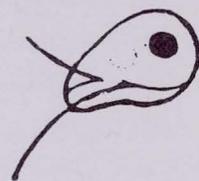


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LETTER



Volume 17, Number 1

Winter 1989

## KEN WOLF'S BOOK A REALITY

**Fish Viruses and Fish Viral Diseases.** Ken Wolf. Cornell University Press, Ithaca, New York. 1988. 476 pp. \$57.50.

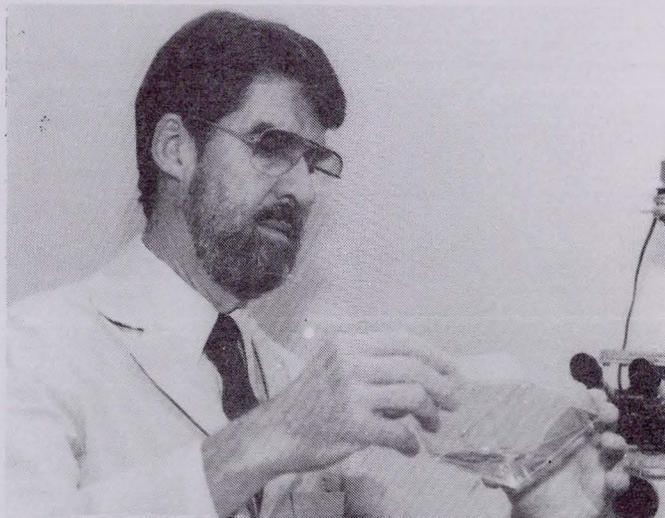
Those with an interest in virus diseases of fish have been awaiting this book with great anticipation. This book is a landmark in fish virology and probably represents the last time that a single volume will be able to encompass the entire field. The book is unique, not only because it serves as a comprehensive and up-to-date reference work in fish virology, but also because it was written by someone who participated so intimately in the development of the field. As most readers are aware, Ken has done much of the pioneering work in fish cell culture, isolated and characterized several important fish viruses, trained many U.S. and foreign scientists in techniques for the isolation and identification of fish viruses, and maintained a virtual repository of information and stock cultures of fish viruses and cell lines. In addition, the book is written in a clear and concise style with excellent organization making it a pleasure to read.

The introduction to the book serves as a review of the development of fish virology. This informative history could only be written by someone who was "there when it happened". The chapter gives the reader the feeling that it is a discussion about close friends and the feeling is strengthened by the inclusion of several photographs of the pioneers in fish disease research. The body of the book contains 6 major parts titled: isolated viruses and resulting diseases; viral infections of indeterminate pathogenicity; viruses visualized but not yet isolated; virus-like particles; chlamydial infections; and nonviral conditions, agents, and artifacts. Within each of the six sections, individual chapters are devoted to each of the 63 agents discussed in this work. The chapters are organized using a common format that includes: definition, history, signs and pathologic changes, etiology, diagnosis, isolation, identification, transmission and incubation, source, host and geographic range, immunity, control, and references. Naturally, for the unusual or newly characterized agents not much is known, but for others (e.g. IPNV), over 200 references are cited and discussed.

It is clear that each of the viruses or viruslike agents included in this volume has received careful and complete treatment; however, there are two aspects of this book that deserve mention because they are so exceptional. The first is the extraordinarily comprehensive list of references cited (including many in 1987). This makes the book a virtual starting point for any future research on fish viruses. The second impressive feature is the wealth of detail provided about the viruses. Much of this information (some of which appears nowhere else) is the product of unpublished research and personal communications with colleagues, but the remainder is the product of long and exhaustive scholarship.

Ken's intimate association with fish virology provides him a scientific perspective that few others could bring to this work. While he maintains a dispassionate approach to the many accomplishments of his own laboratory, Ken's ability to critically evaluate various aspects of fish virus biology provides that unusual synthesis that comes from personal involvement and extensive knowledge. While there are fish virologists who many not agree with some of these "judgement calls" (e.g. separating EVE from IPNV but lumping NeVTA, YTV, and OMV), this personal view, by one of the truly knowledgeable scientists in the field, is an invaluable addition to this book. Without question, this

volume will remain the standard reference in fish virology for years to come and serves as a fitting culmination to an outstanding career in science. The book is required reading and belongs on the bookshelf of every person who claims even a passing interest in fish diseases.



Publication of Ken's text marked the culmination of a long and distinguished career in fish health research which began in 1954 at the Eastern Fish Disease Laboratory, Leetown, West Virginia. His early investigations showed that blue sac disease was caused by a buildup of metabolic wastes and that the antibiotic erythromycin was effective in control of bacterial kidney disease. However, it was in the fields of fish virology and fish cell culture that Ken made major contributions. Using primary cell cultures Ken and co-workers isolated IPN virus in 1957 and developed the first continuously cultured fish cell line, RTG-2, in 1960. Over the next 15 years Ken's research contributions included establishment of additional fish cell lines, development of methods to detect IPN virus in carrier fish, determination of the viral cause of lymphocystis and isolation of viruses from eel and bluegill.

He received international recognition and influenced other investigators to come to the Eastern Fish Disease Laboratory to study fish virology and fish cell culture. Two Japanese and one German scientist each studied for a year, one Japanese for 2 years, and numerous other investigators studied for periods of several days or months. From 1972 to 1977 Ken served as Director of the Eastern Fish Disease Laboratory. During that time he not only directed research but also provided the scientific requirements for the new National Fish Health Research Laboratory. In 1977 Ken returned to full time research as a senior research scientist and together with his co-worker, Mrs. Maria Markiw, began research on solving the life cycle of whirling disease. Ken has received many awards for his work; among them are the Department of Interior's highest honor, the Distinguished Service Award; Emeritus Membership Award from the Wildlife Disease Association; Distinguished Alumni Award from Utah State University; and the S.F. Snieszko Distinguished Service Award.

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## FHS OFFICERS AND COMMITTEES 1988-1989

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 Ron Hedrick, Immediate Past President  
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\*Emmett Shotts, Chair  
 (*Streptococcus, Lactobacillus*)  
 \*John Hawke  
 (*Edwardsiella ictaluri*)  
 \*Yolanda Brady (CCV)  
 Phyllis Barney  
 \*Cliff Starlipper (*Flexibacter*,  
 gill diseases)  
 Howard Jackson  
 Ron Hedrick  
 \*Diane Elliott  
 (*Aeromonas salmonicida*)  
 \*Robert Durborow  
 (Warmwater parasites)  
 \*Roselynn Stevenson  
 (*Yersinia ruckeri*)  
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#### Long Range Projects & Planning

Ron Hedrick  
 Standing Committee Chairs

#### Scientific Journal

Bill Rogers, Chair  
 John Plumb  
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#### Blue Book Field Advisory

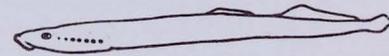
John Thoesen, Chair  
 \*Scott LaPatra (IHNV)  
 \*Jack Frimeth  
 (Coldwater parasites)  
 Chris Horsch  
 Diane Elliott  
 Steve Roberts (*Renibacterium*)  
 Jack Ganzhorn (*Vibrio*)

## SNIESZKO WILL NAME FISH HEALTH SECTION

In their will, Dr. and Mrs. S.F. Snieszko have named the Fish Health Section as benefactor to a cash gift of approximately \$30,000. Dr. Snieszko, former Director of the Eastern Fish Disease Laboratory — now the National Fish Health, Research Laboratory in Leetown, West Virginia, was born near Krakow, Poland, and immigrated to the United States in 1939. A renowned scientist and popular administrator throughout his life, Dr. Snieszko was active in helping students and fellow scientists around him. His writings are widely cited in the scientific literature. He and Mrs. Snieszko also were active supporters of wildlife conservation societies.

The will, administered by the Bank of Charles Town In West Virginia, states that the principal is to be divided equally among 5 benefactors including the Charles Town General Hospital, the University of West Virginia, Father Flanagan's Home, Boys Town, Nebraska, The Berkeley County Human Society for the dog shelter and care of the dogs, and the American Fisheries Society for the use of the Fish Health Section.

The Fish Health Section members will make decisions on the allocation of our portion of the bequest. At the annual FHS meeting this year in July at Annapolis, Maryland, the EXCOM committee will discuss options for appropriate distribution of the interest from a permanent fund. Suggestions from EXCOM members include a trust for funding foreign memberships to the AFS/FHS, scholarships for support of undergraduate and graduate students, and assistance for student travel to the FHS meetings. Members of the FHS are encouraged to give constructive suggestions concerning the direction of these funds. Please write or talk to a member of the EXCOM if you want to express your views on the use of this fortunate gift.



### PASSAGES

Keith Johnson has moved from Alaska and now is a member of the pathology group for the Idaho Department of Fish and Game. His new address is: Rt. 1, Trout Road, Eagle, ID 83616. Telephone 208-939-2413.

\*\*\*\*\*

Jerri Bartholomew changed her name (from Hoffmaster) and her address. She is now employed by USIWS at the National Fisheries Research Center, Building 204, Naval Support Activity, Seattle, WA 98115. Telephone 206-526-6592.

\*\*\*\*\*

Leni Oman is now employed by the Northwest Indian Fisheries Commission. Her new address is 6730 Martin Way East, Olympia, WA 98506. Telephone 206-438-1180.

\*\*\*\*\*

Louis Leibovitz has retired from his position with the New York State College of Veterinary Medicine, Cornell University. During his early career, Dr. Leibovitz was active in avian medicine, primarily in the area of infectious diseases of ducks. In 1973, he moved from the Long Island Duck Research Laboratory to the Cornell University campus to initiate a program in Aquatic Animal Medicine. In 1981, he became Director of the Laboratory for Marine Animal Health, located at the Marine Biological Laboratory, Woods Hole, MA. This laboratory, operated jointly by the College of Veterinary Medicine, Cornell University and the School of Veterinary Medicine, University of Pennsylvania under an NIH grant, provides a center of expertise to the Woods Hole scientific community and elsewhere, particularly to those individuals using marine invertebrates as their biomedical research animal. Lou remained at this position until his retirement on January 1, 1989. Also, on that date, Lou was conferred the title of Professor Emeritus of Aquatic Animal Medicine by the Board of Trustees of Cornell University. We wish Lou all the best in his retirement years.

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\*Designates Disease Committee Network Chairs

## NOMINATIONS SOUGHT FOR THE S.F. SNIESZKO DISTINGUISHED SERVICE AWARD

The S.F. Snieszko Distinguished Service Award is the highest award presented by the Fish Health Section of the American Fisheries Society. Dr. S.F. Snieszko was dedicated to excellence in research, teaching and service in the fish health sciences and the first recipient of the Distinguished Service Award. The purpose of this award is to recognize a fish health scientist(s) for their continued outstanding contributions to the field of fish health.

The individual(s) to be considered for the Distinguished Service Award must be nominated by a current member of the Fish Health Section. The person nominating the individual for the award is expected to solicit and obtain six letters of recommendation from fish health scientists in support of the nominee. These letters shall address the candidate's dedication to research, teaching and service. Letters of recommendation should be sent to the nominator. These six letters should be accompanied by a letter of nomination that clearly states the qualities of the candidate and the specific reason(s) he or she is being nominated for this award. A current curriculum vitae for the candidate should also be obtained and sent with the nomination packet directly to the current chairperson of the Awards Committee. It is suggested that persons nominating or providing letters of support for candidates maintain confidentiality throughout the process.

Nominations should be submitted before March 30, 1989 to this year's Award Committee Chair, Dr. G.L. Bullock, National Fish Health Research Laboratory, Box 700, Kearneysville, WV 25430.



## NEW ENGLAND PROCEEDS TOWARD FISH HEALTH POLICY

Rod Getchell  
Maine Department of Marine Resources  
Fish Research Laboratory  
West Boothbay Harbor, ME 04575

The New England Atlantic Salmon Committee adopted the "Proposed New England Salmonid Health Guidelines" at its last meeting on October 4, 1988. Further action on these guidelines will be taken at a future date, after each natural resources agency has had an opportunity for public or further in-house review.

The proposed guidelines are the product of the best fish health technical people from all the New England agencies and, while there were disagreements on certain specific points, the proposed draft represents a consensus of the fish health subcommittee. It will be the responsibility of the individual natural resources agencies in the various states that have authority over importations, transfers, fish stocking, etc. to implement and enforce these guidelines. The proposed guidelines will affect every producer, no matter how small, if they hold, rear, or release live salmonids in either marine or fresh waters of New England. Currently, many of the small producers do not have annual fish health inspections unless they transfer fish to or from the marine environment or ship to other states where inspections are required.

With the exception of Maine, all of the other New England states currently depend upon the USFWS to provide fish health inspection services. At present, these services in the New England area may not be adequate to meet the future needs in the commercial/private sector.

Hopefully, the public review process now mandated will allow input from the aquaculture industry. Only with the support of the industry and committed agencies will these guidelines become meaningful. Also, a clear understanding of the fish health inspection workloads, who will provide the services, and the costs must be made before individual agencies can begin to implement the provisions of these proposed guidelines.

## NEW JOURNAL OF AQUATIC ANIMAL HEALTH PROGRESSING

Bill Rogers, John Grizzle, and John Plumb, the co-editors of the new Journal of Aquatic Animal Health, report that 91 manuscripts have been submitted for publication. Approximately 80 of these were a result of the international meeting which was held in Vancouver, B.C. Of the 91 manuscripts, 59 have had peer review and 34 of them returned to the authors for revision. Five manuscripts have already been sent to Bob Kendall of the AFS for final editing; an additional seven papers will be submitted to Kendall soon. Rogers anticipates that Volume 1(1) of the quarterly journal will be available in June 1989.

The editors request additional manuscripts which may be submitted to Dr. W.A. Rogers, Department of Fisheries and Allied Aquacultures, Auburn University, Auburn, AL 36849 USA.

## THE USE OF FISH MUCUS IN THE DETECTION OF INFECTIOUS HEMATOPOIETIC NECROSIS VIRUS (IHNV)

Scott LaPatra  
Oregon Department of Fish and Wildlife  
Department of Microbiology  
Oregon State University  
Corvallis, Oregon 97331

In the fall of 1987 an infectious hematopoietic necrosis (IHN) epizootic was detected in yearling chinook salmon (*Oncorhynchus tshawytscha*) at Clackamas Hatchery (Clackamas River, Oregon). Mortality was moderate and chronic and fish displayed erratic swimming behavior not previously observed. Typical pathological signs associated with IHN were not observed and virus could not be detected in any organs other than gill and brain and concentrations of  $10^4$  to  $10^6$  plaque forming units (pfu)/g were routinely detected in nervous tissue. Because IHNV is a rhabdovirus similar to rabies virus, the tropism of IHNV for nervous tissue has been examined by many investigators. Rabies is usually transmitted from an infected animal to another by inoculation of saliva-containing virus as a result of bites. Analogous to the pathogenesis of rabies, we speculated that possibly IHNV was being shed in mucus. Examining mucus from naturally and experimentally infected juveniles of different species and virus strains, IHNV was routinely detected in concentrations ranging from  $10^1$  to  $10^5$  pfu's. Experimental evidence showed this to be a result of the pathogenesis of IHNV and not a result of virus present in the water being concentrated by mucus. Recent immunohistochemical studies of waterborne virus exposed juveniles showed viral antigen present in the epidermis but not in the gill and 1 and 2 d post-infection and the portal of entry of virus was questioned (Yamamoto et al. 1988). Examination of gills and mucus from juvenile fish exposed to waterborne IHNV ( $10^3$  pfu/mL) or intraperitoneally injected ( $10^3$  virions) showed virus present 24 h post-infection in 100% of mucus specimens and 50% of gill tissues in the group which was exposed via the water. The injected group had a 60% prevalence of virus in both. Concentrations up to  $10^5$  pfu's were detected in mucus. Other investigators have shown feces and urine contain low virus concentrations relative to virus detected in water (Nishimura et al. 1988). Possibly the mucus shed from infected fish could be a source of this waterborne virus.

Detection of IHNV in carrier adults is usually done by inoculating susceptible cell cultures with reproductive fluids (e.g. ovarian fluid) or tissues (e.g. kidney or spleen). Examination of reproductive fluids and tissues collected from a population of infected adult salmon showed 50% of the females and males to be IHNV carriers. When mucus collected from the external surface of the same fish was tested, 96% of the females and 83% of the males were IHNV-positive. Concentrations of virus detected in mucus ranged from  $10^1$  to  $10^5$  pfu's. These observations were repeated in other wild and hatchery salmonid adults infected with IHNV. Current investigations are designed to determine the role of the integument in the pathogenesis of IHNV and to examine the potential of fish mucus to be a simple and non-lethal source of virus for health monitoring.

## TWO MORE SPECIES ARE SUSCEPTIBLE TO EXPERIMENTAL INFECTIONS WITH *EDWARDSIELLA ICTALURI*

D. V. Baxa and R. P. Hedrick  
Department of Medicine  
School of Veterinary Medicine  
University of California  
Davis, CA 95616

The first reported epizootic in channel catfish (*Ictalurus punctatus*) caused by the Gram-negative bacterium *Edwardsiella ictaluri* in California was reported by Chen (Cal. Fish and Game, in press) in the summer of 1987. Further studies on the bacterium were initiated in our laboratory following this outbreak. Of particular interest were the potential effects of the bacterium on nonictalurid fishes that might be affected in a mixed sport fisheries.

Three species of commercial aquaculture importance were tested in parallel immersion challenges with juvenile channel catfish. Groups of white sturgeon (*Acipenser transmontanus*) with a mean weight of 30.0 g, striped bass (*Morone saxatilis*) of 6.6 g, and chinook salmon (*Oncorhynchus tshawytscha*) 36.6 g, and channel catfish of 2.0 g were exposed to  $10^8$  bacterial cells/ml for 30 seconds and then placed into 35 gal aquaria receiving well water. Water temperature was held constant at 25 C except for the salmon which were held at 20 C, a temperature often experienced in rearing facilities in California. An identical group of each species was treated in the same manner but exposed only to uninoculated BHI broth.

Mortalities were picked daily and the kidneys cultured on BHI to detect the presence of bacteria. Isolates recovered from the kidney cultures were subsequently tested for their biochemical properties and agglutination with hyperimmune rabbit serum prepared to *E. ictaluri*.

No deaths were observed in the control groups of any of the four species of fish or among white sturgeon or striped bass exposed to the bacterium. In contrast, 32% of the channel catfish and 75% of the chinook salmon succumbed to infections with *E. ictaluri* over a 14 d period. The bacterium was recovered in pure cultures from a majority of the mortalities in both groups. Gross external signs of a severe gram negative septicemia were evident in both the catfish and chinook salmon that died during the experiment.

In a second study, chinook salmon (38 - 40 g) were challenged by immersion in serial log dilutions of the bacterium. A concentration of  $2.8 \times 10^6$  cells was sufficient to kill 50% of the chinook (LD<sub>50</sub>). Similar results were obtained when 5 - 8 g rainbow trout (*O. mykiss*) were exposed to the bacterium.

Microscopic changes detected in salmonids infected with *E. ictaluri* were most evident in the liver and kidney. Hepatocytes were condensed and necrotic and the interstitium of the kidney showed diffuse, moderate to severe necrosis suggestive of an acute infection.

These results clearly indicate that *E. ictaluri* is a potential pathogen of salmonid fishes. The implications are of significance for fisheries management since certain reservoirs which lie above salmon hatcheries are stocked with channel catfish for sport fishing. Sanchez and Plumb (1983) injected five selected warm water species of fish with the bacterium. Although tilapia (*Sarotherodon aureus*) were infected, they concluded that of the five species tested only channel catfish are reproducibly susceptible to *E. ictaluri* infections. Our observations extend the experimental host range to at least two additional species of salmonid fish.

## BLUE BOOK REVISION

Revision of *The Procedures for the Detection and Identification of Certain Fish Pathogens*, of the *Fish Health Blue Book* is progressing. The enclosed questionnaire was developed to gain a wide input from the Section's membership. Your thoughts and ideas will influence the form and scope of the *Blue Book*. Please respond soon.

## UNIFORMITY OF *EDWARDSIELLA ICTALURI* ISOLATES IS CORROBORATED BY GENETIC ANALYSIS

C.E. Starliper and W.B. Schill  
National Fish Health Research Laboratory  
Box 700  
Kearneysville, WV 15430

Genetic analysis of isolates of *Edwardsiella ictaluri* was conducted by measuring genetic variation of enzymes. Results indicated a high degree of genetic homogeneity among this species.

Although it is less than two decades since *E. ictaluri* was originally described as a distinct species, this bacterium has already evolved into a serious pathogen of cultured catfish. The bacterium has also been isolated from such fishes as the danio (*Danio devario*), green knife fish (*Eigenmannia virescens*), and the tetra (*Rasbora heteromorpha*), but the disease caused by this agent, enteric septicemia of catfish, remains a disease principally of ictalurids. Because the bacterium has a significant economic impact on the intensive culture of catfish throughout the southern United States, research has been conducted to establish a more comprehensive understanding of the mechanisms by which this pathogen causes disease. Characterization of the inherent genetic variability of this bacterium is imperative to this understanding.

Genetic analysis revealed a high degree of genetic uniformity among different isolates of this species. The multilocus isozyme electrophoresis (MIE) technique provides a method to distinguish minor genotypic variations which may not be apparent in conventional biochemical and serological studies. Whole-cell sonicates were prepared from 33 *E. ictaluri* isolates, electrophoresed in hydrolyzed starch gels, and stained to detect activity of a particular enzyme or enzyme system (locus). Genetic diversity was estimated by observing the presence or absence of enzyme activity. If activity was noted, the relative migration distances of the enzyme in test strains were quantified. Twenty-three enzyme loci were assayed. Identical enzyme mobility patterns were noted at eight of the loci. No activity was detected among the isolates at another eight of the loci, and minimal variation in activity was observed at the remaining seven loci.

The extremely low amount of genetic variability detected among isolates of *E. ictaluri* suggests that different isolates of the bacterium are highly uniform regardless of their source of isolation. These results corroborate and even explain the studies of other researchers who have reported that little variation exists in plasmid, biochemical, protein, and serological profiles of *E. ictaluri*. Uniformity among *E. ictaluri* isolates is encouraging for future research directed at: registration of new chemotherapeutants to control the disease; development of a vaccine to prevent the disease; and the use of serodiagnostic methods that allow rapid and reliable identification of the pathogen.

## FISH HEALTH MANAGEMENT COURSE

Haywood Community College, in cooperation with the U.S. Fish and Wildlife Service will sponsor a course in Fish Health management, March 13-17, 1989. Instruction is designed to give trout growers and hatchery workers basic information needed to maintain a practical fish health management program on their operation.

Included in the course will be basic prevention of infectious and noninfectious diseases through wise management during all stages of fish growth, recognition of fish health problems, treatment calculations and applications, and the use of chemicals and antibiotics. "Hands on" fish health evaluation will be conducted during lab and field sessions.

The course instructor will be Rick Nelson, Regional Fish Health Biologist in charge of the U.S. Fish and Wildlife Service's Fish Disease Control Center in LaCrosse, WI.

A \$15.00 registration fee will be charged on March 13. For more information or to pre-register, contact: Charles W. Johnson, Fishery Training Specialist, Haywood Community College, Freedlander Dr., Clyde, NC 28721. (704) 627-2821.

## SPHAEROSPORES OBSERVED IN THE KIDNEY OF CHANNEL CATFISH (*ICTALURUS PUNCTATUS*)

J.M. Groff, T. McDowell and R.P. Hedrick  
 Department of Medicine  
 School of Veterinary Medicine  
 University of California  
 Davis, CA 95616

At least 36 species of myxosporeans in the genus *Sphaerospora* have been described. A majority are found as parasites in the kidney and urinary bladder of freshwater fishes although six species are known from marine fish. The vegetative or extrasporogonic stages of certain species can cause severe inflammatory responses in the host. Swim bladder inflammation and gill sphaerosporosis in cyprinid fishes and proliferative kidney disease in salmonids are examples of the severity of the host's immune response associated with the vegetative or extrasporogonic (trophozoite) stages of *Sphaerospora* spp. There are currently no reports of sphaerospores in ictalurid fishes although MacMillan et al. (AFS/FHS abstract of Vancouver meeting, 1988) suggested affinities between stages of the myxosporean found in channel catfish with "proliferative gill disease" and those of *Sphaerospora renicola* of cyprinid fishes.

In October of 1988, channel catfish (*Ictalurus punctatus*) alevins (mean wt. 1.0 g) were submitted to the Fish Disease Diagnostic Laboratory at the University of California, Davis. The fish were suffering a mortality attributed to a monogenetic trematode infestation. An examination of stained tissue sections of these catfish however, showed a concurrent infection with a *Sphaerospora* sp. Blood stages similar to those described by Lom et al. (J. Fish Dis. 8:221-232, 1985) for other species of genus *Sphaerospora* were observed (Fig 1). Similar forms were found in the vasculature in the liver (Fig. 2), the interstitium of the kidney (Fig. 2), brain, skin, spleen and gut (not shown). Sporogonic forms were observed in the lumen of the kidney tubules of four or five fish examined (Fig. 3). One, and occasionally, two spores were present within the

enveloping cell or pseudoplasmodia. The thin valves of the spore surrounded two spherical polar capsules lying in a plane perpendicular to the suture line. Mature spores released from the pseudoplasmodium were seen in wet mounts of kidney on a second examination of fish from the same population 10 d later (Fig. 4). The typical sphaerospores were 6.5  $\mu$  (n = 6, std. dev. = 0.3) in width by 5.8  $\mu$  (n = 6 std. dev. = 0.7) in length which are comparable to the sizes of sphaerospores found in the kidneys of many species of fish including those from trout (Hedrick et al. J. Protozool. 35: 13-18, 1988) and cyprinid fishes (Lom et al. 1985). Blood stages similar to those reported here were described by MacMillan et al. (1988) but they did not report seeing sporogonic forms or spores.

The catfish we examined were hatched in well water and then moved to circular tanks receiving pond water (temperature approximately 72 F). The fish were sent to the laboratory two weeks after exposures to pond water according to the grower. Several fish from this same population also had trophozoites of a myxosporean in the gill stroma eliciting an inflammatory response identical to that associated with "ham-burger" or "proliferative gill disease", PGD (Fig. 5 and 6). As MacMillan et al. (1988) reported, the early stages of a myxosporean parasite can be found in the gills of catfish within 10 h after exposure to water known to contain the infectious stages of PGD. The stages they described from the kidney, liver and spleen of catfish are similar to the "unidentified blood organisms" (UBOs) Csaba (1976) found in common carp that were later shown by Lom et al. (1983) to be vegetative stages of the myxosporean, *S. renicola*. We suspect that the sporogonic stages of the parasite observed in alevin catfish we examined may have been more abundant because of the young age of the fish on exposure to the parasite and the cooler water temperatures. Both of these factors may reduce the severity of the immune response that under other circumstances might prevent sporulation of this myxosporean. Although we have no direct proof of the relationship of the various stages of the *Sphaerospora* sp. we observed in our channel catfish, the similarities and coincidence of these forms to the parasite causing PGD are remarkable.

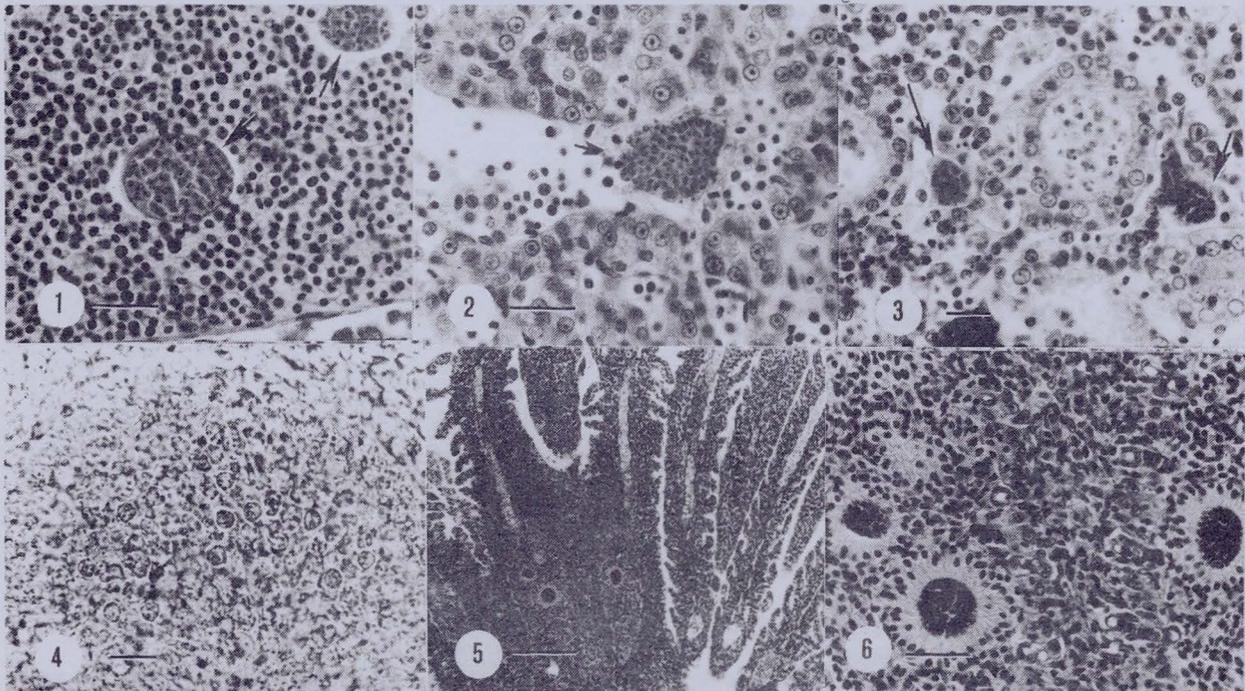


Figure 1 and 2. Myxosporean developmental stages, presumed to be stages of a *Sphaerospora* sp., found in blood sinuses of kidney and liver of alevin channel catfish. H and E stain.

Figure 3. Developmental stages and sporogonic forms in the kidney interstitium (arrows) and lumen of the tubules of channel catfish. H and E stain.

Figure 4. Spores of a *Sphaerospora* sp. found in the lumen of the kidney tubules of alevin channel catfish. Wet mount.

Figure 5 and 6. Stages of proliferative gill disease found in alevin catfish with *Sphaerospora* sp. infections. H and E stain.

Bars = 20  $\mu$ m except for Figure 5 where the bar = 100  $\mu$ m.

## ACRIDINE ORANGE AS A DIFFERENTIAL STAIN FOR BLOOD CELL VIRUSES

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Acridine orange (3,6-bis dimethylaminoacridine) was used to stain blood smears from fish with erythrocytic inclusion body syndrome (EIBS) and viral erythrocytic necrosis (VEN). Blood smears were fixed in 1:1 ethanol/methanol for 10 min, followed by successive rinses (2 min each) in 100%, 70%, and 50% ethanol. The smears were then stained with a 0.1% aqueous solution of acridine orange for 5 min, washed with water, and optionally mounted with glycerol and a coverslip. Acridine orange differentiates double stranded nucleic acid, which fluoresces bright green, from single stranded nucleic acid which fluoresces scarlet. Because of this property, the stain differentiated VEN from EIBS without the use of electron microscopy. The inclusion bodies resulting from EIBS stained bright red while VEN inclusions, containing the double stranded DNA virus, were bright green. The stain could also be used to differentiate cellular debris and artifacts, which do not fluoresce, from EIBS inclusion bodies.

In addition to its use as a diagnostic reagent, acridine orange has provided some insight to questions concerning EIBS and its causative agent. Because the inclusion bodies, which electron microscopy has shown to contain mature virions, fluoresce red, it can be concluded that the genome of the EIBS virus is single stranded nucleic acid. This evidence, in addition to data which indicate that the virus is enveloped and observed only in the cytoplasm, suggests the single stranded nucleic acid is RNA.

## BRIEF REPORTS

A bait-fish farmer lost several thousand golden shiner minnow (*Notemigonus crysoleucas*) two days after treatment of a pond with Karmex (Diuron) to control aquatic vegetation. Gross examination revealed severe swelling of the gills. Histologic examination of specimens showed severe inflammation and hyperplasia of the gills with numerous Myxosporean-like cysts containing spores, in addition to fungal hyphae and spores resembling *Ichthyophonus* sp. These hyphae stained positive with PAS and GMS stains. No other organs appeared to be affected. Mortalities ceased without treatment and subsequent samples taken 14 days later showed nearly total regression of the gill lesions. Evidence of parasitic and mycotic infection was also greatly reduced. This represents the first reported case of *Ichthyophonus* in Mississippi. Chris Wilson, College of Veterinary Medicine, Drawer V, Mississippi State, MS 39762. Telephone 601-325-3432.

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AFS members with a computer terminal and a modem can connect to the American Fisheries Society Computer User Section electronic bulletin board and read bulletins, announcements, and mail as soon as they are posted. The Board has been running as a service to Section members since January 1988.

The Board is available by dialing (313) 996-1456 with your computer modem and signing on. If you have problems connecting, voice help is available from SYSOP Tony Frank weekdays at (313) 994-3331 from 8 am to 4 pm (Central Time). There is a questionnaire for new users to fill out online, and then you are free to explore the board, read and respond to messages, or to download files. Help is available at any junction by typing H at the prompt. An upcoming issue of FISHERIES will feature full instructions in moving around the board, but with help readily available online, the board is easy to explore on your own.

Any AFS subunits who wish to open a conference area should contact the SYSOP. This offers the opportunity for a private conference, away from the main traffic areas, to conduct Excom, Division or Section business. Phyllis Barney, Newsletter Editor, AFSCUS.

## A PICORNA-LIKE VIRUS FROM SALMONID FISHES IN CALIFORNIA

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Examinations of salmonid fish from several locations in northern California have revealed the presence of a newly recognized virus. The virus has been isolated from ovarian fluids of cutthroat trout (*Oncorhynchus clarkii*), rainbow trout (*O. mykiss*), brown trout (*Salmo trutta*) and brook trout (*Salvelinus fontinalis*). There were no signs of disease in any of the adult fish examined nor was the virus isolated from progeny examined from any of the adults from which the agent was recovered.

The cytopathic effect (CPE) of the virus is characterized by areas of diffuse necrosis in CHSE-214 cells. The virus shares many physical properties with a recently discovered picorna-like viruses from smelt (*Osmerus mordax*) and Atlantic salmon (*Salmo salar*) but does not induce syncytia in CHSE-214 cells as the latter two agents. Viral particles appear to have an icosahedral symmetry and have a mean diameter of 37.5 nm (n = 10, st. dev. 0.41).

Initial studies in young rainbow trout, kokanee salmon (*O. nerka*) and chinook salmon (*O. tshawytscha*) show the virus does not cause any detectable disease. The agent is being further examined for its biochemical properties and antigenic relationship to the other two known picorna-like agents from fish.

## STRESS IN LAKE TROUT REARED IN GAS SUPERSATURATION CAN BE MEASURED BY IMMUNE RESPONSE

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Gas supersaturation (sum total of the individual dissolved gases exceeding 100% saturation) has been implicated as a fish stressor at sublethal (nonacute) levels; however, determination of threshold levels cannot be clearly determined by growth and survival measurements. To measure the effect of chronic stress on fish, we tested the antigen:antibody reaction of lake trout (*Salvelinus namaycush*) by injecting them with a nonfish pathogen, *Salmonella* H antigen. We found that the lake trout's ability to produce antibody was reduced when they were held in supersaturated water.

Lake trout were reared in glass aquaria for 7 months with water temperature maintained at 8.5 C and water flow maintained at 1L/min. After 5 months exposure, 40 fish 7-cm long were injected with 0.1 ml of 0.85% saline as a control and placed in one side of a divided 80-L tank. Another 40 fish were injected with *Salmonella* H antigen suspended 1:10 in 0.85% saline and were placed in the other side of the tank. Each treatment included 3 replicate tanks at 10 levels of supersaturation. Pooled serum samples were serially diluted, from  $\Delta$ P13 to  $\Delta$ P81, and *Salmonella* antibody was added, forming antigen:antibody agglutination reactions. Antibody titers peaked 60 days after injection.

Ability of lake trout to produce antibody was reduced at gas levels above  $\Delta$ P29. Immune response seems to be a useful tool for measuring the general state of health of lake trout exposed to chronic levels of gas supersaturation. These results indicate an inability of lake trout to form high antibody titers in response to *Salmonella* H antigen at prolonged exposures to supersaturation above  $\Delta$ P29.

## PUBLICATIONS AVAILABLE

**Fish Vaccination.** A.E. Ellis (Ed.). Academic Press, London. 1988. 255 pp. \$39.95.

This book represents a very successful effort to review the current state of the art in fish vaccination. The first five chapters summarize knowledge about general principles of vaccination, the ontogeny of the fish immune system, optimizing vaccination for fish, commercial production of fish vaccines, and strategies for vaccination of fish. The body of the book consists of 14 chapters on vaccine development for: vibriosis, enteric redmouth, furunculosis, *Aeromonas hydrophila*, bacterial kidney disease, *Edwardsiella tarda*, *Edwardsiella ictaluri*, infectious pancreatic necrosis, virah haemorrhagic septicemia, infectious hematopoietic necrosis, spring viremia of carp, channel catfish virus, protozoan and helminth parasites of fish, and a final chapter on immune control of sexual maturation. In all, the chapters are well written by authorities in the field and present what is known regarding the current status of vaccines for each of the specific diseases.

In general, the chapters follow a standard format that includes information about: the disease problem, the ideal vaccine and its strategic use, the nature of the present vaccines, overcoming the limitations, commercial prospects, conclusions, and references. Naturally, the extent of this material varies greatly for the various diseases as does the status of vaccine development. While commercially successful vaccines exist for the control of vibriosis and enteric redmouth, vaccines for preventing bacterial kidney disease are a long way in the future. Although the book is extremely current (including 1988 references), it seems likely that it will have to be updated within five years to keep pace with the rapid progress in this important field. In summary, the book provides a well-organized review of the current knowledge of fish vaccines and should be of interest to researchers and to fish culturists alike.

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**Textbook of Fish Health, Revised and Expanded Edition.** George Post. 1987. T.F.H. Publications, Neptune City, New Jersey. 288 pp. \$29.95.

This 1987 revision of the first edition of this book has helped to correct the major deficiency of the previous volume. Because the manuscript for the first edition was submitted in 1978 but the book was not released until 1983, the first edition was effectively out of date at time of publication. This new revision, submitted in early 1987, contains additional material, and, more importantly, is more current (some 1986 references). There are 38 more pages in this revised and expanded edition and the new color plates are of much better quality providing 178 useful color photographs. The book now includes material on proliferative kidney disease and a better section on whirling disease. While improved, the lack of certain references in several areas suggests that not all of the book was revised (e.g. the discussion of the role of the Fish Health Section cites the second edition of the "Blue Book" instead of the 1985 third edition, the overview of fish viruses fails to cite a 1984 review by Wolf, and no mention is made of the papers, published since 1981 by Kimura et al., on *Oncorhynchus masou* virus, one of the more important viral diseases of salmonid fish). This criticism aside, in all, the book has been substantially improved and should serve as an appropriate textbook for undergraduate students in Fisheries courses involving fish health.

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**Fish Disease Leaflets (FDL)** are available from Technical Information Services, at the National Fisheries Center, U.S. Fish and Wildlife Service, Box 700 Kearneysville, WV 25430. For those that are not familiar with the FDL's, they are comprehensive descriptions of most of the commonly recognized fish diseases. These leaflets, which are usually less than 10 pages long, contain a description of the disease, its etiologic agent, host and geographic range, methods of control, etc. Currently, 78 have been published.

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The North Central Division - AFS has just published the proceedings of the Missouri River Symposium — The Missouri River The Resources Their Uses and Values, Special Publication No. 8. This publication describes the past history of the Missouri River, energy flow, economic development, regulation of mainstem reservoirs, mitigation, and more. The publication is available for \$7.00 from Vaughn L. Pragman, Dept. of Natural Resources, Manchester Trout Hatchery, R.R. #2, Box 269, Manchester, IA 52057

## EMPLOYMENT OPPORTUNITIES

POSTDOCTORAL POSITION available immediately to assist in defining host responses to parasites, with the goal of developing a vaccine against an ectoparasitic protozoan of fish. Applicants with relevant experience in cell culture, immunological and biochemical techniques will be given preference. Knowledge of fish parasitic diseases desirable, but not essential. Position is renewable annually, for up to three years. Send a CV and three letters of reference to: Dr. Edward J. Noga, College of Veterinary Medicine, North Carolina State University, 4700 Hillsborough Street, Raleigh, NC 27606 USA

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RESEARCH MICROBIOLOGIST. The National Fish Health Research Laboratory is planning to hire a Research Microbiologist in the near future. A Ph.D. in Microbiology and in-depth training in Biochemistry and Immunology are desired. Interested individuals should contact Dr. G.L. Bullock, National Fish Health Research Laboratory, Box 700, Kearneysville, WV 25430

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POSTDOCTORAL POSITION IN MOLECULAR VIROLOGY/BIOLOGY. Successful applicant will work on two existing funded projects in which we are: 1) developing diagnostic procedures for several virus diseases of cultured marine shrimp with immunological and recombinant DNA methods; and 2) using traditional microbiological methods with molecular methods to determine the fate and persistence of genetically altered biological control agents (insect pathogens) in multispecies aquatic laboratory aquaria. Salary is \$19,193 to \$23,032 per calendar year. Closing date: when suitable candidate is found or March 1, 1989, whichever is sooner. Contact or send curriculum vitae and three references to Dr. D.V. Lightner or S. Jurmain, Environmental Research Lab, Univ. of Arizona, 2601 E. Airport Dr., Tucson, AZ 85706. Telephone: (602) 741-1990. The Univ. of Arizona is an Equal Opportunity Employer.

## BLUE BOOK REVISION

Revision of *The Procedures for the Detection and Identification of Certain Fish Pathogens*, of the *Fish Health Blue Book* is progressing. The enclosed questionnaire was developed to gain a wide input from the Section's membership. Your thoughts and ideas will influence the form and scope of the *Blue Book*. Please respond soon.

## FUTURE EVENTS

### WESTERN FISH DISEASE WORKSHOP

The 30th Annual Western Fish Disease Workshop will be held at the Rosario Resort and Spa on Orcas Island in Washington on June 21-23, 1989. The meeting will be hosted by Wayne Brunson of the Washington Department of Wildlife. Sessions will commence at 1 pm on the 21st and end at noon on the 23rd. Further information on accommodations and program format will be available soon.

### ANNUAL MEETING OF THE FISH HEALTH SECTION and EASTERN FISH HEALTH WORKSHOP

The Maryland Department of Natural Resources—Annapolis, the University of Maryland, Department of Microbiology—College-Park, and the National Fish Health Research Laboratory—Leetown, WV are the hosts of this year's national meeting. The joint meeting will be held at the Ramada Hotel in Annapolis, MD on July 17-20, 1989. Registration forms, hotel information and call for papers have been sent to the membership of the Fish Health Section. If you have not received this information and would like it, contact Dr. Frank Hetrick, Department of Microbiology, University of Maryland, College Park, MD 20742 USA. Telephone 301-454-5411.

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## FISH HEALTH NEWSLETTER

The Fish Health 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 and should be addressed to one of the editorial staff or to a member of the publication committee.

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**FHS NEWSLETTER**

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