences of external and internal lesions in herring following stress from various factors including, VEN infections, spawning, commercial fishing or nutritional deficiency through lack of forage. The latter condition was suggested this year by the smaller size of herring returning to PWS. Some unknown amount of

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American VHSV was isolated from one pool of 4 of these fish, although herring returns and the commercial fishery there had been successful. In mid-May, the same VHSV was isolated from over

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herring mortality must occur during these epizootics primarily from the progressive ulcerating skin lesions that would result in osmoregulatory failure and/or act as portals of entry for secondary microbial infections. Such osmoregulatory collapse would result in shock and vasodilation, which may have contributed to some of the passive congestion we observed in tissues.

Our discovery of VHSV in Pacific herring strongly suggests that this fish species may be a major marine reservoir of the virus. A marine reservoir for VHSV has been suggested previously and is more likely now as an explanation for the isolations of VHSV from adult coho, *Oncorhynchus kisutch*, and chinook, *O. tshawytscha*, salmon returning to Washington State during 1988, 1989 and 1991 (Brunson et al. 1989, Hopper 1989, Winton et al. 1989 & 1991, Eaton & Hulett 1990, Stewart et al. 1990). Should North American VHSV be indigenous in Pacific herring populations then it is possible that the virus will be detected again in salmonids. Additional studies need to be conducted to determine the distribution of the virus in Pacific herring in other Pacific Northwest waters.

Rhabdoviruses are noted for their potential of rapid evolution. Hence, North American VHSV should be a concern to salmonid aquaculturists in the Pacific Northwest and Canada due to its potential to become a significant pathogen of trout and salmon. Currently, North American VHSV appears to be largely adapted to marine fish species but this could be altered if the virus is subjected to strong selective pressures as might occur during the course of intensive fish culture practices. It is very plausible that the European strain of VHSV also came from the marine environment where it became a virulent pathogen of rainbow trout after unpasteurized marine fish were used as a food source for hatchery fish. This exact route of adaptation is not as likely today in North America with the use of high quality

processed fish foods, but nonetheless conservative methods should be employed to eradicate the virus whenever detected in salmonids.

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ANNOUNCEMENTS and MEETINGS

Uses and Effects of Cultured Fishes in Aquatic Ecosystems. **March 12-17, 1994.** Albuquerque, NM. Contact: Gary Carmichael, 213 Bryn Mawr Drive SE, Albuquerque, NM 87106.

Pathology in Marine Aquaculture, 6th International Colloquium. **April 28-30, 1994.** Palais des Congres, Montpellier, France. Contact: Conference Secretariat, BIOCIM/IMIM, Parc Euromedecine, 34198, Montpellier Cedex 5, France.

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

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

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Susceptibility of Crayfish and Tadpoles to Experimental Infection with *Edwardsiella ictaluri*

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Crayfish and frogs are common inhabitants of commercial catfish ponds, and could move *Edwardsiella ictaluri* between ponds, or be a reservoir of infection between outbreaks of enteric septicemia. Crayfish are known to be asymptomatic carriers of *Yersinia ruckeri*, the bacterium which causes enteric redmouth of salmonids (Hunter et al., 1980). Alternately, endangered or sport frog and crayfish species could be negatively impacted by *E. ictaluri* as a result of stocking of infected channel catfish, *Ictalurus punctatus*. Frogs and crayfish are important prey items for catfish and other gamefish. We conducted an experiment to determine if crayfish or the most aquatic life stage of the frog, the tadpole, could be infected by a severe waterborne exposure to *E. ictaluri*.

Methods

Challenge Procedure: *Edwardsiella ictaluri*, strain 87-88, derived from diseased channel catfish (Baxa et al., 1990), was grown in Brain Heart Infusion (BHI) broth three days at 28° C. Twenty bullfrog, *Rana catesbeiana*, tadpoles (11.0 g) and twenty White River crayfish, *Procambarus (ortmanicus) spp.*, (13.0 g) obtained from a baitfish farm were immersed for 60 s in full strength broth culture (1.6×10^9 cfu/ml). Twenty control animals of each species were immersed in sterile BHI broth. After immersion, animals were gently rinsed 60 s in well water and placed in 600 L circular tanks maintained at 24° C, with a flow of well water at 0.2 L/min. Crayfish were provided with individual shelters made by cutting 10 cm segments of 7.5 cm diameter plastic pipe lengthwise, and fed once daily with raw potato slices, trout pellets and lettuce. Tadpoles were fed once daily with trout pellets, frozen minced spinach and goldfish flake food. Each tank was examined daily for mortalities.

Recovery of *E. ictaluri*: One week after challenge, 10 animals from each group were anesthetized with tricaine methanesulfonate. A piece of kidney (3 mm diameter) and a 5 mm segment of intestine were aseptically removed from each tadpole. The kidney was swabbed onto a BHI agar plate. The intestine segment was agitated in 0.1 ml sterile phosphate-buffered saline (PBS). The sample was then inoculated onto *Edwardsiella ictaluri* medium (EIM) agar (Shotts and Waltman, 1990). Hemolymph (0.1ml) was obtained from each crayfish at the base of the fifth leg with a 22 gauge needle and syringe. Hemolymph was inoculated onto BHI agar. A segment of crayfish intestine was excised, agitated in PBS and inoculated onto EIM agar. Agar plates were incubated at 28°

C for two days. Colonies isolated were confirmed as *E. ictaluri* after reisolation in pure culture, and examination of biochemical properties. At two weeks, all remaining animals were sacrificed and examined for *E. ictaluri* as above.

Results

Signs of disease or mortality was not observed in any tadpole and *E. ictaluri* was not recovered from these animals. Diseased or dead crayfish were not observed during daily observations, however six animals were missing by the end of the experiment. We believe this was caused by nocturnal cannibalism of crayfish which shed their shells, as crayfish grew rapidly under our experimental conditions. *Edwardsiella ictaluri* was not recovered from the 10 crayfish sampled at one week, or from the four crayfish sampled at week two.

Discussion

Edwardsiella ictaluri did not cause disease in, or was able to infect bullfrog tadpoles or papershell crayfish. The strain of *E. ictaluri*, 87-88, has been previously used as a standard waterborne challenge producing severe mortality in channel catfish at the same passage level and using the same procedures employed in this study (Baxa et al., 1990). Because the tadpoles and crayfish were obtained from a baitfish farm, it is not likely they had any significant previous exposure to *E. ictaluri*. Stocking of catfish which have survived an outbreak of enteric septicemia does not appear to pose a disease threat to these two species.

Acknowledgments This research was partially supported by funds from Federal Aid in Sport Fish Restoration. The challenge strain of *E. ictaluri* was provided by D. Baxa-Antonio, School of Veterinary Medicine, University of California at Davis.

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Observations on Repeated Bacterial Gill Disease Outbreaks and Amoeba Gill Infestation in Rainbow Trout (*Oncorhynchus mykiss*) in a Recycle Culture System

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Bacterial gill disease (BGD) causes serious losses among cultured salmonids. A variety of yellow pigmented bacteria have been implicated as the cause of the disease, and Wakabayashi et al., (1989) reported that an organism they named *Flavobacterium branchiophila*, caused BGD. More recently Ferguson et al. (1991) transmitted BGD by exposing healthy trout to infected trout and by adding pure cultures of a bacterium similar or identical to *F. branchiophila* to water containing healthy trout. Although BGD has been transmitted with a *Flavobacterium*, unfavorable environmental or physiological factors may predispose salmonids to the disease. Also, as suggested by Ferguson et al., (1991) transmission of the disease may depend, at least in part, on the ability of the *Flavobacterium* to colonize gill tissue or produce toxin.

Through a grant from the Agricultural Research Service of the USDA, the Freshwater Institute at Shepherdstown, West Virginia, is conducting research on a semiclosed trout culture system (Figure 1). The goal of the project is to determine optimum conditions for the system, evaluate the economics of the system, determine water quality of the culture system and effluent and evaluate continuous production techniques. The research protocol requires that 15-20 g rainbow trout (*Oncorhynchus mykiss*) be added to the system every two to three months while market-size trout (340 g) are selectively removed every two weeks. We found that in each of five cohorts of fingerling trout stocked into the system from September 1991 through July 1992, BGD occurred within six to eight days after stocking. The disease did not occur in previously stocked cohorts that had recovered from earlier outbreaks. The BGD outbreaks were controlled with one hour treatments of 12-15 ppm chloramine-T, but a secondary infection of gills with an amoeba occurred. The predictable

occurrence of BGD in the newly stocked trout afforded a chance to examine culture conditions for their impact on outbreaks.

In each of the five BGD outbreaks, the disease pattern was similar (Figure 2). Rainbow fingerlings from a commercial source were kept in single pass spring water for at least 30 days prior to stocking into the recycle system. Although some trout showed hyperplasia of gills before stocking there was no evidence of BGD in these trout. Within six to eight days after being stocked into the system BGD occurred. The disease was presumptively diagnosed by observing masses of long thin Gram negative bacteria on gills and confirmed by indirect fluorescent antibody test (IFAT) using rabbit anti-*F. branchiophila* antiserum (obtained from Von Ostland, Pathology Dept., U. of Guelph). The initial BGD outbreak and recurrences were controlled by chloramine-T, but eventually most trout died from amoeba infestation of gills (Figure 3). Overall cohort mortality attributed to amoeba was 71-91% and only 9-29% was attributed to BGD. Daily examination of gills from two lots of trout by Gram stain and IFAT showed increasing numbers of *F. branchiophila* until trout began dying from BGD, and a dramatic decrease in these bacteria after the chloramine-T treatments. We were also able to demonstrate that BGD occurred in 230 gram trout raised in single pass water and stocked into the recycle system which indicates BGD occurred in juvenile and yearling trout when initially stocked into our recycle system. Attempts to culture *F. branchiophila* from infected gill tissue on Ordal's agar were not successful, but the bacterium was cultured from two liters of tank water concentrated by centrifugation to five ml before culturing. Because BGD did not occur in stocked trout after the original outbreak, but only occurred in newly stocked trout, it

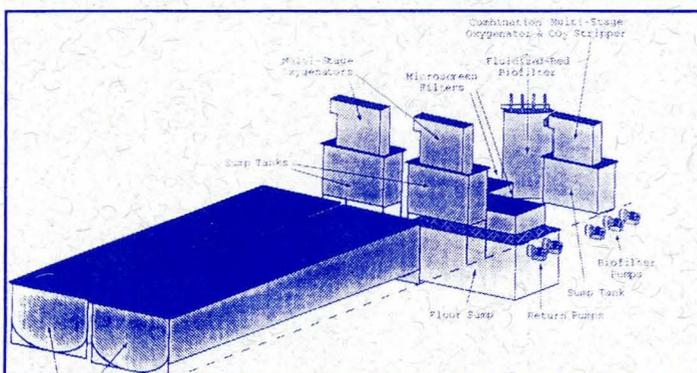


Figure 1. System diagram of recirculating system at the Freshwater Institute. Plumbing has been eliminated for simplicity.

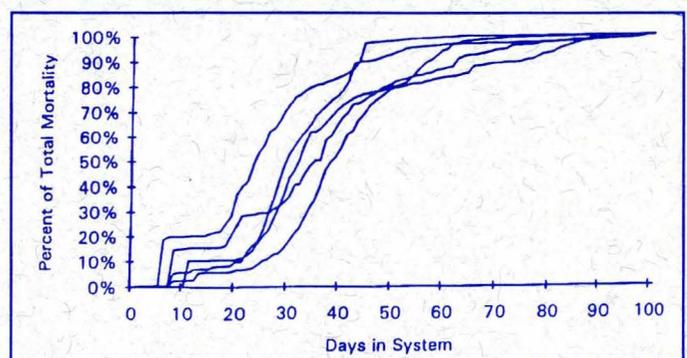


Figure 2. Mortality patterns among five groups of rainbow trout introduced into the semiclosed culture system from September 1991 through July 1992.

is possible that recovered trout were resistant or possibly immune to further infections. Two groups of fingerling trout kept in single pass tanks were exposed for two weeks to effluent from the recycle system, then kept two weeks in spring water before stocking into the system. Non exposed fin clipped trout were also stocked to serve as controls. In one group BGD failed to develop in either the exposed or control trout. In the other group control trout died at three times the rate of exposed trout, but these trout were not examined for BGD or the amoeba.

The predictability of BGD outbreaks in the recycle culture system would indicate conditions were present that predisposed newly stocked trout to BGD. Many water quality parameters of the system were normal. Temperature ranged 13-16 C; dissolved oxygen was 8-10 ppm; alkalinity 230-240 ppm; pH 7.1-7.3; and total ionized and un-ionized ammonia, and nitrite levels below toxic levels. However the design of the cross-flow tanks resulted in suspended solids. These tanks have rounded bottoms (Figure 1) with water entering through holes just above the bottom along one side and exiting through similar holes on the other side. Incoming water causes water to rotate about the long axis of the tank, up-welling on one side and down-welling on the other side, which propels feces and uneaten food toward the outlet. With sufficient fish numbers no debris settles on the tank bottom. However waste particles that do not exit the effluent holes are carried back into the water column, creating

a level of suspended solids which would be a source of gill irritation. In addition trout were heavily stocked. Newly stocked fingerling trout were segregated from other fish by a divider screen so initial densities in the segregated section were only 7-31 kg/m³, but overall densities in the system were 77-121 kg/m³. Although our observations indicate trout develop some type of resistance or immunity after the initial BGD outbreak, attempts to demonstrate resistance or immunity by exposing trout to system water before trout were stocked into the recycle system were not successful. More sophisticated research procedures may show such resistance. It is possible that cell mediated immunity may develop after initial exposure.

There have been several reports of amoeba infestations of salmonid gills. Roubal et al. (1989) reported the normally free-living *Paramoeba sp.* attached to gills of Atlantic salmon (*Salmo salar*) and were associated with necrosis of epithelial cells and cytoplasmic processes that passed into and between surface cells of hyperplastic gill epithelium. Kent et al. (1988) reported *Paramoeba pemaquidensis* infestation on gills of coho salmon (*Oncorhynchus kisutch*) reared in net pens. The source of the amoeba in our system is not known, but they were probably introduced with the juvenile trout. To our knowledge this is the first report of BGD and an amoeba infestation occurring at the same time in a semiclosed system. At present, attempts are being made to culture and identify the amoeba from our system.

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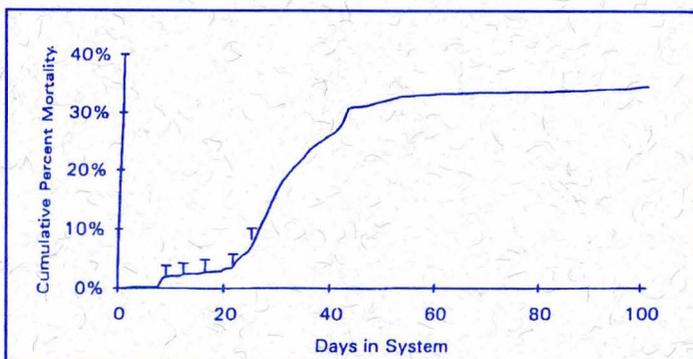


Figure 3. Effect of Chloramine-T treatments (T) on control of bacterial gill disease among introduced rainbow trout in the semiclosed system. Mortality after day 20 caused by amoeba infestation

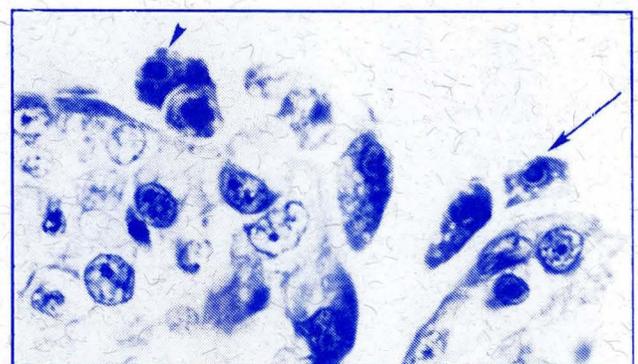


Figure 4. Histological section of rainbow trout gill showing presence of amoebas (arrows). Hematoxylin and eosin stain, 400X.

SECTION REPORT

PROFESSIONAL STANDARDS COMMITTEE



The Professional Standards Committee (PSC) has finally completed a draft (see insert) of the proposed changes to the document "Standards and Procedures for the Certification of Fish Pathologists." This document has already been reviewed and revised numerous times by selected individuals, members of the PSC, and EXCOM. Most of the major issues pertinent to the document's revision have been addressed and incorporated, however, the PSC has felt that the AFS/FHS membership should have an opportunity to also review and comment on it.

Those wishing to make comments may do so by phone, fax, or mail until February 28, 1994, after which final action will be taken to make the document official. Once approved, there will be a time table put in place as to when all or part of the document will be in effect. Please send your comments to the following:

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EDITORS REPORT

This issue marks the last edition for 1993, and with any luck you will receive it before the holidays. I'd like to thank everyone who has made submissions to the newsletter of any kind. We especially thank those who send their submissions by way of computer diskette - that saves a lot of wear and tear and the editors! Speaking of computers, we now have the capacity to import files from most major PC word processors - including WordPerfect, Microsoft Word, Microsoft Works and Word Star. There is also access through the Compuserve network (71024,2467). Hopefully this will make it easier to submit articles.

From time to time, we receive notices of change of address or failure to receive issues. In general, these problems should be addressed to membership services with the AFS office. If your newsletter is damaged in some way, please let us know and we'll be glad to replace it.

Your comments, criticisms and suggestions are always welcome. Best wishes for the holiday season, we'll see you next year.



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FISH HEALTH SECTION AMERICAN FISHERIES SOCIETY

STANDARDS AND PROCEDURES FOR THE CERTIFICATION OF FISH PATHOLOGISTS

I. INTRODUCTION

The Fish Health Section (FHS) has recognized the need for a peer review system to identify professionals possessing the competence, training, and ethics required to effectively serve fisheries programs and aquaculture by meeting their fish health needs. Individuals meeting the requirements which follow shall be eligible for certification by the Fish Health Section as "Fish Pathologists."

The FHS/AFS certified Fish Pathologist is a professional who specializes in the causes, nature, and control of the diseases of finfish, (hereinafter referred to as "fish.") Through a regimen of academic education, specialized training, and years of experience, the individual has developed a thorough understanding of the fish, its environment, infectious and non-infectious diseases, and the interrelationships involved.

The Fish Pathologist is an individual in the fish health field who utilizes various disciplines including fisheries biology, microbiology, parasitology, toxicology, pharmacology, and histopathology to provide an accurate evaluation and diagnosis of fish health problems. When necessary, the Fish Pathologist is capable of seeking appropriate specialized assistance in determining the etiology of a health problem. If a definitive diagnosis is not possible, the Fish Pathologist must have the ability to utilize all available information to establish the most logical cause of the problem.

The Fish Pathologist is competent to conduct fish health inspections, process samples, perform or supervise laboratory work, and interpret in-house results, as well as those from specialized reference laboratories. The Fish Pathologist is also capable of providing responsible recommendations and/or prescriptions (licensed veterinarians only) for control measures for fish diseases within legal constraints.

Technical skills, experience, and high ethical standards enable the Fish Pathologist to serve fisheries programs and aquaculture through the evaluation and diagnosis of fish health problems, through responsible recommendations for disease control, and through the administration of programs designed to enhance the health of cultured and free-ranging fishes.

II. OBJECTIVES

- A. To establish a peer review system within the FHS which can efficiently and judiciously evaluate the basic academic training, specialized training, and work experience required for certification as a Fish Pathologist.
- B. To identify individuals possessing technical and professional competence and demonstrating high ethical standards which qualifies them to evaluate and diagnose disease problems, recommend and/or prescribe control measures within legal constraints, and administer programs designed to enhance the health of cultured and free ranging fishes.
- C. To provide individuals, employing organizations, regulatory agencies, the courts, and the general public with definitive minimum standards for education, experience, and ethics required by the FHS for certification as a Fish Pathologist.
- D. To guide educators in the development of qualifying curricula and to assist employers with the development of position classifications commensurate with the requirements for certification as a Fish Pathologist.

III. QUALIFICATIONS

A. Basic academic education

- 1. Bachelor's Degree, or advanced degree, in a biological science from an accredited college or university. Applicant must provide documentation of degree earned.
- 2. A minimum of eighteen (18) quarter or twelve (12) semester hours of fisheries courses at an accredited college or university. Course work must include fish anatomy and physiology (laboratory required), ichthyology/fish biology, and fish culture.

B. Specialized training

- 1. **Finfish Health:** A minimum of five (5) quarter or three (3) semester hours at an accredited college or university; or 100 lecture hours at a Professional Standards Committee (PSC) approved or accredited training center. Laboratory required. Credit may be calculated on the basis of three (3) quarter hours or two (2) semester hours earned for each 40 hours of formal lecture-laboratory training. (NOTE: Each hour of

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laboratory training counts as 1/2 hour for calculation purposes.) Applicant must submit certified transcript(s) and document course content.

- Academic Science:** The applicant must have taken a minimum of 37 semester hours or 51 quarter hours in the following required and elective courses at an accredited college or university. Applicant must submit certified transcript(s) and document course content.

<u>Required Courses</u>	<u>Lab Required</u>	<u>Minimum Hours</u>	
		<u>Semester</u>	<u>Quarter</u>
1. Pathogenic bacteriology	Yes	3	4
2. Parasitology	Yes	3	4
3. Virology	Yes	3	4
4. Immunology	Yes	3	4
5. Histology	Yes	3	4
6. Pathology/Histopathology	Yes	3	4
7. Nutrition	No	2	3
8. Biochemistry	No	4	4
9. Vertebrate physiology	Yes	3	4
10. Pharmacology	No	3	4
11. Toxicology	No	2	3
12. Water Quality/ Pollution Biology/ Limnology	Optional	2	3

Elective Courses (2 are required)

1. Fish Genetics	Optional	2	3
2. Epidemiology	No	2	3
3. Clinical Chemistry	Optional	2	3
4. Hematology	Optional	2	3
5. Mycology	Optional	2	3

A grade of "C" or greater is required in all course work. Courses taken "Pass/Fail" are not acceptable.

C. Professional work experience

- Definition:** Full-time fish health work experience is defined as a minimum of seventy-five percent (75%) of the applicant's professional work time during a 12-month period engaged in fish health activities which may include: (1) disease diagnostics and control; (2) fish disease/health research; (3) fish disease/health instruction at the university level or its equivalent; or (4) administrative work directly related to fish disease diagnostics and control.
- Requirement:** The applicant must have a minimum of three (3) years of professional level full-time fish health work experience during the five (5) years preceding application.
- Restriction:** Work experience gained prior to meeting the Basic Academic Education (III.A.1 & 2) and Specialized Training/Finfish Health (III.B.1.) requirements does not qualify as professional work experience. All Specialized Training/Academic Science (III.B.2.) requirements must be completed at least one year prior to applying for certification.

D. Work status at time of application

The applicant must be engaged in fish health activities at the minimum rate of seventy-five (75%) percent of the total work time.

IV. APPLICATION PROCEDURES

- Applicants must be members of the American Fisheries Society and the Fish Health Section.
- Application forms may be obtained from the Chairperson of the Professional Standards Committee or Board of Certification.
- Individuals seeking certification as Fish Pathologists must file completed application forms, including required supporting documents, and a \$50.00 non refundable application fee with the Chairperson of the Board of Certification. The applicant is responsible for an accurate and complete application as well as arrangements for the timely submission of letters of recommendation. Applications with discrepancies will be returned.

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- D. At the time of application, applicants shall promptly arrange for the forwarding of three letters of recommendation directly to the Board Chairperson from the following:
1. The applicant's immediate supervisor or employer must submit a letter of recommendation directly to the Board of Certification Chairperson which documents the applicant's current work status (III.,D.). If appropriate, this letter may also document the applicant's previous professional work experience (III.,C.,2.) as defined in (III.,C.,1.), technical proficiency and total years and months of professional level fish health work experience of which he or she has direct knowledge.
 2. Letters of recommendation on the applicant's behalf must be submitted directly to the Board of Certification Chairperson by a minimum of two (2) fish health professionals. Applicants are encouraged to obtain letters of recommendation from AFS/FHS certified pathologists or inspectors, or individuals recognized as authorities in the fish health field. These letters must attest to the applicant's professional work experience (III.,C.,2.) as defined in (III.,C.,1.), technical proficiency and, if appropriate, total years and months of professional level fish health work experience of which he or she has direct knowledge.
- E. The Board of Certification shall review all applications. Upon satisfactory completion of all application requirements, the Chairperson of the Board shall notify successful applicants, in writing, that they are eligible to take the written examination and must do so within one year of the notification.
- F. Applications not approved by the Board of Certification shall be returned to the applicant with a summary explanation.
- G. Applicants desiring a review of a negative decision by the Board may file a request with the Chairperson of the Professional Standards Committee for a formal review of their application by the committee. Applicants have 3 months to request a review of a negative decision. The review panel convened for such considerations shall consist of all PSC Committee members and the Chairperson of the Board of Certification. The decision of the review panel shall be final and shall be completed within 3 months of the formal review request.

V. EXAMINATION

- A. All applicants who have fulfilled the qualification requirements in A through D above shall be required to take a written examination administered by a member of the PSC or an agent appointed by the PSC. The examination will cover topics such as fish disease etiology, diagnostic procedures, pathology of fish diseases, fish disease therapy, fish pond management, fish disease control, general fisheries, fish culture, and other items essential to a thorough knowledge of the care and health of fish. The examination will also include 35 mm slides. A minimum score of 75 percent will be required to pass the written examination.
- B. Prior to taking the examination, the applicant must submit a non refundable **\$100.00** examination fee to the Secretary/Treasurer by personal or certified check made payable to the FHS/AFS. The Secretary/Treasurer will notify the Chairperson of the Professional Standards Committee who will notify the applicant of the site, date, and time of the examination.
- C. Upon successful completion of the written examination, the PSC Chairperson shall notify the applicant, the Board of Certification Chairperson, and the President of the Fish Health Section. The Section President, when assured that all certification procedures have been completed, shall then officially notify the applicant of his/her certification as a Fish Pathologist by a congratulatory letter and a certificate indicating the period of certification, which will be five (5) years.
- D. Applicants failing the examination must wait one year before retaking the exam. Applicants failing the exam a second time must reapply for certification under the current certification requirements.

VI. RECERTIFICATION

- A. FHS certified Fish Pathologists must be recertified five (5) years after initial certification and every five (5) years thereafter.
- B. Two months prior to each Fish Pathologist's five-year anniversary date, the Chairperson of the Board of Certification will mail to the Fish Pathologist notification of the recertification requirements and appropriate forms. When so notified, the individual seeking recertification must submit a non refundable **\$50.00** recertification fee to the Chairperson of the Board of Certification and payable to the AFS/FHS.
- C. To be recertified, the Fish Pathologist must meet the following criteria:
1. During the previous five years, the individual must have been engaged in fish health activities at the minimum rate of fifty (50) percent of the total work time (full-time employment) for a minimum of three (3) years.

DRAFT

- 2.** Each individual must remain a member of the AFS/FHS for each of the five years prior to recertification.
- 3.** When Continuing Education becomes available, each individual must participate in the program, and must attend workshops, seminars, meetings, classes, etc. approved and specified by the PSC in order to meet the total CE units required for the 5-year period prior to recertification. The AFS/FHS newsletter, provided as a part of membership, will contain information regarding continuing education opportunities and recertification requirements.
- D.** The requirements for recertification (VI.,C.,1.) must be substantiated in letters of recommendation from the individual's immediate supervisor or employer and from three (3) fish health professionals. The requirements for recertification in VI.,C., 2 & 3, must be documented in the application for recertification. Letters of recommendation are to be mailed directly to the Chairperson, Board of Certification.
- E.** Failure to comply with any of the recertification criteria will result in the loss of certification and the need to reapply under current application requirements. If extenuating circumstances arise that do not allow a Fish Pathologist to meet any of these criteria, a letter, describing the circumstances, must be forwarded to the PSC for review.
- F.** Upon recertification by the Board of Certification, the Fish Pathologist will be so notified in writing by the Chairperson. With this notification, the Fish Pathologist will also receive an official seal, indicating the year of recertification, which is to be affixed to the original certificate where indicated.
- G.** Any Fish Pathologist denied recertification shall be so notified in writing by the Chairperson of the Board, within 2 months, with reasons for denying recertification.
- H.** Fish Pathologists desiring a review of a negative decision by the Board regarding their recertification may file a request with the Chairperson of the Professional Standards Committee for a formal review of their recertification credentials by the PSC committee. The review panel convened for such considerations shall consist of all PSC committee members and the Chairperson of the Board of Certification. The decision of the review panel shall be final and shall be completed within 3 months of the formal review request.

VII. REVOCATION

- A.** Fish Pathologist certification may be revoked by the Board of Certification for reasons such as gross negligence, incompetence, falsification of data or reports, misrepresentation, acceptance of a bribe or any other action determined by the Board of Certification to be professionally unethical.
- B.** Information concerning unethical action as indicated in A above should be filed with the Chairperson of the Professional Standards Committee.



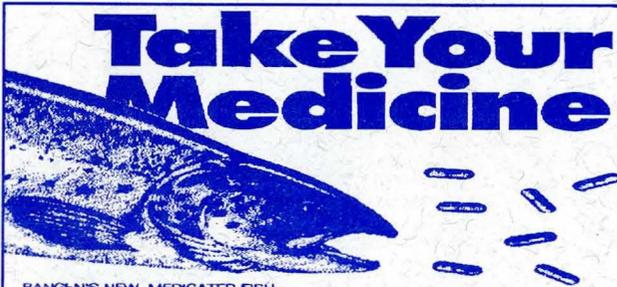
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Aquatic Pathobiology Videotapes. A set of eight tapes and a study guide (287 pp.) are available, while supplies last, at a cost of **\$175.00** which includes postage and handling. Each videotape consists of a one hour lecture followed by a thirty minute question and answer session. Topics covered include: Aquatic Environment, Clinical Examination, Bacterial Diseases, Protozoan and Metazoan Diseases, Viral Diseases, Nutrition, Husbandry and Therapeutics. Please send check, money order, or institutional voucher made out to "Texas Agricultural Experiment Station" to Department of Veterinary Pathobiology, Texas Veterinary Medical Center, Texas A&M University, College Station, TX 77843, ATTN: Ms. Betty Suehs.

South Atlantic Snapper-Grouper Poster. South Atlantic Fishery Management Council (SAFMC). 1993. Poster depicts 60 snapper-grouper illustrations. **FREE** (one per person). Contact Carrie Knight, SAFMC, One South Circle, Suite 306; Charleston SC 29407-4699, 803-571-4366; FAX: 803-769-4520.

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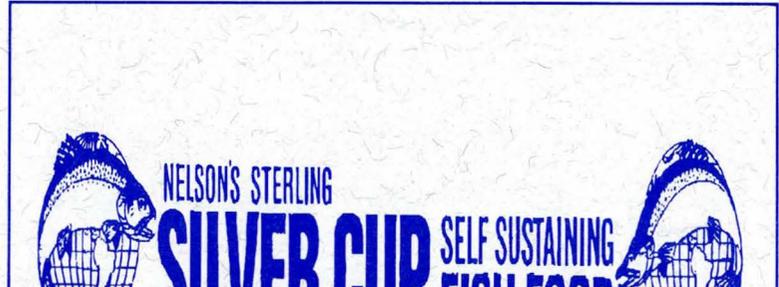


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Review of a Workshop of Comparative Immunologists on: Modulators of Fish Immune Responses Models for Environmental Toxicology/biomarkers, Immunostimulators

Christopher J. Bayne

Department of Zoology, Oregon State University, Corvallis, Oregon 97331-2914, USA.

*The time has come,
The fish immunologist said
To talk of many things
Of lakes and rivers,
Of streams and springs
Of oceans and fishy things.
Of how we do modulate and stimulate,
And what pollution brings.*

Joanne Stolen

Breckenridge, Colorado was at its autumnal peak September 17-21, 1993, when 45 fish and shellfish immunologists and toxicologists gathered to exchange information on **Modulators of Fish Immune Responses**. The meeting was sponsored by SOS Publications with grant money from the U.S. Army Biomedical Research and Development Laboratory and organized by Joanne Stolen (SOS Publications) with help from Judith Zelikoff (NYU Medical Center), Douglas Anderson (National Fish Health Research Lab), Lorraine Twerdok (GEO-CENTERS Inc.) and Stephen Kaattari (Virginia Institute of Marine Science - VIMS). It was conceived as a workshop at which investigators using and planning to use fish immune systems as sensitive indicators of environmental contaminants would interact with others engaged in more basic studies. Videotapes made by M. Faisal (VIMS), D. Anderson, L. Twerdok and S. Kaattari not only illustrated various techniques (such as how to obtain immunocytes from the tiny medaka and how to do ELISAs and plaque assays for antibody-secreting cells) but also catalyzed discussions of real world predicaments such as the basis on which to select field sampling strategies in studies of immunotoxic damage in feral fish populations.

Non-Ig-mediated ('innate') immunity surfaced as a predominant theme. Since innate immune mechanisms are available for essentially immediate assay, whereas lymphoid immunity takes days or weeks to yield responses, the former provides suitable systems (e.g. phagocytosis, superoxide anion production, chemotaxis) for assaying xenobiotic influences. Assays of innate immune mechanisms are often less technically demanding than assays of lymphoid immunity. β -glucans are being used not only to potentiate phagocytosis and superoxide production, but to elevate serum lysozyme and lead fish to higher and longer lasting antibody responses to bacterial vaccines (B. Robertson, Tromso; J. Ainsworth, Mississippi State). Such effects can be very dose-sensitive and time-dependent, and may have undesirable consequences such as hypersensitivity and auto-immune reactions, though these have not surfaced in fish work.

Consistent with the interest of the U.S. Army Biomedical R and D laboratory in fish as sentinels for pollution in association with ongoing hazardous waste remediation efforts, a good deal was heard about the effects of xenobiotics on fish immune systems. Many fish species are well placed ecologically, physiologically and in terms of life history traits to meet this need, and the sensitivity of their immune systems to xenobiotic damage (Zelikoff) adds to their utility. Assay systems are still being developed, and will need to be validated for lab-to-lab consistency. Much of this work relies on running cells from feral fish through panels of assays like phagocytosis, bacterial killing, oxidative burst and lysozyme titrations. As the chemical mixtures in contaminated waters and sediments are usually complex, more laboratory-based work was called for on the influences of known, individual xenobiotics. An example of this was reported by S. Kaattari, who has found that embryonic exposure of salmonids to aflatoxin B induces long term immune dysfunction: following secondary challenge with antigen-treated fish produce less antibody, and with less specificity, than their siblings.

Cytokines are being explored in fish, and C. Secombes (Aberdeen) described advances that bring planned intervention in immune responses within the realms of possibility. While inter-species differences in cytokines and/or their receptors have slowed progress, recombinant human TNF, for example, synergizes with a fish macrophage-activating factor in conditioned

media from salmon cell cultures. Salmon macrophages given β ,1-3 glucan *in vitro* yielded more potent respiratory bursts, and this was inhibited by anti-mammalian TNF (Robertson, Tromso). Cytokines generated *in vivo* were illustrated by H. Kodama (Osaka) who found that sera from fish injected with muramyl dipeptide or LPS contained elevated colony-stimulating activity when assayed *in vitro*.

Phagocytes of both fish and invertebrates generate superoxide anions. Labs in the U.S. (R. Anderson, Maryland) and France (M. Auffret, CNRS, Brest) have exploited this with bivalve mollusks and fish (M. Dunier, INRA, Lyon). Biphasic effects were reported for some heavy metals, organic pesticides and oil-derived xenobiotics: low concentrations may be enhancing while higher levels have the expected toxic suppressive effects. The phagocytes of some bivalve mollusks evidently contain the metabolic machinery to process xenobiotics, similar to the vertebrate liver (Faisal), and primary cell cultures from such animals have been used profitably in such investigations. The development of melano-macrophage aggregates is a good histopathological indicator of contaminant exposure (V. Blazer, West Virginia), however the pathways by which xenobiotics induce such responses remain unclear. Similarly, increased concentrations of specific plasma proteins (including C3) in serum immediately (i.e. ahead of the acute phase response) following stress (N. Demers, Oregon State) remain as possible indicators of adaptive changes in innate immune effectors, with the activation pathways remaining in need of study.

Economic losses in the aquaculture industry are often due to diseases, and much excellent basic research has been and is being done in the pursuit of effective and affordable vaccines. However, mirroring the complexities of novel vaccine development being experienced in human medicine, the next generation of fish vaccines are proving both more expensive and more technically complex to develop.

The proceedings and abstracts are available from SOS Publications, 43 DeNormandie Avenue, Fair Haven, NJ 07704-3303. FAX: 908/530-5896. The next meeting will be held July 8-15, 1995 in Breckenridge, Colorado.

The views, opinions, and/or findings contained in this article are those of the author(s) and should not be construed as official Department of the Army position, policy or decision, unless so designated by other official documentation. Citations of commercial organizations or trade names in this article do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations. Research was conducted in compliance with the Animal Welfare Act, and other Federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the *Guide for the Care and Use of Laboratory Animals*, NIH publication 86-23, 1985 edition.

LETTERS TO THE EDITOR

Yes, Virginia, someone is reading the newsletter and cared enough to write!



I would like to see the FHS Newsletter continue to serve its function as a newsletter, which is to keep its readers informed. Timely information in a condensed format is essential to many who have little time and/or access to scientific literature from a variety of sources. Therefore I think the Newsletter should continue to publish informal, preliminary research, and case studies which may not find their way into scientific journals for a considerable time or possibly not at all.

I want a newsletter that keeps me up to date with what is happening in the fish health field now.
John G. Hnath Fish Health Laboratory Mattawan, MI 49071

I read with interest the recent comments in the newsletter about the direction of newsletter material. As a strong supporter of the administration of both the newsletter and the *Journal of Aquatic Animal Health*, I decided to offer my opinion.

Clearly, there is a place for both publications within the fish health community. Each has its role and they serve well. The Journal is available for refereed articles that report rigorous science. As a result of the significant time required to prepare and review candidate articles, we all know that the Journal cannot be used to report time-sensitive material.

On the other hand, the time between submission of material and publication in the newsletter is much shorter. A newsletter is just that - a vehicle to present news. Additionally, readers understand that the material has not necessarily been peer reviewed and rigorously criticized and edited.

If authors, editors and readers continue to make a clear distinction between the roles of these two publications, then they both can peacefully coexist and be complementary. I like what I read in the Journal and the newsletter. Keep up the good work.
William E. Mancini, C.F.S. Senior Biologist Fisheries Technology Associates, Inc. Ft. Collins, CO 80522-0080

Seawater Transmission of *Loma salmonae* (Microsporea)

M.L. Kent and S.C. Dawe

Biological Sciences Branch, Dept. of Fisheries and Oceans, Pacific Biological Station, Nanaimo, B.C. V9R 5K6 Canada

Loma salmonae is a well recognized gill pathogen of salmonid fishes reared in freshwater. Recently, the microsporidean has been recognized as a cause of gill disease in pen-reared coho (*Oncorhynchus kisutch*) and chinook (*O. tshawytscha*) salmon.

The limited studies on transmission of fish microsporeans indicate that they are transmitted directly from fish to fish. However, it has been unclear if infections in salmon reared in seawater netpens are a result of infection prior to seawater introduction, or if the parasite is transmitted horizontally in netpens. At one netpen farm in British Columbia, a high prevalence of *L. salmonae* infection has been observed in chinook that had been reared solely on ground water during their freshwater phase of development, which suggested seawater transmission.

A laboratory study was conducted to determine if *L. salmonae* could be transmitted in seawater by direct exposure to infected tissues. Parasite-free chinook smolts were transferred to a flow-through seawater tank. Approximately 5 grams of *L. salmonae* infected gill tissue was finely chopped and placed into the tank. Fish were maintained for 5 wk at 11-15° C, after which the gills of five fish were examined histologically. Four of the five exposed fish exhibited *L. salmonae* infection, whereas controls maintained under identical conditions were uninfected.

The transmissibility of *L. salmonae* in seawater should be considered in management decisions for netpen farms. For example, uninfected smolts should not be placed on a site where a high prevalence of the infection exists.

(Continued from page 2)

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

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

Western Fish Disease Workshop: June 22-24, 1994, Holiday Inn, Bozeman, Montana. Room rates for the meeting are \$64 single, \$72 double at the Holiday Inn. Dorm rooms at Montana State University will also be available (for approximately \$10 single, \$15 double). An informal and affordable workshop is planned. For those interested (or who need to stay over Saturday night to take advantage of reduced airfares) a raft trip will be arranged for Saturday June 25, 1994. First mailing will be sent out in January. Please contact Beth MacConnell, USFWS, Fish Technology Center, 4050 Bridger Canyon Road, Bozeman, MT 59715 (phone 406-587-9265) if you have any questions or suggestions for the agenda and/or activities.

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

Fish Parasite Roundtable Discussion/Symposium As part of the VIII International Congress of Parasitology (ICOPA VIII) to be held at **Izmir, Turkey (10-14 October, 1994)** will be a roundtable discussion/symposium pertaining to fish parasites. The fish parasite symposium for ICOPA VIII will be organized into two parts. The first part will be given by invited speakers who will address major fish parasite problems in a given geographical region including recent research centered on the parasitic problems. The second part will be an open session for those wishing to participate in the roundtable but were not invited speakers. Those interested in the symposium and ICOPA VIII should write to:

Professor, Dr. M. Ali Ozel
ICOPA VIII
P.K. 81 35042
Bornova, Izmir TURKEY

Fourth Biennial Fish Diagnosticians Workshop: March 17-18, 1994. Fish Farming Experimental Laboratory, Stuttgart, Arkansas. For more info, call Andrew Mitchell, (501) 673-4483.

The Asian Fish Tapeworm, *Bothriocephalus acheilognathi*, is Alive and Thriving in Utah Ponds and Streams.

Richard Heckmann, Department of Zoology, Brigham Young University, Provo, Utah 84602.

To date, the Asian fish tapeworm, *Bothriocephalus acheilognathi*, is present in one river and three ponds in Utah. *B.acheilognathi* was first reported from Utah in the lower Virgin River (Heckmann et al., 1986) and then appeared in introduced and endemic fishes in the Virgin River next to St. George, Utah (Heckmann et al., 1987). It is also found in states bordering Utah due to the introduction of grass carp, *Ctenopharyngodon idella*, and the use of live minnows for fish bait (Heckmann et al., 1993).

The Asian fish tapeworm has been introduced into the United States through shipments of grass carp which were brought to this country from China to control aquatic vegetation (Hoffman and Schubert, 1984). The cestode has spread from its initial introduction in the southern part of the United States to the western part due to infected fish introductions (Heckmann et al., 1986). This species, first described from fish in Japan, is common in the intestine of grass carp cultured in South China. From China, cestode infections have followed grass carp imports into Europe, Russia and the United States (Hoffman and Schubert, 1984). The best known carp parasite transported to the fish ponds of many countries with the Chinese carp is *B.acheilognathi* (= *B. gowkongensis*, = *B. opsalichthydis*) (Bauer et al., 1981). All European countries that culture carp in large quantities now have this pathogen. The spread of this parasite to new localities usually results in heavy infection of young fishes during the first years after it appears. It is a thermophilic parasite and can infect many fish species.

B.acheilognathi is characterized by viper-like or arrow shaped scolex and numerous microtriches. Excellent reviews of the histopathology, biology, life history, control and management of *Bothriocephalus* are found in a series of papers by Nakajima and Egusa (1974 a,b) It has been a dangerous parasite for cultured grass carp and German carp fingerlings in Europe (Bauer et al., 1981). In Europe, it has also been found in European catfish, guppies, mosquito fish and other species (Hoffman, 1983; Hoffman and Schubert, 1984).

In the western United States, *B. Acheilognathi* has been found in *Notemigonus crysoleucas*, *Pimephales promelas*, *C. idella*, *Gambusia affinis*, *Gila robusta*, *Rhinichthys osculus*, *Lepidomeda molispinis*, *Plagopterus argentissimus*, and *Ptychocheilus lucis* (Heckmann et al., 1986) and recently in *Cyprinella lutrensis* (Heckmann et al., 1993). Fish in the Virgin River infected with the Asian fish tapeworm include: woundfin minnow (*Plagopterus argentissimus*), Virgin roundtail chub (*Gila robusta*), speckled dace (*Rhinichthys osculus*), red

shiner (*Notropis lutrensis*), and Virgin spinedace (*Lepidomeda mollispinis*). Recently, grass carp were introduced into Salem Pond (Utah Valley) and a small pond near Salem Pond. One fish from Salem Pond died and the author found 18 adult cestodes, *B.acheilognathi*, in the intestine of the dead fish. There is a potential of the introduced Asian fish tapeworm in Utah Valley to spread to other fish.

The major management problem for fish infected with *B.acheilognathi*, is that this cestode is non-host specific (Heckmann et al., 1986) and uses two hosts (copepod, fish) in its life history (Hoffman, 1983) instead of the usual three hosts for cestodes. The adult stage occurs in fish and the larval stage in copepods. There are other cestodes in fish that look like the Asian fish tapeworm. Suspected infections of the Asian fish tapeworm should be confirmed by a qualified fish parasitologist.

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Fish Health Section Newsletter

The Fish Health Section Newsletter is a quarterly publication of the Fish Health Section of the American Fisheries Society. Submissions of any length on a topic of interest to fish health specialists are encouraged with the understanding that material is not peer reviewed. Submissions (files on diskette from a PC word processors preferred) should be addressed to the editors listed below:

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